SOIL CHEMISTRY OF HEAVY METALS UNDER CONTRASTING VEGETATION COVERS

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Pollok Park – The site of investigation

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Author's Declaration

Except where specific reference is made to other sources, the work presented here is the original work of the author. It has not been submitted, in part or in whole, for any other degree. Some of the results may have been published elsewhere.

Vicki MacIver

Abstract

The site of study (Pollok Park, Glasgow) features soil under both a coniferous canopy (predominantly Corsican Pine) and a deciduous canopy (predominantly Beech). There is a clear segregation between the two vegetation types which enables a direct comparison into the contribution of canopy type to the heavy metal distribution in soil.

Average total metal contents of cores extracted from the two soil types indicated that the deciduous cores have a greater total content of Cr, Cu, Fe, and Mn, and that the two core types contain approximately the same Pb and Zn content. Concentration profiles for Cr, Cu, Fe, Mn, Pb and Zn were established for the two soil types. They showed enrichment of Cr, Cu, Pb and Zn at the surface. Correlation graphs (LOI vs. metal (Cr, Cu, Pb and Zn) showed there to be a correlation between organic matter content and concentrations of these metals. Molar ratios (carbon : metal (Cr, Cu, Pb and Zn)) were estimated from the correlation graphs. In each case, these were found to be of considerable value, indicating that perhaps these metals are complexed by large organic molecules. Mn and Fe showed no enrichment to the surface and no correlation with organic matter.

Speciation studies (Modified BCR sequential extraction procedure and cupric ISE investigations) were conducted on the surface soil (top 5cm), litter and leaves taken from below both vegetation types (deciduous and coniferous) in order to gain an understanding of soil-metal associations. The deciduous and coniferous soils were found to have virtually identical fractionation patterns of Cr, Cu, Pb and Zn (Modified BCR sequential extraction). Cr was distributed between the oxidisable and residual phases, Cu was found predominantly in the oxidisable fraction, Pb existed mainly in the reducible fraction, and Zn was mainly found in the residual fraction. The fractionation patterns corresponded well with those reported in the literature.

Cupric ISE investigations involved adding Cu to soil, litter and leaf samples. All samples were measured for their total carbon content. Molar ratios (carbon : Cu) were established from these investigations, and were found to be of considerable size, thereby indicating that each Cu atom is probably complexed by a large organic molecule. The cupric ISE investigations enabled a picture of Cu complexation in the litter-leaves-soil system. The following order (starting with greatest affinity) was established: soil > litter (quickly saturated) > leaves (virtually no complexation).

The development of agarose gel electrophoresis for the fractionation and isolation of organic-metal complexes was studied with the aim of isolating components from the two soil types. However, attempts proved to be unsuccessful.

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List of Abbreviations

Atomic Absorption Spectroscopy	AAS
Background Corrected Atomic Absorption Spectroscopy	BCAAS
Cumulative, Anthropogenic, Atmospheric Pb	CAAPb
Certified Reference Material	CRM
Community Bureau of Reference	BCR
Dissolved Organic Matter	DOM
Effective Cation Exchange Capacity	ECEC
Flame Atomic Absorption Spectroscopy	FAAS
Fulvic Acid	FA
Hubbard Brook Experimental Forest	HBEF
Humic Acid	HA
Hydrochloric Acid	HCl
Hydroxylammonium hydrochloride	NH_2OH HCl
Inductively Coupled Plasma-Mass Spectrometry	ICP-MS
Ionic Strength Adjustor	ISA
Ion Selective Electrode	ISE

James McLean Oliver Ecological Centre	JMOE
Laboratory of the Government Chemist	LGC
Low Molecular Weight	LMW
Loss On Ignition	LOI
Municipal Soil Waste	MSW
Polyethylene	PE
Polytetrafluoroethylene	PTFE
PolyAcrylamide Gel	PAG
PolyAcrylamide Gel Electrophoresis	PAGE
Sodium nitrate	$NaNO_3$
Sodium Dodecyl Sulfate	SDS
Tris-borate-EDTA, pH 8.5	TBE
Tris(hydroxymethyl)aminomethane	TRISMA
Tris(hydroxymethyl)aminomethane hydrochloride	TRIZMA

All website references are referenced by a superscripted number.

Chapter 1 - Introduction

1.1 Heavy Metals in the Environment

Natural heavy metal sources such as weathering tend to introduce heavy metals in small quantities only. High concentrations of heavy metals are normally only found when anthropogenic sources are responsible (Alloway, 1990).

The main anthropogenic sources of heavy metals are various industrial point sources, including present and former mining activities, foundries and smelters, and diffuse sources such as combustion. Relatively volatile heavy metals and those that become attached to air-borne particles can be widely dispersed on very large scales. Heavy metals conveyed in aqueous and sedimentary transport enter the normal coastal biogeochemical cycle and are largely retained within near-shore and shelf regions.

As trace elements, some heavy metals are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination, high ambient air concentrations near emission sources, or intake via the food chain.

Soil fulfils a multiple role in most ecosystems. It provides plants with essential major and minor nutrients, with water and with firm anchorage. It acts as a sink for organic detritus and for natural and pollution inputs from the atmosphere. It regulates the solute chemistry of freshwaters to make the appropriate environments to support aquatic life. If properly managed, soil continues to fulfil all these roles. Proper management requires an understanding of soil chemistry (Cresser et al., 1993).

The heavy metal concentrations inherited from the soil parent material are modified by pedogenic and biogeochemical processes, by natural inputs such as dust particles derived

from soil, rocks and volcanic ash and most importantly, by anthropogenic inputs (Alloway, 1990). As mentioned previously, soil is a sink for many substances, including heavy metals. However, it is important to understand the chemistry of soils in the event that the soil conditions change and pollutants such as heavy metals cease to be retained within the soils, and are instead mobilized.

Heavy metals can be associated with various soil components that differ in their ability to retain or release metals. In general, there are six soil components which are classified according to their physico-chemical behaviour (McBride, 1994). These components are:

- Soluble ions and inorganic and organic complexes in soil solution.
- Exchangeable.
- Stable organic complexes in humus.
- Adsorption by hydrous oxides of Mn, Fe and Al.
- Adsorption on the clay-humus colloidal complex.
- Crystal lattice-bound in soil minerals.

The above points apply, in varying degree, for all metals. For example, Cu can be complexed with organic matter, adsorbed onto the surfaces of clays, Fe and Mn oxides, and present in the lattice of primary silicate minerals or secondary minerals. The distribution of Cu among these various components can be defined as the fractionation of soil Cu. This fractionation will strongly influence the mobility and hence, the bioavailabilty of Cu. These will thus depend on those chemical properties of the soils that are likely to govern the fractionation of Cu, such as pH, redox potential, the content and

nature of organic matter, clays and metal oxides, and cation exchange capacity (Chaignon et al., 2003; McBride, 1989).

1.2 Chemistry of Heavy Metals Studied

1.2.1 Chemistry of chromium (Cr)

Chromium exists as either Cr(III) or Cr(VI) in the environment. The main human activities that increase the concentrations of Cr(III) are steel, leather and textile manufacturing.[1] The common range for Cr in soils is 1-1,000 mg kg^{-1} (Lindsay, 2001). However, in areas of contamination, values can reach up to 10,000 mg kg^{-1} (McGrath & Smith, 1990).

Cr(VI) is toxic to both plants and animals, being a strong oxidising agent, corrosive and a potential carcinogen. Concentrations as low as 0.50 mg l^{-1} in solution and 5 mg kg^{-1} in soil can be toxic to plants. In contrast, Cr(III) is not toxic to plants and is necessary in animal nutrition for glucose metabolism (Fendorf, 1995).

As stated previously, chromium exists as either Cr(III) or Cr(VI) in the environment; these two ions have sharply contrasting chemical properties. The toxic Cr(VI) ion exists as an anion such as chromate or dichromate and is very mobile in soils. Whereas Cr(III) is much less mobile in soils and adsorbs to particulates more strongly, and precipitates as Cr(III) oxides (McGrath & Smith, 1990).

In the trivalent state, chromium readily forms compounds such as $Cr(OH)_3$. The solubility of Cr(III) decreases above pH 4, and above pH 5.5 complete precipitation occurs. The incorporation of Fe(II) or Fe(III) into a Cr(III)-containing compound lowers the solubility of Cr even further. Chromium(VI) is the more stable form in equilibrium with atmospheric oxygen. However, Cr(VI), with its high positive reduction potential, is a strongly

oxidising species, and in the presence of organic matter and ferrous iron (common soil reductants), Cr(VI) is reduced to Cr(III). Reduction is more rapid in acid than alkali soils. Thus in the majority of soils, the relatively insoluble and less mobile Cr(III) form predominates and it generally occurs as insoluble hydroxides and oxides. Manganese oxides are the only inorganic oxidants found in the environment that cause the rapid oxidation of Cr(III) to Cr(VI) (McGrath & Smith, 1990; Rai et al., 1989).

The mobility of Cr(III) can be enhanced by complexing Cr(III) with soluble organic acids, especially those containing carboxyl groups (e.g. oxalic, citric and tartaric acids). These chelates maintain Cr(III) in solution above the pH at which uncomplexed Cr precipitates. However, in highly organic or sewage sludge amended soils this is not the case, as the molecular complexes which result are very stable and insoluble, therefore making Cr(III) extremely unavailable (McGrath & Smith, 1990).

1.2.2 Chemistry of copper (Cu)

The main human activities that contribute to copper release are mining, landfills and waste disposal. The world's use of Cu is still rising, and as a result the incidences of Cu pollution are increasing.[1] The common range for Cu in soils is 2-100 mg kg^{-1} (Lindsay, 2001). However, in areas of contamination, values can reach up to 1,200 mg kg^{-1} (Ross, 1994).

Cu is one of the important, essential elements for plants and animals. In plants, Cu functions as part of the prosthetic group of enzyme systems, and as a facultative activator of enzyme systems. However, high Cu concentrations can have a detrimental effect on many plants, and as a result, only a limited number of plants have a chance of survival on Cu-rich soils. This is why there is not much plant diversity near Cu-disposing factories. Due to the effects upon plants, Cu is a serious threat to the productions of farmlands. It is difficult to construct a human diet with less than 1 mg Cu / day. Under normal conditions Cu is a benign agent to humans. However, for humans with hereditary Cu toxicosis known

as Wilson's disease, the Cu-binding ligands involved in Cu homeostasis are deficient and toxic levels accumulate in several tissues (Baker, 1990).[1]

There are two forms of available ions; $[Cu(H_2O)_6]^{2+}$ in acid soils, and $[Cu(OH)_2]^0$ in neutral and alkali soils (Baker, 1990). The associations of Cu with soluble and insoluble organic matter are the strongest, followed by those with Fe and Mn oxides, soil silicate clays and other minerals.

The COO^- groups present in the solid and liquid phase organic matter form stable ligands with Cu. Solid phase ligand formation is considered responsible for Cu deficiencies in organic soils and for decreases in Cu toxicity to plants from the addition of a high organic matter source to a high Cu-containing substrate. Clearly the rate of decomposition of organic matter is a significant consideration, due to soil organic matter being the dominant factor controlling Cu retention. The situation is complicated further by the fact that the activities of bacteria and actinomycetes are suppressed as soil pH declines, as this results in organic matter accumulating in acidic soils and complexing strongly with available Cu (Cresser et al., 1993).

1.2.3 Chemistry of iron (Fe)

The majority of Fe present in soils originates from the parent materials (Bodek, 1988). However, Fe can also be released into the environment as a result of human activities such as coal mining. Compared to other trace elements, the concentration of Fe in soils is considerable, and commonly ranges from 7,000-550,000 mg kg^{-1} (Lindsay, 2001).

Fe is one of the important, essential elements for plants and animals. In plants it functions as part of the prosthetic group of cytochromes which function as electron carriers.[1] In humans it is an essential part of haemoglobin; the red colouring agent of the blood that transports oxygen through our bodies. Fe occurs naturally in soils as oxides. Fe oxides are

not hazardous, but they do affect other metals, as they are involved in the attenuation of most trace and heavy metals. In soils and sediments where conditions are not strongly reducing, adsorption by solid Fe oxides is one of the most important controls on the distribution of trace elements. In an aerobic sediment or water system, the metal bonding may be almost irreversible. If the oxides are dissolved by reduction, the adsorbed metal may be released and appear in solution, or they may be precipitated as some other phase, such as a sulfide (Bodek, 1988).

Redox potential and pH are the main controls on the solubility of Fe in soils:

- Fe(III) is only soluble at pH <3 and stable under aerobic conditions (>120 mV).

- Fe(II) is only soluble at pH <7 and stable under anaerobic conditions (<120 mV).

However, the concentration of Fe in soils also depends on the formation of Fe-organic complexes, which can raise the solution concentration of Fe (Bodek, 1988; Wild, 1988).

1.2.4 Chemistry of manganese (Mn)

Soils derive virtually all their Mn content from the parent materials. In soils, Mn commonly occurs as oxides. At elevated levels, Mn is toxic in plant and animal systems. However, environmental pollution problems are relatively insignificant compared with those associated with some other heavy metals. For animals, Mn is an essential component in enzymes that are used for carbohydrate, protein and fat metabolism. In plants, Mn deficiency causes disturbances in a number of mechanisms.[1] The concentration of Mn in soils commonly ranges from 20-3000 mg kg^{-1} (Lindsay, 2001).

Manganese has three possible oxidation states in soils: +2, +3, and +4. The most reduced form of Mn, the Mn^{2+} ion, is the only stable form in soil solution. Both Mn^{3+} and Mn^{4+} are stable only in the solid phase of soils, where they form insoluble oxides and hydroxides.

The Mn^{2+} ion is released from these solids by spontaneous dissolution or cation exchange, especially under acidic or reducing conditions (McBride, 1994).

Manganese solubility is controlled by the redox potential and pH of the soil:

- Mn^{2+} is soluble at low pH (< 4.5) and under anaerobic conditions (< 200 mV).

Small changes in the soil redox potential or pH can shift the Mn^{2+}-Mn precipitate reaction. Clearly there is a close link between Mn solubility and the redox potential of the soil. Low pH or low electrode potential favours the reduction of insoluble Mn oxides and an increased solubility of Mn^{2+}. As a result, Mn solubility within any particular soil can fluctuate tremendously over time, sometimes ranging from deficient to toxic levels (McBride, 1994; Smith, 1990).

1.2.5 Chemistry of lead (Pb)

Most Pb concentrations that are found in the environment are a result of human activities such as mining and smelting, sewage sludge and vehicle exhausts. Lead is poisonous and there is no significant evidence that Pb plays any essential role in either plants or animals (Davies, 1990).[1] The concentration of Pb in soils commonly ranges from 2-200 ppm (Lindsay, 2001). In areas of contamination, values can reach up to 20,000 mg kg^{-1} (Ross, 1994).

Pb exists principally in the +2 oxidation state in soils and is typically found as an impurity in minerals. Under oxidising conditions, the Pb^{2+} ion becomes less soluble as soil pH is raised. Complexation with organic matter, chemisorption on oxides and silicate clays, and precipitation as the carbonate, hydroxide, or phosphate are all favoured at higher pH. In alkaline soils, solubility may increase by formation of soluble Pb-organic and Pb-hydroxy complexes. The Pb^{2+} ion has a particularly high affinity for Mn oxides. Pb is one of the

the least mobile heavy metal in soils. As expected from the strong complexation of Pb^{2+} by organic matter, Pb bioaccumulates in the humus-rich surface layer of soils (McBride, 1994).

1.2.6 Chemistry of Zinc (Zn)

The main human activities that contribute to the increasing incidences of Zn pollution are human activities such as mining, coal and waste combustion, and steel processing.[1] The common range for Zn in soils is 10-300 mg kg^{-1} (Lindsay, 2001). In areas of contamination, values can reach up to 80,000 mg kg^{-1} (Ross, 1994).

Zinc is an essential trace element for humans, animals and higher plants. As a component of a large number of enzymes, it is involved in carbohydrate and protein metabolism.[1]

Zn exists in the +2 oxidation state in soils and is typically found as an impurity in minerals. In acid, aerobic soils, Zn has medium mobility, held in exchangeable forms on clays and organic matter. At higher pH, however, chemisorption on oxides and aluminosilicates and complexation with humus lower the solubility of Zn^{2+} markedly. Consequently, Zn mobility in neutral soils is very low. If soils are slightly alkaline, Zn-organic complexes can become soluble and raise mobility. In strongly alkaline soils, Zn-hydroxy anions may form to increase solubility (Kiekens, 1990; McBride, 1994).

1.3 Biological Processes Influencing / Affected by Heavy Metals in Soil

In natural environments the elements follow cycles from soil to plant to soil (De Nicola et al., 2003). There are biological processes involved throughout, and these can be subdivided into the following:

- Vegetation.

- Litter.

- Microorganisms.

1.3.1 The biological influences of vegetation on metal distribution

Vegetation can influence the fate of metals in ecosystems in three ways (Ross, 1994):

- The canopy characteristics of plants influence the way that metal aerosols are trapped, and the shape and geometric configuration of leaves influence how wet and dry metal deposition is accumulated.

- Plant roots alter their immediate environment through exudation, and small changes in soil pH and organic matter status in the rhizophere.

- Different species, genera and families of plants show differential metal uptake.

1.3.1.1 Canopy characteristics

In general, canopies can capture metals by either interception of wet and dry deposition or by trapping of particles and aerosols by leaves. Several characteristics of vegetation canopies, leaves and stems will influence the way that they trap incoming metal aerosols. For example, conifer canopies are better aerosol trapping structures because their fine needle leaves, dense canopy branching and year-round foliage provide a very large surface area. Similarly, dry deposition to a forest canopy is much higher than to an open pasture because of the greater leaf surface area. Leaf surface characteristics also play an important role in capturing and trapping metal aerosols (Ross, 1994).

There are two components of canopy concentration:

- Accumulation of leaf deposition from the atmosphere, followed by washing.

- Leaching of ions from within the leaves.

The flux of elements in throughfall (atmospheric deposition and leachate from leaves) is an important pathway in the cycling of nutrients within forest stands (Parker, 1983; Turkey, 1980). Different forest stands vary in their nutritional requirements, and as a result, different canopies will have varying effects on the elemental concentration of atmospheric deposition / rainfall; due to leaching concentrations of trace metals from within their leaves which are dependent on their nutritional requirements. For example, the effect of the tree canopy on element concentrations in throughfall varies between conifers and broadleaves as well as between individual species (Hyvarinen, 1990; Nieminen et al., 1999; Turkey, 1980)

Compared to above canopy concentrations in bulk rainfall, it was found that Pb, Cd, Cu and Fe were all concentrated below the canopy, in throughfall, by a factor of around 2.5-5. This indicates that the contribution of Pb, Cd, Cu and Fe to the throughfall from the leaves leachate was considerable, when compared to the bulk rainfall. Mn and Cr above : below canopy concentration factors were higher, at 8-10 and 15 respectively. This indicates that the contribution of Mn and Cr to the throughfall from the leaves leachate was very considerable, when compared to the bulk rainfall (Grosch, 1986). Throughfall under conifers frequently shows higher acidity than under deciduous trees and this may be the reason for enhanced throughfall concentrations of trace metals under spruce canopies (Nihlgard, 1970; Ross, 1994).

Estimations of the contribution of dry deposition and canopy interaction processes to the deposition associated with throughfall content have been attempted by several authors.

However, the mechanisms involved in the processes are still not well known (Hansen, 1996). The primary source of an element in net throughfall will be influenced by local and regional sources of dry deposition as well as individual forest characteristics. In general, dry deposition is important for elements with known atmospheric sources resulting from combustion processes, mines, smelters, etc. These include Cu, Zn and Pb (Rea et al., 2001). La, Ce and and Al are crustal elements which have no nutritional value in plants and, as such, their source in throughfall is attributed to wash-off of wind blown soil dust. Foliar leaching is thought to be the primary source of the biologically active elements such as Mn and Cu, and elements that behave similarly to nutrients such as Rb, Sr and Ba (Potter, 1991).

1.3.1.2 Rhizosphere Characteristics

The roots of plants and trees influence the availability and uptake of metals from soil through small changes to soil conditions in the rhizosphere (Ross, 1994). The two main rhizosphere influences are slightly lower pH and increased quantities of different organic molecules in the zone of exudation.

It has been shown that small changes to pH in the rhizosphere can make trace metals more available for uptake (Sarkar, 1982). In general, the pH tends to be lower in the rhizosphere than in the bulk soil, (Hinsinger, 2003; Seguin, 2004). A major process that contributes to root-induced pH changes in the rhizosphere is the release of charges carried by H^+ and OH^- to compensate for an unbalanced cation-anion uptake at the soil-root interface.

The influence of different organic compounds in the rhizophere is complex. Mycorrhizal fungi and bacteria, as well as plant roots, all contribute organic molecules to the rhizophere, by exudation (Ross, 1994). Examples of organic molecules exuded from roots include organic-acid anions such as citrate, malate and oxalate, and amino acids. Siderophores are examples of organic molecules secreted by rhizosphere bacteria and

mycorrhizal fungi, they are iron-binding organic molecules. These exuded organic compounds can bind with metals in the rhizosphere and make them unavailable for uptake (Cumming, 1990; Morselt, 1986), increase the availability of trace metals for uptake by roots (Grayston et al., 1997; Ryan, 2001; Shen et al., 2002) and release other essential nutrients such as phosphorus from metal complexes, in order for plant uptake of these essential nutrients through the roots.

Siderophores bind with metals in the rhizosphere and make them unavailable for uptake. The following study by Shen et al. (2002) highlights how organic compound exudates can function in soil-plant systems to make metals and other nutrients available for uptake. Although soils normally contain very high levels of phosphorus, the majority is normally unavailable to plants as it is held in insoluble complexes. Shen et al. (2002) studied the common bean and how it combats low levels of phosphorus by exuding citrate. The exuded citrate mobilizes phosphates from Fe and Al complexes by forming stable complexes with Fe and Al, therefore releasing the phosphate for uptake by the roots of the common bean.

1.3.1.3 The effect of species on metal uptake

Different plant species display significant variation in their ability to uptake metal. This has been shown in various studies (Dahmani-Muller et al., 2000; Nedelkoska & Doran, 2000; Pulford & Watson, 2003). For examples, numerous experiments have shown that food crops take up different quantities of potentially toxic metals when grown under identical conditions. The results of some of these experiments are displayed in Table 1-1.

Metal	Relative metal uptake	Source
Cd	lettuce > radish > carrot > spinach > cauliflower > oats > pea	(John, 1973)
Cu	kidney beans > lettuce > peas > potatoes	(Purves, 1985)
Pb	lettuce > cabbage > carrots > radish	(Alloway & Morgan, 1986)
Zn	lettuce > kidney beans > peas > potatoes	(Purves, 1985)

Table 1-1 Relative metal uptake by a range of different crop plants growing in contaminated soil

In general, the studies on food crops found that many dicotyledenous crop plants such as spinach or lettuce, absorbed more heavy metals than monocotlyedonous crop plants such as oats or wheat (Ross, 1994).

Other studies have found a wide range of higher plant species to possesss adaptations that enable them to survive and to reproduce in soils heavily contaminated with Zn, Cu, Pb, Cd, Ni and As (Baker, 1987; Dahmani-Muller et al., 2000). Such species are divided into two main groups: the so-called pseudometallophytes that grow on both contaminated and non-contaminated soils, and the absolute metallophytes that grow only on metal-contaminated and naturally metal-rich soils (Baker, 1987). Depending on plant species, metal tolerance may result from two basic strategies: metal exclusion and metal accumulation (Baker, 1981; Baker, 1987; Baker & Walker, 1990; Taylor, 1987). The exclusion strategy, comprising avoidance of metal uptake and restriction of metal transport to the shoots (De Vos et al., 1991), is usually used by pseudometallophytes. These plants are therefore currently used to re-vegetate bare soil areas (e.g. in phytostabilisation technology), i.e. where the lack of vegetation results from excessively high metal concentrations. The accumulation strategy consists of strong concentrations of metals in plant tissues. This implies a highly specialized plant physiology. This extreme level of metal tolerance in vascular plants is known as hyperaccumulation. Hyperaccumulators are defined as higher plants whose shoots contain > 100 mg Cd kg^{-1}, > 1000 mg Ni, Pb and Cu kg^{-1}, or > 10,000 mg Zn, and Mn kg^{-1} (dry wt.) when grown in metal-rich soils (Baker & Brooks, 1989; Baker et al., 1994). The use of hyperaccumulators for phytoextraction relies on their

ability to absorb metal contaminants from the soil and to translocate them to aerial plant parts.

1.3.2 The biological processes associated with litter

Hydrologically, litter at the soil surface provides a filter and controlling mechanisms for water infiltration into the mineral soil profile (Ross, 1994). Thick leaf litter layers absorb incoming rainfall and throughfall, slowing down water percolation into soil and providing time for metals in percolating water to be adsorbed by decaying litter and organic matter. Water absorption by dead leaves and litter thus reduces runoff and soil erosion. An intact layer at the soil surface, particularly in forest soils, provides soil protection and helps to prevent disruption of soil particles which is the start of soil erosion (Ross, 1990). The litter layer is also the repository for metals accumulated in leaves prior to shedding.

A number of authors have reported a build-up of litter on the forest floor in woodlands adjacent to metal pollution sources (Coughtrey et al., 1979; Jackson & Watson, 1977; Strojan, 1978) caused by reduced rates of organic matter decomposition. Strojan (1978) observed that metal contamination more strongly affected the later stages of litter decomposition. In an experiment to study the decomposition of contaminated Scots pine needles near a brass works, Berg et al. (1991) found that lignin decay was more sensitive to metal contamination than was whole litter. As in acidic forest litter layers, the lignin component of contaminated forest floors accumulates relative to the non-lignin tissues and becomes the rate limiting factor in litter decomposition (Berg et al., 1991).

1.3.3 The biological processes associated with soil microbes and mycorrhizal fungi

Studies have found possible damaging effects on soil microbes, due to long term addition of heavy metals to soil. For example, long term addition of heavy metal contaminated

sewage sludge to soil, results in the inhibition of soil processes such as nitrification and production of foliage with high metal content; processes that are normally maintained and regulated by microbes (Lepp & Eardley, 1978). Another study found that the abnormally large organic matter accumulation on the soil surface of an oak woodland ecosystem was the result of aerially deposited Cd and Zn. Under normal conditions, microbes normally ensure that organic matter is decomposed and therefore no accumulation results (Martin & Bullock, 1994).

Mycorrhizal fungi operate in a symbiotic relationship with plant roots. However, reports have found that the mycorrhizal fungi do not operate as well in areas of heavy metal contamination. One group sampled fungi-innoculated willow and poplar roots from trees grown in Cu mine tailings and found no mycorrhizal associations had developed. Elevated quantities of Cu may have inhibited fungal infection (Harris & Jurgensen, 1977). Another group observed that heavy metals, particularly Cd and Ni, reduced mycorrhizal fungal colonisation rates of pine and spruce seedlings (Dixon & Buschena, 1988). Chappelka et al. (1991) investigated the effects of Pb on the mycorrhizal fungi colonisation of pot-grown loblolly pine. The fungitoxicity of Pb to certain strains of mycorrhizal fungi, as a result of increasing concentrations of soil-applied Pb, was demonstrated (Chappelka et al., 1991).

1.4 Forest Soils

A number of research groups have conducted studies which have shown forest soils to have certain characteristics associated with them. Taking two of these as specific examples:

- The Friedland group have found evidence for Pb mobility in forest floors (Kaste et al., 2003).

- The Andersen group have found that afforestation decreases soil pH and thus increases the solubility of Cd in the soil (Andersen et al., 2002).

1.4.1 Friedland group

1.4.1.1 Aim of the study

The aim of the study was to research findings that atmospherically deposited Pb is leaching from the forest floor to the mineral soil (Kaste et al., 2003).

1.4.1.2 Background to the study

Earlier investigations have documented the forest floor as being a net sink of atmospherically deposited Pb and suggested that Pb leaching from the forest floor to the mineral soil and thus to groundwaters and streams would be negligible over the next few decades (Friedland & Johnson, 1985). These studies estimated Pb residence times in the forest floor to be in the order of hundreds of years due to the high affinity of Pb to organic complexes. However, more recent investigations have documented a decrease in Pb concentrations and amounts in the forest floor (Friedland et al., 1992; Johnson et al., 1995) and suggested a net loss of Pb from the forest floor, presumably to the mineral soil below or to streams. It was reported that at five previously ploughed forested sites in New England, approximately 35% of the anthropogenically derived Pb was in the forest floor, with the remaining in the upper mineral soil (Marsh & Siccama, 1997).

The research by the Friedland group was conducted in the remote forest soils of the northeastern United States (Kaste et al., 2003). A significant amount of anthropogenic Pb was deposited on soils and surface waters in the northeastern United States throughout most of the 20th century. The dominant source of this Pb was the combustion of gasoline lead additive in automobile engines (Nriagu & Pacyna, 1988). Pb was emitted to the

atmosphere associated with aerosols and introduced to terrestrial ecosystems via rainfall and dry deposition.

The following points characterise the research site:

- The total deposition of Pb to remote forests in the northeastern United States during the 20th century is estimated at 10-40 kg ha^{-1}, depending on elevation and forest canopy type (Johnson et al., 1995; Miller & Friedland, 1994).

- Concentrations of Pb in the forest floor sampled across the north-eastern United States in the early 1980s ranged from 50 to 330 mg kg^{-1}, which is typically 1-2 orders of magnitude higher Pb concentration than resides in the parent material at these sites (Friedland & Johnson, 1985; Smith & Siccama, 1981).

- Atmospheric deposition of Pb in the United States declined rapidly after amendments to the Clean Air Act in 1977.

- Between 1975 and 1989, the annual volume-weighted mean concentration of Pb in bulk precipitation decreased from 23 $\mu g\ l^{-1}$ to 0.85 $\mu g\ l^{-1}$ at the Hubbard Brook Experimental Forest (HBEF) in New Hampshire (Johnson et al., 1995).

- Although atmospheric loading of Pb on surfaces in the United States has reduced considerably, a large pool of potentially labile Pb remains in these soils.

1.4.1.3 Study description

The intention of the study was to investigate findings from other studies which indicated that Pb in forest soils is residing in the mineral soil after being transported from the forest floor (Miller & Friedland, 1994; Wang & Benoit, 1997; Wang et al., 1995). The study used the isotopic composition (^{206}Pb / ^{207}Pb) of soil Pb and measurements of ^{210}Pb and

^{226}Ra to directly trace the transit of atmospherically deposited Pb in the soils profile. Pb isotopes provide an elegant way to trace the migration of Pb in soils, and at the same time, to distinguish between the migration patterns of natural (rock) lead vs. anthropogenic Pb (Erel et al., 1997).

1.4.1.4 Study findings

The study findings were as follows:

- In low elevation deciduous forests, approximately 65% of the original atmospheric Pb load has migrated from the forest floor to the upper 10 cm of the mineral soil.

- Higher elevation sites with coniferous vegetation have thicker forest floors, which have prevented significant amounts of Pb from entering the mineral soil. After seventeen years, the soil organic horizon in the coniferous zone prevented any penetration of the applied Pb into the mineral soil.

- Using ^{210}Pb budgets in different soil compartments, the forest floor response times for atmospherically delivered Pb to be approximately sixty years in the low elevation deciduous forest zone, and one hundred and fifty years for the high elevation sites with coniferous vegetation.

They concluded that a dispersed release of anthropogenic Pb to groundwater and surface water is possible this century, based on its distribution in the soils profile.

It has been reported that the high concentrations of Pb in the forest floor are attributed to the strong affinity of Pb for organic matter, and that it is this strong correlation which is thought to be responsible for the loss of Pb from the forest floor to the mineral soil (as reported by the Friedland group) (Bergkvist et al., 1989). This is due to the movement of Pb appearing to be strongly associated with the solubility and turnover of organic matter

(Bergkvist et al., 1989), for as the organic matter breaks down, perhaps the Pb is released. For example, the rapid breakdown of litter at the JMOEC (James McLean Oliver Ecological Centre) appears to promote an extremely rapid transfer of Pb to the underlying mineral soil (Watmough & Hutchinson, 2004).

1.4.2 Andersen group

1.4.2.1 Aim of the study

The aim of the study was to research the effects of afforestation on soil pH and thus Cd solubility in the soil (Andersen et al., 2002).

1.4.2.2 Background to the study

Large areas of intensively cultivated agricultural land are afforested in northern Europe to reduce both the agricultural production and the release of nutrients to the surrounding aquatic environment. Unfortunately, soil acidification following afforestation may cause mobilization of heavy metals in the soil, especially the toxic Cd that might be leached to streams and groundwater (Egli et al., 1999; Jug et al., 1999). In the afforested areas, lime is no longer applied and in addition an increased production of organic acids and atmospheric acid deposition causes a decline in pH, which increased Cd solubility. Although increased Cd solubility as a consequence of complexation with dissolved organic matter in the afforested soils seems negligible (Strobel et al., 2001).

Cadmium can be bound in soil by simple electrostatic forces or intimately associated with metal oxides, carbonates and organic matter. It is also found that the Cd solubility increases as pH decreases (Chlopecka et al., 1996; Christensen, 1989). A number of investigations have shown Cd solubility to be dependent on the cation exchange capacity (CEC), clay content, organic matter, and other metal ions present (Christensen, 1989; Ma

et al., 1997; McBride et al., 1997; Szakova et al., 1999; Wilkens & Loch, 1997). Cadmium release from soil increases substantially when pH drops below 4.5 (Bergkvist et al., 1989; McBride et al., 1997). This is especially true for sandy soils with low CEC, low acid neutralization capacity, and low ability of the subsoil to sorb Cd ions. Such soils may be vulnerable to Cd after afforestation.

The following points characterise the research sites:

- Arable land receives Cd from the application of fertilizers, lime, and to some extent from sewage sludge (Bak et al., 1997).

- Atmospheric deposition is also a substantial source of Cd to the soil. Atmospheric deposition of Cd is higher in forest areas compared with arable land (Wilcke et al., 1999).

- A considerable reduction of the atmospheric Cd deposition in Denmark has taken place during the last decade, but years of high atmospheric Cd deposition may have increased the total Cd content in the soil.

- Whether the Cd added to from the various sources results in a net loss or accumulation of Cd from the soil profile will depend on the soil parameters important to Cd solubility, for example pH and the amount of sorption sites available for Cd.

1.4.2.3 Study description

The intention of the study was to determine the effects of afforestation on Cd concentrations, solubility and distribution in soil. The Cd concentration in both soil and soil solution were determined in eleven pairs of Danish arable and afforested soil profiles.

1.4.2.4 Study findings

The study findings were as follows:

- The soil pH did not change or decrease with depth through the arable profiles, but did increase with depth in the forest profiles.

- Significantly higher Cd contents were found in the upper 30 cm of the arable soil compared with that of the forest soil.

- The total soil Cd concentrations correlated with the effective cation exchange capacity (ECEC), clay content, and organic matter content, but not the soil pH.

- The soil solution pH was unchanged or decreasing downwards through the arable profiles, but increasing with depth in the forest profiles.

- The soil solution concentration of Cd was significantly higher in the forest soils than in the arable soils.

- The Cd concentration in the soil solution decreased as pH increased.

It was concluded that afforestation may lead to higher soil solution concentrations of Cd as decreasing pH and ECEC diminish Cd retention and reduces Cd concentrations in the forest topsoils.

1.4.3 Summary

The Friedland and Andersen groups have demonstrated two specific characteristics associated with soils having a forest canopy. The Friedland group have shown that release of Pb from forest soils is a distinct possibility this century (e.g. Kaste et al. (2003)), and

the Andersen group have indicated that afforestation of soils can lead to increased concentrations of mobile Cd (e.g. Andersen et al. (2002)).

1.5 Monitoring Heavy Metal Contamination

1.5.1 Atmospheric emissions

Country	Year	Cr	Cu	Pb	Web reference
UK	1970	260	305	7500	2
	1984	170	150	7200	2
	1986	180	175	3500	2
	2002	45	50	100	2

Table 1-2 Heavy metal emissions (tonnes) per year in the UK

Table 1-2 presents emission data for Cr, Cu and Pb over the last thirty years in the UK. Clearly the emissions of these heavy metals have declined considerably.[2] This is due to the introduction of tight regulations that force industries to reduce heavy metal emissions. Pb atmospheric emissions will be focussed on to give an overview of the effect of these regulations.

The environmental distribution of Pb has been altered greatly by human activity. Since the inception of metal smelting over five thousand years ago, Pb has been widely disseminated throughout the environment due to its low boiling point and hence its large emissions to the atmosphere and potential for long-range transport (Urban et al., 1990). The increase in coal combustion and metal smelting associated with the industrial revolution (1750-1900) greatly increased Pb emissions and its subsequent dissemination throughout the environment (Murozumi et al., 1969; Shirahata et al., 1980). The use of Pb as a gasoline additive since 1920 caused a dramatic increase in atmospheric emission of Pb (Eddington & Robbins, 1976). The detrimental effects to human health of environmental contamination with Pb are now generally realised and substantial progress has been made in reducing emissions of Pb, and consequently in reducing concentrations and burdens in the environment.

For example, since 1970, Pb emissions have declined by 98%. The largest source is Pb from anti-knock Pb additives in petrol and it is here where the most significant reductions have been made. The Pb content of leaded petrol was reduced from around 0.34 g l^{-1} to 0.143 g l^{-1} in 1986, and since 1987, sales of unleaded petrol have increased, particularly as a result of the increased use of cars fitted with catalytic converters. Leaded petrol was phased out from general sale at the end of 1999, and consequently a decline in the road transport sector is seen. Other major sources are industrial processes, and iron and steel production. There has been some reduction in emissions from iron and steel production processes due to improved abatement measures. Emissions have also declined as a result of the decreasing use of coal. The large reduction in waste emissions is due to improved controls on municipal waste incinerators from 1997 onwards.

Other countries have introduced similar regulations with regard to heavy metal emissions, and as a result their emissions have reduced also.

Country	Year	Pb (tonnes)	Web reference
USA	1982	53,600	3
	2002	4,500	3
Finland	1990	325	4
	2001	40	4
Sweden	1990	480	4
	2001	10	4
Denmark	1990	120	4
	2001	4	4
Germany	1990	2300	4
	2001	500	4
Poland	1990	1400	4
	2001	600	4
Estonia	1990	230	4
	2001	30	4
Russia	1990	3500	4
	2001	2200	4

Table 1-3 Heavy metal emissions (tonnes) per year from various countries

Table 1-3 presents Pb emission data for various countries. Each country displays a considerable decline in Pb emissions as a result of heavy metal regulations. For example, Sweden emitted 480 tonnes of Pb in 1990 and only 10 tonnes of Pb in 2001.

The measurement of heavy metals in natural substances is a common means of monitoring heavy metal contamination, as illustrated in the following sections.

1.5.2 ^{210}Pb-dating and ^{206}/^{207}Pb ratios

^{210}Pb–dated cores from accumulating ombrotrophic peat deposits have been employed to characterise historical trends in atmospheric deposition of heavy metals and contaminants as far back as 300 years (Appleby et al., 1997; Clymo et al., 1990; Espi et al., 1997; Jones & Hao, 1993; Lee & Tallis, 1973; Livert et al., 1979; Martinez Cortizas et al., 2002; Martinez Cortizas et al., 2002; Shotyk, 1996; Shotyk et al., 1997; Shotyk et al., 1992; Weiss et al., 2002). As stated by Mackenzie et al. (1998), this approach is supported by:

- Well matched results from a wide range of peat deposits.

- Consistency of peat-derived data with corresponding information from lake sediment studies and from analysis of archived herbage samples (Bacon et al., 1996; Brannvall et al., 1997; Farmer et al., 1996; Farmer et al., 1997; MacKenzie et al., 1997; Sugden et al., 1993).

- Agreement between temporal variations in atmospheric depositional fluxes of contaminant metals inferred from ^{210}Pb-dated peat core data and known historical trends in industrial activity (Brannvall et al., 1997; Farmer et al., 1996; Lee & Tallis, 1973; MacKenzie et al., 1997; Martinez Cortizas et al., 1997; Shotyk, 1996; Weiss et al., 1997).

- Agreement between ^{210}Pb dating and independent peat chronologies (Appleby et al., 1997; Clymo et al., 1990; Shotyk, 1996; Shotyk et al., 1997).

- Consistency of peat core stable lead isotope data with trends in the use of lead, with a characteristic isotopic signature, as a petrol additive (Brannvall et al., 1997; Delves & Campbell, 1993; MacKenzie et al., 1997; Shotyk, 1996).

The use of peat concentration profiles for reconstruction of historical trends in depositional fluxes of contaminants is a widely used method for monitoring heavy metal contamination.

The use of variations in stable $^{206}Pb/^{207}Pb$ ratios has become a well established, diagnostic technique for characterising sources of contaminant Pb. In Scotland, most geological materials have $^{206}Pb/^{207}Pb$ atom ratios in the range 1.16 – 1.18 (MacKenzie & Pulford, 2002). For example, Pb from the Leadhills ore deposit in SW Scotland have a ratio of 1.173 (Sugden et al., 1993) and Scottish coals have an average ratio of 1.181 (Farmer et al., 1999). Thus in the period from the eighteenth century to the early twentieth century, when atmospherically deposited Pb was derived predominantly from coal burning and processing of indigenous ores, $^{206}Pb/^{207}Pb$ ratios were typically in the range 1.16 - 1.18 (Farmer et al., 1996; MacKenzie et al., 1997; MacKenzie et al., 1998). In contrast, Pb in tetra alkyl Pb, which was used as an antiknock additive to petrol from the 1920s until the end of 1999, was characterised by an average $^{206}Pb/^{207}Pb$ ratio of 1.09 (Sugden et al., 1993). Consequently, in the period from the 1920s to the late twentieth century, there was a general decrease in the $^{206}Pb/^{207}Pb$ ratio of atmospherically deposited Pb in response to the input of petrol derived Pb, with typical ratios in the late twentieth century in the range 1.12 – 1.14 (Farmer et al., 1996; MacKenzie et al., 1997; MacKenzie et al., 1998).

1.5.3 Atmospheric Deposition of Heavy Metals

Terrestrial lichens and mosses have been used successfully over the past thirty years to monitor and map atmospheric deposition of contaminants in different parts of the world (Adamo et al., 2003; Farmer et al., 2002; Fernandez et al., 2002; Grodzinska et al., 1990; Grodzinska et al., 1999; Ruhling & Tyler, 1970). Lichens and mosses are cryptogamic

organisms which occur in almost all terrestrial ecosystems and by virtue of their ability to tolerate long periods of drought may even colonise areas with extreme environmental conditions. Because of their high surface : volume ratio, the simple anatomy and absence of a cuticle, they accumulate heavy metals, concentrating them in tissues. Due to this ability, they may show an elemental composition which reflects, over the long term, the dissolved gases, particulate matter and metal ions of the atmosphere and can be considered important biomonitors of environmental pollution.

Table 1-4 (Cr, Cu and Fe) and Table 1-5 (Mn, Pb and Zn) present average concentrations ($mg\ kg^{-1}$) in mosses from countries around the world.

Location	Site Description	Cr (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Reference
Italy	Power plant	5.01	13.5	280	(Genoni et al., 2000)
Spain	Power plant	1.24	9.12	626.8	(Carballeira & Fernandez, 2002)
South-eastern US	Regional study (rural)	0.685	8.955		(Schilling & Lehman, 2002)
Czech Republic	Industrial area	6.61	10.15	1146	(Sucharova & Suchara, 1998)
Northern Spain	Regional study	2.68	6.86	868.2	(Fernandez et al., 2002)
Hungary	National study	2.8	11.8	2070	(Otvos et al., 2003)
Finland, 1985	National study	1.49	5.99	379	(Poikolainen et al., 2004)
Finland, 2000	National study	1.25	3.96	259	(Poikolainen et al., 2004)
Argentina	Cordoba city		15.5	186	(Carreras & Pignata, 2002)
Portugal	National study	3.86	9.10	1934.29	(Figueira et al., 2002)
China, 1960s	National study	28.6		6861	(Zhang et al., 2002)
China, 1990s	National study	1.68		805	(Zhang et al., 2002)
Italy	Naples city	4.1	12.9		(Adamo et al., 2003)
Poland	National study	1.50	7.6	362	(Szczepaniak & Biziuk, 2003)
Germany	National study	1.40	9.4	447	(Szczepaniak & Biziuk, 2003)
Czech Republic	National study	1.38	7.2	399	(Szczepaniak & Biziuk, 2003)
Sweden	National study	0.57	4.5	182	(Szczepaniak & Biziuk, 2003)
Norway	National study	1.05	5.2	331	(Szczepaniak & Biziuk, 2003)
Sweden, 1975	National study	1.62	7.5	430	(Ruhling & Tyler, 2004)
Sweden, 2000	National study	0.79	4.5	138	(Ruhling & Tyler, 2004)
France	National study	3.0	8	534	(Galsomies et al., 1999)
Poland	National study	1.8	10.7	448	(Grodzinska et al., 1999)

Table 1-4 Average Cr, Cu and Fe concentrations (mg kg^{-1}) in mosses from countries around the world

Location	Site Description	Pb (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Reference
Italy	Power plant	12.4	68	68.8	(Genoni et al., 2000)
Spain	Power plant	4.27		60.6	(Carballeira & Fernandez, 2002)
South-eastern US	Regional study (rural)	7.450			(Schilling & Lehman, 2002)
Czech Republic	Industrial area	20.44		115	(Sucharova & Suchara, 1998)
Northern Spain	Regional study	9.35		48.8	(Fernandez et al., 2002)
Hungary	National study	19.5		52	(Otvos et al., 2003)
Finland, 1985	National study	15.50		38.1	(Poikolainen et al., 2004)
Finland, 2000	National study	3.37		28.8	(Poikolainen et al., 2004)
Argentina	Cordoba city	2.58	48.2	29.0	(Carreras & Pignata, 2002)
Portugal	National study	21.77	187.51	52.48	(Figueira et al., 2002)
China, 1960s	National study			37.2	(Zhang et al., 2002)
China, 1990s	National study			18.0	(Zhang et al., 2002)
Italy	Naples city	9.1			(Adamo et al., 2003)
Poland	National study	13.6		43.0	(Szczepaniak & Biziuk, 2003)
Germany	National study	7.8		53.9	(Szczepaniak & Biziuk, 2003)
Czech Republic	National study	11.0		41.8	(Szczepaniak & Biziuk, 2003)
Sweden	National study	6.0		39.9	(Szczepaniak & Biziuk, 2003)
Norway	National study	5.8		37.2	(Szczepaniak & Biziuk, 2003)
Sweden, 1975	National study	47.5	290	58.4	(Ruhling & Tyler, 2004)
Sweden, 2000	National study	4.2	289	32.3	(Ruhling & Tyler, 2004)
France	National study			42	(Galsomies et al., 1999)
Poland	National study	17.3		48	(Grodzinska et al., 1999)

Table 1-5 Average Pb, Mn and Zn concentrations (mg kg^{-1}) in mosses from countries around the world

The values obtained from studies such as those presented in Tables 1-4 and 1-5 can be used to compare levels of contamination throughout the world.

1.5.4 Heavy Metal Concentrations in Soils

Tables 1-6, 1-7, 1-8 and 1-9 present heavy metal concentration values for urban (Table 1-6 and 1-7) and rural (Table 1-8 and 1-9) soils from around the world.

City	Cr ($mg\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Reference
Warsaw, Poland	32	31		(Czarnowska, 1980)
Hamburg, Germany	95.4	146.6	3600	(Lux, 1986)
Salamanca, Spain				(Sanchez-Camazano et al., 1994)
La Coruna, Spain	39	60		(Cal-Prieto et al., 2001)
Madrid, Spain	74.7	71.7	23078	(De Miguel et al., 1998)
Bangkok, Thailand	26.4	41.7	16100	(Wilcke et al., 1998)
Aberdeen, Scotland	23.9	27		(Paterson et al., 1996)
Glasgow, Scotland		97		(Gibson & Farmer, 1986)
London Boroughs, England		49		(Culbard et al., 1988)
Hong Kong	23.1	23.3		(Li et al., 2004)
Harjavalta, Finland		3528		(Nieminen et al., 2002)
Nanjing, China	88.6	117.3		(Lu et al., 2003)
Seville, Spain	39.4	68.2	20100	(Madrid et al., 2002)

Table 1-6 Average Cr, Cu and Fe concentrations ($mg\ kg^{-1}$) in urban soils from different cities in the world

City	Mn ($mg\ kg^{-1}$)	Pb ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	Reference
Warsaw, Poland	337	57	166	(Czarnowska, 1980)
Hamburg, Germany	750	218.2	516	(Lux, 1986)
Salamanca, Spain		53.1		(Sanchez-Camazano et al., 1994)
La Coruna, Spain		309	206	(Cal-Prieto et al., 2001)
Madrid, Spain	437	161	210	(De Miguel et al., 1998)
Bangkok, Thailand	340	47.8	118	(Wilcke et al., 1998)
Aberdeen, Scotland	286	94.4	58.4	(Paterson et al., 1996)
Glasgow, Scotland		216	207	(Gibson & Farmer, 1986)
London Boroughs, England		294	183	(Culbard et al., 1988)
Hong Kong		94.6	125	(Li et al., 2004)
Harjavalta, Finland		204	500	(Nieminen et al., 2002)
Nanjing, China		151.4	280.3	(Lu et al., 2003)
Seville, Spain	471	137	145	(Madrid et al., 2002)

Table 1-7 Average Mn, Pb and Zn concentrations ($mg\ kg^{-1}$) in urban soils from different cities in the world

City	Cr ($mg\ kg^{-1}$)	Cu ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Reference
Hong Kong		9.14		(Chen et al., 1997)
Hietajarvi, Finland		8		(Nieminen et al., 2002)
Switzerland	69.3	7.9		(Blaser et al., 2000)
Loiret, central France	13.92	2.01		(Hernandez et al., 2003)
Landes, south-west France	8.21	1.12		(Hernandez et al., 2003)
Ontario, Canada				(Watmough & Hutchinson, 2004)
China	21.89	25.43		(Lu et al., 2003)
Scotland				(MacKenzie et al., 1998)
Scotland		2	6470	(MacKenzie et al., 1998)

Table 1-8 Average Cr, Cu and Fe concentrations ($mg\ kg^{-1}$) in rural soils from around the world

City	Mn ($mg\ kg^{-1}$)	Pb ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)	Reference
Hong Kong		40.6	51.0	(Chen et al., 1997)
Hietajarvi, Finland		4	50	(Nieminen et al., 2002)
Switzerland		43	36.6	(Blaser et al., 2000)
Loiret, central France		29.77	18.46	(Hernandez et al., 2003)
Landes, south-west France		3.34	3.95	(Hernandez et al., 2003)
Ontario, Canada		24		(Watmough & Hutchinson, 2004)
China		17.49	75.15	(Lu et al., 2003)
Scotland		24		(MacKenzie et al., 1998)
Scotland	133		62	(MacKenzie et al., 1998)

Table 1-9 Average Mn, Pb and Zn concentrations ($mg\ kg^{-1}$) in rural soils from around the world

The values in tables 1-6, 1-7, 1-8 and 1-9 are based on total dissolution. Overall, these tables show a distinct trend between urban soils and rural soils, in that urban soils possess much greater heavy metal concentrations than rural soils. For example:

- An urban soil in Scotland was found to contain 216 mg kg^{-1} Pb (Gibson and Farmer, 1986), whereas a rural soil in Scotland was found to contain 24 mg kg^{-1} Pb (MacKenzie et al., 1998).

- An urban soil in Finland was found to contain 500 mg kg^{-1} Zn (Nieminen et al., 2002), whereas a rural soil in Finland was found to contain 50 mg kg^{-1} Zn (Nieminen et al., 2002).

1.6 Speciation

Contamination of soils with trace metals is a world-wide problem, affecting the safety of food and drinking water, the production of food, and the stability of ecosystems (Scheinost et al., 2002). Total concentrations of metals in soils are a poor indicator of metal toxicity since metals exist in different solid-phase forms that can vary greatly in terms of their availability (Krishnamurti & Naidu, 2002; Morera et al., 2001; Nolan et al., 2003; Peijnenburg et al., 1997; Schramel et al., 2000). Current government guidelines are based on total soil heavy metal concentrations rather than on heavy metal speciation in soils, this is due to speciation being difficult to assess (McLaughlin et al., 2000; Rieuwerts et al., 1998). However, regulators are moving towards a rationale based on speciation.

Numerous analytical techniques have been used to determine heavy metal speciation in soils. These include:

- Selective extraction.

- Ion selective electrodes (ISEs).

- Electrophoresis.

1.6.1 BCR extraction

There are a number of selective extraction techniques, one example is the BCR (Community Bureau of Reference) method. It is the standard European method and is very simple to follow.

1.6.1.1 Aim of the technique

The aim of the technique is to assess how strongly metals are retained in soil, what phases they are retained within and how easily they may be released into soil solution.

1.6.1.2 Background to the technique

The determination of specific chemical species or binding forms is difficult, and therefore methods for determination of broader forms depending on operationally-defined procedures can be a good compromise to give information on environmental risk (Quevauviller et al., 1997). As a result of this practicality, single and sequential extraction schemes have been designed for the determination of binding forms of trace metals in soils and sediment (Kaasalainen & Yli-Halla, 2003; Tessier et al., 1979); and have been increasingly used over the last 10 years. This concept has been described in detail (Forstner, 1993). The lack of uniformity of these schemes, however, did not allow the results to be compared world-wide or the procedures to be validated, which led to critical comments. All extractions are operationally defined in that the 'forms' of metals are defined by the determination of extractable elements using a given procedure (Ure, 1991). Therefore the analytical results obtained are directly related to the extraction scheme used. A further problem associated with extractions was the lack of suitable reference materials, which meant that the quality of measurements could not be controlled (Griepink, 1993).

Owing to the many pitfalls likely to occur in the use of extraction protocols for soil analysis, the European Commission through the Community Bureau of reference (BCR, now superseded by the Standards, Measurements and Testing Programme), launched a programme aimed at harmonising single and sequential extraction schemes for the determination of extractable trace metals in soil and sediment, respectively (Davidson et al., 1994).

This programme started in 1987 with the comparison of existing procedures tested in two inter-laboratory exercises (Ure et al., 1993). A series of inter-laboratory-studies followed on from these, and were successfully concluded by:

- The development of the BCR sequential extraction protocol.
- The development of single extraction procedures.
- The certification of soil and sediment reference materials (Quevauviller et al., 1997; Rauret et al., 2000).

1.6.2 Original and modified BCR extraction

The main outcomes set by the BCR when harmonising extraction methods were (Mossop & Davidson, 2003):

- To develop a simple, three-step sequential extraction protocol for the fractionation of trace elements in sediment (Ure et al., 1993).
- To develop a lake sediment reference material (BCR CRM 601) certified for metals extractable by the protocol (Quevauviller et al., 1997).

Step	Fraction	Nominal target phase(s)	Original BCR method	Modified BCR method
1	Exchangeable, water and acid soluble	Soluble species, carbonates, cation exchange sites	0.11 mol l^{-1} acetic acid	0.11 mol l^{-1} acetic acid
2	Reducible	Iron and manganese oxyhydroxides	0.1 mol l^{-1} hydroxylammonium chloride at pH 2	0.5 mol l^{-1} hydroxylammonium chloride at pH 1.5
3	Oxidisable	Organic matter and sulfides	Hydrogen peroxide followed by 1.0 mol l^{-1} ammonium acetate at pH 2	Hydrogen peroxide followed by 1.0 mol l^{-1} ammonium acetate at pH 2
4	Residual		Aqua-regia	Aqua-regia

Table 1-10 Comparing the original BCR selective extraction method with the modified BCR selective extraction method

Table 1-10 presents the original and modified BCR methods of extraction.

The original BCR procedure was also applied to soil (Vidal & Rauret, 1993) and, later, to a range of matrices, including sewage sludge (PerezCid et al., 1996). However, during certification of BCR CRM (certified reference material) 601, significant inter-laboratory variability was apparent, particularly in step 2 of the extraction. This resulted in there being only a few elements for which this certified soil was viable. As a result, this step of the protocol was re-evaluated (Sahuquillo et al., 1999) using CRM 601. This re-evaluation study lead to the development of a modified BCR sequential extraction procedure (Rauret et al., 2000; Rauret et al., 1999). The revised protocol involves use of an increased concentration of NH_2OH HCl (hydroxylammonium hydrochloride), 0.5 mol l^{-1} rather than 0.1 mol l^{-1}; and lower pH, 1.5 instead of 2. It is thought that this alteration improves reproducibility due to a more efficient dissolution of the reducible fraction of the soil matrix, most probably the iron oxyhydroxide phase.

A comparative study was conducted on a variety of soils and sediment substrates, to compare the performance of original and modified BCR protocols (Mossop & Davidson, 2003). The study found that step 2 of the revised BCR sequential extraction extracts more Fe than the equivalent step of the original BCR extraction, for several soil and sediment

substrates. This suggests that, although developed on the basis of one certified reference material (CRM 601), the modified procedure should provide an improved attack on the Fe-based components of the reducible matrix for a range of soils and sediments. This is supported by Ure who found that acidified 0.1 M acidified hydroxylamine hydrochloride (NH_2OH HCl) releases metals from the manganese oxide phase with little attack on the Fe oxide phase, but increasing the hydroxyalamine concentration increases the dissolution of metals associated with the iron oxide phases (Ure, 1996). No clear improvement in precision was obtained with use of the modified protocol. However, it is possible that this would be more evident in inter-laboratory trials where potential sources of variability are greater than in the present work.

The BCR sequential extraction protocol is now a widely used technique in defining speciation in a range of matrices, as illustrated by the increasing number of scientific papers making reference to it (Andersen et al., 2002; Davidson et al., 1998; Davidson et al., 1994; Rauret et al., 2000). Bacon et al. (2005) made a key find when they were studying the reproducibility of the BCR sequential extraction procedure in a long-term study of the association of heavy metals with soil components in an upland catchment in Scotland. They found that the distribution of metals between each of the extracted fractions varied not only between each metal, but also between each of the three soils studied; clearly indicating that both metal and soil / sediment influence the metal distribution (Bacon et al., 2005). Their finding is supported in the literature by data resulting from the BCR sequential extraction procedure (Andersen et al., 2002; Davidson et al., 1994; Fernandez et al., 2004; Fuentes et al., 2004; Rauret et al., 2000; Sutherland & Tack, 2002).

1.6.3 BCR extraction in use

The BCR sequential extraction consists of three steps and an additional step, which gives rise to four different fractions (Mossop & Davidson, 2003; Tokalioglu et al., 2000) as shown in Table 1-11:

Step	Reagents	Target phase/s
1	0.11 M Acetic Acid (HOAc)	Soil solution, carbonates, exchangeable metals
2	0.5 M Hydroxylammonium Chloride (NH_2OH HCl) at pH 1.5	Iron / manganese oxyhydroxides
3	8.8 M hydrogen peroxide then 1 M Ammonium Acetate (NH_4OAc) at pH 2-3	Organic matter and sulfides
Residual	Aqua Regia	Remaining non-silicate bound metals

Table 1-11 BCR sequential extraction scheme

- Step 1: Acetic acid – the exchangeable and bound to carbonates fraction.

Trace metals are adsorbed on the soils / sediments or on their essential components, namely clays, Fe- and Mn-hydrated oxides, and humic acids. The adsorption of trace metals is related to changes in water ionic composition which probably affect sorption-desorption processes. It is known that the carbonates of sediment contain significant trace metal concentrations and the concentrations are sensitive to pH. Fairly large amounts of Mg and Ca are normally extracted in this step, and this is a clear indication of the dissolution of carbonates (Arunachalam et al., 1996).

- Step 2: Hydroxylammonium chloride – the reducible or bound to Fe- and Mn-oxides fraction.

It is well known that Fe- and Mn-oxides are present as cement, concretions or nodules between particles or only as a coating on particles. These oxides bind the trace metals and have strong scavenging efficiency for trace metals, but they are thermodynamically unstable under the anoxic circumstances.

- Step 3: Oxidisable fraction or bound to organic / sulfidic matter fraction.

The trace metals may be associated with various forms of organic / sulfidic matter such as living organisms, detritus, or coatings on mineral particles. The complexation and peptization characteristics of the natural organic substances are well known. Under oxidising conditions, the organic / sulfidic substances may be broken up, freeing the soluble trace metals in the natural waters.

- Step 4: Residual fraction. The residual solids mainly contain primary and secondary solids that occlude the metals in their crystalline structure.

Sequential extractions have been criticized for being based on 'operationally defined host phases' (Arunachalam et al., 1996). Despite these restrictions, sequential extractions are at present, the experimentally easier schemes to identify trace element partitioning into the various solid phases and to contrast the labile fractions of trace elements.

1.6.4 Ion Selective Electrodes (ISEs)

1.6.4.1 Aim of the technique

Ion selective electrodes (ISEs) are employed for the determination of various ions in aqueous solutions.[5]

1.6.4.2 Background to the technique

A pH electrode is an ion selective electrode for H^+ ions.[5,6] The chief difference between the pH electrode and other electrodes is that the latter, generally speaking, are not as selective as the pH electrode and some account must be taken of possible interferences in an analytical situation.

An ion selective electrode generates a difference in electrical potential between itself and a reference electrode. The output potential is proportional to the amount or concentration of the selected ion in solution.

The concentration is a measure of the number of ions in a specific volume. The definition assumes that all of those ions behave in the same manner. However, ions do not always behave similar to one another: some are effective, i.e. exhibit properties associated with that ion, and some are not effective. The number of effective ions is called the activity of the solution. It is therefore reasonable to assume that the electrode will measure the activity rather than the finite concentration of the ions. In dilute solutions though, the ionic activity and concentration are practically identical, but in solutions containing many ions, activity and concentration may differ. This is why dilute samples are preferred for measurement with ISEs.

The ISE (Ion Selective Electrode) measures concentration directly. The activity of a solution is described by the following equation: $a = cf$ (a = activity, c = concentration and f = the activity coefficient). Clearly, the total ionic strength of a sample affects the activity coefficient. By adding a constant concentration of an inert electrolyte (known as an Ionic Strength Adjustor (ISA)) to the solutions under test, the ISE will measure concentration directly. The adjustment in concentration resulting from the addition of an ISA is large, compared to the ionic strength of the sample. This results in variations between samples becoming small and the potential for error being reduced.

There are a number of advantages associated with ISEs compared to other analytical techniques. ISEs:

- Are simple to use. They can be used very rapidly and easily when measuring ions in relatively dilute aqueous solutions and where interfering ions are not a problem.

- Are highly accurate and precise, ±0.69% for some elements, thus comparing favourably with analytical techniques that require far more complex and expensive instruments.

- Are one of the few techniques which can measure both positive and negative ions.

- Can be used in aqueous solutions over a wide range of temperatures.

- Have a wide range of applications, including:

  - Agriculture: NO_3^-, Cl^-, NH_4^+, K^+, Ca^{2+}, I^-, Cu^{2+}, Pb^{2+} etc. in soils, plant material, fertilisers and feedstuffs.

  - Pollution monitoring: CN^-, F^-, S^{2-}, Cl^-, NO_3^- etc. in effluents.

- Are able to measure over a wide concentration range.

The three main problems with ion selective electrode measurements are the effect of interference from other ions in solution, the effect of the ionic strength of the solution reducing the measured activity relative to the true concentration at high concentrations, and the drift in electrode potential during a sequence of measurements. For each of these problems, there are simple steps which can be taken to overcome them.

In summary, as long as the difficulties associated with using ISEs are recognized when they arise and the appropriate steps to overcome them are taken, ISEs are a very useful and cost-effective analytical tool

1.6.5 Ion selective electrodes in use

ISEs are a commonly used tool for measuring the free ion in solution, as reported by many authors (Kaschl et al., 2002; Logan et al., 1997; McBride, 2001; Merritt & Erich, 2003;

Saar & Weber, 1979; Sauve et al., 1996; Sauve et al., 1995; Smith et al., 2004; Town & Powell, 1993; Yin et al., 1997). The most prevalent study using ISEs is the quantification of complexation of different trace metals by organic ligands. Two such studies were conducted:

- To determine copper complexation by low molecular weight (LMW) (<1 kDa) water-extractable carbon (Merritt & Erich, 2003).

- To study the relative importance of different types of organic ligands in municipal solid waste (MSW) compost for the binding of Cd (Kaschl et al., 2002).

Merritt & Erich (2003) collected low molecular weight (LMW) (<1 kDa) water-extractable carbon compounds from fresh and microbially degraded wheat straw and crimson clover. A cupric ISE was used to determine copper complexation for LMW water-extractable carbon compounds for both plant materials. The concerns regarding LMW soluble Cu complexes which prompted the study included percolation through soils or runoff into adjacent water bodies, as well as effects on plant root development.

Kaschl et al. (2002) studied the importance of different types of organic ligands in MSW (municipal soil waste). The agricultural practice of amending soils with composted MSW adds significant amounts of organic matter and trace metals, including Cd. Under these conditions, soluble organic complexes of Cd formed in the compost may be more significant than previously thought, due to Cd bioavailability and mobility in the soil environment. To study the relative importance of different types of organic ligands in MSW compost for the binding of Cd, six fractions of the dissolved organic matter (DOM) in addition to humic acid (HA) and fulvic acid (FA) were extracted and their complexation of Cd quantified at pH 7 using an ISE.

These two studies illustrate the importance of using ISEs for quantifying the complexation of different heavy metals by organic ligands.

1.6.6 Agarose Gel Electrophoresis

1.6.6.1 Aim of the technique

Gel electrophoresis may be able to differentiate metal-organic complexes.

