University of Strathclyde
Department of Pure and Applied Chemistry

Determination and Fractionation of potentially toxic elements in urban particulates

by

Balarabe S. Sagagi

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy

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This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Dedication

To the memory of my late father Alhaji Sarki Yaro who died at a critical time when I was just about to submit this thesis. May your gentle soul rest in perfect peace.

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A lot of people have contributed toward the successful completion of this work; they are so numerous that I cannot mention all their names. However their contributions are highly appreciated and acknowledged.

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ABSTRACT

Urban environmental pollution is of increasing concern. There is a need for improved analytical methods to assess risk from potentially toxic elements in urban soils and related material. This thesis focuses on development and application of such methods, notably the modified BCR sequential extraction procedure. The analytes studied were Cr, Cu, Fe, Mn, Ni, Pb and Zn, and quantification was by ICP-AES and ICP-MS.

Results obtained by BCR extraction of urban allotment soils were compared with those from *aqua regia* digestion, 0.05 M EDTA extraction, and the SBET. Correlation analysis and principal component analysis (PCA) grouped analytes into a set mainly of lithogenic origin (Cr, Fe, Mn,Ni) and another of mainly anthropogenic origin (Cu, Pb, Zn). The PCA indicated relationship between amounts of analytes released in step 1 of the sequential extraction and EDTA-extractable analytes, and between results of the SBET method and those obtained with EDTA.

The BCR extraction was then miniaturised for application to urban airborne particles. The fractionation pattern was not affected by reducing the sample mass from 1 g to 0.0625 g for particles < 45 µm and 500 – 1000 µm in diameter, but changing the size of vessel used did have an effect. When the miniaturised extraction was applied to test samples presented on filter dynamics measurement system (FDMS) filters a significant Zn blank was found. Despite this, recoveries within 30 % of certified values were obtained when 0.1 g test portion of BCR CRM 701, loaded onto FDMS filters, were extracted.

Finally, the chemometric identification of substrates and element distribution (CISED) method was miniaturised and adapted for application to particulate samples on FDMS filters. Four distinct physicochemical components were extracted from BCR 701 using the traditional CISED method, but 11 components when the CRM was loaded onto a filter. The mobilities of the PTE as predicted by the CISED were in good agreements with fractionation patterns obtained using the BCR sequential extraction protocol.

1 Introduction

Some potentially toxic elements (PTE) are biologically essential for living organisms including humans.[1-3] but the presence of these elements at a higher concentration may cause toxic effects if human populations are exposed to them.[4]

According to the World Urbanisation Prospect: 2011 Revisions[5] (figure 1.1), published by the United Nations, more than half of the world population is now living in urban areas. It is projected that by 2050, the world urban population will increase by 75 % (from 3.6 billion in 2011 to 6.3 billion in 2050).

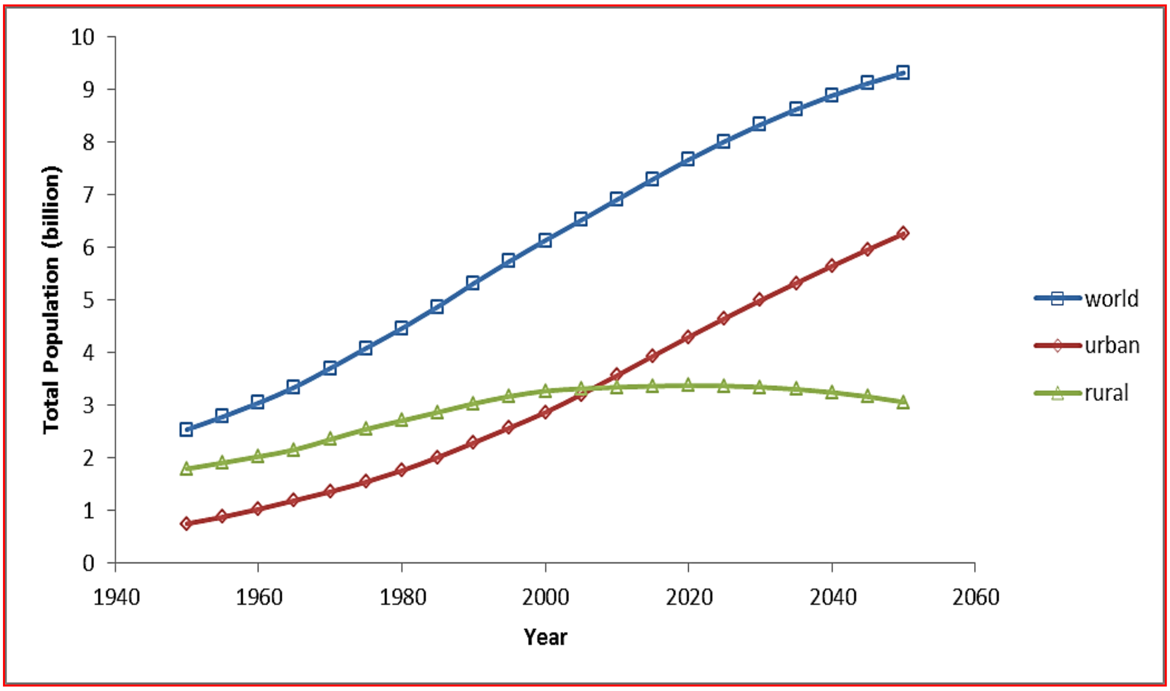


***Figure 1.1** World population and urbanisation prospect*[5]

Due to accelerated urbanisation, anthropogenic activities have become more intense, thereby increasing the amount of contaminants being released into the urban environment. This leads to various forms of environmental problems of which PTE pollution is a major issue especially in urban soils and roadside dust.[6, 7] Because of their non-biodegradability and their long biological half-lives for elimination, the accumulation of these elements in the food chain can have a significant effect on human health in the long term.[1, 2] Human beings are exposed to the risk of these elements through a direct link via inhalation,[8, 9] oral ingestion[10, 11] and dermal contact.[12] Children are more vulnerable to the adverse effect of soil ingestion than adults due to their developing nervous system and high absorption rate[13, 14] and are likely to ingest greater quantity of soil or dust by pica and hand or finger sucking which are considered to be the main pathways of exposure to metals in soils by children.[15, 16] These elements can also be taken in through the indirect paths; the elements are transported into receiving bodies of water through urban runoff causing the enrichment of PTE in the sediments.[17, 18] These PTE can be taken up by aquatic organisms and thereby potentially causing metal contamination of the food chain, or the element can be taken up by plants from contaminated soils, and this too is a route for PTE entry into the food chain.

Owing to the growing dominance of urban living and the potential danger posed by these elements, there is a pressing need to investigate the behaviors of PTE in urban environments, especially the different forms in which they exist (speciation), since this governs their transport, fate, bioavailability and ultimately toxicity to humans.[19]

1.1 Urban Particulates[20-22]

Particulate matter is a term used for the mixture of solid particles and liquid droplets suspended in the air. Qualitatively it can be classified according to size into coarse and fine particles. Some common names are used to describe atmospheric particles:

Dust, Smoke and Soot: solid particles

Mist and Fog: liquid particles or droplets

Aerosols: collections of particulates, whether solid particles or liquid droplets, dispersed in air

1.1.1 Coarse Particles (PM_{10})

These are particles with aerodynamic diameter between 2.5 µm and 10 µm. They are formed by break-up of larger pieces of materials (e.g. crushing, grinding, pulverizing of materials by vehicles and pedestrian, wind erosion, etc), evaporation of sprays and suspension of dust. The composition of PM_{10} varies from place to place and from different sources. Atmospheric dust, especially from rural areas, consist of elements similar to that of the Earth's crust, i.e. high concentration in Al, Ca, Si and O in the form of aluminum silicates, some of which also contain calcium ion. High concentration of solid sodium chloride is found near and above oceans, as sea spray leaves salt particles when the water evaporates. Pollen released from plants and volcanic ash particles are mostly of coarse size. The main sources of coarse particles include natural ones such as volcanic eruption and those from anthropogenic activities such as agriculture, construction and demolition and fly ash from fossil fuel combustion. The residence time of PM_{10} in the atmosphere is from minutes to hours and its travel distance varies from less than 1 km to 10 km.

1.1.2 Fine Particles ($PM_{2.5}$)

These have aerodynamic diameter of less than 2.5 µm. They are formed mainly by chemical reactions and by the coagulation of smaller species including molecules in the vapour state. They are composed of various combinations of sulfate compounds, nitrate compounds, carbon compounds, ammonium ions, hydrogen ions, organic compounds, metals and particles bound to water. The major sources of $PM_{2.5}$ are fossil fuels combustion, vegetation burning and the smelting and processing of metals. The residence time for $PM_{2.5}$ is from days to weeks and travel distance from hundreds to thousands of km.

1.1.3 Deposition of Particulate Matter

Particles emitted either naturally or by human activities to the atmosphere are ultimately deposited on to the Earth's surface by two main deposition methods: dry deposition and wet deposition.

In dry deposition the particles are deposited by the act of gravitation (sedimentation), or by inertia impaction where the particles strike an obstacle and are deposited. They can also be deposited by diffusion; in this case the particles migrate to, and collide with, a surface where they remain.

In wet deposition, the particles are washed out by falling rain or snow on to the Earth's surface. The rates at which the particles fall depend on the size of the particle. For particles whose diameter is greater than 1 µm, the rate can be found quantitatively by Stoke's law:

$$\upsilon = gd^2 (\rho_1 - \rho_2)/18\eta$$

Where g = the acceleration due to gravity
d = diameter of the particle
ρ_1 = density of the particle
ρ_2 = density of air
η = viscosity of air

The dispersion and distribution of the PTEs associated with particulate matter are highly dependent on the size of the particles and on the surface properties of the substrate surface on which they are deposited. Those deposited on the land in an urban environment can be readily relocated and dispersed by wind, rain, and surface runoff.[23]

1.2 Potentially Toxic Elements in Urban Particulates

Various research works[7, 24-33] have shown that the major sources of PTE in urban soils and road side dust are from anthropogenic activities, and that they tend to accumulate within the top 30 cm of soil even with decades of exposure to traffic[34] (although their penetration into the soil may increase due to tilling of the soil[35]).

1.2.1 Soil[1, 2]

Soil is defined as "dynamic natural body composed of mineral and organic solids, gases, liquids and living organisms which have properties resulting from integrated effects of climate, organisms, parent material and topography over periods of time and which can serve as a medium for plant growth".[36] The mineral component is mainly composed of rock fragments formed by weathering and secondary minerals such as clay minerals and oxides of Fe, Mn and Al, while the organic component consists of living organism, dead plant materials (litters) and decomposed plant material (humus). Soil is an important component of the ecosystem not only as a sink for contaminants but also because it acts as a natural buffer, controlling the transport of chemical elements and compounds to the atmosphere, hydrosphere and biota. It is the main source of trace elements for plants both as micronutrients and as pollutants; it is also a direct source of these elements to humans through ingestion, inhalation and dermal contact.

1.2.1.1 Sources of PTE in soils

The major sources of PTE in soils are lithogenic and anthropogenic.