1.6.6.2 Background to the technique

Many studies have emphasised the importance of organic matter in determining the vertical distribution and associations of heavy metals in forest soils (Wang & Benoit, 1996). It has been reported that humic substances are the active metal-binding component of soil organic matter implicated in these associations (Graham et al., 1995). Traditional methods for the isolation of humic substances are very harsh and involve extremes of pH which are thought to alter the structure of humic compounds. Alternative, milder methods for the isolation of humic substances have been sought with the aim of causing minimal alteration to the structure of humic compounds. Gel electrophoresis has been studied as a suitable method (Farmer et al., 2002; Graham et al., 2000; Higney, 2003; Trubetskoj et al., 1991; Trubetskoj et al., 1992) .

Gel electrophoresis has been extensively used for the isolation of nucleic acids and proteins (Dunn, 1986; Hames, 1998; Hawcroft, 1997; Jones, 1995). Electrophoresis of nucleic acids is different in principle from that of proteins due to differences in their nature. For instance, while most simple proteins range in size from say 3 kDa to 200 kDa (perhaps 26-1650 amino acids), nucleic acids (10 bases to 300 kilobases (kb) of single stranded DNA) cover a much wider range, perhaps from 3 kDa to 93 000 kDa.

Electrophoresis isolates charged molecules by use of an electric field. Any charged ion or group will migrate when placed in an electric field. The strong charge of nucleic acids at neutral pH values ensures that they will migrate towards the cathode; however, their constant charge / mass ratio will mean that in open-pored systems they would tend to move together and not separate. Separation has to rely, therefore, on sieving effects acting on the differently sized molecules and the wide range of molecular sizes requires the availability of a wide range of gel pore sizes. Proteins carry a net charge at any pH other than their isoelectronic point, and as a result will also migrate. Their rate of migration will depend on the charge density (the ratio of charge to mass) of the proteins concerned.

Humic matter is thought to be a mixture of polymerized organic compounds (Kononova, 1966; Schnitzer & Khan, 1972). The presence of charge resulting from the ionization of functional groups is a fundamental property of humic substances. It is this property which is exploited when humic substances are fractionated by electrophoresis

There is a considerable amount of literature on the use of polyacryalmide gel (PAG) electrophoresis as a means of fractionating humic substances (Trubetskoj et al., 1991; Trubetskoj et al., 1992). Polyacrylamide gel electrophoresis is the standard electrophoretic method for isolating proteins. However, this method is relatively invasive as far as the reagents used are concerned, and as a result will alter the native structure of humic substances considerably.

There have been recent reports on the use of agarose gel electrophoresis to separate organic substances (Farmer et al., 2002; Graham et al., 2000). Agarose gel electrophoresis is the standard electrophoretic method for the isolation of nucleic acids. The use of agarose gel electrophoresis for the isolation of humic substances is an undeveloped area, as reflected by the small number of publications on the subject. However, it is definitely an area worth

developing as it is less intrusive than the PAG method and as a result should yield more representative results as to the true structure and properties of humic substances.

1.6.7 Agarose gel electrophoresis in use

Graham et al. (2000) recently reported successfully fractionating humic substances in a coniferous forest soil using gel electrophoresis. They found that after each electrophoretic run, the gel displayed visible bands which they were able to isolate. As a result of fractionating the humic substances, they were able to study the association of ^{238}U (uranium-238) with each fraction.

Higney (2003) recently reported using agarose gel electrophoresis to fractionate humic substances from a road dust sample. The publication indicated that the gel was cut into 1 cm section for analysis, rather than relying on bands being visible. As a result of fractionating the humic substances, they were able to study the association of Pt (platinum) with each 1 cm section.

Both agarose and polyacryalmide gel electrophoresis have been reported in the literature as methods for the fractionation of humic substances. These studies indicate agarose gel electrophoresis to be the most promising, due to it to being less invasive than the PAG method. However, it is clear that considerable development is still required to determine a standard procedure with minimal interferences to samples.

1.6.8 Summary

The development of a method which will enable direct determination of metal speciation in soil is an unrealistic aim. However, by using a variety of methods which provide information on metal speciation, such as the modified BCR extraction and various ion selective electrodes, it should be possible to develop an accurate overview.

The modified BCR extraction procedure and ion selective electrodes are clearly well exploited methods for gaining information on metal speciation in soils. Agarose gel electrophoresis is in the rudimentary stages of development as a means of determining information on metal speciation in soils, however, the information this technique will provide, once sufficiently developed, will be invaluable.

The benefits gained from such speciation techniques are significant, so it clearly makes sense to continue improving current methods and developing new ones in order to devise a more sophisticated picture of speciation within soils.

On of the aims of the investigation was to use these techniques (BCR extraction, ion selective electrode studies and gel electrophoresis) in order to obtain a picture of heavy metal speciation in the soils taken from below the two vegetation types (deciduous and coniferous).

1.7 Introduction Overview

Soil clearly plays a very important role in our environment as a sink for heavy metals. However, it appears as though it is not a permanent sink and that heavy metals have the potential to become mobilized. The potential for mobilization depends on a number of factors, including soil type, heavy metal type and environmental conditions. It is therefore very important to study each soil type in a variety of climates in order to ascertain trends and standard information for a specific soil under specific environmental conditions. This includes studying heavy metals, their interactions with the soil and the form they take under each type of environmental condition.

The trees over forest soils clearly provide the soil with specific characteristics. These characteristics are attributed to such things as the canopy, the rhizosphere and the high

input of vegetation. Studies by the Friedland (Friedland et al., 1992; Kaste et al., 2003) and Anderson (Andersen et al., 2002) groups have demonstrated these characterisitcs.

The first stage in understanding heavy metal distribution in different climates, is to obtain information on a global scale. There are many research groups throughout the world, which have reported such information, by analysing natural substances using standard techniques. By gathering such information together, valuable information can be obtained. For example, there are clearly much higher levels of heavy metal contaminants in areas associated with anthropogenic emissions.

The next stage is to focus on a specific soil type in a particular climate. Information should be obtained, by using standard procedures, which enables a comparison with the global information on metal distribution. Once this information is obtained, established speciation techniques can be used to obtain vital information on the interactions heavy metals have with these soils. A reliable picture should be obtained by using as many techniques as possible. The findings from such studies will hopefully provide data that enables the development of hypotheses that can be tested out on subsequent studies.

Chapter 2 - Materials and Methods

2.1 Sample Site Description

Pollok Park, an inner city country park in Glasgow was the site of investigation (Ordinance Survey grid reference 550 620). All studies, except for the electrophoresis study (Chapter 5), were conducted using samples collected from this site only.

The site features soil under both a coniferous canopy (predominantly Corsican Pine) and a deciduous canopy (predominantly Beech). The important feature of the site is the clear segregation between the two vegetation types that enable a comparative study into the contribution of canopy type to the heavy metal distribution in the soils.

Figure 2-1 Photograph of the Pollok Park sampling site showing the clear division between the deciduous canopy (left of photograph) and the coniferous canopy (right of photograph)

Figure 2-1 clearly shows the division between the two types of vegetation in Pollok Park. Figure 2-2 shows a map of the site, taken from multimap (www.multimap.com).[7]

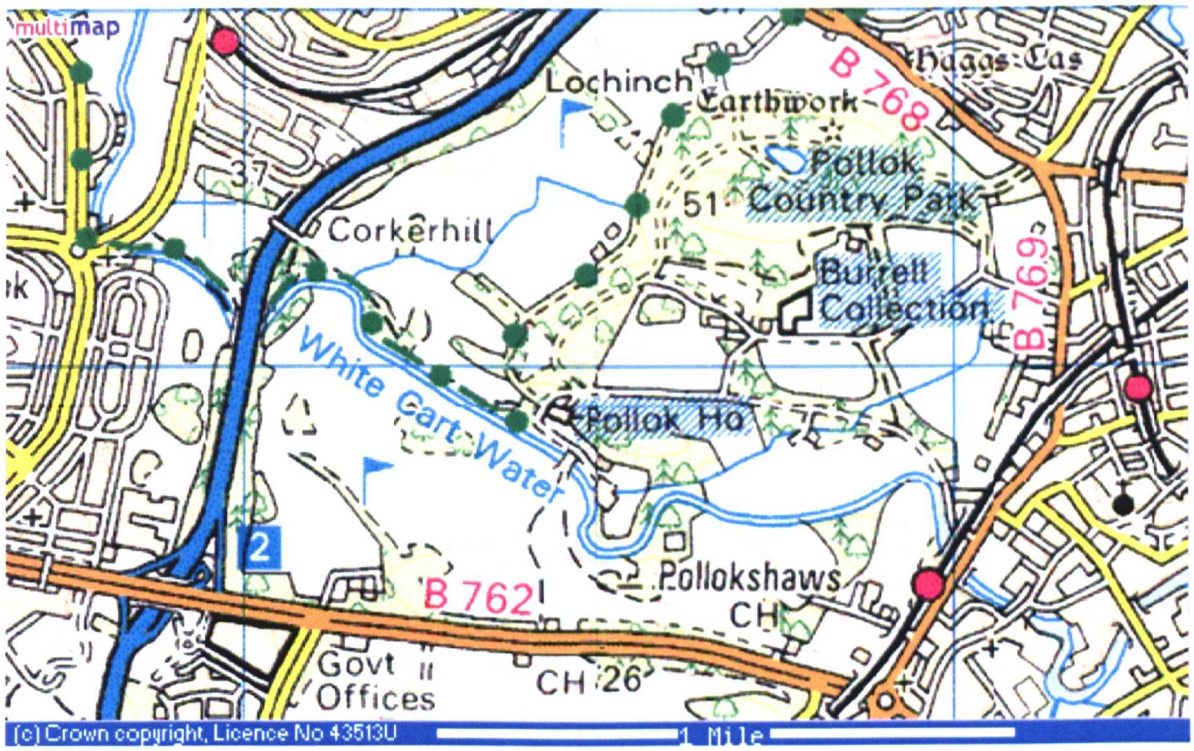


Figure 2-2 Map of Pollok Park sampling site

The site of study has been undisturbed for approximately two hundred and fifty years.

2.2 Description of Soils

2.2.1 Deciduous soil

In general, the deciduous profile consists of a black organic layer (approximately 4 cm), followed by a grey / brown layer (approximately 2 cm), followed by another darker layer (approximately 1 cm), followed by another grey / brown layer (approximately 7 cm), followed by an orange / brown layer (approximately 12 cm) and then another grey /brown layer.

2.2.2 Coniferous soil

In general, the coniferous profile consists of a black organic layer (approximately 9 cm), followed by a dark brown layer with dark streaks through it (approximately 7 cm), followed by a light brown layer (approximately 10 cm) and then a medium brown layer.

2.2.3 Soil series

The Soil Survey of Scotland has published large-scale soil maps (Dickson et al., 2000). Unfortunately, the Soil Survey of Scotland does not include built-up areas, and as a result of this, a soil survey of the urban area was carried out using the same classification system as the Soil Survey of Scotland. The soil survey was focused on soils surviving relatively unchanged from before the development of the city, rather than on artificial substrates for plant growth that have resulted from dereliction or demolition. The major unit of soil classification is the Soil Association. A Soil Association is a group of soils that are all formed on geologically similar parent materials. Each Soil Association contains a number of Soil Series, which differ in the processes that have contributed to their formation. In practice most of the Soil Associations correspond roughly with the major divisions of the solid geology of the Glasgow area, with the exception of the Clyde and its tributaries, where water has modified the deposits. Soil Associations and Soil Series are named after the place where they were first mapped by the Soil Survey. Unfortunately, the site of study (Pollok Park) was never evaluated by the soil survey, and as a result of this a Soil Association and Soil Series can only be assigned by estimation, based on findings for nearby areas which were assigned.

On consulting soil maps for the outskirts of Glasgow near the site of study (Dickson et al., 2000), one of the Soil Associations found to be most prevalent was the Kirktonmoor Association (BGS South-East of Scotland large scale Soil Map , 1 : 250,000) (OS, 1982).

This Soil Association is present in the district of Kilmarnock, which has been surveyed as part of the urban Soil Survey (Mitchell & Jarvis, 1956). As a result of this publication, an accurate description of this Soil Association can be given. The kirktonmoor Series beongs to the Kirktonmoor Association and is described in the following paragraph:

*'The Kirktonmoor Series can be described (*in general terms*) as a medium granular structure which is usually associated with the sandy loam textured surface horizon. The B horizon also textures to a sandy loam but the structure is seldom well-defined. Normally there is no ochreous mottling. The organic matter content, particularly in the B horizon, is often higher than colour would indicate. A gritty sandy loam, loose and structureless, typifies the C horizon. The stone content is very high with the proportion of angular stones higher than in either the A or the B horizon. Again there is seldom evidence of mottling. The morphology of the profile is similar to that of the brown forest soil of low base status'* (Mitchell & Jarvis, 1956).

2.3 Sample Collection

Four collections were made from Pollok Park and these are detailed below. On collection, all samples were stored at 4 °C prior to preparation for analysis.

2.3.1 Collection 1

Two soil cores were taken from the site in October 2001, one from below the deciduous canopy and one from below the coniferous canopy. These cores were taken using metal trays (length 32 cm, breadth 14.7 cm and depth 7.2 cm). In order to obtain each core, a soil pit was carefully dug, the tray was pushed into the face and then dug out of the face. Both the deciduous core and the coniferous core did not contain the litter layer. After collection, each tray was labelled and sealed in a PE bag. Both the deciduous and coniferous cores did not contain the litter layer. Prior to preparing the soil for analysis, each core was

extruded and cut into 1 cm sections. Each section was thoroughly mixed by hand in order to ensure homogenisation of the samples.

2.3.2 Collection 2

Organic soil samples (the top 5 cm) were taken from the site in June 2002; from below the deciduous canopy and from below the coniferous canopy. Each of these was obtained by digging a soil pit, carefully measuring 5 cm and then digging out the top 5 cm into PE bags using trowels. After collection, each bag was labelled and sealed. Prior to preparing the soil for analysis, each batch (deciduous and coniferous) was thoroughly mixed by hand in order to ensure homogenisation of the samples.

2.3.3 Collection 3

In October 2003, four soil cores (two from below the deciduous canopy and two from below the coniferous canopy) were extracted and two bags of litter (one from below the deciduous canopy and one from below the coniferous canopy) were collected. The cores were taken using metal trays (length 32 cm, breadth 14.7 cm and depth 7.2 cm). In order to obtain each core, a soil pit was dug, the tray was pushed into the face and then dug out from the face. After collection, each tray was labelled and sealed in a PE bag. Prior to preparing the soil for analysis, each core was extruded and cut into 1 cm sections for the first 5 cm and then into 5 cm sections thereafter. The litter samples were collected by hand into PE bags. After collection, each bag was labelled and sealed.

From these litter samples, leaves and needles were picked out in order to obtain samples of leaves from below each canopy type (deciduous and coniferous). The samples were collected in PE bags. After collection, each bag was labelled and sealed.

2.3.4 Collection 4

In December 2003, ten soil cores (five from below the deciduous canopy and five from below the coniferous canopy) were taken from the site. The cores were taken using pre-cut PTFE pipes (length 18 cm and diameter 10 cm). In order to obtain each core, a pre-cut pipe was pushed into the ground and then carefully dug out. After collection, each pipe was labelled and sealed in a PE bag. Prior to preparing the soil for analysis, each core was extruded and cut into approximately 5 cm sections.

2.4 Standard Preparation of Samples

2.4.1 Soil preparation

Prior to all procedures and analyses, unless otherwise stated, soils were weighed, air-dried at room temperature, re-weighed, sieved to 2 mm, then re-weighed and stored at room temperature. Also, for each air-dried soil sample, a pH value and loss on ignition value was obtained.

2.4.2 Litter preparation

Prior to all procedures and analyses, unless otherwise stated, litter samples were weighed, air-dried at room temperature, re-weighed, broken up manually and stored at room temperature.

2.4.3 Preparation of leaves

After collection, samples of leaves were kept at 4 °C prior to all procedures.

2.5 Glassware and Plasticware Preparation

All glassware and plasticware used was soaked overnight in 2-5% solution of Decon 90, rinsed in tap water repeatedly, washed 5 times with de-ionised water and dried prior to use. All reagents used, unless otherwise stated, were analytical grade.

2.6 Determining Metal Concentrations

2.6.1 Atomic Absorption Spectrometry (AAS): Determining metal concentration of samples

Metal concentrations of samples were determined by Flame Atomic Absorption Spectroscopy (FAAS). The instrument model used was the PerkinElmer AAnalyst 100 for all the metals, except for Cr which was analysed by the PerkinElmer AAnalyst 1100B. All standards solutions for calibration were prepared from certified stock standards. All certified stock solutions used were at a concentration level of 1000 mg l^{-1}. The FAAS instrumental parameters for each element are shown in Table 2-1.

Element	Cr	Cu	Fe	Mn	Pb	Zn
Wavelength (nm)	357.9	324.8	248.3	279.5	283.3	213.9
Slit width (nm)	0.70	0.70	0.20	0.20	0.70	0.70
Signal Type	AAS	AAS	AAS	AAS	AAS	BCAAS
Signal Measurement	TA	TA	TA	TA	TA	TA
Flame Type	A/C	A/C	A/C	A/C	A/C	A/C
Oxidant Flow ($L\ min^{-1}$)	3.5	2.5	2.4	2.5	2.5	2.5
Fuel Flow ($L\ min^{-1}$)	8.0	1.8	2.0	1.8	2.0	2.0

Table 2-1 AAS conditions used for determining Cr, Cu, Fe, Mn, Pb and Zn concentrations: AAS= Atomic Absorption Spectroscopy, BCAAS= Background Corrected Atomic Absorption Spectroscopy, TA= Time Average, A / C= Air /C_2H_2

2.6.2. Standard solutions for calibration

The details of the standard solutions used for calibrating the flame atomic absorption spectrometer for the measurement of samples obtained from the digestion / extraction of soils are presented in Tables 2.2 and 2.3. In any calibration, the matrix of the standard solutions always matched the matrix of the sample solutions being measured.

Metal	Concentration range of standard solutions (ppm)
Cr	0, 2.0, 3.0, 4.0, 5.0
Cu	0, 2.0, 3.0, 4.0, 5.0
Fe	0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0
Mn	0, 0.5, 1.0. 1.5, 2.0
Pb	0, 5.0, 10.0, 15.0, 20.0
Zn	0, 0.20, 0.40, 0.80, 1.00

Table 2-2 Concentration range of standard solutions (ppm): Cr, Cu, Fe, Mn, Pb and Zn

Standard matrices used for the measurement of soil digestion samples
Aqua-regia – dilute. 6 M HCl : 15.8 M HNO_3 (ratio 3:1)
Aqua-regia – concentrated. 11.65 M HCl : 15.8 M HNO_3 (ratio 3:1)
0.11 M Acetic Acid (HOAc)
0.5 M Hydroxylammonium Chloride (NH_2OH HCl)
1 M Ammonium acetate (NH_4Oac) at pH 2 –3

Table 2-3 Standard solution matrices: soil digestion samples

In the preparation of all standard solutions (details presented in Tables 2-2 and 2-3), (Cr, Cu, Fe, Mn, Pb and Zn), a stock solution of 1000 ppm in 0.5 M HNO_3 was used. In general, a 100 ppm standard solution in the required matrix would be prepared from the stock solution, and then all other standards would originate from this 100 ppm solution (by using dilution). It was always ensured that the standard matrix matched that associated with the sample being measured. For example, a soil sample may be digested in 10 ml aqua-regia – diluted and then collected in a 50 mL volumetric flask which is made up to 50 mL using de-ionised water. Essentially, this is a 20% dilution of the digesting solution (aqua-regia – diluted) in de-ionised water. All standards used for measuring metal concentrations in this sample would be made from a 20% aqua-regia - diluted solution in de-ionised water.

When calibrating the flame atomic absorption spectrometer, the authenticity of the standards was initially ensured by checking that the absorption values compared favourably with those obtained by previous users under the same conditions. Certified or indicative reference materials were run through the procedure, and the accuracy of standards can be checked further by ensuring the values obtained for these are what they should be.

2.6.3 Pseudo-Total Digestion of Soil

Psuedo-total (i.e. aqua-regia extractable) metal concentrations were obtained by either block digestion or microwave digestion. For each procedure, blank samples (i.e. containing no sample, but containing digestion reagent) were run through alongside samples to ensure that no errors were being introduced into the final values from the procedure.

2.6.3.1 Heating block-assisted digestion

Digestion was conducted using aqua-regia prepared from 6 M HCl and HNO_3 (conc.) added together on a 3 : 1 basis. Approximately 0.25 g of each sample (air-dried, three replicates) was placed in a block digestion tube, and then 10 mL of aqua-regia solution was added to each tube The tubes were left to stand overnight in order for the acid to equilibrate with the soil. The tubes were then placed in the digest block and the temperature set at 125 °C. They were left for approximately 3 hours. The tubes were allowed to cool, 10 mL of deionised water was added and the digests were filtered with washings, using 125 mm Whatman No. 50 hardened filter papers, into 50 mL volumetric flasks. All digests were stored at 4 °C prior to analyses

2.6.3.2 Microwave-assisted digestion

Digestion was conducted using aqua-regia prepared from HCl (conc.) and HNO_3 (conc.) added together on a 3 : 1 basis. Approximately 1 g of each sample (air-dried, three replicates) was added to an advanced composite vessel (ACV), along with 20 mL aqua-regia. The vessels were left to stand overnight, before being digested using a CEM-MDS-2000 system (CEM Corporation, North Carolina, USA). The microwave conditions were: set to 60 psi, hold for 20 minutes once at 60 psi, then set to 120 psi and hold for 30 minutes once at 120 psi. After cooling, 10 mL of deionised water was added and the digests were filtered with washings, using 125 mm Whatman No. 50 hardened filter papers, into 100 mL volumetric flasks. All digests were stored at 4 °C prior to analyses.

2.6.3.3 Quality control

Soil-Hackney Brick-Works certified reference material LGC 6135 was used to ensure the quality of the results. Table 2-4 presents the found and certified values for Cr, Cu, Fe, Mn, Pb and Zn following aqua-regia digestion. In each case, the found values are in good agreement with the indicative values reported by the Laboratory of the Government Chemist (LGC, 2001).

Certified reference material LGC 6135	**Measured value**	**Certified values**	
	(mg kg^{-1})	Constituent (total metals) (mg kg^{-1})	Constituent (leachable metals) (mg kg^{-1})
Cr	372 (11)	455 (59)	336 (28)
Cu	106 (3)	107 (5)	105 (5)
Fe	50322 (2709)	47500 (4600)	40900 (2700)
Mn	369 (9)	390 (40)	348 (18)
Pb	397 (12)	411 (26)	391 (16)
Zn	313 (8)	345 (49)	316 (41)

Table 2-4 Extractable contents of Cr, Cu, Fe, Mn, Pb and Zn in certified reference material LGC 6135 following the aqua-regia digestion procedure (standard deviations are given in brackets)

2.7 Measurement of Soil pH

Two buffer solutions were used to calibrate the pH meter, a pH 4 buffer and a pH 7 buffer. Soil suspensions were made up (5 g soil : 25 mL H_2O) in 30 mL screw cap bottles. The bottles were stoppered and shaken intermittently for 15 minutes. The pH of each soil-water suspension was measured by swirling the suspension, lowering the pH electrode into the suspension and then allowing 30 seconds for equilibration before taking the reading.

2.8 Measurement of Conversion Factor

Air-oven conversion factor measurements were obtained using silica basins. Firstly, the silica basins were placed in the 110 °C oven for 1 hour before being cooled in a desiccator and then weighed. Approximately 0.25 g of each sample (air-dried, two replicates) was weighed into a silica basin. The silica basins were then placed in the 110 °C oven overnight, cooled in a desiccator and re-weighed.

Fresh-dry conversion factor measurements were obtained using porcelain basins. Firstly, the porcelain basins were placed in the 110 °C oven for 1 hour before being cooled in a desiccator and then weighed. Approximately 5 g of each sample (fresh, two replicates) was weighed into a porcelain basin. The porcelain basins were then placed in the 110 °C oven overnight, cooled in a desiccator and re-weighed.

2.9 Measurement of Organic Matter by Loss on Ignition

The samples used for measuring the conversion factors for each soil sample were used for measuring loss on ignition. The silica basins containing the oven-dry samples were furnaced for 6 hours at 550 °C. When the basins were cooled, they were placed in the 110 °C oven overnight, cooled in desiccators and weighed.

2.10 Measurement of Organic Carbon

Organic carbon was measured using the dichromate oxidation method set out in the Analysis of Agricultural Materials manual, published by the Ministry of Agriculture, Fisheries and Food (Gough, 1981). The soil samples used were from collection 2 (Section 2.3.2). The litter and leaf samples were from collection 3 (Section 2.3.3). The water extracts from the soil, litter and leaf samples are the same extracts used in the ISE studies (Section 4.3.2, Tables 4-3, 4-4 and 4-5). Blanks (i.e. no sample present) were measured in order that the possibility of errors in results could be reduced. All water extracts were stored at 4 °C prior to analyses.

2.11 Modified BCR Sequential Extraction

2.11.1 Method

The modified BCR sequential extraction procedure was conducted as set out in the report EUR 19502, published by the European Commission (Rauret et al., 2000). The only difference being in the pseudo-total digestion method. The report EUR 19502 recommends a reflux-assisted digestion, whereas microwave-assisted digestion was the digestion method used (as previously detailed in section 2.6.3.2). The soil samples used were from collection 2 (section 2.3.2).

Table 2-5 presents the modified BCR sequential extraction method used.

Step 1	0.11 M Acetic Acid (HOAc) (solution A)	Soil solution, carbonates, exchangeable metals
Step 2	0.5 M Hydroxylammonium Chloride (NH_2OH HCl) at pH 1.5 (solution B)	Iron / manganese oxyhydroxides
Step 3	8.8 M hydrogen peroxide (Solution C) then 1 M Ammonium Acetate (NH_4OAc) at pH 2-3 (solution D)	Organic matter and sulfides
Residue digestion	Aqua Regia	Remaining non-silicate bound metals

Table 2-5 Modified BCR sequential extraction scheme

All laboratory-ware used for the BCR procedure was borosilicate glass, polypropylene or PTFE. Vessels which came in contact with samples or reagents, were soaked in 4 M HNO_3 overnight and rinsed with distilled water before use. The blanks were determined as follows: to one vessel from each batch, taken through the cleaning procedure, the reagents were added at each step, and then collected for analyses with the samples. A mechanical, end-over-end shaker at a speed of 23 rpm was used for shaking samples. Centrifugation was conducted at 3000 g.

The sequential extraction procedure was followed according to the steps described below and summarised in Table 2-5.

Step 1: Add 40 mL of 0.11 M acetic acid to 1 g of soil in a 100 mL centrifuge tube and extract by shaking for 16 hours at ambient temperature (overnight). No delay should occur between the addition of the extractant solution and the beginning of the shaking. Separate the extract from the solid residue by centrifugation for 20 minutes and decantation of the supernatant liquid into a high pressure polyethylene container. Stopper the container and analyse the extract immediately or store at 4 °C prior to analysis. Wash the residue by adding 20 mL of distilled water, shaking for 10 minutes and centrifuging for 10 minutes Decant the supernatant and discard, taking care not to discard any of the solid residue.

Step 2: Add 40 mL of 0.5 M hydroxylammonium chloride (pH 1.5) to the residue from step1 in the centrifuge tube, shake the centrifuge tube gently to ensure re-suspension of the solid material and extract by shaking for 16 hours at ambient temperature (overnight). No

delay should occur between the addition of the extractant solution and the beginning of the shaking. Separate the extract from the solid residue by centrifugation for 20 minutes and decantation as in Step 1. Retain the extract in a stoppered polyethylene tube, as before, for analysis. Wash the residue by adding 20 mL of distilled water, shaking for 10 minutes, and centrifuging for 10 minutes. Decant the supernatant liquid and discard, taking care to avoid discarding any of the solid residue. Retain the residue for Step 3.

Step 3: Add carefully, in small aliquots to avoid losses due to violent reaction, 10 mL of 8.8 M hydrogen peroxide to the residue in the residue tube and shake the centrifuge tube gently to ensure re-suspension of the solid material. Cover the centrifuge tube with a watch glass and digest at room temperature for 1 hour with occasional manual shaking. Continue the digestion for 1 hour at 85 °C and reduce the volume to a few mL by further heating of the uncovered vessel in a steam bath or equivalent. Add a further aliquot of 10 mL of 8.8 M hydrogen peroxide. Heat the covered vessel again to 85 °C and digest for 1 hour. Remove the cover and reduce the volume of the liquid to a few mL.

Add 50 mL of ammonium acetate (pH 2-3) to the cool moist residue and shake for 16 hours at ambient temperature (overnight). No delay should occur between the addition of the extractant solution and the beginning of the shaking. Separate the extract by centrifugation and decant into a high pressure polethylene tube. Stopper and retain as before for analysis.

The residue in each centrifuge tube underwent pseudo-total digestion, using the microwave-assisted digestion as detailed in Section 2.6.3.2.

2.11.2 Quality control

When a certified reference material is prepared, the values for the various parameters are normally identified by inter-laboratory comparison exercise, i.e. lots of different laboratories (say 10 to 20 of them) analyse the material and then get together and try to decide on what the true values are (Davidson, 2005). If agreement for a particular parameter is sufficiently good in that the certifying body (e.g. BCR) is really confident they have the true value and uncertainty (standard deviation), that parameter will normally appear on the CRM description as a 'certified' value. If the result of the inter-comparison for a particular parameter is not so good, then the parameter is considered not to be well enough defined to be 'certified'. However, if there is some idea about the correct value, the value may appear as 'indicative' on the CRM description (often with a large RSD value, or sometimes no RSD quoted at all). So in effect, 'indicative' values have gone through the same procedure as 'certified' values, but the outcome wasn't good enough for the parameter to be certified. For CRM 601, the values provided are 'indicative'.

CRM 601	Indicative value (mg kg^{-1}) (Rauret et al., 2000)	Measured value (mean) ($mgkg^{-1}$)
Cr (Step 1)	0.35 (0.08)	0.77 (0.02)
Cr (Step 2)	10.6 (0.9)	9.2 (0.8)
Cr (Step 3)	14.4 (2.6)	17.5 (0.4)
Cr (aqua-regia, residue)	78.2 (6.5)	63.7 (0.06)
Cr (aqua-regia, pseudo-total)	112 (9.5)	121 (26)
Cu (Step 1)	10.5 (0.8)	12.3 (0.3)
Cu (Step 2)	72.8 (4.9)	72.2 (0.9)
Cu (Step 3)	78.6 (8.9)	92.4 (2.7)
Cu (aqua-regia, residue)	60.4 (4.9)	52.5 (0.4)
Cu (aqua-regia, pseudo-total)	230 (15)	222 (4.1)

Table 2-6 Extractable contents (standard error in brackets) of Cr and Cu in CRM 601 following the modified three-step sequential and aqua-regia extraction protocols (indicative and found values)

Table 2-6 presents measured and indicative values for Cr and Cu following the modified three-step sequential and aqua-regia extraction protocols.

In each case, the found values are in good agreement with the indicative values as presented in report EUR 19502 (Rauret et al., 2000).

CRM 601	Indicative value (mg kg^{-1}) (Rauret et al., 2000)	Found value (mean) ($mgkg^{-1}$)
Pb (Step 1)	2.28 (0.44)	5.90 (0.6)
Pb (Step 2)	205 (11)	220 (1.6)
Pb (Step 3)	19.7 (5.8)	23.6 (0.4)
Pb (aqua-regia, residue)	38.0 (8.7)	40.1 (0.4)
Pb (aqua-regia, pseudo-total)	288 (52)	284 (7.1)
Zn (Step 1)	261 (13)	264 (5.4)
Zn (Step 2)	266 (17)	289 (3.3)
Zn (Step 3)	106 (11)	117 (1.9)
Zn (aqua-regia, residue)	161 (14)	151 (4.6)
Zn (aqua-regia, pseudo-total)	833 (17)	118 (11)

Table 2-7 Extractable contents (standard error in brackets) of Pb and Zn following the modified three-step sequential and aqua-regia extraction protocols (indicative and found values)

Table 2-7 presents found and indicative values for Pb and Zn following the modified three-step sequential and aqua-regia extraction protocols. In each case, with the exception of Zn, (aqua-regia, pseudo-total), the found values are in good agreement with the indicative values as presented in EUR 19502 (Rauret et al., 2000).

2.12 Water Washing Methods

The washing of soil, litter and leaf samples with water was carried out in order for preparation of samples for Cu ISE studies and organic carbon measurements.

2.12.1 Water washing of soil

The water washings of soil were obtained by weighing 150 g soil into a suitable container, and then adding 500 mL water to the container. The container was then shaken on a reciprocating shaker for 6 hours and filtered into PTFE containers using Whatman No. 50 hardened filter papers. All samples were stored at 4 °C prior to analyses.

2.12.2 Water washing of litter

The water washings of litter were obtained by weighing 50 g litter into a suitable container, and then adding 600 mL water to the container. The container was then shaken on a reciprocating shaker for 6 hours and filtered into PTFE containers using Whatman No. 50 hardened filter papers. All samples were stored at 4 °C prior to analyses.

2.12.3 Water washing of leaves

The water washings of leaves were obtained by weighing 0.5 g of fresh leaves into a petri dish. The leaves were washed with 20 mL water using a dropper. All extracts were stored in PTFE containers and stored at 4 °C prior to use.

2.13 Cupric Ion Selective Electrode Method

The soil samples used were from collection 2 (Section 2.3.2). The litter and leaf samples used were from collection 3 (Section 2.3.3). The water washings and solid materials were used in titrations adding known concentrations of Cu^{2+}. A cupric ion selective electrode (ISE) (model 9629 ionplus, Thermo Orion) was used to measure the Cu^{2+} concentrations. Prior to taking any readings, the following were conducted:

- The electrode was prepared, following the recommendations reported in the Thermo Orion instruction manual (ThermoOrion, 2001).
- Standard solutions were prepared from certified stock standards.
- Samples were prepared (Section 4.3.2, Tables 4-3, 4-4 and 4-5).
- 5 M $NaNO_3$ (the recommended ISA for cupric electrodes) was added in a ratio of 50 : 1 (solution : ISA) to all standards and samples.

- The electrode was tested by running standards and ensuring that for every 10-fold change in concentration, there was approximately a 28 mV difference in the readings.

- On preparation, all standards and samples were stored at 4 °C prior to analyses.

Once all of the above were completed, each of the samples was run through the procedure. In summary, the procedure involved loading each sample with a known concentration of copper solution in increments of volume. The procedure operated as follows:

- Stir sample and take reading once a constant value is reached

- Add a known volume of the copper solution, stir sample and take reading once a constant value is reached.

- The above was repeated until a satisfactory volume of Cu solution had been added to the sample.

- Once a sample had a satisfactory amount of Cu added, each standard was measured.

- The readings for each standard were plotted and the values for the sample loading were obtained as a result.

The Thermo Orion manual (model 9629 ionplus) indicates that the cupric ISE operates with an error of ± 4%. However, on operation it was found to have an error of ± 3% (ThermoOrion, 2001).

2.14 Statistical Analysis

The statistical package Minitab 13 for Windows was used to compare total metal contents (in Section 3.3) using a paired t-test.

2.15 General Electrophoresis Set Up

A horizontal gel electrophoresis unit with power pack Apelex P5 304 minipac 11 and Edvoteck unit M12 (dimensions: 6 inches (length) and 3 inches (wide) was used for all electrophoretic runs.

All electrophoretic runs followed the same basic set-up as that reported by Higney (2003). The Higney (2003) electrophoretic set-up:

Agarose/TRIZMA gel: 1 g of agarose powder (to provide a 2% agarose content) was weighed onto a clean watch glass, the balance was re-zeroed and 0.788 g of TRIZMA salt weighed. This agarose powder and TRIZMA were added to 50 mL of distilled water heated to 50 – 60 C on a hotplate. The solution was mixed well and allowed to reflux for ten minutes on the hotsplate, until it became transparent. The solution was allowed to cool and when the temperature fell to less than 50 C, the gel was poured into the trevi tray (with combs in place) and was placed in a cool fridge for 30 – 40 minutes. The final agarose / TRIZMA gel had an agarose content of 2 % and a TRIZMA concentration of 100 mM.

TRIZMA buffer: 15.76 g of TRIZMA buffer was weighed into a clean dry beaker along with 250 mL of distilled water and stirred until the buffer dissolved. The solution was transferred to a 1 litre volumetric flask, rinsing the washings into the volumetric flask before making up to volume.

For each electrophoretic run (Chapter 5), the specific details associated are given when the run is described (e.g. buffer type and concentration, sample type and amount of sample applied). Each electrophoretic run (Chapter 5) used a gel based on a volume of 50 mL. For the majority of electrophoretic runs (Chapter 5), the entire gel mixture (based on a 50 mL volume) was applied to the trevi tray.

Chapter 3 - Metal Distribution

3.1 Introduction

The work presented in this chapter aimed to:

- Ascertain the distribution of Cr, Cu, Fe, Mn, Pb and Zn through the profile of both the soil below the deciduous canopy and the soil below the coniferous canopy.

- Determine the total metal content (for Cr, Cu, Fe, Mn, Pb and Zn) of all the cores extracted.

- Determine the organic matter content and pH of both soil types.

- Assess whether there are any distinct differences or similarities between the soil taken from below the deciduous canopy and the soil taken from below the coniferous canopy.

There is a vast amount of published information on soils from all over the world, which can be compared with the soils from Pollok Park (Bergkvist et al., 1989; Bindler et al., 1999; Cal-Prieto et al., 2001; Chen et al., 1997; Czarnowska, 1980; Erel, 1998; Hernandez et al., 2003; Paterson et al., 1996; Pietrzak & McPhail, 2004; Watmough & Hutchinson, 2004).

3.2 Background

For a long time it was assumed that the elemental composition of soil could be explained solely on the basis of local geology (Steinnes et al., 1997). However, it is now clear that the concentration and distribution of metals in soils can be significantly affected by a variety of external factors. Possible external factors include aerial deposition (long-range

and short-range), the contribution of vegetation to the enrichment of a trace element in a soil and interactions with ground water (Blaser et al., 2000; MacKenzie et al., 1998).

There is considerable evidence of long-range transport of air pollution throughout the world, even in remote regions (Brannvall et al., 1999). For example, there is great concern for contamination of the Arctic environment by atmospheric emissions derived from lower latitudes (Pacyna, 1995). There are several studies of ice cores from Greenland (Hong et al., 1994; Hong et al., 1996; Rosman et al., 1997) and lake sediments and peat in Sweden (Brannvall et al., 1997; Renberg et al., 1994) which indicate that large scale pollution of high latitudes by emissions from cultural centres in Europe has occurred for several thousands of years, and there is also convincing evidence of early pollution from the British Isles (Lee & Tallis, 1973) and continental Europe (Kempter et al., 1997; Martinez Cortizas et al., 1997; Shotyk et al., 1998; Weiss et al., 1997). The diagnosis of trace element soil contamination requires knowledge of the original contents of these elements in soil, in other words, the pedogeochemical background content. It has been reported that this may be sought either by analysing the corresponding horizons of non-contaminated soils of the same type, or by analysing the deep horizons of surface contaminated soils (Sterckeman et al., 2000). However, soils not exposed to deposition of atmospheric pollution no longer exist. For example, present lead pollution conditions in northern European soils are a consequence of nearly 4,000 years of atmospheric pollution (Bindler et al., 1999). Probably the best means of studying contamination and natural levels are to obtain long cores (soils, sediments and snow etc.) and combine available study methods such as Pb isotope measurements (^{206}Pb / ^{207}Pb) and total metal concentration (Bindler et al., 1999; Brannvall et al., 1999; Eades et al., 2002; Farmer et al., 1996; Martinez Cortizas et al., 2002; Schwikowski et al., 2004). Environmental archives of atmospheric metal pollution include polar snow and ice, soils, marine and lacustrine sediments, various biological media (corals, tree rings, herbarium specimens) and peat bogs (Shotyk et al.,

2000). These archives have proven to be very valuable for reconstructing, in detail, the chronology of environmental contamination across the globe.

For example, published details of peat bog archives of atmospheric metal deposition, have been summarized by several authors (Glooschenko, 1986; Jones & Hao, 1993; Shotyk, 1996; Shotyk et al., 1997; Stewart & Fergusson, 1994). One of the most significant questions regarding the use of peat bogs as archives of atmospheric metal deposition has been the possible importance of post-depositional migration of metals (Shotyk, 1996; Urban et al., 1990). Peat bogs are naturally acid, and organic-rich, and may vary between oxic and anoxic, depending on the depth of the water table which fluctuates seasonally. Thus, there is considerable opportunity for a variety of chemical and biochemical transformations of metals, which could liberate them from the solid phase (peat) (Shotyk et al., 2000). If a metal supplied to a bog surface was to migrate vertically upward or downward subsequent to atmospheric deposition, the observed relationship between measured metal concentration and time of atmospheric deposition (as determined using an appropriate dating method) would not be in agreement with the original relationship between these two parameters. Until recently, the authenticity and reliability of the peat bog records of atmospheric metal deposition was poorly known. Now, however, there is a growing body of evidence which indicates that Pb, at least, is effectively immobile in peat profiles, and that ombrotrophic bogs are capable of preserving the changing rates of atmospheric deposition (MacKenzie et al., 1997; MacKenzie et al., 1998; Shotyk et al., 1997; Vile et al., 1999). Due to the ability of ombrotrophic peat bogs to efficiently retain Pb, a complete record of atmospheric deposition can be reconstructed using long peat cores (Shotyk et al., 1998). This geochemical data can be combined with the use of conservative, lithogenic, reference elements such as Sc and Zr to calculate cumulative, anthropogenic, atmospheric Pb (CAAPb) (Shotyk et al., 2000).

Vegetation can contribute to the enrichment of a trace element in soil. For example, the process is expected to play a certain role for trace elements such as Zn and Cu which are essential for plant growth and are strongly recycled within the rooting zone (Blaser et al., 2000; Goldschmidt, 1954). When considering the metals being studied (Cr, Cu, Fe, Mn, Pb and Zn), Cu, Fe, Mn and Zn, are all essential to plants.[1] As a result, vegetation can be considered as a possible means by which these can be added to the soil.

Another possible external factor which can affect the distribution and amount of metals in a soil profile is that of interaction with groundwater. It seems inevitable that the interaction of a soil profile with groundwater will have some effect on metal distribution, even though the actual outcome remains ambiguous. Reports have indicated that it may result in diagenetic mobility of metals, including lead, iron, aluminium, copper, and zinc (Damman, 1978; Jones & Hao, 1993; Urban et al., 1990), while other investigations have found no evidence of this (Gorres & Frenzel, 1997).

The content and distribution of heavy metals in soils is a very important subject which has resulted in many publications (Bindler et al., 1999; Brannvall et al., 1999; Farmer et al., 1996; Hernandez et al., 2003). This vast amount of published information from all over the world has enabled an understanding of natural and contaminant levels in soils.

The work presented in this chapter involved studying sixteen cores from Pollok Park, the site of study. Eight cores were taken from below the deciduous canopy and eight cores were taken from below the coniferous canopy. These cores were obtained in Collection 1 (Section 2.3.1), Collection 3 (Section 2.3.3) and Collection 4 (Section 2.3.4). For each of these cores, total metal contents (Cr, Cu, Fe, Mn, Pb and Zn), metal distribution profiles (Cr, Cu, Fe, Mn, Pb and Zn), pH profiles and loss on ignition (LOI) profiles were established. A description of the study site is given in Section 2.1. The soil sampling

regime is given in Section 2.3, the method for standard preparation of samples is given in Section 2.4 and the procedure for heavy metal extraction is given in Section 2.6.

3.3 Total Metal Contents: Cr, Cu, Fe, Mn, Pb and Zn

Cr, Cu, Fe, Mn, Pb and Zn total metal contents were found for each core (all cores taken to a depth of 16 cm for total metal contents). The original data (based on three replicates) for determining the total metal contents is given in the Appendix section.

Core	Vegetation type / Total Cr content	
	Deciduous (g m^{-2})	Coniferous (g m^{-2})
Core 1	4.39	3.00
Core 2	3.58	1.81
Core 3	5.61	3.41
Core 4	5.57	5.19
Core 5	8.20	5.06
Core 6	8.76	4.47
Core 7	5.97	5.15
Core 8	6.41	3.63
Average of 8 cores	6.06	3.97

Table 3-1 Total core content of Cr in the deciduous cores and the coniferous cores

Table 3-1 presents the total core content of Cr for each of the sixteen cores. Figure 3-1 presents the average core content of Cr for the deciduous cores and the coniferous cores.

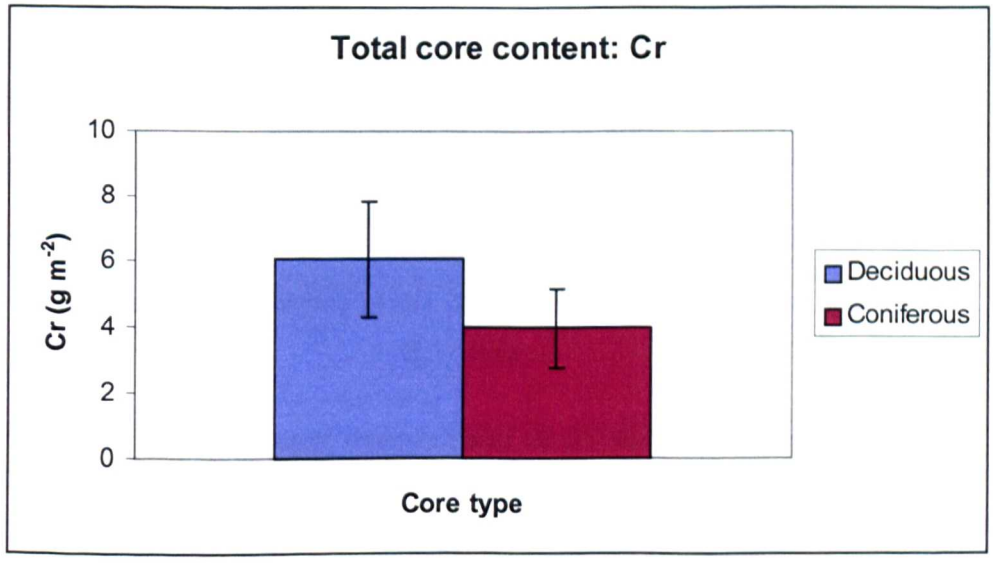


Figure 3-1 Average total core content of Cr in the deciduous cores and the coniferous cores (standard deviations are included)

When comparing the two columns in Figure 3-1, the cores taken from below the deciduous canopy have a greater average Cr content than the cores taken from below the coniferous canopy. This observation is verified by a t-test on the data in Table 3-1. The t-test suggested that the cores taken from below the coniferous canopy are considerable dissimilar to the cores taken from below the deciduous canopy ($p = 0.002$).

Core	Vegetation type / Total Cu content	
	Deciduous (g m^{-2})	Coniferous (g m^{-2})
Core 1	3.82	3.97
Core 2	5.27	2.32
Core 3	4.76	3.66
Core 4	4.65	3.66
Core 5	5.11	4.85
Core 6	4.08	2.33
Core 7	3.92	4.08
Core 8	5.06	4.14
Average of 8 cores	4.58	3.63

Table 3-2 Total core content of Cu in the deciduous cores and the coniferous cores

In Table 3-2 the total core content of Cu for each of the sixteen cores is displayed. The average core content of Cu in the coniferous cores and the deciduous cores is shown in Figure 3-2.

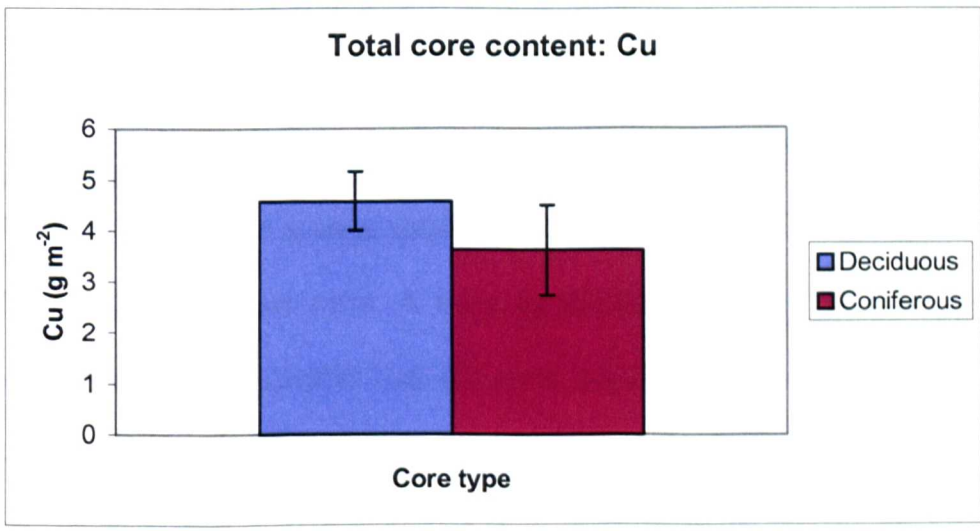


Figure 3-2 Average total core content of Cu in the deciduous cores and the coniferous cores (standard deviations are included)

Figure 3-2 shows that the core taken from below the coniferous core possesses a smaller total core content of Cu than the core taken from below the deciduous canopy. This is supported by a t-test on the data presented in Table 3-2 ($p = 0.035$).

Core	Vegetation type / Total Fe content	
	Deciduous (kg m^{-2})	Coniferous (kg m^{-2})
Core 1	7.59	4.28
Core 2	5.75	2.15
Core 3	4.17	3.50
Core 4	4.83	4.85
Core 5	7.06	5.50
Core 6	6.00	4.84
Core 7	7.45	4.40
Core 8	9.52	5.77
Average of 8 cores	6.55	4.41

Table 3-3 Total core content of Fe in the deciduous cores and the coniferous cores

The total Fe content for each of the sixteen cores is presented in Table 3-3. In Figure 3-3, the average core content of Fe in the deciduous cores and the coniferous cores is shown in Figure 3-3.

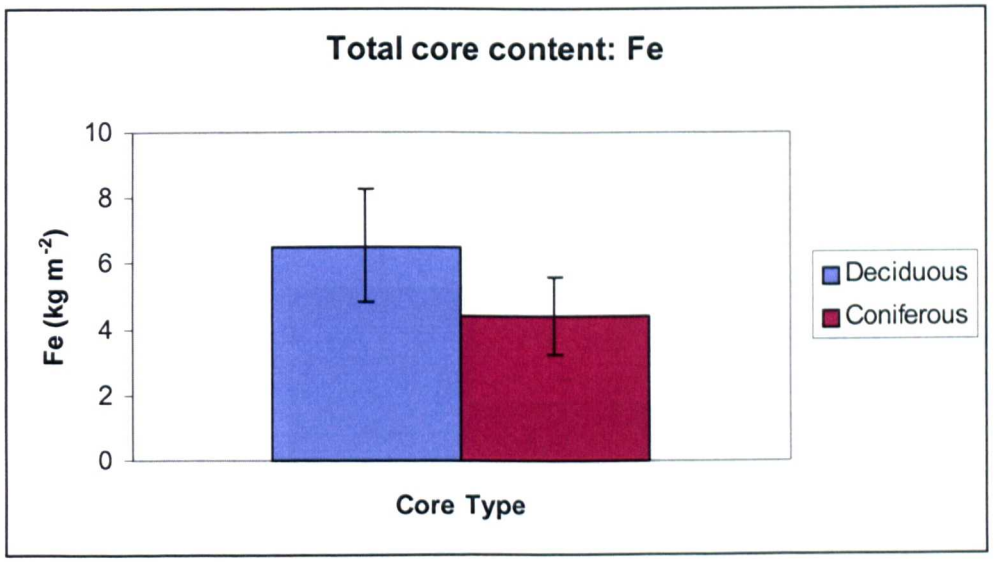


Figure 3-3 Average total core content of Fe in the deciduous cores and the coniferous cores (standard deviations are included)

Figure 3-3 shows the average total core content of Fe in the deciduous cores to be greater than in the coniferous cores. A t-test on the data presented in Table 3-3 supports this observation, as it indicated that the cores taken from below the deciduous canopy are significantly different than the cores taken from below the coniferous canopy ($p = 0.004$).

Core	Vegetation type / Total Mn content	
	Deciduous (g m^{-2})	Coniferous (g m^{-2})
Core 1	138	47.8
Core 2	98.3	21.4
Core 3	67.3	36.1
Core 4	78.9	61.2
Core 5	124	60.0
Core 6	80.8	34.5
Core 7	103	53.3
Core 8	205	34.1
Average of 8 cores	112	43.5

Table 3-4 Total core content of Mn in the deciduous cores and the coniferous cores

The total core content of Mn for each of the sixteen cores is given in Table 3-4. The average core content of Mn (for the deciduous cores and the coniferous cores) is shown in Figure 3-4.

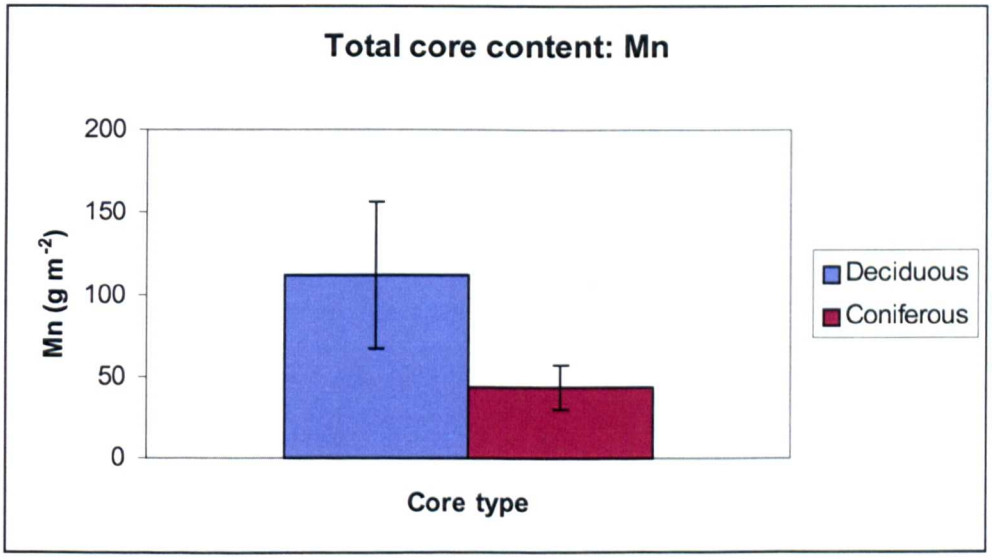


Figure 3-4 Average total metal content of Mn in the deciduous cores and the coniferous cores (standard deviations are included)

Figure 3-4 presents the deciduous core as having a greater Mn content compared to the coniferous core, the difference being approximately 2:1. This observation is substantiated by a t-test on the data in Table 3-4, which clearly showed there to be a difference between the two core types ($p= 0.005$).

Core	Vegetation type / Total Pb content	
	Deciduous (g m^{-2})	Coniferous (g m^{-2})
Core 1	13.1	17.8
Core 2	16.0	9.54
Core 3	22.1	18.3
Core 4	20.0	17.4
Core 5	10.8	22.8
Core 6	17.8	14.2
Core 7	14.3	19.9
Core 8	19.5	20.5
Average of 8 cores	16.7	17.6

Table 3-5 Total core content of Pb in the deciduous cores and the coniferous cores

The total core content of Pb for each of the sixteen cores is given in Table 3-5. The average total core content of Pb for the deciduous cores and coniferous cores is presented in Figure 5-5.

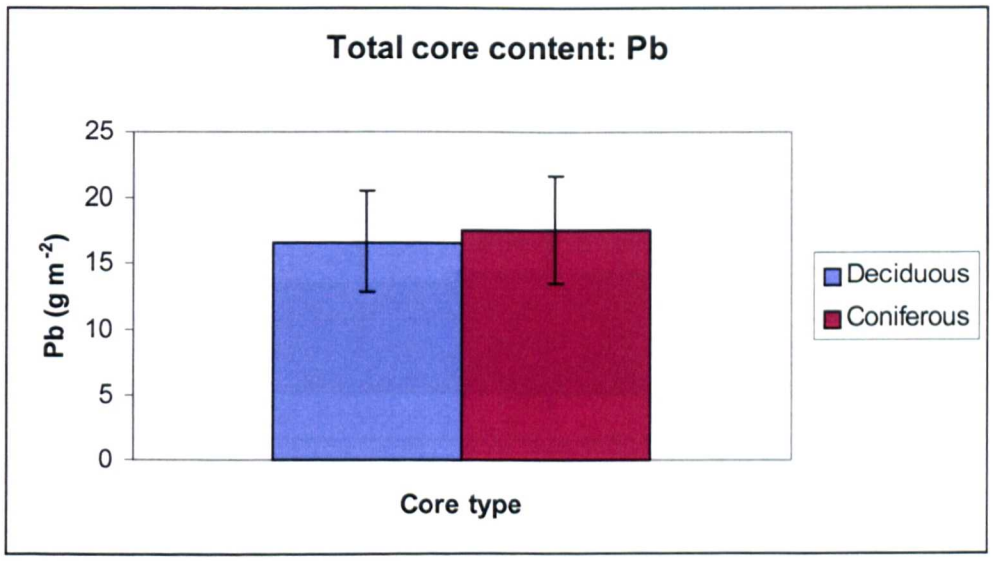


Figure 3-5 Average total core content of Pb in the deciduous cores and the coniferous cores (standard deviations are included)

Figure 3-5 shows the deciduous cores and the coniferous cores as having a virtually identical total Pb content. This is supported by a t-test on the data in Table 3-5, as it implies that there is no significant difference between the cores taken from below the coniferous canopy and the cores taken from below the deciduous canopy ($p = 0.702$).

Core	Vegetation type / Total Zn content	
	Deciduous (g m^{-2})	Coniferous (g m^{-2})
Core 1	11.3	12.4
Core 2	12.4	3.32
Core 3	8.46	6.92
Core 4	9.07	8.66
Core 5	11.1	11.9
Core 6	9.41	5.16
Core 7	9.27	6.59
Core 8	22.7	45.2
Average of 8 cores	11.7	12.5

Table 3-6 Total core content of Zn in the deciduous cores and the coniferous cores

The total Zn content for each of the sixteen cores is displayed in Table 3-6. The average total core content of Zn for the deciduous cores and the coniferous cores is presented in Figure 3-6.

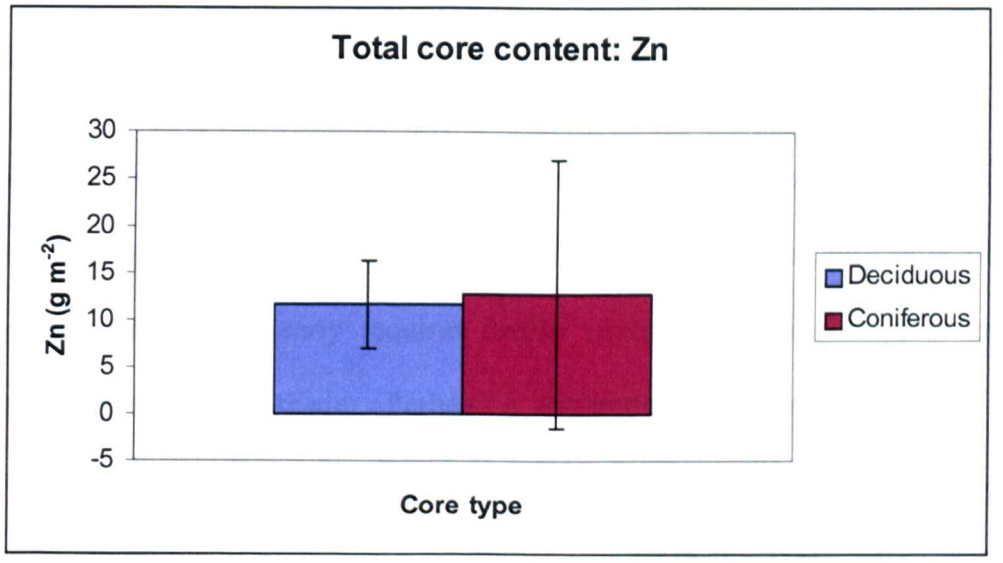


Figure 3-6 Average total core content of Zn in the deciduous cores and the coniferous cores (standard deviations are included)

On observing Figure 3-6, there appears to be no significant difference between the total Zn content in the deciduous cores and the total Zn content in the coniferous cores. This observation is supported by a t-test on the data in Table 3-6 ($p = 0.778$).

It is clear that the deciduous cores contain a greater Cr, Cu, Fe and Mn content than the coniferous cores. However, there is no significant difference between the coniferous and deciduous cores when comparing total Pb and Zn contents. On comparing the pH of the deciduous soil with the pH of the coniferous soil, the coniferous soil is clearly more acidic (Section 3.6). The more acidic nature of the coniferous soil could explain the difference

between the metal content of the cores. Cr exists as $Cr(OH)_3$ in soil, and the solubility of Cr(III) increases with decreasing pH (McGrath & Smith, 1990; Rai et al., 1989). Fe / Mn oxides dissolve at low pH (Bendell-Young & Harvey, 1992; Bryant et al., 1997; McBride, 1994; White & Driscoll, 1987). When they do dissolve, they not only release Mn and Fe, but they also release other heavy metals, such as Cu (Baker, 1990), which are known to associate with Fe / Mn oxides in soils.

Bryant et al. (1997) determined Mn concentrations in sediments and pore waters of five freshwater lochs in Scotland. While doing so, they provided evidence to support the findings that Mn oxides dissolve with decreasing pH. They found that the acidified loch, Round Loch of Glenhead, showed Mn loss from the sediment, when compared to other lochs with higher pH values.

Perhaps the reason for Pb and Zn not showing any differences between the two core types is that they have a strong affinity for matrices which are not significantly affected by acidic conditions. This clearly requires further investigation in order that more convincing conclusions can be made. Perhaps a sequential extraction such as the modified BCR sequential extraction could be used to determine the types and proportion of soil-metal associations involved through each profile (Rauret et al., 2000).

3.4 Metal Distribution Profiles: Cr, Cu, Fe, Mn, Pb and Zn

Three sets of Cr, Cu, Fe, Mn, Pb and Zn metal distribution profiles were established. The numerical means and accompanying standard deviations (based on three replicates) are presented in the Appendix section. Each set was based on a different soil collection. The first set of metal distribution profiles was derived using cores obtained in Collection 1 (Section 2.3.1). The second set of metal distribution profiles were achieved using cores

from Collection 3 (Section 2.3.3). The third set of metal distribution profiles were obtained using cores from Collection 4 (Section 2.3.4).

Set 1 profiles were obtained from cores which were cut into 1 cm sections. Set 2 profiles were obtained from cores which were cut into 1 cm sections for the first 5 cm and then 5 cm sections thereafter. Set 3 profiles were obtained from cores which were cut into 3 sections which were of approximately 5 cm depth.

3.4.1 Metal distribution profiles: Cr

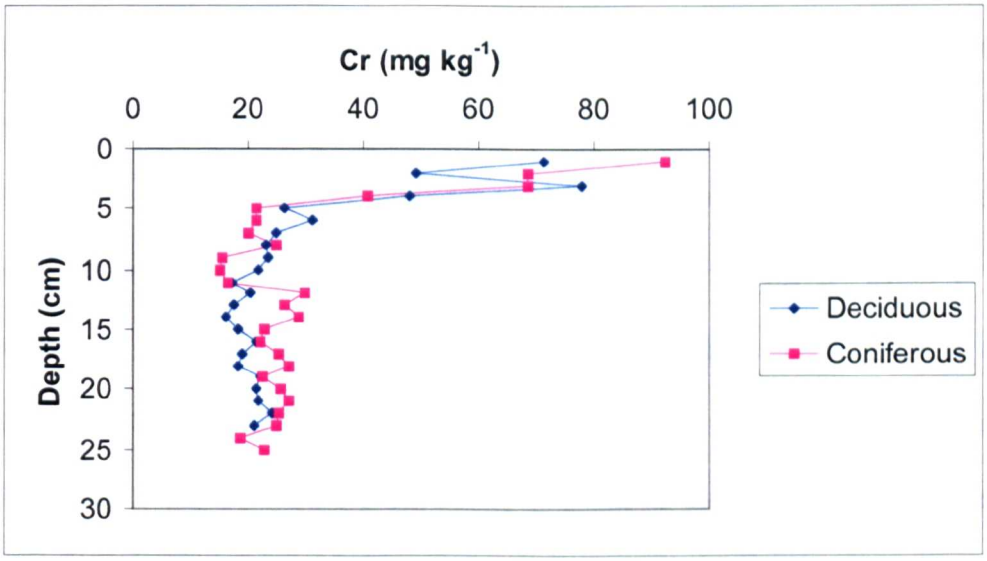


Figure 3-7 Cr concentration profile for the deciduous core and the coniferous core: Set 1

Figure 3-7 shows the Cr concentration profiles (Set 1) for the deciduous core and the coniferous core to be very similar. Both profiles have a considerable high Cr concentration at the surface (approximately 70 to 90 mg kg^{-1}), which declines until a depth of 5 cm is reached. Greater depths than 5 cm indicate no further decline in Cr concentration. Instead it evens out to give the regional background soil value (approximately 20 mg kg^{-1}).

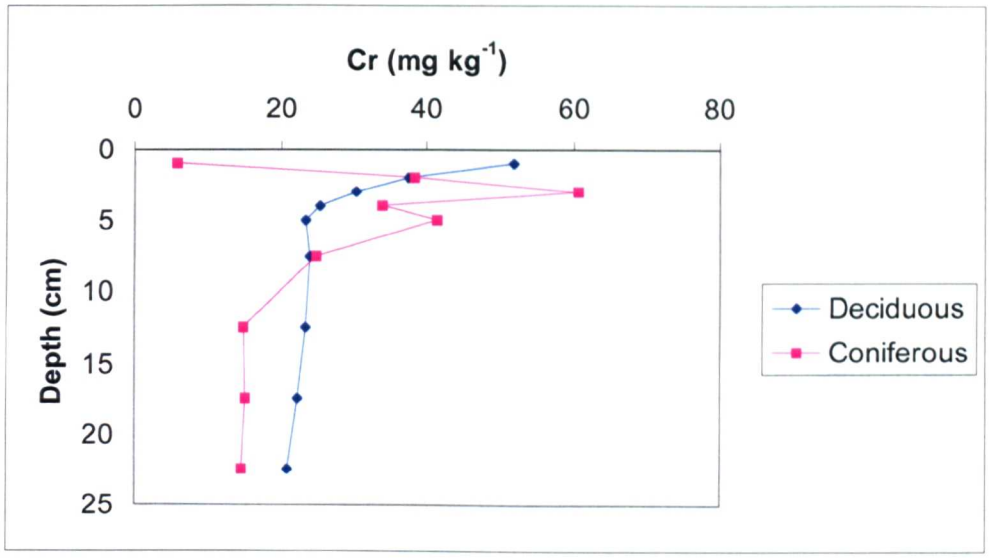


Figure 3-8 Average Cr concentration profiles for the deciduous cores and the coniferous cores: Set 2

The Set 2 concentration profiles are indicated in Figure 3-8. The coniferous profile shows a concentration of approximately 2 mg kg^{-1} at the surface. This could be due to the surface of the cores used to obtain this profile containing litter material, instead of soil at the surface, as was the case for the cores used to obtain Set 1 profiles. It appears that soil is not reached in the core until a depth of 3 cm (60 mg kg^{-1}). From 3 cm depth, the coniferous profile follows a similar pattern to the coniferous profile for Set 1; as it declines until a depth of 5 cm is reached and then maintains an approximately constant Cr value of 20 mg kg^{-1}. The deciduous profile follows a similar pattern to the one in Set 1, as it starts at a high Cr concentration and shows a decline until 5 cm is reached. Once a depth of 5 cm is reached, it maintains an approximately constant value of 20 mg kg^{-1}.

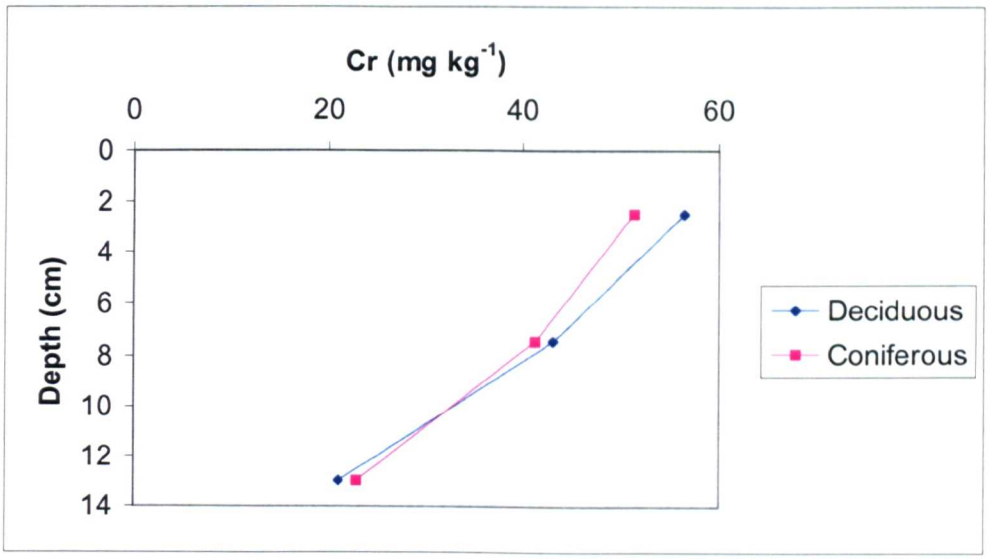


Figure 3-9 Average Cr concentration profiles for the deciduous cores and the coniferous cores: Set 3

Figure 3-9 (Set 3) repeats the trend shown in Figures 3-7 (Set 1) and 3-8 (Set 2), whereby the surface shows a high concentration of Cr for each profile, then a decline in concentration, until eventually a constant value of approximately 20 mg kg^{-1} is obtained.

The Pollok Park soils clearly show enrichment of Cr to the surface. Considering the fact that Cr is not an essential element to plants, vegetation is probably not one of the main sources of enrichment to the soil surface. However, it should not be ruled out as a minor contributor, as uptake by organisms has been reported for elements such as Pb which have no known biological role (Blaser et al., 2000). Elements such as Pb or As may be taken up passively with water flow. In addition, vegetation may contribute to a biological enrichment by superficial deposition of pollutants on leaves and needles, a process which is important in forest sites (Blaser et al., 2000; Kabata-Pendias & Pendias, 1992). Aerial deposition of atmospheric Cr is the most likely cause.[1]

When comparing average Cr concentrations in urban soils around the world, the surface values of Cr (55 mg kg^{-1}, 0-5 cm) in the Pollok Park soils are very similar (Cal-Prieto et al., 2001; Czarnowska, 1980; De Miguel et al., 1998; Lux, 1986; Manta et al., 2002; Paterson et al., 1996; Wilcke et al., 1998). For example, Cal-Priesto et al. (2001) reported on urban soils in Spain containing Cr concentrations which average at 39 mg kg^{-1}.

A study into surface Cr concentration values at various sites in Guanajuato (Mexico) which are in the vicinity of a chromate factory, found concentrations of over 500 mg kg^{-1} (in the immediate vicinity of the factory) (Armienta et al., 1996). These values contrast with the much lower surface Cr values found in the Pollok Park soils (approximately 70 mg kg^{-1}, 0-1 cm).

An investigation into Cr distribution in industrial, residential and 'unpolluted' sites in Spain reported Cr soil (0-3 cm) values of 13.8 ± 3.9 mg kg^{-1} in industrial sites, 10.2 ± 3.2 mg kg^{-1} in residential sites and 8.6 ± 0.9 mg kg^{-1} in 'unpolluted' sites (Nadal et al., 2004). On comparing with the Pollok Park soils (both approximately 55 mg kg^{-1}, 0-5 cm), it is clear that all the sites in the study by Nadal et al. (2004) are significantly lower. Perhaps there are no sources of Cr emission in the vicinity of any of the study sites. Indeed, when comparing the Cr concentration data with the soil quality guidelines for the study region, it can be seen that Cr is well within the limits (Nadal et al., 2004).

A survey into Cr distribution in French forest soils has been published (Hernandez et al., 2003). In a north of France site a Cr (0-4 cm) concentration of 83.08 mg kg^{-1} was found, in a central France site a Cr (0-4 cm) concentration of 48.52 mg kg^{-1} was found, and in a south of France site a Cr (0-4 cm) concentration of 8.21 mg kg^{-1} was found. The Cr surface concentration values in the north and centre of France compare favourably with the Pollok Park soils (both approximately 55 mg kg^{-1}, 0-5 cm). However, the Cr surface concentration value in the south of France is significantly less. The site in the south of France is clearly exposed to very little contaminant Cr.

The mean Cr content of crustal rocks is 100 mg kg^{-1} (Sposito, 1989). The regional background value for the Pollok Park soils is approximately 20 mg kg^{-1}. A forest soil in central France was found to have a regional background value of 58.36 mg kg^{-1}, and a coniferous forest soil in Switzerland was found to have a regional value of 99.7 mg kg^{-1}

(Blaser et al., 2000; Hernandez et al., 2003). The regional Cr background value for the Pollok Park soils is in good agreement with the values for the forest soils in both France and Switzerland.

3.4.2 Metal distribution profiles: Cu

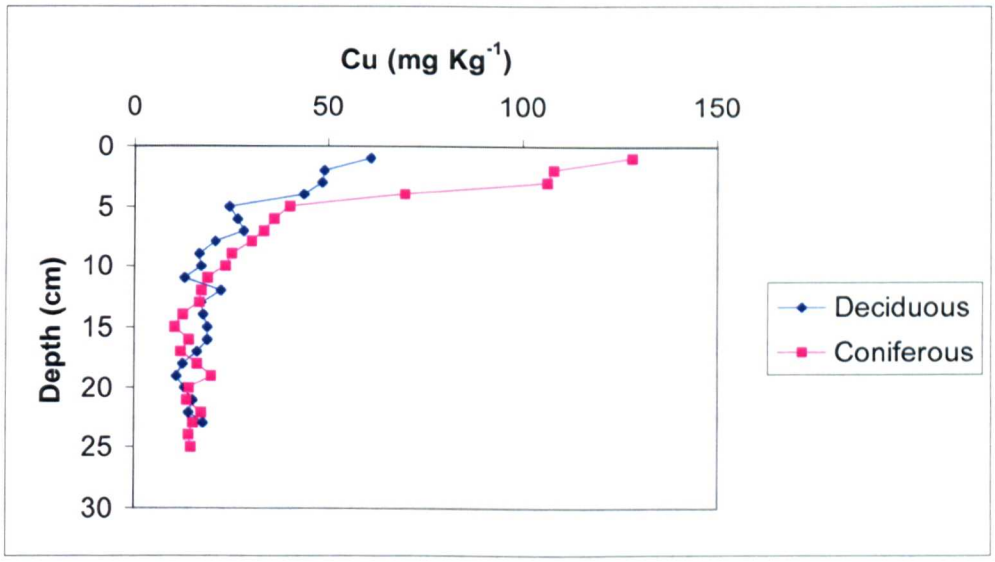


Figure 3-10 Cu concentration profile for the deciduous core and the coniferous core: Set 1

Figure 3-10 shows the Cu concentration profiles (Set 1) for the deciduous and the coniferous profiles to be different. There is a distinct difference between the two profiles when comparing the top 5 cm. The Cu concentration for the surface of the coniferous profile (approximately 125 mg kg^{-1}), is more than twice that of the deciduous profile (approximately 60 mg kg^{-1}). From the surface to a depth of 5 cm, both cores present a considerable decline in Cu concentration, to approximately 30 mg kg^{-1}. From 5 cm to 10 cm there is a further decline from 30 mg kg^{-1}, to approximately 20 mg kg^{-1}. Depths greater than 10 cm indicate no further decline in Cu concentration, instead there is an evening out in Cu concentration (approximately 20 mg kg^{-1}), to give the regional background soil value.

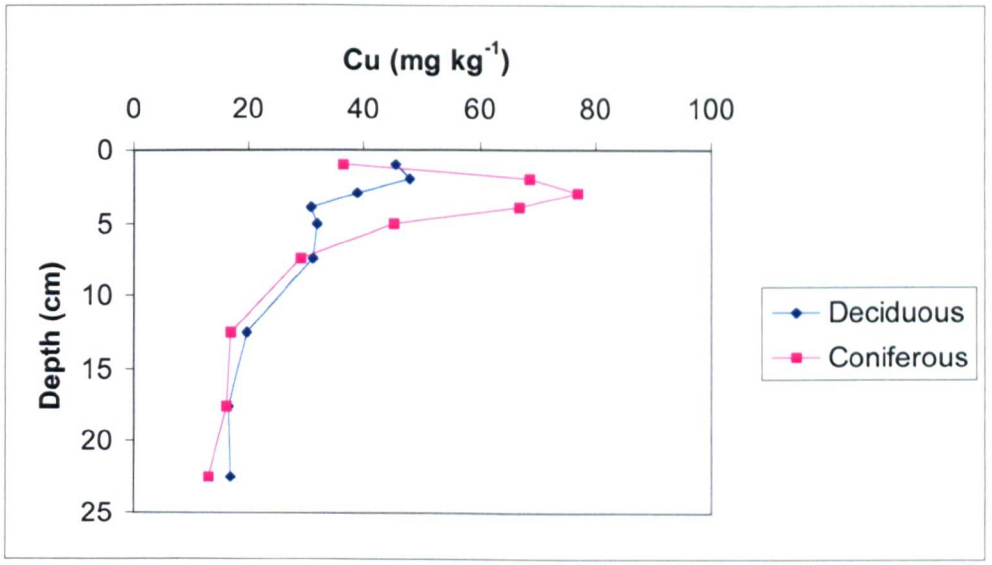


Figure 3-11 Average Cu concentration profiles for the deciduous cores and the coniferous cores: Set 2

Figure 3-11 presents Set 2 Cu concentration profiles for the deciduous cores and the coniferous cores. As was the case for the Set 2 coniferous profile for Cr, the Set 2 coniferous profile does not reach its highest concentration value until a depth of 3 cm (approximately 80 mg kg^{-1}) is attained. This reinforces the observation that perhaps the surface of the cores used to obtain the Set 2 coniferous concentration profile contained litter material at the surface, and not soil. Soil appears to be apparent only from a depth of 3 cm.

From a depth of 3 cm, the coniferous profile follows a similar pattern to the coniferous profile for Set 1; as it declines until a depth of 10 cm is reached and then maintains an approximately constant Cu value of 20 mg kg^{-1}. The deciduous profile follows a similar pattern to the one in Set 1, as it starts at a high Cu concentration and shows a decline until 10 cm is reached. Once a depth of 10 cm is reached, it maintains an approximately constant value of 20 mg kg^{-1}.

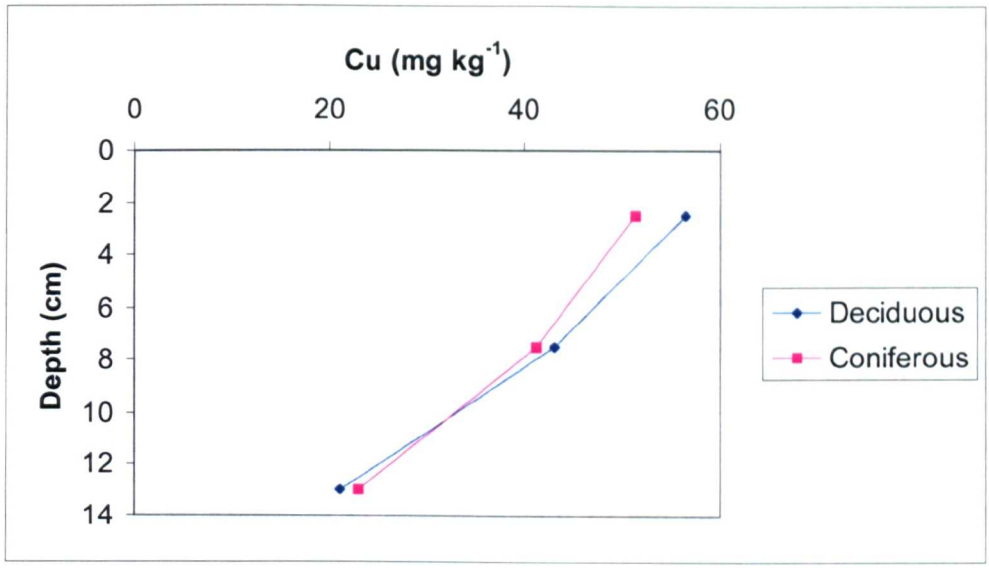


Figure 3-12 Average Cu concentration profiles for the deciduous cores and the coniferous cores: Set 3

Figure 3-12 presents a similar pattern for Cu concentration profiles, when compared to Figure 3-10 (Set 1) and Figure 3-11 (Set 2). The surface of both profiles presents a high Cu concentration profile which eventually decreases to a constant concentration of 20 mg kg^{-1}.

The enrichment of Cu to the surface is probably due to a combination of aerial deposition and vegetation (as Cu is an essential element to plants) (Blaser et al., 2000; Goldschmidt, 1954).

Cu concentration in the surface of the Pollok Park soils (approximately 60 mg kg^{-1}, 0-5 cm) compares favourable with average Cu concentrations in urban soils from different cities in the world (Cal-Prieto et al., 2001; Chen et al., 1997; Czarnowska, 1980; De Miguel et al., 1998; Li et al., 2004; Lux, 1986; Manta et al., 2002; Paterson et al., 1996; Thornton, 1991; Wilcke et al., 1998). For example, Cal-Priesto et al. (2001) reported that soils in Coruna contain an average Cu content of 60 mg kg^{-1} and Thornton et al. (1991) reported values of 73 mg kg^{-1} in London.

A study into Cu concentrations in the surface soils surrounding a Cu smelter and industrial complex found Cu values (0-5 cm) ranging from 12 mg kg^{-1} to 599 mg kg^{-1} (Martley et al., 2004). The high Cu concentrations were found close to the contamination sources.

Clearly, the Pollok Park soils (Cu: 60 mg kg^{-1}, 0-5 cm) are within the lower side of this range.

Cu concentration profiles for vineyard soils of Victoria, Australia, which had undergone the application of copper to them as a means of counteracting Cu deficiency have been established (Pietrzak & McPhail, 2004). They follow a similar outline to those in Figure 3-10. Cu concentrations at one vineyard site gave a surface concentration (0-1 cm) of 80.3 mg kg^{-1} which decreased to approximately 12 mg kg^{-1} at a depth of 20-25 cm, with no significant change at depths greater than 25 cm. Evidently, there are Cu deposits associated with the surface of this profile. However, when comparing with the Pollok Park site, the coniferous soil has a higher surface concentration (approximately 125 cm, 0-1 cm) and the deciduous soil has a lower surface concentration (approximately 60 mg kg^{-1}, 0-1 cm). Perhaps this is a result of the vegetation associated with each of these soils, in that the coniferous soil has the greatest organic matter content, followed by the vineyard soil and then the deciduous soil; as Cu is known to form complexes with organic compounds.

Cu distribution was studied in French forest soils (Hernandez et al., 2003). In a north of France site, 23.07 mg kg^{-1} of Cu was found in the soil surface (0-4 cm), in a central France site, 23 mg kg^{-1} of Cu was found in the soil surface (0-4 cm), and in a south of France site 1.12 mg kg^{-1} of Cu was found in the surface. All these sites contain smaller surface Cu concentration values than the Pollok Park soils (60 mg kg^{-1}, 0-5 cm). Evidently, the north site and central site are exposed to lower levels of Cu than the Pollok Park soils and the south site is exposed to virtually none.

The distribution of Cu in four Forest sites was conducted by Andersen et al. (2004). They reported Cu values in the soil surface (e.g. 4.45 mg kg^{-1}, 0-5 cm) as being much lower than those found for the Pollok Park soils (60 mg kg^{-1}, 0-5 cm). Perhaps this is due to these sites being exposed to much lower anthropogenic Cu inputs than the Pollok Park soils.

The regional background Cu value for the Pollok Park soils is approximately 20 mg kg^{-1}. The mean Cu content in crustal rocks is 50 mg kg^{-1} (Sposito, 1989). A forest soil in central France has a background value of 9.48 mg kg^{-1}, a forest soil in Switzerland had a background value of 17 mg kg^{-1} and a soil in a relatively unpolluted area in Finland has a background value of 4 mg kg^{-1} (Blaser et al., 2000; Hernandez et al., 2003; Nieminen et al., 2002). These soils from nearby regions have background regional values for Cu which are in good agreement with the Pollok Park soils.

3.4.3 Metal distribution profiles: Fe

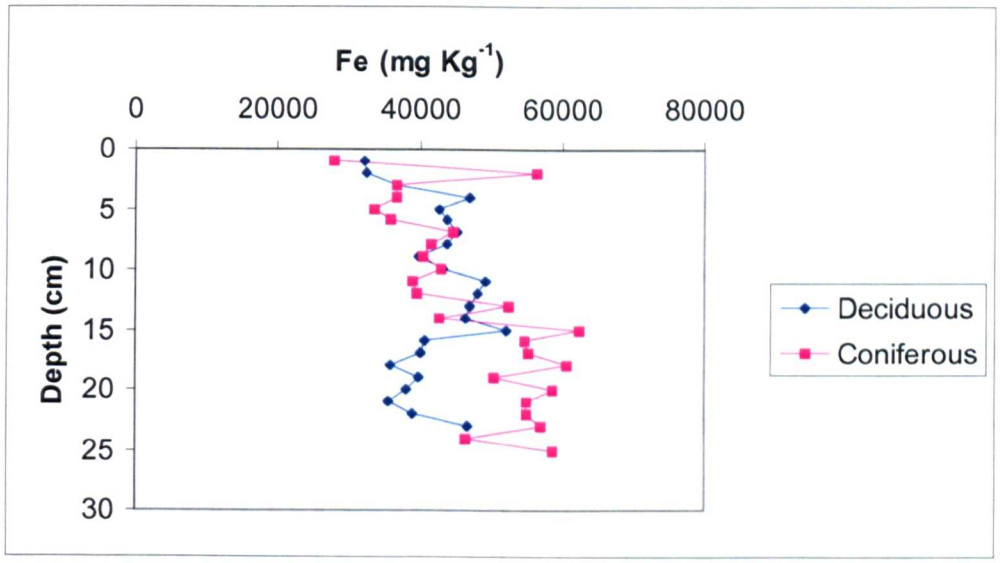


Figure 3-13 Fe concentration profile for the deciduous core and the coniferous core: Set 1

Figure 3-13 shows the Fe concentration profiles for the deciduous core and the coniferous core to be very similar in that they average around 40,000 mg kg^{-1} from the surface to a depth of 15 cm. From a depth of 15 cm, there is a pronounced decrease in the deciduous profile to a depth of approximately 20 cm. However, from 20 cm depth to the base of the core, the concentration averages out to 40,000 mg kg^{-1} once again. From a depth of 15 cm, there is a marked increase in the coniferous profile to the base of the core where a value approaching 60,000 mg kg^{-1} is attained.

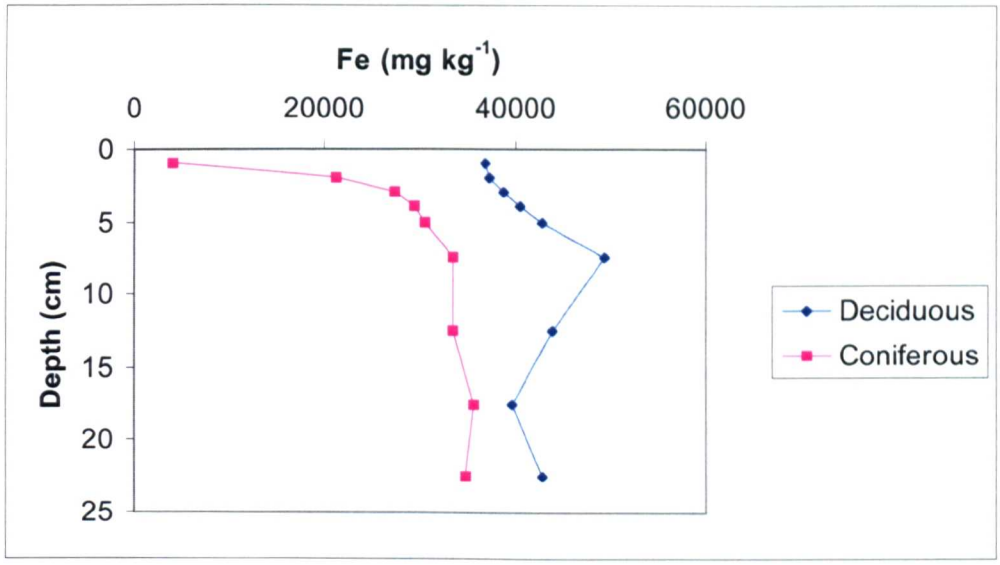


Figure 3-14 Average Fe concentration profiles for the deciduous cores and the coniferous cores: Set 2

Figure 3-14 shows there to be a marked difference between the deciduous profile and the coniferous profile. This difference was not evident in Set 1, it is perhaps an artefact of sampling. The deciduous concentration profile for Set 2 is very similar to the deciduous concentration profile for Set 1, as overall, it averages out at 40,000 mg kg^{-1}. The finer details observed in Set 1 below a depth of 5 cm are not evident in Set 2. This is probably due to the larger sections (from 5 cm depth) which the cores were cut into in order to obtain the concentration profiles for Set 2. The coniferous Fe concentration profile for Set 2 possesses the same feature as the coniferous concentration profiles for Cr and Cu; in that it appears as though the surface of the cores used to obtain these profiles was litter and not soil. The soil surface does not appear until 3 cm depth has been reached. From 3 cm depth, the coniferous concentration profile maintains an average Fe concentration of 30,000 mg kg^{-1} to the base of the core. This is very similar to the coniferous Fe concentration profile in Set 1, with the exception that the average concentration for Set 1 is nearer to 40,000 mg kg^{-1}. This could be due to differences in the sampling procedures used to obtain the cores for Set 1 and Set 2 concentration profiles.

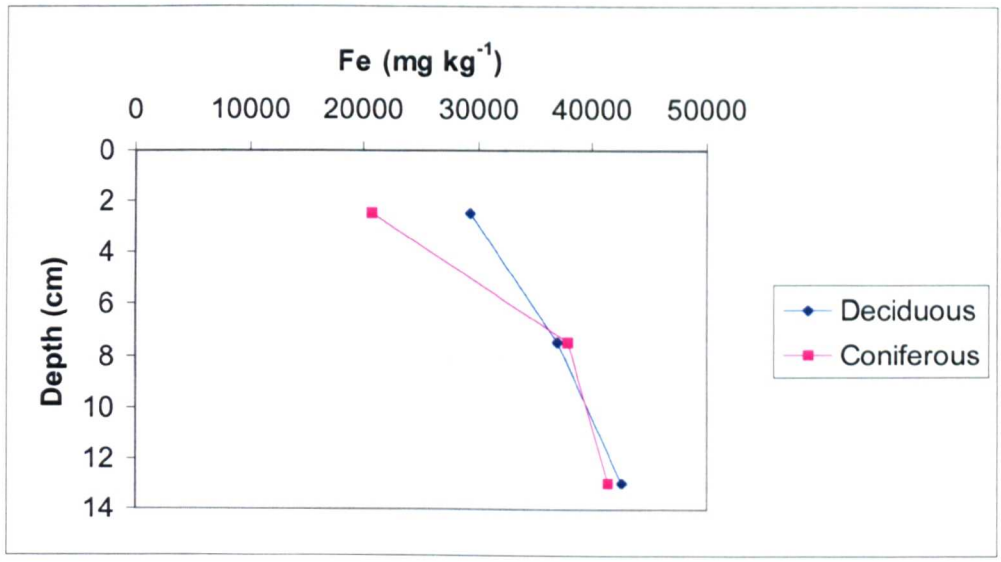


Figure 3-15 Average Fe concentration profiles for the deciduous cores and the coniferous cores: Set 3

The concentration profiles in Figure 3-15 support the findings from Set 1 and Set 2, as there is no evidence of Fe enrichment to the surface of the profiles. Due to the cores used to obtain these profiles only being cut into three section (approximately 5 cm), the details observed in the profiles for Set 1 and Set 2 are not evident.

On studying the Fe profiles for the Pollok Park soils, there appears to be no enrichment to the surface. This indicate that perhaps there is no aerial deposition and no vegetation input to the surface. Considering that Fe is an essential plant element, it would be expected that vegetation has a part to play in enriching the surface with Fe.[1] However, another possibility is that Fe is deposited to the surface by the vegetation, and the reason this is not evident in the concentration profiles is that the Fe is redistributed due to redox driven processes in the soil (MacKenzie et al., 1998).

The Fe concentration profiles for the Pollok Park soils are similar to those reported by Madrid et al. (2002) and Wilcke et al. (1998). Madrid et al. (2002) studied the distribution of Fe in urban soils in parks in Seville. As was the case with the Pollok Park soils, these soils showed no enrichment of Fe to the surface of the soil (mean: 20,100 mg kg^{-1}) when compared to the background value of the soil (mean: 21,400 mg kg^{-1}). Wilcke et al. (1998) reported a mean Fe value of 16,100 mg kg^{-1} in the surface of soils in Bangkok.

The mean crustal abundance for Fe is 41,000 mg kg^{-1} (Sposito, 1989). The Pollok Park soils have an approximate background regional value for Fe of 40,000 mg kg^{-1}, which is approximately double the background value reported for urban soils in Seville (21,400 mg kg^{-1}) (Madrid et al., 2002).

3.4.4 Metal distribution profiles: Mn

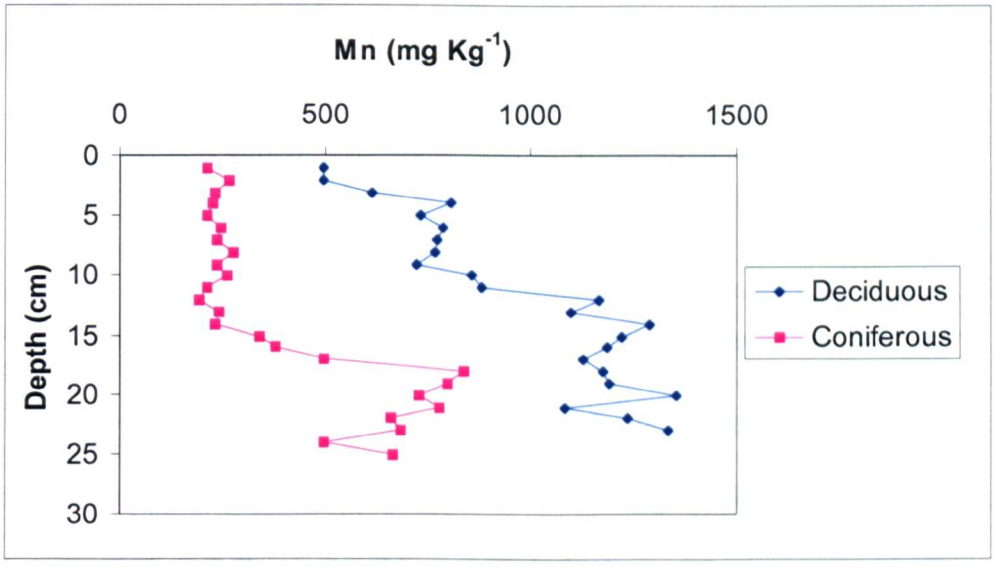


Figure 3-16 Mn concentration profile for the deciduous core and the coniferous core: Set 1

Figure 3-16 shows the Mn concentration profiles for the deciduous core and the coniferous core to be very different. Neither profile shows evidence of Mn enrichment to the surface. The Mn concentration in the coniferous concentration profile is significantly lower than the Mn concentration in the deciduous concentration profile. The coniferous core profile has a surface concentration of approximately 250 mg kg^{-1} and the deciduous core profile has a surface concentration of approximately 500 mg kg^{-1}. The deciduous profile concentration remains constant from the surface to a depth of approximately 15 cm. From 15 cm to 20 cm, it rapidly increases in concentration to a concentration of 750 mg kg^{-1} (20 cm). From approximately 20 cm depth to the base of the deciduous core there is a slight decrease in concentration to approximately 400 mg kg^{-1}. The coniferous concentration profile gradually increases to a value of 1250 mg kg^{-1} from the surface to a depth of approximately

15 cm. From 15 cm to the base of the core, the coniferous profile remains at approximately 1250 mg kg^{-1}.

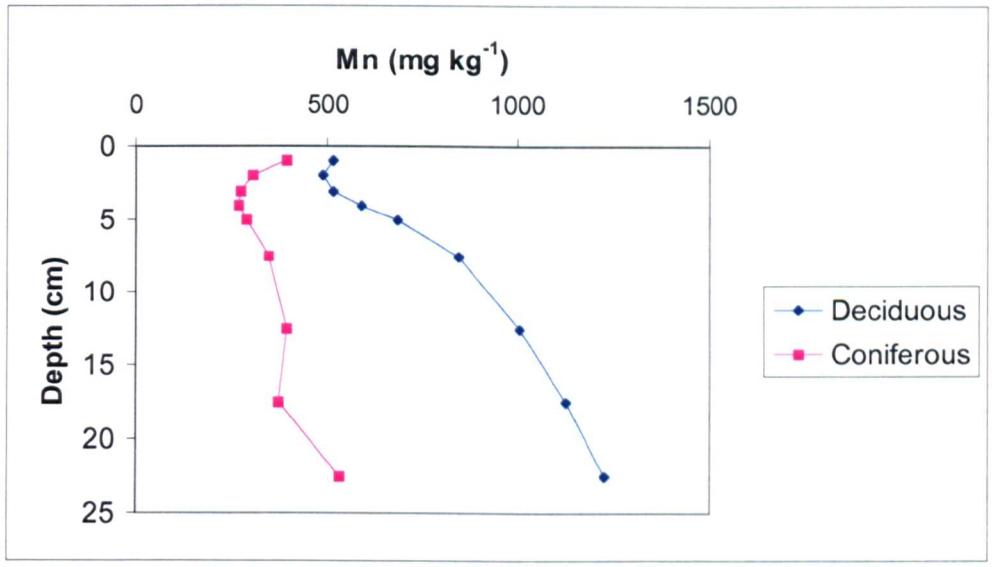


Figure 3-17 Average Mn concentration profiles for the deciduous cores and the coniferous cores: Set 2

Figure 3-17 supports the findings from Set 1 Mn concentration profiles, in that the coniferous Mn concentration profiles are very different from the deciduous Mn concentration profiles and neither profile shows enrichment to the surface. Also, the Mn concentration in the coniferous profile is significantly lower than the Mn concentration in the deciduous concentration profile.

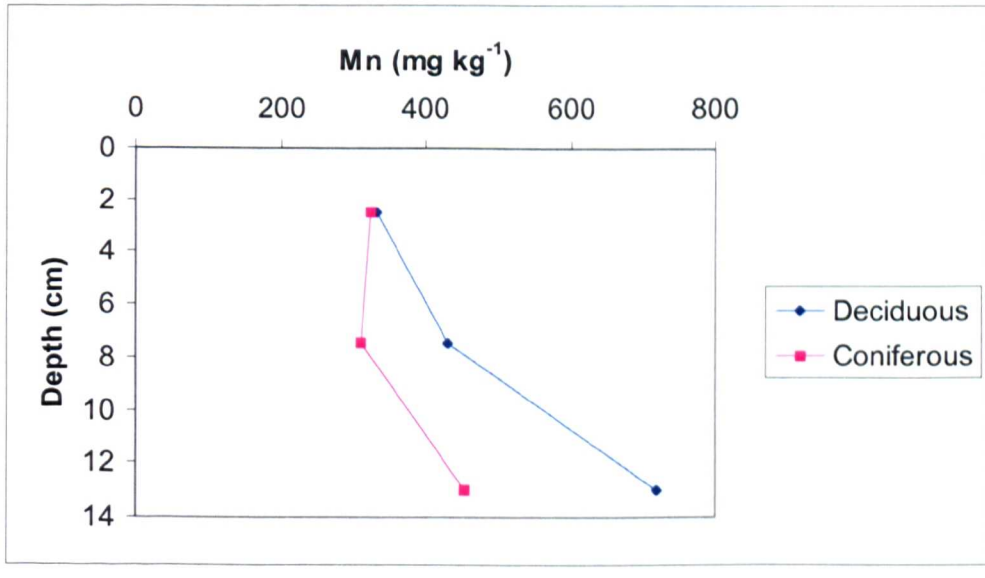


Figure 3-18 Average Mn concentration profiles for the deciduous cores and the coniferous cores: Set 3

Figure 3-18 (Set 3) concentration profiles support the information obtained from Set 1 (Figure 3-16) and Set 2 (Figure 3-17) concentration profiles.

Mn showed no signs of enrichment to the surface. However, the situation is very similar to that found for Fe. Mn is an essential element to plants, so it would be expected that this factor would play a part in the enrichment of Mn to the soil surface (Gorres & Frenzel, 1997).[1] Mn could very possible have been redistributed through the soil due to redox influences (MacKenzie et al., 1998).

The results obtained for the Pollok Park soils compare favourably with work by Wilcke et al. (1998) and Madrid et al. (2002) (Mn distribution in urban soils). Wilcke et al. (1998) found a mean Mn value of 340 mg kg^{-1} in urban soils. Madrid et al. (2002) reported Mn values of 471 mg kg^{-1} (0-10 cm) (Pollok Park soils: approximately 500 mg kg^{-1}, 0-5 cm) and regional background values of 510 mg kg^{-1} (Pollok Park soils: approximately 500 mg kg^{-1}).

The mean Mn crustal value is 950 mg kg^{-1} (Sposito, 1989). The regional background value for the Pollok Park soils is approximately 500 mg kg^{-1}. This value is in good agreement with the regional background value reported by Madrid et al. (2002) (510 mg kg^{-1}).

3.4.5 Metal distribution profiles: Pb

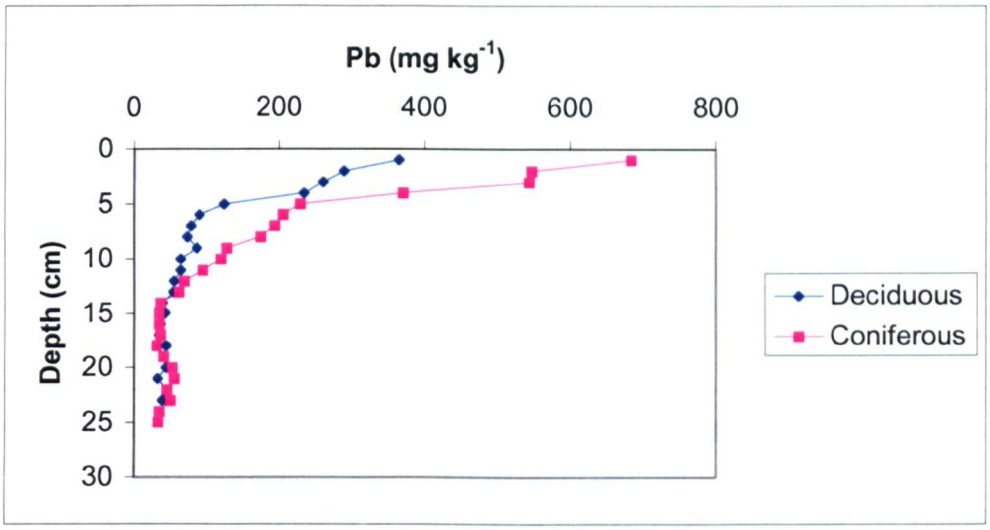


Figure 3-19 Pb concentration profile for the deciduous core and the coniferous core: Set 1

Figure 3-19 (Set 1) shows there to be a difference between the coniferous Pb concentration profile and the deciduous Pb concentration profile. When comparing the top 5 cm, there is a distinct difference. The Pb concentration for the surface of the coniferous profile (approximately 700 mg kg^{-1}) is almost twice that of the deciduous profile (approximately 400 mg kg^{-1}). From the surface to a depth of 5 cm, both cores show a decline in Pb concentration; the coniferous profile to approximately 200 mg kg^{-1} and the deciduous profile to approximately 125 mg kg^{-1}. From 5 cm to 10 cm depth, there is a further decline, as both cores fall to approximately 50 mg kg^{-1} From 10 cm depth to the base of the core, both profiles maintain a constant concentration of approximately 50 mg kg^{-1}.

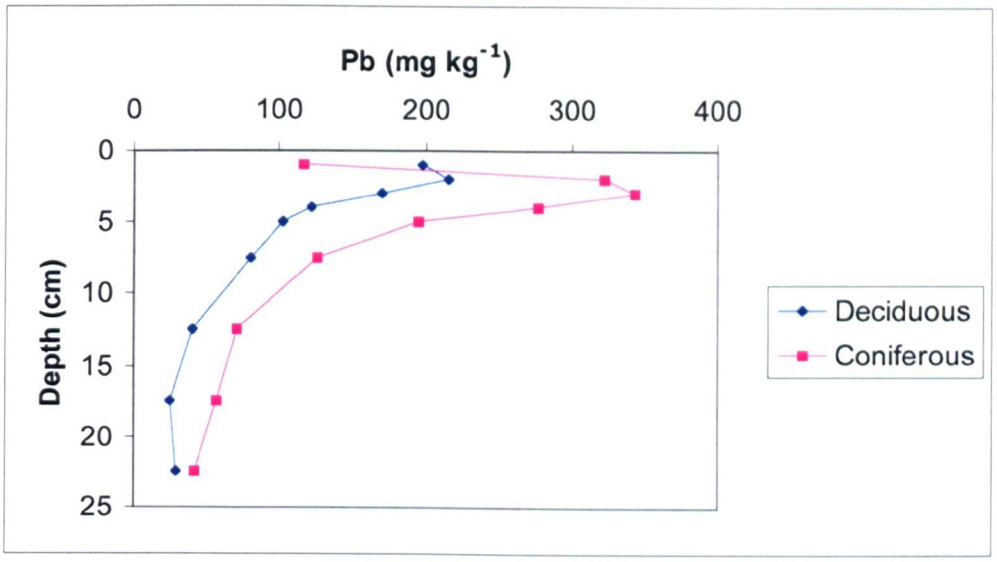


Figure 3-20 Average Pb concentration profiles for the deciduous cores and the coniferous cores: Set 2

Set 2 average concentration profiles for the deciduous cores and the coniferous cores are presented in Figure 3-11. As was noted for Set 2 coniferous concentration profiles for Cr, Cu and Fe, the coniferous profile does not reach its highest concentration value until a depth of 3 cm (approximately 350 mg kg^{-1}) is reached. This supports the finding that perhaps the surface of the cores used to obtain the Set 2 coniferous concentration profiles contained litter material at the surface, not soil, as soil only appears to be present from 3 cm depth.

The coniferous profile from Set 2, when considered from a depth of 3 cm, follows a similar pattern to the coniferous profile from Set 1. From 3 cm to 10 cm depth, it declines to 50 mg kg^{-1} Pb and then maintains this value. A similar pattern is found when comparing the deciduous profile from Set 1 with the deciduous profile from Set 2. The deciduous profile from Set 2 starts with a high Pb concentration and shows a decline until 10 cm depth is reached. From 10 cm depth, it maintains an approximately constant value of 50 mg kg^{-1}.

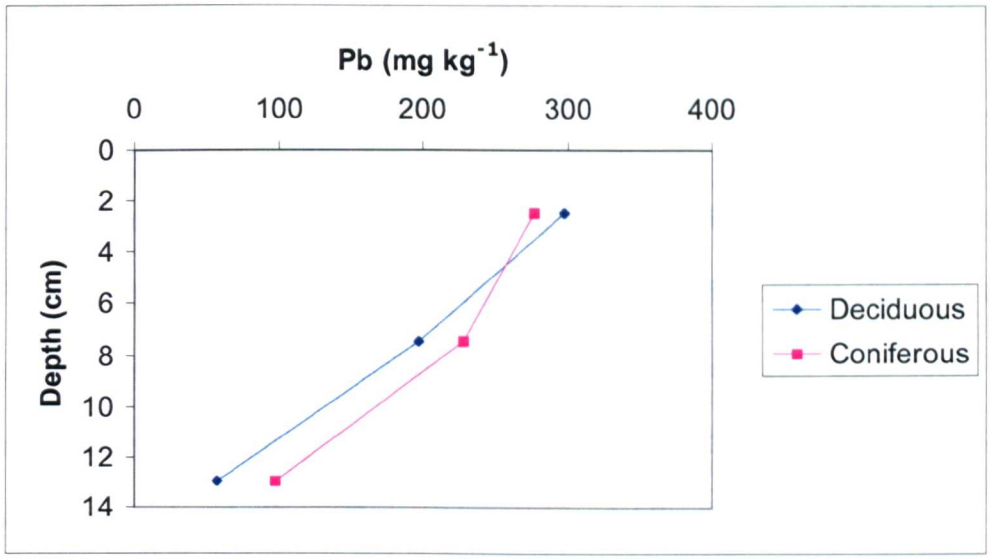


Figure 3-21 Average Pb concentration profiles for the deciduous cores and the coniferous cores: Set 3

Figure 3-21 (Set 3) supports the findings from Set 1 and Set 2 that there is Pb enrichment to the surface of both profiles and eventually a constant value of approximately 50 mg kg^{-1} is attained.

There is clearly a considerable degree of enrichment to the surface and this is very likely due to atmospheric deposition as a result of the widespread use of Pb in industry etc. There is a large body of evidence conducted in a great number of different environmental archives (e.g. polar ice, lake sediments, bogs and moss samples), providing convincing evidence of lead pollution due to long range transport (Martinez Cortizas et al., 2002). It has been demonstrated that industrial activities are responsible for global-scale Pb pollution and established that there is a link between world-wide Pb production and atmospheric pollution (Martinez Cortizas et al., 2002; Murozumi et al., 1969).

However, vegetation should be considered as a contributor to surface enrichment of Pb. As stated previously, there have been reports of uptake of elements such as Pb by vegetation, despite the fact that they have no known biological role (Blaser et al., 2000; Kabata-Pendias & Pendias, 1992).

There have been many studies into Pb distribution in all types of soils due to its known toxicity (Bergkvist et al., 1989; Bindler et al., 1999; Erel, 1998; Erel et al., 1997; Friedland

et al., 1992; Friedland & Johnson, 1985; Kelly & Thornton, 1996; Marsh & Siccama, 1997; Nowack et al., 2001; Watmough & Hutchinson, 2004).

On comparing average Pb values in urban soils world-wide with the surface layer of the Pollok Park soils (both approximate at 300 mg kg^{-1}, 0-5 cm), it is clear that the Pollok Park soil values are much lower than those reported for samples from some large and / or industrialised cities (e.g. Boston, central Madrid, central London). The Pollok Park soil values are more similar to those measured in smaller cities (e.g. Hamburg, Aberdeen) and residential areas of London (London Borough) (Angelone et al., 1995; Cal-Prieto et al., 2001; Chen et al., 1997; Culbard et al., 1988; Czarnowska, 1980; De Miguel et al., 1998; Gibson & Farmer, 1986; Li et al., 2001; Lux, 1986; Manta et al., 2002; Paterson et al., 1996; Rundle & Duggan, 1980; Sanchez-Camazano et al., 1994; Thornton, 1991; Wilcke et al., 1998). For example, Rundle and Duggan (1980) reported that soils in central London contain an average of 647 mg kg^{-1} Pb. This is over two times the values found in the soils from Pollok Park. However, Lux (1986) reported that soils in Hamburg average at approximately 218.2 mg kg^{-1}.

Kelly and Thornton (1996) found concentrations of Pb with values in excess of 1000 mg kg^{-1} in an area associated with busy road junctions and old housing. They reported that the highest concentrations of Pb generally occur close to major junctions or roundabouts on main roads with a high traffic density, and that most of the Pb deposited from traffic sources occurs where the traffic density is high and vehicles are required to stop and accelerate (Lyons et al., 1990). Concentrations of Pb were also seen to be high in locations parallel to busy roads (from 342-581 mg kg^{-1}). However, Pb in roadside soils typically decreases exponentially, with most of the metal deposited within 30-50 m of the road (Davies, 1990). When Kelly and Thornton (1996) compared Pb values in this area with an industrial area with less busy road junctions, they found a mean for topsoils (0-15 cm) of

158 mg kg^{-1} in the area with busy road junctions, compared to a mean of 106 mg kg^{-1} in the industrial area.

The Pb value for the site of study is approximately 300 mg kg^{-1} (0-5 cm) for both soils. Running nearby and parallel to the sampling site (Pollok Park) is a busy road, which is probably one of the main sources of Pb depostion to the soils being studied. Although not in excess of 1000 mg kg^{-1}, the value associated with sites containing busy road junctions, the value is greater than the average of both sites (industrial site and busy roads site); thereby implying the degree of pollution associated with the site to be moderate but not overly pronounced.

Watmough and Hutchinson (2004) studied the distribution of Pb in woodland (maple and pine) in south-central Ontario, 150 km northeast of Toronto. Pb concentrations at the maple site decreased from approximately 24 mg kg^{-1} (0-1 cm) in the surface horizon, to around 6 mg kg^{-1} at 7.5-10 cm depth, with little change thereafter. A similar pattern was found at the pine stand, with Pb concentrations decreasing from approximately 22 mg kg^{-1} (0-1 cm) to around 7 mg kg^{-1} at 10-20 cm depth. The decrease in Pb concentration with soil depth in the pine stand was more gradual than in the maple stand, reflecting the distribution of organic matter in the soil. In comparison with the Pollok Park deciduous soil (approximately 700 mg kg^{-1}, 0-1 cm) and coniferous soil (approximately 400 mg kg^{-1}, 0-1 cm), the degree of aerial deposition associated with the soils below the maple and pine stands is considerably smaller. Evidently, the Pollok Park site is either exposed to considerably higher levels of Pb pollution, or this difference is a reflection of differences in properties of the forest floor, or indeed a combination of these two factors play a part. When comparing the background value for Pb in the deciduous (approximately 50 mg kg^{-1}) and coniferous (approximately 50 mg kg^{-1}) soils with those found for the soils below the maple (6 mg kg^{-1}) and pine (7 mg kg^{-1}) canopies, the background value of Pb in Pollok Park is approximately ten times that found in the south-central Ontario site.

Kaste et al. (2003) also studied the distribution of Pb in forest soils. They focussed on montane forest soils. Average Pb concentrations at the surface were approximately 59.8 mg kg^{-1} (0-4 cm). This value, although considerably higher than the surface values reported by Watmough and Hutchinson (2004), is still considerably smaller than the values for the Pollok Park site (approximately 300 mg kg^{-1} for both soils, 0-5 cm). This implies, (as did the results reported by Watmough and Hutchinson, 2004), that the Pollok Park site is either exposed to considerably higher levels of Pb pollution, or this difference is a reflection of differences in properties of the forest floor, or indeed a combination of these two factors play a part.

Andersen et al. (2002) also reported Pb surface values (8.2 mg kg^{-1}, 0-10 cm) in forest soils as being much lower than those found for the Pollok Park soils (approximately 300 mg kg^{-1}, 0-5 cm) (Andersen et al., 2002).

The regional background Pb concentration for the Pollok Park soils is 50 mg kg^{-1}. This fits in well with the mean Pb background values reported in the literature: Hernandez et al. (2003) reported a value of 34.99 mg kg^{-1} in a French forest soil, Blaser et al. (2000) reported a value of 26.8 mg kg^{-1} in a Swiss forest soil, Nieminen et al. (2002) reported a value of 20 mg kg^{-1} in a rural Finnish soil and Farmer et al. (1996) reported a value of 15 ± 4 mg kg^{-1} in a Scottish loch sediment (Blaser et al., 2000; Farmer et al., 1996; Hernandez et al., 2003; Nieminen et al., 2002). The mean Pb content in crustal rocks is 14 mg kg^{-1} (Sposito, 1989). This value clearly indicates that the background Pb levels found for these soils involve a considerable degree of pollutant Pb.

Studies into the true natural background level of Pb have been conducted in Sweden (Bindler et al., 1999; Brannvall et al., 1999). Brannvall et al. (1999) combined concentration and stable lead isotope analysis of varved sediments from four lakes to differentiate between natural and pollution lead to study the detailed atmospheric pollution

history of the last 3000 years in northern Sweden. The results indicate that approximately 3000 years ago, background values were approximately 1 mg kg^{-1} Pb. These results indicate that pollution lead became the totally dominant source of lead in the remote north Scandinavain environment, long prior to the Industrial Revolution. The same must have applied to areas closer to the main sources in old cultural areas of Europe. Bindler et al. (1999) combined the analyses of Pb concentrations and stable Pb isotopes ($^{206}Pb/^{207}Pb$) of ombrotrophic peat and forest soils from southern Sweden with a model for Pb cycling in forest soils to derive an estimate for the prepollution concentration of lead in the mor layer of boreal forest soils, and to back-calculate Pb concentrations for the last 5,500 years. While the present-day concentrations of the mor layer are typically 40-100 mg kg^{-1}, they found that Pb concentrations of pristine forest mor layers in Sweden were quite low ($\leq$0.1 mg kg^{-1}). They also reported that large-scale atmospheric pollution from the Greek and Roman cultures (ca. 0 AD) increased Pb concentrations to about 1 mg kg^{-1}, and that Pb concentrations increased to about 4 mg kg^{-1} following the increase of metal production and atmospheric pollution in medieval Europe (ca. 1000 AD).

3.4.6 Metal distribution profiles: Zn

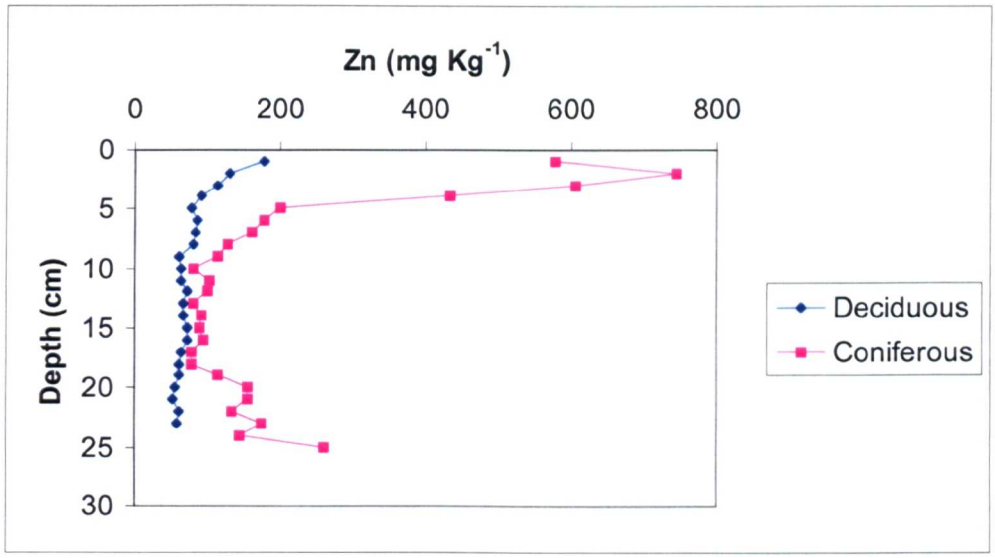


Figure 3-22 Zn concentration profile for the deciduous core and the coniferous core: Set 1

Figure 3-22 shows the Set 1 Zn concentration profiles for the deciduous core and the coniferous core to be very different. When the top 5 cm are compared there is a distinct difference. The Zn concentration for the surface of the coniferous profile (approximately 700 mg kg^{-1}) is around three times that of the deciduous profile (approximately 200 mg kg^{-1}). From the surface to a depth of 5 cm, both cores show a decline in Zn concentration; the coniferous profile to approximately 200 mg kg^{-1} and the deciduous profile to around 100 mg kg^{-1}. From 5 cm to 10 cm depth, there is a further decline, as both cores fall to approximately 75 mg kg^{-1}. For the deciduous core, a concentration of 75 mg kg^{-1} is maintained from 10 cm depth to the core base. For the coniferous core, a concentration of 75 mg kg^{-1} is maintained from 10 cm to 20 cm depth. From 20 cm depth to the core base, there is a pronounced increase to approximately 225 mg kg^{-1}.

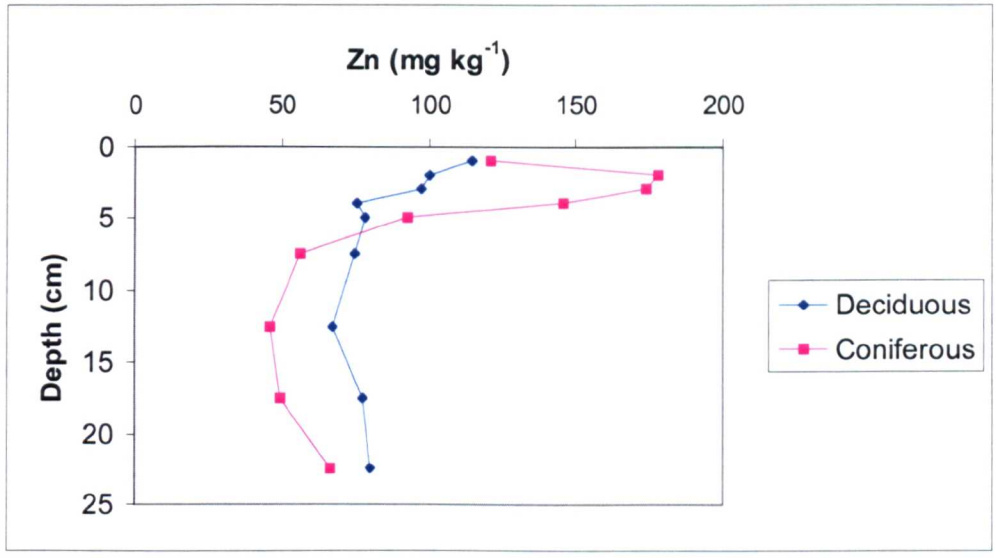


Figure 3-23 Average Zn concentration profiles for the deciduous cores and the coniferous cores: Set 2

The average Zn concentration profiles for the deciduous cores and the coniferous cores are presented in Figure 3-23. The coniferous profile does not reach its highest concentration value until a depth of 2 cm. This compares with Cr, Cu, Fe and Pb Set 2 concentration profiles, as they did not reach their highest value until 3 cm. The readings at 2 cm and 3 cm are very similar, as they approximate at 175 mg kg^{-1}. As a result of these two findings, it was decided to continue with the assumption that the soil surface is at 3 cm for Set 2 coniferous profiles, and the previous two sections (1 cm and 2 cm) are surface litter.

When considered from a depth of 3 cm, the coniferous profile from Set 2 follows a similar pattern to the coniferous profile from Set 1. A high Zn concentration is found from 3 cm depth, and from 3 cm to 10 cm depth, the concentration declines to approximately 75 mg kg^{-1}. This value of 75 mg kg^{-1} is maintained from 10 cm depth to the base of the core. When comparing the deciduous profile from Set 2 with Set 1, they both start with a high concentration which declines to a depth of 10 cm, where 75 mg kg^{-1} is reached. From 10 cm to the base of both cores (Set 1 and 2), an approximate value of 75 mg kg^{-1} is maintained.

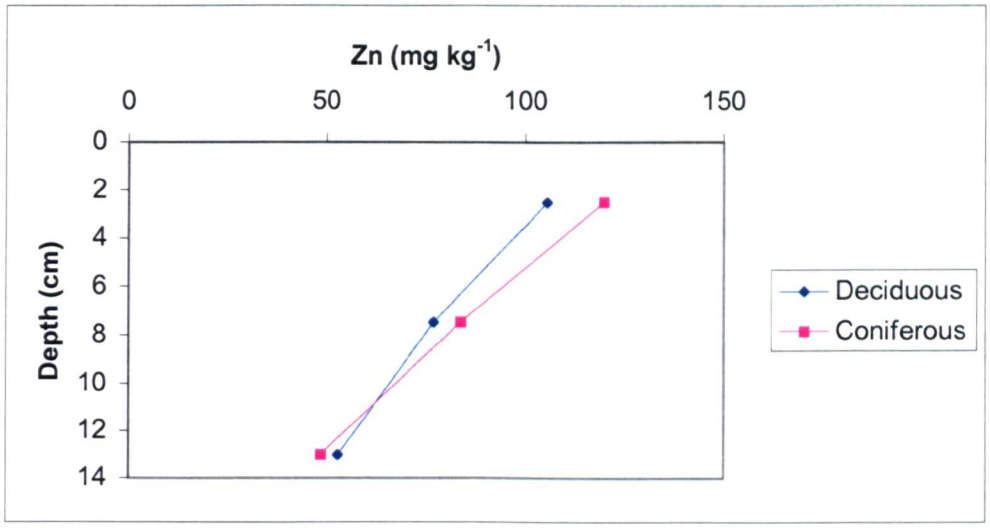


Figure 3-24 Average Zn concentration profiles for the deciduous and the coniferous cores: Set 3

Set 3 Zn concentration profiles (Figure 3-24) substantiate the findings from Set 1 and Set 2. All profiles clearly indicate Zn enrichment to the surface. The enrichment of Zn to the surface of the coniferous and deciduous profiles is probably caused by both aerial deposition and contributions from vegetation (as Zn is an essential element for plants) (Blaser et al., 2000; Goldschmidt, 1954).[1]

By comparing the surface Zn concentration of both Pollok Park soils (approximately 100-125 mg kg^{-1}, 0-5 cm) with the average Zn concentration in urban soils from different cities in the world, an overview can be obtained on the degree of Zn pollution in the Pollok Park soils (Cal-Prieto et al., 2001; Chen et al., 1997; Culbard et al., 1988; Czarnowska, 1980; De Miguel et al., 1998; Li et al., 2001; Lux, 1986; Manta et al., 2002; Paterson et al., 1996; Thornton, 1991; Wilcke et al., 1998). The Pollok Park soils comply with the average for Zn concentrations in city soils around the world. For example, Wilcke et al. (1998) found that soils in Bangkok contain an average of 118 mg kg^{-1} Zn, and Li et al. (2001) found that soils in Hong Kong contain an average of 168 mg kg^{-1} Zn.

Kelly and Thornton (1996) found very high Zn concentrations in topsoils (0-15 cm) when studying an area which has suffered contamination from the Fe industry (54.2-6740 mg kg^{-1}). When they compared this value to topsoils (0-15 cm) in a non-industrial area, they

found, in general, much reduced Zn concentrations (11.4-1810 mg kg^{-1}). Typically, topsoils in the industrially affected area contain two times the Zn concentration found in the non-industrial area. The Zn values for the Pollok Park samples (approximately 125 mg kg^{-1} for both soils, 0-5 cm) clearly indicate very low levels of pollution.

Hernandez et al. (2003) reported concentration values for Zn in the surface (0-4 cm) of French forest soils. A Zn value of 45.01 mg kg^{-1} was found in a north of France site, 116 mg kg^{-1} was found in a central France site and 3.95 mg kg^{-1} was found for a south of France site. When comparing these values with the Zn concentration (100-125 mg kg^{-1} 0-5 cm) for the Pollok Park soils, it is clear that the site in central France contains a very similar level. However, the north of France site value is considerable less and the south of France value is such that it appears to be exposed to virtually no Zn inputs.

Andersen et al. (2002) found significantly lower Zn values in forest surface soils (7.1 mg kg^{-1}, 0-10 cm) than those found for the Pollok Park soils (approximately 125 mg kg^{-1}, 0-5 cm). This contrast in surface soil values is probably a reflection of the different aerial Zn inputs each site is exposed to.

The average regional background Zn value for the Pollok Park soils is 50 mg kg^{-1}. The mean crustal Zn content is 75 mg kg^{-1}. Background values reported for nearby regions (Sposito, 1989) are compatible with the background value found for the Pollok Park soils. Blaser et al. (2000) reported a values of 80.7 mg kg^{-1} in a Swiss forest soil, Hernandez et al. (2003) reported a value 84.73 mg kg^{-1} in a French forest soil and Nieminen et al. (2002) reported a value of 61 mg kg^{-1} in a rural Finnish soil (Blaser et al., 2000; Hernandez et al., 2003; Nieminen et al., 2002).

3.5 Loss on Ignition

3.5.1 Loss on ignition profiles

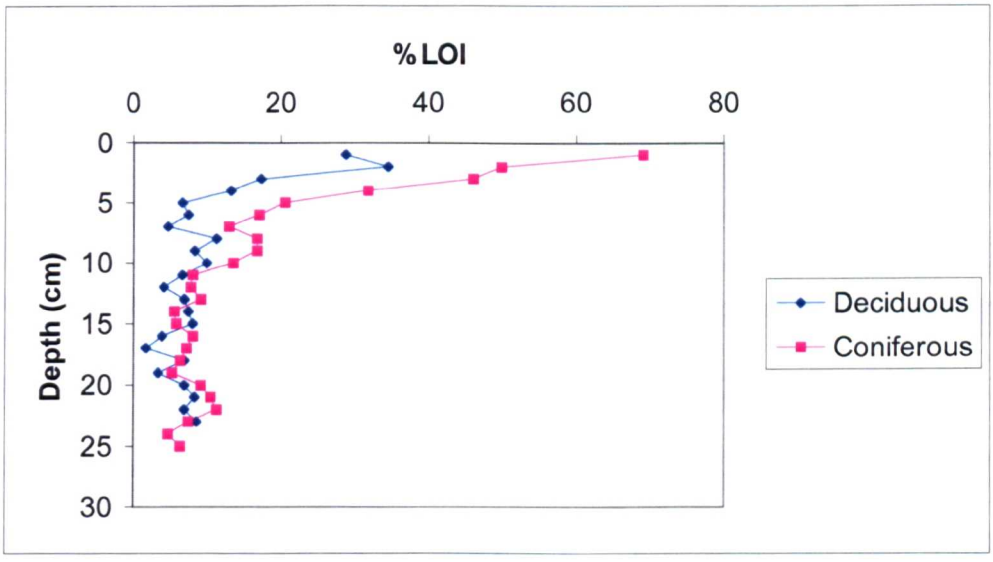


Figure 3-25 Loss on ignition profile for the deciduous core and the coniferous core: Set 1

Figure 3-25 shows the loss on ignition profiles for the deciduous core and the coniferous core to be different. When the top 5 cm are compared, there is a distinct difference. The LOI (loss on ignition) value for the coniferous profile is approximately 70%, whereas the LOI for the deciduous profile is approximately 30%. From the surface to a depth of 5 cm, both profiles show a sharp decline in LOI value; the coniferous profile has a value of approximately 20% and the deciduous profile has a value of approximately 15%. From 5 cm to 10 cm depth, both profiles show a further decline, as both reach a value of approximately 10%. From a depth of 10 cm to 25 cm, both profiles maintain a steady LOI value of approximately 10%.