Lithogenic sources

The geological minerals in the soil parent material are the key sources of the PTE in soils: the types and concentrations of PTE depend on the type of the parent material. The elements are released from these minerals into the soils during weathering and pedogenic processes. Soils developed on ultramafic rocks are known to contain high concentrations of Co, Cr and Ni. For example, a survey of rural areas in Northern Ireland showed that the mean concentrations of Cr and Ni in soils from those areas were higher than the rest of UK, which was attributed to a greater occurrence of mafic and ultramafic soil parent materials.[37] Serpentine soils from northern Spain were found to contain up to 1,160 mg/kg of Cr and 940 mg/kg of Ni compared with up to 50 -100 mg/kg of Cr and Ni for control soils on other parent materials.[38] Sedimentary ironstone are found to accumulate a high amount of As, Ba, Cu, Mn, Mo, Ni, Pb, V and Zn,[39] while due to their high organic and clay contents, black shales have elevated concentrations of a variety of PTE. Black shales have been found to be a source of high Cd concentrations in soils in different parts of the world, e.g. 60 mg/kg in China, [40] 11 mg/kg in Korea.[41]

Anthropogenic sources

In this case the PTE are introduced into the soils through some human activities. They are often referred to as contaminants or pollutants. The contamination can either be extensive (covering large area) or localized. In extensive contamination the

PTE are transported from the source of pollution to other areas far from it. Atmospheric deposition is an example of extensive contamination. The PTE emitted from different sources such as industrial emissions from smelters and foundries, coal electricity generating stations, traffic related emissions, domestic heating etc. are released into the atmosphere and can be transported over large distances. They are deposited onto the soil surface by either dry or wet deposition. Flooding and sediment deposition are another example of extensive contamination. This involves the transportation of the PTE particles from sources such as mine wastes discharged into rivers in a form of suspension and, when flooding occurs, these particles are deposited onto soil thereby contaminating it.

Other anthropogenic sources lead to localized contamination, these include; application of inorganic fertilisers, sewage sludge, livestock manures, and fungicides to agricultural soils. Phosphate fertilisers produced from phosphate rock contain contaminants such as As, Cd, Cr, Hg, Pb, Se, U and V and are considered to be the most important diffuse source of PTE in agricultural soils.[42] In Europe, livestock manures are found to contain high concentration of metals. For example, in the UK an average of 870 and 600 mg/kg of Cu and Zn respectively were found in pig and poultry manure.[43] These high concentrations are due to the addition of these elements to the feeds which helps improve food conversion efficiency in the animals.

1.2.1.2 Mechanisms of adsorption of metal ions by soils

The availability of elements in soils for uptake by plants and to the environment depends on the interaction between the element ions in solution and the soil solid phase. The ions are removed from the solution and accumulated on the surface of the soil solid phase by a process known as adsorption. This process controls the availability of these ions in soil solution for uptake by plants and supply to the environment in general. There are various mechanisms involved in the adsorption of metal ions onto the soil particles. These involve both physical and chemical processes and these include: cation exchange (or non-specific adsorption), specific adsorption, co-precipitation and organic complexation.

Cation exchange

This mechanism involves exchange between the metal ions in the soil solution and the counter-ions balancing the surface charges on the soil. It is usually described in terms of the formation of outer-sphere complexes with the surface functional groups to which the ions are bound electrostatically. In this process the ion is in hydrated form, one or two molecules of water are between the surface and the ion. This masking by the water molecules makes the properties of the ions less significant in determining the nature of the binding. This bonding - being an electrostatic coulombic interaction - is very weak and ions involved in this mechanism can be easily available. The process is reversible, stoichiometric and sometimes selective and occurs only on surfaces of opposite charge to the adsorbate.

Specific adsorption

The mechanism involves exchange between ligands on the soil surface and metal cations or even anions with the formation of covalent or ionic bond. This mechanism occurs through inner-sphere complexation, and there is no water molecule between the ions and the surface. The mechanism is specific (dependant on the properties of the metal ion and the surface group), usually irreversible and can occur on a surface regardless of its charge. The ionic and covalent bonds are stronger than the electrostatic binding involved in the non-specific cation exchange, so ions adsorbed by specific adsorption are less available than those held by cation exchange. Beside adsorption, metal ions can also diffuse into minerals such as illites and goethite.[44]

Co-precipitation

This is the simultaneous precipitation of a chemical element with other elements by any mechanism and at any rate[45]. Elements such as Ni, Cu and Zn are found co-precipitated with secondary minerals like Fe oxides and clay minerals in soils.[45]

Organic complexation

Organic materials in soils such as humic acids, lignin and other organic compounds with high molecular weight can adsorb metal ions from soil solution by forming chelate complexes. The stability constants of the chelates of the metals with humic substances follow the following trend: Cu > Fe = Al > Mn = Co > Zn. Soluble complexes are also formed with low molecular weight organic ligands such as short-chain organic acids, amino acids and other organic compounds which prevents the precipitation and adsorption of the ions.

1.2.2 Urban Soil

Urban soils differ greatly from rural soils in many respects,[46, 47] including composition, physical properties and metal content.

The composition of urban soils differs from that of rural soils, especially with regard to the probability of it having originated from several different locations and having been mixed with non-soil materials.[48]Most urban soils have more coarse-grained materials than typical rural soils[48] and they also contain more building wastes and other waste materials.[49]

Unlike rural soils, which show a vertical stratum of soil formation, urban soils have more vertical variability due to excavations and backfilling and also a more pronounce horizontal variability due to different land use.[50]

Urban soil have a more compact structure than rural soils, which is due to treading and soil compaction by vehicles and heavy machinery.[51] Soil organisms are responsible for the incorporation and decomposition of organic matter in soil, and compaction can result in a decrease in population and diversity of these organisms thereby affecting some processes such as litter incorporation.[52] Compacted surface soil may result in sparse vegetation. As a result of the above, urban soils typically contain less organic materials than rural soils.[52]

In terms of the metal content, urban soils differ from rural soils in that they receive additional metals from anthropogenic activities. Since more anthropogenic activities take place in urban areas, urban soils receive a higher load of metals from the immediate surroundings than rural soils.[53]

In recent years, studies of PTE contamination have been carried out in urban soils in many cities around the world,[13, 29, 54-66] further details are provided in section 1.6. These studies have given an insight into the chemistry, mobility and bioavailability of PTE in urban soils and also the possible contamination of these elements in urban soils and risk they pose to human health.

1.2.3 Street Dust

Street dust is another important component of the urban environment; it contributes significantly to urban pollution. The major sources of heavy metal contamination in street dust are water-transported materials from surrounding soils and slope, dry and wet deposition, road paint degradation, vehicle wear (tyres, brake lining, body) and vehicular fluid and particulate emissions.[67] Many studies have been conducted on street dust and most attention was paid to the identification of sources and concentrations of the elements present.[25, 28, 68-72] Topsoil and street dusts in urban areas are indicators of heavy metal contamination from atmospheric deposition. Some studies of street dust have shown that the total metal concentrations are related to industrial and residential areas[73, 74] as well as traffic movements, numbers of vehicles and their speed.[75-77] Charlesworth *et al,* [71] compared the population of some cities and the heavy metal concentrations and they found that, in general, the larger the population, the higher the heavy metal concentration in the street dust. Other studies by Fergusson *et al.*[78] and Ellis and Revitt[76] noted a trend for higher PTE concentration to be found on streets where traffic was more likely to undergo stop-start maneuvers such as at traffic lights. Street dust are characterized by short atmospheric resident times. The metal contents represent only recent accumulation of pollutants[79] and also - due to the variation in local processes - is site specific.[80]

1.3 PTE and their health implications[1, 2, 81]

Potentially toxic elements play fundamental roles in the normal development and health of organisms. These elements can be taken up by humans directly or indirectly through the food chain. Factors such as agricultural practices, variable soil parameters and anthropogenic pollution may influence the content of these elements in food crops and other plants, which leads to deficiencies and excesses of these elements.

Briefly, the chemistry and the health implications of the PTEs under study are discussed below:

1.3.1 Chromium[81, 82]

Chromium is the seventh most abundant element on Earth. The main mineral ores of economic importance are chromite, $FeCr_2O_4$ and crocoite, $PbCrO_4$. Chromium is mainly used for stainless steel and chromate plating; it is also used in pigments, leather tanning and wood preservatives. The main source of Cr pollution is the dyestuffs and leather tanning industry when wastes are discharged directly into streams, either as liquids or solids. Others are phosphate fertilisers, sewage sludge and poultry manures applied to agricultural soils.[83, 84] Chromium is also emitted in large quantities into the atmosphere from ferrochrome production. Chromium exhibits a variable oxidation state but Cr^{3+} and Cr^{6+} are the most common forms in soils. These forms are pH dependent: within the pH range 4-8 Cr^{6+} exists mainly in anionic forms, $HCrO_4^-$ or CrO_4^{2-}, while Cr^{3+} exists as cationic species such as $Cr(OH)_2^+$.[85] At $pH > 5$, Cr^{3+} is precipitated and strongly adsorbed to surfaces of soil particles and this limits its mobility and bioavailability in the soil. In contrast Cr^{6+} as chromate is very soluble in water and is a strong oxidizing agent. This makes it the more mobile and toxic form of Cr.[82, 85, 86]

The world median content of Cr in soils has been established as 54 mg/kg and it mainly occurs in the immobile fraction (> 80 % of total content).[2] Human deficiency of Cr results in increased cholesterol levels, high blood sugar level and coronary dysfunction, while high Cr concentration results in liver and kidney failure, anemia, muscle breakdown and abnormalities in blood clotting. When an excess of Cr compounds is inhaled, lung, nasal, and possible stomach cancer may develop.

1.3.2 Copper[87]

The main minerals of Cu are chalcopyrite, $CuFeS_2$, bornite, Cu_5FeS_4, and chalcocite, Cu_2S. Its main use is in the production of conductor materials, wire, rod and bar; it is also used in coins, art objects and ammunition. Copper is also widely used in agriculture, in pesticides and as a feed additive in livestock and poultry nutrition. Copper is emitted into the atmosphere from both natural processes e.g. volcanic eruption and forest and bush fires, and anthropogenic processes such as fuel combustion and non-ferrous metal production. Other anthropogenic sources of Cu release into the environment include agricultural practices (e.g. application of mineral fertilisers, manure, pesticides and sewage sludge), and traffic related processes (e.g. corrosion of overhead wires in rail transportation and brake and tyre wear in road transportation).

Copper exhibits variable oxidation state, Cu^+ and Cu^{2+} are the most common forms. In aqueous media Cu^+ is unstable and readily converts to Cu^{2+}. In soil the Cu^{2+} binds to both organic and inorganic components in varying affinities, the general order is Mn oxides > organic matter > Fe oxide > clay minerals. However, in general organic matter controls specific Cu adsorption in soil and is generally responsible for holding adsorbed Cu.[88]

The world mean value for the total Cu content in soil has been reported to be between 20 and 30 mg/kg.[1] In humans Cu is an essential element, it is a component of several proteins and metalloenzymes. Copper deficiency in human can result in slow growth, anemia, and hair lost, disorders of the central nervous system, cardiovascular problems, osteoporosis and several other metabolic dysfunction.[89] High concentration of copper in human body as a result of Wilson’s disease in most cases lead to death. Copper is also an essential element for plants and plays a vital role in photosynthesis, cell wall metabolism, respiration and hormone signaling.[90] Copper deficiency normally affects young leaves and reproductive organs with visible symptoms such as malformed leaves and chlorosis.[90] In excess, Cu can be toxic to plants by slowing down their growth.

1.3.3 Iron

Iron is one of the major constituents of the lithosphere and constitutes about 5 % of the Earth’s crust. The main iron ore minerals are hematite, $Fe_2O_3.xH_2O$, siderite, $FeCO_3$, pyrite, FeS_2, and ilmenite, $FeO.TiO_2$. The use of Fe has been known since early human civilization and, it is the most commonly used metal. It is mainly used for different types of tools, and in the transportation and construction industry.