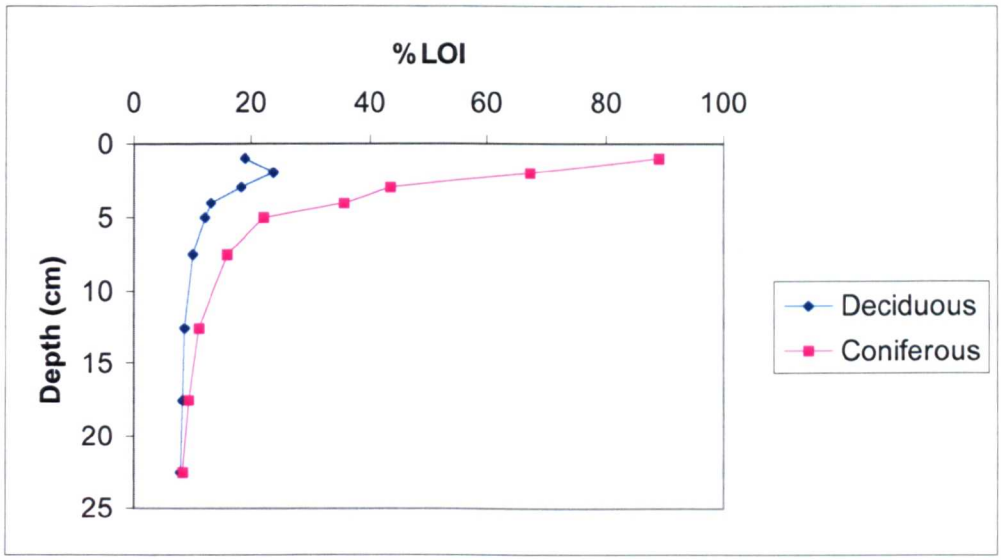


Figure 3-26 Average loss on ignition for the deciduous cores and the coniferous cores: Set 2

The Set 2 LOI profiles show a similar pattern to the Set 1 LOI profiles. The coniferous profile has a significantly higher organic matter content in the surface (approximately 90%) than the deciduous surface (approximately 20%). This declines for both profiles with depth, and once a depth of 10 cm is reached, a value of 10% is attained by both profiles. For both profiles, this value remains constant from 10 cm to the base of the profile.

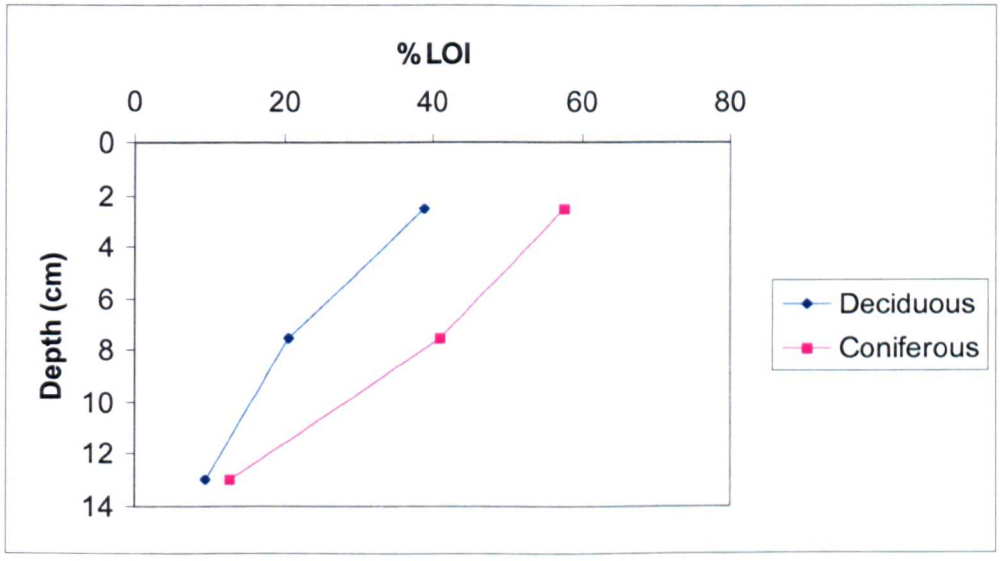


Figure 3-27 Average LOI profiles for the deciduous cores and the coniferous cores: Set 3

Both profiles clearly indicate significant organic matter at the surface, however, this appears to be more pronounced for the coniferous profile.

Loss on ignition values vs. Cr, Cu, Pb and Zn concentration values for each Set are shown in Sections 3.5.2 (Cr), 3.5.3 (Cu), 3.5.4 (Pb) and 3.5.5 (Zn). All LOI ignition vs. metal concentration graphs include the best line of fit (equation for the line and R^2 value) for the data points, in order that any correlation can be highlighted. All data points are included in the best line of fit in Sets 1 and 3 graphs. However, for graphs involving coniferous Set 2 metal concentrations, the first two data points are not included as they have been shown to be litter and not soil. In page 42 of Soil Science Methods and Applications, it is stated that a feasible approximation of LOI values is to take 58% as the amount of carbon (Rowell, 1994). Molar ratios (carbon : metal (Cr, Cu, Pb or Zn)) have been established for each set of data (deciduous and coniferous). These molar ratios are based on 40% LOI. The amount of C involved is derived from the assumption that 60% LOI gives the approximate amount of C. The metal value is obtained by using the best line of fit equation (deciduous and coniferous). The reference to molar ratio in this chapter represents the metal to organic carbon ratio. The proportion of the organic carbon which is actually involved in the complexation of Cu is unknown, as is the nature and size of the individual molecules which are involved in Cu complexation.

3.5.2 Loss on ignition vs. Cr concentration

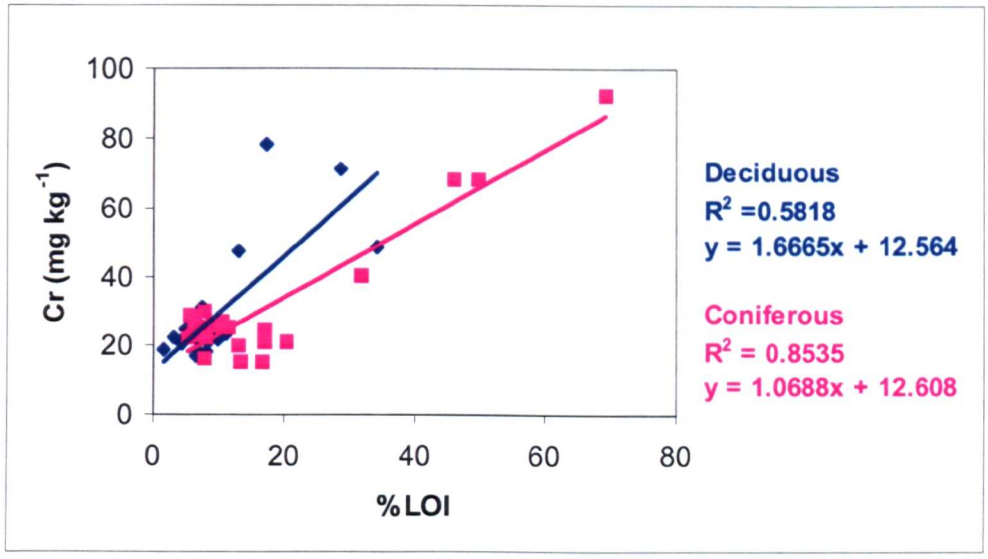


Figure 3-28 LOI vs. Cr concentration for the deciduous core and the coniferous core: Set 1

Figure 1-28 shows there to be a correlation between Cr and % LOI; the greater the % LOI, the greater the Cr concentration is likely to be. Approximate molar ratios (carbon : Cr) were established for each set of data in Figure 3-28: deciduous (6058) and coniferous (8671). The molar ratios indicate that for each Cr atom there is a large amount of carbon. The deciduous core shows an enhanced affinity for Cr per % LOI when compared to the coniferous core. Perhaps the type of organic matter below the deciduous canopy has a greater ability for binding Cr than the type of organic matter below the coniferous canopy. A possible reason is that the deciduous soil is less resistant to breakdown into Cr-binding forms of organic matter than the coniferous soil. As it has been reported that decomposition rates of organic matter below a Norway Spruce canopy are reduced when compared to organic matter decomposition below broad leaves (such as Beech) (Bergkvist, 1987).

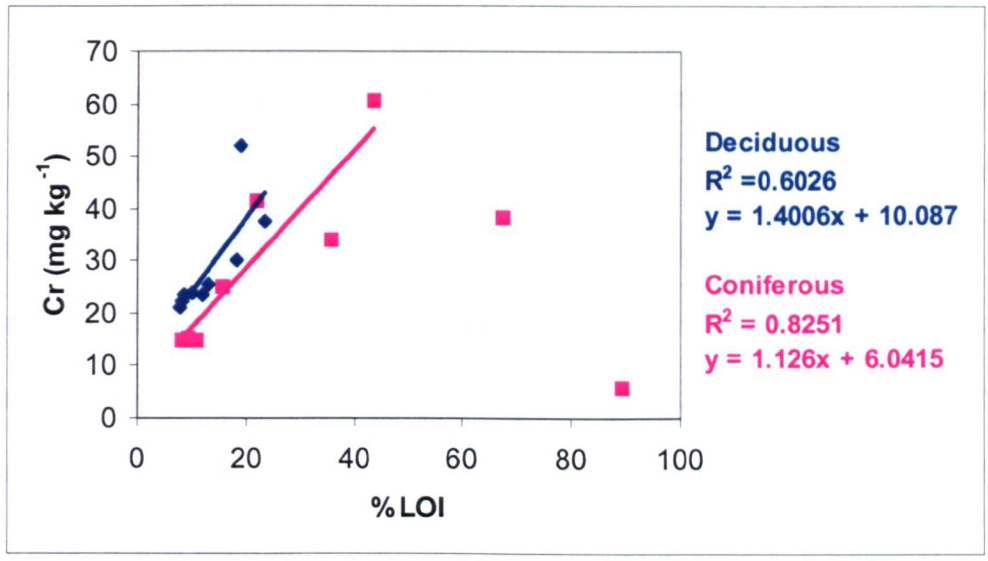


Figure 3-29 LOI vs. average Cr concentration for the deciduous cores and the coniferous cores: Set 2

Figure 3-29 (Set 2) supports the findings from Figure 3-28 (Set 1). The approximate molar ratios (carbon : Cr) were established for the deciduous data (7261) and the coniferous data (9397). The largest two % LOI values correspond to what is assumed to be litter material, as found through previous results in this Chapter. Clearly, these sections contain the greatest amount of organic matter, but have a lesser ability to retain Cr. This makes sense, for once soil is reached (the third largest % LOI value), organic matter has been broken down into a form which is more likely to interact with metals.

3.5.3 Loss on ignition vs. Cu concentration

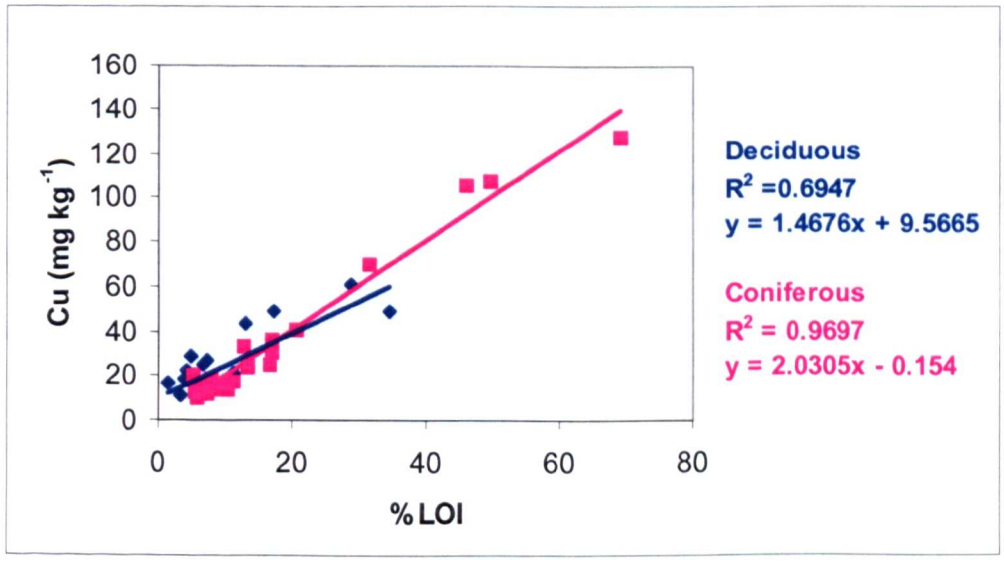


Figure 3-30 LOI vs. Cu concentration for the deciduous core and the coniferous core: Set 1

There is clearly a link between % LOI and Cu concentration (Figure 3-30); as the % LOI increases, the Cu concentration increases also. The approximate molar ratios (carbon : Cu) for the deciduous (18600) and the coniferous (17300) data were obtained, and they clearly indicate that there is a huge number of carbon atoms for every Cu atom. The coniferous soil and deciduous soil follow the same correlation line, this indicates that they are very similar with respect to their % LOI values and affinity for Cu. These findings are in good agreement with Banerjee (2003) who reported on finding a significant correlation between organic matter and Cu.

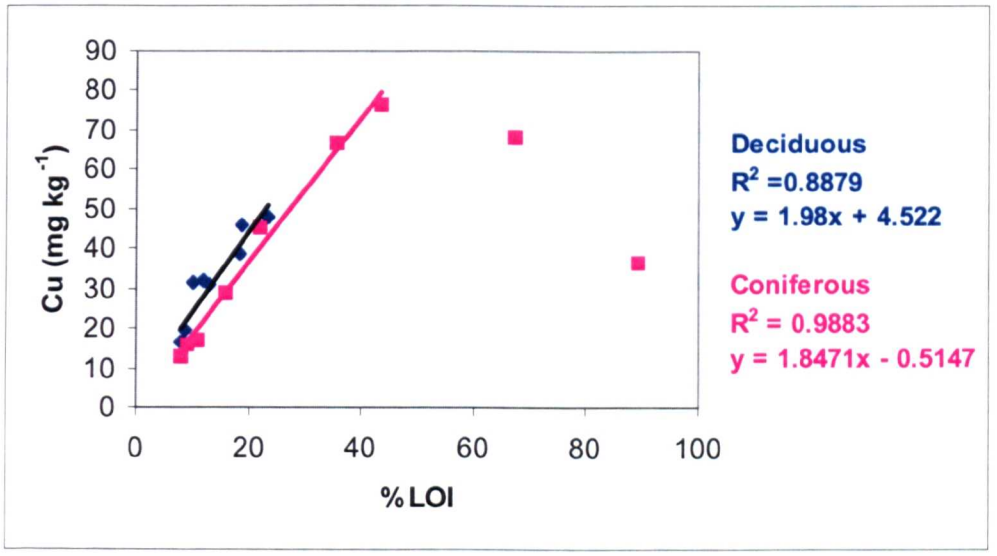


Figure 3-31 LOI vs. average Cu concentration for the deciduous cores and the coniferous cores: Set 2

Figure 3-31 (Set 2) supports the findings from Figure 3-30 (Set 1). Molar ratios were estimated: deciduous (15200) and coniferous (17300). As found for Cr (Figures 3-28 and 3-29) and Set 2 concentration profiles (Section 3.4), the first two coniferous points relate to litter values. These first two points for the coniferous data contain the greatest amount of organic matter, but have a much reduced affinity for Cu when compared to the data points which follow (corresponding to soil).

3.5.4 Loss on ignition vs. Pb concentration

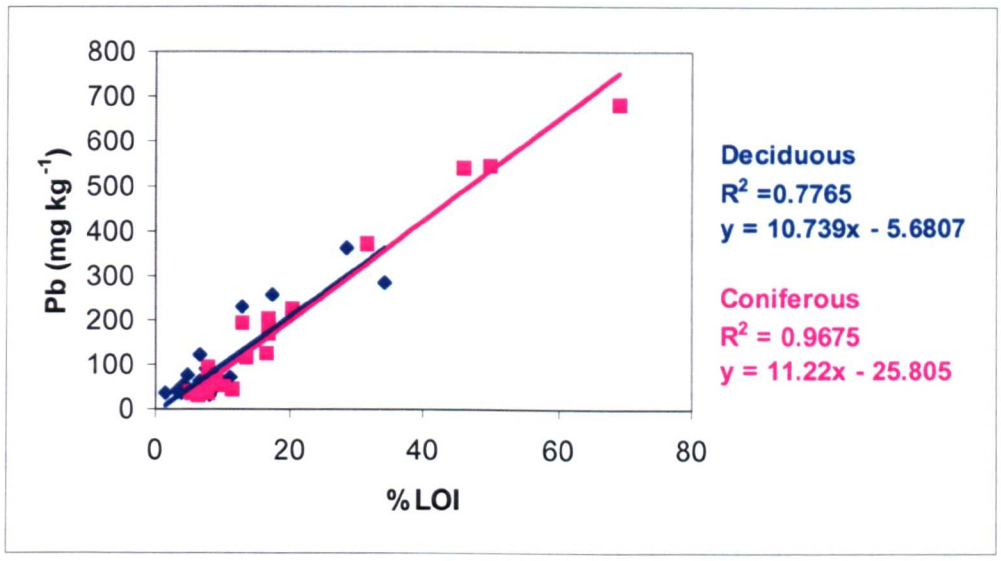


Figure 3-32 LOI vs. Pb concentration for the deciduous core and the coniferous core: Set 1

In Figure 3-32, the deciduous and coniferous cores appear to be very similar in terms of their % LOI and affinity for Pb. The estimated molar ratios for the deciduous (9780) and the coniferous (9800) data support this. As with Cr and Cu, there appears to be a high Pb content when high % LOI values are present.

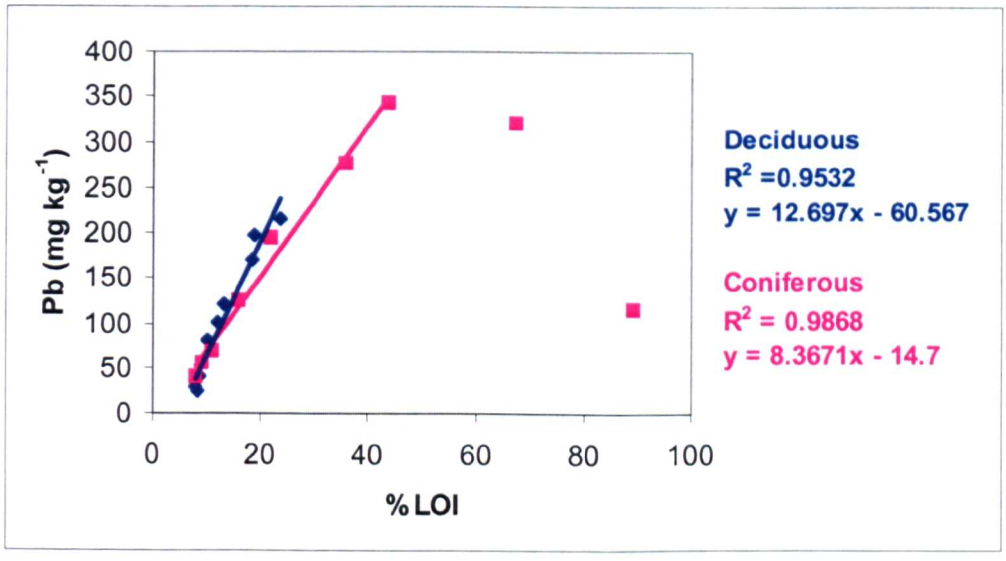


Figure 3-33 LOI vs. average Pb concentration for the deciduous cores and the coniferous cores: Set 2

Approximate molar ratios (carbon : Pb) were obtained for the coniferous (13000) and the deciduous (9260) data. The Set 1 (Figure 3-32) data is substantiated by the Set 2 (Figure 3-

33) data. As was found for the LOI vs. Cr Set 2 graph (Figure 3-29) and the LOI vs. Cu Set 2 graph (Figures 3-31), the largest two coniferous % LOI values are litter material.

These findings are supported by Watmough and Hutchinson (2004), as they reported strong linear relationships between organic matter (% LOI) and Pb concentration in soils below maple canopies. It is well documented that Pb from atmospheric deposition accumulates in organic soil horizons in both polluted ((Erel, 1998; Erel et al., 1997; Marsh & Siccama, 1997) and 'unpolluted' (Bindler et al., 1999; Nowack et al., 2001) soils, although Pb concentrations in the forest floor are usually far greater than concentrations in underlying mineral soils (Erel & Patterson, 1994; Marsh & Siccama, 1997; Martin & Coughtrey, 1987; Miller & Friedland, 1994; Semlali et al., 2001). The high concentrations generally reported for Pb in the forest floor are attributed to the strong affinity of Pb for organic matter. Indeed, it is this strong correlation that is thought to be responsible for the loss of Pb from the forest floor to the mineral soil, as reported by the Friedland group (Friedland et al., 1992; Miller & Friedland, 1994). This is due to the movement of Pb appearing to be strongly associated with the solubility and turnover of organic matter (Bergkvist et al., 1989), for as the organic matter breaks down, perhaps the Pb is released. For example, the rapid breakdown of litter at the JMOEC (James McLean Oliver Ecological Centre) appears to promote an extremely rapid transfer of Pb to the underlying mineral soil (Watmough & Hutchinson, 2004).

3.5.5 Loss on ignition vs. Zn concentration

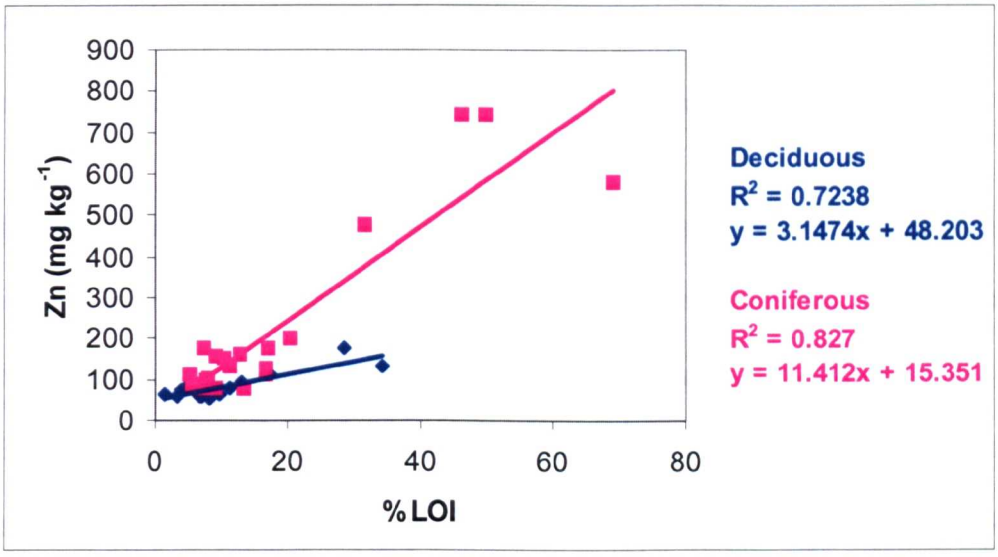
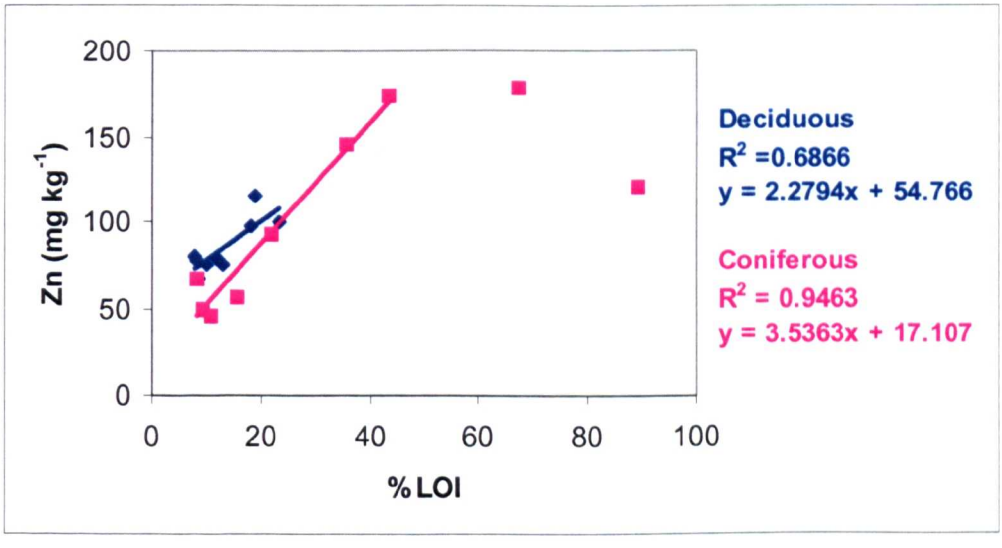


Figure 3-34 LOI vs. Zn concentration for the deciduous core and the coniferous core: Set 1

There is obviously an association between organic matter and Zn concentration (Figure 3-34); the greater the organic matter content, the higher the Zn concentration value is likely to be. The coniferous soil appears to have a greater affinity for Zn per % LOI. The estimated molar ratios (carbon : Zn) for the deciduous data (7510) and the coniferous data (2770) support this. Perhaps the organic matter below the coniferous canopy contains a greater content of Zn-binding organic matter.



Figures 3-35 LOI vs. average Zn concentration for the deciduous cores and the coniferous cores: Set 2

Once again, as shown in Figure 3-34 (Set 1), a direct association is presented between Zn concentration and % LOI. The molar ratios were estimated for the deciduous data (8960)

and the coniferous data (8250). As found in all the metal concentration vs. LOI coniferous Set 2 graphs, the first two coniferous data points in Figure 3-35 are obviously litter. However, the observation from Set 1 (Figure 3-34) that the organic matter in the deciduous soil contains a reduced affinity for Zn per % LOI is not supported here.

There was no correlation between organic matter and Mn, and organic matter and Fe evident in the concentration profiles. This can be explained by the fact that Mn and Fe exist primarily as oxides in soil (Bodek, 1988; McBride, 1994; Smith, 1990; Wild, 1988). Cr, Cu, Pb and Zn all show a strong correlation with organic matter. It is a known fact that each of these metals form associations with organic matter (Baker, 1990; Kiekens, 1990; McBride, 1994; McGrath & Smith, 1990). However, it should be noted that the observed correlations between the organic matter and certain metals may not be evidence of direct associations (between each metal and organic matter), there is also a possibility that something else follows the same path and is the cause of the evidence for the associations.

3.6 pH

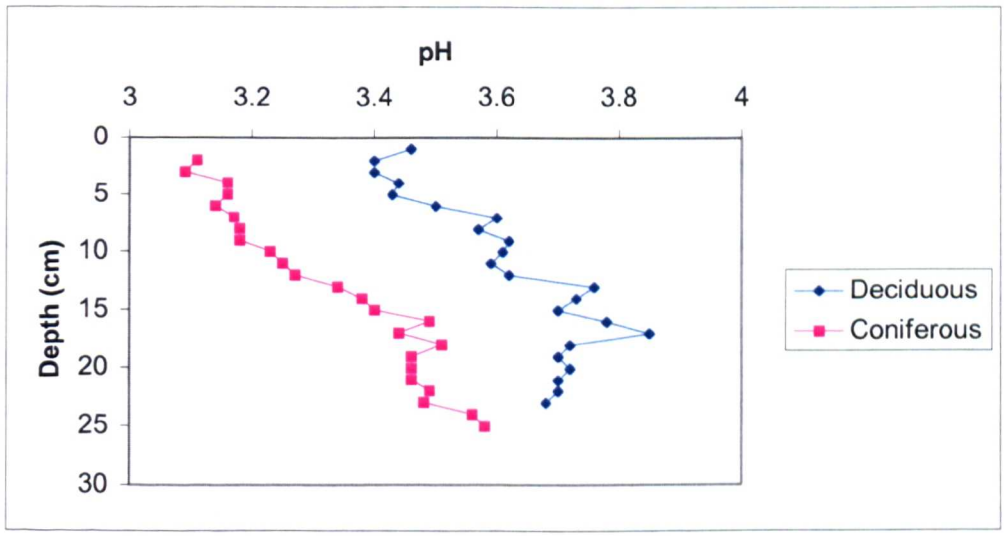


Figure 3-36 pH profile for the deciduous core and the coniferous core: Set 1

Figure 3-36 (Set 1) shows the coniferous profile to be more acidic than the deciduous profile; the coniferous profile has a surface pH of approximately 3.1 and the deciduous

profile has a surface pH of approximately 3.4. Both profiles show an increase in pH with depth.

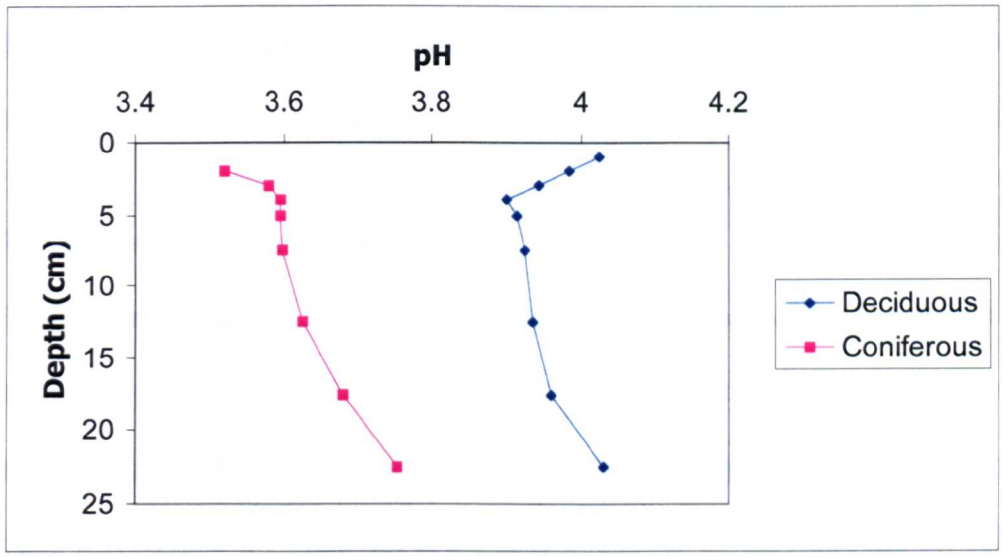


Figure 3-37 Average pH profile for the deciduous cores and the coniferous cores: Set 2

Figure 3-37 (Set 2) supports Figure 3-36 (Set 1), in that the coniferous profile is more acidic than the deciduous profile; the coniferous profile has a surface pH of approximately 3.5 and the deciduous profile shows a surface pH of approximately 4. The coniferous profile shows an increase in pH with depth. However, the deciduous profile shows a marked decrease from the surface to a depth 4 cm. From 4 cm, the profile shows an increase to the base of the core.

Many research groups have reported on the pH of coniferous soils being lower than the pH of deciduous soils (Ahokas, 1997; Andersen et al., 2004; Binkley & Valentine, 1991; Brown & Iles, 1991; Norden, 1994; Raulund-Rasmussen & Vejre, 1995).

3.7 Summary

Sixteen cores (eight deciduous and eight coniferous) were collected from the Pollok Park site (Collections, 1, 3 and 4: Section 2.3). The total metal contents (Cr, Cu, Fe, Mn, Pb and Zn) of these cores were established. The average total metal contents for these cores (supported by t-tests) showed that the deciduous cores contained more Cr, Cu, Fe and Mn

than the coniferous cores. There was found to be no difference in total metal content of Pb and Zn (supported by t-tests) when comparing the deciduous and coniferous cores. There appears to be no obvious reason for these differences in total metal content. Further investigations are required into the associations involved between the soil and metals through each profile, e.g. modified BCR sequential extraction scheme (Rauret et al., 2000).

Three sets of concentration profiles were obtained from the three core collections (Collection 1,3 and 4: Section 2.3). They provided evidence of enrichment of Cr, Cu, Pb and Zn to the surface of both soils (Coniferous and deciduous). There appeared to be no enrichment of Mn or Fe to the surface of either soil type. However, it is possible that if there was any, both Fe and Mn could have been redistributed through the soil due to redox influences (MacKenzie et al., 1998).

The regional background values found for each of the metals studied (Cr, Cu, Fe, Mn, Pb and Zn) comply with those reported for nearby regions (e.g. Blaser et al. (2002) and Hernandez et al. (2003)).

The coniferous soil and the deciduous soil were both found to have large LOI values at the surface. Correlation graphs (LOI values vs. metal concentration) for Cr, Cu, Pb and Zn showed that each of these metals have a strong correlation with organic matter content. However, it is possible that these correlations may be indirect. Molar ratios (carbon : metal) were estimated for each of these metals. These estimated molar ratios indicated that for each metal atom, there is a large number of carbon atoms; indicating that perhaps each metal atom held by organic matter is complexed by a large organic molecule.

Both soils are very acidic, however, the coniferous soil is more acidic than the deciduous soil.

Important information was obtained on soil collection methods and soil preparation (for analyses) procedures. Methods on soil collection should detail such things as whether cores include the litter layer. Without such information, results can be misinterpreted. When it comes to splitting cores into sections for analyses, the number of sections will dictate the degree of information obtained. For example, fine splitting shows more details, whereas coarse splitting is useful for obtaining an overview of profile trends.

The concentration profiles presented in this section revealed the top 5 cm of the cores (the organic layer) to be the most interesting. This was due to the elevated heavy metal concentrations and elevated organic matter content of the cores. As a result, it was decided that future studies would focus on speciation techniques which would enable an understanding of the organic-metal interactions contained within the top 5 cm of soil (deciduous and coniferous), and also the litter and leaves which were contributing to both soil types.

Chapter 4 - Speciation Studies

4.1 Introduction

The work presented in this chapter aimed to use established speciation techniques which would enable an understanding of the organic-metal interactions within the soil (top 5 cm), litter and leaves taken from the Pollok Park site. This work comprises two separate studies as summarised below:

- Speciation Study 1 involved using the modified BCR sequential extraction technique (Rauret et al., 2000). This technique enabled an assessment on how strongly Cr, Cu, Pb and Zn are retained in soil, what phases they are retained within and how easily they may be released into soil solution.

- Speciation Study 2 entailed using a Cu ion selective electrode (ISE) to measure the loading of soil, litter and leaf samples with Cu. This study also included measuring the organic carbon content of these samples in order that any trends in Cu loading of samples and organic carbon content of samples could be identified.

These studies involved using soil, litter and leaves collected from Pollok Park, the site of study. A description of the study site is given in Section 2.1. The soil, litter and leaf sampling regime is given in Section 2.3, the method for standard preparation of soil, litter and leaf samples is given in Section 2.4, and the procedure for heavy metal extraction from soils is given in Section 2.6.

4.2 Speciation Study 1: Modified BCR Sequential Extraction

4.2.1 Background

Knowledge of the total contents of heavy metals present in soil horizons provides limited information about their potential behaviour and bioavailability. Heavy metals are associated with various soil components in different ways, and these associations determine their mobility and availability (Ahumada et al., 1999; Kabata-Pendias & Pendias, 1992; Singh, 1997). As a result, it is essential when studying a certain metal in a certain soil, that an investigation is conducted into the soil phases responsible for retaining that metal. Water-soluble and exchangeable forms are considered readily mobile and available to plants, while metals incorporated into crystalline lattices or clays appear relatively inactive. The other forms, precipitated as carbonate, occluded in Fe, Mn and Al oxides, or complexed with organic matter, could be considered relatively active or firmly bound, depending upon the actual combination of chemical and physical properties of soil (Shuman, 1985; Sposito et al., 1982). Soil texture (clay content), pH, organic matter, and Fe-Mn oxides have been found to be the most important soil properties and components influencing the lability and biological uptake of heavy metals (Iyengar et al., 1981; Ma & Rao, 1997; Narwal & Singh, 1998).

Generally, under 'natural' conditions, only a small fraction of trace metals is present in plant-available form (Kabata-Pendias & Pendias, 1992), the majority are found in the 'unavailable' (residual) fraction (Davidson et al., 1994; Koleli, 2004; Lu et al., 2003). However, in some natural soils developed from metal-rich parent materials, as well as in contaminated soils, up to 30 to 60% of heavy metals can occur in easily labile forms (Karczewska et al., 1998; Kuo et al., 1983). For example, Lu et al. (2003) reported that the non-residual fractions of Cu, Zn, Pb and Cr in urban soils average 33.89%, 39.79%,

43.25% and 7.08%, respectively, which suggests that the mobility and bioavailability of the four metals probably declined in the following order: Pb > Zn > Cu > Cr. This is supported by other reports which have found that heavy metals in the soils of more contaminated sites are more easily available for leaching (Chlopecka et al., 1996; Wilcke & Kaupenjohann, 1997; Wilcke et al., 1998; Xu & Yang, 1995).

Single and sequential extractions provide information on potential mobility as well as bioavailability and plant uptake of trace elements (Christensen & Huang, 1999; He & Singh, 1995; Iyengar et al., 1981; Narwal et al., 1999; Shuman, 1990; Singh, 1997). Studies on the speciation of heavy metals in polluted soils using sequential extraction techniques have increased in recent years, because these simple techniques provide knowledge about metal affinity to the soil components and the strength with which they are bound to the matrix (Narwal et al., 1999). Unlike the single extraction technique, sequential extraction gives information about both mobile and stable fractions of metals in soil, which evaluates the actual and potential mobility of metals. Numerous fractionation techniques have been used for sequential extraction of heavy metals in soils (Rauret et al., 2000; Shuman, 1985; Sposito et al., 1982; Tessier et al., 1979). The techniques vary in the number of fractions extracted, as well as the order and kind of reagents used. In general, the fractionation schemes start with the weakest extractants and end up with the strongest, and separate three to seven metal fractions as follows (in order of extraction sequence): exchangeable > carbonate > Fe-Mn oxide bound > organic > residual.

Tessier et al. (1979) described the five fractions from their sequential extraction procedure as follows: '*the exchangeable fraction was the first to be brought into solution and is considered to be easily available for plant uptake, the carbonate fraction was susceptible to pH changes, the Fe-Mn oxide fraction was unstable under low Eh conditions, the organic fraction could be degraded under oxidizing conditions and the residual fraction was not considered to create a bioavailable pool since it was not expected to be solubilized*

over a reasonable period of time under natural conditions' (Tessier et al., 1979). This general description applies to the majority of sequential extraction schemes. Selective extraction methods for trace metals have been widely studied and implemented (Armienta et al., 1996; Banerjee, 2003; Davidson et al., 1994; Fernandez et al., 2004; Gibson & Farmer, 1986; Koleli, 2004; Lu et al., 2003; Miller et al., 1986; Morera et al., 2001; Oughton et al., 1992; Rauret et al., 2000; Tessier et al., 1979).

This study used the organic layer of soil (top 5 cm) taken from below the coniferous canopy and the organic layer of soil (top 5 cm) taken from below the deciduous canopy. These samples were obtained in Collection 2 (Section 2.3.2). This study used the modified BCR extraction technique (Section 2.11) (Rauret et al., 2000).

4.2.2 Results and Discussion

The modified BCR sequential extraction procedure was used to obtain the results presented in Figures 4-1 and 4-2 (Rauret et al., 2000). Figure 4-1 shows the distribution of metals (Cr, Cu, Pb and Zn) in the deciduous soil and Figure 4-2 shows the distribution of metals (Cr, Cu, Pb and Zn) in the coniferous soil. Each soil sample was run in quadruplicate.

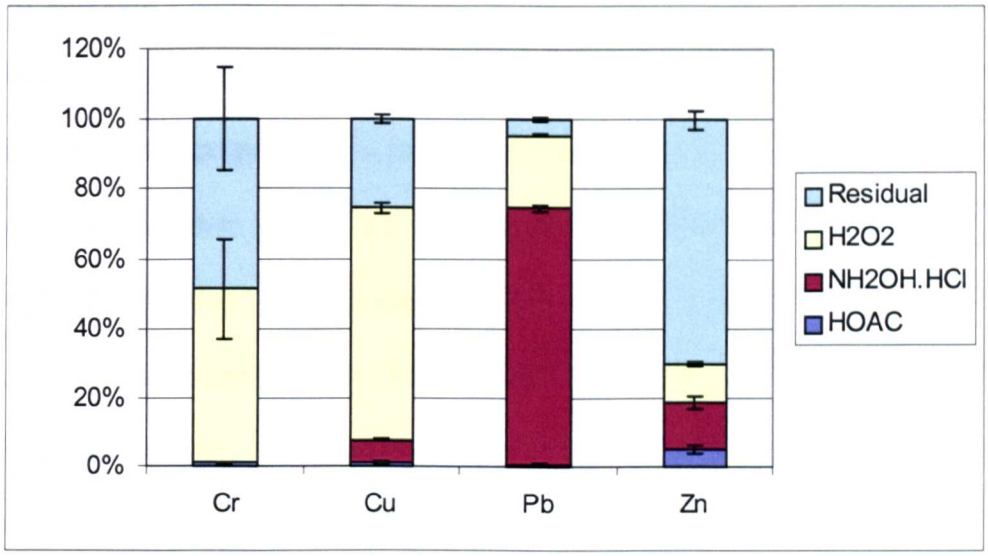


Figure 4-1 Fractionation of Cr, Cu, Pb and Zn in soil taken from below the deciduous canopy (expressed as % of the total sum of each step, standard deviations are included), following the modified BCR sequential extraction procedure (Rauret et al., 2000)

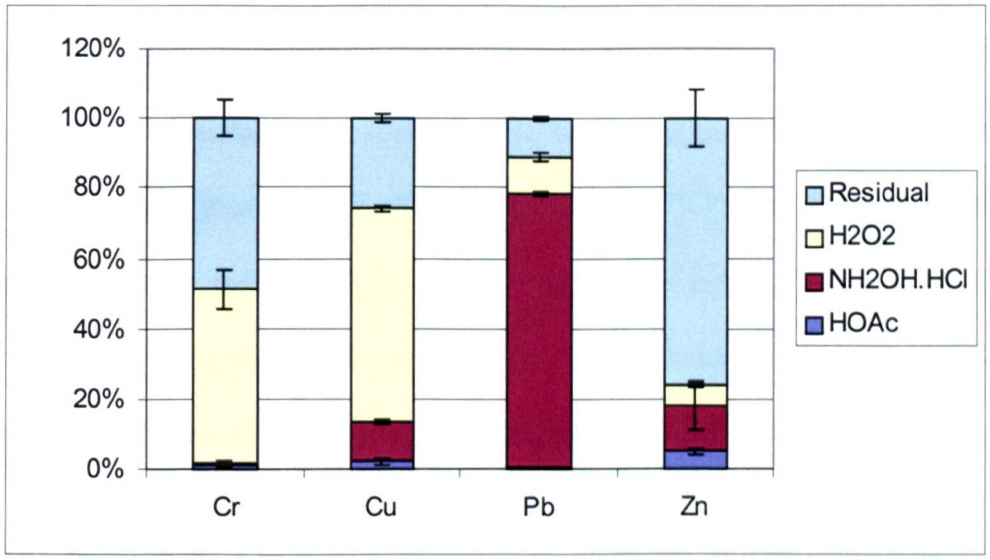


Figure 4-2 Fractionation of Cr, Cu, Pb and Zn in soil taken from below the coniferous canopy (expressed as % of the total sum of each step, standard deviations are included), following the modified BCR sequential extraction procedure (Rauret et al., 2000)

The modified BCR sequential extraction procedure according to Rauret et al. (2000) divides metals into acid soluble / exchangeable, reducible, oxidisable and residual fractions (Section 1.6.2 and Section 2.11). The deciduous soil (Figure 4-1) and the coniferous soil (Figure 4-2) display almost identical fractionation patterns for Cr, Cu, Pb and Zn.

Chapter 3 indicates that Cr, Cu, Pb and Zn are all retained by organic interactions, as the correlation graphs (metal concentration vs. organic matter content, Section 3-5) show that, in general, the greater the organic matter content, the greater the concentrations of these metals.

On studying Figures 4-1 and 4-2, it is clear that the oxidisable (organic matter) fraction does play a part in the distribution of these metals (Cr, Cu, Pb and Zn) within the soil. However, the proportion of total metal associated with the oxidisable fraction varies considerable when comparing the fractionation patterns for Cr, Cu, Pb and Zn. The oxidisable fraction is the predominant fraction for Cu only; Cr, Pb and Zn display more significant associations with other fractions. For Pb, approximately 80% is found in the reducible fraction. This indicates that the majority of Pb is associated with Fe oxides and / or Mn oxides Approximately 80% of Zn is found in the residual fraction, indicating that any other Zn is likely to have been very mobile and leached out of the soil. Cr is retained equally between the oxidisable and residual fractions. The presence of Cr in the oxidizable phase has been reported previously and is probably the preferred association for Cr inputs to the soil (Thomas et al., 1994). The correlation between organic matter content and metal concentration (Cr, Cu, Pb and Zn) (Section 3-5) can probably be explained by the fact that these metals are retained by organic complexation to varying degrees when organic matter is present. In the absence of organic matter content, there may be a reduced retention of these metals due to this additional association being absent.

The acid soluble / exchangeable fraction is thought to indicate the bioavailable fraction (Harrison et al., 1981; Li et al., 2001; Tessier et al., 1979). In both soil types (deciduous and coniferous), as indicated by Figures 4-1 and 4-2, this is negligible for each of the metals analysed. This is most likely due to the acidic nature of these soils (Section 3.6) resulting in the metals associated with this fraction being mobilized and leached through the soil. In the literature, many examples of sequential extractions on soils and sediments etc. with considerable higher pH values (6-8) than the Pollok Park soils can be found. These fractionation patterns tend to include the exchangeable fraction. This supports the assumption that low pH is the reason behind this fraction being absent from the fractionation patterns established for the Pollok Park soils (Kabala & Singh, 2001; Koleli, 2004; Sipos et al., 2005). The fractionation patterns (Cr, Cu, Pb and Zn) displayed in

Figure 4-1 (deciduous soil) and Figure 4-2 (coniferous soil) are in good agreement with those reported in the literature (e.g. Davidson et al., 1994), as discussed below.

Figures 4-1 and 4-2 show the fractionation pattern of Cr in the deciduous soil (Cr concentration approximately 60 mg kg^{-1}, 0-5 cm) to be: 1.01% exchangeable, 0.34% reducible, 50% oxidisable and 48.7% residual; and the coniferous soil (Cr concentration approximately 60 mg kg^{-1}, 0-5 cm) to be: 0.91% exchangeable, 0.60% reducible, 49.8% oxidisable and 48.7% residual.

Davidson et al. (1994) studied the distribution of Cr in the surface of a freshwater sediment collected from the River Clyde, Glasgow, using the BCR sequential extraction scheme. The Cr concentration of the sediment was found to average at 17.1 mg kg^{-1}. The following fractionation pattern was found for Cr: 0.67% exchangeable, 1.72% reducible, 42.39 oxidisable and 54.94% residual. This fractionation pattern is very similar to the one found for the Pollok Park soils which showed Cr as being proportioned between the residual and oxidisable fraction, in the approximate ratio 55% to 45%. Davidson et al. (1994) stated that the high proportion of metals in the residual fraction and the generally low levels of extractable metals indicate that the sediment is relatively unpolluted. This is supported by other reports which indicated that pollutant metals tend to associate with the non-residual fractions (Chlopecka et al., 1996; Wilcke & Kaupenjohann, 1997). It can be assumed, given the very similar fractionation patterns (between the Pollok Park soils and the sediments studied by Davidson et al. (1994)), that the Pollok Park soils are relatively unpolluted by Cr.

Lu et al. (2003) studied the chemical speciations of Cr in urban topsoils. The sequential extraction scheme proposed by Tessier et al. (1979) was adopted to partition the soil. The mean total Cr concentration of the topsoils studied was found to be in the range 87.1–108.9 mg kg^{-1}. The following mean fractionation pattern was found for Cr: 0.037%

exchangeable, 0.20% carbonates, 2.69% oxides (reducible), 4.16% organics (oxidisable) and 92.92% residual. Most of the Cr is found in the residual fraction, indicating that these soils are possibly exposed to much smaller anthropogenic inputs of Cr than the Pollok Park soils.

Koleli et al. (2004) studied the speciation of chromium in 12 agricultural surface soils (Cr: 101-158 mg kg^{-1}). The extraction scheme of Tessier et al (1979) was used to determine the Cr speciation in these samples. Mean values of the extractable forms of Cr, expressed in percentages of total soil contents were: 0.94% exchangeable, 0.80% carbonates, 2.13% oxides, 7.08% organics and 89.81% residual. The Cr fractionation pattern reported by Koleli et al. (2004) produced a very similar outcome to the Lu et al. (2003) study, in that most of the Cr was found in the residual fraction. It appears as though the Pollok Park soils are exposed to a greater proportion of Cr pollution (per total Cr content) than the soils in the Koleli et al. (2004) study, going by the proportion of residual ('unavailable') Cr.

Armienta et al. (1996) studied the distribution of Cr in soils (Cr concentrations up to 12,000 mg kg^{-1}) which were in the vicinity of a chromate factory, using a method based on the Tessier et al. (1979) chemical sequential extraction, to determine the distribution of Cr (Tessier & Campbell, 1988). The average values found for Cr in each of the fractions were as follows: 0.12% exchangeable, 1.34% carbonates, 44.79% oxides, 34.14% organics and 18.1 residual. This fractionation pattern conflicts, in part, with the Pollok Park findings (approximately 45% oxides and approximately 55% residual). Their findings are in agreement with the Pollok Park findings with respect to the exchangeable and carbonate fraction / fractions being virtually negligible. However, they found the majority of Cr was obtained in the reducible (oxides) fraction. This ties in well with findings that indicated polluted sediments as having a more significant association between Cr and the reducible phase than 'unpolluted' sediments (Rauret et al., 1989). Armienta et al. (1996) stated that the association of Cr with the reducible (oxides) fraction was observed between highly

polluted (around 12,000 mg kg^{-1} of total Cr) and less polluted (less than 2,500 mg kg^{-1} of total Cr) samples. The proportion of Cr in the reducible (oxides) fraction was greater in the highly polluted soils than in the less polluted soils. Also, a high percentage of Cr was in the oxidisable (organic) phase for the less polluted soils, as supported by other studies (Armienta et al., 1996; Davidson et al., 1994; Koleli, 2004; Lu et al., 2003).

The fractionation patterns found for Cu in the deciduous soil (1.22% exchangeable, 6.62% reducible, 67% oxidisable and 25.1% residual) and the coniferous soil (2.29% exchangeable, 11.12% reducible, 60.7% oxidisable and 25.9% residual) are displayed in Figures 4-1 and 4-2. Both surface soils (0-5 cm) contain approximately 60 mg kg^{-1} of Cu.

The associations of Cu in the surface sediment (Cu concentration: 14.5 mg kg^{-1}) of a river in Glasgow were studied by Davidson et al. (1994), using the BCR sequential extraction method. They found associations as shown: 14.9% exchangeable, 5.75% reducible, 32.6% oxidisable and 46.9% residual. Davidson et al. (1994) reported that the significant proportion of Cu associated with the residual fraction indicated that the sediment did not contain a high input of pollutant Cu. High contents of Cu in the residual fraction are often described in soils (Hickey & Kittrick, 1984; McLaren & Crawford, 1973; Ramos et al., 1994) and dusts (Harrison et al., 1981).

The majority of non-residual Cu was associated with the oxidisable phase. This compares favourably with previous reports on polluted sediments which stated that non-residual Cu is mainly associated with the oxidisable phase, where it is likely to occur as organically complexed metal species (Thomas et al., 1994; Ure et al., 1993). The significant presence of Cu in the organic fraction is also in strong agreement with that observed in many earlier studies on soil (Hickey & Kittrick, 1984; Kuo et al., 1983; Levy et al., 1992; Ma & Rao, 1997; McLaren & Crawford, 1973; Ramos et al., 1994) and street dusts (Gibson & Farmer, 1984; Hamilton et al., 1984; Harrison et al., 1981; Stone & Marsalek, 1996; Sutherland et

al., 2000). On comparing with the Pollok Park findings, there is agreement in that the majority of the non-residual Cu is associated with the oxidisable phase. However, the proportion of Cu in the residual phase in the Pollok Park soils (both approximately 25%) is considerable smaller than the sediment studied (46.94%) by Davidson et al. (1994). Perhaps this is an indication of the degree of pollutant Cu in the Pollok Park soils.

Lu et al. (2003) studied the chemical speciation of Cu in urban surface soils using the Tessier et al. (1979) sequential extraction procedure. The Cu concentrations of the soils studied ranged from 84.4-111.7 mg kg^{-1}. Lu et al. (2003) found the following fraction pattern for Cu: 0.38% exchangeable, 4.7% carbonates, 5.4% oxides, 23.4% organics and 66.1% residual. The non-residual fractions of Cu averages at 33.89% in urban soils and the non-residual fraction of Cu in non-urban soils averages at 6.7%. This implies that Cu in urban soils has a much higher mobility and bioavailability than in non-urban soils. It has been reported that anthropogenic inputs, such as atmospheric deposition, street dusts, etc., are the main sources of Cu in the urban soils being studied (Lu, 2000). For atmospheric deposition and street dust, the major portion of Cu is associated with Fe- and Mn-oxide or organic fractions, with small percentages in the residual fraction (Harrison et al., 1981; Wang et al., 1998), which result in the different distribution of Cu between urban and non-urban soils. This supports other reports which indicate that anthropogenic inputs of metals associate with non-residual phases in soils and sediments etc (e.g. Chlopecka et al., 1996; Davidson et al. (1994)).

Morera et al. (2001) reported on the distribution of Cu in 'natural' surface soils and 'natural' surface soils spiked with Cu. The Cu concentration in the 'natural' soils studied ranged from 24.11–65.35 mg kg^{-1}. The metal distribution in soils was evaluated by using the sequential extraction procedure developed by Tessier et al. (1979). The following mean fractionation pattern was found in 'natural' surface soils: 0% exchangeable, 0% carbonates, 8.90% oxides, 11.53% organics and 79.58% residual. Clearly, the majority of

Cu is associated with the residual fraction with smaller concentrations extracted from oxides, organic matter, and carbonate fractions. These findings are very similar to results reported by other authors, including those looked at in detail in this section (Chlopecka et al., 1996; Davidson et al., 1994; Hickey & Kittrick, 1984; Lu et al., 2003; McLaren & Crawford, 1973; Miller et al., 1986; Morera et al., 2001; Shuman, 1979; Singh et al., 1988). The significant residual fraction indicates that the soils being studied have not been subjected to high levels of pollutant Cu.

The mean fractionation pattern which resulted for the 'natural' soil spiked with Cu was: 14.35% exchangeable, 44.82% carbonates, 28.70% oxides, 10.79% organics and 1.34% residual. In the spiked 'natural' soils, most of the metals were extracted from more mobile fractions, which contrasted with the partitioning in the 'natural ' soils, where the total sum of metals associated with less mobile fractions (oxides, organic matter and residual) was larger than 90% of the total amount extracted from the five fractions. This compares with the Pollok Park soils as the organic fraction is the most significant, which indicates a certain amount of pollutant Cu input when considering various other reports on the subject (Davidson et al., 1994). If the 'natural' soils had a lower pH (approximately 3), it is very likely that a fractionation pattern similar to the one found for the Pollok Park soils would have resulted. At pH 3 (the approximate pH of the two Pollok Park soils, Section 3.6), the exchangeable and carbonate fraction would not be a significant consideration as any metals retained in these fractions would be released at such a low pH. However, the soils in the Morera et al. (2001) study fell into the higher pH range of 5-8, and as a result these fractions would have been more stable in these soils. The spiking of the 'natural' soils by Morera et al. (2001) was done in a laboratory situation and any fractionation results should be viewed with caution, given that the soils are not in their true environment. The results found by Morera et al. (2001) are important as they support previous reports on Cu retention in soils, in that they show that Cu inputs to soils and sediments etc. are found in the non-residual fractions (Davidson et al., 1994).

Banerjee (2003) studied Cu distribution in street dusts (Cu concentrations: 130–1904 mg kg^{-1}) using the sequential extraction scheme designed by Tessier et al. (1979). The amount of Cu in each fraction followed the order: organic > residual > Fe-Mn oxide >> carbonate > exchangeable. The organic phase (44.26%) is the dominant fraction in the non-residual fraction, with Fe-Mn oxide bound (12.08%), carbonate bound (2.94%) and exchangeable (1.68%) of secondary importance. The residual proportion was 39.04%. These findings support the studies reviewed so far and the findings for the Pollok Park soils, in that the Cu is mainly proportioned between the residual and oxidisable fractions. The high Cu concentrations (130 to 1904 mg kg^{-1}) and the fact that more Cu is found in the oxidisable fraction than in the residual fraction point to these streets dusts as being subjected to a certain degree of Cu pollution.

The Pb fractionation patterns for the Pollok Park deciduous (0.97% exchangeable, 73.78% reducible, 21% oxidisable and 4.35% residual) and coniferous (0.58% exchangeable, 77.71% reducible, 10.82% oxidisable and 10.9% residual) soils (both with Pb concentrations of approximately 300 mg kg^{-1}, 0-5 cm) show that Pb is predominantly associated with the reducible fraction (approximately 75%).

Sipos et al. (2005) studied the distribution of Pb in a natural forest soil profile (Pb surface concentration: 27 mg kg^{-1}), using a modified Tessier chemical extraction (Li et al., 1995; Sipos et al., 2005). The largest portion of Pb is bound to the residual phase (42%). This is consistent with reports that heavy metals are resident primarily in the residual phase in soils, sediments and road-dusts containing low levels of heavy metal pollution (Chlopecka et al., 1996; Davidson et al., 1994). To the organic matter and Fe-Mn oxides fraction, 19% and 27% of Pb is associated respectively. The role of exchangeable and specifically adsorbed Pb is the least significant (12%). Sipos et al. (2005) reported that besides the residual fraction, there are two main factors affecting the distribution of Pb among soil constituents, Fe-Mn oxides and organic matter of soil samples. This is supported by other

reports, both in uncontaminated (Arunachalam et al., 1996; Palumbo et al., 2000) and polluted soils (Li et al., 2000). They found that the importance of the residual phase in the binding of Pb increases with depth. This makes sense, as there will be less pollutant Pb at depth when compared with the surface.

Wong and Li (2004) studied Pb speciation in urban surface soils (Pb concentrations: 54.4-185 mg kg^{-1}) using a modified chemical extraction procedure as described in Li et al. (1995) (Wong & Li, 2004). Despite differences in Pb concentrations at the sites studied, the distribution of Pb in the five chemical fractions displayed a similar pattern. In general, the results showed that Pb was predominantly associated with the reducible phase, accounting for approximately 60% of the total Pb in soils. This is in good agreement with previous studies (Harrison et al., 1981; Li et al., 2001; Wong et al., 2002), where Fe-Mn oxides acted as important scavengers of Pb in soils. The second largest fraction of Pb was found in the carbonate fraction (10-20% of the total Pb). Pb in the organic and residual fraction collectively accounted for approximately 20% of the total amount of Pb. The chemical partitioning of the Pb generally showed that the majority of the Pb in the soils was relatively stable. Nonetheless, the comparatively significant association of the Pb with the carbonate fractions indicated that approximately 20% of the total Pb (~15-35 mg kg^{-1}) could be easily soluble and potentially bioavailable in these urban soils.

While the chemical distribution of Pb indicated that a large percentage of Pb was associated with the Fe-Mn oxide fraction, the isotopic composition of Pb in the five chemical fractions demonstrated that anthropogenic Pb in contaminated soils tended to reside in all four of the non-residual fractions, especially in the carbonate fractions, suggesting the potential solubility and mobility of anthropogenic Pb in soils. This study supports the Pollok Park findings for Pb in that the dominant fraction is the reducible phase. However, the considerable presence of the carbonate fraction in this study and its virtual absences from the Pollok Park study could be explained if the soils used are of a

significantly higher pH than those used in the Pollok Park study (approximately pH 3, Section 3.6).

Lu et al. al. (2003) studied the distribution of Pb in urban topsoils (84.6-118.9 mg kg^{-1} Pb) using the sequential extraction procedure developed by Tessier et al. (1979): residual (56.8%) > Fe-Mn oxide (30.9%) > organic (6.3%) > carbonate (5.2%) > exchangeable (0.8%). The Pb in urban soils is mainly associated with residual and Fe-Mn oxide fractions, whereas the exchangeable fraction is the lowest. These findings are in good agreement with the Pollok Park soils in that the dominant non-residual fraction is the reducible phase. However, the residual phase is much more significant than the Pollok Park soils, this can probably be explained by the lower inputs of pollutant Pb to the soils studied by Lu et al. (2003).

The fractionation patterns for Zn were obtained for the deciduous (5.7% exchangeable, 13.6% reducible, 11.1% oxidisable and 70.1% residual) and coniferous (5.0% exchangeable, 13% reducible, 6.3% oxidisable and 75.7% residual) soils which contain approximately 120 mg kg^{-1} Zn.

Davidson et al. (1994) used the BCR sequential extraction scheme to evaluate the distribution of Zn in a sediment (95 mg kg^{-1} Zn): 22.1% exchangeable, 14.1% reducible, 11.9% oxidisable and 51.9% residual. As was the case for the Pollok Park soils, the greatest proportion of Zn is found within the residual fraction. However, the proportion of Zn found in the non-residual fractions is greater than within the Pollok park soils, perhaps this is due to the presence of the exchangeable fraction in the sediment. As stated previously, the low exchangeable fraction associated with the Pollok Park soils is due to the low pH (Section 3.6) of these soils causing any metals associated with the exchangeable fraction to be released and washed through the soil.

Morera et al. (2001) studied Zn associations in surface soils (Mean Zn concentration: 62.8 mg kg^{-1}) using the sequential extraction procedure developed by Tessier et al. (1979): 0% exchangeable, 1.35% carbonates, 12.1% oxides, 4.79% organics and 81.8% residual. These findings support those for the Pollok Park soils with the residual fraction being the most considerable phase associated with Zn.

Lu et al. (2003) studied the speciation of Zn in urban topsoils (Zn concentration range: 81.1-118.9 mg kg^{-1}) using the sequential extraction method developed by Tessier et al. (1979): residual (60%) > Fe-Mn oxides (19.1%) > carbonate (11.6%) > organic (8.8%) > exchangeable (0.5%). Most of the total Zn is present in the residual fraction. In non-urban topsoils, the residual fraction of Zn accounts for 90.33%. The percentage of Zn in the residual fraction is much higher in the non-urban topsoil. These findings follow the same outcome as that found for the Pollok Park soils.

Perhaps a future study in this area could focus on the contribution of soil components such as organic matter content and clay content to the retention of these metals through the profile. For example, it has been reported that soils with a predominance of inorganic surfaces and low organic matter content retain Zn with a higher affinity than Ni and Cd; but in soils with a high organic matter content, organic matter favoured interaction of Cd and Ni over Zn (Elliot et al., 1986; Uren, 1992). Also, Sipos et al. (2005) reported that with depth, the residual phase becomes increasingly important in the binding of Pb.

The proportions involved in the fractionation of each soil are linked to metal concentrations, pH of soil and organic matter content: If the pH is very low, certain fractions will not exist. If the soil contains a high organic matter content, the chances of metals associating with this fraction are significantly enhanced. If the concentration of a certain metal is very high, it will saturate all possible sites of association possible.

4.2.3 Recovery

Calculating the recovery (%) enables a comparison between the amount of metal removed by aqua-regia digestion and the amount of metal removed by the sequential extraction procedure. Table 4-1 and Table 4-2 present recovery values for each element (Cr, Cu, Pb and Zn) from the deciduous soil and from the coniferous soil.

	Element / mg kg^{-1}			
	Cr	Cu	Pb	Zn
Step 1	0.39 (0.01)	0.66 (0.23)	3.54 (0.77)	5.38 (1.0)
Step 2	0.13 ($3x10^{-3}$)	3.54 (0.21)	299 (6.47)	13.9 (1.7)
Step 3	19.8 (1.2)	35.9 (1.8)	85.2 (5.2)	11.4 (1.0)
Residual	21.5 (11)	13.5 (0.26)	17.7 (2.9)	72.1 (5.8)
Σ3 steps + residual	41.9 (12)	53.6 (1.5)	406 (15)	103 (5.8)
Pseudo-total	49.4 (22)	46.8 (8.4)	320 (41)	46.8 (8.4)
Recovery (%)	84.8	114	127	220

Table 4-1 Comparison of results (standard error in bracket) obtained following sequential and aqua-regia extraction protocols of soil taken from below the deciduous canopy

Table 4-1 (deciduous soil) shows that the sum of the amount of Cr, Cu and Pb removed by the sequential extraction procedure, plus that released when the residue was digested, is approximately equivalent to the amount liberated by aqua-regia digestion alone. For Zn, these values are not in agreement as there is a discrepancy of 56.2 mg kg^{-1} (recovery: 220%).

On consulting Chapter 3 (Section 3.4.6), the pseudo-total values for Zn in the surface (top 5 cm) of the cores taken from the deciduous soil are all approximately 100 mg kg^{-1}. If this approximate value were the pseudo-total value in Table 4-1, a recovery value of approximately 100% would result. This indicates that the pseudo-total value in Table 4-1 is inaccurate, in that it is considerably lower than what is expected. Perhaps the Zn pseudo-total value is an artefact of the digestion method, as the pseudo-total value in Table 4-1 was obtained via microwave-assisted digestion (Section 2.6.3.2), whereas the pseudo-total value obtained in Section 3.4.6 was obtained by heating block-assisted digestion

(2.6.3.1). However, this mismatch of totals and pseudototals for Zn is found for the reference material too (Chapter 2, Table 2-5) and thus looks like a real effect.

	Element / mg kg^{-1}			
	Cr	Cu	Pb	Zn
Step 1	0.39 ($1x10^{-3}$)	1.2 (0.4)	1.86 (0.23)	4.65 (1.4)
Step 2	0.27 (0.46)	5.85 (0.41)	249 (8.1)	12.2 (7.6)
Step 3	21.8 (0.84)	31.9 (1.0)	34.6 (2.9)	5.73 (1.0)
Residual	21.6 (4.77)	13.6 (1.0)	34.9 (2.6)	68.8 (3.6)
Σ3 steps + residual	44.1 (4.7)	52.6 (1.8)	320 (8.0)	91.4 (8.9)
Pseudo-total	96.5 (14.4)	62.2 (3.0)	352 (28)	119 (8.3)
Recovery (%)	45.6	84.6	90.9	76.8

Table 4-2 Comparison of results (standard error in brackets) obtained following sequential and aqua-regia extraction protocols of soil taken from below the coniferous canopy

Table 4-2 (coniferous soil) shows that the sum of the amount of Cu, Pb and Zn removed by the sequential extraction procedure, plus that released when the residue was digested, is approximately equivalent to the amount liberated by aqua-regia digestion alone. For Cr, these values are not in agreement as there is a discrepancy of 52.5 mg kg^{-1} (recovery: 45.6%).