In soils the Fe contents ranges between 0.1 and 10 % and its distribution in the soil profile is variable and controlled by many soil parameters. Despite the extensive use of iron, high natural concentrations in the environment mean that its emission from anthropogenic sources has little relative impact. In humans, lack of Fe causes mainly anemia. It also reduces the availability of other nutrients. When taken in excess, Fe can results in haematochromatosis, siderosis, and liver damage.

1.3.4 Manganese[91]

Manganese is one of the most abundant trace elements in the lithosphere. It belongs to the same family as Fe and it is closely associated with Fe in geochemical processes. It is mainly used in the metallurgical industry for steel and various alloys. It is also used in the production of glass, ceramic and pigments and in agriculture as a component of fungicides and fertilizer.

Its content in soil is variable but the world mean value has been calculated as 437 mg/kg.[2] Manganese is an essential element to both plants and animals. It is involved in many physiological and biochemical processes in plants, so its deficiency can seriously inhibit their growth and health. In humans Mn deficiency results in skeletal and cartilage deformities, while excess Mn can lead to liver cirrhosis, manganism and neurological disorders.

1.3.5 Nickel

Nickel is the 24th most abundant element in the Earth’s crust, and is twice as abundant as Cu. The major ores of Ni are pentlandite, $(Ni,Fe)_9S_8$, millerite, NiS, and niccolite, NiAs. Nickel is mainly used in production of a variety of alloys for aircraft; most the alloys produced have the ability to resist oxidation and corrosion by acids and salts. It also used in the manufacture of permanent magnets and electrical equipment. The major anthropogenic sources in the environment are Ni mining and smelting and burning of fossil fuel.[82] Phosphate fertilisers and livestock manure are also important.[82] Its compounds are widely used as dyes in ceramic and glass manufacture and in batteries containing Ni-Cd compounds. Nickel has a variable oxidation states, but Ni^{2+} is the stable form over the pH range and redox conditions found in soils.[82] Nickel forms complexes with both organic and inorganic ligands and suspended mineral colloids.[92]

The Ni content in soils varies widely throughout the world but the common background average range has been calculated to be between 19 and 22 mg/kg.

Nickel is an essential element for animals and beneficial for plants.[93] A very small amount of Ni is needed for normal metabolism in plants and thus deficiency is very rare. In excess, Ni can cause a decrease in photosynthesis, inhibit growth and may also cause leaf chlorosis and wilting.[93] High concentration of Ni in humans leads to gastric, liver and kidney defects, neurological effects, and emphysema and lung cancer.

1.3.6 Lead[94]

Lead is a non-essential element to both plants and animals and a well-known harmful element to human and other organisms. In nature it exists in different mineral ores, the most important one is galena, PbS; others are cerussite, $PbCO_3$, minium, Pb_3O_4 and anglesite, $PbSO_4$. The main use of lead is in the production of lead-acid batteries. Other uses are in solder, cables, alloy, pipes, roofs, pigments in paints, lead weights, and lead wool. It is also used as an additive in petrol as an anti-knock agent. In developed countries this has been phased out in an effort to reduce the atmospheric Pb concentration, but in some countries, especially the developing ones, it is still being used. Lead is also released into the environment from coal burning and Pb ore smelting. Lead is mainly in +2 oxidation state in soils and its behavior depends strongly on the amount of organic matter in the soil. At $pH \geq 4$ lead is strongly adsorbed to humic matter[95] and it is thought to bind mainly as a monodentate complex.[96] However in the absence of appreciable amount of organic matter, Pb is strongly adsorbed on clay minerals and Fe oxides.[97]

The world mean concentration of Pb in surface of soil has been calculated to be 25 mg/kg.[2] Excess Pb in humans may cause several health effects such as: kidney damage, impaired reproduction, carcinogenicity and genotoxicity, impaired mental development of young children, inhibition of haem formation and damage to the nervous system.

1.3.7 Zinc[98]

Zinc an essential element for humans, animals and plants. The major mineral ore in which Zn is found is sphalerite, ZnS; other minerals are smithsonites, $ZnCO_3$, willemite, Zn_2SiO_4 and zincite, ZnO. Zinc is mainly used in industries as corrosion protection on steel and other metals. It is used also in the manufacture of batteries, automotive equipment, pipes and household devices. Its compounds are used in dental and medical applications. The major anthropogenic sources of zinc in the environment include burning of coal and other fossil fuels, smelting of non-ferrous metals, and the application of sewage sludge, fertilisers and pesticides to agricultural soils. Natural sources such as volcanic eruption and aeolian dust also contribute to the amount of Zn in the environment.

In soil, the total concentration of Zn varies widely, from 10 to 300 mg/kg with a mean value of 50 mg/kg. Deficiency of zinc leads to anorexia, anemia, impaired keratosis and teratogenic effects. The ingestion or inhalation of large doses of Zn can cause damage to the alimentary tract and metal fume fever; it can also cause chills, excessive sweating and weakness.

1.4 The determination of PTE in environmental solids[99-101]

Determination of the different forms in which elements exist in environmental solids gives more information on their mobility, availability and toxicity than the total concentrations. These forms can be determined either by direct (or nondestructive) or indirect methods.

1.4.1 Direct methods

Some analytical methods involve direct measurements of the PTE content in the solid sample without decomposition of the sample. One of the major advantages of these methods is that, they do not require complicated sample handling as such problems related to decomposition and separation such as dissolution, contamination of samples and analyte losses are avoided. During analysis in these methods the speciation also remained unchanged. Some of the direct instrumental techniques used for soil analysis include synchrotron-based X-ray radiation fluorescence (SXRF) spectroscopy,[102, 103] particle-induced X-ray emission (PIXE)[103] and extended X-ray absorption fine structure (EXAFS).[102, 103] In the X-ray spectroscopic methods, an electron is ejected from an inner shell of an atom and an electron from an outer shell falls down to replace it by emitting X-ray radiation equal in energy to the difference between the energies of the two shells. An X-ray spectrum is produced in this way with radiation of different wavelengths and intensities that are characteristic to the atoms present in the sample and their concentrations. Beside the X-ray based techniques, other nondestructive methods used include: instrumental neutron activation analysis (INAA), laser-induced breakdown spectroscopy (LIBS) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS).[104] Most of these methods are less commonly used because they are less sensitive for some environmentally important trace elements, such as Cd, Cu and Zn when compared with other techniques (for example ICP-MS). Also some of these methods require some highly sophisticated and expensive components that are normally very rare to be found in most common laboratories. For the example, for INAA an access to nuclear reactor is required while a method like PIXE a very expensive component (particle accelerator) is required.

1.4.2 Indirect methods

In the indirect methods of analysis, the elements are extracted from the solid substrates using appropriate reagents and analysed using a suitable analytical technique. There are some disadvantages of using these methods, such contamination of samples and loss of analytes during the extraction process. Also the speciation in the sample may change during the extraction. In this thesis very sensitive analytical techniques (ICP-AES and ICP-MS) were employed, these techniques allow the determination of the PTE under study at very low concentrations. These techniques require the analytes in solution and for these reasons the indirect methods of analysis were used in this work.

Many extraction procedures have been developed and the choice of the procedure depends on the solid substrates and the information required from the analysis. Some different approaches used in extraction are illustrated in figure 1.2.

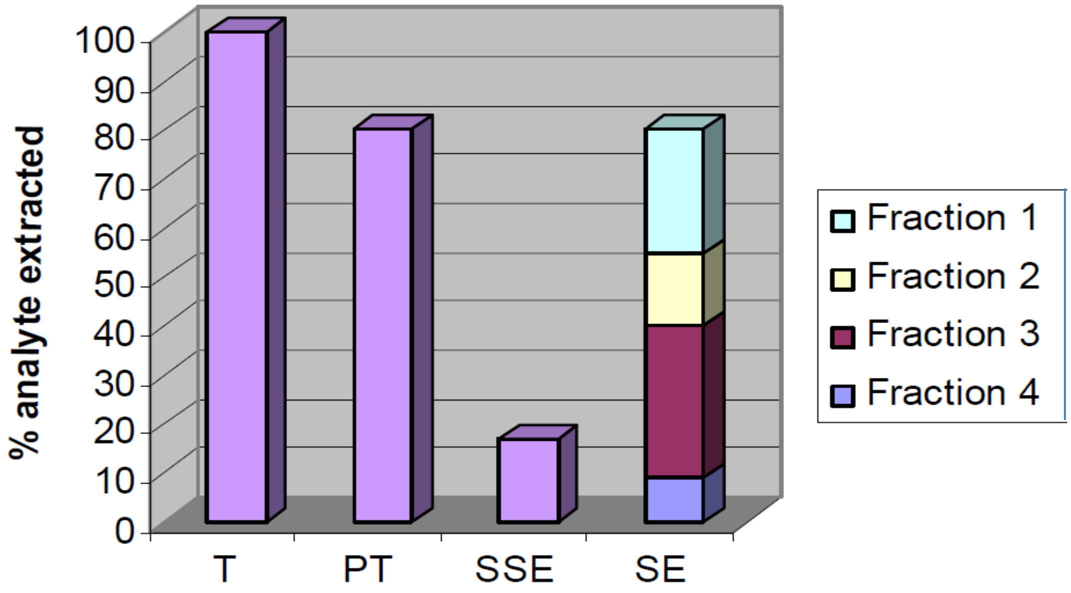


Figure 1.2 Approaches to PTE extraction (T-total, PT- pseudototal, SSE- selective single extraction, SE- sequential extraction) (after[105])

Total Digestion

Total digestion is used when a complete liberation of metals from an environmental solid is required; hydrofluoric acid is usually used as it has the ability to liberate even the silicate-bound metals. In some cases HF is combined with oxidising acids such nitric or perchloric in order to help in dissolving organic components of soils. Total digestion gives information on both the geological origins of soils and the anthropogenic inputs. However, for environmental studies, it is often the amount that is added from the anthropogenic sources that is of interest, since under natural condition it is very unlikely for silicate bound metals to leach.

Pseudototal Digestion

In pseudototal digestion all metals bound to environmental solids are liberated except the silicate-bound ones. This is the fraction that is of more interest to environmental analyst as it represents the maximum amounts of metals that are potentially available to organisms under natural conditions. *Aqua regia*, a mixture of hydrochloric and nitric acids in a ratio 3:1 is mainly used in this digestion method. The total and pseudototal digestions can both be carried out in an open vessel e.g. in a beaker covered with watch glass and heated on a hot plate in a fume cupboard or in a closed vessel such as in bomb digestors or in microwave digestors. Microwave assisted digestion is now extensively used. It exhibits the following advantages: it is a very fast method, it gives uniform heating throughout the mixtures, and contamination is minimised.

Single Extraction: reagents other than acids can be used to extract metals from phases of interest in environmental solids. Soil scientists used single extraction methods to assess plant-available element contents in soils using different reagents such as water[106, 107], 1 M $CH_3COO\ NH_4$[107, 108], 1 M NH_4NO_3[107-109], 0.01 M $CaCl_2$[107, 110], 0.1 M $Ca(NO_3)_2$[111], 0.005 M diethylene triamine pentaacetic acid (DTPA)[112, 113], 0.05 M ethylenediaminetetraacetic acid (EDTA)[114, 115] and 0.01 M HNO_3[107]. These single extractants are also used to extract difference mineral phases in soils, though phase specificity cannot always be guaranteed.

Sequential Extraction: instead of using a single reagent to extract metals associated with a particular phase, a series of reagents can be used to partition the metals into various phases. In sequential extraction, series of reagents are added sequentially to the same substrate in the order of increasing “harshness”. The information obtained from this method can be used to estimate the mobility, bioavailability and toxicity of the metals.

1.5 Chemical Speciation

This term has been widely used by different researchers with different meanings; however in this work the definition put forward by Ure[116] is used. He defined chemical speciation as either “the *active process* of identification and quantification of the different *defined species, forms or phases* in which an element occurs in a material” or “the *description* of the amounts and kind of species, forms or phases present in the mineral”. He proposed the classification of speciation into:

Classical speciation: which refers to specific compounds or oxidation states of an element, e.g. Cr^{3+} and $Cr^{6+?}$

Functional speciation: this refers to the observed role of behaviour or the element, e.g. “plant available, exchangeable cations”

Operational speciation: which refers to the speciation where the reagent used to extract the sample defines the species, e.g. water /acid soluble, oxidisable, residual fractions.

Sequential extraction is an example of operation speciation.

1.5.1 Sequential Extraction

Sequential extraction involves use of series of reagents to leach out metals in different phases in a solid substrate. The phases that are leached out are actually operationally-defined phases and that means specific mineral phases are not necessarily removed. The reagents are applied in an increasing order of harshness, from mild extractants like water to strong mineral acids, so that the successive fractions obtained correspond to metal association forms with lesser mobility. This is normally intended to simulate various possible natural and anthropogenic modifications of the environmental conditions. The approach provides information on reservoirs of metal that are likely to respond in different ways to changes in ambient conditions such as pH or redox potential.[19]

Numerous sequential extraction schemes have been developed in the past 35 years, this can be traced back to the work of Tessier *et al.*[117] where a 5-step scheme was used to fractionate some PTE from river sediment. This scheme, even though developed for fluvial bottom sediments, has since been widely used in soils. The schemes developed use different kinds of extraction reagents, numbers of steps and extraction conditions on varieties of environmental substrates such as soils, sediments, sludge, road dust, mining waste, fly ash, etc. Table 1.1 gives a few recent examples of the sequential extraction procedures developed.

Rao *et al.*[118] reviewed several sequential extraction schemes by different workers; they gave a full details of the different methodologies adopted for different varieties of solid samples (soils, industrially contaminated soils, sewage sludge and sludge amended soils, road dust and runoff and waste materials). The application of sequential extraction schemes for metals in various environmental solids was also reviewed by Filgueiras *et al.*[119] The different schemes used, elements analysed and analytical technique used were given. Gleyzes *et al.*[120] in their review discussed the various reagents used in different schemes with their advantages and disadvantages. An excellent critical review was given by Bacon and Davidson.[121] Their review focused on some key issues surrounding use of sequential extraction procedures such as nomenclature, methodologies, and presentation of data. The review also discussed the emerging developments in sequential extraction such as the use of ultrasound and microwave-assisted extraction to speed up extraction processes, dynamic extractions, the use of chemometrics and the extension of the procedure to non-traditional analytes such as arsenic, mercury, selenium and radionuclides.

From the various sequential extractions procedures reviewed, the modified BCR sequential extraction protocol was chosen for work in this thesis. This is because, results from this work can be easily compared with others as the method harmonises various sequential extraction methods. A certified reference material is also available for this procedure to evaluate the accuracy of the work.

Table 1.1 Examples of sequential extraction schemes recently developed

S/N	Author	Matrix	Elements determined
1	Jamali *et al.*[122]	Untreated sewage sludge from Pakistan	Cd, Cr, Cu ,Ni, Pb and Zn
2	Banerjee[24]	Street dust from Delhi	Cd, Cr, Cu, Ni, Pb and Zn
3	Arain *et al.*[123]	Fresh water sediment	Cd, Cr, Cu, Ni, Pb and Zn
4	Meza-Figueroa[124]	Dust from Mexico	Ba, Cd, Cr, Cu, Co, Fe, Ni, Pb,V and Zn
5	Soco and Kalembkiewicz[125]	Industrial fly ash	Co and Ni
6	Rigol *et al.*[126]	Mineral and organic soils	Radiostrontium and radio cesium
7	Silveira *et al.*[127]	Tropical soils from Brazil	Cu and Zn
8	Benitez and Dubois[128]	Soil samples of the Swiss Jura	Cd,Cu,Ni, Pb and Zn
9	Kim and McBride[129]	Sludge amended soils	Cd,Cu Pb and Zn
10	Nakamaru *et al.*[130]	Agricultural soil from Japan	Se
11	Lerouge *et al.*[131]	Callovian-Oxfordian claystone	Sr

1.5.1.1 Reagents and targeted phases in environmental solid substrates

Water soluble fraction

Water extracted PTEs are the most labile and therefore most bioavailable. This fraction consists of all water soluble species in the soil and includes free ions and ions complexed with soluble organic matter and other constituents. Deionised water at a certain soil-water ratio is typically used to extract this phase. The extract can be separated from the solid phase by centrifugation,[132] filtration,[44] displacement,[133] or dialysis.[19] The amounts of metals extracted in this fraction is usually very low, often below the detection limits of available routine analytical techniques (FAAS, ICP-OES).[134] In most cases the water soluble and the exchangeable fractions are determined together.

Exchangeable fraction

This fraction consists of metals that are weakly adsorbed on the surface of solid substrate by weak electrostatic interaction, those that can be released by ion-exchange processes. Changes in the ionic composition, influencing adsorption-desorption reactions, or lowering pH could cause remobilisation of metals from this fraction[19, 67, 135].

The exchangeable fraction can be released by displacement of the metals from the organic and inorganic sites of the substrate with cations such as K^+, Ca^{2+}, Mg^{2+} or NH_4^+. Salt solutions of these cations are usually used in the extraction of this fraction, such as 0.1 M $CaCl_2$[136], $MgCl_2$[117], 1 M KNO_3[137], 1 M NH_4Cl[126], 1 M CH_3COONH_4.[138] The most widely used reagent is 1 M $MgCl_2$, it combines the strong ion-exchange capacity of Mg^{2+} and the weak complexing power of the Cl^-. It has the advantage that is does not attack silicates, metal sulfides and organic matter. Although it slightly dissolves carbonate (2 -3 %), this can be avoided by reducing the contact time[117]. Another reagent that is commonly used is CH_3COONH_4, the metal complexing power of acetate helps in the prevention of readsorption or precipitation of the leached elements onto the surface of the solid substrate, but the difficulties in reliable analytical determination is the major setback.[139]

Acid soluble fraction

This fraction contains metals which are precipitated or coprecipitated with carbonates.[140]The carbonate fraction is a loosely bound phase and liable to change with environmental conditions.[141]This fraction is pH sensitive, and metal release is attained through dissolution of a fraction of the solid material at pH ≤ 5.

The reagent widely used in the extraction of this fraction is a buffered 1 M acetic acid/ sodium acetate solution at pH 5.[117, 142] However at this pH some of the metals specifically sorbed on the substrate may also dissolve and complexation with reagent must also be assumed.[135] The reagent is capable of dissolving carbonates without significant attack on organic matter, Fe and Mn oxides and aluminosilicate.[117]

This fraction and the exchangeable are considered to be more potentially bioavailable, while the reducible and oxidisable fractions are relatively stable under normal soil condition.[143]

Reducible fraction

Iron and manganese oxides are excellent scavengers of metals. In reducing conditions such as water flooding Fe^{3+} and Mn^{4+} are reduced and their subsequent dissolution could release the PTE in to the environment.[140, 144] The reducible fraction can be subdivided into 3 groups: easily reducible (Mn oxides); moderately reducible (amorphous Fe oxides); and poorly-reducible (crystalline Fe oxides) fractions. In some sequential extraction methods these sub-fractions are differentiated[145, 146] while in others are considered as just a single reducible fraction to simplify the procedure.[147]

The easily reducible fraction is widely extracted using hydroxylamine hydrochloride which is a reducing agent (E^o = - 1.87V) in nitric acid medium. However this reagent releases some amount of elements bound to organic matter, and this will leads to the overestimation of this fraction at the expense of the oxidisable fraction.[135] By using 1 M hydroxylamine hydrochloride in acetic acid medium, the amorphous Fe oxides fraction is released without attacking the silicate or organic fractions,[148] but at $pH < 1.5$, some of the silicate fraction is released.[117]

Oxalate/oxalic acid buffered solution at pH 3 is another widely used reagent. It is fairly selective for dissolving amorphous Fe oxides, so it has been used for leaching of the moderately reducible fraction. The leaching of organically bound metals is likely to occur due to the complexing capacity of the oxalate, and so extraction of the organic fraction using sodium hypochlorite prior to extraction using oxalate/oxalic acid solution has been recommended.[149]

Sodium citrate/sodium dithionite buffer (DCB) has also been used and can adequately dissolve the crystalline Fe oxide, but most of the commercially available dithionite salts can contain Zn and a complex purification steps are required.[117] Also, due to the high salt content, analysis of the extract using FAAS becomes a problem.[117]

Oxidisable fraction

Trace elements may be integrated in many forms of organic matter including organisms, organic coatings on inorganic particles and biotic detritus.[150] Potentially toxic elements in this fraction tend to stay longer in the soil but are mobilised by decomposition processes.[151] Under oxidising conditions, these organic materials tend to be degraded, resulting in the release of sorbed metals.[140] Thus, oxidising agents such as H_2O_2 (E^o = 1.77V) or NaClO (E^o = 0.90V) are used to extract elements from this fraction. Some oxidising agents tend to simultaneously oxidise any sulfides present.

Hydrogen peroxide in acidic medium has been applied in some schemes.[117, 142, 145] This reagent applied at 85^{o}C for several hours is preferred as a compromise between dissolving the organic matter and minimum alteration of the silicates.[117] The major problem with using this reagent is the metal readsorption in the residual fraction which requires an additional extraction step with ammonium acetate at pH 2. Many schemes now use a combination of H_2O_2 and NH_4OAc to extract the metals associated with organic matter and sulfides, where the NH_4OAc prevents the readsorption of the metals onto the oxidised substrate.

For the extraction of this fraction, NaClO at pH 9.5[149] is also used. This reagent does not dissolve Fe/Mn oxides or carbonate, and it found to be superior to H_2O_2 in removing organic carbon. Other reagents used are $Na_4P_2O_7$ at pH 9.5,[152] and $K_4P_2O_7$[153, 154]The pyrophosphates solubilise organic matter through complexation. The setback of these reagents is their poor extraction efficiencies.[155]

Residual fraction

This normally consists of metals contained in the crystal lattice of the primary and secondary minerals. They are usually extracted by digestion with strong acids that do not usually dissolve the silicate matrix, such as $HClO_4$, HCl, HNO_3 and *aqua regia*. This gives an estimate of the maximum amounts of elements that are potentially bioavailable with changing environmental conditions, and so can be used to assess the long term potential risk of the elements going into the biosphere.

Digestion using *aqua regia* is now a widely used procedure and has been used as a reference procedure in the preparation of soil and sediment reference materials certified for extractable content by the Commission of European communities, Community Bureau of Reference (BCR), later superceded by the Standards, Measurement and Testing Program (SM&T).

1.5.1.2 Harmonisation of sequential extraction schemes: BCR Procedure

Many sequential extraction schemes were developed using different steps, reagents and extraction conditions. Comparing results obtained from these schemes became difficult and, in 1987, the BCR, launched a project to harmonise the measurements of extractable metal content in soils and sediment.[156]This led to the development of a harmonised 3-step sediment sequential extraction protocol[156] together with the production of a certified reference material (CRM 601).[157]

There were reports of problems of irreproducibility of the BCR scheme especially the step 2. It was shown that the pH adjustment could be the major source of the irreproducibility, as the amount of the nitric acid added varied from laboratory to laboratory. The same was found by Davidson *et al.*[158] in a study of polluted soil. It was then proposed that a fixed volume of dilute nitric acid be added to adjust the pH, and the concentration of the reagent was changed from 0.1 to 0.5 M. A revised version, known as the modified BCR procedure was recommended, [159] and a new reference material (CRM 701) was certified for the extractable metals using this modified procedure.[160] In the modified scheme, it was recommended that an additional step be added, in which the residue from step 3 is digested using *aqua regia* and the sum of all the steps be compared with the result of a separate *aqua regia* of the whole soil (pseudototal content). This serves as an internal check to assess the overall effectiveness of sequential extraction and element recoveries.