In Chapter 3 (Section 3.4.1), the pseudo-total values for Cr in the surface (top 5cm) of the cores taken from the coniferous soil are all approximately 50 mg kg^{-1}. If this value were the pseudo-total value in Table 4-2, a recovery value of approximately 100% would result. This suggests that the pseudo-total value in Table 4-2 is inaccurate, as it is significantly higher than the expected value, when compared to the Σ3 steps + residual step value and the heating block-assisted digestion value. However, perhaps the pseudo-total microwave-assisted digestion method is more efficient at recovering Cr. As the microwave-assisted digestion value, when compared to the heating block-assisted digestion value and the Σ3 steps + residual step value, is considerable higher. If this is the case, clearly it is the Σ3 steps + residual step value which is too low.

When comparing the outcome for the Zn values and the Cr values between the coniferous soil and the deciduous soil there is clearly a contradictory situation. The Zn values add up in the coniferous soil, but not in the deciduous soils, and the Cr values add up in the deciduous soil, but not in the coniferous soil. There appears to be no obvious reason as to why these contradictions should occur.

4.3 Speciation Study 2: Cu ISE Studies

4.3.1 Background

Cu distribution profiles (Section 3.4.2), LOI vs. Cu correlation graphs (Section 3.5), and Speciation Study 1 (modified BCR sequential extraction, Section 4.2), collectively indicate that Cu is mainly retained by the organic components of the surface soils (deciduous and coniferous). Humic substances have been shown as the active metal-binding component of soil organic matter (Graham et al., 1995). Humic substances are ubiquitous in soil and aquatic environments, being formed through random reactions that presumably occur during microbial breakdown of biomass (Perdue & Lytle, 1983). Despite the fact that elemental analyses and functional group analyses etc. indicate that some bulk properties of humic substances are relatively invariant, all attempts to fractionate humic substances into simpler subfractions have been unsuccessful. For example, it has been found that the infrared spectra of molecular size fractions of aquatic humus do not decrease in complexity with decreasing molecular weight, even for fractions with number-average molecular weights as low as 340 g mol^{-1} (Reuter & Perdue, 1981). The complexity of the mixture of organic ligands in humic substances is significantly greater than the complexity of ligand sites on pure solids (metal oxides and clays), and this fact alone would be sufficient to explain the greater difficulty in characterizing the ligands in humic substances. When the inherent complexity of the ligand mixture is combined with the reported tendency of humic substances to undergo aggregation reactions and configurational changes and with the

electrostatic problems that arise from interactions between two or more ligand sites on the same molecule, it is reasonable to conclude that defining the ligand mixture is impossible (Chen & Schnitzer, 1976; Perdue & Lytle, 1983).

Crude isolation methods, based on solubility in acid and alkali reagents, have been developed (Wild, 1988). The major materials separated are humic acid (extracted by alkali and precipitated by acid), fulvic acid (extracted by alkali and not precipitated by acid) and humin (not extracted by dilute acid or alkali). Each of these are complex mixtures, where no two humic molecules are exactly alike, and cannot be crystallized or otherwise separated into classes of homogeneous molecules. There are no sharp distinctions between humic acid, fulvic acid and humin. For example, the proportion of the humic matter that is precipitated by acid from an alkaline extract depends on the type of acid used and its concentration.

Many studies have been conducted into the complexation of heavy metals with soil, humic acid and fulvic acid, using a variety of procedures (Smith et al., 2004; Tye et al., 2004). Free metal ion activity can be measured using techniques such as anodic stripping voltametry (ASV), ISEs, competitive chelation and the Donnan membrane method (Holm et al., 1995; Salam & Helmke, 1998; Sauve et al., 1995; Sauve et al., 1997). Alternatively (M^{2+}) can be estimated from standard chemical analysis of solutions combined with the use of speciation programs such as WHAM (Tipping, 1998; Tipping, 1994) which include consideration of inorganic complexes and metal binding by humic and fulvic acids.

Cu has a very high affinity for soil organic matter, but it also has a strong affinity to complex with dissolved organic matter (Sauve et al., 2003; Temminghoff et al., 1998). As a result, the sorption of Cu to the solid phase is counterbalanced in part by solubilization by dissolved organic matter (50 to > 99% of soil solution dissolved metals are bound to soluble organic ligands) (Krishnamurti & Naidu, 2002; Sauve, 2003; Sauve et al., 1997).

The binding of Cu to dissolved organic carbon (DOC) in natural water and humus substances or their fractions (humic acid (HA), fulvic acids (FA)) extracted from soil has been studied extensively (Cao et al., 2004; Filella & Town, 2000; Joaquim et al., 2002; Krajnc et al., 1995; Nierop et al., 2002; Town & Filella, 2000; Witter et al., 1998).

The cupric ion-selective electrode was chosen to study the addition of Cu to samples originating from both the deciduous site and the coniferous site. As discussed previously (Sections 1.6.4 and 1.6.5), ISEs have been reported as a cheap and effective means for measuring the free ion in solution (Kaschl et al., 2002; Logan et al., 1997; McBride, 2001; Merritt & Erich, 2003; Saar & Weber, 1979; Sauve et al., 1996; Sauve et al., 1995; Smith et al., 2004; Town & Powell, 1993; Yin et al., 1997). The cupric ion-selective electrode is specific for the free ion, is very sensitive, and free from significant interferences from other metals (Gulens, 1987; Sauve et al., 1995).

The study used soil (organic soil, top 5 cm), litter and leaves taken from below the deciduous canopy and from below the coniferous canopy. The samples were obtained in Collection 2 and 3 as given in Section 2.3.2 and Section 2.3.3. The study used a Cu ISE to study how the samples interacted with Cu. Each sample was also measured for its organic carbon content.

4.3.2 Sample preparation

Three types of sample were used for Speciation Study 2:

- Soil samples as presented in Table 4-3
- Litter samples as presented in Table 4-4
- Leaf samples as presented in Table 4-5

Sample collection	Sample type	Sample preparation	Starting sample description
2 (Section 2.3.2)	Soil taken from below a deciduous canopy	Air-dried soil, no preparation	1 g soil in 40 mL water (suspension)
2 (Section 2.3.2)	Soil taken from below a coniferous canopy	Air-dried soil, no preparation	1 g soil in 40 mL water (suspension)
2 (Section 2.3.2)	Soil taken from below a deciduous canopy	150 g soil washed in 500 mL water	20 mL of extract
2 (Section 2.3.2)	Soil taken from below a coniferous canopy	150 g soil washed in 500 mL water	20 mL of extract

Table 4-3 Soil sample preparation for Cu ISE studies and organic carbon measurements (Cu concentration of deciduous soil: 58.8 mg kg^{-1}, and Cu concentration of coniferous soil: 61.3 mg kg^{-1})

The washing method in Table 4-3 is given in Section 2.12.1. Table 4-3 illustrates the preparation of the four soil samples which underwent Cu ISE studies and organic carbon measurements.

Sample collection	Sample type	Sample preparation	Starting sample description
3 (Section 2.3.3)	Litter taken from below a deciduous canopy	Air-dried litter, no preparation	1 g litter in 40 mL water (suspension)
3 (Section 2.3.3)	Litter taken from below a coniferous canopy	Air-dried litter, no preparation	1 g litter in 40 mL water (suspension)
3 (Section 2.3.3)	Litter taken from below a deciduous canopy	50 g litter washed in 600 mL water	20 mL of extract
3 (Section 2.3.3)	Litter taken from below a coniferous canopy	50 g litter washed in 600 mL water	20 mL of extract

Table 4-4 Litter sample preparation for Cu ISE studies and organic carbon measurements (Cu concentration of deciduous litter: 10.3 mg kg^{-1}, and Cu concentration of coniferous litter: 33 mg kg^{-1})

The washing method in Table 4-4 is given in Section 2.12.2. Preparation of the four litter samples (which underwent Cu ISE studies) are described in Table 4-4. Organic carbon measurements for the litter samples are also given in Table 4-4.

Sample collection	Sample type	Sample preparation	Starting sample description
3 (Section 2.3.3)	Litter taken from below a deciduous canopy	0.5 g fresh leaves were picked from the litter, washed with 20 ml water and the extract collected	Extract from washing
3 (Section 2.3.3)	Litter taken from below a coniferous canopy	0.5 g fresh pines were picked from the litter, washed with 20 ml water and the extract collected	Extract from washing

Table 4-5 Leaf sample preparation for Cu ISE studies and organic carbon measurements (Cu concentration of deciduous leaves: 0.90 mg kg^{-1}, and Cu concentration of coniferous leaves: 15.3 mg kg^{-1})

The washing method in Table 4-5 is given in Section 2.12.3. Table 4-5 presents the preparation method for the two leaf samples which underwent Cu ISE studies (organic carbon measurements for these samples are given also).

4.3.3 Organic carbon measurements of samples

All samples were measured for their organic carbon content, using the method stated in Section 2.10.

Sample	Organic carbon content
	mg g^{-1}
Soil taken from below the deciduous canopy in water	199
Soil taken from below the coniferous canopy in water	203
	µg mL^{-1}
Water washings of the soil taken from below the deciduous canopy	360
Water washings of the soil taken from below the coniferous canopy	290

Table 4-6 The organic carbon content of the four soil samples which underwent Cu ISE studies (Sections 4.3.3.1 and 4.3.3.2)

Table 4-6 shows that the deciduous soil (199 mg g^{-1}) has a slightly smaller organic carbon content than the coniferous soil (203 mg g^{-1}). Table 4-6 also shows that the water

washings of the deciduous soil (360 μg mL^{-1}) contain a slightly greater organic carbon content than the water washings of the coniferous soil (290 μg mL^{-1}).

Sample	Organic carbon content
	mg g^{-1}
Litter taken from below the deciduous canopy in water	424
Litter taken from below the coniferous canopy in water	379.4
	μg mL^{-1}
Water washings of the litter taken from below the deciduous canopy	190
Water washings of the litter taken from below the coniferous canopy	170

Table 4-7 The organic carbon content of the four litter samples which underwent Cu ISE studies (Sections 4.3.3.3 and 4.3.3.4)

Table 4-7 shows that the deciduous litter (424 mg g^{-1}) contains a greater organic carbon content than the coniferous litter (379.4 mg g^{-1}). Table 4-7 also shows that the water washings of the deciduous litter (190 μg mL^{-1}) have a greater organic carbon content than the water washings of the coniferous litter (170 μg mL^{-1}).

Organic carbon content values were calculated for the two leaf water washing samples (deciduous leaves and coniferous pines). However, the results were negligible and showed no measurable organic carbon content.

When comparing the four soil samples (Table 4-6) with the four litter samples (Tables 4-7), a couple of trends were observed. The organic carbon content values for the litter suspension samples in water are significantly higher than the organic carbon content values for the soil suspension samples in water, by approximately 2:1. The organic carbon content values for the soil water washings are significantly higher than the organic carbon content values for the litter water washings.

4.3.4 Loading samples with Cu

Each of the samples illustrated in Section 4.3.2 underwent two separate Cu loading investigations, one with a 0.50 ppm Cu solution and one with a 1.00 ppm Cu solution. In this chapter and the Conclusion chapter (Chapter 6), the term loading is used to describe the adding of Cu to samples (deciduous and coniferous) The two concentrations were used to ensure reproducibility of results. The 0.50 ppm Cu solution was added in increments of approximately 10 mL and the 1.00 ppm Cu solution was added in increments of approximately 5 mL. Prior to Cu addition, water washings all started with a volume of 20 mL, with the exception of the water washings of the leaves, which all started with a volume of 15 mL. Before addition of Cu, samples suspended in water all started with a volume of 40 mL. On each addition of Cu, the solution volumes increased, in general, by either 5 mL (1.00 ppm Cu solution) or 10 mL (0.50 ppm Cu solution), depending on the concentration of Cu solution being added. The method used is described in Section 2.13. On each addition of Cu, the solution was stirred for thirty seconds and then the Cu^{2+} concentration was measured using a Cu ISE (readings were taken when stabilised). All Cu loading investigations were accompanied by loading water, this was to gain an insight into Cu loading in the absence of sample. Prior to the addition of any Cu to samples, each sample was measured for Cu^{2+} using the cupric ISE. All of the samples were negligible for Cu^{2+} prior to addition of Cu. The organic carbon measurements (Section 4.3.2) for the water washings undergoing Cu loading are incorporated in this section also.

After each Cu loading investigation, standard solutions (10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 10 and 100 mg l^{-1}) were measured for Cu^{2+} in order to obtain Cu concentration values for the measurements and to test that the electrode was operating according to the manufacturer's instructions (ThermoOrion, 2001). Standard solutions were prepared from 1000 mg l^{-1} stock solution in dilute HNO_3.

In the following sections of Chaper 4, there are references to molar ratio, which refers to the metal to organic carbon ratio. The proportion of the organic carbon which is actually involved in the complexation of Cu is unknown, as is the nature and size of the individual molecules which are involved in Cu complexation.

4.3.4.1 Cu loading of soil water washings

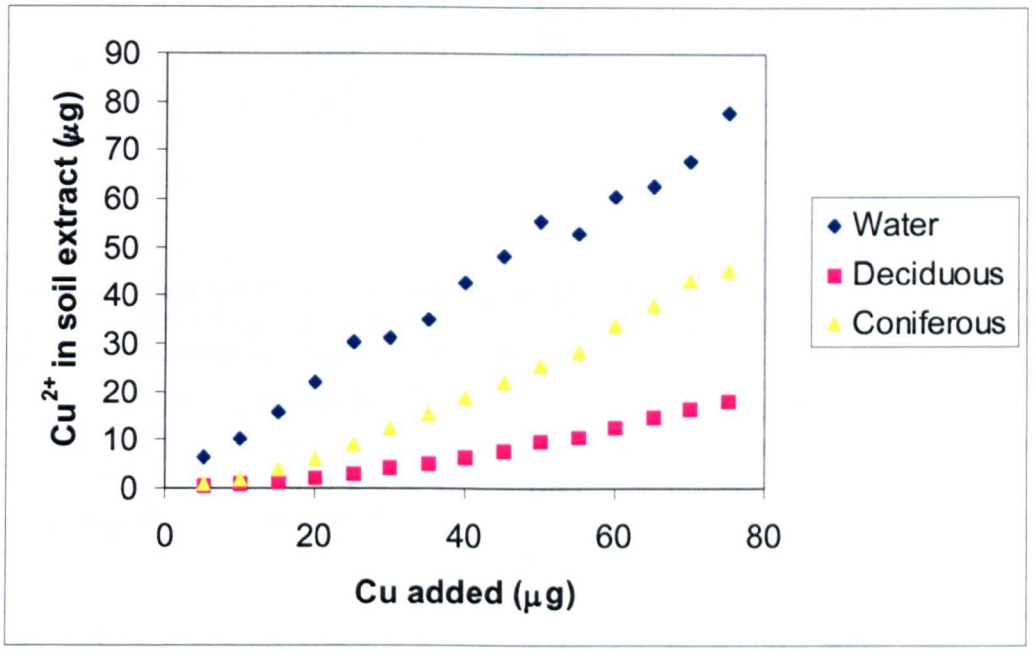


Figure 4-3 Loading water and water washings of soil (taken from below the deciduous canopy and from below the coniferous canopy) with 0.50 ppm Cu solution

Figure 4-3 shows that the water washings sample of the deciduous soil has a greater affinity for Cu than the water washings sample of the coniferous soil. Tables 4-8 (deciduous soil) and 4-9 (coniferous soil) show the amount of Cu complexed and the organic carbon to Cu complexed molar ratio data which accompanies the information in Figure 4-3.

Amount of Cu added (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Organic carbon / Cu complexed (molar ratio)
5	0.33	4.67	8220
10	0.68	9.32	4120
15	1.21	13.8	2780
20	2.00	18.0	2130
25	2.75	22.2	1730
30	4.08	25.9	1480
35	5.18	29.8	1290
40	6.43	33.6	1140
45	7.78	37.2	1030
50	9.58	40.4	950
55	10.7	44.3	866
60	12.8	47.2	814
65	14.7	50.3	764
70	16.6	53.4	718
75	18.2	56.8	676

Table 4-8 Data obtained from loading water washings of soil (organic carbon mass: 7.26 mg) taken from below the deciduous canopy with 0.50 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

Amount of Cu added (µg) (ISE measured)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Organic carbon / Cu complexed (molar ratio)
5	0.81	4.19	7270
10	1.73	8.27	3680
15	3.63	11.4	2680
20	6.00	14.0	2170
25	8.76	16.2	1870
30	12.1	17.9	1700
35	15.4	19.6	1550
40	18.5	21.5	1410
45	21.8	23.2	1310
50	25.2	24.8	1230
55	28.3	26.7	1140
60	33.8	26.2	1160
65	38.1	26.9	1130
70	43.2	26.8	1140
75	45.1	29.9	1020

Table 4-9 Data obtained from loading water washings of soil (organic carbon mass: 5.75 mg) taken from below the coniferous canopy with 0.50 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

When comparing Table 4-8 (deciduous soil) with Table 4-9 (coniferous soil), it is clear that in each case, the system tends towards saturation. Both the deciduous water washings sample and the coniferous water washings sample show very high molar ratios, indicating that the organic molecules binding with each Cu molecule are of considerable bulk. The

water washings sample of the deciduous soil (Table 4-8) has a greater complexing ability than the water washings sample of the coniferous soil. For example, on addition of 75 μg of Cu to the water washings sample of the soil taken from below the deciduous canopy (Table 4-8), 56.8 μg of the Cu is complexed. Whereas on addition of 75 μg of Cu to the water washings sample of the soil taken from below the coniferous canopy (Table 4-9), 29.9 μg of the Cu is complexed. The water washings sample of the deciduous soil (7.26 μg) contains a slightly greater organic carbon content to the water washings sample of the coniferous soil (5.75 μg). Perhaps this contributes to the deciduous sample having a greater affinity for Cu than the coniferous canopy. It has been reported that a lower microbial activity under the Norway spruce (when compared to below broad leaves such as beech) can result in a build up of organic substances due to them not being readily decomposed (Andersen et al., 2004; Bergkvist, 1987). Therefore, it is possible that the deciduous sample has been broken down to a greater degree than the coniferous sample, and as a result contains a greater content of organic material with an affinity for Cu.

The different build up of organic matter in the mineral soil and in the litter layer, as well as differences in the microbial activity sustained by different tree species affects the amounts of dissolved organic carbon (DOC) in the soil solution (Andersen et al., 2004). Many authors have found a higher DOC under conifers than under broad leaves (Raulund-Rasmussen et al., 1998; Robertson et al., 2000), others have reported on a higher DOC concentration in the A-horizon under spruce than under Grand Fir, Beech and Oak (Strobel et al., 1999). These reports do not agree with the results for the organic carbon contents for the water washing samples of the deciduous and coniferous soils (Pollok Park), as higher organic carbon values are found in the deciduous sample. Perhaps this variability is due to these samples (water washing of deciduous and coniferous samples) being water washings and not the actual soil solution (method: Section 2.12.1). The soil solution can be obtained by the centrifugation of natural moist soil samples (Andersen et al., 2004; Davies & Davies, 1963). It has been reported that approximating the soil solution concentration by

the water extractable fraction of the soil samples may underestimate the true soil solution (Blaser et al., 2000). A future study could incorporate the technique developed by Davies and Davies (1963) and other authors in order that a comparison of results can be made, based on methods for obtaining the actual soil solution.

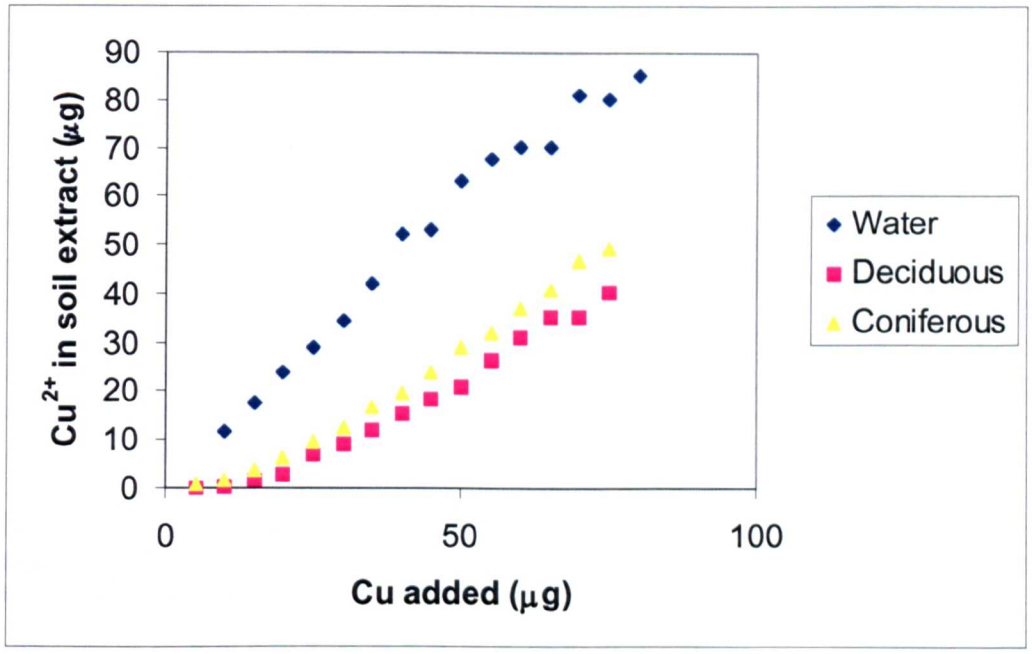


Figure 4-4 Loading water and water washings of soil (taken from below the deciduous canopy and from below the coniferous canopy) with 1.00 ppm Cu solution

Figure 4-4 presents an identical study to the one shown in Figure 4-3, with the exception of a Cu solution of 1 ppm (Figure 4-4) being added instead of a 0.50 ppm (Figure 4-3) solution. As was the case in Figure 4-3, Figure 4-4 shows that the water washing sample of the deciduous soil has a greater affinity for Cu than the water washing sample of the coniferous soil. Table 4-10 (deciduous soil) and Table 4-11 (coniferous soil) present the data which accompanies Figure 4-4: the amount of Cu complexed and the organic carbon to Cu complexed molar ratios.

Amount of Cu added (μg)	Amount of Cu detected (μg) (ISE measured)	Amount Cu complexed (μg)	Organic carbon / Cu complexed (molar ratio)
10	0.58	9.4	4070
15	1.77	13.2	2900
20	3.02	17.0	2260
25	7.02	18.0	2130
30	9.31	20.7	1860
35	12.1	22.9	1680
40	15.4	24.6	1560
45	18.4	26.6	1440
50	20.8	29.2	1320
55	26.4	28.6	1340
60	31.1	28.9	1330
65	35.1	29.9	1290
70	35.3	34.7	1110
75	40.3	34.7	1110
80	43.6	36.4	1050
85	49.1	35.9	1070
90	50.1	39.9	963
95	55.8	39.2	978
100	57.2	42.8	896

Table 4-10 Data obtained from loading water washings of soil (organic carbon mass: 7.26 mg) taken from below the deciduous canopy with 1.00 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

Amount of Cu added (μg)	Amount of Cu detected (μg) (ISE measured)	Amount Cu complexed (μg)	Organic carbon / Cu complexed (molar ratio)
10	1.81	8.19	3720
15	3.86	11.1	2730
20	6.29	13.7	2220
25	9.57	15.4	1970
30	12.8	17.2	1770
35	16.8	18.2	1670
40	19.9	20.1	1510
45	24.2	20.8	1460
50	28.9	21.1	1440
55	31.8	23.2	1310
60	37.1	22.9	1330
65	40.8	24.2	1260
70	46.8	23.2	1310
75	49.0	26.0	1170
80	51.6	28.4	1070
85	57.1	27.9	1090
90	60.9	29.1	1050
95	64.8	30.2	1010
100	68.2	31.8	958

Table 4-11 Data obtained from loading water washings of soil (organic carbon mass: 5.75 mg) taken from below the coniferous canopy with 1.00 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

The accompanying data for Figure 4-4 (Tables 4-10 and 4-11) is in good agreement with the accompanying data for Figure 4-3 (Tables 4-8 and 4-9). When comparing Table 4-10 (deciduous) with Table 4-11 (coniferous), they both show systems which are moving towards saturation and have high molar ratio values. Also, the water washings sample of the deciduous soil (Table 4-10) has a greater complexing ability than the water washings sample of the coniferous soil (Table 4-11). On adding 100 μg of Cu to the water washings sample of the soil taken from below the deciduous canopy (Table 4-10), 42.8 μg of the Cu is complexed. However, on adding 100 μg Cu to the coniferous water washings sample (Table 4-11), 31.8 μg of the Cu is complexed.

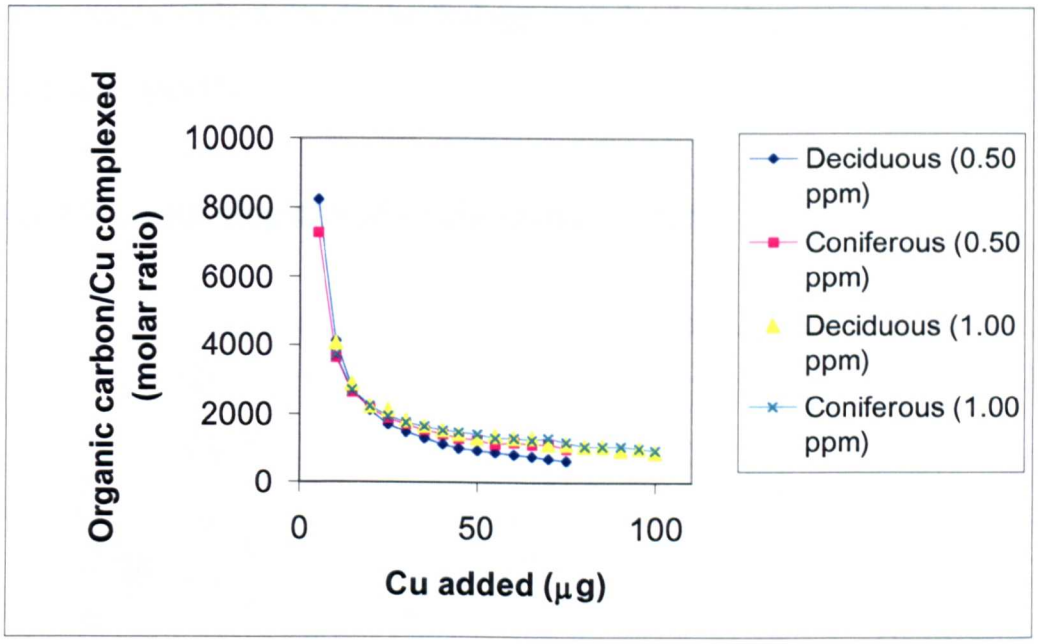


Figure 4-5 Organic carbon / Cu complexed (molar ratio) vs. Cu added for the four investigations into Cu loading of soil water washings: deciduous (0.50 ppm and 1.00 ppm) and coniferous (0.50 ppm and 1.00 ppm)

Figure 4-5 combines the data for the four investigations (organic carbon / Cu complexed (molar ratio) vs. Cu added) (Figures 4-3 and 4-4), and clearly indicates that the variation in complexing ability between the deciduous water washings and coniferous water washings is due to organic carbon content. The deciduous water washings (7.26 mg) contain a greater organic carbon content than the coniferous water washings (5.75 mg), and as a result show a greater affinity for Cu in Figures 4-3 (0.50 ppm Cu addition) and 4-4 (1.00 ppm Cu addition).

McBride (2001) conducted a similar investigation, which involved adding Cu to peat soil leachates and measuring the resulting Cu^{2+} concentration with a cupric ISE. He reported that dissolved Cu in the unamended (control) peat was more than 98% in complexed form, presumably with dissolved organic matter. In the study, this fraction decreased systematically with higher Cu loadings, so that more than one-half the soluble Cu in the peat with 4000 mg kg^{-1} added was free ionic Cu^{2+}. There was therefore a clear trend in the more toxic (free-ionic) form of Cu to increase more markedly in response to Cu addition to the soil than all of the dissolved forms. McBride (2001) reported that this response could indicate a limited capacity of dissolved organic matter to complex Cu addition to solution.

This study clearly supports the findings from loading the water washings of the Pollok Park soils with Cu.

4.3.4.2 Cu loading of soil suspended in water

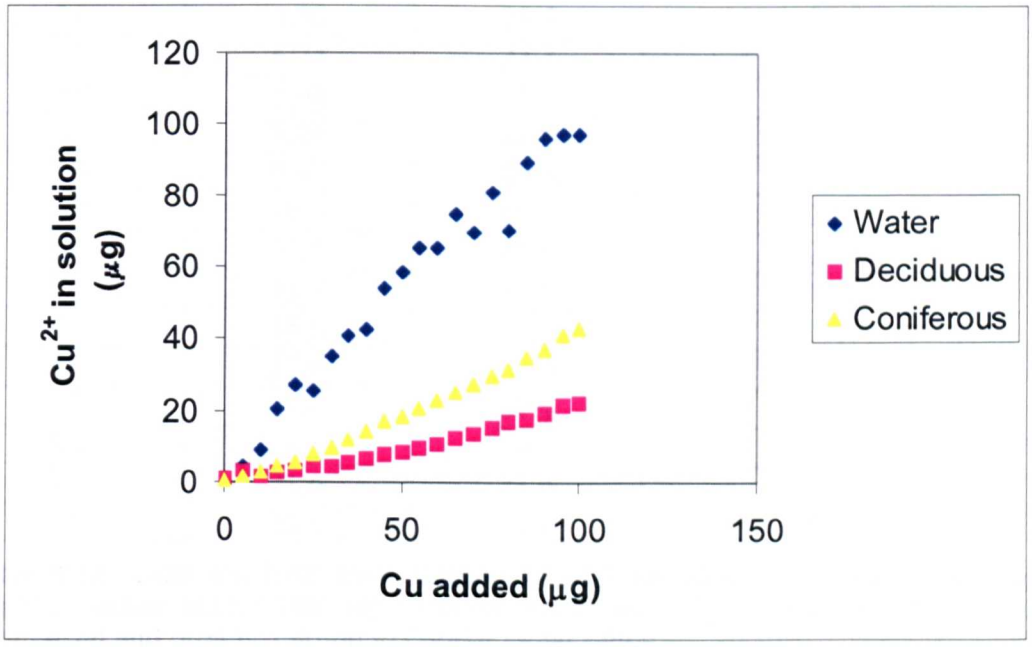


Figure 4-6 Loading water and soil suspensions in water (taken from below the deciduous canopy and from below the coniferous canopy) with 0.50 ppm Cu solution

Figure 4-6 shows that the deciduous soil has a greater affinity for Cu than the coniferous soil. Tables 4-12 and 4-13 show the amount of Cu complexed and organic carbon / Cu complexed (molar ratio) data which accompanies Figure 4-6.

Amount of Cu added (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Organic carbon / Cu complexed (molar ratio) ($x10^3$)
10	1.95	8.05	131
15	2.62	12.4	85.0
20	3.50	16.5	63.8
25	4.35	20.6	51.0
30	4.38	25.6	41.1
35	5.50	29.5	35.7
40	6.74	33.3	31.7
45	7.94	37.1	28.4
50	8.48	41.5	25.4
55	9.47	45.5	23.1
60	10.9	49.1	21.4
65	12.5	52.5	20.0
70	13.7	56.3	18.7
75	15.5	59.5	17.7
80	17.0	63.0	16.7
85	17.6	67.4	15.6
90	19.5	70.5	14.9
95	21.6	73.4	14.3
100	22.2	77.8	13.5

Table 4-12 Data obtained from loading soil (taken from below the deciduous canopy, organic carbon mass: 199 mg) suspended in water with 0.50 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

Amount of Cu added (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Organic carbon / Cu complexed (molar ratio) ($x10^3$)
10	3.00	7.00	154
15	4.42	10.6	102
20	5.62	14.4	74.7
25	7.72	17.3	62.2
30	9.72	20.3	53.0
35	11.8	23.2	46.3
40	14.1	25.9	41.5
45	16.9	28.1	38.2
50	18.3	31.7	33.9
55	20.5	34.5	31.1
60	22.8	37.2	28.9
65	24.8	40.2	26.7
70	27.4	42.6	25.2
75	29.4	45.6	23.6
80	31.2	48.8	22.0
85	34.7	50.3	21.4
90	37.0	53.0	20.3
95	40.6	54.4	19.8
100	42.4	57.6	18.7

Table 4-13 Data obtained from loading soil (taken from below the coniferous canopy, organic carbon mass: 203 mg) suspended in water with 0.50 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

Both sets of data (coniferous and deciduous) are indicating that nearly all Cu-binding sites have been saturated. When comparing the data for the deciduous soil (Table 4-12) and the coniferous soil (Table 4-13), it is clear that the deciduous sample has a greater complexing ability than the coniferous sample. When 100 μg Cu is added to the coniferous sample, 57.6 μg of Cu is complexed. However, when 100 μg Cu is added to the deciduous sample, 77.8 μg Cu is complexed. As was the case for the soil water washingss, it appears as though the samples originating from below the deciduous canopy contain a greater degree of Cu-binding components. This is not reflected in the organic carbon values, as the deciduous soil (199 mg g^{-1}) contains less organic carbon than the coniferous soil (203 mg g^{-1}). However, as reported by others, the organic carbon value does not necessarily relate to the Cu-binding organic components (Buffle et al., 1977). It has been reported that more than 98% of Cu in soil is complexed by DOC (Andersen et al., 2004; Sauve et al., 1997). Another possibility is that the coniferous soil has not being broken down to the same extent as the deciduous soil in order to provide Cu-binding organic components, as it has been reported that there are low levels of microbial activity in the soil below a canopy of Norway spruce (when compared to below broad leaves such as beech) which results in organic substances not being readily decomposed (Andersen et al., 2004; Bergkvist, 1987).

The molar ratios for the soil suspended in water samples (Tables 4-12 and 4-13) are considerable larger than those for the water washings (Tables 4-8 to 4-11). This perhaps supports reports that a considerable amount of the organic carbon in soil does not relate to Cu-binding carbon, and that most Cu is complexed by DOC (Buffle et al., 1977; Sauve et al., 1997). These molar ratios (Tables 4-12 and 4-13) tie in very well with the LOI vs. Cu concentration graphs (Section 3.5.3). From these graphs, approximate molar ratios (carbon : Cu) were established. The molar ratios were large in value and therefore indicated that for every Cu atom there are many carbon atoms. For example, from Set 1 graphs (Section 3.5.3), the deciduous soil had an approximate molar ratio of 1.86 $x10^2$ and the coniferous soil had an approximate molar ratio of 173 $x10^2$. In terms of values, the estimated molar

ratios for carbon : Cu (Section 3.5.3) correspond well to those reported in Tables 4-12 and 4-13.

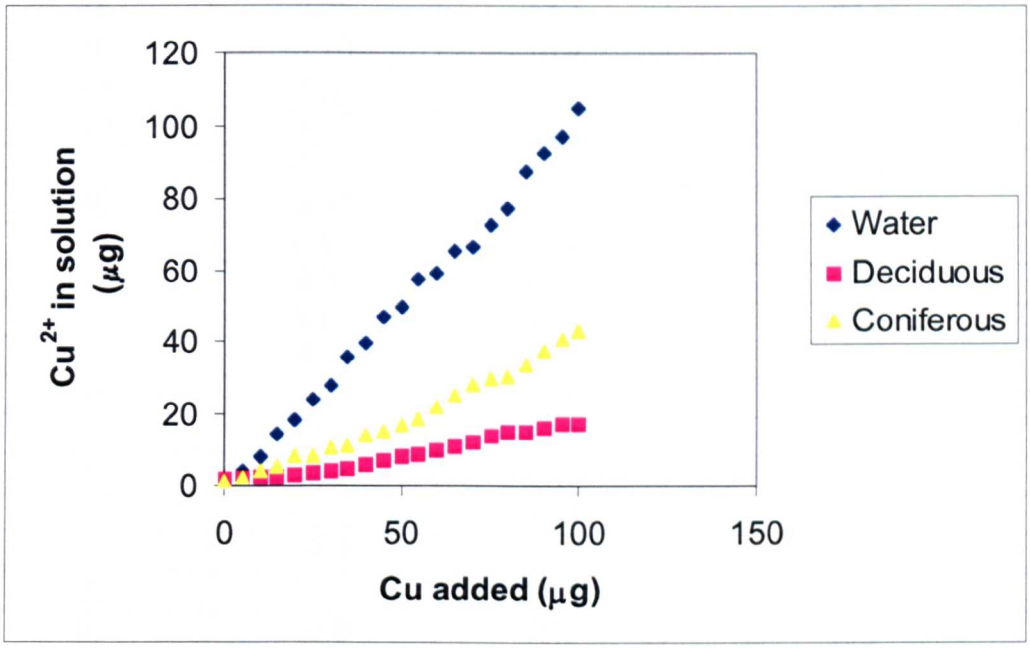


Figure 4-7 Loading water and soil suspended in water (taken from below the deciduous canopy and from below the coniferous canopy) with 1.00 ppm Cu solution

Figure 4-7 presents the same study as that shown in Figure 4-6, with the exception of a 1.00 ppm Cu solution being used in place of a 0.50 ppm solution. Figure 4-6 and Figure 4-7 resulted in a very similar outcome, therefore showing good reproducibility of results. The amount of Cu complexed and molar ratio data which accompanies Figure 4-7 is presented in Tables 4-14 and 4-15.

Amount of Cu added (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Organic carbon / Cu complexed (molar ratio) ($x10^3$)
10	2.25	7.75	136
15	2.26	12.7	82.6
20	3.10	16.9	62.3
25	3.33	21.7	48.6
30	3.73	26.3	40.1
35	4.72	30.3	34.8
40	5.42	34.6	30.4
45	6.62	38.4	27.4
50	8.05	41.9	25.1
55	8.50	46.5	22.6
60	9.60	50.4	20.9
65	10.9	54.1	19.5
70	12.1	57.9	18.2
75	13.3	61.7	17.1
80	14.6	65.4	16.1
85	14.9	70.1	15.0
90	15.9	74.1	14.2
95	16.8	78.2	13.5
100	16.7	83.3	12.6

Table 4-14 Data obtained from loading soil (taken from below the deciduous canopy, organic carbon mass: 199 mg) suspended in water with 1.00 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

Amount of Cu added (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Organic carbon / Cu complexed (molar ratio) ($x10^3$)
10	3.96	6.04	178
15	5.21	9.79	110
20	7.64	12.4	87.0
25	7.88	17.1	62.8
30	10.0	20.0	53.7
35	10.5	24.5	43.9
40	13.4	26.6	40.3
45	14.8	30.2	35.6
50	16.5	33.5	32.0
55	17.8	37.2	28.9
60	21.5	38.5	27.9
65	24.6	40.4	26.6
70	27.5	42.5	25.3
75	29.4	45.6	23.6
80	30.0	50.0	21.5
85	33.3	51.7	20.8
90	37.0	53.0	20.3
95	40.4	54.6	19.7
100	42.9	57.1	18.8

Table 4-15 Data obtained from loading soil (taken from below the coniferous canopy, organic carbon mass: 203 mg) suspended in water with 1.00 ppm Cu: amount Cu complexed and organic carbon to copper molar ratios

As was the case for loading soil suspended in water with 0.50 ppm Cu solution (Tables 4-12 and 4-13) and loading water washings with 0.50 ppm and 1.00 ppm Cu solution (Tables 4-8 to 4-11), Tables 4-14 and 4-15 indicate that the samples originating from below the deciduous canopy have a greater Cu complexing ability than the sample originating from below the coniferous canopy. When 100 μg Cu is added to the coniferous soil suspended in water, 57.1 μg Cu is complexed, and when 100 μg Cu is added to the deciduous soil suspended in water, 83.3 μg Cu is complexed.

Also, as was the case for the loading of soil suspended in water with 0.50 ppm Cu solution (Figure 4-6), the molar ratios are considerable larger than those obtained for the loading of the water extracts (Figures 4-3 and 4-4).

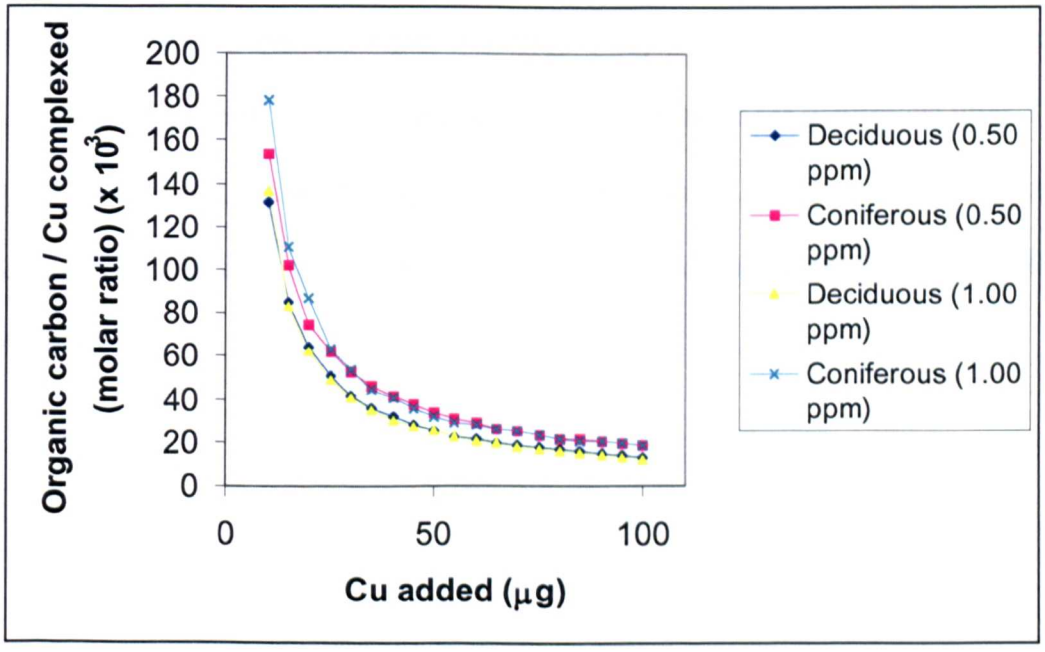


Figure 4-8 Organic carbon / Cu complexed (molar ratio) ($x10^3$) vs. Cu added for the four investigations into Cu loading of soil suspended in water: deciduous (0.50 ppm and 1.00 ppm) and coniferous (0.50 ppm and 1.00 ppm)

Figure 1-8 shows that the organic carbon / Cu complexed molar ratio ($x10^3$) is approximately the same in every investigation (Figures 4-5 and 4-6). This indicates that the proportion of organic carbon with Cu-binding affinity in both the deciduous soil and the coniferous soil organic carbon is approximately equal, as both have very similar organic carbon values (deciduous: 199 mg g^{-1} and coniferous: 203 mg g^{-1}).

4.3.4.3 Cu loading of litter water washings

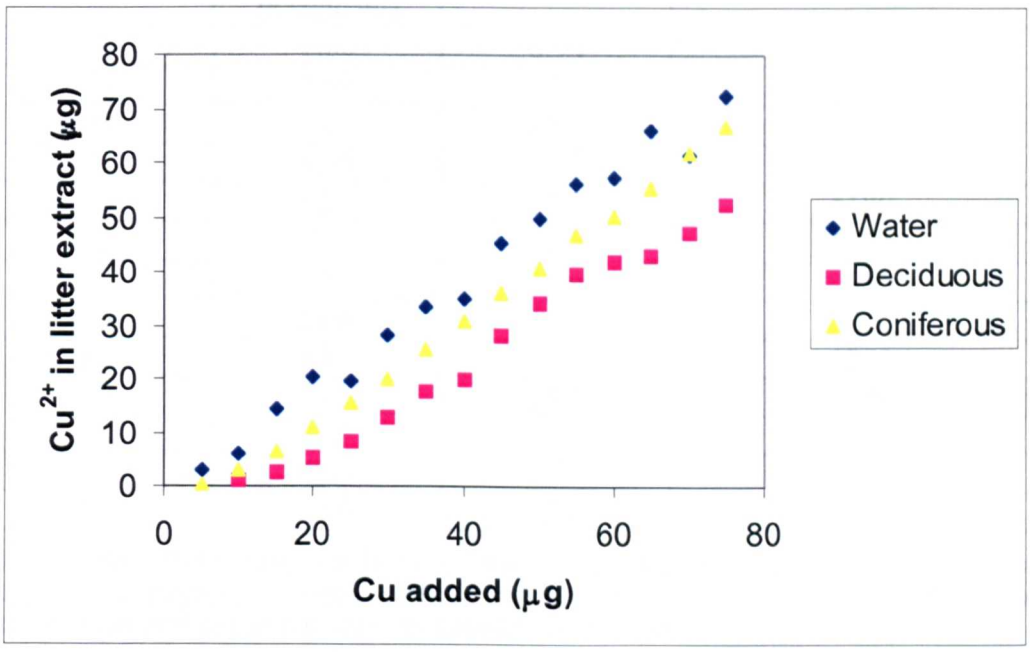


Figure 4-9 Loading water and water washings of litter (taken from below the deciduous canopy and from below the coniferous canopy) with 0.50 ppm Cu solution

Figure 4-9 shows that the water washings sample of the deciduous litter has a slightly greater affinity for Cu than the water washings sample of the coniferous litter. Tables 4-16 (deciduous litter) and 4-17 (coniferous litter) present the accompanying data (amount of Cu complexed and the organic carbon to Cu complexed molar ratios) to the information in Figure 4-9.

Amount of Cu added (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Organic carbon / Cu complexed (molar ratio)
10	1.06	8.94	2250
15	2.66	12.3	1630
20	5.26	14.7	1360
25	8.26	16.7	1200
30	12.9	17.1	1180
35	17.8	17.2	1170
40	20.1	19.9	1010
45	28.4	16.6	1210
50	34.2	15.8	1280
55	39.5	15.5	1300
60	41.7	18.3	1100
65	43.2	21.8	922
75	52.6	22.4	896

Table 4-16 Data obtained from loading water washings of litter (taken from below the deciduous canopy, organic carbon mass: 3.80 mg) with 0.50 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

Amount of Cu added (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Organic carbon/ Cu complexed (molar ratio)
10	2.98	7.02	2600
15	6.48	8.52	2150
20	11.1	8.94	2040
25	15.4	9.56	1910
30	20.0	10.0	1830
35	25.5	9.52	1920
40	31.1	8.90	2050
45	36.1	8.87	2060
50	40.7	9.34	1960
55	46.9	8.11	2260
60	50.3	9.69	1890
65	55.5	9.54	1920
75	66.9	8.05	2270

Table 4-17 Data obtained from loading water washings of litter (taken from below the coniferous canopy, organic carbon mass: 3.46 mg) with 0.50 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

On comparing Tables 4-16 (deciduous litter) with Table 4-17 (coniferous litter), it is clear that both the water washings of the deciduous litter and the water washings of the coniferous litter quickly become saturated with Cu. However, this is more evident for the coniferous sample (Table 4-17) as it is certainly saturated after the first three additions, indicating that perhaps the deciduous sample (table 4-16) has a greater proportion of Cu-binding organic compounds. The two samples have a very similar organic carbon content (deciduous: 3.80 mg, coniferous: 3.46 mg), indicating that perhaps the deciduous sample

has been broken down to a greater degree and hence contains a greater proportion of Cu-binding matter for every mg of organic carbon. If this is the case, this will probably tie in well with reports on reduced decomposition rates of organic matter below a Norway spruce canopy when compared to organic matter below broad leaves (such as Beech) (Andersen et al., 2004; Bergkvist, 1987).

As was the case for the water washings of the soil (deciduous and coniferous, Figures 4-3 and 4-4), the water washings of the litter (Figure 4-9) have considerable molar ratio values. These high values imply that each Cu atom is bound by an organic molecule of considerable size.

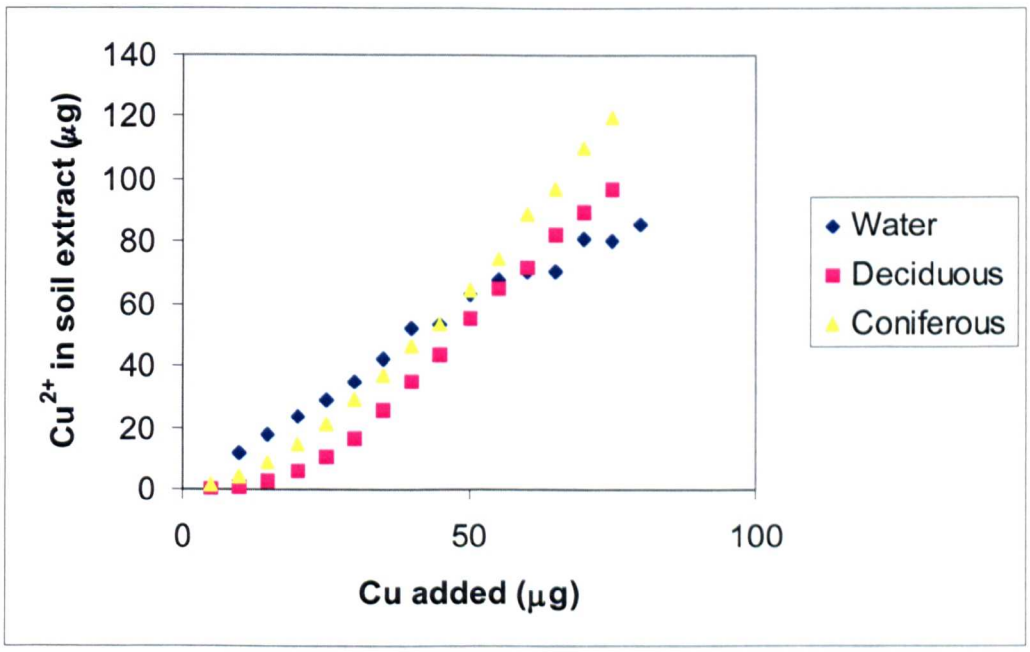


Figure 4-10 Loading water and water washings of litter (taken from below the deciduous canopy and from below the coniferous canopy) with 1.00 ppm Cu solution

Figure 4-10 (loading with 1.00 ppm Cu solution) is in agreement with Figure 4-9 (loading with 0.50 ppm Cu solution) in that the water washings of the litter (deciduous and conifeorus) quickly become saturated with Cu. Table 4-18 (deciduous litter) and 4-19 (coniferous litter) present the accompanying data (amount of Cu complexed and the organic to Cu complexed molar ratios) to the information in Figure 4-10.

Amount of Cu added (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Organic carbon / Cu complexed (molar ratio)
10	0.92	9.08	2210
15	2.84	12.2	1650
20	6.15	13.8	1450
25	10.7	14.3	1400
30	16.7	13.3	1510
35	25.5	9.49	2120
40	34.8	5.18	3880
45	43.6	1.43	nd
50	55.1	nd	nd
55	64.9	nd	nd
60	71.5	nd	nd
65	82.3	nd	nd
70	89.3	nd	nd
75	96.5	nd	nd

Table 4-18 Data obtained from loading water washings of the litter (taken from below the deciduous canopy, organic carbon mass: 3.80 mg) with 1.00 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios (nd = non-detectable)

Amount of Cu added (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Organic carbon / Cu complexed (molar ratio)
10	1.02	8.98	2040
15	3.84	11.2	1640
20	8.36	11.6	1570
25	14.4	10.6	1720
30	20.9	9.12	2000
35	29.0	5.98	3060
40	36.9	3.14	5830
45	46.1	nd	nd
50	53.2	nd	nd
55	64.6	nd	nd
60	74.4	nd	nd
65	88.8	nd	nd
70	96.6	nd	nd
75	110	nd	nd

Table 4-19 Data obtained from loading water washings of the litter (taken from below the coniferous canopy, organic carbon mass: 3.46 mg) with 1.00 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios (nd = non-detectable)

Tables 4-18 and 4-19 are in good agreement with the data for the investigation into the addition of 0.50 ppm Cu to water washings of litter (Tables 4-16 and 4-17), in that they show that the water washings quickly become saturated. These findings clearly indicate that the litter water washings do not contain as much Cu-binding matter as the samples originating from soil. This makes sense, considering that soil is the next stage of

breakdown after litter, and will contain more Cu-binding matter as a result of this. Also, it ties in well with the fact that the soil water washings have greater organic carbon values associated with them than the litter water washings (Section 4.3.2). The values indicated as non-detectable (Tables 1-18 and 1-19) are due to the experimental error associated with using the cupric ISE. Once again, the large molar ratio values hint at each Cu molecule being bound by a considerably large organic molecule.

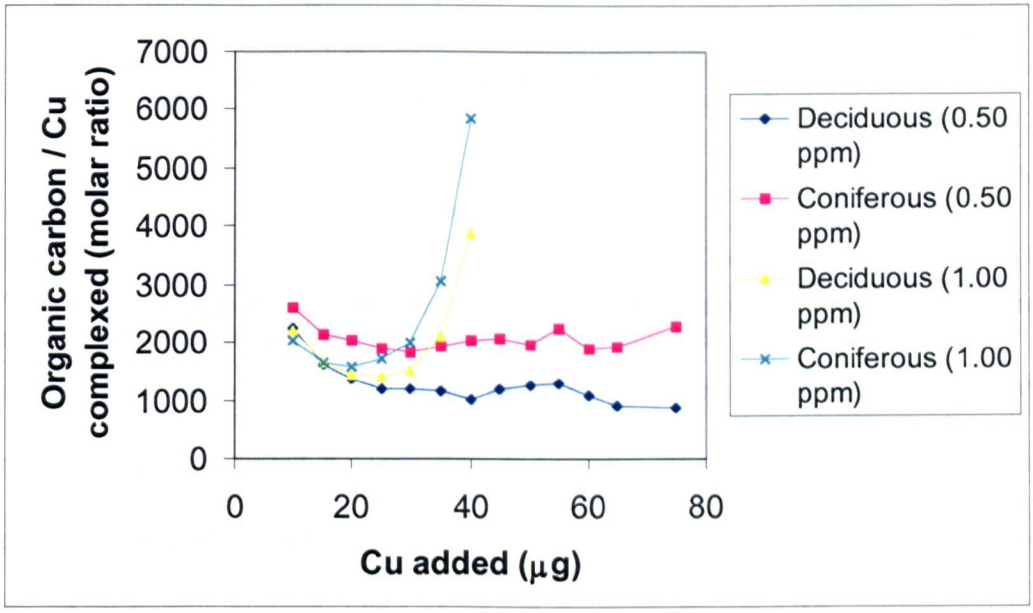


Figure 4-11 Organic carbon / Cu complexed (molar ratio) vs. Cu added for the four investigations into Cu loading of litter water washings: deciduous (0.50 ppm and 1.00 ppm) and coniferous (0.50 ppm and 1.00 ppm)

Figure 4-11 supports the findings from Figures 4-9 and 4-10 in that it clearly shows that the water washings of the litter (deciduous and coniferous) are very quickly saturated with Cu. Also, the experimental error involved with using the cupric ISE is evident for the deciduous 1.00 ppm and the coniferous 1.00 ppm (Figure 4-11).

4.3.4.4 Cu loading of litter suspended in water

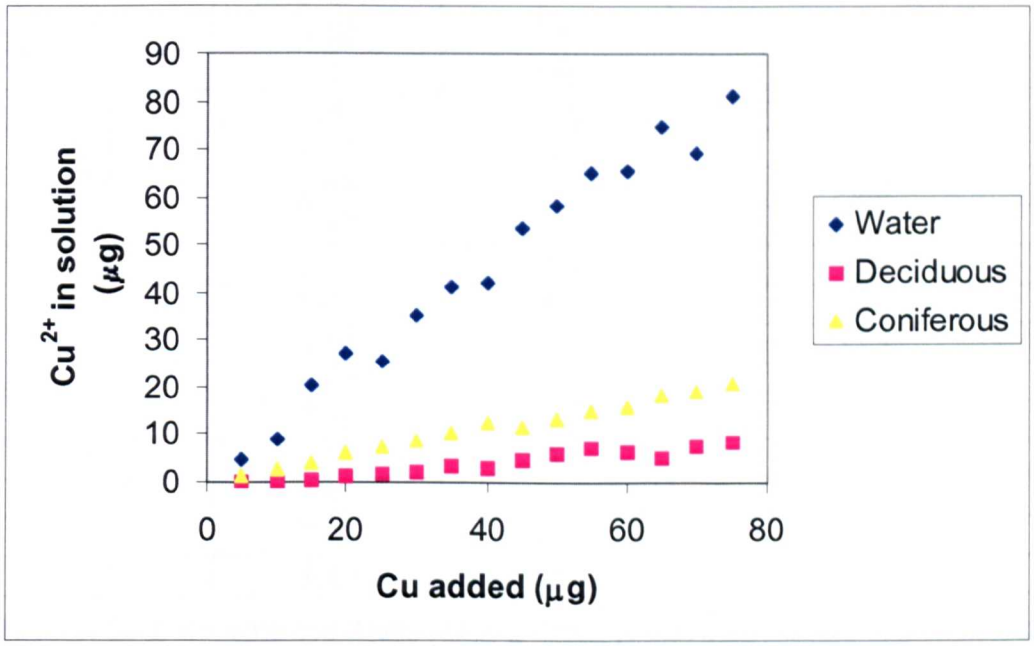


Figure 4-12 Loading water and litter suspensions in water (taken from below the deciduous canopy and from below the coniferous canopy) with 0.50 ppm Cu solution

Figure 4-12 shows that both litter samples (deciduous and coniferous) have a strong complexing ability for Cu. However, the sample taken from below the deciduous canopy exhibits the greatest affinity for Cu. Tables 4-20 and 4-21 show the amount of Cu complexed and molar ratio data which accompanies Figure 4-12.

Amount of Cu added (μg)	Amount of Cu detected (μg) (ISE measured)	Amount Cu complexed (μg)	Organic carbon / Cu complexed (molar ratio) (x 10^3)
5	0.12	4.88	459
10	0.20	9.80	229
15	0.40	14.6	154
20	1.09	18.9	119
25	1.67	23.3	96.2
30	2.32	27.7	81.0
35	3.34	31.7	70.9
40	3.05	36.9	60.7
45	4.60	40.4	55.5
50	5.82	44.2	50.8
55	7.01	48.0	46.7
60	6.55	53.5	42.0
65	5.01	60.0	37.4
70	7.79	62.2	36.1
75	8.45	66.5	33.7

Table 4-20 Data obtained from loading litter (taken from below the deciduous canopy, organic carbon mass: 424 mg) suspended in water with 0.50 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

Amount of Cu added (μg)	Amount of Cu detected (μg) (ISE measured)	Amount Cu complexed (μg)	Organic carbon / Cu complexed (molar ratio) (x 10^3)
5	1.14	3.86	520
10	2.53	7.47	269
15	3.93	11.1	181
20	5.84	14.2	142
25	7.41	17.6	114
30	8.68	21.3	94.2
35	10.2	24.8	81.0
40	12.3	27.7	72.4
45	11.4	33.6	59.7
50	13.3	36.7	54.7
55	14.8	40.2	49.9
60	15.8	44.2	45.4
65	18.1	46.9	42.8
70	19.0	51.0	39.3
75	20.9	54.1	37.1

Table 4-21 Data obtained from loading litter (taken from below the coniferous canopy, organic carbon mass: 379 mg) suspended in water with 0.50 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

On comparing the data in Tables 4-20 and 4-21, it is clear that the sample originating from below the deciduous canopy has a greater complexing ability than the sample originating from below the coniferous canopy. On addition of 75 μg Cu to the coniferous litter, 54.1 μg Cu is complexed. However, on addition of 75 μg Cu to the coniferous litter, 66.5 μg Cu

is complexed. This could be the result of the deciduous sample (424 mg) containing a greater organic carbon content than the coniferous sample (379 mg). Also, the data indicates that both systems are showing signs of saturation. High molar ratio values are evident here also, and are greater than those found for loading soil in water with Cu (Tables 4-12 to 4-15). This could be a reflection of the larger organic carbon values associated with the litter samples, when compared to those associated with the soil samples (Section 4.3.2), or it could be that the organic carbon in soil has a greater affinity for Cu per μg than the organic carbon in litter. For example, in Table 4-12, when 70 μg Cu are added to deciduous soil suspended in water, a molar ratio of 18.7 $x10^3$ is found. However, when 70 μg Cu are added to deciduous litter (Table 4-20), a molar ratio of 36.1 $x10^3$ is found.

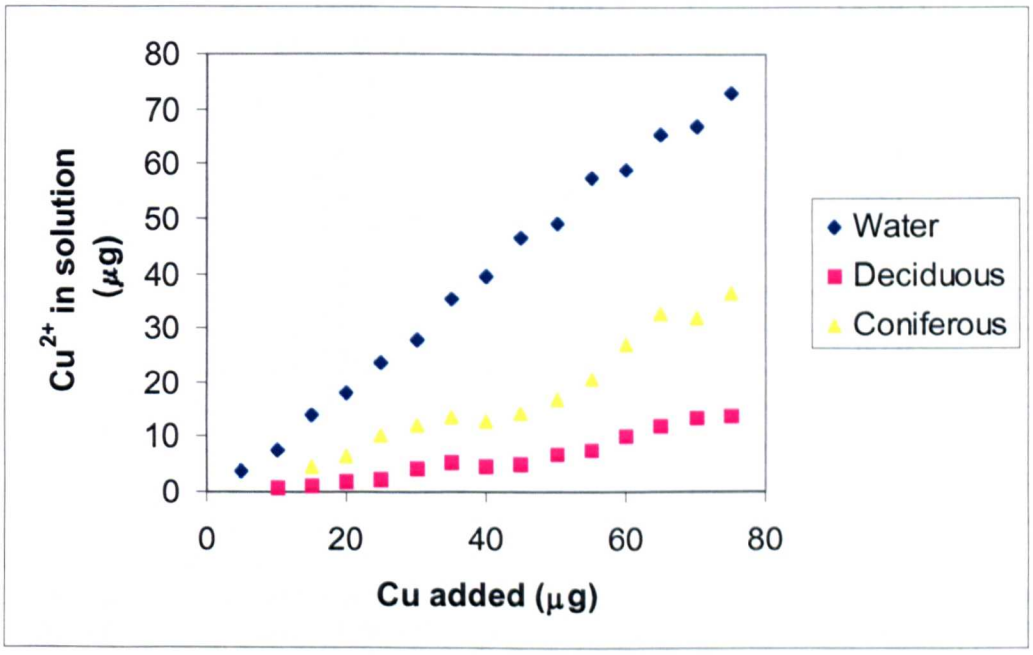


Figure 4-13 Loading water and litter suspended in water (taken from below the deciduous canopy and from below the coniferous canopy) with 1.00 ppm

Figure 4-13 involves the same study as that reported in Figure 4-12, with the exception of a Cu solution of 1.00 ppm being added in place of a 0.50 ppm solution. Figure 4-13 supports the findings found for Figure 4-12, as it indicates that both samples (deciduous and conniferous) have a strong complexing ability for Cu, and that this is greatest for the deciduous sample. Tables 4-22 and 4-23 present the data for Figure 4-13: the percentage of Cu complexed and the molar ratio.

Amount of Cu added (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Organic carbon / Cu complexed (molar ratio) (x 10^3)
15	1.10	13.9	161
20	1.88	18.1	124
25	2.22	22.8	98.5
30	4.11	25.9	86.6
35	5.21	29.8	75.3
40	4.39	35.6	63.0
45	4.90	40.1	55.9
50	6.58	43.4	51.7
55	7.50	47.5	47.2
60	10.1	49.9	44.9
65	11.9	53.1	42.3
70	13.6	56.4	39.8
75	14.0	61.0	36.8

Table 4-22 Data obtained from loading litter (taken from below the deciduous canopy, organic carbon mass: 424 mg) suspended in water with 1.00 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

Amount of Cu added (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Organic carbon / Cu complexed (molar ratio) (x 10^3)
15	4.35	10.6	189
20	6.44	13.6	148
25	10.2	14.8	136
30	11.9	18.1	111
35	13.4	21.6	93.1
40	12.8	27.2	73.8
45	14.2	30.8	65.1
50	17.0	33.0	60.9
55	20.8	34.2	58.7
60	27.1	32.9	61.1
65	32.7	32.3	62.1
70	31.8	38.2	52.6
75	36.3	38.7	51.9

Table 4-23 Data obtained from loading litter (taken from below the coniferous canopy, organic carbon mass: 379 mg) suspended in water with 1.00 ppm Cu: amount of Cu complexed and organic carbon to copper molar ratios

Tables 4-22 and 4-23 supports the data in Tables 4-20 and 4-21 (Figure 4-12, adding 0.50 ppm Cu solution). The both show a tendency towards saturation, the deciduous sample exhibits a greater affinity for Cu than the coniferous sample and the molar ratio values are large.

The litter suspended in water samples (Figures 4-12 and 4-13) appear to have a similar affinity for Cu to the soil suspended in water samples (Figures 4-6 and 4-7). For example,

in Table 4-14 (deciduous soil), addition of 70 μg Cu resulted in 57.9 μg being retained, and in Table 4-22 (deciduous litter), addition of 70 μg resulted in 56.4 μg being retained. This contrasts with the comparison of the water washing samples, as the soil water washings (Figures 4-3 and 4-4) clearly have a greater affinity for Cu than the litter water washings (Figures 4-9 and 4-10). Perhaps the similarity in solid samples suspended in water is due to litter containing a higher organic carbon content than soil (Section 4.3.3).