1.5.1.3 Limitation of sequential extraction

The major problems associated with the use of sequential extraction schemes are:

- Non-selectivity of reagents for target phase
- Incomplete extraction
- Re-adsorption and re-distribution of analytes among phases during the extraction process
- Formation of artefacts (new mineral phases)

Despite these limitations, sequential extraction has proved to be an important and widely applied tool for gaining information on the potential mobility and hence bioavailability and toxicity of PTEs in the environment. The application of this method is more likely to be successful if some basic conditions are met.[121]These include the use of ‘standard’ sequential extraction method (e.g. revised BCR protocol), validating the experimental work using CRM or mass balance. The ‘standard method should be strictly adhered to; otherwise any deviations should be reported. The scope and limitations of the approach should be taking into cognizance.

1.6 Previous studies on PTE in environmental urban particulates

Due to accelerated industrialisation and urbanisation, intense anthropogenic activities are taking place within urban environments this leads to increase in the amounts of contaminants being discharge, of which PTE are of great interest because of their toxicological effects. This topic has raised a lot of interest in the last few decades. This section will briefly discuss some studies of interest found in the literature.

1.6.1 Total metal contents in urban soils

The total metal content of some urban soils has been determined for different cities in the world. Table 1.2 shows the total concentration of some metals in urban soils from some cities.

Table 1.2 Mean metal content (mg/kg) in urban soils from different cities in the world

City	Cr	Cu	Ni	Pb	Zn	Ref
Islamabad, Pakistan		18	91	208	1643	Ali and Malik[54]
Izmit, Turkey	35	25	40	32	74	Canbay *et al*[55]
Ulaanbaatar, Mongolia	20	36	19	64	159	Batjargal *et al*[161]
Damascus, Syria	57	34	39	17	103	Moller *et al*[62]
Glasgow	45	85	35	307	199	Rodrigues *et al*[162]
Hangzhou, China		52		88	207	Lu and Bai[163]
Ibadan, Nigeria	64.4	47	20.2	95	229	Odewande and Abimbola[66]
Ljubljana, Slovenia	34	39	26	87	148	Biasioli *et al*[65]
Napoli, Italy	11	74		262	251	Imperator *et al*[58]
Palermo, Italy	39	77	19	253	151	Manta *et al*[13]
Sevilla, Spain	34	55	28	123	105	Biasioli *et al*[65]
Shanghai, China	108	59	31	71	301	Shi *et al*[164]
Torino, Italy	191	90	209	149	183	Biasioli *et al*[63]

These values were used in most cases to evaluate the extent of contamination of the soils and this was sometimes done by determining the enrichment factor, EF which is calculated by comparing the metal content in the surface and deep layers. The approach is based on the fact that contaminants from anthropogenic activities are supposed to accumulate in the surface layers. If EF > 1, then the concentration of the element is higher than the background value and this may show the influence of an anthropogenic contribution to the soil. However in urban environments this may not necessarily be true as the soils are frequently mixed up (e.g. in road construction), new soil is added and other construction materials incorporated into the soils. A new approach is now sometimes used to evaluate the degree of contamination by comparing the metal content in the urban samples with that of rural samples, and this give the Pollution Index (PI). Lu and Bai[163] reported PI values which varied greatly between different metals and land uses, for example they reported the average PI values for Cd, Cu, Pb and Zn as 7.4, 2.9, 3.4 and 3.0 respectively. These data indicate that the Cd, Cu, Pb and Zn pollution widespread in the urban soils they studied.

1.6.2 Total metal content in street side dust

Street side dust represents a dangerous carrier of PTE and organic compounds which can easily be re-suspended by passing vehicles, trams and wind, resulting in an important source of atmospheric particulate matter. In urban areas the re-suspension of street dust is an important carrier of vehicle-related pollutants into atmospheric PM. The metal contents of some street dusts were investigated by various workers during the last few decades. Table 1.3 gives a summary of some findings.

Table 1.3 mean level of the metal concentrations (mg/kg) in street dust samples in different cities of the world.

City	Cr	Cu	Fe %	Mn	Ni	Pb	Zn	Ref.
Amman, Jordan		177			88.0	236	358	Al-Khashman[165]
Baoji, China		123		804	49.0	408	715	Lu *et al.*[166]
Hermosillo, Mexico	11.2	26.3			4.70	36.2	388	Meza-Figueroa *et al.*[124]
Honolulu/Hawaii, USA	273	167	6.59	1040	177	106	434	Sutherland and Tolosa[18]
Islamabad, Pakistan		52.0			23.0	104	116	Faiz *et al.*[25]
Kavala, Greece	196	124			58.0	301	272	Christoforidis and Stamatis[70]
Luanda, Angola	26.0	42.0	1.15	258	10.0	351	317	Baptista and De Miguel[33]
Ottawa, Canada	43.3	65.8	1.9	4315	15.2	39.1	113	Rasmussen *et al.*[16]
Shanghai, China	159	197			84.0	295	734	Shi *et al.*[164]
Urumqi, China		94.5			43.3	53.5	295	Wei *et al.*[167]

Just as in the case of the urban soils, some of these values were used to evaluate the extent of contamination of the environment by calculating the Pollution Index (PI). For example Faiz *et al.*[25] calculated the PI for Cd, Cu, Ni, Pb and Zn in street dust of the Islamabad Expressway. They found that the pollution level of this site is in the category of "lower to intermediate level", this is based on the classification PI reported by Lu *et al.*[166]

1.6.3 Sequential extractions on urban soils

Table 1.4 gives some previous studies involving the use of sequential extraction methods to urban soils.

Table 1.4 Examples of sequential extraction schemes applied to urban soils

Author	Extraction scheme	Element	Summary of findings
Davidson *et al*[168]	Modified BCR	Cr, Cu, Fe, Mn, Ni, Pb and Zn	Cr, Fe, Ni, at most sites were found mainly in association with the residual phase. Cu was present in the reducible, oxidisable and residual fractions, whilst Zn was found in all four sequential extracts. Mn was strongly associated with reducible material as, in some cities, was Pb. No overall relationships were, found between analyte concentrations and land use, nor between analyte partitioning and land use.
Gibson and Farmer[142]	6 steps sequential extraction	Cd, Cu, Pb and Zn	32 % of Cd was in exchangeable and carbonate fraction, 51 % of Pb in moderately reducible fraction and 41 % of Cu and 29 % Zn were in organic fraction. The mobility of the metal was in order of Cd >> Pb > Zn > Cu

Imperator *et al*[58]	4 steps sequential extraction	Cr, Cu, Pb and Zn	Cu and Cr existed in soil mainly in organic forms (68%), whereas Pb occurs essentially as residual mineral phases (77%). The considerable presence of Zn in the soluble, exchangeable and carbonate bound fraction (23%) suggests this element has high potential bioavailability and leachability through the soil.
Li and Feng[169]	Modified BCR	As ,Ba, Co, Cr, Cu, Mn,, Ni, Pb, V, and Zn	The mobility sequence based on the sum of the BCR sequential extraction steps was: Pb (53.8 %) > Zn (51.8 %) > Cu (51.0 %) > Ba (42.6 %) > Cr (18.5%). Pb was predominantly associated (46.7 %) with the form bound to Fe/Mn oxides, and the highest percentage of Zn was exchangeable and carbonate-bound fraction. Cu was present mainly in organic fraction, while the residual fraction was the most dominant solid phase pool of Cr (81.5 %) and Ba (57.4 %).
Lu *et al*[170]	Modified BCR	Cd, Cu, Fe, Mn, Ni, Pb, and Zn	Cu, Fe, Ni, and Zn were predominately located in the residual fraction, Pb in the reducible fraction, and Cd and Mn within the HOAc extractable fraction. The order of Cd in each fraction was generally HOAc extractable > reducible > residual > oxidizable; Cu and Fe were residual > reducible > oxidizable > HOAc extractable; Mn was HOAc extractable >

			residual > reducible > oxidizable; Ni and Zn were residual > reducible > HOAc extractable > oxidizable; and Pb was reducible > residual > oxidizable > HOAc extractable. Cd was identified as being the most mobile of the elements, followed by Mn, Zn, Ni, Cu, Pb and Fe.
Waterlot *et al*[171]	4 steps sequential extraction	Cd, Cu, In, Pb, and Zn	The results showed that Cd and Zn were mainly in the acid-extractable and reducible forms in the urban soils studied. In contrast, Pb and In were largely in the reducible fraction. However, in some samples, the amount of In extracted in the residual or exchangeable fraction was higher than that in the reducible fraction. Copper was mainly found in the reducible and residual fractions.

There is a general elevation of the amount of PTE in urban environment due to the intense human activities. With the majority of world population living in urban areas there is the need to investigate the amounts of these elements in the environment, more especially their concentrations in various operationally defined fractions as determined by the sequential extraction methods. This will allow scientists to ascertain the mobility and availability of PTE in the environment, and their potential health risks to humans.

In this thesis, chapter 4 describes an investigation of the mobility, availability and human bioaccessibility of PTE in soils from an urban allotment using the modified BCR sequential extraction method, EDTA extraction and simplified bioaccessibilty extraction test SBET. Correlation studies were carried out to establish relationship between results of these different extraction methods using a chemometric treatment.

Chapter 5 describes the miniaturisation of the BCR method to allow application to samples with limited mass. Chapter 6 describes the application of the minuaturised BCR sequential extraction method to particulate matter on filters. Chapter 7 describes the modification of a non-specific reagent extraction method for the determination solid phase distribution of PTE in airborne urban particulate matter.

1.7 Aims

The aims of this work were to develop and evaluate extraction methods to determine potential availability of PTE in urban particulate matter, in order to provide improved information on their urban geochemistry and risks to human and environmental health with particular emphasis on sequential extraction. Seven elements Cr, Cu, Fe, Mn, Ni, Pb and Zn were chosen for this study. Their choices were based on their high natural abundance (e.g. Fe and Mn) while others are considered widespread pollutants in urban environments. Element such as Cd which is considered very toxic was not included in this study because previous studies of soils from Glasgow showed that its concentrations were below the detection limits of the ICP-AES and ICP-MS used.

The specific objectives were:

- Fractionating PTE using the modified BCR protocol in urban soil samples and comparing the fractions with EDTA extractable and SBET extracted fractions
- Miniaturisation of the BCR sequential extraction method
- Application of the miniaturised methods to airborne particulate matter on standard air quality monitoring filters
- Modification of the chemometric identification of substrates and element distribution (CISED) extraction protocol for application to standard air quality monitoring filters.

2 Theory of atomic spectrometry [172-175]

2.1 Introduction

Atomic spectrometry is a widely used technique for the determination of trace elements in environmental samples. This technique involves electromagnetic radiation that is absorbed by and/or emitted from atoms of a sample. The technique is used for both qualitative and quantitative analysis, the intensities of the radiations emitted or absorbed are related to the quantitative information while the qualitative information is given by the wavelength at which the radiation is emitted or absorbed.