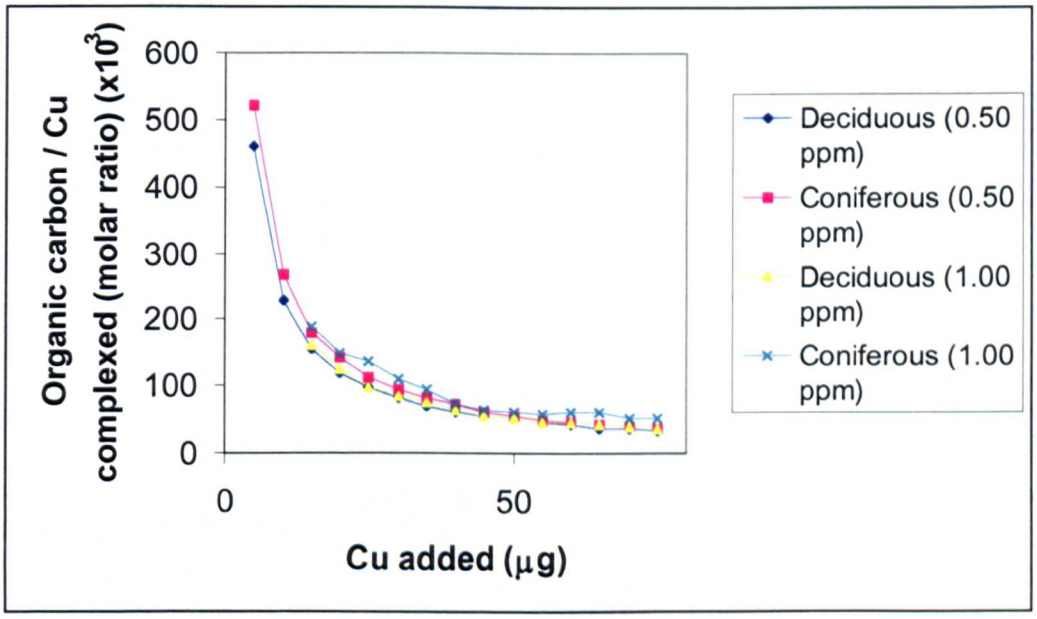


Figure 4-14 Organic carbon / Cu complexed (molar ratio) (x10^3) vs. Cu added for the four investigations into Cu loading of litter suspended in water: deciduous (0.50 ppm and 1.00 ppm) and coniferous (0.50 ppm and 1.00 ppm)

Figure 1-14 clearly indicates that the molar ratio is the same in each of the four investigations. This means that the proportion of Cu-binding organic matter in the organic carbon associated with each soil, is approximately equal. The difference evident between the two litter types (Figure 4-13), in that the deciduous sample appears to have a greater affinity for Cu than the coniferous sample, is due to the deciduous soil containing a greater organic carbon mass (424 mg) than the coniferous soil (379 mg).

On comparing the molar ratio vs. Cu added graphs for litter suspended in water (Figure 4-14) with the molar ratio vs. Cu added graphs for soil suspended in water (Figure 4-8), it is clear that the organic carbon in soil has a greater affinity for Cu than the organic carbon in litter. Therefore, the reason it appears as though litter samples suspended in water have a

similar affinity for Cu to the soil samples suspended in water is due to litter containing a greater organic carbon content (Section 4.3.3).

4.3.4.5 Cu loading of leaf water washings

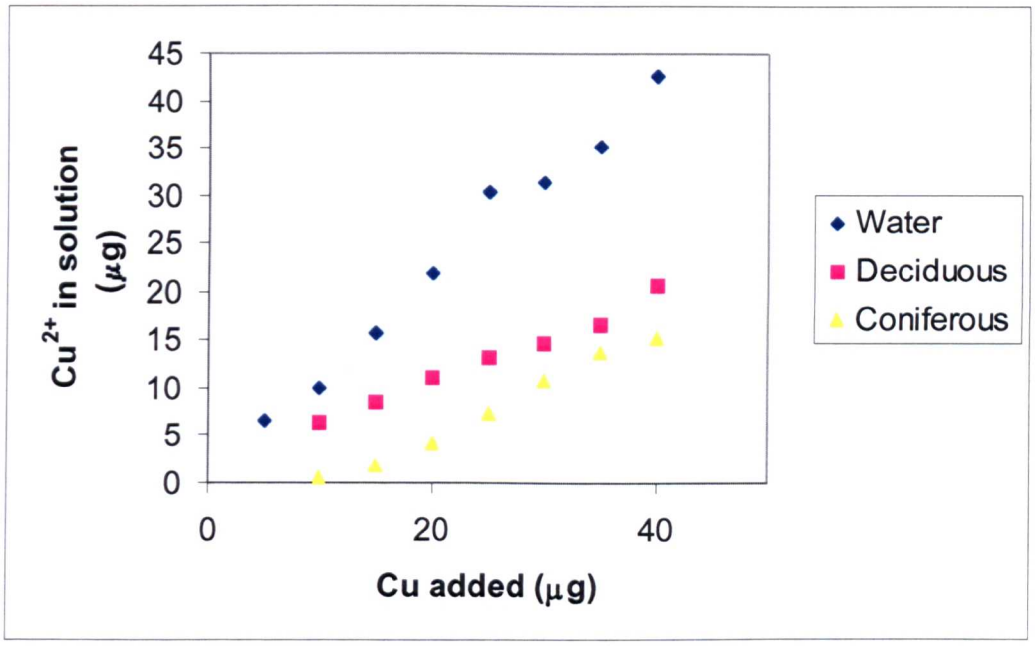


Figure 4-15 Loading water and water extracts of leaves (taken from below the deciduous canopy and from below the coniferous canopy) with 0.50 ppm Cu solution

Figure 4-15 shows that the coniferous sample has a greater affinity for Cu than the deciduous sample. This finding clearly conflicts with previous Cu loading studies of water extracts. Tables 4-24 shows the amount of Cu complexed data which accompanies Figure 4-15.

Amount of Cu added (µg)	Amount of Cu detected (µg) in deciduous sample (ISE measured)	Amount Cu complexed in deciduous sample (µg)	Amount of Cu detected (µg) in coniferous sample (ISE measured)	Amount Cu complexed in coniferous sample (µg)
10	6.37	3.63	0.47	9.53
15	8.49	6.51	1.68	13.3
20	11.1	8.91	3.95	16.0
25	13.1	11.9	7.29	17.7
30	14.6	15.4	10.7	19.3
35	16.5	18.5	13.7	21.3
40	20.5	19.5	15.0	25.0

Table 4-24 Data obtained from loading water washings of leaves (taken from below the deciduous and coniferous canopies) with 0.50 ppm Cu solution: amount of Cu complexed

The data displayed in Table 4-24 contradicts previous findings on loading water washings of deciduous and coniferous samples, in that the coniferous sample exhibits the greatest ability to complex Cu. However, there is clearly a tendency towards saturation in both systems.

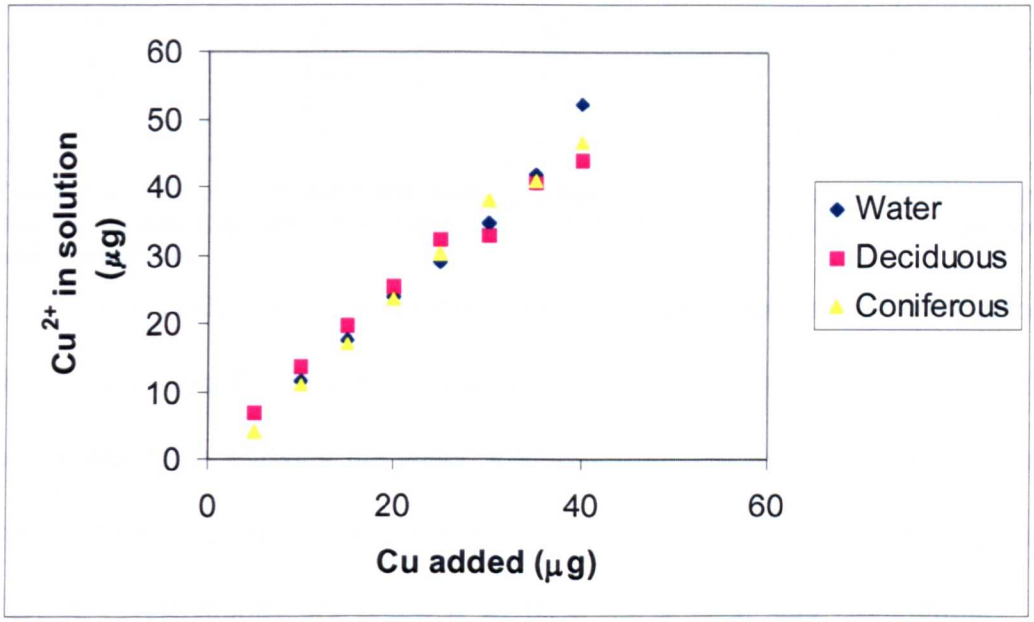


Figure 4-16 Loading water and water washings of leaves (taken from below the deciduous canopy and from below the coniferous canopy) with 1.00 ppm Cu solution

The methods used to obtain Figure 4-15 (0.50 ppm Cu solution) and Figure 4-16 (1.00 ppm Cu solution) were the same, except for the concentration of Cu solution added. Figures 4-15 and 4-16 do not show good reproducibility of results. Figure 4-15 shows the sample taken from below the coniferous canopy to have the greatest affinity for Cu. However, Figure 4-16 shows both samples as having no affinity for Cu, in that they follow the same path as water (i.e. no sample present). The variability between Figures 4-15 and 4-16, and the fact that neither support the previous findings from the Cu loading of water washings, could be due to experimental error. For example, the method for obtaining water washings of the leaves (Section 2.12.3) could be responsible for this error; as it was different to the method used for obtaining water washings of the soil (Section 2.12.1) and the litter (Section 2.12.2). The organic carbon measurements (Section 4.3.3) found these samples to contain negligible amounts of organic carbon, indicating that there is not much Cu-binding matter in these samples, thereby reinforcing the conclusion that these results are suspect.

Table 4-25 shows the amount of Cu complexed data which accompanies Figure 4-16.

Amount of Cu added (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)	Amount of Cu detected (µg) (ISE measured)	Amount Cu complexed (µg)
10	13.6	nd	11.0	nd
15	19.8	nd	17.0	nd
20	25.3	nd	23.6	nd
25	32.3	nd	30.0	nd
30	32.7	nd	37.8	nd
35	40.5	nd	40.9	nd
40	43.8	nd	46.7	nd

Table 4-25 Data obtained from loading water washings of leaves (taken from below the deciduous and the coniferous canopies) with 1.00 ppm Cu: amount of Cu complexed (nd = non-detectable)

The data in Table 4-25 supports that there is probably experimental error associated with the results from Cu loading of the water washings from leaves. Overall, the data established from studying the water washings from leaves (loading with Cu and organic carbon measurements), indicates that these samples do not have a great affinity for Cu. Further investigations are required in order to confirm this assumption. Perhaps an alternative water extraction method, similar to the one used for obtaining the water washings from both soil (2.12.1) and litter (2.12.2).

On comparing the Cu loading of soil, litter and leaves, the following order of affinity for Cu has been found in both the deciduous and coniferous systems: soil > litter (quickly saturated) > leaves (virtually no affinity). This makes sense, considering that the soil is broken down to a greater degree than litter and leaves, and is likely to contain more organic matter in a form for Cu-binding (Ross, 1994).

There have been a vast number of studies into the complexation of Cu with soils and soil extracts, including investigations into the nature of the organic components directly responsible for Cu complexation (Bhat et al., 1981; Bresnahan et al., 1978; Buffle et al., 1977; Cabaniss & Shuman, 1986; Cao et al., 2004; Carballeira et al., 2000; Logan, 1995;

Logan et al., 1997; Plaza et al., 2005; Ramos et al., 2002; Robertson & Leckie, 1999; Smith et al., 2004).

As reported previously, DOC is thought to be the main component of soil responsible for complexing with Cu (Andersen et al., 2004; Sauve et al., 1997; Yin et al., 2002). There are many investigation in the literature reporting on the complexation of Cu(II) by dissolved organic matter (Luster et al., 1996). It would be of interest to conduct a study on the Pollok Park soils whereby the various literature methods of isolating DOC from soils could be compared. Also, the report as to the significant contribution DOC has towards the soils contribution to Cu complexation could be investigated.

The complexation of Cu^{2+} by peat and humic acid was studied by Logan et al. (1997) by using a cupric ISE. They reported that significantly less metal was bound to the unextracted peat than to the humic acid, indicating that the isolation either exposed reactive sites or chemically altered the material to give reactive groupings. They derived formation constants and found that the formation constants for the metals reacting with the unextracted peat also showed differences between the behaviour and stability of the interactions. The Cu^{2+} ion reacted to form more stable complexes with un-extracted peat than with humic acid, indicating that in the unextracted material Cu^{2+} can react with other components of the soil, not just the humic substances, to give stable interactions. It would be of interest to conduct similar studies on the Pollok Park soils. For example, organic fractions (e.g. humic and fulvic acids) could be isolated from the Pollok Park soils and their complexation with the cupric ISE could be measured. Such studies have been reported by a number of authors (Cabaniss & Shuman, 1986; Logan, 1995; Saar & Weber, 1980; Sahu & Banerjee, 1990). Using these on the Pollok Park soils would hopefully enable an insight in to the components of soil responsible for the binding of Cu^{2+}.

A considerable amount of the literature on Cu complexation by organic matter involves the use of metal binding models. Several approaches have been proposed for modelling the binding of metals by humic material (Carballeira et al., 2000; Logan et al., 1997). For example, Carballeira et al. (2000) used an electrostatic model in the analysis of fulvic acid-copper ion complexation. The aim of the investigation was to estimate the contribution of the electrostatic effect to metal binding, and to calculate the intrinsic complexing parameters in solutions with a dissolved organic carbon (DOC) analogous to that in natural media such as aqueous environments and soil solution.

In the literature it is very common to see such models used alongside analytical methods (e.g. anodic stripping voltammetry and ISEs), as these combinations maximise the information obtained (Carballeira et al., 2000; Logan et al., 1997). In the future, it would be advantageous to combine the use of these models with the findings from ISE investigations etc. on the Pollok Park soils. Carballeira et al., 2000

4.4 Summary

The distribution of Cr, Cu, Pb and Zn in surface soil (deciduous and coniferous, top 5cm) was analysed using the modified BCR sequential extraction procedure. The deciduous and coniferous soils were found to have virtually identical fractionation patterns. The findings from Chapter 3, which indicated strong correlations between organic matter and Cu were confirmed in this chapter, as Cu was found predominantly in the oxidisable fraction. However, the findings which indicated strong correlations between organic matter and Cr, Pb, and Zn, are shown not to be as significant as the Cu-organic matter correlations implied. Pb was found mainly in the reducible fraction, Zn existed mainly in the residual fraction, while Cr was found to be distributed, on the whole, between the oxidisable and residual phases. These fractionation patterns comply with those reported in the literature (e.g. Armienta et al. (1996), Davidson et al. (1994) and Lu et al. (2003)).

Soil, litter and leaf samples were loaded with Cu. The loadings were monitored with a cupric ISE. The soil and litter samples were water washings, and solid soil or litter suspended in water. The leaf samples were water washings. The samples were analysed for their organic carbon content, and all were found to contain organic carbon, with the exception of the leaf samples which proved to have a negligible organic carbon content. In all cases (with the exception of the leaf samples), the deciduous samples were found to contain a greater organic carbon value than the equivalent coniferous sample. It is thought that this is due to deciduous samples being more readily broken down than coniferous samples (Andersen et al., 2004; Bergkvist, 1987).

On loading the samples with Cu, the deciduous samples, in each case, appeared to have a greater affinity for Cu. However, on plotting all the data for each investigation on the same graph, it was clear that the reason behind this greater affinity was due to the deciduous samples containing a greater amount of organic carbon (or Cu-binding material). As the organic carbon in the deciduous and coniferous samples appears to have equal affinity for Cu (per weight of organic matter). Molar ratios were established for each set of data (loading samples with Cu), and in each case these values were large. The considerable size of these molar ratios point to the fact that when a Cu atom is complexed by organic matter, it could be by a large organic molecule. However, it should also be noted that this is speculation only, as a lot is not known about the complexing species, including their molecular weight and the percentage (which may be small) of the total organic matter that they comprise The solid samples (soil and litter) suspended in water provided considerably larger molar ratios than the water washings (soil and litter). It has been reported in the literature that a considerable amount of organic carbon in soil does not relate to Cu-binding carbon, and that most Cu is complexed by DOC (Buffle et al., 1977; Sauve et al., 1997). This may explain these larger molar ratios associated with the solid samples suspended in water. The molar ratios data obtained from the soil suspended in

water investigations, corresponds well with the estimated molar ratios from the LOI vs. Cu concentration graphs in Chapter 3 (Section 3.5.3).

The loading of water washings of leaves (deciduous and coniferous), although not conclusive, indicate that the leaves have no real affinity for Cu. The negligible organic carbon values support this (Section 4.3.3). However, further investigations are required to confirm this.

The loading of soil, litter and leaves with Cu has enabled a picture of Cu complexation in the litter-leaves-soil sytem. The following order (starting with greatest affinity) was established: soil > litter (quickly saturated) > leaves (virtually no complexation). Future investigations should incorporate similar studies on the affinity of the other metals for soil, litter and leaf samples.

Chapter 5 - Electrophoresis: Method Development

5.1 Introduction

The work presented in this chapter aimed to apply electrophoresis as an effective means of isolating the humic substances in forest soils, with the intent of obtaining a more accurate picture of the organic compounds involved in retaining metals.

The isolation of humic substances is very important as they have been shown as the active metal-binding component of soil organic matter (Graham et al., 1995). As discussed previously (Sections 1.6.6 and 1.6.7), gel electrophoresis has been reported in the literature as a means of simultaneously extracting and fractionating humic material (e.g. (Farmer et al., 2002; Graham et al., 2000; Trubetskoj et al., 1991). However, there is still considerable development of gel electrophoresis required in order for it to be an efficient technique for the isolation of humic substances. Clearly, successfully developing this method for use on soils will provide invaluable information on metal speciation.

In order to develop gel electrophoresis as a viable technique for the electrophoresis of soils, it is important to understand the theory behind the technique and previous uses of the technique. Gel electrophoresis was originally developed for use in biological fields. It has been extensively developed and used for the isolation of nucleic acids and proteins (Dunn, 1986; Hames, 1998; Hawcroft, 1997). As a result of these developments, much information has been published on how electrophoresis operates (Chrambach et al., 1992; Dunn, 1986; Hames, 1998; Hawcroft, 1997; Martin, 1995; Wilson & Walker, 2000). Some of the most important points are elaborated on in Section 5.2.

This chapter comprises three separate sections as summarised below:

- General principles of gel electrophoresis as found through the development of gel electrophoresis in biological fields

- Existing literature on the use of gel electrophoresis on soils

- Developing gel electrophoresis as an efficient technique for use on soils

These studies involved using various soil types (as discussed in Section 5.4, Table 5.2), including the two soil types from Pollok Park, the site of study (soil taken from below the deciduous site and soil taken from below the coniferous site).

5.2 The general principles of electrophoresis

Electrophoresis is defined as the migration of a charged particle in an electric field (Chrambach et al., 1992; Dunn, 1986; Hames, 1998; Hawcroft, 1997; Martin, 1995; Wilson & Walker, 2000). Many important biological molecules such as proteins and nucleic acids possess ionisable groups and, therefore, at any given pH, exist in solution as electrically charged species, either as cations or anions. Under the influence of an electric field these charged particles will migrate either to the cathode or the anode, depending on the nature of their net charge.

Under conditions of a constant velocity, the driving force on a particle is the product of the charge on the particle and the applied field strength. The force is counteracted by the frictional resistance of the separation medium, which is proportional to its sheer velocity. The situation is complicated further if a gel medium is used in place of a solution medium, as the frictional resistance will depend on additional factors such as gel density and particle size.

Much information has been provided from the development of electrophoresis as a means of isolating biological molecules (Chrambach et al., 1992; Dunn, 1986; Hames, 1998; Hawcroft, 1997; Martin, 1995; Wilson & Walker, 2000), including the following important points:

- Electrophoretic mobility.
- Effects of pH and ionic strength.
- Joule heating.
- Zone electrophoresis.
- Gel-based support media.

Below, these points are elaborated on from the perspective of those who found this information as a result of developing electrophoresis for the purpose of separating proteins and nucleic acids.

5.2.1 Electrophoretic mobility

An important concept in electrophoresis is electrophoretic mobility, which is defined as the velocity of the charged species per unit field. Therefore, the choice of applied voltage and separation path length, which determine the field strength, together with the time of the run, are important parameters in optimizing an electrophoretic separation.

5.2.2 Effects of pH and ionic strength

If a substance has a variable charge which is pH dependent, the pH of the medium will exert a profound influence on its mobility during electrophoresis. For example, proteins

possess both negatively and positively charged groups as part of their primary structure, and as a result they act as zwitterions.

The ionic strength of the separation medium also exerts a major influence on electrophoretic mobility. Buffers of low ionic strength permit higher rates of migration than do those of higher ionic strength, while the latter generally result in sharper zones of separation. Therefore, choice of buffer ionic strength is an important parameter in determining the time and resolution of an electrophoretic separation.

5.2.3 Joule heating

During every electrophoretic separation, electrical energy is transformed into heat, termed Joule heating. This can result in severe deleterious effects such as increased diffusion of protein molecules (which degrades resolution). The limitation and removal of this generated heat is, therefore, a major consideration in the implementation of a particular electrophoretic separation, and in the design of the equipment used. The choice of buffer strength is crucial here, as the higher its ionic strength, the greater its conductivity, and the greater the amount of heat generated.

It is desirable to use power supplies which can be regulated to provide a constant output during electrophoresis. Nearly all commercially available power supplies provide for use of either constant current, to be used in systems with decreasing or constant resistance, or constant voltage, to be used where resistance increases during the separation. There are also more expensive power packs which have the capability to monitor voltage and current continuously, thereby performing electrophoresis at constant power. This approach provides constant heat generation during electrophoresis, but other factors such as the choice of buffer and applied voltage are critical for the minimization and removal of that heat. In any case, reproducibly controlled separating can only be achieved by carrying out electrophoresis at a constant temperature.

5.2.4 Zone electrophoresis

As proteins are charged at any pH other than their pI (isoelectric point), they will migrate in an electric field at a rate which is dependent on their charge density. Charge density is defined as the ratio of charge to mass (kDa). The higher this ratio, the faster the molecule will migrate. Thus, if an electric field is applied to a solution of proteins, the different molecules will migrate at different rates (dependent on the magnitude of their charge density) towards either the anode or the cathode (dependent on whether their net charge is negative or positive). Little or no separation of the proteins is possible if they are present throughout the separation medium. However, if the sample is initially present as a narrow zone, proteins of different mobilities will travel as discrete zones and thus separate during electrophoresis. This approach is known as zone electrophoresis.

5.2.5 Gel-based support media

A major advancement in electrophoresis occurred with the advent of polyacrylamide gels. They are non-ionic polymers which are chemically inert, stable over a wide range of pH, temperature and ionic strength, and are transparent. Moreover, polyacrylamide gels can be produced with a wide range of pore sizes, optimized for the separation of proteins of different size ranges. These advantages, coupled with the fact that very high resolution protein separation can be obtained, have resulted in polyacrylamide gels being the support medium of choice for zone electrophoresis of proteins.

Agarose gels are used for the analysis of large molecules or complexes such as nucleic acids, as very large molecules cannot be separated satisfactorily using polyacrylamide gels.

In the case of gel-based media it is important to understand that their relatively small pore size, approximately of the same order as the size of protein molecules, will result in a

molecular sieving effect during electrophoresis, so that the resulting separation will depend on both the charge density and size of the proteins being analyzed.

5.2.6 Electrophoresis in practice

Simple electrophoresis on paper or cellulose is nowadays quite uncommon; gel-based systems are virtually universal. Agarose and polyacrylamide have found widespread use in electrophoresis and this is largely as a result of their reproducibility, reliability and versatility. Table 5-1 compares the advantages of polyacrylamide and agarose gels.

Polyacrylamide gel	Agarose gel
Chemically and physically inert	Some slight and variable electric charge
Complex preparation since it requires chemical polymerization	Preparation easy: simply involves cooling of liquefied suspension
Will take additives, including incorporation by copolymerization	Additives less useful and less common
Monomers have significant toxicity	No toxicity problems
Incomplete polymerization creates run variability	No residual polymerization by-products or monomers to create variability
Forms pores of an easily controlled size or gradient of sizes	Pore sizes less easily controlled
Pores are in a low size range (therefore suitable for smaller molecules)	Pores are of larger size range (therefore suitable for larger molecules)
Low pore size means gels run, stain, destain, and elute relatively slowly	These processes tend to be faster
Gel is relatively robust	Gel is relatively fragile
Can tolerate high field strengths and gives reasonably rapid separations	Needs lower field strengths

Table 5-1 Comparing the advantages of polyacrylamide and agarose gels

5.2.7 Electrophoresis under native conditions

Electrophoresis under native-systems is used in circumstances where it is desired to maintain the native conformation of the molecules being separated. This should ensure that the biological activity of the separated components is preserved, so that properties such as enzyme activity (in the case of proteins) can be studied after electrophoresis.

Unfortunately, native electrophoresis techniques can only be applied to protein samples which are soluble and which will not precipitate or aggregate.

5.2.8 Electrophoresis in the presence of additives

For insoluble proteins, effective electrophoretic separation can only be achieved if suitable additives are present in the gel to increase protein solubility and minimize aggregate formation. Such additives include urea and detergents.

Urea is a commonly used gel additive, designed to increase sample solubility and minimize protein aggregation. Proteins unfold and are denatured progressively as they are exposed to increasing concentrations of urea, and different proteins have different sensitivities to denaturation by urea. Thus, low concentrations of urea are used if it is desired to maintain proteins in a native state, whereas high concentrations of urea (typically 8 M or higher) are used if the proteins are to be separated in a denatured state. Urea should be added to the gel, but it is not necessary to add urea to the electrolyte buffer solution.

The use of detergents in both the sample and in the gel is a popular and effective approach to the solubilization and disaggregation of proteins to be analysed by electrophoresis. A major factor that must be considered when choosing an appropriate detergent for a particular electrophoretic separation is whether the biological properties are to be preserved. Sodium dodecyl sulfate (SDS), an anionic detergent, is undoubtedly one of the most common detergents. SDS is known to solubilize, dissociate and denature the majority of oligomeric proteins into their constituent subunits. In addition, the resulting SDS-complexes migrate towards the cathode during electrophoresis in gels containing SDS in accordance with molecular size of the subunits. These properties have made SDS-polyacrylamide gel electrophoresis (SDS-PAGE) the most commonly used procedure for the analysis of proteins.

5.2.9 Summary

The basic concept of electrophoresis is simple, but it should now be clear that there are a wide range of factors which can influence and modify an electrophoretic separation process. Indeed, these various factors have been exploited to generate a diversity of electrophoretic techniques able to separate proteins according to size, mobility and net charge. This information, derived as a result of the development of electrophoresis for separation of proteins and nucleic acids, can be used when developing electrophoresis for the separation of other substances. For example, in the separation of humic substances in soil.

5.3 Applications of Gel Electrophoretic Techniques to the Fractionation of Humic Substances in Soils

The following points were reported in Sections 1.6.6 and 1.6.7, with regard to gel electrophoresis of soils:

- There is a considerable amount of literature on the use of polyacryalmide gel electrophoresis (PAGE) as a means of fractionating humic substances (Trubetskoj et al., 1991; Trubetskoj et al., 1992). However, this method is relatively invasive as far as the reagents used are concerned (i.e. the native structure of the humic substances is not maintained).

- Recently, Graham et al. (2000) have studied agarose gel electrophoresis as a suitable alternative means of gel electrophoresis of soils. It is an undeveloped area, but definitely worth developing as it is far less intrusive then PAG electrophoresis.

- Higney (2003) recently attempted to use agarose gel electrophoresis to fractionate humic substances from a road dust sample.

5.4 Gel Electrophoresis of Soils: Method Development

The aim of the work in this chapter was to develop an effective method of gel electrophoresis for the isolation and fractionation of the humic substances within forest soils. Humic matter has so far not been unequivocally defined. On the contrary, it has been recognized as a mixture of polymerized organic compounds (Flaig et al., 1975; Kononova, 1966; Schnitzer & Khan, 1972). However, the presence of charge resulting from the ionization of functional groups is known to be a fundamental property of humic substances. It is this property, along with molecule size (as in the case of proteins and nucleic acids), which is exploited in electrophoresis.

The work presented in this chapter involved twenty eight electrophoretic runs. Each of these was based on previously published gel electrophoretic work on soils, and any variations introduced were inspired from information gained from the successful development of gel electrophoresis for proteins and nucleic acids. For these runs, various samples were used and they are presented in Table 5-2. The reasoning behind using the variety of samples indicated in Table 5-2 is explained throughout the chapter, as new soil types are introduced to the procedure.

Sample Description
Deciduous soil (Collection 2, Section 2.3.2)
Coniferous soil (Collection 2, Section 2.3.2)
Humic acid sodium salt (Humic acid)
Garden soil
Agricultural soil
Ombrotrophic peat
Inter-tidal sediment
River-side sample

Table 5-2 Various samples placed in wells for the electrophoretic runs

Along with the variety of samples used, a variety of buffers were also used, and these are presented in Table 5-3.

Buffer Abbreviation	Buffer name and description
TRISMA	Tris(hydroxymethyl)aminomethane
TRIZMA	Tris(hydroxymethyl)aminomethane hydrochloride
TBE	Tris-borate-EDTA, pH 8.5

Table 5-3 Description of various buffers used in the electrophoretic runs

The concentrations of these buffers are indicated in the description for each individual run.

The basic electrophoresis set-up is given in Section 2.15.

5.4.1 Electrophoresis study 1

Electrophoresis Study 1 focussed on the Higney (2003) procedure and three samples:

- Humic acid sodium salt (technical grade, Aldrich: H1, 675-2)
- Soil taken from below the deciduous canopy (Collection 2, Section 2.3.2).
- Soil taken from below the coniferous canopy (Collection 2, Section 2.3.2).

In Table 5-4, the method developed by Higney (2003) was utilized, with the only difference being TRISMA buffer used in place of TRIZMA buffer. The was due to TRISMA being readily available. Initially, Higney (2003) defined the electrophoretic conditions by using humic acid sodium salt. Runs 1-5 (Table 5-4 and Table 5-5) present the process of optimising with humic acid sodium salt.

	Run 1	Run 2	Run 3
Agarose (g, %)	1.00 g, 2%	1.00 g, 2%	0.50 g, 1%
Sample suspended	Humic acid	Humic acid	Humic acid
Sample mass suspended (g)	0.01 g	0.01 g	0.01 g
Solution type / volume sample suspended in (mL)	0.1 M TRISMA / 1 mL	0.1 M TRISMA / 1 mL	0.1 M TRISMA / 1 mL
Buffer	TRISMA	TRISMA	TRISMA
Buffer (M)	0.1 M	0.1 M	0.1 M
Constant voltage (V)	56V	56V	56V
Time of run	2 h	1 h	1 h

Table 5-4 Electrophoresis runs 1 to 3

For each electrophoretic run in Table 5-4:

- There were 6 individual wells
- Approximately 0.01 mL of suspended sample was added to each well.
- The direction of movement was from anode to cathode.
- A buffer volume of 50 mL was used for the gel.
- The result was a negative one as no band resulted, just a smudge.

The conditions used in Run 1 are those reported by Higney (2003), with the exceptions of TRISMA being used in place of TRIZMA and the run time being 2 hours, not I hour. At the end of Run 1 there was a smudge, but also discolouration in the cathode chamber. It was thought that Run 1 may have over-run (as indicated by the discolouration in the cathode chamber), therefore, Run 2 had a reduced run time. At the end of Run 2, the same smudge which resulted at the end of Run 1 was found. As a result, the percentage of agarose used in Run 3 was reduced (from 2% to 1%) in order to increase the pore size, as it was though that perhaps the pores in Runs 1 and 2 were too small (and the cause of the resulting smudge for both Runs). Increasing the pore size had no effect on the outcome, the same smudge occurred.

The next stage in the investigation involved changing the buffer from TRISMA to TRIZMA, due to TRIZMA being available and the choice buffer in the Higney (2003) procedure.

	Run 4	**Run 5**	**Run 6**	**Run 7**
Agarose (g, %)	1.00 g, 2%	1.00 g, 2%	1.00 g, 2%	1.00 g, 2%
Sample suspended	Humic acid	Deciduous	Deciduous	Deciduous
Sample mass suspended (g)	1 g	1 g	1 g	1 g
Solution type / volume sample suspended in (mL)	0.1 M TRIZMA / 1 mL	0.1 M TRIZMA / 1 mL	1 M NH_3 (ammonia) / 10 mL	1 M NH_3 (ammonia) / 10 mL
Buffer	TRIZMA	TRIZMA	TRIZMA	TRIZMA
Buffer (M)	0.1 M	0.1 M	0.1 M	0.05 M
Constant Voltage (V)	56 V	56 V	56 V	56V
Time of Run	1 h	1 h	2 h	1 h

Table 5-5 Electrophoresis runs 4 to 7

Regarding the runs in Table 5-5:

- There were 6 individual wells.
- Approximately 0.01 mL of suspended sample was added to each well in Runs 4 and 5.
- Approximately 0.02 mL of suspended sample was added to each well in Runs 6 and 7.
- The direction of movement was from anode to cathode.
- A buffer volume of 50 mL was used for the gel.

Run 4 (Table 5-5) involved the same conditions as Run 2 (Table 5-4), except for using TRIZMA buffer in place of TRISMA buffer (the conditions recommended by Higney (2003)) and the amount of sample has been increased by a factor of 100. After 1 hour, Run 4 (Table 5-5) presented a relatively distinct band which was dark brown in colour with another band slightly further ahead which was slightly faded and light brown in colour. The observed band could indeed be due to the change in amount of sample (an increase by

a factor of 100). As a result of the relatively successful outcome of Run 4, in that bands were identified, the humic acid sodium salt was replaced with the deciduous soil sample.

All soil / sample suspensions in the following electrophoretic runs were prepared, just prior to well loading, using the same method. This preparation involved adding the required mass of soil / sample to a glass container and then adding the required volume of buffer. Once both soil and buffer were added to the glass container, they were stirred with a glass rod, before the glass container was stoppered and shaken for approximately 2 minutes.

When preparing for Run 5 (Table 5-5), transferring the suspension of soil in buffer to the well using a Gilson pipette (the method of transfer for Runs 1 to 4) proved to be very difficult. Instead, a spatula was used to place TRIZMA moistened soil into the wells. Run 5 (Table 5-5) was very unsuccessful as:

- There was no real movement from the wells, just a slight smudge in the direction of both the anode and the cathode.

- There was a high degree of condensation within the system, indicating that perhaps the system voltage was too high for the sample type (deciduous soil) and conditions being used.

It was clear that a better means of applying the sample to the wells was required. It was thought that perhaps the use of varying concentrations of various bases to dissolve the organic matter in the soil, prior to applying the soils, may prove successful; and as a result, provide an insight into which area the method development should be focussed.

Run 6 (Table 5-5) involved the same overall procedure as Run 5 (Table 5-5) with the exceptions of:

- A 2 hour run instead of a 1 hour run in order see whether there would be more movement with a longer run time.

- 10 mL of solvent being used in place of 1 mL. This ten-fold increase was an attempt at improving the breakdown of soil components in order to optimise the possibility of a successful fractionation.

- The sample was suspended in ammonia (NH_3) and not TRIZMA.

- Approximately 0.02 mL was added to each well.

Run 6 provided a positive result in that an individual band was produced. However, it only ran a few centimetres from the well and was slightly diffuse. A more successful outcome was sought in the form of a discrete band (however, with hindsight, it would have been advantageous to perhaps attempt isolation and characterisation of this slightly diffuse band). It was thought that in order to obtain a more discrete band, perhaps the percentage of agarose could be decreased in order to increase the pore size, as the pore size could be hampering the run outcome by preventing a greater progression from the well. However, this is only one of a number of possibilities (as presented by Hawcroft (1997)), a range of factors can be altered in an attempt to improve the electrophoresis outcome:

- Buffer concentration: normally in the range of 0.01 to 0.1 M.

- Constant current, not constant voltage.

- Increasing pore size by decreasing the percentage of agarose.

- For normal electrophoresis, sample ionic strength and pH ought in general to be close to that of the gel buffer.

- Buffers often have a pH in the range of 8 to 9.

- Decrease gel thickness.

- Better separation occurs with a longer time and smaller voltage (although care has to be taken not to allow a time which results in increased diffusion).

Some of the above were elaborated upon in Section 5.2. It was decided to work on increasing the distance the band travelled in Run 6 (Table 5-5). Therefore the following factors were focussed on:

- Buffer concentration.

- Pore size.

- Ensuring the sample ionic strength and pH are close to that of the gel buffer.

Run 7 (Table 5-5) involved the same overall procedure as Run 6 (Table 5-5), with the exceptions of an altered TRIZMA buffer concentration from 0.1 M to 0.05 M, and a reduced run time (1 hour instead of 2 hours). The result appeared to be two diffuse bands which had a degree of overlap.

Due to the limited success in reproducing the Higney (2003) procedure, the Graham et al. (2000) procedure was referred to for the next stage in the investigation (Electrophoresis Study 2, Section 5.4.2).

5.4.2 Electrophoresis study 2

Electrophoresis Study 2 focussed on the Graham et al. (2000) procedure and the sample taken from below the deciduous canopy.

	Run 8	Run 9	Run 10	Run 11	Run 12
Agarose (g, %)	1.00 g, 2%	0.50 g, 1%	1.00 g, 2%	1.00 g, 2%	1.00 g, 2%
Sample suspended	Deciduous	Deciduous	Deciduous	Deciduous	Deciduous
Sample mass suspended (g)	1 g	3 g	3 g	10 g	10 g
Solution type / volume sample suspended in (mL)	1 M NH_3 / 10 mL	1 M NH_3 / 10 mL	1 M NH_3 / 10 mL	0.1 M NH_3 / 100 mL	0.1 M NH_3 / 100 mL
Buffer (M)	0.045 TBE	0.045 TBE	0.045 TBE	0.1 TBE	0.1 TBE
Start Voltage	56 V	78 V	71 V	42 V	120 V
End Voltage	56 V	92 V	75 V	46 V	120 V
Start Current	N/A	10 mA	10 mA	10 mA	30 mA
End Current	N/A	10 mA	10 mA	10 mA	30 mA
Time of Run	1 h	3 h	2 h	2 h	2 h

Table 5-6 Electrophoretic runs 8 to 12

Table 5-6 and the accompanying bullet points were based on the Graham et al. (2000) procedure, however, there were a number of important differences. In the Graham et al. (2000) procedure, TBE was not used as the running buffer, NH_3 was not used to suspend the soil, the soil-solvent ratio was 0.1 g in 2 mL (not 1 g in 10 mL), and the whole (rather than part) of the sample was transferred to the gel well.

Regarding the runs in Table 5-6:

- There were 6 individual wells.
- Approximately 0.02 mL of suspended sample was added to each well in Runs 8, 10 and 11.
- Approximately 0.01 mL of suspended sample was added to each well in Run 9.
- Approximately 0.04 mL of suspended sample was added to each well in Run 12.
- The direction of movement was from anode to cathode.
- A buffer volume of 50 mL was used for the gel.

Run 8 (Table 5-6) was exactly the same as Run 7 (Table 5-5), with the exception of 0.045 M TBE buffer being used in place of 0.05 M TRIZMA buffer. The outcome was unsuccessful in that diffuse bands resulted.

For Run 9, when compared to Run 8 (Table 5-6) (1 g sample), a more concentrated soil sample (3 g) was applied to the wells for Run 9 (Table 5-6) in an effort to combat the diffuse band problem. It was thought that by increasing the sample applied, any bands which were produced would be more concentrated, and as a result easier to observe. Also, a decrease in agarose was used for Run 9 (1%) when compared to Run 8 (2%), as it was thought that having the larger pore size for the higher sample mass would be a more advantageous starting point (if it was found that the pore size was too large, then a smaller pore size could be tried in future runs). Initially, Run 9 (Table 5-6) ran more rapidly than Run 8 (this was probably due to a greater voltage being applied in Run 9). A higher voltage was observed for Run 9 because instead of the voltage being fixed for that run (as had been the case in all previous runs), the current was fixed at 10 mA, which meant that the voltage was able to fluctuate to greater values than it had been at before. The decision to fix the current and not the voltage was an attempt at improving the run outcome (it was one of the factors recommended by Hawcroft (1997) to improve the outcome of an electrophoretic run). However, at the end of the run there was nothing to be seen in the gel. This could either indicate that:

- All the sample had run straight through the gel.

- The resulting bands are too dilute to see.

In Run 10 (Table 5-6), the same procedure was used as for Run 9 (Table 5-6), with the exception of the agarose content, which was increased from 1% to 2%. This was aimed at preventing the sample running through the gel too fast. There was evidence of some separation from the well in the form of a smear which appeared to incorporate several

bands (found by observation). The smear stretched from 3 cm to 7 cm away from the well. Clearly, a method for moving the sample has been achieved, but a means of obtaining separate bands is now required.

Run 11 (Table 5-6) involved ensuring the pH of the applied sample was close to that of the gel buffer. Hawcroft (1997) indicated that for normal electrophoresis this ought to be the case. The sample was adjusted to 8.34 and this compared favourable with the 8.52 pH of the buffer.

The end of the run indicated that a method of obtaining a separate band from the sample loaded into the well had been found. Up until Run 11 (Table 5-6), in all of the runs where movement of sample had occurred, the entire sample had left the wells. However, in this case, a distinct yellow band appeared, leaving a black residue in the wells. Unfortunately, after approximately one hour, the band which had been very distinct, became diffused through the gel. Evidently, a means of retaining a distinct band is required. The final diffuse band stretched from approximately 1 cm to 4 cm away from the well. No other bands emerged from the black material in the well. It was thought that applying a large voltage to the system, as high as the system could take, should be the next step as this may improve band sharpness (Hawcroft, 1997). Prior to this, only a relatively small voltage had been applied to the system. As indicated by Hawcroft (1997), the maximum capacity that an electrophoretic system can manage is deduced by using 5 V cm^{-1}. The maximum voltage for the system was found to be 122.5 V.

For Run 12 (Table 5-6), a voltage of 120 V was applied to the system. Run 12 began the same way as Run 11, with a yellow band separating from the black material. However, the yellow band moved rapidly and diffusion through the gel resulted after approximately thirty minutes of time had lapsed. At the end of the run, the band had run to the very edge

of the gel and had obviously over-run. A means of maintaining a more definite band throughout is clearly required.

	Run 13	Run 14	Run 15	Run 16	Run 17
Agarose (g, %)	1.00 g, 2%	0.50 g, 1%	0.50, 1%	0.50 g, 1%	0.50 g, 1%
Sample suspended	Deciduous	Deciduous (ground)	Deciduous (ground)	Deciduous (ground)	Deciduous (ground)
Sample mass suspended (g)	0.5 g	0.5 g	0.5 g	0.5 g	0.5 g
Solution type / volume sample suspended in (mL)	0.045 M TBE / 2 mL	0.045 M TBE / 2 mL	0.045 M TBE / 2 mL	0.045 M TBE / 2 mL	0.045 M TBE / 2 mL
Buffer (M)	0.045 TBE	0.045 TBE	0.045 TBE	0.045 TBE	0.045 TBE
Gel Additives	N/A	N/A	N/A	7 M urea	7 M urea
Start Voltage	100 V	75 V	68 V	Varied	108 V
End Voltage	100 V	74 V	68 V	N/A	84 V
Start Current	13 mA	10 mA	10 mA	Varied	30 mA
Run Time	2 h	2 h	2 h	2 h	2 h
End Current	12 mA	10 mA	10 mA	N/A	30 mA

Table 5-7 Electrophoretic runs 13 to 17

Regarding the runs in Table 5-7:

- A mono-well was used in place of the 6 wells used in Runs 1 to 12 (except Run 15, where a mono-well was used).
- 7 M urea was added to the gel in Runs 16 and 17.
- Approximately 0.20 mL of suspended sample was added to the mono-well in Run 13.
- Approximately 0.15 mL of suspended sample was added to the mono-well in Runs 14 and 16.
- Approximately 0.02 mL of suspended sample was added to each well in Runs 15 and 17.
- The direction of movement was from anode to cathode.

- A buffer volume of 50 mL was used for the gel.

Based on the fact that placing soil samples into the wells in a compatible solution was proving very difficult, Margaret Graham at Edinburgh University was contacted for advice (Graham, 2003). She stated that her group normally place soil samples in suspension with minimum volume of buffer. Her advice was followed for Run 13 (Table 5-7), whereby 2 ml of 0.045 M TBE buffer was used to suspend 0.5 g of the soil sample taken from below the deciduous canopy. At the start of Run 13 (Table 5-7), a large yellow band of approximately 3 cm developed from the well. However, as the run progressed, it continued to expand and became more and more diffuse. Most of the sample remained in the well.

It was decided that in order to improve the outcome of Run 13 (Table 5-7), Run 14 (Table 5-7) would involve the same procedure with the exceptions of the soil sample being ground, to aid the degree of suspension, and the agarose content being decreased from 2% to 1%. A yellow band emerged from the well and stretched to approximately 3 cm. It appeared as though the sample had been unevenly distributed to the mono-well, for in parts where it had been more sparsely distributed, bands could be seen. Those areas which resulted from the heavily loaded parts of the mono-well presented smears rather than distinct bands. As a result, it was decided to focus on sample preparation and loading in an attempt to remove smears.

Run 15 (Table 5-7) followed the same procedure as Run 14 with the exception that six individual wells were used in place of the mono-well. This was to aid in loading minimal amounts of sample to the gel in an effort to eliminate smears and encourage distinct bands. The outcome for Run 15 was very similar to that found for Run 14 (which is what would be expected, given that the only variation between the two was the use of six smaller wells rather than one large well). In the first ten minutes, yellow bands began to emerge from

the individual wells. However, as the run progressed, they increased in length rather than becoming discrete bands. Clearly, a method for band distinction is required.

On referring to methods for polyacryalmide gel electrophoresis (e.g. (Trubetskoj et al., 1991; Trubetskoj et al., 1992), it was decided that perhaps the addition of additives may improve the outcome and/or provide an insight as to the direction the investigations should take due to the following:

- Gel electrophoresis of proteins under native conditions generally works well for samples of soluble proteins.

- Problems arise for gel electrophoresis of proteins if any of the proteins present in the sample are insoluble or are likely to form multimolecular complexes or aggregates under the conditions used for the separation.

- These problems can be overcome if suitable additives are present in the gel to increase protein solubility and minimize aggregate formation.

- Trubetskoj et al. (1991) overcame the problem of smears with the addition of additives.

Run 16 (Table 5-7) followed exactly the same procedure as Run 15 (Table 5-7) except that 7 M urea was added to the gel. Urea is a commonly used gel additive designed to increase sample solubility and minimize aggregation. For Run 16:

- The current was varied throughout as it did not appear strong enough to move any bands from the well.

- It was thought that the use of a six individual wells in place of the mono-well may help with the loading process as perhaps the well was overloaded previously.

- A yellow band which stretched from approximately 1 cm to 3 cm away from the well resulted.

- There was a significant proportion of the sample remaining in the well at the end of the run.

The observation of the current being insufficient complies with Hawcroft's (1997) findings, as he indicated that the presence of urea would require greater strengths of current and voltage.

Run 17 (Table 5-7) followed the same procedure as Run 16, with the exception of a fixed current of 30 mA being used throughout. This compares to previous runs where no urea was present in the gel, as a current of approximately 10 mA was sufficient. At the end of the run, two smeared bands were present:

- One that ranged from approximately 2.5 cm to 3 cm away from the well.

- A faint one that was approximately 5 cm from the well.

The two bands were attached by a smear. The addition of urea has improved the outcome, for instead of the individual smear found for Run 16 (in the absence of urea), two smaller smeared bands were found for Run 17 (in the presence of urea), although they were not completely distinct from each other. It was thought that the addition of urea to the sample as well as to the gel would improve the outcome of Run 17 (Table 5-7).

	Run 18	Run 19
Agarose(g, %)	0.50 g, 1%	1.00 g, 2%
Sample suspended	Deciduous (ground)	Deciduous (ground)
Sample mass suspended (g)	0.5 g	0.5 g
Sample additions	7 M urea	7 M urea
Solution type / volume sample suspended in (mL)	0.045 M TBE / 2 mL	0.045 M TBE / 2 mL
Buffer (M)	0.045 TBE	0.045 TBE
Additives	7 M urea	7 M urea
Start Voltage	98 V	97 V
End Voltage	71 V	83 V
Start Current	30 mA	30 mA
End Current	30 mA	30 mA
Run Time	2 h	2 h

Table 5-8 Electrophoretic runs 18 to 19

Regarding the runs in Table 5-8:

- A mono-well was used.
- 7 M urea was added to the gel and the sample in Runs 18 and 19.
- Approximately 0.20 ml of suspended sample was added to the mono-well in Runs 18 and 19.
- The direction of movement was from anode to cathode.
- A buffer volume of 50 ml was used for the gel.

Run 18 (Table 5-8) followed exactly the same procedure as Run 17 (Table 5-7), except that:

- The sample applied to the well contained 7 M urea.
- A mono-well was used in place of the six individual wells.

At the end of Run 18 (Table 5-8) the following was noted:

- There appeared to be four bands within a smear that ranged from 2.5 cm to 5 cm away from the well.

- Perhaps the well had been overloaded, and this may be the reason for the smearing.

For Runs 1 to 18 the following has been found:

- Any bands present are either totally smeared or too faint, there never appears to be an in-between outcome.

- Only half of the gel was ever required, as past the half-way mark, things become more and more faded.

Run 19 (Table 5-8) followed the same procedure as Run 18 (Table 5-8), with the exception of the agarose content being increased from 1% to 2%. This increase in agarose content was an attempt to improve the resolution of the bands. However, at the end of Run 19 (Table 5-8), no improvement resulted. Although there were indications of bands being present, they were within one smear. The lack of any change between Runs 18 and 19 when increasing the agarose content from 1% to 2% indicates that the size of the molecules being separated are larger than the pores provided by an agarose content of 2%.

It was thought that perhaps the lack of success was due to the sample type (deciduous soil) being applied to the system, in that it does not contain the type of organic components which would separate via gel electrophoresis. As a result, the next stage in the investigation was to incorporate a number of other sample types and see if they provide a more successful outcome.

	Run 20	Run 21	Run 22	Run 23
Agarose(g, %)	1.00 g, 2%	1.00 g, 2%	1.00 g, 2%	1.00 g, 2%
Sample suspended	Garden soil (ground)	Humic acid (ground)	Agricultural soil (ground)	Humic acid (ground)
Sample mass suspended (g)	0.5 g	0.5 g	0.5 g	0.5 g
Solution type / volume sample suspended in (mL)	0.045 M TBE / 2 mL	0.045 M TBE / 1 mL	0.045 M TBE / 2 mL	0.045 M TBE / 2 mL
Buffer (M)	0.045 TBE	0.045 TBE	0.045 TBE	0.045 TBE
Additives	7 M urea	7 M urea	7 M urea	7 M urea
Start Voltage	95 V	96 V	96 V	109 V
End Voltage	72 V	71 V	78 V	92 V
Start Current	30 mA	30 mA	30 mA	30 mA
End Current	30 mA	30 mA	30 mA	30 mA
Run Time	1 h	1 h	1 h	2 h

Table 5-9 Electrophoretic runs 20 to 23

Regarding the runs in Table 5-9:

- A mono-well was used for Runs 20 to 22.
- Six individual wells were used for Run 23.
- For Runs 20 to 23, 7 M urea was added to the gel and sample.
- Approximately 0.10 mL of suspended sample was added to the mono-well in Runs 20 and 21.
- Approximately 0.20 mL of suspended sample was added to the mono-well in Run 22.
- Approximately 0.001 mL of suspended sample was added to each individual well in Run 23.
- The direction of movement was from anode to cathode.
- A buffer volume of 50 mL was used for the gel.

Run 20 (Table 5-9) followed the same procedure as Run 19 (Table 5-8), with the exception of using a garden soil sample. Run 20 was stopped after an hour as very little was coming off the well. There was a very faint smeared band ranging from approximately 0.5 cm to 1 cm away from the well, and a lot of sample left in the well. Going by the band produced, there appeared to be even less material isolated from the garden soil, than with the deciduous soil sample.

Run 21 (Table 5-9) followed the same procedure as Run 20 (Table 5-9), with the exception of using humic acid sodium salt in place of the garden soil. A the end of Run 21 (table 5-9):

- It appeared that the well may have been overloaded, as a huge smear stretching from 1 cm to 3.5 cm away from the well resulted.

- There was virtually no sample remaining in the well at the end of the run.

It was thought that in order to improve the outcome of this run, individual wells would be more appropriate, as they are more difficult to overload.

Run 22 (Table 5-9) followed the same procedure as Run 21 (Table 5-9), except that an agricultural soil was used in place of the humic acid sodium salt. At the end of Run 22:

- It appeared that the well may have been overloaded, as a huge smear stretched from the well to approximately 7 cm away from the well.

- There was virtually no sample remaining in the well at the end of the run.

Run 23 (Table 5-9) followed the same procedure as Run 21, except with six individual wells in place of one mono-well. It is more difficult to overload the six individual wells than it is the mono-well, so the aim was to minimize overloading by using six individual

wells. At the end of the run, the same outcome resulted as Run 21, in that there was a smear present with faint bands within it.

Clearly, Runs 20 to 23 were unsuccessful in showing that alternative samples are more suited to agarose gel electrophoresis than the sample taken from below the deciduous canopy. However, perhaps trying a more extensive variety of samples would yield more positive results.

A popular and effective approach to the solubilization and disaggregation of proteins to be analysed by electrophoresis is to include a detergent both in the sample and in the gel. In the gel electrophoresis of proteins, SDS (sodium dodecyl sulfate) is the most popular. SDS is known to solubilize, dissociate and denature the majority of oligomeric proteins into their constituent subunits.

It was thought that the next step towards improving the electrophoretic outcome would be the addition of a detergent, in an attempt to reduce the significant degree of smearing occurring. Sodium hydroxide is a detergent and is known to have a similar effect to SDS when used for gel electrophoresis. As a result, Run 24 (5-10) incorporated 2% NaOH into both the sample and the gel. The same general procedure was used for Run 19 (Table 5-8), with the exception of:

- 2% agarose being used in place of 1% agarose.

- The incorporation of NaOH.

	Run 24
Agarose(g, %)	0.50 g, 1%
Sample suspended	Deciduous (ground)
Sample mass suspended (g)	0.50 g
Solution type / volume sample suspended in (mL)	0.045 M TBE / 2 mL
Buffer (M)	0.045 TBE
Start Voltage	96 V
End Voltage	72 V
Start Current	30 mA
End Current	30 mA
Run Time	3 h

Table 5-10 Electrophoretic run 24

Regarding the run in Table 5-10:

- A mono-well was used.
- Approximately 0.30 mL of suspended sample was added to the mono-well The direction of movement was from anode to cathode.
- A buffer volume of 50 mL was used for the gel.

Unfortunately the outcome for Run 24 (Table 5-10) was no more successful than previous runs.

It was decided, as a final investigation, to study the outcome of four different sample types (including the deciduous soil studied previously) being applied to the agarose gel electrophoresis method developed by Higney (2003). The four soil types were:

- Deciduous soil.
- Coniferous soil.
- A river-side soil sample.

- An inter-tidal sediment sample.

The conditions for these runs are given in Table 5-11.

	Run 25	Run 26	Run 27	Run 28
Agarose(g, %)	0.50 g, 1%	0.50 g, 1%	0.50 g, 1%	0.50 g, 1%
Sample suspended	Coniferous (ground, 0.50 mm sieved)	Deciduous (ground, 0.50 mm sieved)	Inter-tidal (ground, 0.50 mm sieved)	River-side (ground, 0.50 mm sieved)
Sample mass suspended (g)	0.25 g	0.25 g	0.25 g	0.25 g
Solution type / volume sample suspended in (mL)	0.045 M TBE / 1 mL	0.045 M TBE / 1 mL	0.045 M TBE / 1 mL	0.045 M TBE / 1 mL
Buffer (M)	0.045 TBE	0.045 TBE	0.045 TBE	0.045 TBE
Start Voltage	66 V	55 V	58 V	48 V
End Voltage	65 V	54 V	57 V	46 V
Start Current	10mA	10 mA	10 mA	10 mA
End Current	10 mA	10 mA	10 mA	10 mA
Run Time	3 h	3 h	3 h	3 h

Table 5-11 Electrophoretic runs 25 to 28

Runs in Table 5-11 presented the following:

- Runs 27 and 28 showed no visible movement from the well.

- Runs 25 and 26 showed a faint yellow smear, this was more prominent for the sample taken from below the coniferous canopy (Run 25).

In addition to Runs 1 to 28, other investigative routes were considered or attempted:

- The water washings from soil and litter used in the Cu ISE studies in Chapter 4 (Section 4.3) would be a good material to use in an electrophoretic run due to the organic carbon content present. However, the amount of metal present would have been well below the detection limits of FAAS.

- It was thought that if organic material existed in the gel plates at the end of an electrophoretic run, these would perhaps be visible under UV. Gel plates were placed

under a UV light at the end of numerous electrophoretic runs, but on each occasion no sign of organic matter was found through this detection method.

- Higney (2003) sliced gels into 1 cm sections after each electrophoretic run, and analysed each of these sections (after digestion etc.) by ICP-MS analysis. This slicing procedure was not conducted in Runs 1 to 28 due to the samples being below the detection limits of FAAS.

5.5 Summary

Clearly the development of agarose gel electrophoresis for the fractionation and isolation of organic-metal complexes has been limited. However, this is probably due to the many factors which can affect the outcome of an electrophoretic run. It appears that the next logical plan of action would be to conduct an extensive study which involves varying all these factors based on work to date.

5.6 Post Method Development: Discussion and Future Work

The information and publications which were brought to my attention from discussion with Dr M.C. Graham during my viva (Graham, 2005), have enabled me to develop a better understanding of what would be required in future developments of agarose gel electrophoresis as a successful technique for the isolation of humic substances (Cavani et al., 2003; Janos, 2003; Shirshova & Osterberg, 1998; Trubetskoj et al., 1991; Trubetskoj et al., 1992; Trubetskoj et al., 1999; Vinogradoff et al., 1998).

In general, when working step by step through the procedure, the first recommendation would be to ensure that prior to application of the soil to the well, a considerable period of

soil suspension in buffer takes place. A period of at least eight hours of soil suspension (via shaker) in buffer is recommended (Graham, 2005). Through the method development reported in this chapter, it has been found that a more complete mixing can be obtained from soil which is ground prior to being suspended in buffer. On application to the well, it is recommended that all of the suspended soil is transferred. Clearly, the greater the amount of soil in the gel well, the greater the concentration of any fractions in the gel (which results in them being easier to observe). In practice, this is very difficult to attain, with the result being varying amounts of soil (solution phase and solid phase) being applied to the well/s from run to run. However, perhaps the lengthy period of soil suspension will improve the application process and enable a uniform amount of soil (solution phase and solid phase) to be transferred to the well/s for each run. The Graham et al. procedure which is mentioned throughout this Chapter and in Chapter 1 (Graham et al., 2000), used buffer Tris-borate and not TBE (as was indicated in both Chapter 1 and Chapter 5), so in future attempts of this procedure it would be recommended to use Tris-borate (Dr M. C. Graham found very positive results through the use of tris-borate). A number of very important points which have come to my attention through discussion of the development of agarose gel electrophoresis are as follows (Graham, 2005):

- The pH of all buffers used and slurries / suspensions applied to wells should be noted in all instances. The pH of any buffers used in an electrophoresis run should be as close as possible to the pH of what is applied to the well/s. It has been found that this produces a more successful outcome.

- Any movement from wells should be noted (length, shape and colour) through either / both photograph and text.

- In all instances where there is movement from the well/s, methods should be incorporated to try and analyse what has moved from the well (e.g. cutting any visible distinctions in the well and analysing their form).

- A mixture of proteins of different sizes (blue coloured) could be run alongside in a separate mini-well. This could give a good indication of how effective each systematic change had been for well-defined molecules.

By taking notes of pHs and movements from the well/s through the method development stages, much more information can be gleamed in the long term.

The publications provide additional information which could aid significantly in the method development stages (Trubetskoj et al., 1991). For example, freeze-dried soil could provide another route of investigation if air-dried soil does not prove to be successful (Vinogradoff et al., 1998), and if an investigation requires alternative buffers, a considerable list of possible buffers can be obtained (e.g. Janos (2003)). The publications also provide photographs of the successful outcomes which are useful for comparison purposes (e.g. Shirshova & Osterberg (1998) and Cavani et al. (2003)).

The viva discussion should provide any future investigations into the development of agarose gel electrophoresis (based on the results in this chapter) with invaluable information.

Chapter 6 - Conclusions

6.1 Introduction

The Pollok Park investigation is a study into the distribution and associations of heavy metals (Cr, Cu, Fe, Mn, Pb and Zn) in the soils of an inner city park. The Pollok Park site features soil under both a coniferous canopy (predominantly Corsican Pine) and a deciduous canopy (predominantly Beech). There is a clear segregation between the two vegetation types which enables a direct comparison into the contribution of canopy type to the heavy metal distribution of soil.

The investigation comprised:

- Establishing heavy metal total content and heavy metal distribution profiles (Chapter 3).

- Studying heavy metal distribution in the two soil types (modified BCR sequential extraction) and loading soil, litter and leaf samples with Cu (using a cupric ISE) (Chapter 4).

- Method development of electrophoresis (Chapter 5).

Conclusions from Chapters 3 to 5 are considered below.

6.2 Conclusions: Chapters 3 and 4

The deciduous cores have a greater average total core content of Cr, Cu, Fe and Mn than the coniferous cores (supported by t-tests) (Section 3.3). One obvious difference between the two profiles (deciduous and coniferous) is the lower pH values associated with the

coniferous soil (Section 3.6). The lower pH associated with coniferous soil (when compared to deciduous soil) is supported by numerous reports in the literature (Ahokas, 1997; Andersen et al., 2004; Binkley & Valentine, 1991; Brown & Iles, 1991; Norden, 1994; Raulund-Rasmussen & Vejre, 1995). Under acidic conditions, certain phases are susceptible to dissolution (e.g. Fe and Mn oxides) (Bendell-Young & Harvey, 1992; Bryant et al., 1997; McBride, 1994; White & Driscoll, 1987). Perhaps there is increased dissolution of acid-sensitive phases in the coniferous profile (when compared to the deciduous profile). If this is the case, increased levels of metals will be released from the coniferous soil and leached through the profile, with the resulting observed difference between the two profiles.

Bryant et al. (1997) conducted a study which provides support for pH being the cause of the differences in total metal contents between the two cores. They found that when comparing the sediment of various lochs for Mn content, the most acidified loch showed evidence of Mn loss. Further investigation into associations between the soil and the metals, will hopefully provide some insightful information as to whether pH is the cause, or whether there are other factors responsible. A possible route of investigation would be to use a sequential extraction procedure such as the modified BCR sequential extraction procedure (Rauret et al., 2000). This technique enables the assessment of how strongly metals are retained in soil, what phases they are retained within and how easily they may be released into soil solution (Section 1.6.1).

Many authors have reported on the use of sequential extraction procedures (Armienta et al., 1996; Banerjee, 2003; Davidson et al., 1994; Fernandez et al., 2004; Fuentes et al., 2004; Gibson & Farmer, 1986; Koleli, 2004; Lu et al., 2003; Miller et al., 1986; Morera et al., 2001; Oughton et al., 1992; Rauret et al., 2000; Tessier et al., 1979). Collectively, these results show that the absence or presence of certain phases is dependent on pH.

There was no significant difference in the total metal content of Pb and Zn between the coniferous and deciduous cores (supported by t-tests) (Section 3.3). It would be of benefit to conduct a BCR sequential extraction on these metals also, as a means of comparison with those metals that do show a difference (between the deciduous and the coniferous cores).

The concentration profiles established for Cr, Cu, Pb, and Zn, all showed enrichment to the soil surface. These surface values are more pronounced in the coniferous soil (when compared to the deciduous soil). On comparing the Cr, Cu, Pb, and Zn values for other forest soils with the Pollok Park values, it is clear that the Pollok Park soils have greater inputs of these metals to the surface (Andersen et al., 2002; Andersen et al., 2004; Blaser et al., 2000; Hernandez et al., 2003; Kaste et al., 2003; Shparyk & Parpan, 2004; Sipos et al., 2005; Tichy, 1996; Watmough et al., 2005; Watmough & Hutchinson, 2004; Watmough et al., 2004). Evidently, forest soils reported in the literature have low heavy metal contents, due to not having been impacted by high metal deposition. The Pollok Park site is located in the city of Glasgow, and as a result is exposed to elevated levels of heavy metals (when compared to forest soils reported in the literature (e.g. Andersen et al. (2002)). In effect, it is a unique site, as it is forested and subjected to elevated heavy metal inputs. However, on comparison with soils which are located next to direct sources of pollution, it is clear that although the Pollok Park site has been exposed to a certain level of heavy metal contamination, this level is not excessive (Armienta et al., 1996; Kabala & Singh, 2001; Karczewska, 1996; Maiz et al., 1997; Martley et al., 2004; Nieminen et al., 2002; Sterckeman et al., 2000; Wilcke et al., 1998).