According to quantum theory, the electrons of an atom occupy quantized energy levels and under normal conditions the electrons prefer to occupy the lowest energy level, this is referred as the *ground state*. When energy is added to the atom as a result of the absorption of electromagnetic radiation, the electrons are promoted to a higher energy level and the atom is said to be in an *excited state*. In an excited state the atom is less stable, so the promoted electron returns to the ground state by losing energy either through collision with other particles or by emission of a particle of electromagnetic radiation, known as a *photon*. These processes are shown in figure 2.1.

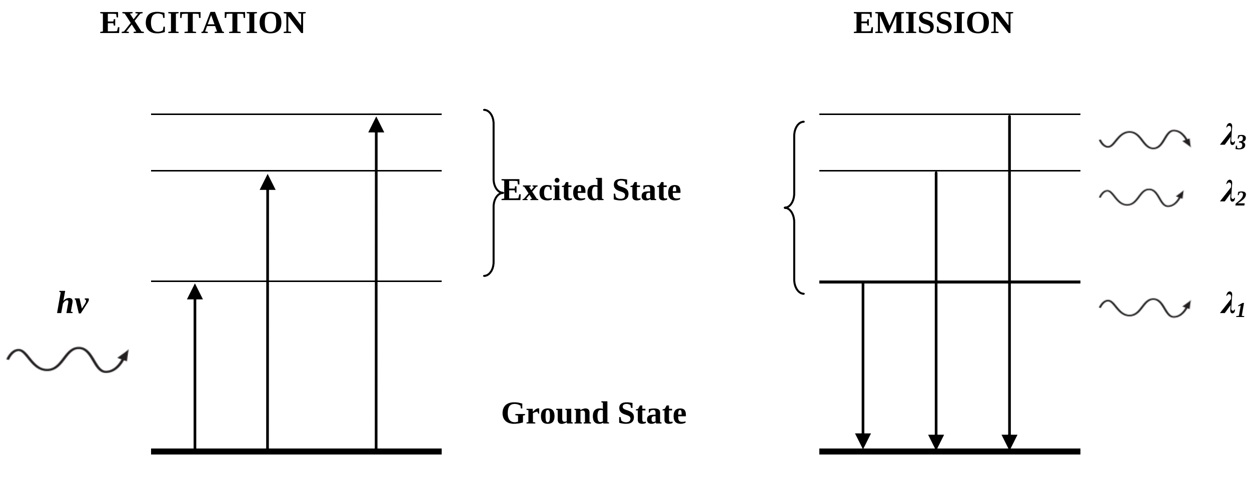


Figure 2.1 Energy level diagram showing energy transitions (after[175])

The energy absorbed or emitted during this transition is equal to the difference in energy of the levels involved and the wavelength of the radiation is given by the Planck equation (Equation 2.1)

$$\Delta E = E_0 - E_1 = h\upsilon \qquad \textbf{\textit{Equation 2.1}}$$

Where ΔE is energy difference between two levels, h is Planck's constant and υ is the frequency of the radiation.

Substituting c/λ for υ, equation 2.1 becomes equation 2.2

$$\Delta E = hc/\lambda \qquad \textbf{\textit{Equation 2.2}}$$

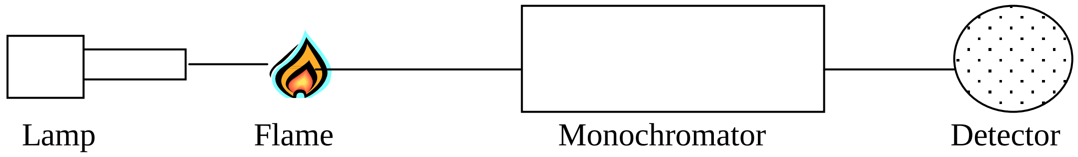
Where c is the velocity of light and λ is the wavelength of the radiation.

Equation 2.2 shows that there is an inverse relation between energy and wavelength, that is as the energy increases the wavelength decreases and *vice versa*.
There are different instrumental analytical techniques that are based on the measurements of these radiations absorbed or emitted. The most common are:

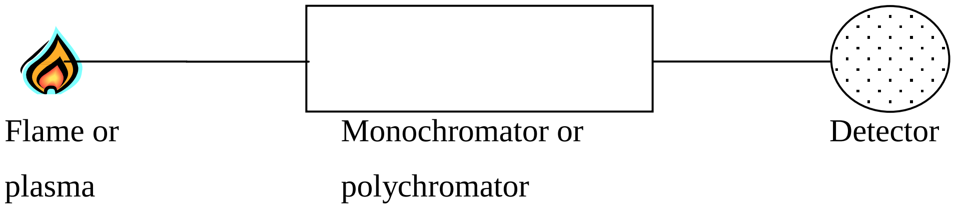
Atomic absorption spectrometry (AAS). In this technique, light of the same wavelength as an absorption line of the element of interest is shone through its atomic vapour. Some of this light is absorbed by the atoms of the element and the amount absorbed is directly proportional to the number of atoms present in the sample. This can then be used to determine the concentration of element in the sample. Figure 2.2a shows a diagram of a typical instrument system for AAS.

Atomic emission spectrometry (AES). In this method, the sample is subjected to a high temperature, enough not only to cause dissociation into atoms but also cause the excitation of the atoms. The electrons in the excited atoms, on returning to lower energy level, emits radiation of a specific wavelength which is measured and used to determine the concentration of the elements of interest in the sample. Figure 2.2b shows a diagram of a typical instrument system for AES.

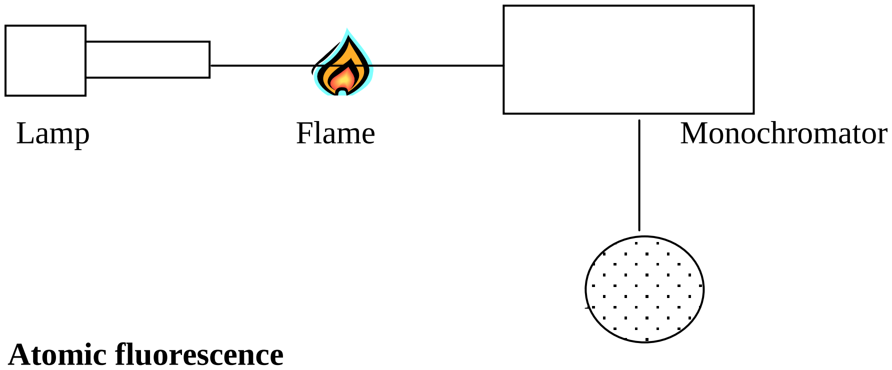
Atomic fluorescence spectrometry (AFS). In this technique, a light source similar to the one in AAS is used for the excitation of the atoms of the element of interest. Just as in AES, the radiation emitted by the atoms when the electrons return to a lower energy level is measured and can be used to determine the concentration of the elements in the sample. Figure 2.2c shows a diagram of a typical instrument system for AFS.



a) Atomic absorption



b) **Atomic emission**



c) **Atomic fluorescence**

Figure 2.2 Instrumentation typically used in atomic spectrometry techniques

In this research work neither AAS nor AFS were used and so only AES and elemental mass spectrometry are discussed in detail below.

2.2 Inductively coupled plasma atomic emission spectrometry (ICP-AES)

In atomic emission spectrometry, a sample is excited by exposing it to high temperatures. Different heating sources have been used, and these include flame, electric arc or spark. Nowadays, plasma is commonly used, since it provides a much higher temperature in the range 6000 to 8000K. In general this technique involves aspirating the sample solution into the plasma in the form of an aerosol where it is desolvated, vapourised, atomised and excited. The excited atoms then undergo decay by emitted a characteristic radiation which is collected and sorted according to the wavelengths. These are then detected, measured and converted to concentrations of the analytes.

2.2.1 Inductively Coupled Plasma

In inductively coupled plasma atomic emission spectrometry (ICP-AES) the heating source is the inductively coupled plasma (figure 2.3). The torch consists of three concentric quartz tubes known as outer, intermediate and inner gas tubes respectively, through which argon gas is passed. Around the top of the torch is a water cooled induction coil which is powered by a radio-frequency (RF) generator producing typically 700 -1500 W of energy, at a frequency of 27 or 40 MHz. This sets up a high frequency oscillation of electric and magnetic fields around the top of the torch. The ionisation of the flowing argon is initiated by a spark from a Tesla coil. These argon ions and the associate electrons interact with the fluctuating magnetic field forcing them to flow rapidly in a circular way thereby colliding with more argon atoms and stripping them of electrons and causing further ionisation in a chain reaction pattern. The resistance produces tremendous heat which forms the plasma. This addition of energy to the electrons by the coil is known as *inductive* *coupling*, and the plasma formed is maintained by the continuous addition of energy from the RF field through this process.

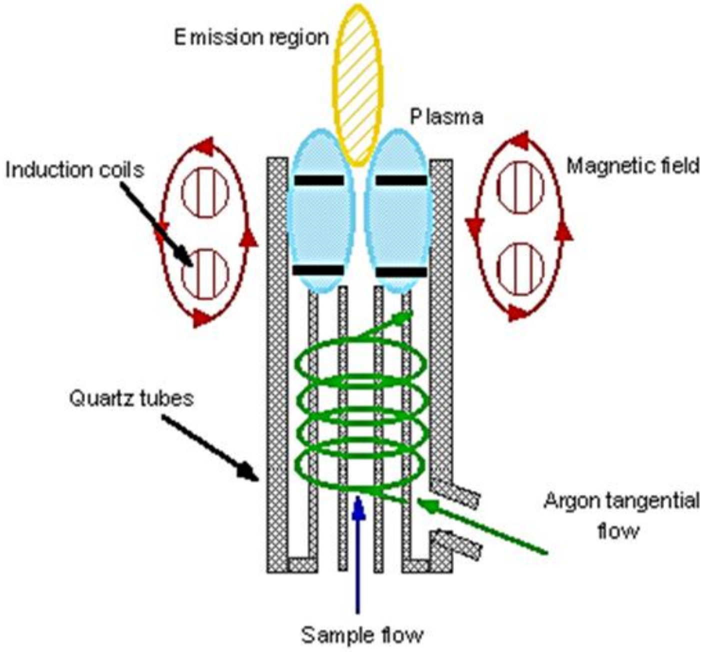


***Figure 2.3 Inductively Coupled Plasma*[176]**

The plasma can be clasified into diffent regions as suggested by Koirtyohann *et al* [177] (figure 2.4). The induction region is at the base of the plasma.It is “toroidal shape” which allows the passage of the sample in form of aerosol, and is where the inductive energy transfer from the coil takes place. The next region up is the preheating zone, the processes of desolvation, vapourisation and atomisation of the sample predominately takes place here. The exitation and ionisation of the atoms takes place predominantely in the initial radiation zone and the normal analytical zone from which analyte emission is measured.

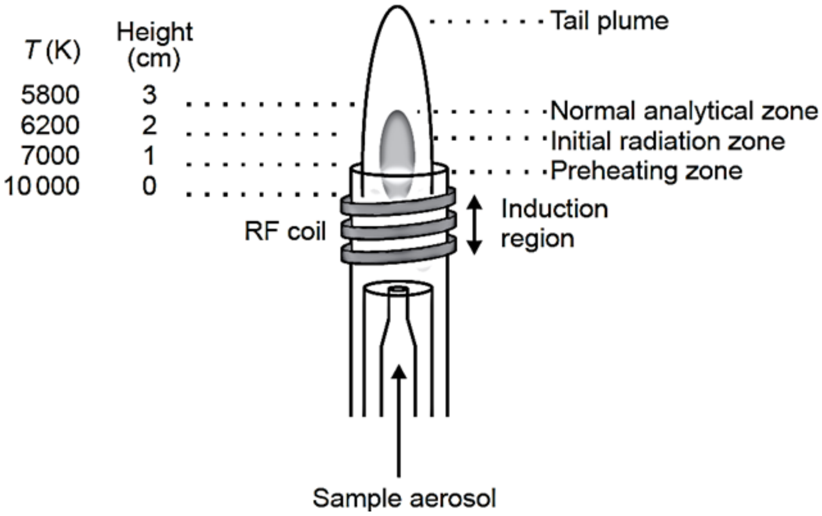


Figure 2.4 Regions of the Inductively Coupled Plasma [178]

2.2.2 Sample introduction

Different methods for sample introduction in ICP-AES have been developed, such as nebulisation, hydride generation, electrothermal vaporisation, and laser ablation[179]. Nebulisation is the commonly used method.