On establishing the LOI profiles for the deciduous and coniferous soils, it was evident that both profiles have enhanced LOI values at the surface (Section 3.5). On comparing the average surface LOI values for the deciduous and coniferous cores, the value associated with the coniferous soil is considerably larger. Correlation graphs (LOI value vs. metal)

were established for those metals which showed enhancement at the surface: Cr, Cu, Pb and Zn (Section 3.5). In general, these showed that the greater the organic matter content (LOI value), the higher the concentrations of these metals are likely to be within the soil. These correlations are supported by the literature (Baker, 1990; Banerjee, 2003; Kiekens, 1990; McBride, 1994; McGrath & Smith, 1990; Watmough et al., 2004). For each correlation graph, an approximate molar ratio (organic carbon : metal) was established. These approximate molar ratios were found to be large, indicating that perhaps the organic molecules complexing with these metals are large. Although it should be noted that this is speculation alone and that there are many things about the complexing sspecies which are unknown, including their molecular weight and the percentage of the total organic matter that they comprise.

The concentration profiles (Section 3.4) not only provided a valuable insight into the distribution of the metals being studied (Cr, Cu, Fe, Mn, Pb and Zn), they also provided important information on sampling procedures and sample preparation.

Set 2 concentration profiles indicated that the first 2 cm of the core was litter and not soil due to the very high LOI values and the low metal concentrations associated with these soils (when compared to the metal values at 3 cm), and the Set 1 concentration profiles. This observation lead the conclusion that noting whether collection of a core includes litter or not is crucial. Without this being detailed in a procedure, comparisons with other work could result in misinterpretation of data. On observing concentration profiles reported in the literature, there are many authors which do not specify whether litter is included in collection of cores (Blaser et al., 2000; Kaste et al., 2003). However, there are some that do, and as a result, comparisons with their work will be accurate in terms of knowing that all the data is based on soil alone (Andersen et al., 2004).

Concentration profiles Sets 1 to 3 highlighted the importance of splitting cores into sections in preparation for analyses. Set 1 concentration profiles resulted from cores which were split into 1 cm sections. Due to the fine splitting, these concentration profiles provided very detailed information. Set 2 concentration profiles came from cores which were split into 1cm sections for the first 5 cm and then approximately 5 cm sections thereafter. From 5 cm, the details associated with the Section 1 concentration profiles were not present. Finally, the Set 3 concentration profiles were split into 3 sections of approximately 5 cm. The concentration profiles obtained from these cores provided only crude detail on distribution. The coarse splitting of cores is sufficient for obtaining an overview of profile trends and can be justified as a method for large scale surveys. Many authors have reported using this approach for surveys (Kabala & Singh, 2001; Madrid et al., 2002; Pietrzak & McPhail, 2004; Shotyk, 2002; Sterckeman et al., 2000). However, there are many reports in the literature of fine splitting of cores being used when detailed information on soil profiles is required (MacKenzie et al., 1997; MacKenzie et al., 1998; MacKenzie et al., 1998; Nieminen et al., 2002; Shotyk et al., 2000; Weiss et al., 2002).

As a result of the elevated levels of organic matter and metals (Cr, Cu, Pb and Zn) in the surface layer (top 5cm) of both soil types, studies into the associations and distributions of these metals were conducted. A modified BCR sequential extraction was conducted on the soils (Section 4.2). The outcome contradicted, in part, with the findings from Chapter 3, which found strong correlations between Cr, Cu, Pb, and Zn, with organic matter. The fractionation patterns do indeed show that these metals associate with the oxidisable (organic) phase. However, they are only minor associations for all except Cu (where the oxidisable fraction is the dominant one, approximately 65%). Pb was found mainly in the reducible fraction (approximately 80%), Cr was distributed between the oxidisable (45%) and residual phase (55%), and Zn existed mainly in the residual phase (approximately 80%).

The fractionation patterns for each of the metals studied complies with reports on, and related to, sequential extractions (Armienta et al., 1996; Banerjee, 2003; Chlopecka et al., 1996; Davidson et al., 1994; Hickey & Kittrick, 1984; Koleli, 2004; Lu et al., 2003; Ma & Rao, 1997; McLaren & Crawford, 1973; Miller et al., 1986; Morera et al., 2001; Ramos et al., 1994; Shuman, 1979; Singh, 1997; Sipos et al., 2005; Thomas et al., 1994; Ure et al., 1993; Wang et al., 1998; Wilcke & Kaupenjohann, 1997; Wong & Li, 2004). On comparing the Pollok Park site fractionation pattern with those reported in the literature, the conclusion can be drawn that the Pollok Park site contains levels of pollution which are moderate, but not excessive, as a result of the proportion of these metals in the non-residual phase. This is due to the numerous reports in the literature indicating that the proportion of non-residual metal is indicative of the input of metals to soil (Chlopecka et al., 1996; Wilcke & Kaupenjohann, 1997; Wilcke et al., 1998; Xu & Yang, 1995). This ties in well with the findings in Chapter 3, where the Pollok Park soils were compared with forested sites and sites influenced by direct sources of pollution etc., as the conclusion from these observations also indicated that the Pollok Park site is moderately polluted.

The associations of Cu with organic matter were studied in more detail using a cupric ISE. This time, as well as studying deciduous and coniferous soil samples (top 5 cm), samples of the litter and leaves contributing to the soils were also studied. The soil, litter and leaf samples were loaded with Cu, and the affinity each sample had for the Cu added was measured using a cupric ISE. In addition to these measurements, all samples were measured for their organic carbon content. All samples were found to contain organic carbon, with the exception of the water extracts of leaves (deciduous and coniferous), they were found to have a negligible content. All deciduous samples were found to contain a greater organic carbon value than the corresponding deciduous sample. This is probably due to deciduous samples being broken down more readily than coniferous samples (Andersen et al., 2004; Bergkvist, 1987). Many authors have found a higher DOC under conifers than under broad leaves (Raulund-Rasmussen et al., 1998; Robertson et al., 2000).

This clearly does not agree with the Pollok Park soil findings. Perhaps the method for obtaining the water extractable fraction could have resulted in this discrepancy (method: Section 2.12.1). There are reports which state that approximating the soil solution with the water extractable fraction may result in an underestimation of the true soil solution (Blaser et al., 2000). A future study could incorporate the same Cu loading method, but with a literature method for achieving the true soil solution. The true soil solution can be obtained by the centrifugation of natural moist soil samples ((Andersen et al., 2004; Davies & Davies, 1963).

On loading the deciduous samples with Cu, they clearly had a greater affinity for Cu (when compared to the corresponding coniferous samples). However, this was shown by the molar ratio vs. Cu added graphs (Figures 4-5, 4-8, 4-11 and 4-14) as being due to the greater organic carbon content associated with these samples (Section 4.3.3). Per weight of carbon, deciduous and coniferous samples of the same sample type have equal affinity for Cu. Molar ratios (carbon : metals) were derived for each data set and in each investigation these were found to be large. This supports the estimated molar ratios in Chapter 3 (Section 3.5), as they too were found to be large. The considerable size of these molar ratios points to the fact that each complexed Cu atom probably does so with a large organic molecule. The molar ratios associated with the solid samples (soil and litter) suspended in water, were considerably larger than those associated with the water extraction samples. This can probably be explained by reports stating that a significant amount of organic carbon in soil does not relate to Cu-binding carbon, and that the majority of complexed Cu is done so by DOC (Buffle et al., 1977; Sauve et al., 1997).

The results from loading the leaf water extracts with Cu were not totally conclusive, but they did indicate that these samples had virtually no affinity for Cu. If this is the case, this ties in well with the organic carbon data which showed these samples as containing virtually no organic carbon (Section 4.3.3). Further investigations into the affinity of these

samples (water extracts of leaves) for Cu are required in order to firm up conclusions. Perhaps an alternative extraction method similar to the one used to obtain the soil and litter extracts (Section 2.12) could be utilized, and then the resulting samples could be run through the same Cu loading procedure.

The Cu loading investigations have enabled a picture (in both the deciduous and coniferous systems) to be created of Cu's affinity for soil, litter and leaves. The leaves seem to have no real affinity for Cu and instead of being retained by the leaves, Cu is washed through to the litter and soil which do have an ability to retain Cu. The following order (staring with greatest affinity) was established: soil > litter (quickly saturated) > leaves (virtually no complexation). These findings tie in well with reports stating that Cu accumulates in soil roots and is not readily transferred to shoots (McBride, 2001; Minnich, 1987). Future investigations could incorporate studies on other metals in soil, litter and leaves. Very different results could be found for the affinity of these metals. For example, Zn tends to accumulate in foliage (Watmough et al., 2005) and may show enhanced affinity for leaves.

In addition to using analytical methods such as ISEs, metal binding models could be used, as such combinations have been shown to maximise the information obtained (Carballeira et al., 2000; Logan et al., 1997).

6.3 Conclusions: Chapter 5

Agarose gel electrophoresis was applied to deciduous and coniferous organic (top 5 cm) soil samples as a means of isolating the humic substances in these soils, in order to obtain a more accurate picture of the organic compounds involved in retaining metals in forest soils. Unfortunately, the application of this procedure (based on the work by Graham et al. (2000) and Higney (2003)) to these soils was unsuccessful. An extensive investigative

study (based on work to date) which encompasses the many factors affecting the outcome of an electrophoretic run is required.

Appendix

This section contains the original data for Tables 3-1 to 3-6 and Figures 3-1 to 3-24 in Chapters 3 (Cr, Cu, Fe, Mn, Pb and Zn in the 8 cores studied) (all results are mean values from three replicates). For each metal there are four tables. The first two tables give the raw data for the total metal contents (including weight of each section and metal concentration for each section). The total metal contents for each core were obtained by taking each core to a depth of 16 cm. However, some of the cores were greater than 16 cm in depth, and the additional metal concentration data for these cores can be found in the final two tables.

For each metal studied, the following applies:

- Cores 1 and 2 in the deciduous table, and Cores 1 and 2 in the coniferous table were obtained in Collection 3 (Section 2.3.3)

- Cores 3 to 7 in the deciduous table, and Cores 3 to 7 in the coniferous table were obtained in Collection 4 (Section 2.3.4)

- Core 8 in the deciduous table and Core 8 in the coniferous table were obtained in Collection 1 (Section 2.3.1)

Cr, Cu, Fe, Mn, Pb and Zn:

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets
Cr	Core 1	0-1	77.9	0.79	10.8	60.1 (44.2)
	(Deciduous)	1-2	144	0.81	10.8	42.1 (2.89)
		2-3	168	0.83	10.8	26.6 (0.80)
		3-4	135	0.85	10.8	30.7 (4.95)
		4-5	212	0.85	10.8	25.5 (0.76)
		5-10	687	0.87	10.8	25.9 (3.45)
		10-16	587	0.87	10.8	22.3 (2.28)
	Core 2	0-1	147	0.82	10.2	43.9 (2.89)
	(Deciduous)	1-2	120	0.83	10.2	33.1 (4.20)
		2-3	98.9	0.81	10.2	33.8 (3.69)
		3-4	222	0.85	10.2	19.9 (1.95)
		4-5	160	0.85	10.2	21.4 (3.81)
		5-10	519	0.87	10.2	22.0 (1.90)
		10-16	401	0.88	10.2	24.4 (7.54)
	Core 3	0-4	171	0.29	7.85	33.0 (3.13)
	(Deciduous)	4-10	389	0.44	7.85	66.7 (0.48)
		10-16	853	0.77	7.85	47.2 (15.7)
	Core 4	0-5	369	0.60	7.85	56.1 (2.84)
	(Deciduous)	5-8	258	0.73	7.85	44.2 (1.97)
		8-16	748	0.81	7.85	38.1 (2.38)
	Core 5	0-4	347	0.50	7.85	57.2 (3.02)
	(Deciduous)	4-9	581	0.75	7.85	46.6 (3.72)
		9-16	1135	0.80	7.85	37.6 (1.66)
	Core 6	0-5	309	0.43	7.85	46.1 (2.23)
	(Deciduous)	5-10	446	0.68	7.85	65.8 (7.70)
		10-16	843	0.74	7.85	67.9 (4.17)
	Core 7	0-5	422	0.65	7.85	44.2 (6.69)
	(Deciduous)	5-10	526	0.71	7.85	31.3 (3.74)
		10-16	967	0.76	7.85	31.2 (0.90)
	Core 8	0-1	121	0.48	10.3	71.4 (22.9)
	(Deciduous)	1-2	81	0.61	10.3	49.1 (3.74)
		2-3	106	0.72	10.3	78.1 (33.7)
		3-4	122	0.71	10.3	47.9 (3.14)
		4-5	159	0.78	10.3	26.4 (5.06)
		5-6	159	0.79	10.3	31.3 (10.2)
		6-7	213	0.79	10.3	24.9 (1.47)
		7-8	243	0.78	10.3	23.3 (70.9)
		8-9	180	0.77	10.3	23.3 (3.90)
		9-10	171	0.77	10.3	21.8 (1.82)
		10-11	189	0.78	10.3	17.2 (16.0)
		11-12	210	0.78	10.3	20.4 (0.14)
		12-13	255	0.79	10.3	17.5 (2.11)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration ($mg\ kg^{-1}$) (3 replicates) (Standard deviation in brackets
Cr	Core 8	13-14	202	0.80	10.3	16.2 (1.88)
	(Deciduous)	14-15	213	0.81	10.3	18.2 (3.34)
		15-16	262	0.81	10.3	21.4 (0.52)

Table A Original data: Total core content of Cr in the deciduous cores (Table 3-1 & Figure 3-1) and Cr distribution profiles (Figures 3-7 to 3-9) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration ($mg\ kg^{-1}$) (3 replicates) (Standard deviation in brackets)
Cr	Core 1	0-1	182	0.24	14.4	10.2 (1.62)
	(Coniferous)	1-2	156	0.32	14.4	42.8 (2.42)
		2-3	273	0.31	14.4	86.1 (53.2)
		3-4	240	0.44	14.4	37.8 (1.31)
		4-5	260	0.54	14.4	44.7 (37.8)
		5-6	406	0.63	14.4	19.4 (1.54)
		6-11	810	0.67	14.4	15.0 (1.23)
		11-16	994	0.70	14.4	14.7 (1.40)
	Core 2	0-1	72.5	0.44	16.0	1.31 (1.13)
	(Coniferous)	1-2	70.9	0.49	16.0	34.1 (9.09)
		2-3	82.1	0.63	16.0	35.0 (2.68)
		3-4	111	0.71	16.0	30.0 (2.25)
		4-5	107	0.73	16.0	37.9 (21.7)
		5-10	588	0.72	16.0	34.7 (24.6)
		10-16	484	0.81	16.0	15.0 (0.05)
	Core 3	0-5	220	0.26	7.85	33.4 (1.64)
	(Coniferous)	5-10	288	0.53	7.85	52.8 (4.58)
		10-16	643	0.72	7.85	36.7 (1.07)
	Core 4	0-5	232	0.49	7.85	101 (77.0)
	(Coniferous)	5-10	403	0.73	7.85	41.7 (3.16)
		10-16	724	0.78	7.85	30.0 (1.38)
	Core 5	0-5	321	0.24	7.85	36.1 (7.62)
	(Coniferous)	5-10	528	0.57	7.85	56.8 (1.44)
		10-16	770	0.73	7.85	35.5 (3.47)
	Core 6	0-6	636	0.52	7.85	36.2 (3.27)
	(Coniferous)	6-10	405	0.67	7.85	24.4 (2.73)
		10-16	937	0.73	7.85	24.1 (1.95)
	Core 7	0-5	285	0.43	7.85	84.4 (26.5)
	(Coniferous)	5-10	458	0.67	7.85	38.4 (1.34)

Table continued on next page

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Cr	Core 7 (Coniferous)	10-16	781	0.73	7.85	32.3 (0.40)
		0-1	108	0.28	10.3	92.3 (3.26)
		1-2	121	0.32	10.3	68.3 (6.29)
		2-3	91	0.44	10.3	68.4 (4.41)
		3-4	144	0.51	10.3	40.7 (1.87)
		4-5	132	0.58	10.3	21.5 (1.97)
		5-6	130	0.62	10.3	21.2 (2.09)
		6-7	125	0.62	10.3	20.1 (0.99)
		7-8	129	0.65	10.3	24.8 (7.23)
		8-9	128	0.66	10.3	15.5 (1.73)
		9-10	147	0.65	10.3	15.2 (1.39)
		10-11	111	0.68	10.3	16.6 (1.37)
		11-12	181	0.72	10.3	29.8 (8.80)
		12-13	146	0.73	10.3	26.3 (0.98)
		13-14	138	0.80	10.3	28.8 (5.13)
		14-15	149	0.76	10.3	22.7 (2.92)
		15-16	162	0.77	10.3	22.1 (0.95)

Table B Original data: Total core content of Cr in the coniferous cores (Table 3-1 & Figure 3-1) and Cr distribution profiles (Figures 3-7 to 3-9) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (mg kg^{-1}) (3 replicates)	Standard deviation
Cr	Core 1	16-20	28.4	2.94
	(Deciduous)	20-25	23.5	0.01
	Core 2	16-20	16.3	2.28
	(Deciduous)	20-23	18.4	1.01
	Core 8	16-17	18.9	0.77
	(Deciduous)	17-18	18.2	1.24
		18-19	22.1	1.28
		19-20	21.5	1.06
		20-21	21.6	0.42
		21-22	24.2	1.84
		22-23	21.2	0.91

Table C Original data: Cr distribution profiles (deciduous cores) (Figures 3-7 to 3-9) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (mg kg^{-1}) (3 replicates)	Standard deviation
Cr	Core 1	16-21	15.1	1.70
	(Coniferous)	21-24	15.2	1.60

Table continued on next page

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (mg kg^{-1}) (3 replicates)	Standard deviation
Cr	Core 2	16-20	15.2	0.46
	(Coniferous)	20-25	14.2	3.60
		25-30	12.4	1.71
	Core 8	16-17	25.4	1.68
	(Coniferous)	17-18	27.0	2.84
		18-19	22.4	0.51
		19-20	25.6	0.51
		20-21	26.9	2.28
		21-22	25.3	1.28
		22-23	24.9	3.66
		23-24	18.6	1.69
		24-25	22.6	4.80

Table D Original data (coniferous cores): Cr distribution profiles (Figures 3-7 to 3-9) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets
Cu	Core 1	0-1	77.9	0.79	10.8	37.4 (2.17)
	(Deciduous)	1-2	144	0.81	10.8	46.5 (1.21)
		2-3	168	0.83	10.8	30.1 (4.29)
		3-4	135	0.85	10.8	25.1 (1.67)
		4-5	212	0.85	10.8	23.8 (0.65)
		5-10	687	0.87	10.8	22.0 (1.90)
		10-16	587	0.87	10.8	17.5 (1.59)
	Core 2	0-1	147	0.82	10.2	53.7 (5.26)
	(Deciduous)	1-2	120	0.83	10.2	49.3 (8.07)
		2-3	98.9	0.81	10.2	47.5 (0.51)
		3-4	222	0.85	10.2	36.3 (4.19)
		4-5	160	0.85	10.2	39.8 (3.23)
		5-10	519	0.87	10.2	40.5 (2.80)
		10-16	401	0.88	10.2	21.7 (1.96)
	Core 3	0-4	171	0.29	7.85	45.3 (3.91)
	(Deciduous)	4-10	389	0.44	7.85	85.7 (6.64)
		10-16	853	0.77	7.85	31.1 (9.20)
	Core 4	0-5	369	0.60	7.85	70.4 (5.68)
	(Deciduous)	5-8	258	0.73	7.85	40.1 (3.63)
		8-16	748	0.81	7.85	22.1 (2.06)
	Core 5	0-4	347	0.50	7.85	51.4 (2.92)
	(Deciduous)	4-9	581	0.75	7.85	31.3 (7.91)
		9-16	1135	0.80	7.85	19.3 (6.75)
	Core 6 (Deciduous)	0-5	309	0.43	7.85	74.2 (3.88)

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Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets
Cu	Core 6	5-10	446	0.68	7.85	35.2 (0.29)
	(Deciduous)	10-16	843	0.74	7.85	18.1 (1.39)
	Core 7	0-5	422	0.65	7.85	41.2 (2.04)
	(Deciduous)	5-10	526	0.71	7.85	22.6 (1.92)
		10-16	967	0.76	7.85	15.0 (1.29)
	Core 8	0-1	121	0.48	10.3	60.9 (8.08)
	(Deciduous)	1-2	81	0.61	10.3	48.9 (1.13)
		2-3	106	0.72	10.3	48.5 (6.82)
		3-4	122	0.71	10.3	43.7 (4.48)
		4-5	159	0.78	10.3	24.5 (2.71)
		5-6	159	0.79	10.3	26.4 (3.67)
		6-7	213	0.79	10.3	28.4 (4.00)
		7-8	243	0.78	10.3	21.0 (0.53)
		8-9	180	0.77	10.3	16.5 (3.15)
		9-10	171	0.77	10.3	17.1 (1.09)
		10-11	189	0.78	10.3	13.2 (1.87)
		11-12	210	0.78	10.3	22.5 (2.08)
		12-13	255	0.79	10.3	17.3 (0.20)
		13-14	202	0.80	10.3	17.6 (0.68)
		14-15	213	0.81	10.3	18.7 (2.34)
		15-16	262	0.81	10.3	18.5 (2.20)

Table E Original data: Total core content of Cu in the deciduous cores (Table 3-2 & Figure 3-2) and Cu distribution profiles (Figures 3-10 to 3-12) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Cu	Core 1	0-1	182	0.24	14.4	43.2 (3.67)
	(Coniferous)	1-2	156	0.32	14.4	73.3 (7.25)
		2-3	273	0.31	14.4	89.1 (3.11)
		3-4	240	0.44	14.4	79.4 (4.22)
		4-5	260	0.54	14.4	42.9 (1.97)
		5-6	406	0.63	14.4	32.0 (1.59)
		6-11	810	0.67	14.4	22.7 (2.15)
		11-16	994	0.70	14.4	13.7 (0.41)
	Core 2	0-1	72.5	0.44	16.0	29.3 (1.91)
	(Coniferous)	1-2	70.9	0.49	16.0	63.8 (3.31)

Table continued on next page

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Cu	Core 3	2-3	82.1	0.63	16.0	64.7 (2.75)
	(Coniferous)	3-4	111	0.71	16.0	54.2 (5.13)
		4-5	107	0.73	16.0	47.2 (1.08)
		5-10	588	0.72	16.0	35.1 (1.14)
		10-16	484	0.81	16.0	20.2 (1.16)
	Core 3	0-5	220	0.26	7.85	47.5 (2.30)
	(Coniferous)	5-10	288	0.53	7.85	70.0 (7.26)
		10-16	643	0.72	7.85	33.5 (2.17)
	Core 4	0-5	232	0.49	7.85	57.9 (3.04)
	(Coniferous)	5-10	403	0.73	7.85	37.3 (1.19)
		10-16	724	0.78	7.85	19.7 (1.41)
	Core 5	0-5	321	0.24	7.85	41.2 (1.79)
	(Coniferous)	5-10	528	0.57	7.85	55.9 (1.18)
		10-16	770	0.73	7.85	32.4 (1.60)
	Core 6	0-6	636	0.52	7.85	31.6 (3.18)
	(Coniferous)	6-10	405	0.67	7.85	10.3 (0.65)
		10-16	937	0.73	7.85	7.40 (1.11)
	Core 7	0-5	285	0.43	7.85	78.4 (4.08)
	(Coniferous)	5-10	458	0.67	7.85	32.3 (0.96)
		10-16	781	0.73	7.85	22.0 (1.97)
	Core 8	0-1	108	0.28	10.3	128 (2.30)
	(Coniferous)	1-2	121	0.32	10.3	108 (2.95)
		2-3	91	0.44	10.3	106 (1.65)
		3-4	144	0.51	10.3	70.0 (3.89)
		4-5	132	0.58	10.3	40.1 (1.24)
		5-6	130	0.62	10.3	36.0 (1.47)
		6-7	125	0.62	10.3	33.3 (0.99)
		7-8	129	0.65	10.3	30.2 (2.12)
		8-9	128	0.66	10.3	24.8 (1.53)
		9-10	147	0.65	10.3	23.7 (2.74)
		10-11	111	0.68	10.3	18.6 (0.65)
		11-12	181	0.72	10.3	17.4 (1.54)
		12-13	146	0.73	10.3	16.7 (1.47)
		13-14	138	0.80	10.3	12.7 (2.09)
		14-15	149	0.76	10.3	10.3 (1.64)
		15-16	162	0.77	10.3	13.9 (2.69)

Table F Original data: Total core content of Cu in the coniferous cores (Table 3-2 & Figure 3-2) and Cu distribution profiles (Figures 3-10 to 3-12) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (mg kg^{-1}) (3 replicates)	Standard deviation
Cu	Core 1	16-20	16.6	1.15
	(Deciduous)	20-25	14.2	0.79
	Core 2 (Deciduous)	16-20	16.3	1.06

Table continued from previous page				
Cu	Core 2 (Deciduous)	20-23	19.2	2.28
	Core 8	16-17	16.2	1.90
	(Deciduous)	17-18	12.3	0.41
		18-19	11.2	1.71
		19-20	13.3	1.87
		20-21	15.4	1.46
		21-22	13.9	2.09
		22-23	17.8	0.35

Table G Original data: Cr distribution profiles (deciduous cores) (Figures 3-10 to 3-12) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (mg kg^{-1}) (3 replicates)	Standard deviation
Cu	Core 1	16-21	14.5	4.72
	(Coniferous)	21-24	12.8	2.43
	Core 2	16-20	17.9	2.09
	(Coniferous)	20-25	13.3	0.14
		25-30	14.9	1.69
	Core 8	16-17	11.7	1.83
	(Coniferous)	17-18	16.0	2.75
		18-19	20.0	4.84
		19-20	14.0	3.48
		20-21	13.7	1.95
		21-22	17.4	1.16
		22-23	15.1	2.33
		23-24	14.3	0.71
		24-25	14.5	0.58

Table H Original data (coniferous cores): Cr distribution profiles (Figures 3-10 to 3-12 (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (g kg^{-1}) (3 replicates) (Standard deviation in brackets ($x10^3$))
Fe	Core 1	0-1	77.9	0.79	10.8	34.0 (7.04)
	(Deciduous)	1-2	144	0.81	10.8	32.8 (1.74)
		2-3	168	0.83	10.8	38.0 (7.02)
		3-4	135	0.85	10.8	44.8 (1.38)
		4-5	212	0.85	10.8	45.8 (2.81)
		5-10	687	0.87	10.8	55.8 (9.11)
		10-16	587	0.87	10.8	46.8 (5.08)
	Core 2	0-1	147	0.82	10.2	39.8 (2.48)
	(Deciduous)	1-2	120	0.83	10.2	42.0 (4.89)
		2-3	98.9	0.81	10.2	39.7 (3.89)
		3-4	222	0.85	10.2	36.4 (3.77)
		4-5	160	0.85	10.2	40.0 (7.88)

Table continued on next page

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration ($g\ kg^{-1}$) (3 replicates) (Standard deviation in brackets ($x10^3$))
Fe	Core 2	5-10	519	0.87	10.2	43.0 (6.96)
	(Deciduous)	10-16	401	0.88	10.2	40.8 (2.89)
	Core 3	0-4	171	0.29	7.85	11.0 (1.17)
	(Deciduous)	4-10	389	0.44	7.85	37.9 (3.61)
		10-16	853	0.77	7.85	39.2 (2.20)
	Core 4	0-5	369	0.60	7.85	36.4 (2.88)
	(Deciduous)	5-8	258	0.73	7.85	35.1 (2.53)
		8-16	748	0.81	7.85	38.6 (3.30)
	Core 5	0-4	347	0.50	7.85	31.0 (0.36)
	(Deciduous)	4-9	581	0.75	7.85	36.9 (3.21)
		9-16	1135	0.80	7.85	37.4 (4.88)
	Core 6	0-5	309	0.43	7.85	36.2 (7.84)
	(Deciduous)	5-10	446	0.68	7.85	37.7 (2.15)
		10-16	843	0.74	7.85	49.1 (4.57)
	Core 7	0-5	422	0.65	7.85	32.2 (2.28)
	(Deciduous)	5-10	526	0.71	7.85	37.3 (2.41)
		10-16	967	0.76	7.85	48.4 (2.87)
	Core 8	0-1	121	0.48	10.3	32.1 (1.71)
	(Deciduous)	1-2	81	0.61	10.3	32.4 (0.23)
		2-3	106	0.72	10.3	36.8 (2.98)
		3-4	122	0.71	10.3	47.0 (2.37)
		4-5	159	0.78	10.3	42.8 (8.00)
		5-6	159	0.79	10.3	43.9 (0.32)
		6-7	213	0.79	10.3	45.3 (5.41)
		7-8	243	0.78	10.3	43.8 (2.38)
		8-9	180	0.77	10.3	39.8 (3.41)
		9-10	171	0.77	10.3	43.4 (2.96)
		10-11	189	0.78	10.3	49.2 (1.17)
		11-12	210	0.78	10.3	48.2 (3.87)
		12-13	255	0.79	10.3	47.0 (5.98)
		13-14	202	0.80	10.3	46.5 (6.72)
		14-15	213	0.81	10.3	52.2 (4.08)
		15-16	262	0.81	10.3	40.8 (1.08)

Table I Original data: Total core content of Fe in the deciduous cores (Table 3-3 & Figure 3-3) and Fe distribution profiles (Figures 3-13 to 3-15) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration ($g\ kg^{-1}$) (3 replicates) (Standard deviation in brackets ($x10^3$))
Fe	Core 1	0-1	182	0.24	14.4	6.07 (1.13)
	(Coniferous)	1-2	156	0.32	14.4	24.0 (3.70)
		2-3	273	0.31	14.4	26.4 (1.69)
		3-4	240	0.44	14.4	28.2 (2.23)
		4-5	260	0.54	14.4	30.2 (2.84)
		5-6	406	0.63	14.4	39.0 (5.48)
		6-11	810	0.67	14.4	36.0 (5.46)
		11-16	994	0.70	14.4	30.7 (3.94)
	Core 2	0-1	72.5	0.44	16.0	2.20 (0.99)
	(Coniferous)	1-2	70.9	0.49	16.0	18.5 (2.47)
		2-3	82.1	0.63	16.0	28.3 (3.72)
		3-4	111	0.71	16.0	30.7 (1.00)
		4-5	107	0.73	16.0	30.9 (4.67)
		5-10	588	0.72	16.0	31.2 (3.10)
		10-16	484	0.81	16.0	36.3 (7.88)
	Core 3	0-5	220	0.26	7.85	13.2 (2.31)
	(Coniferous)	5-10	288	0.53	7.85	42.9 (9.07)
		10-16	643	0.72	7.85	43.9 (3.18)
	Core 4	0-5	232	0.49	7.85	25.3 (3.24)
	(Coniferous)	5-10	403	0.73	7.85	42.6 (4.70)
		10-16	724	0.78	7.85	40.1 (5.30)
	Core 5	0-5	321	0.24	7.85	15.6 (1.56)
	(Coniferous)	5-10	528	0.57	7.85	39.1 (5.84)
		10-16	770	0.73	7.85	53.9 (8.41)
	Core 6	0-6	636	0.52	7.85	24.8 (3.34)
	(Coniferous)	6-10	405	0.67	7.85	29.0 (7.53)
		10-16	937	0.73	7.85	32.0 (4.09)
	Core 7	0-5	285	0.43	7.85	24.2 (0.85)
	(Coniferous)	5-10	458	0.67	7.85	35.2 (3.99)
		10-16	781	0.73	7.85	36.6 (2.56)
	Core 8	0-1	108	0.28	10.3	27.8 (1.48)
	(Coniferous)	1-2	121	0.32	10.3	56.4 (12.5)
		2-3	91	0.44	10.3	36.9 (4.78)
		3-4	144	0.51	10.3	36.8 (5.17)
		4-5	132	0.58	10.3	33.6 (4.63)
		5-6	130	0.62	10.3	36.0 (3.14)
		6-7	125	0.62	10.3	44.8 (4.96)
		7-8	129	0.65	10.3	41.7 (3.48)
		8-9	128	0.66	10.3	40.4 (11.9)
		9-10	147	0.65	10.3	42.9 (5.98)
		10-11	111	0.68	10.3	39.1 (4.29)
		11-12	181	0.72	10.3	39.6 (2.94)
		12-13	146	0.73	10.3	52.3 (2.88)
		13-14	138	0.80	10.3	42.8 (4.53)

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Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration ($g\ kg^{-1}$) (3 replicates) (Standard deviation in brackets ($x10^3$))
Fe		14-15	149	0.76	10.3	62.3 (6.77)
		15-16	162	0.77	10.3	54.8 (6.01)

Table J Original data: Total core content of Fe in the coniferous cores (Table 3-3 & Figure 3-3) and Fe distribution profiles (Figures 3-13 to 3-15) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration ($g\ kg^{-1}$) (3 replicates) ($x10^3$)	Standard deviation ($x10^3$)
Fe	Core 1	16-20	39.6	0.16
	(Deciduous)	20-25	45.3	2.53
	Core 2	16-20	39.6	3.00
	(Deciduous)	20-23	40.3	2.11
	Core 8	16-17	40.1	6.62
	(Deciduous)	17-18	35.9	3.42
		18-19	39.7	2.37
		19-20	38.0	3.46
		20-21	35.7	2.46
		21-22	39.0	2.38
		22-23	46.8	1.25

Table K Original data: Fe distribution profiles (deciduous cores) (Figures 3-13 to 3-15) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration ($g\ kg^{-1}$) (3 replicates) ($x10^3$)	Standard deviation ($x10^3$)
Fe	Core 1	16-21	33.2	3.07
	(Coniferous)	21-24	35.5	2.58
	Core 2	16-20	38.0	4.62
	(Coniferous)	20-25	34.1	2.45
		25-30	37.4	0.88
	Core 8	16-17	55.3	4.60
	(Coniferous)	17-18	60.8	1.73
		18-19	50.4	3.04
		19-20	58.6	4.22
		20-21	54.9	11.3
		21-22	54.9	5.75
		22-23	56.8	6.57

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Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (g kg^{-1}) (3 replicates) ($x10^3$)	Standard deviation ($x10^3$)
Fe	Core 8	23-24	46.5	1.59
	(Coniferous)	24-25	58.6	10.5

Table L Original data (coniferous cores): Fe distribution profiles (Figures 3-13 to 3-15) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Mn	Core 1	0-1	77.9	0.79	10.8	563 (138)
	(Deciduous)	1-2	144	0.81	10.8	451 (36.2)
		2-3	168	0.83	10.8	511 (64.4)
		3-4	135	0.85	10.8	637 (18.4)
		4-5	212	0.85	10.8	778 (9.81)
		5-10	687	0.87	10.8	1009 (109)
		10-16	587	0.87	10.8	1013 (49.6)
	Core 2	0-1	147	0.82	10.2	472 (9.22)
	(Deciduous)	1-2	120	0.83	10.2	533 (53.8)
		2-3	98.9	0.81	10.2	527 (53.0)
		3-4	222	0.85	10.2	547 (66.2)
		4-5	160	0.85	10.2	597 (42.2)
		5-10	519	0.87	10.2	683 (23.0)
		10-16	401	0.88	10.2	997 (90.0)
	Core 3	0-4	171	0.29	7.85	519 (41.3)
	(Deciduous)	4-10	389	0.44	7.85	337 (19.9)
		10-16	853	0.77	7.85	679 (21.6)
	Core 4	0-5	369	0.60	7.85	313 (15.2)
	(Deciduous)	5-8	258	0.73	7.85	496 (48.3)
		8-16	748	0.81	7.85	758 (70.0)
	Core 5	0-4	347	0.50	7.85	312 (6.32)
	(Deciduous)	4-9	581	0.75	7.85	545 (36.4)
		9-16	1135	0.80	7.85	752 (54.2)
	Core 6	0-5	309	0.43	7.85	343 (82.1)
	(Deciduous)	5-10	446	0.68	7.85	415 (32.6)
		10-16	843	0.74	7.85	736 (40.2)
	Core 7	0-5	422	0.65	7.85	299 (18.7)
	(Deciduous)	5-10	526	0.71	7.85	449 (127)
		10-16	967	0.76	7.85	760 (93.0)
	Core 8	0-1	121	0.48	10.3	499 (132)
	(Deciduous)	1-2	81	0.61	10.3	496 (49.6)
		2-3	106	0.72	10.3	616 (62.4)
		3-4	122	0.71	10.3	807 (51.0)

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Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Mn	Core 8	4-5	159	0.78	10.3	733 (149)
	(Deciduous)	5-6	159	0.79	10.3	786 (39.3)
		6-7	213	0.79	10.3	770 (71.5)
		7-8	243	0.78	10.3	766 (52.3)
		8-9	180	0.77	10.3	722 (89.8)
		9-10	171	0.77	10.3	854 (86.2)
		10-11	189	0.78	10.3	879 (67.1)
		11-12	210	0.78	10.3	1164 (152)
		12-13	255	0.79	10.3	1097 (51.7)
		13-14	202	0.80	10.3	1286 (105)
		14-15	213	0.81	10.3	1220 (38.4)
		15-16	262	0.81	10.3	1184 (79.6)

Table M Original data: Total core content of Mn in the deciduous cores (Table 3-4 & Figure 3-4) and Mn distribution profiles (Figures 3-16 to 3-18) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Mn	Core 1	0-1	182	0.24	14.4	459 (5.66)
	(Coniferous)	1-2	156	0.32	14.4	382 (63.3)
		2-3	273	0.31	14.4	301 (3.52)
		3-4	240	0.44	14.4	270 (12.7)
		4-5	260	0.54	14.4	293 (18.5)
		5-6	406	0.63	14.4	376 (19.1)
		6-11	810	0.67	14.4	416 (104)
		11-16	994	0.70	14.4	337 (48.8)
	Core 2	0-1	72.5	0.44	16.0	337 (15.6)
	(Coniferous)	1-2	70.9	0.49	16.0	231 (6.06)
		2-3	82.1	0.63	16.0	252 (36.6)
		3-4	111	0.71	16.0	272 (20.5)
		4-5	107	0.73	16.0	283 (39.9)
		5-10	588	0.72	16.0	278 (23.6)
		10-16	484	0.81	16.0	379 (99.1)
	Core 3	0-5	220	0.26	7.85	502 (16.0)
	(Coniferous)	5-10	288	0.53	7.85	346 (25.2)
		10-16	643	0.72	7.85	440 (25.0)
	Core 4	0-5	232	0.49	7.85	357 (14.1)
	(Coniferous)	5-10	403	0.73	7.85	413 (45.4)

Table continued on next page

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Mn	Core 4 (Coniferous)	10-16	724	0.78	7.85	563 (63.7)
	Core 5	0-5	321	0.24	7.85	458 (5.67)
	(Coniferous)	5-10	528	0.57	7.85	315 (13.1)
		10-16	770	0.73	7.85	609 (97.9)
	Core 6	0-6	636	0.52	7.85	181 (26.8)
	(Coniferous)	6-10	405	0.67	7.85	159 (14.8)
		10-16	937	0.73	7.85	245 (13.9)
	Core 7	0-5	285	0.43	7.85	270 (14.1)
	(Coniferous)	5-10	458	0.67	7.85	375 (58.1)
		10-16	781	0.73	7.85	476 (7.68)
	Core 8	0-1	108	0.28	10.3	213 (5.42)
	(Coniferous)	1-2	121	0.32	10.3	266 (53.2)
		2-3	91	0.44	10.3	232 (5.37)
		3-4	144	0.51	10.3	226 (6.65)
		4-5	132	0.58	10.3	213 (17.5)
		5-6	130	0.62	10.3	246 (26.6)
		6-7	125	0.62	10.3	236 (21.8)
		7-8	129	0.65	10.3	274 (18.6)
		8-9	128	0.66	10.3	237 (67.2)
		9-10	147	0.65	10.3	259 (49.6)
		10-11	111	0.68	10.3	210 (6.54)
		11-12	181	0.72	10.3	193 (21.4)
		12-13	146	0.73	10.3	239 (14.8)
		13-14	138	0.80	10.3	232 (15.1)
		14-15	149	0.76	10.3	337 (37.1)
		15-16	162	0.77	10.3	378 (30.7)

Table N Total core content of Mn in the coniferous cores (Table 3-4 & Figure 3-4) and Mn distribution profiles (Figures 3-16 to 3-18) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (mg kg^{-1}) (3 replicates)	Standard deviation
Mn	Core 1	16-20	1141	36.7
	(Deciduous)	20-25	1195	18.5
	Core 2	16-20	1107	92.1
	(Deciduous)	20-23	1254	44.1
	Core 8	16-17	1124	72.4
	(Deciduous)	17-18	1178	255
		18-19	1189	120
		19-20	1354	255
		20-21	1084	51.1

Table continued on next page

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (mg kg^{-1}) (3 replicates)	Standard deviation
Mn	Core 8	21-22	1237	81.8
	(Deciduous)	22-23	1331	106

Table O Original data: Mn distribution profiles (deciduous cores) (Figures 3-16 to 3-18) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (mg kg^{-1}) (3 replicates)	Standard deviation
Mn	Core 1	16-21	526	63.0
	(Coniferous)	21-24	626	18.1
	Core 2	16-20	411	11.4
	(Coniferous)	20-25	437	47.3
		25-30	453	15.5
	Core 8	16-17	499	36.9
	(Coniferous)	17-18	837	35.4
		18-19	799	181
		19-20	727	111
		20-21	778	95.7
		21-22	660	43.5
		22-23	685	27.0
		23-24	497	15.3
		24-25	662	50.4

Table P Original data (coniferous cores): Mn distribution profiles (Figures 3-16 to 3-18) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Pb	Core 1	0-1	77.9	0.79	10.8	186 (18.4)
	(Deciduous)	1-2	144	0.81	10.8	246 (7.10)
		2-3	168	0.83	10.8	165 (11.9)
		3-4	135	0.85	10.8	118 (3.60)
		4-5	212	0.85	10.8	103 (2.12)
		5-10	687	0.87	10.8	62.3 (4.00)
		10-16	587	0.87	10.8	16.9 (2.27)
	Core 2	0-1	147	0.82	10.2	209 (26.1)
	(Deciduous)	1-2	120	0.83	10.2	186 (7.72)
		2-3	98.9	0.81	10.2	175 (6.27)
		3-4	222	0.85	10.2	125 (17.3)
		4-5	160	0.85	10.2	101 (2.79)
		5-10	519	0.87	10.2	99.2 (4.08)
		10-16	401	0.88	10.2	63.7 (6.01)
	Core 3	0-4	171	0.29	7.85	287 (21.1)
	(Deciduous)	4-10	389	0.44	7.85	485 (24.2)

Table continued from previous page

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Pb	Core 3 (Deciduous)	10-16	853	0.77	7.85	116 (5.20)
	Core 4	0-5	369	0.60	7.85	400 (22.3)
	(Deciduous)	5-8	258	0.73	7.85	164 (4.72)
		8-16	748	0.81	7.85	62.3 (2.86)
	Core 5	0-4	347	0.50	7.85	219 (19.1)
	(Deciduous)	4-9	581	0.75	7.85	71.6 (4.19)
		9-16	1135	0.80	7.85	16.6 (2.45)
	Core 6	0-5	309	0.43	7.85	378 (23.5)
	(Deciduous)	5-10	446	0.68	7.85	176 (4.82)
		10-16	843	0.74	7.85	56.1 (3.00)
	Core 7	0-5	422	0.65	7.85	204 (15.5)
	(Deciduous)	5-10	526	0.71	7.85	87.5 (6.93)
		10-16	967	0.76	7.85	32.7 (1.89)
	Core 8	0-1	121	0.48	10.3	365 (30.8)
	(Deciduous)	1-2	81	0.61	10.3	289 (30.9)
		2-3	106	0.72	10.3	261 (33.2)
		3-4	122	0.71	10.3	234 (9.67)
		4-5	159	0.78	10.3	123 (14.8)
		5-6	159	0.79	10.3	89.8 (5.16)
		6-7	213	0.79	10.3	78.3 (3.00)
		7-8	243	0.78	10.3	72.4 (0.44)
		8-9	180	0.77	10.3	86.6 (28.4)
		9-10	171	0.77	10.3	63.7 (4.54)
		10-11	189	0.78	10.3	64.4 (5.39)
		11-12	210	0.78	10.3	55.1 (3.57)
		12-13	255	0.79	10.3	54.1 (7.83)
		13-14	202	0.80	10.3	39.4 (3.69)
		14-15	213	0.81	10.3	42.7 (2.94)
		15-16	262	0.81	10.3	36.7 (5.91)

Table Q Original data: Total core content of Pb in the deciduous cores (Table 3-5 & Figure 3-5) and Pb distribution profiles (Figures 3-19 to 3-21) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Pb	Core 1	0-1	182	0.24	14.4	177 (10.3)
	(Coniferous)	1-2	156	0.32	14.4	367 (10.5)
		2-3	273	0.31	14.4	426 (2.05)
		3-4	240	0.44	14.4	335 (13.2)

Table continued on next page

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Pb	Core 1	4-5	260	0.54	14.4	206 (7.40)
	(Coniferous)	5-6	406	0.63	14.4	150 (10.4)
		6-11	810	0.67	14.4	111 (12.8)
		11-16	994	0.70	14.4	46.7 (2.65)
	Core 2	0-1	72.5	0.44	16.0	56.1 (0.36)
	(Coniferous)	1-2	70.9	0.49	16.0	277 (9.38)
		2-3	82.1	0.63	16.0	261 (11.7)
		3-4	111	0.71	16.0	218 (10.7)
		4-5	107	0.73	16.0	184 (5.54)
		5-10	588	0.72	16.0	140 (15.1)
		10-16	484	0.81	16.0	94.6 (7.89)
	Core 3	0-5	220	0.26	7.85	244 (20.3)
	(Coniferous)	5-10	288	0.53	7.85	371 (35.4)
		10-16	643	0.72	7.85	160 (8.08)
	Core 4	0-5	232	0.49	7.85	274 (5.28)
	(Coniferous)	5-10	403	0.73	7.85	193 (3.44)
		10-16	724	0.78	7.85	85.2 (4.80)
	Core 5	0-5	321	0.24	7.85	291 (21.9)
	(Coniferous)	5-10	528	0.57	7.85	324 (19.4)
		10-16	770	0.73	7.85	107 (12.6)
	Core 6	0-6	636	0.52	7.85	182 (21.1)
	(Coniferous)	6-10	405	0.67	7.85	68.2 (4.82)
		10-16	937	0.73	7.85	48.5 (7.91)
	Core 7	0-5	285	0.43	7.85	396 (12.7)
	(Coniferous)	5-10	458	0.67	7.85	184 (19.6)
		10-16	781	0.73	7.85	90.0 (4.68)
	Core 8	0-1	108	0.28	10.3	684 (5.09)
	(Coniferous)	1-2	121	0.32	10.3	547 (13.7)
		2-3	91	0.44	10.3	543 (10.4)
		3-4	144	0.51	10.3	371 (6.63)
		4-5	132	0.58	10.3	228 (2.49)
		5-6	130	0.62	10.3	205 (11.9)
		6-7	125	0.62	10.3	194 (4.03)
		7-8	129	0.65	10.3	174 (7.03)
		8-9	128	0.66	10.3	127 (3.66)
		9-10	147	0.65	10.3	119 (3.57)
		10-11	111	0.68	10.3	94.6 (1.80)
		11-12	181	0.72	10.3	68.1 (10.0)
		12-13	146	0.73	10.3	61.4 (5.31)
		13-14	138	0.80	10.3	36.2 (3.76)
		14-15	149	0.76	10.3	34.7 (1.00)
		15-16	162	0.77	10.3	34.6 (2.83)

Table R Total core content of Pb in the coniferous cores (Table 3-5 & Figure 3-5) and Pb distribution profiles (Figures 3-19 to 3-21) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (mg kg^{-1}) (3 replicates)	Standard deviation
Pb	Core 1	16-20	13.3	3.57
	(Deciduous)	20-25	19.5	0.22
	Core 2	16-20	35.4	4.81
	(Deciduous)	20-23	38.4	5.70
	Core 8	16-17	34.2	10.2
	(Deciduous)	17-18	44.6	16.6
		18-19	41.3	2.17
		19-20	44.6	3.16
		20-21	32.7	1.84
		21-22	45.3	13.8
		22-23	39.0	4.81

Table S Original data: Pb distribution profiles (deciduous cores) (Figures 3-19 to 3-21) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (mg kg^{-1}) (3 replicates)	Standard deviation
Pb	Core 1	16-21	36.0	1.45
	(Coniferous)	21-24	23.8	2.34
	Core 2	16-20	77.4	1.38
	(Coniferous)	20-25	58.0	4.28
		25-30	50.1	2.20
	Core 8	16-17	36.5	1.72
	(Coniferous)	17-18	31.2	3.93
		18-19	40.5	1.95
		19-20	52.8	5.52
		20-21	55.4	14.1
		21-22	45.7	0.89
		22-23	50.1	13.8
		23-24	34.4	1.24
		24-25	32.7	4.26

Table T Original data (coniferous cores): Pb distribution profiles (Figures 3-19 to 3-21) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Zn	Core 1	0-1	77.9	0.79	10.8	94.4 (7.49)
	(Deciduous)	1-2	144	0.81	10.8	95.8 (0.31)
		2-3	168	0.83	10.8	68.3 (7.21)
		3-4	135	0.85	10.8	68.7 (2.86)
		4-5	212	0.85	10.8	61.6 (7.09)
		5-10	687	0.87	10.8	66.0 (9.42)
		10-16	587	0.87	10.8	71.9 (14.0)
	Core 2	0-1	147	0.82	10.2	135 (4.88)
	(Deciduous)	1-2	120	0.83	10.2	104 (5.81)

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Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Zn	Core 2	2-3	98.9	0.81	10.2	127 (3.41)
	(Deciduous)	3-4	222	0.85	10.2	82.5 (6.95)
		4-5	160	0.85	10.2	95.2 (15.3)
		5-10	519	0.87	10.2	84.4 (8.50)
		10-16	401	0.88	10.2	62.9 (6.00)
	Core 3	0-4	171	0.29	7.85	113 (10.1)
	(Deciduous)	4-10	389	0.44	7.85	164 (14.8)
		10-16	853	0.77	7.85	49.8 (0.83)
	Core 4	0-5	369	0.60	7.85	138 (4.92)
	(Deciduous)	5-8	258	0.73	7.85	57.4 (3.59)
		8-16	748	0.81	7.85	49.7 (3.62)
	Core 5	0-4	347	0.50	7.85	83.2 (3.91)
	(Deciduous)	4-9	581	0.75	7.85	55.1 (3.25)
		9-16	1135	0.80	7.85	53.5 (3.93)
	Core 6	0-5	309	0.43	7.85	133 (8.19)
	(Deciduous)	5-10	446	0.68	7.85	58.7 (7.70)
		10-16	843	0.74	7.85	60.8 (3.47)
	Core 7	0-5	422	0.65	7.85	60.3 (5.26)
	(Deciduous)	5-10	526	0.71	7.85	50.1 (2.42)
		10-16	967	0.76	7.85	50.8 (3.71)
	Core 8	0-1	121	0.48	10.3	178 (15.1)
	(Deciduous)	1-2	81	0.61	10.3	131 (2.83)
		2-3	106	0.72	10.3	114 (9.26)
		3-4	122	0.71	10.3	93.0 (5.51)
		4-5	159	0.78	10.3	78.3 (3.14)
		5-6	159	0.79	10.3	84.7 (8.77)
		6-7	213	0.79	10.3	84.6 (2.88)
		7-8	243	0.78	10.3	79.6 (3.01)
		8-9	180	0.77	10.3	62.4 (5.66)
		9-10	171	0.77	10.3	64.6 (6.40)
		10-11	189	0.78	10.3	64.9 (4.19)
		11-12	210	0.78	10.3	71.9 (8.41)
		12-13	255	0.79	10.3	67.6 (11.8)
		13-14	202	0.80	10.3	68.0 (5.87)
		14-15	213	0.81	10.3	73.1 (5.06)
		15-16	262	0.81	10.3	72.6 (3.81)

Table U Original data: Total core content of Zn in the deciduous cores (Table 3-6 & Figure 3-6) and Zn distribution profiles (Figures 3-22 to 3-24) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Section Weight (g)	Conversion Factor (air dry – oven dry)	Sample Area (m^2) ($x10^3$)	Mean Concentration (mg kg^{-1}) (3 replicates) (Standard deviation in brackets)
Zn	Core 1	0-1	182	0.24	14.4	185 (22.2)
	(Coniferous)	1-2	156	0.32	14.4	241 (13.5)
		2-3	273	0.31	14.4	240 (46.6)
		3-4	240	0.44	14.4	206 (12.3)
		4-5	260	0.54	14.4	124 (6.75)
		5-6	406	0.63	14.4	73.0 (15.5)
		6-11	810	0.67	14.4	72.2 (6.80)
		11-16	994	0.70	14.4	59.4 (13.9)
	Core 2	0-1	72.5	0.44	16.0	56.4 (8.10)
	(Coniferous)	1-2	70.9	0.49	16.0	116 (7.21)
		2-3	82.1	0.63	16.0	108 (7.16)
		3-4	111	0.71	16.0	85.9 (14.6)
		4-5	107	0.73	16.0	61.6 (3.35)
		5-10	588	0.72	16.0	41.0 (1.86)
		10-16	484	0.81	16.0	32.8 (1.66)
	Core 3	0-5	220	0.26	7.85	114 (3.01)
	(Coniferous)	5-10	288	0.53	7.85	130 (17.1)
		10-16	643	0.72	7.85	61.0 (2.11)
	Core 4	0-5	232	0.49	7.85	136 (5.01)
	(Coniferous)	5-10	403	0.73	7.85	73.9 (4.47)
		10-16	724	0.78	7.85	54.3 (4.18)
	Core 5	0-5	321	0.24	7.85	157 (23.1)
	(Coniferous)	5-10	528	0.57	7.85	150 (16.1)
		10-16	770	0.73	7.85	65.4 (5.08)
	Core 6	0-6	636	0.52	7.85	55.3 (8.34)
	(Coniferous)	6-10	405	0.67	7.85	20.2 (2.24)
		10-16	937	0.73	7.85	24.5 (1.86)
	Core 7	0-5	285	0.43	7.85	137 (1.48)
	(Coniferous)	5-10	458	0.67	7.85	45.2 (1.18)
		10-16	781	0.73	7.85	37.2 (2.33)
	Core 8	0-1	108	0.28	10.3	578 (43.8)
	(Coniferous)	1-2	121	0.32	10.3	744 (135)
		2-3	91	0.44	10.3	607 (2.39)
		3-4	144	0.51	10.3	432 (77.4)
		4-5	132	0.58	10.3	201 (11.6)
		5-6	130	0.62	10.3	178 (25.2)
		6-7	125	0.62	10.3	162 (14.2)
		7-8	129	0.65	10.3	129 (5.67)
		8-9	128	0.66	10.3	113 (7.19)
		9-10	147	0.65	10.3	79.5 (5.29)
		10-11	111	0.68	10.3	104 (1.91)
		11-12	181	0.72	10.3	101 (8.96)
		12-13	146	0.73	10.3	80.1 (4.06)
		13-14	138	0.80	10.3	90.8 (27.3)
		14-15	149	0.76	10.3	88.4 (10.4)

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Zn	Core 8 (Deciduous)	15-16	162	0.77	10.3	95.7 (13.7)

Table V Original data: Total core content of Zn in the coniferous cores (Table 3-6 & Figure 3-6) and Zn distribution profiles (Figures 3-22 to 3-24) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (mg kg^{-1}) (3 replicates)	Standard deviation
Zn	Core 1	16-20	86.1	18.6
	(Deciduous)	20-25	80.9	23.4
	Core 2	16-20	68.6	6.73
	(Deciduous)	20-23	80.0	14.6
	Core 8	16-17	65.0	4.23
	(Deciduous)	17-18	60.4	4.31
		18-19	61.0	7.82
		19-20	56.9	2.45
		20-21	51.8	1.67
		21-22	59.8	5.48
		22-23	59.2	2.48

Table W Original data: Zn distribution profiles (deciduous cores) (Figures 3-22 to 3-24) (each sample was run in triplicate)

Metal	Core (Deciduous / Coniferous)	Sections (cm)	Mean Concentration (mg kg^{-1}) (3 replicates)	Standard deviation
Zn	Core 1	16-21	65.6	15.1
	(Coniferous)	21-24	101	9.32
	Core 2	16-20	33.6	4.87
	(Coniferous)	20-25	33.2	2.28
		25-30	33.6	1.52
	Core 8	16-17	79.1	9.10
	(Coniferous)	17-18	78.7	2.47
		18-19	115	24.7
		19-20	155	29.0
		20-21	154	14.0
		21-22	133	14.1
		22-23	175	36.8
		23-24	144	10.4
		24-25	262	21.0

Table X Original data (coniferous cores): Zn distribution profiles (Figures 3-22 to 3-24) (each sample was run in triplicate)

References

Adamo, P., Giordano, S., Vingiani, S., Cobianchi, R. C. & Violante, P. (2003). Trace element accumulation by moss and lichen exposed in bags in the city of Naples (Italy). *Environmental Pollution*, **122**, 91-103.

Ahokas, H. (1997). Acidification of forest top soils in 60 years to the southwest of Helsinki. *Forest Ecology and Management*, **94**, 187-193.

Ahumada, I., Mendoza, J. & Ascar, L. (1999). Sequential extraction of heavy metals in soils irrigated with wastewater. *Communications in Soil Science and Plant Analysis*, **30**, 1507-1519.

Alloway, B. J. (1990). *Heavy metals in soils*. Blackie, Glasgow.

Alloway, B. J. & Morgan, H. (1986). The behaviour and availability of Cd, Ni and Pb in polluted soils. In *Contaminated soil*, eds. J. W. Assink & W. J. van den Brink, Martinus Nijhoff Publishers. Dordrecht, pp. 101-113.

Andersen, M. K., Raulund-Rasmussen, K., Hansen, H. C. B. & Strobel, B. W. (2002). Distribution and fractionation of heavy metals in pairs of arable and afforested soils in Denmark. *European Journal of Soil Science*, **53**, 491-502.

Andersen, M. K., Raulund-Rasmussen, K., Strobel, B. W. & Hansen, H. C. B. (2004). The effects of tree species and site on the solubility of Cd, Cu, Ni, Pb and Zn in soils. *Water Air and Soil Pollution*, **154**, 357-370.

Andersen, M. K., Refsgaard, A., Raulund-Rasmussen, K., Strobel, B. W. & Hansen, H. C. B. (2002). Content, distribution, and solubility of cadmium in arable and forest soils. *Soil Science Society of America Journal*, **66**, 1829-1835.

Angelone, M., Corrado, T. & Dowgiallo, G. (1995). Lead and cadmium distribution in urban soil and plants in the city of Rome: a preliminary study. *Third International Conference on the Biogeochemistry of Trace Elements*. 23-24.

Appleby, P. G., Shotyk, W. & Fankhauser, A. (1997). Lead-210 age dating of three peat cores in the Jura Mountains, Switzerland. *Water Air and Soil Pollution*, **100**, 223-231.

Armienta, M. A., Rodriguez, R., Cencieros, N., Juarez, F. & Cruz, O. (1996). Distribution, origin and fate of chromium in soils in Guanajuato, Mexico. *Environmental Pollution*, **91**, 391-397.

Arunachalam, J., Emons, H., Krasnodebska, B. & Mohl, C. (1996). Sequential extraction studies on homogenized forest soil samples. *Science of The Total Environment*, **181**, 147-159.

Bacon, J. R., Hewitt, I. J. & Cooper, P. (2005). Reproducibility of the BCR sequential extraction procedure in a long-term study of the association of heavy metals with soil components in an upland catchment in Scotland. *Science of The Total Environment*, **337**, 191-205.

Bacon, J. R., Jones, K. C., McGrath, S. P. & Johnston, A. E. (1996). Isotopic character of lead deposited from the atmosphere at a grassland site in the United Kingdom since 1860. *Environmental Science & Technology*, **30**, 2511-2518.

Bak, J., Jensen, J., Larsen, M. M., Pritzl, G. & Scott-Fordsmand, J. (1997). A heavy metal monitoring-programme in Denmark. *Science of The Total Environment*, **207**, 179-186.

Baker, A. J. M. (1981). Accumulators and excluders - strategies in the response of plants to heavy metals. *Journal of Plant Nutrition*, **3**, 643.

Baker, A. J. M. (1987). Metal tolerance. *New Phytologist*, **106**, 93-111.

Baker, A. J. M. & Brooks, R. R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements - a review of their distribution, ecology and phytochemistry. *Biorecovery*, **1**, 81-126.

Baker, A. J. M., Reeves, R. D. & Hajar, A. S. M. (1994). Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens J- and C-Presl* (Brassicaceae). *New Phytologist*, **127**, 61-68.

Baker, A. J. M. & Walker, P. L. (1990). Ecophysiology of metal uptake by tolerant plants. In *Heavy metal tolerance in plants*, ed. A. J. Shaw, CRC Press. Boca Raton, pp. 155-178.

Baker, D. E. (1990). Copper. In *Heavy metals in soils*, ed. B. J. Alloway, Blackie. Glasgow, pp. 151-176.

Banerjee, A. D. K. (2003). Heavy metal levels and solid phase speciation in street dusts of Delhi, India. *Enviromental Pollution*, **123**, 95-105.

Bendell-Young, L. I. & Harvey, H. H. (1992). Geochemistry of Mn and Fe in lake sediments in relation to lake acidity. *Limnology Oceanography*, **37**, 603-613.

Berg, B., Ekbohm, G., Soderstrom, B. & Staaf, H. (1991). Reduction of decomposition rates of Scots pine needle litter due to heavy-metal pollution. *Water Air and Soil Pollution*, **59**, 165-177.

Bergkvist, B. (1987). Leaching of metals from forest soils as influenced by tree species and management. *Forest Ecology and Management*, **22**, 29-56.

Bergkvist, B., Folkeson, L. & Berggren, D. (1989). Fluxes of Cu, Zn, Pb, Cd, Cr, and Ni in temperate forest ecosystems - a literature review. *Water Air and Soil Pollution*, **47**, 217-286.

Bhat, G. A., Saar, R. A., Smart, R. B. & Weber, J. H. (1981). Titration of soil-derived fulvic acid by copper(II) and measurement of free copper(II) by anodic stripping voltammetry and copper(II) selective electrode. *Analytical Chemistry*, **53**, 2275-2280.

Bindler, R., Brannvall, M. L., Renberg, I., Emteryd, O. & Grip, H. (1999). Natural lead concentration in pristine boreal forest soils and past pollution trends: a reference for critical load models. *Envionmental Science and Technology*, **33**, 3362-3367.

Binkley, D. & Valentine, D. (1991). Fifty-year biogeochemical effects of green ash, white pine, and Norway spruce in a replicated experiment. *Forest Ecology and Management*, **40**, 13-25.

Blaser, P., Zimmermann, S., Luster, J. & Shotyk, W. (2000). Critical examination of trace element enrichments and depletions in soils: As, Cr, Cu, Ni, Pb, and Zn in Swiss forest soils. *Science of The Total Environment*, **249**, 257-280.

Bodek, I. (1988). *Environmental inorganic chemistry : properties, processes, and estimation methods*. Pergamon Press, New York.

Brannvall, M. L., Bindler, R., Emteryd, O., Nilsson, M. & Renberg, I. (1997). Stable isotope and concentration records of atmospheric lead pollution in peat and lake sediments in Sweden. *Water Air and Soil Pollution*, **100**, 243-252.

Brannvall, M. L., Bindler, R., Renberg, I., Emteryd, O., Bartnicki, J. & Billstrom, K. (1999). The medieval metal industry was the cradle of modern large-scale atmospheric lead pollution in northern Europe. *Envionmental Science and Technology*, **33**, 4391-4395.

Bresnahan, W. T., Grant, C. L. & Weber, J. H. (1978). Stability constants for the complexation of Cu(II) ions with water and soil fulvic acids measured by ion selective electrodes. *Analytical Chemistry*, **50**, 1675-1679.

Brown, A. H. F. & Iles, M. A. (1991). Water chemistry profiles under our tree species at Gisburn, NW England. *Forestry*, **64**, 169-187.

Bryant, C. L., Farmer, J. G., MacKenzie, A. B., Bailey-Watts, A. E. & Kirika, A. (1997). Manganese behaviour in the sediments of diverse Scottish freshwater lochs. *Limnology Oceanography*, **42**, 918-929.

Buffle, J., Greter, F.-L. & Haerdl, W. (1977). Measurement of complexation properties of humic and fulvic acids in natural waters with lead and copper ion-selective electrodes. *Analytical Chemistry*, **49**, 216-221.

Cabaniss, S. E. & Shuman, M. S. (1986). Combined ion selective electrode and fluorescence quenching detection for copper-dissolved organic matter. *Analytical Chemistry*, **58**, 398-401.

Cal-Prieto, M. J., Carlosena, A., Andrade, J. M., Martinez, M. L., Muniategui, S., Lopez-Mahia, P. & Prada, D. (2001). Antimony as a tracer of the anthropogenic influence on soils and estuarine sediments. *Water Air and Soil Pollution*, **129**, 333-348.

Cao, J., Lam, K. C., Dawson, R. W., Liu, W. X. & Tao, S. (2004). The effect of pH, ion strength and reactant content on the complexation of Cu(II) by various natural organic ligands from water and soil in Hong Kong. *Chemosphere*, **54**, 507-514.

Carballeira, A. & Fernandez, J. A. (2002). Bioconcentration of metals in the moss Scleropodium purum in the area surrounding a power plant - A geotopographical predictive model for mercury. *Chemosphere*, **47**, 1041-1048.

Carballeira, J. L., Antelo, J. M. & Arce, F. (2000). Analysis of the Cu(II)-soil fulvic acid complexation by anodic stripping voltammetry using an electrostatic model. *Environmental Science & Technology*, **34**, 4969-4973.

Carreras, H. A. & Pignata, M. L. (2002). Biomonitoring of heavy metals and air quality in Cordoba City, Argentina, using transplanted lichens. *Environmental Pollution*, **117**, 77-87.