Nebulisers

These are devices that break the sample liquid into aerosols for onward transportation into the plasma. The most commonly used are pneumatic nebulisers which uses high speed argon gas to break the liquids into the aerosols. There are three basically types of pneumatic nebulisers used in ICP-AES: concentric nebuliser, cross-flow nebuliser and the Babington nebuliser (figure 2.5).

In concentric nebuliser, the sample solution is introduced through the capillary tube to a low-pressure region at the tip of the capillary which is created by a high velocity argon gas. This low pressure and high velocity gas break the liquid into aerosol. The advantages of this nebuliser are excellent sensitivity and stability due to the small orifices but these are prone to clogging. To reduce the clogging problems of the concentric nebulisers, cross-flow nebulisers were designed. In this design, a high velocity argon gas is passed perpendicular to the tip of the sample capillary; the sample liquid is broken into aerosol by this gas. The problem with this nebuliser is low sensitivity and the possibility of misalignment of the capillary. In Babington nebulisers the liquid is allowed to flow over a smooth surface with a tiny orifice. A high velocity argon gas coming out through the hole shears the film of the liquid into aerosols. This is the least affected by clogging and it can be used to nebulised viscous liquids[179].

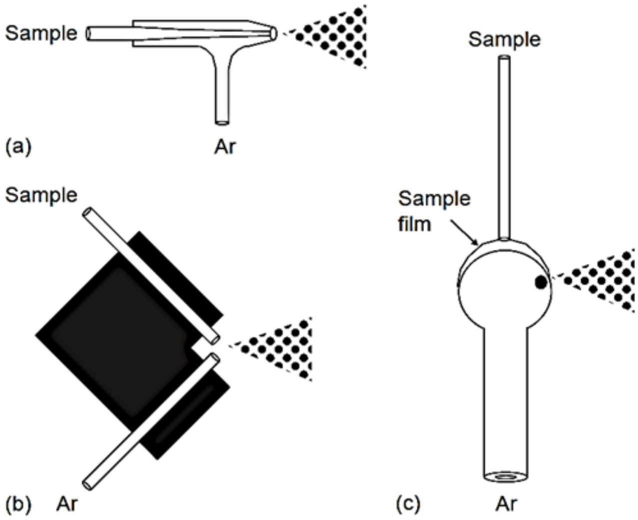


Figure 2.5 Schematic diagrams of three types of pneumatic nebulisers; (a) the concentric nebuliser; (b) cross-flow nebuliser; and (c) the Babington nebuliser[178]

Peristaltic pump are used in ICP-AES to pump the sample solution at a constant flow rate, they use a series of rollers to push the solution through the tubing.

Spray chambers

These are placed between the nebulisers and the torch to remove large droplets from the aerosol, as only very fine droplets (< 8 µm) are suitable for the plasma. They also smooth out any pulses that may occur during the process of nebulization. Only about 1 - 5 % of the sample introduced to the nebuliser is transported into the plasma, the remaining 95 - 99 % is drained into the waste.

2.2.3 Excitation

The ICP is not in thermal equilibrium, because the various collisional processes which occur in the plasma such as ionization, recombination, excitation and de-excitation are not in equilibrium. However, the ICP is thought to *approach* thermal equilibrium (TE), a condition termed local thermal equilibrium (LTE). One of the consequences of this is that ICP cannot be characterised by a single temperature (figure 2.4). On entering the plasma, the aerosol is desolvated, vapourised, atomized and excited. When an electron absorbs energy it is excited to a higher level; as it comes down to the ground state it emits radiation which is characteristic for that particular transition. If the electron absorbs sufficient energy, equal to the first ionisation energy, it escapes the hold of the nucleus completely and anion is formed. In the simplest case where TE or LTE holds, the number of atoms or ions in the excited state is given by the Boltzmann distribution equation (equation 2.3).

$$\frac{N_1}{N_0} = \frac{g_1}{g_0} e^{\left(\frac{-\Delta E}{kT}\right)} \qquad \textbf{\textit{Equation 2.3}}$$

Where N_1 is the number of atoms or ions in the upper (excited) state

N_0 is the number of atoms or ions in the lower (ground) state

g_1 is the number of energy level having the same energy for excited state

g_0 is the number of energy level having the same energy for ground state

ΔE is the difference in energy between the upper and lower states

k is the Boltzmann constant

T is the temperature

Different mechanisms have been proposed to explain the mechanism of excitation in the ICP. The major ones are:

Electron collision - the high kinetic energy electrons (e^-_1) transfer their energy to the atoms.

$$A + e^-_1 \rightleftharpoons A^* + e^-_2 \qquad E_{e-1} > E_{e-2}$$

Molecular collision - the molecules (XY) transfer rotation or vibrational energy to the atoms.

$$A + XY^* \rightleftharpoons A^* + XY$$

Collision with meta stable species (Ar^*_m) - transfer its energy to the atoms there by relaxing to the ground state.

$$A + Ar^*_m \rightleftharpoons A^{+*} + Ar + e^-$$

The electrons in the excited atoms and ions relax to the ground state by releasing radiation characteristic of a particular transition.

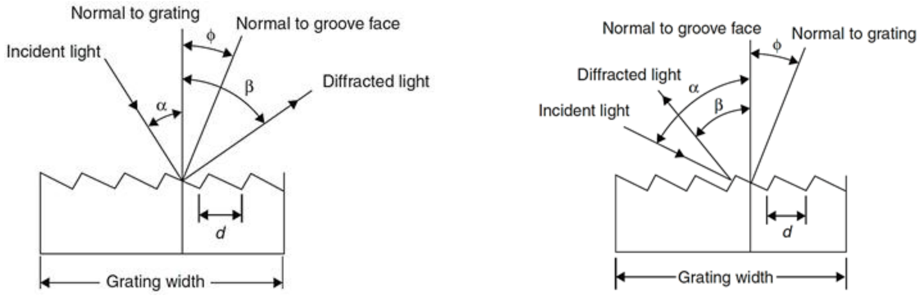
2.2.4 Spectrometer

This is the device used to separate, isolate and measure the radiation according to wavelength. The radiation can be viewed from the side of the NAZ of the plasma; this is known as radial view, or from the end of the plasma and this is known as axial view. Recent commercial ICP-AES combine the two configurations in a single unit, known as dual view.

The radiation is focused into the spectrometer using the focusing optics, such as a convex lens or concave mirrors and it is dispersed into different wavelengths using a *diffraction grating.* The most commonly used is the reflection diffraction grating, which simply consists of a mirror with many finely spaced grooves etched or ruled onto its surface. The light is diffracted at an angle which depends on its wavelength (λ) and the line density of the grating; this is given by the grating formula (equation 2.4).

$$m\lambda = d \sin \phi$$ ***Equation 2.4***

where ϕ is the angle of groove, d is the groove spacing and m the order of diffraction (figure2.6).



a **b**

Figure 2.6 Diagram of (a) plane-ruled grating (b) echelle grating: d, distance between grooves; ϕ, angle of a groove (blazed angle); α, angle of reflection; β, angle of reflection.[180]

One of the advantages of ICP-AES is the ability to measure many elements from the same sample *i.e. multielement analysis*; this can be done using the conventional dispersion devices in two ways. When multiple exit slits and detectors are used on the same spectrometer, this device is called a *polychromator*, each slit allows a specific wavelength to pass through it, thereby allowing a simultaneous multielement analyses. The most common polychromator design is the Paschen-Runge mount (figure 2.7).

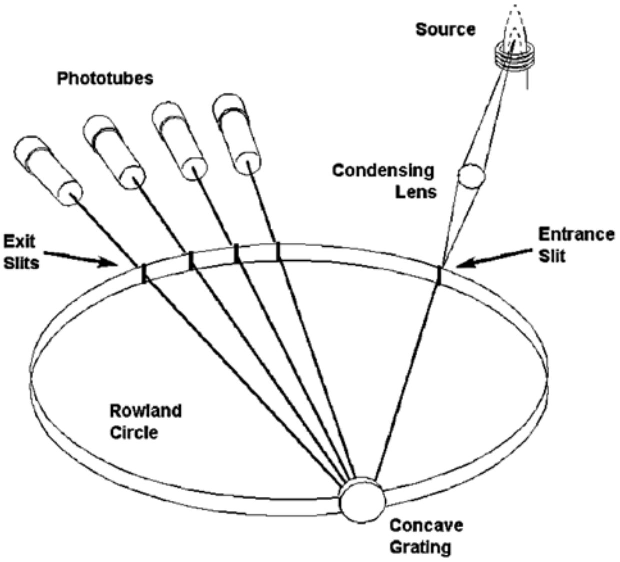


Figure 2.7 Paschen-Runge mount used in a Rowland circle polychromator [179]

The other way is the use of a monochromator which consist of a single slit and a detector. In this case, the multielement analyses can be done by rapidly scanning from one wavelength to another. This is done by either rotating the diffraction grating or moving the detector at the exit slit while keeping the grating fixed. The most popular monochromator designs are Czerny-Turner (figure 2.8a) and Ebert (figure 2.8b) mounts, both of which contain movable grating.

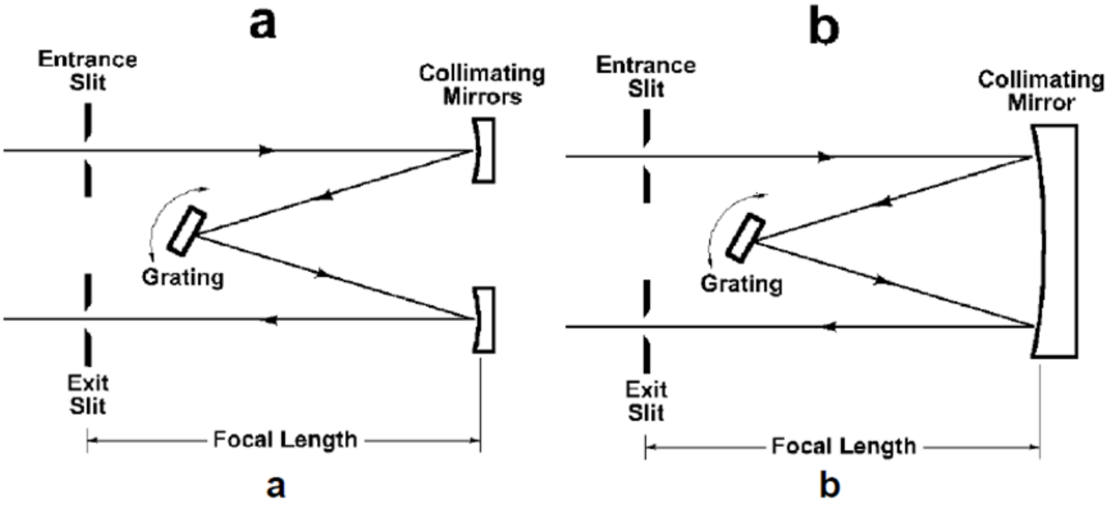


Figure 2.8 Czerny-Turner (a) and Ebert (b) monochromator mounts[179]

In recent times, most modern instruments are using another type of spectrometer known as an *echelle spectrometer* (figure 2.9). It gives better resolution for the same size or even smaller "box" when compared with the conventional spectrometers, and the basic difference between the two spectrometers is an echelle grating (figure 2.8b) used in echelle spectrometer. The echelle grating is similar to the normal blazed grating except that the light is reflected off the 'short side' of the grove, so the blaze angle is greater than 45^o, leading to higher resolution.