Cavani, L., Ciavatta, C., Trubetskaya, O. E., Reznikova, O. I., Afanaseva, G. V. & Trubetskoj, O. A. (2003). Capillary zone electrophoresis of soil humic acid fractions obtained by coupling size-exclusion chromatography and polyacrylamide gel electrophoresis. *JOurnal of Chromatography A*, **983**, 263-270.

Chaignon, V., Sanchez-Neira, I., Herrmann, P., Jaillard, B. & Hinsinger, P. (2003). Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine-growing area. *Environmental Pollution*, **123**, 229-238.

Chappelka, A. H., Kush, J. S., Runion, G. B., Meier, S. & Kelley, W. D. (1991). Effects of soil-applied lead on seedling growth and ectomycorrhizal colonization of lobolly pine. *Environmental Pollution*, **72**, 307-316.

Chen, T. B., Wong, J. W. C., Zhou, H. Y. & Wong, M. H. (1997). Assessment of trace metal distribution and contamination in surface soils of Hong Kong. *Environmental Pollution*, **96**, 61-68.

Chen, Y. & Schnitzer, M. (1976). Viscosity measurements on soil humic substances. *Soil Science Society of America Journal*, **40**, 866-872.

Chlopecka, A., Bacon, J. R., Wilson, M. J. & Kay, J. (1996). Forms of cadmium, lead, and zinc in contaminated soils from southwest Poland. *Environmental Quality*, **25**, 69-79.

Chrambach, A., Dunn, M. J. & Radola, B. J. (1992). *Advances in electrophoresis*. VCH Publishers, New York.

Christensen, T. H. (1989). Cadmium soil sorption at low concentrations .8. correlation with soil parameters. *Water Air and Soil Pollution*, **44**, 71-82.

Christensen, T. H. & Huang, P. M. (1999). Solid phase cadmium and the reaction of aqueous cadmium with soil surfaces. In *Cadmium in soils and plants*, eds. M. J. McLaughlin & B. R. Singh, Kluwer Academic Publishers. Dordrecht, the Netherlands, pp. 65-96.

Clymo, R. S., Oldfield, F., Appleby, P. G., Pearson, G. W., Ratnesar, P. & Richardson, N. (1990). The record of atmospheric deposition on a rainwater-dependent peatland. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **327**, 331-338.

Coughtrey, P. J., Jones, C. H., Martin, P. J. & Shales, S. W. (1979). Litter accumulation in woodlands contaminated with Pb, Zn, Cd and Cu. *Oecologia (Berlin)*, **39**, 51-60.

Cresser, M. S., Killham, K. & Edwards, A. (1993). *Soil chemistry and its applications*. Cambridge University Press, Cambridge ; New York.

Culbard, E. B., Thornton, I., Watt, J., Wheatley, M., Moorcroft, S. & Thompson, M. (1988). Metal contamination in British urban dusts and soils. *Journal of Environmental Quality*, **17**, 226-234.

Cumming, J. R., Weinstein, L. H. (1990). Aluminium-mycorrhizal interactions in the physiology of pitch pine seedlings. *Plant and Soil*, **125**, 7-18.

Czarnowska, K. (1980). Akumulacja metali ciezicck w glebach, oslinach i niektoych zwieretach na erenie. *Warzawy Rocz Glebozn*, **31**, 77-115.

Dahmani-Muller, H., van Oort, F., Gelie, B. & Balabane, M. (2000). Strategies of heavy metal uptake by three plant species growing near a metal smelter. *Environmental Pollution*, **109**, 231-238.

Damman, A. W. H. (1978). Distribution and movement of elements in ombrotrophic peat deposits. *Oikos*, **30**, 480-495.

Davidson, C. (2005). The distinction between indicative reference materials and certified reference materials (communication via e-mail).

Davidson, C. M., Duncan, A. L., Littlejohn, D., Ure, A. M. & Garden, L. M. (1998). A critical evaluation of the three-stage BCR sequential extraction procedure to assess the potential mobility and toxicity of heavy metals in industrially-contaminated land. *Analytica Chimica Acta*, **363**, 45-55.

Davidson, C. M., Thomas, R. P., McVey, S. E., Perala, R., Littlejohn, D. & Ure, A. M. (1994). Evaluation of a sequential extraction procedure for the speciation of heavy-metals in sediments. *Analytica Chimica Acta*, **291**, 277-286.

Davies, B. E. (1990). Lead. In *Heavy metals in soils*, ed. B. J. Alloway, Blackie. Glasgow, pp. 177-196.

Davies, B. E. & Davies, R. I. (1963). A simple centrifugation method for obtaining small samples of soil solution. *Nature*, **198**, 216-217.

De Miguel, E., Jimenez de Grado, M., Llamas, J. F., Martin-Dorado, A. & Mazadiego, L. F. (1998). The overlooked contribution of compost application to the trace element

load in the urban soil of Madrid (Spain). *Science of The Total Environment*, **215**, 113-122.

De Nicola, F., Maisto, G. & Alfani, A. (2003). Assessment of nutritional status and trace element contamination of holm oak woodlands through analyses of leaves and surrounding soils. *Science of The Total Environment*, **311**, 191-203.

De Vos, C. H. R., Schat, H., De Waal, M. A. M., Voojs, R. & Ernst, W. H. O. (1991). Increased resistance to copper-induced damage of root cell plasmalemma in copper tolerant *Silene cucubalus*. *Physiologia Plantarum*, **82**, 523-528.

Delves, H. T. & Campbell, M. J. (1993). Identification and apportionment of sources of lead in human tissue. *Environmental Geochemistry and Health*, **15**, 75-84.

Dickson, J. H., Jardine, G. & Jarvis, M. C. (2000). Topography, geology and soils. In *The changing flora of Glasgow*, eds. J. H. Dickson, P. Macpherson & K. J. Watson, Edinburgh University Press. Edinburgh.

Dixon, R. K. & Buschena, C. A. (1988). Response of ectomycorrhizal *Pinus banksiana and Picea glauca* to heavy metals in soil. *Plant and Soil*, **105**, 265-271.

Dunn, M. J. (1986). *Gel electrophoresis of proteins*. Wright, Bristol.

Eades, L. J., Farmer, J. G., MacKenzie, A. B., Kirika, A. & Bailey-Watts, A. E. (2002). Stable lead isotopic characterisation of the historical record of environmental lead contamination in dated freshwater lake sediment cores from northern and central Scotland. *The Science of The Total Environment*, **292**, 55-67.

Eddington, D. N. & Robbins, J. A. (1976). Records of lead deposition in Lake Michigan sediments since 1800. *Envionmental Science and Technology*, **10**, 266-274.

Egli, M., Fitze, P. & Oswald, M. (1999). Changes in heavy metal contents in an acidic forest soil affected by depletion of soil organic matter within the time span 1969-93. *Environmental Pollution*, **105**, 367-379.

Elliot, H. A., Liberati, M. R. & Huang, C. P. (1986). Competitive adsorption of heavy metals by soils. *Journal of Environmental Quality*, **15**, 214-219.

Erel, Y. (1998). Mechanisms and velocities of anthropogenic Pb migration in Mediterranean soils. *Environmental Research*, **78**, 112-117.

Erel, Y. & Patterson, C. C. (1994). Leakage of industrial lead into the hydrocycle. *Geochimica et Cosmochimica Acta*, **58**, 3289-3296.

Erel, Y., Veron, A. & Halicz, L. (1997). Tracing the transport of anthropogenic lead in the atmosphere and in soils using isotopic ratios. *Geochimica et Cosmochimica Acta*, **61**, 4495-4505.

Espi, E., Boutron, C. F., Hong, S., Pourchet, M., Ferrari, C., Shotyk, W. & Charlet, L. (1997). Changing concentrations of Cu, Zn, Cd and Pb in a high altitude peat bog from Bolivia during the past three centuries. *Water Air and Soil Pollution*, **100**, 289-296.

Farmer, J. G., Eades, L. J., Atkins, H. & Chamberlain, D. F. (2002). Historical trends in the lead isotopic composition of archival Sphagnum mosses from Scotland (1838-2000). *Environmental Science & Technology*, **36**, 152-157.

Farmer, J. G., Eades, L. J. & Graham, M. C. (1999). The lead content and isotopic composition of British coals and their implications for past and present releases of lead to the UK environment. *Environmental Geochemistry and Health*, **21**, 267-272.

Farmer, J. G., Eades, L. J., Mackenzie, A. B., Kirika, A. & BaileyWatts, T. E. (1996). Stable lead isotope record of lead pollution in Loch Lomond sediments since 1630 AD. *Environmental Science & Technology*, **30**, 3080-3083.

Farmer, J. G., Mackenzie, A. B., Sugden, C. L., Edgar, P. J. & Eades, L. J. (1997). A comparison of the historical lead pollution records in peat and freshwater lake sediments from central Scotland. *Water Air and Soil Pollution*, **100**, 253-270.

Farmer, J. G., Thomas, R. P., Graham, M. C., Geelhoed, J. S., Lumsdon, D. G. & Paterson, E. (2002). Chromium speciation and fractionation in ground and surface waters in

the vicinity of chromite ore processing residue disposal sites. *Journal of Environmental Monitoring*, **4**, 235-243.

Fendorf, S. E. (1995). Surface reactions of chromium in soils and waters. *Geoderma*, **67**, 55-71.

Fernandez, E., Jimenez, R., Lallena, A. M. & Aguilar, J. (2004). Evaluation of the BCR sequential extraction procedure applied for two unpolluted Spanish soils. *Environmental Pollution*, **131**, 355-364.

Fernandez, J. A., Ederra, A., Nunez, E., Martinez-Abaigar, J., Infante, M., Heras, P., Elias, M. J., Mazimpaka, V. & Carballeira, A. (2002). Biomonitoring of metal deposition in northern Spain by moss analysis. *Science of The Total Environment*, **300**, 115-127.

Figueira, R., Sergio, C. & Sousa, A. J. (2002). Distribution of trace metals in moss biomonitors and assessment of contamination sources in Portugal. *Environmental Pollution*, **118**, 153-163.

Filella, M. & Town, R. M. (2000). Determination of metal ion binding parameters for humic substances. Part 1. Application of a simple calculation method for extraction of meaningful parameters from reverse pulse polarograms. *Journal of Electroanalytical Chemistry*, **485**, 21-33.

Flaig, W., Beutelspacher, H. & Reitz, E. (1975). Chemical composition and physical properties of humic substances. In *Soil components: Volume 1. Organic components*, ed. J. E. Gieseking, Springer. New York, pp. 1-211.

Forstner, U. (1993). Metal speciation - general concepts and applications. *International Journal of Environmental Analytical Chemistry*, **51**, 5-23.

Friedland, A. J., Craig, B. W., Miller, E. K., Herrick, G. T., Siccama, T. G. & Johnson, A. H. (1992). Decreasing lead levels in the forest floor of the northeastern USA. *Ambio*, **21**, 400-403.

Friedland, A. J. & Johnson, A. H. (1985). Lead distribution and fluxes in a high-elevation forest in northern Vermont. *Journal of Environmental Quality*, **14**, 332-336.

Fuentes, A., Llorens, M., Saez, J., Soler, A., Aguilar, M. I., Ortuno, J. F. & Meseguer, V. F. (2004). Simple and sequntial extractions of heavy metals from different sewage sludges. *Chemosphere*, **54**, 1039-1047.

Galsomies, L., Letrouit, M. A., Deschamps, C., Savanne, D. & Avnaim, M. (1999). Atmospheric metal deposition in France: initial results on moss calibration from the 1996 biomonitoring. *Science of the Total Environment*, **232**, 39-47.

Genoni, P., Parco, V. & Santagostino, A. (2000). Metal biomonitoring with mosses in the surroundings of an oil-fired power plant in Italy. *Chemosphere*, **41**, 729-733.

Gibson, M. J. & Farmer, J. G. (1984). Chemical partitioning of trace metal contaminants in urban street dirt. *Science of The Total Environment*, **33**, 49-57.

Gibson, M. J. & Farmer, J. G. (1986). Multistep sequential chemical-extraction of heavy-metals from urban soils. *Environmental Pollution Series B-Chemical and Physical*, **11**, 117-135.

Glooschenko, W. A. (1986). Monitoring the atmospheric deposition of metals by use of bog vegetation and peat profiles. In *Toxic metals in the atmosphere*, eds. J. O. Nriagu & D. I. Davidson, John Wiley and Sons. New York, pp. 508-533.

Goldschmidt, V. M. (1954). In *Geochemistry*, ed. A. Muir, Clarendon Press. Oxford, pp. 730.

Gorres, M. & Frenzel, B. (1997). Ash and metal concentrations in peat bogs as indicators of anthropogenic activity. *Water Air and Soil Pollution*, **100**, 355-365.

Gough, H. C. (1981). Organic Matter in Soil. In *The Analysis of Agricultural materials*, Her Majesty's Stationary Office. London, pp. 148-150.

Graham, M. C. (2003). Method development discussion: agarose gel electrophoresis for the isolation of humic substances (communication via e-mail).

Graham, M. C. (2005). Viva discussion: agarose gel electrophoresis as a successful method for the isolation of humic substances.

Graham, M. C., MacKenzie, A. B., Scott, R. D. & Livens, F. R. (1995). Geochemical associations of actinide elements in marine influenced soils and sediments. *Heavy Metals in the Environment*, Hamburg. CEP Consultants Ltd. 73-76.

Graham, M. C., Vinogradoff, S. I., Abbott, A. & Farmer, J. G. (2000). Application of gel electrophoretic techniques to the investigation of actinide-humic interactions in soils. *Radiochimica Acta*, **88**, 775-778.

Grayston, S. J., Vaughan, D. & Jones, D. (1997). Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *Applied Soil Ecology*, **5**, 29-56.

Griepink, B. (1993). Some considerations with regard to the quality of results of analysis of trace element extractable contents in soils and sediments. *International Journal of Environmental Analytical Chemistry*, **51**, 123.

Grodzinska, K., Szarek, G. & Godzik, B. (1990). Heavy-metal deposition in Polish National-Parks - changes during 10 years. *Water Air and Soil Pollution*, **49**, 409-419.

Grodzinska, K., Szarek-Lukaszewska, G. & Godzik, B. (1999). Survey of heavy metal deposition in Poland using mosses as indicators. *Science of the Total Environment*, **229**, 41-51.

Grosch, S. (1986). Wet and dry deposition of atmospheric trace elements in forest areas. In *Atmospheric pollutants in forest areas*, ed. H. W. Georgii, D. Reidel. Dordrecht.

Gulens, J. (1987). Assessment of research on the prepration, response and application of solid-state copper ion-selective electrodes. *Ion-Selective Electrode Reviews*, **9**, 127-171.

Hames, B. D. (1998). *Gel electrophoresis of proteins : a practical approach. 3rd ed.* Oxford University Press, Oxford ; New York.

Hamilton, R. S., Revitt, D. M. & Warren, R. S. (1984). Levels and physico-chemical associations of Cd, Cu, Pb and Zn in road sediments. *Science of The Total Environment*, **33**, 59-74.

Hansen, K. (1996). In-canopy throughfall measurements of ion fluxes in Norway spruce. *Atmospheric Environment*, **30**, 4065-4076.

Harris, M. M. & Jurgensen, M. F. (1977). Development of salix and populus mycorrhizae in metallic mine tailings. *Plant and Soil*, **47**, 509-517.

Harrison, R. M., Laxen, D. P. H. & Wilson, S. J. (1981). Chemical associations of lead, copper and zinc in street dusts and roadside soils. *Environmental Science & Technology*, **15**, 1378-1383.

Hawcroft, D. M. (1997). *Electrophoresis. 1st ed.* IRL Press at Oxford University Press, Oxford New York.

He, Q. B. & Singh, B. R. (1995). Cadmium availability to plants as affected by repeated applications of phosphorus fetilizers. *Acta Agriculturae Scandinavica Section B - Soil and Plant Science*, **45**, 22-31.

Hernandez, L., Probst, A., Probst, J. L. & Ulrich, E. (2003). Heavy metal distribution in some French forest soils: evidence for atmospheric contamination. *Science of The Total Environment*, **312**, 195-219.

Hickey, M. G. & Kittrick, J. A. (1984). Chemical partitioning of copper, nickel and zinc in soils and sediments containing high levels of heavy metals. *Journal of Environmental Quality*, **13**, 372-376.

Higney, E. (2003). Distribution of platinum and other vehicle emitted elements in the urban environment. In *Scottish Universities Environmental Research Centre*, University of Glasgow. East Kilbride.

Hinsinger, P., Plassard, C., Tang, C. X., Jaillard, B. (2003). Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints. *Plant and Soil*, **248**, 43-59.

Holm, P. E., Christensen, T. H., Tjell, J. C. & McGrath, S. P. (1995). Speciation of cadmium and zinc with applications to soil solutions. *Journal of Environmental Quality*, **24**, 183-190.

Hong, S. M., Candelone, J. P., Patterson, C. C. & Boutron, C. F. (1994). Greenland ice evidence of hemispheric lead pollution 2-millennia ago by Greek and Roman civilizations. *Science*, **265**, 1841-1843.

Hong, S. M., Candelone, J. P., Patterson, C. C. & Boutron, C. F. (1996). History of ancient copper smelting pollution during Roman and medieval times recorded in Greenland ice. *Science*, **272**, 246-249.

Hyvarinen, A. (1990). Deposition on forest soils. Effect of tree canopy on throughfall. In *Acidification in Finland*, ed. P. kauppi, Anttila, P., Kenttamies, K., Springer. Berlin, pp. 199-213.

Iyengar, S. S., Martens, D. C. & Miller, W. P. (1981). Distribution and plant availability of soil zinc fractions. *Soil Science Society of America Journal*, **45**, 735-739.

Jackson, D. R. & Watson, A. P. (1977). Disruption of nutrient pools and transport of heavy metals in a forested watershed near a smelter. *Journal of Environmental Quality*, **6**, 331-338.

Janos, P. (2003). Separation methods in the chemistry of humic substances. *Journal of Chromatography A*, **983**, 1-18.

Joaquim, C. G., Silva, E. & Oliveira, C. J. S. (2002). Metal ion complexation properties of fulvic acids extracted from composted sewage sludge as compared to a soil fulvic acid. *Water Research*, **36**, 3404-3409.

John, M. K. (1973). Cadmium uptake by eight food crops as influenced by various soil levels of cadmium. *Environmental Pollution*, **4**, 7-15.

Johnson, C. E., Siccama, T. G., Driscoll, C. T., Likens, G. E. & Moeller, R. E. (1995). Changes in lead biogeochemistry in response to decreasing atmospheric inputs. *Ecological Applications*, **5**, 813-822.

Jones, J. M. & Hao, J. (1993). Ombrotrophic peat as a medium for historical monitoring of heavy-metal pollution. *Environmental Geochemistry and Health*, **15**, 67-74.

Jones, P. (1995). *Gel electrophoresis: nucleic acids*. Wiley, Chichester ; New York.

Jug, A., Makeschin, F., Rehfuess, K. E. & Hofmann-Schielle, C. (1999). Short-rotation plantations of balsam poplars, aspen and willows on former arable land in the Federal Republic of Germany. III. Soil ecological effects. *Forest Ecology and Management*, **121**, 85-99.

Kaasalainen, M. & Yli-Halla, M. (2003). Use of sequential extraction to assess metal partitioning in soils. *Environmental Pollution*, **126**, 225-233.

Kabala, C. & Singh, B. R. (2001). Fractionation and mobility of copper, lead and zinc in soil profiles in the vicinity of a copper smelter. *Journal of Environmental Quality*, **30**, 485-492.

Kabata-Pendias, A. & Pendias, H. (1992). *Trace elements in soils and plants. 2nd ed.* CRC Press, London.

Karczewska, A. (1996). Metal species distribution in top- and sub-soil in an area affected by copper smelter emissions. *Applied Geochemistry*, **11**, 35-42.

Karczewska, A., Szerszen, L. & Kabala, C. (1998). Forms of selected heavy metals and their transformations in soils polluted by the emissions from copper smelters. *Advanced GeoEcology*, **31**, 705-712.

Kaschl, A., Romheld, V. & Chen, Y. (2002). Cadmium binding by fractions of dissolved organic matter and humic substances from municipal solid waste compost. *Journal of Environmental Quality*, **31**, 1885-1892.

Kaste, J. M., Friedland, A. J. & Sturup, S. (2003). Using stable and radioactive isotopes to trace atmospherically deposited Pb in montane forest soils. *Environmental Science & Technology*, **37**, 3560-3567.

Kelly, J. & Thornton, I. (1996). Urban geochemistry: A study of the influence of anthropogenic activity on the heavy metal content of soils in traditionally industrial and non-industrial areas of Britain. *Applied Geochemistry*, **11**, 363-370.

Kempter, H., Gorres, M. & Frenzel, B. (1997). Ti and Pb concentrations in rainwater-fed bogs in Europe as indicators of past anthropogenic activities. *Water Air and Soil Pollution*, **100**, 367-377.

Kiekens, L. (1990). Zinc. In *Heavy metals in soils*, ed. B. J. Alloway, Blackie. Glasgow, pp. 261-279.

Koleli, N. (2004). Speciation of chromium in 12 agricultural soils from Turkey. *Chemosphere*, **57**, 1473-1478.

Kononova, M. M. (1966). *Soil organic matter*. Pergamon Press, New York.

Krajnc, M., Stupar, J. & Milicev, S. (1995). Characterization of chromium and copper complexes with fulvic acids isolated from soils in Slovenia. *Science of The Total Environment*, **159**, 23-31.

Krishnamurti, G. S. R. & Naidu, R. (2002). Solid-solution speciation and phytoavailability of copper and zinc in soils. *Environmental Science & Technology*, **36**, 2645-2651.

Kuo, S., Heilman, P. E. & Baker, A. S. (1983). Distribution and forms of copper, zinc, cadmium, iron and manganese in soils near a copper smelter. *Soil Science*, **135**, 101-109.

Lee, J. A. & Tallis, J. H. (1973). Regional and historical aspects of lead pollution in Britain. *Nature*, **245**, 216-218.

Lepp, N. W. & Eardley, G. T. (1978). Growth and trace metal content of European sycamore seedlings grown in soil amended with sewage sludge. *Journal of Environmental Quality*, **7**, 413-416.

Levy, D. B., Barbarick, K. A., Siemer, E. G. & Sommers, L. E. (1992). Distribution and partitioning of trace metals in contaminated soils near Leadville, Colarado. *Journal of Environmental Quality*, **21**, 189-195.

LGC. (2001). Soil-Hackney Brick Works certified reference material LGC 6135 (LGC: Laboratory of the Government Chemist).

Li, X., Lee, S.-l., Wong, S.-c., Shi, W. & Thornton, I. (2004). The study of metal contamination in urban soils of Hong Kong using a GIS-based approach. *Environmental Pollution*, **129**, 113-124.

Li, X., Poon, C.-s. & Liu, P. S. (2001). Heavy metal contamination of urban soils and street dusts in Hong Kong. *Applied Geochemistry*, **16**, 1361-1368.

Li, X., Shen, Z., Wai, O. & Li, Y. (2000). Chemical partitioning of heavy metal contaminants in sediments of the Pearl River Estuary. **12**, 17-25.

Li, X. D., Coles, B. J., Ramsey, M. H. & Thornton, I. (1995). Sequential extraction of soils for multielement analysis by ICP-AES. *Chemical Geology*, **124**, 109-123.

Lindsay, W. L. (2001). *Chemical equilibria in soils*. Blackburn Press, Caldwell, N.J.

Livert, E. A., Lee, J. A. & Tallis, J. H. (1979). Lead, zinc and copper analyses of British blanket peats. *Ecology*, **67**, 865-891.

Logan, E. M. (1995). The measurement of the complexation of heavy metals and radionuclides with natural humic substances. In *Chemistry Department*, University of Glasgow. Glasgow.

Logan, E. M., Pulford, I. D., Cook, G. T. & MacKenzie, A. B. (1997). Compexation of Cu(II) and lead(II) by peat and humic acid. *European Journal of Soil Science*, **48**, 685-696.

Lu, Y. (2000). The characteristics and environmental significance of urban soils: a case study for Nanjing city. In *Academia Sinica*, Institute of Soil Science. Nanjing, China.

Lu, Y., Gong, Z., Zhang, G. & Burghardt, W. (2003). Concentrations and chemical speciations of Cu, Zn, Pb and Cr of urban soils in Nanjing, China. *Geoderma*, **115**, 101-111.

Luster, J., Lloyd, T. & Sposito, G. (1996). Multi-wavelength molecular fluorescence spectrometry for quantitative characterization of copper(II) and aluminium(III) complexation by dissolved organic matter. *Envionmental Science and Technology*, **30**, 1565-1574.

Lux, W. (1986). Shhwermetallgehalte und isoplethin in Boden, subhydrishen Ablagerung und Pflanzen im Sudosten Hamburgs. *Hamburger Bodenkudliche Arbeiten*, **5**, 249.

Lyons, T. J., Kensworth, J. R. & Newman, P. W. G. (1990). Urban structure and air pollution. *Atmospheric Environment*, **24B**, 43-48.

Ma, L. Q. & Rao, G. N. (1997). Chemical fractionation of cadmium, copper, nickel and zinc in contaminated soils. *Journal of Environmental Quality*, **26**, 259-264.

Ma, L. Q., Tan, F. & Harris, W. G. (1997). Concentrations and distributions of eleven metals in Florida soils. *Environmental Quality*, **26**, 769-775.

MacKenzie, A. B., Farmer, J. G. & Sugden, C. L. (1997). Isotopic evidence of the relative retention and mobility of lead and radiocaesium in Scottish ombrotrophic peats. *Science of the Total Environment*, **203**, 115-127.

MacKenzie, A. B., Logan, E. M., Cook, G. T. & Pulford, I. D. (1998). Distributions, inventories and isotopic composition of lead in ^{210}Pb-dated peat cores from contrasting biogeochemical environments: Implications for lead mobility. *Science of The Total Environment*, **223**, 25-35.

MacKenzie, A. B., Logan, E. M., Cook, G. T. & Pulford, I. D. (1998). A historical record of atmospheric depositional fluxes of contaminants in west-central Scotland derived from an ombrotrophic peat core. *Science of The Total Environment*, **222**, 157-166.

MacKenzie, A. B. & Pulford, I. D. (2002). Investigation of contaminant metal dispersal from a disused mine site at Tyndrum, Scotland, using concentration gradients and stable Pb isotope ratios. *Applied Geochemistry*, **17**, 1093-1103.

Madrid, L., Diaz-Barrientos, E. & Madrid, F. (2002). Distribution of heavy metal contents of urban soils in parks of Seville. *Chemosphere*, **49**, 1301-1308.

Maiz, I., Esnaola, M. V. & Millan, E. (1997). Evaluation of heavy metal availability in contaminated soils by a short sequential extraction procedure. *Science of The Total Environment*, **206**, 107-115.

Manta, D. S., Angelone, M., Bellanca, A., Neri, R. & Sprovieri, M. (2002). Heavy metals in urban soils: a case study from the city of Palermo (Sicily), Italy. *Science of The Total Environment*, **300**, 229-243.

Marsh, A. S. & Siccama, T. G. (1997). Use of formerly plowed land in New England to monitor the vertical distribution of lead, zinc and copper in mineral soil. *Water Air and Soil Pollution*, **95**, 75-85.

Martin, M. H. & Bullock, R. J. (1994). The impact and fate of heavy metals in an oak woodland ecosystem. In *Toxic metals in soil-plant systems*, ed. S. M. Ross, Wiley. New York, pp. 327-365.

Martin, M. H. & Coughtrey, P. J. (1987). Cycling and fate of heavy metals in a contaminated woodland ecosystem. In *Pollution transfer and fate in ecosystems*, eds. P. J. Coughtrey, M. H. Martin & M. H. Unsworth, Blackwell. Oxford, pp. 319-336.

Martin, R. (1995). *Gel electrophoresis: nucleic acids*. BIOS Scientific, Oxford.

Martinez Cortizas, A., Garcia-Rodeja, E., Pombal, X. P., Munoz, J. C. N., Weiss, D. & Cheburkin, A. (2002). Atmospheric Pb deposition in Spain during the last 4600 years recorded by two ombrotrophic peat bogs and implications for the use of peat as archive. *The Science of The Total Environment*, **292**, 33-44.

Martinez Cortizas, A., Gayoso, E. G. R. & Weiss, D. (2002). Peat bog archives of atmospheric metal deposition. *Science of the Total Environment*, **292**, 1-5.

Martinez Cortizas, A., Pontevedra Pombal, X., Munoz, J. C. N. & Garcia-Rodeja, E. (1997). Four thousand years of atmospheric Pb, Cd and Zn deposition recorded by

the ombrotrophic peat bog of Penido Vello (northwestern Spain). *Water Air and Soil Pollution*, **100**, 387-403.

Martley, E., Gulson, B. L. & Pfeifer, H.-R. (2004). Metal concentrations in soils around the copper smelter and surrounding industrial complex of Port Kembla, NSW, Australia. *Science of The Total Environment*, **325**, 113-127.

McBride, M., Sauve, S. & Hendershot, W. (1997). Solubility control of Cu, Zn, Cd and Pb in contaminated soils. *European Journal of Soil Science*, **48**, 337-346.

McBride, M. B. (2001). Cupric ion activity in peat soil as a toxicity indicator for maize. *Journal of Environmental Quality*, **30**, 78-84.

McBride, M. B. (1994). *Environmental chemistry of soils*. Oxford University Press, New York.

McBride, M. B. (1989). Reactions controlling heavy metal solubility in soils. *Advances in Soil Science*, **10**, 1-56.

McGrath, S. P. & Smith, S. (1990). Chromium and nickel. In *Heavy metals in soils*, ed. B. J. Alloway, Blackie. Glasgow, pp. 125-150.

McLaren, R. G. & Crawford, D. V. (1973). Studies on soil copper. I. The fractionation of copper in soils. *Journal of Soil Science*, **24**, 172-181.

McLaughlin, M. J., Hamon, R. E., McLaren, R. G., Speir, T. W. & Rogers, S. L. (2000). Review: A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand. *Australian Journal of Soil Research*, **38**, 1037-1086.

Merritt, K. A. & Erich, M. S. (2003). Influence of organic matter decomposition on soluble carbon and its copper-binding capacity. *Journal of Environmental Quality*, **32**, 2122-2131.

Miller, E. K. & Friedland, A. J. (1994). Lead migration in forest soils - response to changing atmospheric inputs. *Environmental Science & Technology*, **28**, 662-669.

Miller, W. P., Martens, D. C. & Zelazny, L. W. (1986). Effect of the sequence in extraction of trace metals from soils. *Soil Science Society of America Journal*, **50**, 598-601.

Minnich, M. M. (1987). Copper activity in soil solution: II. Relation to copper accumulation in young snapbeans. *Soil Science Society of America Journal*, **51**, 573-578.

Mitchell, B. D. & Jarvis, R. A. (1956). *The soils of the country round Kilmarnock (sheet 22 and part of sheet 21)*. Her Majesty's Stationary Office, Edinburgh.

Morera, M. T., Echeverria, J. C., Mazkiaran, C. & Garrido, J. J. (2001). Isotherms and sequential extraction procedures for evaluating sorption and distribution of heavy metals in soils. *Environmental Pollution*, **113**, 135-144.

Morselt, A. F. W., Smits, W. T. M., Limonard, T. (1986). Histochemical demonstration of heavy metal tolerance in ectomycorrhizal fungi. *Plant and Soil*, **96**, 417-420.

Mossop, K. F. & Davidson, C. M. (2003). Comparison of original and modified BCR sequential extraction procedures for the fractionation of copper, iron, lead, manganese and zinc in soils and sediments. *Analytica Chimica Acta*, **478**, 111-118.

Murozumi, M., Chow, T. J. & Patterson, C. C. (1969). Chemical concentrations of pollutant lead aerosols, terrestrial dusts and sea salts in Greenland and Antarctic snow strata. *Geochimica et Cosmochimica Acta*, **33**, 1247-1294.

Nadal, M., Schuhmacher, M. & Domingo, J. L. (2004). Metal pollution of soils and vegetation in an area with petrochemical industry. *Science of The Total Environment*, **321**, 59-69.

Narwal, R. P. & Singh, B. R. (1998). Effect of organic materials on partitioning, extractability and plant uptake of metals in an alum shale soil. *Water Air and Soil Pollution*, **103**, 405-421.

Narwal, R. P., Singh, B. R. & Salbu, B. (1999). Association of cadmium, zinc, copper and nickel with components in naturally heavy metal-rich soils studied by parallel and

sequential extractions. *Communications in Soil Science and Plant Analysis*, **30**, 1209-1230.

Nedelkoska, T. V. & Doran, P. M. (2000). Characteristics of heavy metal uptake by plant species with potential for phytoremediation and phytomining. *Minerals Engineering*, **13**, 549-561.

Nieminen, T. M., Derome, J. & Helmisaari, H.-S. (1999). Interactions between precipitation and Scots pine canopies along a heavy-metal pollution gradient. *Environmental Pollution*, **106**, 129-137.

Nieminen, T. M., Ukonmaanaho, L. & Shotyk, W. (2002). Enrichment of Cu, Ni, Zn, Pb and As in an ombrotrophic peat bog near a Cu-Ni smelter in Southwest Finland. *Science of The Total Environment*, **292**, 81-89.

Nierop, K. G. J., Jansen, B., Vrugt, J. A. & Verstraten, J. M. (2002). Copper complexation by dissolved organic matter and uncertainty assessment of their stability constants. *Chemosphere*, **49**, 1191-1200.

Nihlgard, B. (1970). Precipitation, its chemical composition and effects on soil and water in a beech and spruce forest in south Sweden. *Oikos*, **21**, 208-217.

Nolan, A. L., McLaughlin, M. J. & Mason, S. D. (2003). Chemical speciation of Zn, Cd, Cu, and Pb in pore waters of agricultural and contaminated soils using Donnan dialysis. *Environmental Science & Technology*, **37**, 90-98.

Norden, U. (1994). Influence of broad-leaved tree species on pH and organic matter content of forest topsoils in Scania, South Sweden. *Scandanavian Journal of Forest Research*, **9**, 1-8.

Nowack, B., Obrecht, J.-M., Schluep, M., Schulin, R., Hansmann, W. & Koppel, V. (2001). Elevated lead and zinc contents in remote alpine soils of the Swiss National Park. *Journal of Environmental Quality*, **30**, 919-926.

Nriagu, J. O. & Pacyna, J. M. (1988). Quantitative assessment of worldwide contamination of air, water and soils by Trace-metals. *Nature*, **333**, 134-139.

OS. (1982). Ordinance Survey (OS) soil map of south east Scotland (1 : 250,000). Made and published by the Ordinance Survey, for the Macaulay Institute for Soil Research, Soil Survey of Scotland. Southampton.

Otvos, E., Pazmandi, T. & Tuba, Z. (2003). First national survey of atmospheric heavy metal deposition in Hungary by the analysis of mosses. *Science of The Total Environment*, **309**, 151-160.

Oughton, D. H., Salbu, B., Riise, G., Lien, H. N., Ostby, G. & Noren, A. (1992). Radionuclide mobility and bioavailability in Norwegian and Soviet soils. *Analyst*, **117**, 481-486.

Pacyna, J. M. (1995). The origin of arctic air-pollutants - lessons learned and future-research. *Science of the Total Environment*, **161**, 39-53.

Palumbo, B., Angelone, M., Bellanca, A., Dazzi, C., Hauser, S., Neri, R. & Wilson, J. (2000). Influence of inheritance and pedogenesis on heavy metal distribution in soils of Sicily, Italy. *Geoderma*, **95**, 247-266.

Parker, G. G. (1983). Throughfall and stemflow in the forest nutrient cycle. *Advances in Ecological Research*, **13**, 58-133.

Paterson, E., Sanka, M. & Clark, L. (1996). Urban soils as pollutant sinks - a case study from Aberdeen, Scotland. *Applied Geochemistry*, **11**, 129-131.

Peijnenburg, W., Posthuma, L., Eijsackers, H. J. P. & Allen, H. E. (1997). A conceptual framework for implementation of bioavailability of metals for environmental management purposes. *Ecotoxicology and Environmental Safety*, **37**, 163-172.

Perdue, E. M. & Lytle, C. R. (1983). Distribution model for binding of protons and metal ions by humic substances. *Environmental Science and Technology*, **17**, 654-660.

PerezCid, B., Lavilla, I. & Bendicho, C. (1996). Analytical assessment of two sequential extraction schemes for metal partitioning in sewage sludges. *Analyst*, **121**(10), 1479-1484.

Pietrzak, U. & McPhail, D. C. (2004). Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. *Geoderma*, **122**, 151-166.

Plaza, C., Orazio, V. D. & Senesi, N. (2005). Copper(II) complexation of humic acids from the first generation of EUROSOILS by total luminescence spectroscopy. *Geoderma*, **125**, 177-186.

Poikolainen, J., Kubin, E., Piispanen, J. & Karhu, J. (2004). Atmospheric heavy metal deposition in Finland during 1985-2000 using mosses as bioindicators. *Science of The Total Environment*, **318**, 171-185.

Potter, C. S., Ragsdale, H. L., Swank, W. T. (1991). Atmospheric deposition and foliar leaching in a regenerating southern appalachian forest canopy. *Ecology*, **79**, 97-115.

Pulford, I. D. & Watson, C. (2003). Phytoremediation of heavy metal-contaminated land by trees - a review. *Environment International*, **29**, 529-540.

Purves, D. (1985). *Trace-element contamination of the environment, revised edition.* Elsevier, Amsterdam.

Quevauviller, P., Rauret, G., Lopez-Sanchez, J. F., Rubio, R., Ure, A. & Muntau, H. (1997). Certification of trace metal extractable contents in a sediment reference material (CRM 601) following a three-step sequential extraction procedure. *Science of the Total Environment*, **205**, 223-234.

Rai, D., Eary, L. E. & Zachara, J. M. (1989). Environmental chemistry of chromium. *Science of The Total Environment*, **86**, 15-23.

Ramos, L., Hernandez, M. & J., G. M. (1994). Sequential fractionation of copper, lead, cadmium and zinc in soils from or near Donana National Park. *Journal of Environmental Quality*, **23**, 50-57.

Ramos, M. A., Fiol, S., Lopez, R., Antelo, J. M. & Arce, F. (2002). Analysis of the effect of pH on Cu(II)-fulvic acid complexation using a simple electrostatic model. *Environmental Science & Technology*, **36**, 3109-3113.

Raulund-Rasmussen, K., Borggaard, O. K., Hansen, H. C. B. & Olsson, M. (1998). Effect of natural orgranic soil solutes on weathering rates of soil minerals. *European Journal of Soil Science*, **49**, 397-406.

Raulund-Rasmussen, K. & Vejre, H. (1995). Effects of tree species and soil properties on the nutrient immobilization in the forest floor. *Plant and Soil*, **168-169**, 345-352.

Rauret, G., Lopez-Sanchez, J. F., Sahuquillo, A., Barahona, E., Lachica, M., Ure, A. M., Davidson, C. M., Gomez, A., Luck, D., Bacon, J., Yli-Halla, M., Muntau, H. & Quevauviller, P. (2000). Application of a modified BCR sequential extraction (three-step) procedure for the determination of extractable trace metal contents in a sewage sludge amended soil reference (CRM 483), complemented by a three-year stability study of acetic acid and EDTA extractable metal content. *Journal of Environmental Monitoring*, **2**, 228-233.

Rauret, G., Lopez-Sanchez, J.-F., Sahuquillo, A., Muntau, H. & Quevauviller, P. (2000). Indicative values for extractable contents (mass fractions) of Cd, Cr, Cu, Ni, Pb and Zn in sediment (CRM 601) following the modified BCR-sequential extraction (three-step) procedure. European Commission.

Rauret, G., Lopez-Sanchez, J. F., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A. & Quevauviller, P. (1999). Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *Journal of Environmental Monitoring*, **1**, 57-61.

Rauret, G., Rubio, R. & Lopez-Sanchez, J. F. (1989). Optimization of Tessier Procedure for Metal Solid Speciation in River Sediments. *International Journal of Environmental Analytical Chemistry*, **36**, 69-83.

Rea, A. W., Lindberg, S. E. & Keeler, G. J. (2001). Dry deposition and foliar leaching of mercury and selected trace elements in deciduous forest throughfall. *Atmospheric Environment*, **35**, 3453-3462.

Renberg, I., Persson, M. W. & Emteryd, O. (1994). Preindustrial atmospheric lead contamination detected in Swedish lake-sediments. *Nature*, **368**, 323-326.

Reuter, J. H. & Perdue, E. M. (1981). Calculation of molecular-weights of humic substances from colligative data - application to aquatic humus and its molecular-size fractions. *Geochimica et Cosmochimica Acta*, **45**, 2017-2022.

Rieuwerts, J. S., Thornton, I., Farago, M. E. & Ashmore, M. R. (1998). Factors influencing metal bioavailability in soils: preliminary investigations for the development of a critical loads approach for metals. *Chemical Speciation and Bioavailability*, **10**, 61-75.

Robertson, A. P. & Leckie, J. O. (1999). Acid / base, copper binding, and Cu^{2+} / H^{+} exchange properties of a soil humic acid, an experimental and modeling study. *Environmental Science & Technology*, **33**, 786-795.

Robertson, S. M. C., Hornung, M. & Kennedy, V. H. (2000). Water chemistry of throughfall and soil water under four tree species at Gisburn, northwest England, before and after felling. *Forest Ecology and Management*, **129**, 101-117.

Rosman, K. J. R., Chisholm, W., Hong, S. M., Candelone, J. P. & Boutron, C. F. (1997). Lead from Carthaginian and Roman Spanish mines isotopically identified in Greenland ice dated from 600 BC to 300 AD. *Environmental Science & Technology*, **31**, 3413-3416.

Ross, S. M. (1994). Sources and forms of potentially toxic metals in soil-plant systems. In *Toxic metals in soil-plant sytems*, ed. S. M. Ross, Wiley. Chichester, West Sussex, England ; New York, pp. 3-25.

Ross, S. M. (1994). *Toxic metals in soil-plant systems*. Wiley, Chichester, West Sussex, England ; New York.

Ross, S. M. (1994). Toxic metals: Fate and Distribution in contaminated ecosystems. In *Toxic metals in soil-plant systems*, ed. S. M. Ross, Wiley. Chichester, West Sussex, England ; New York, pp. 189-243.

Ross, S. M., Thornes, J. B., Nortcliff, S. (1990). Soil hydrology, nutrient and erosional response to the clearance of terra firme forest, Maraca island, Roraima, Northern Brazil. *The Geographical Journal*, **156**, 267-282.

Rowell, D. L. (1994). *Soil science methods and applications*. Longman Scientific and Technical, Marlow.

Ruhling, A. & Tyler, G. (2004). Changes in the atmospheric deposition of minor and rare elements between 1975 and 2000 in south Sweden, as measured by moss analysis. *Environmental Pollution*, **131**, 417-423.

Ruhling, A. & Tyler, G. (1970). Sorption and retention of heavy metals in the woodland moss *Hylocomium splendens* (Hedw). *Br et Sch Oikos*, **21**, 92-97.

Rundle, S. & Duggan, M. (1980). Report DE/SB/EDS/R91. Greater London Council.

Ryan, P. R., Delhaize, E. (2001). Function and mechanism of organic anion exudation from plant roots. *Annual Review of Plant Physiology and Plant Molecular Biology*, **52**, 527-560.

Saar, R. A. & Weber, J. H. (1980). Lead(II) complexation by fulvic acid: how it differs from fulvic acid complexation of copper(II) and cadmium(II). *Geochimica et Cosmochimica Acta*, **44**, 1381-1384.

Saar, R. S. & Weber, J. H. (1979). Complexation of cadmium (II) with water- and soil-derived fulvic acids: Effect of pH and fulvic acid concentration. *Canadian Journal of Chemistry*, **57**, 1263-1268.

Sahu, S. & Banerjee, D. K. (1990). Complexation properties of typical soil and peat humic acids with copper(II) and cadmium(II). *International Journal of Environmental and Analytical Chemistry*, **42**, 35-44.

Sahuquillo, A., Lopez-Sanchez, J. F., Rubio, R., Rauret, G., Thomas, R. P., Davidson, C. M. & Ure, A. M. (1999). Use of a certified reference material for extractable trace metals to assess sources of uncertainty in the BCR three-stage sequential extraction procedure. *Analytica Chimica Acta*, **382**, 317-327.

Salam, A. K. & Helmke, P. A. (1998). The pH dependence of free ionic activities and total dissolved concentrations of copper and cadmium in soil solution. *Geoderma*, **83**, 281-291.

Sanchez-Camazano, M., Sanchez-Martin, M. J. & Lorenzo, L. F. (1994). Lead and cadmium in soils and vegetables from urban gardens of Salamanca (Spain). *Science of The Total Environment*, **146-147**, 163-168.

Sarkar, A. N., Jones, R. G. (1982). Effect of rhizosphere pH on the availability and uptake of Fe, Mn and Zn. *Plant and Soil*, **66**, 361-372.

Sauve, S. (2003). The role of chemical speciation in bioavailability. In *Bioavailability, toxicity and risk relationships in ecosystems*, eds. R. Naidu, V. S. R. Gupta, R. S. Kookana, S. L. Rogers & D. Adriano, Science Publishers. Enfield NH, pp. 21-44.

Sauve, S., Cook, N., Hendershot, W. H. & McBride, M. B. (1996). Linking plant tissue concentrations and soil copper pools in urban contaminated soils. *Environmental Pollution*, **94**, 153-157.

Sauve, S., Manna, S., Turmel, M.-C., Roy, A. G. & Courchesne, F. (2003). Solid-solution partitioning of Cd, Cu, Ni, Pb and Zn in the organic horizons of a forest soil. *Environmental Science & Technology*, **37**, 5191-5196.

Sauve, S., McBride, M., Norvell, W. A. & Hendershot, W. H. (1997). Copper solubility and speciation of in situ contaminated soils: Effects of copper level, pH and organic matter. *Water Air and Soil Pollution*, **100**, 133-149.

Sauve, S., McBride, M. B. & Hendershot, W. H. (1995). Ion-selective electrode measurements of copper(II) activity in contaminated soils. *Archives of Environmental Contamination and Toxicology*, **29**, 373-379.

Sauve, S., McBride, M. B. & Hendershot, W. H. (1997). Speciation of lead in contaminated soils. *Environmental Pollution*, **98**, 149-155.

Scheinost, A. C., Kretzschmar, R. & Pfister, S. (2002). Combining selective sequential extractions, x-ray absorption spectroscopy, and principal component analysis for

quantitative zinc speciation in soil. *Environmental Science & Technology*, **36**, 5021-5028.

Schilling, J. S. & Lehman, M. E. (2002). Bioindication of atmospheric heavy metal deposition in the southeastern US using the moss *Thuidium Delicatulum*. *Atmospheric Environment*, **36**, 1611-1618.

Schnitzer, M. & Khan, S. U. (1972). *Humic substances in the environment*. Dekker, New York.

Schramel, O., Michalke, B. & Kettrup, A. (2000). Study of the copper distribution in contaminated soils of hop fields by single and sequential extraction procedures. *Science of The Total Environment*, **263**, 11-22.

Schwikowski, M., Barbante, C., Doering, T., Gaeggeler, H. W., Boutron, C., Schotterer, U., Tobler, L., Van de Velde, K., Ferrari, C., Cozzi, G., Rosman, K. & Cescon, P. (2004). Post-17th-century changes of European lead emissions recorded in high-altitude Alpine snow and ice. *Envionmental Science and Technology*, **38**, 957-964.

Seguin, V., Gagnon, C., Courchesne, F. (2004). Changes in water extractable metals, pH and organic carbon concentrations at the soil-root interface of forested soils. *Plant and Soil*, **260**, 1-17.

Semlali, R. M., Van Oort, F., Denaix, L. & Loubet, M. (2001). Estimating distributions of endogenous and exogenous Pb in soils by using Pb isotopic ratios. *Envionmental Science and Technology*, **35**, 4180-4188.

Shen, H., Yan, X., Zhao, M., Zheng, S. & Wang, X. (2002). Exudation of organic acids in common bean as related to mobilization of aluminum- and iron-bound phosphates. *Environmental and Experimental Botany*, **48**, 1-9.

Shirahata, H., Elias, R. W., Patterson, C. C. & Koide, M. (1980). Chronological variations in concentrations and isotopic compositons of anthropogenic atmospheric lead in sediments of a remote subalpine pond. *Geochimica et Cosmochimica Acta*, **44**, 149-162.

Shirshova, L. & Osterberg, R. (1998). Electrophoresis of podzol soil humic acids. *Environment International*, **24**, 625-628.

Shotyk, W. (2002). The chronology of anthropogenic, atmospheric Pb deposition recorded by peat cores in three minerogenic peat deposits from Switzerland. *Science of The Total Environment*, **292**, 19-31.

Shotyk, W. (1996). Natural and anthropogenic enrichments of As, Cu, Pb, Sb, and Zn in ombrotrophic versus minerotrophic peat bog profiles, Jura Mountains, Switzerland. *Water Air and Soil Pollution*, **90**, 375-405.

Shotyk, W., Blaser, P., Grunig, A. & Cheburkin, A. K. (2000). A new approach for quantifying cumulative, anthropogenic, atmospheric lead deposition using peat cores from bogs: Pb in eight Swiss peat bog profiles. *Science of The Total Environment*, **249**, 281-295.

Shotyk, W., Cheburkin, A. K., Appleby, P. G., Fankhauser, A. & Kramers, J. D. (1997). Lead in three peat bog profiles, Jura Mountains, Switzerland: Enrichment factors, isotopic composition, and chronology of atmospheric deposition. *Water Air and Soil Pollution*, **100**, 297-310.

Shotyk, W., Nesbitt, H. W. & Fyfe, W. S. (1992). Natural and anthropogenic enrichments of trace-metals in peat profiles. *International Journal of Coal Geology*, **20**, 49-84.

Shotyk, W., Norton, S. A. & Farmer, J. G. (1997). Summary of the workshop on peat bog archives of atmopsheric metal deposition. *Water Air and Soil Pollution*, **100**, 213-219.

Shotyk, W., Weiss, D. & Appleby, P. G. (1998). History of atmospheric lead deposition since 12,370 ^{14}C yr BP recorded in a peat bog profile, Jura Mountains, Switzerland. *Science*, **281**, 1635-1640.

Shparyk, Y. S. & Parpan, V. I. (2004). Heavy metal pollution and forest health in the Ukranian Carpathians. *Enviromental Pollution*, **130**, 55-63.

Shuman, L. M. (1990). Comparison of exchangeable aluminium, extractable aluminium, and aluminium in soil fractions. *Canadian Journal of Soil Science*, **70**, 263-267.

Shuman, L. M. (1985). Fractionation method for soil microelements. *Soil Science*, **140**, 11-22.

Shuman, L. M. (1979). Zinc, manganese, and copper in soil fractions. *Soil Science*, **127**, 10-17.

Singh, B. R. (1997). Soil pollution and contamination. In *Methods for assessment of soil degradation*, ed. R. Lal, CRC Press. Boca Raton, FL, pp. 279-299.

Singh, J. P., Karwasra, S. P. S. & Singh, M. (1988). Distribution and forms of copper, iron, manganese, and zinc in calcareous soils of India. *Soil Science*, **146**, 359-366.

Sipos, P., Nemeth, T., Mohai, I. & Dodony, I. (2005). Effect of soil composition on adsorption of lead as reflected by a study on a natural forest soil profile. *Geoderma*, **124**, 363-374.

Smith, E. J., Rey-Castro, C., Longworth, H., Lofts, S., Lawlor, A. J. & Tipping, E. (2004). Cation binding by acid-washed peat, interpreted with humic ion- binding model VI-FD. *European Journal of Soil Science*, **55**, 433-447.

Smith, K. A. (1990). Manganese and cobalt. In *Heavy metals in soils*, ed. B. J. Alloway, Blackie. Glasgow, pp. 197-221.

Smith, W. H. & Siccama, T. G. (1981). The Hubbard Brook Ecosystem Study - Biogeochemistry of lead in the northern hardwood forest. *Journal of Environmental Quality*, **10**, 323-332.

Sposito, G. (1989). *The chemistry of soils*. Oxford University Press, Oxford.

Sposito, G., Lund, L. J. & Chang, A. C. (1982). Trace metal chemistry in arid zone field soils amended with sewage sludge. 1. Fractionation of Ni, Cu, Zn, Cd and Pb in solid phases. *Soil Science Society of America Journal*, **46**, 260-264.

Steinnes, E., Allen, R. O., Peterson, H. M., Rambaek, J. P. & Varskog, P. (1997). Evidence of large scale heavy-metal contamination of natural surface soils in Norway from long-range atmospheric transport. *Science of The Total Environment*, **205**, 255-266.

Sterckeman, T., Douay, F., Proix, N. & Fourrier, H. (2000). Vertical distribution of Cd, Pb and Zn in soils near smelters in the north of France. *Enviromental Pollution*, **107**, 377-389.

Stewart, C. & Fergusson, J. E. (1994). The use of peat in the historical monitoring of trace metals in the atmosphere. *Environmental Pollution*, **86**, 243-249.

Stone, M. & Marsalek, J. (1996). Trace metal composition and speciation in street sediments: Saultste. Marie, Canada. *Water Air and Soil Pollution*, **87**, 149-169.

Strobel, B. W., Bernhoft, I. & Borggaard, O. K. (1999). Low-molecular weight aliphatic carboxylic acids in soil solutions under different vegetations determined by capillary electrophoresis. *Plant and Soil*, **212**, 115-121.

Strobel, B. W., Hansen, H. C. B., Borggaard, O. K., Andersen, M. K. & Raulund-Rasmussen, K. (2001). Cadmium and copper release kinetics in relation to afforestation of cultivated soil. *Geochimica et Cosmochimica Acta*, **65**, 1233-1242.

Strojan, C. L. (1978). Forest leaf litter decomposition in the vicinity of a zinc smelter. *Oikos*, **31**, 41-46.

Sucharova, J. & Suchara, I. (1998). Atmospheric deposition levels of chosen elements in the Czech Republic determined in the framework of the International Bryomonitoring Program 1995. *Science of the Total Environment*, **223**, 37-52.

Sugden, C. L., Farmer, J. G. & Mackenzie, A. B. (1993). Isotopic-ratios of lead in contemporary environmental material from Scotland. *Environmental Geochemistry and Health*, **15**, 59-65.

Sutherland, R. A. & Tack, F. M. G. (2002). Determination of Al, Cu, Fe, Mn, Pb and Zn in certified reference materials using the optimized BCR sequential extraction procedure. *Analytica Chimica Acta*, **454**, 249-257.

Sutherland, R. A., Tack, F. M. G., Tolosa, C. A. & Verloo, M. G. (2000). Operationally defined metal fractions in road deposited sediment, Honolulu, Hawaii. *Journal of Environmental Quality*, **29**, 1431-1439.

Szakova, J., Tlustos, P., Balik, J., Pavlikova, D. & Vanek, V. (1999). The sequential analytical procedure as a tool for evaluation of As, Cd and Zn mobility in soil. *Fresenius Journal of Analytical Chemistry*, **363**, 594-595.

Szczepaniak, K. & Biziuk, M. (2003). Aspects of the biomonitoring studies using mosses and lichens as indicators of metal pollution. *Environmental Research*, **93**, 221-230.

Taylor, G. J. (1987). Exclusion of metals from the symplasm: a possible mechanism of metal tolerance in higher plants. *Journal of Plant Nutrition*, **10**, 1213.

Temminghoff, E. J. M., Van der Zee, S. & De Haan, F. A. M. (1998). Effects of dissolved organic matter on the mobility of copper in a contaminated sandy soil. *European Journal of Soil Science*, **49**, 617-628.

Tessier, A. & Campbell, P. G. C. (1988). Partitioning of trace metals in sediments. In *Metal speciation: Theory, analysis and application*, eds. J. R. Kramer & H. E. Allen, Lewis Publ. Inc. Chelsea, Mass, pp. 183-195.

Tessier, A., Campbell, P. G. C. & Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry*, **51**, 844-851.

ThermoOrion. (2001). *Cupric electrodes instruction manual, Cu(II) model 9629.*

Thomas, R. P., Ure, A. M., Davidson, C. M., Littlejohn, D., Rauret, G., Rubio, R. & Lopezsanchez, J. F. (1994). 3-stage sequential extraction procedure for the determination of metals in river sediments. *Analytica Chimica Acta*, **286**, 423-429.

Thornton, I. (1991). Metal Contamination of soils in urban areas. In *Soil in the urban environment*, eds. P. Bullock & P. J. Gregory, Blackwell. London, pp. 47-75.

Tichy, J. (1996). Impact of atmospheric deposition on the status of planted Norway spruce stands: a comparative study between sites in southern Sweden and the northeastern Czech Republic. *Enviromental Pollution*, **93**, 303-312.

Tipping, E. (1998). Humic ion-binding model VI: An improved description of thte interactions of protons and metal ions with humic substances. *Aquatic Geochemistry*, **4**, 3-48.

Tipping, E. (1994). WHAM - A chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site / electrostatic model of ion-binding by humic substances. *Computers and Geosciences*, **20**, 973-1023.

Tokalioglu, S., Kartal, S. & Elci, L. (2000). Determination of heavy metals and their speciation in lake sediments by flame atomic absorption spectrometry after a four-stage sequential extraction procedure. *Analytica Chimica Acta*, **413**, 33-40.

Town, R. M. & Filella, M. (2000). Determination of metal ion binding parameters for humic substances. Part 2. Utility of ASV pseudo-polarography. *Journal of Electroanalytical Chemistry*, **488**, 1-16.

Town, R. M. & Powell, H. K. J. (1993). Ion-selective electrode potentiometric studies on the complexation of copper(II) by soil-derived humic and fulvic- Acids. *Analytica Chimica Acta*, **279**, 221-233.

Trubetskoj, O. A., Kudryavceva, L. Y. & Shirshova, L. T. (1991). Characterization of soil humic matter by polyacrylamide-gel electrophoresis in the presence of denaturating agents. *Soil Biology & Biochemistry*, **23**, 1179-1181.

Trubetskoj, O. A., Trubetskaya, O. E. & Khomutova, T. E. (1992). Isolation, purification and some physicochemical properties of soil humic substances fractions obtained by polyacrylamide-gel electrophoresis. *Soil Biology & Biochemistry*, **24**, 893-896.

Trubetskoj, O. A., Trubetskaya, O. E., Reznikova, O. I. & Afanaseva, G. V. (1999). Weight and optical differences between soil humic acid fractions obtained by coupling SEC-PAGE. *Geoderma*, **93**, 277-287.

Turkey, H. B. (1980). Some effects of rain and acid mist on plants, with implications for acid precipitation. In *Effects of acid precipitation on terrestrial ecosystems*, ed. T. C. Hutchinson, Havas, M., Plenum Press. New York, pp. pp. 141-150.

Tye, A. M., Young, S., Crout, N. M. J., Zhang, H., Preston, S., Zhao, F. J. & McGrath, S. P. (2004). Speciation and solubility of Cu, Ni and Pb in contaminated soils. *European Journal of Soil Science*, **55**, 579-590.

Urban, N. R., Eisenrich, N. R., Grigal, D. F. & Schurr, K. T. (1990). Mobility and diagenesis of Pb and ^{210}Pb in peat. *Geochimica et Cosmochimica Acta*, **54**, 3329-3346.

Ure, A. M. (1996). Single extraction schemes for soil analysis and related applications. *Science of The Total Environment*, **178**, 3-10.

Ure, A. M. (1991). Trace-element speciation in soils, soil extracts and solutions. *Mikrochimica Acta*, **2**, 49-57.

Ure, A. M., Quevauviller, P., Muntau, H. & Griepink, B. (1993). Speciation of heavy-metals in soils and sediments - an account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commission-of-the-European-Communities. *International Journal of Environmental Analytical Chemistry*, **51**, 135-151.

Ure, M., Thomas, R. & Littlejohn, D. (1993). Ammonium Acetate Extracts and Their Analysis for the Speciation of Metal-Ions in Soils and Sediments. *International Journal of Environmental Analytical Chemistry*, **51**, 65-84.

Uren, N. C. (1992). Forms, reactions, and availability of nickel in soils. In *Advances in Agronomy*, ed. D. L. Sparks, Vol. 48, Academic Press. New York, pp. 141-203.

Vidal, M. & Rauret, G. (1993). Two approaches for sequential extraction of radionuclides in soils - batch and column methods. *International Journal of Environmental Analytical Chemistry*, **51**, 85-95.

Vile, M. A., Weider, R. K. & Novak, M. (1999). Mobility of Pb in *Sphagnum*-derived peat. *Biogeochemistry*, **45**, 35-52.

Vinogradoff, S. I., Abbott, A., Graham, M. C. & Farmer, J. G. (1998). The influence of microbial alteration of humic macromolecules on humic-actinide binding:

implications for actinide mobility in soils. *Contaminated Soil, Thomas Telford, London*, 931-932.

Wang, E. X. & Benoit, G. (1997). Fate and transport of contaminant lead in spodosols: A simple box model analysis. *Water Air and Soil Pollution*, **95**, 381-397.

Wang, E. X. & Benoit, G. (1996). Mechanisms controlling the mobility of lead in the spodsols of a northern hardwood forest ecosystem. *Envionmental Science and Technology*, **30**, 2211-2219.

Wang, E. X., Bormann, F. H. & Benoit, G. (1995). Evidence of complete retention of atmospheric lead in the soils of northern hardwood forested ecosystems. *Environmental Science & Technology*, **29**, 735-739.

Wang, W. H., Wong, M. H., Leharne, S. & Fischer, B. (1998). Fractionation and biotoxicity of heavy metals in urban dusts collected from Hong Kong and London. *Environmental Geochemistry and Health*, **20**, 185-198.

Watmough, S. A., Dillon, P. J. & Epova, E. N. (2005). Metal partitioning and uptake in central Ontario forests. *Environmental Pollution*, **134**, 493-502.

Watmough, S. A. & Hutchinson, T. C. (2004). The quantification and distribution of pollution Pb at a woodland in rural south central Ontario, Canada. *Environmental Pollution*, **128**, 419-428.

Watmough, S. A., Hutchinson, T. C. & Dillon, P. J. (2004). Lead dynamics in the forest floor and mineral soil in south-central Ontario. *Biogeochemistry*, **71**, 43-68.

Weiss, D., Shotyk, W., Boyle, E. A., Kramers, J. D., Appleby, P. G. & Cheburkin, A. K. (2002). Comparative study of the temporal evolution of atmospheric lead deposition in Scotland and eastern Canada using blanket peat bogs. *Science of The Total Environment*, **292**, 7-18.

Weiss, D., Shotyk, W., Cheburkin, A. K., Gloor, M. & Reese, S. (1997). Atmospheric lead deposition from 12,400 to Ca. 2,000 yrs BP in a peat bog profile, Jura mountains, Switzerland. *Water Air and Soil Pollution*, **100**, 311-324.

White, J. R. & Driscoll, C. T. (1987). Manganese cycling in an acidic Adirondack lake. *Biogeochemistry*, **3**, 87-103.

Wilcke, W., Guschker, C., Kobza, J. & Zech, W. (1999). Heavy metal concentrations, partitioning, and storage in Slovak forest and arable soils along a deposition gradient. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*, **162**, 223-229.

Wilcke, W. & Kaupenjohann, M. (1997). Differences in concentrations and fractions of aluminium and heavy metals between aggregate interior and exterior. *Soil Science*, **162**, 323-332.

Wilcke, W., Muller, S., Kanchanakool, N. & Zech, W. (1998). Urban soil contamination in Bangkok: heavy metal and aluminium partitioning in topsoils. *Geoderma*, **86**, 211-228.

Wild, A. (1988). *Russell's soil conditions and plant growth. 11th ed.* Longman Scientific & Technical, Burnt Mill, Harlow, Essex, England.

Wilkens, B. J. & Loch, J. P. G. (1997). Accumulation of cadmium and zinc from diffuse immission on acid sandy soils, as a function of soil composition. *Water Air and Soil Pollution*, **96**, 1-16.

Wilson, K. & Walker, J. (2000). *Principles and techniques of practical biochemistry.* University Press, Cambridge.

Witter, A. E., Mabury, S. A. & Jones, A. D. (1998). Copper(II) complexation in northern California rice field waters: an investigation using differential pulse anodic and cathodic stripping voltammetry. *Science of The Total Environment*, **212**, 21-37.

Wong, C. S. C. & Li, X. D. (2004). Pb contamination and isotopic composition of urban soils in Hong Kong. *Science of The Total Environment*, **319**, 189-195.

Wong, S. C., Li, X. D., Zhang, G., Qi, S. H. & Min, Y. S. (2002). Heavy metals in agricultural soils of the Pearl River Delta, South China. *Enviromental Pollution*, **119**, 33-44.

Xu, J. L. & Yang, J. R. (1995). *Heavy metals in terrestrial ecosystem.* China Environ. Sci. Press, Bejing, China (in Chinese).

Yin, Y., Impellitteri, C. A., You, S.-J. & Allen, H. E. (2002). The importance of organic matter distribution and extract : solution ratio on the desorption of heavy metals from soils. *Science of The Total Environment*, **287**, 101-119.

Yin, Y. J., Allen, H. E., Huang, C. P. & Sanders, P. F. (1997). Interaction of Hg(II) with soil-derived humic substances. *Analytica Chimica Acta*, **341**, 73-82.

Zhang, Z. H., Chai, Z. F., Mao, X. Y. & Chen, J. B. (2002). Biomonitoring trace element atmospheric deposition using lichens in China. *Environmental Pollution*, **120**, 157-161.

Website References

1. http://www.lenntech.com/Periodic-chart-elements.htm (03/08/04).

2. www.naei.org.uk (16/09/04).

3. www.epa.gov/airtrends (16/09/04).

4. http://www.helcom.fi/environment/indicators2003/hemissions.html (23/09/04).

5. www.nico2000.net/Book/Guide3.html (20/05/02).

6. www.omega.com/techref/ph-5.html (08/10/02).

7. www.multimap.com (12/12/04).