The spectrometer consists of two dispersion devices, a prism and an echelle grating or two gratings. The two dispersion devices are arranged in such a way that they are perpendicular to each other. The prism disperses the light in one plane by wavelengths producing multiple and overlapping spectral order. The echelle grating separates this overlapping orders into a two dimensional pattern called the echellogram.

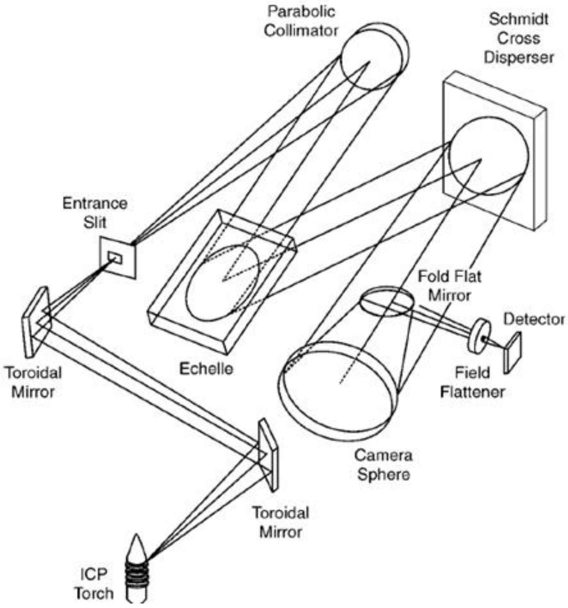


Figure 2.9 An echelle spectrometer[174]

2.2.5 Detectors

After the emitted lines have been isolated by the spectrometer, their intensities are measured using the detectors by converting the radiation energy to electrical energy.

Photomultiplier tube (PMT)

The PMT (figure 2.10) is vacuum tube consisting of electrodes coated with easily ionised material. The light enters the tube through a silica window on to the negative electrode known as the photocathode and removing electrons from it. These electrons are accelerated towards a dynode which ejects two or more secondary electrons for every one electron that strikes it. The secondary electrons eject further electrons from the next dynode. This process continues producing multiple of electrons. Typical PMTs contain 9 to 16 dynodes. The final step is to collect the electrons at the anode. The electric current measured at the anode is proportional to the amount of the light that struck the photocathode.

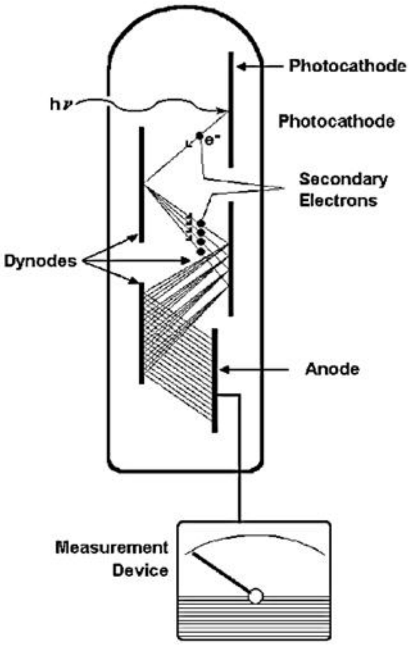


Figure 2.10 Photomultiplier tube [179]

Charge-transfer devices (CTD)

Most modern ICP-AES use the silicon-based CTD. They are semiconductors devices consisting of a series of cells or pixels which accumulate charge when exposed to light. The amount of charge stored gives the measure of the amount of light to which a particular pixel is exposed. A CTD is an array of closely spaced metal-insulator-semiconductor diodes formed on a wafer of semiconductor material (figure 2.11).

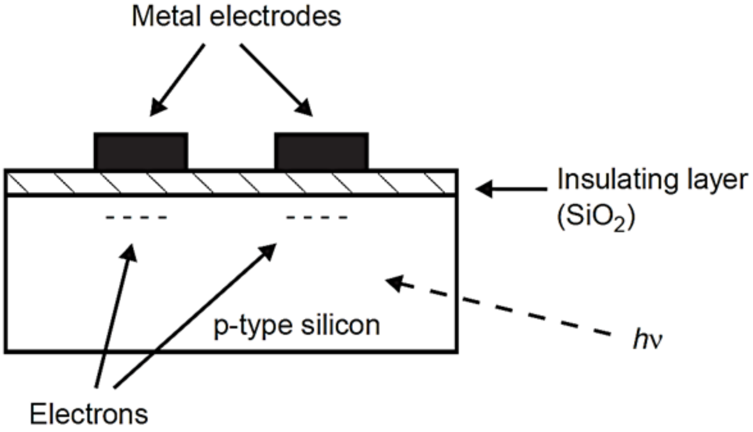


Figure 2.11 Cross-sectional diagram of two adjacent pixels[178]

When light strikes the surface of the silicon substrate electron-hole pairs are produced. If a voltage is applied across the electrodes, the electrons move in one direction relative to the applied electric field while the holes move in the other direction. This movement creates a current which is proportional to the amount of the light striking the surface. There are two common forms of CTD: charge-injection device (CID) and charge-coupled device (CCD), which differ slightly in their mode of operation. In CCD, the charges are accumulated for a specified time then read out on a row by row basis, the charges are transferred from one pixel to another until they reach the read out amplifier. In CID, individual pixels are readout at any time during the exposure.

The major advantages of this detector include; flexibility of wavelength selection, use of several wavelengths for the same element in order to extend the linear dynamic range, use of several wavelengths for the same element to improve accuracy and to identify potential matrix or spectral interferences and also potential for qualitative analysis. However this detector suffers from some limitations related to pixilation, UV response, dynamic range and shot noise.

2.2.6 Interferences

The very high temperature of the plasma and inertness of argon gas make ICP-AES relatively free from chemical interferences. Spectral interference (background interferences) is the most important type of interferences in ICP-AES, and can be classified into the following groups:

Simple background shift:

This occurs when there is a shift of the background intensity which is essentially constant over a range, on both side of the analyte line. The shift can either be upward or downward. This can be corrected by subtracting the background intensity from the intensity of the analyte. An alternative strategy is to measure at a wavelength where the inference does not occur.

Sloping background shift:

This occurs when there is partial overlapping of the emission line of the analyte from an interfering line in its close proximity. This can be avoided by using another line which is free from the interference, by chemical separation or using background correction.

Direct spectral overlap:

This occurs when the monochromator in the spectrometer is not capable of separating an analyte line and a matrix line that have very close wavelengths. For example, Cd (228.802 nm) and As (228.812 nm) coincide when measurement is taken at 228.8 nm. This problem can be solved by either separating the analyte from the interfering matrix by methods such as ion exchange or liquid-liquid extraction, or by choosing another wavelength where the interference does not occur.

Complex background shift:

In this interference, the background intensity is not constant but varies significantly on both sides of the analyte line. It is cause by the existence of a number of intense, closely spaced emission lines which overlap with the analyte line. It is corrected by using an interference-free analyte line.

Matrix interferences are other types of interferences encountered in ICP-AES usually associated with the sample introduction. Uneven nebulisation can occur due to the change of the viscosity or surface tension of the solution, this affect the rate of uptake of the neubuliser and hence its sensitivity. The easily ionized elements (EIE) are elements with low ionization potentials such as the alkaline metals; high concentrations of these metals can suppress or enhance the emission signal of the analytes. This effect is more serious in direct current plasma and microwave induced plasma than in ICP, and the effect can be reduced by diluting the sample to the extent that the EIE effect is not measurable.

2.3 Inductively coupled plasma mass spectrometry (ICP-MS)

As ICP produces high concentrations of ions it can be used as an ion source in elemental MS, this is the basis of the very important technique ICP-MS. The ‘ICP part’ of this instrument is very similar to that in ICP-AES, with just the usual variations between manufacturers.

2.3.1 Sample introduction

The samples are generally introduced as liquids as in ICP-AES but usually in a more diluted form (< 0.1 % dissolved solid); this is to prevent salt build up on the nickel cone (described below). The processes from nebulisation up to atom and ion formation in the plasma are all the same as in ICP-AES.

2.3.2 Ion sampling

The ions produced in the plasma are sampled into a mass spectrometer. The major challenge in this process is sampling the ions from the plasma at atmospheric pressure into a mass spectrometer which requires an extremely low pressure to operate. This is overcome by having an interface that couples the ICP torch and the mass spectrometer. The coupling is accomplished by making use of a series of differentially pumped vacuum chambers. To sample the ions, the torch is positioned horizontally and the interface, which consists of a water-cooled outer sampling cone made from nickel, is brought close to the plasma source (figure 2.12). The pressure behind the sampling cone is reduced to approximately 2.5 mbar using a vacuum pump thereby drawing the plasma gas into a lower pressure area through a small orifice in tip of the cone (≈ 1 mm diameter). The gas now passes through a second cone (skimmer cone) with a smaller orifice diameter of ≈ 0.75 mm. The pressure behind the skimmer cone is maintained at ≈ 10^{-4} mbar using a turbo pump. The ions are focused using a series of ion lens on to the entrance of the mass analyser.

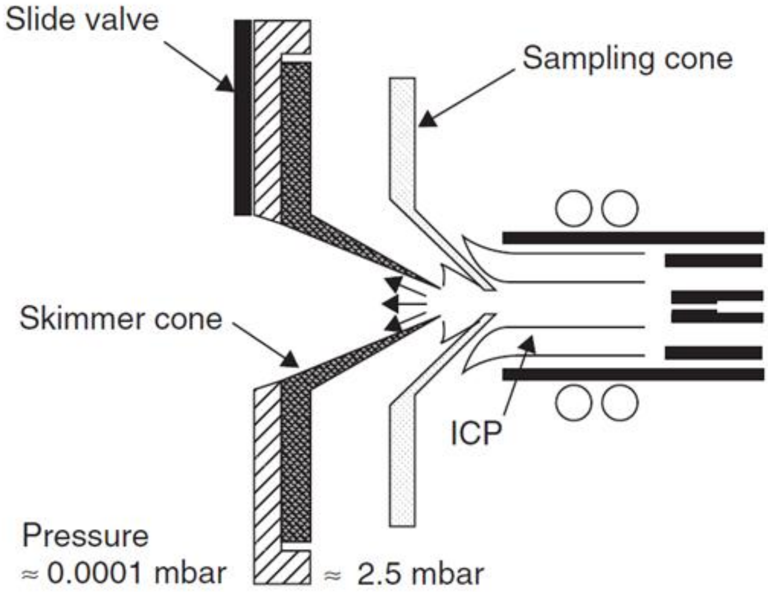


Figure 2.12 Schematic diagram of the ICP-MS interface [172]

2.3.3 Mass analysers

The ions sampled are then separated in the mass analyser based on their mass-to-charge ratio (m/z). There are two main types of mass analysers used in ICP-MS: the quadrupole and magnetic sector. The quadrupole is commonly used and explained below.

Quadrupole mass analyser

This instrument consists of four parallel cylindrical metal rods which are equidistant from a central axis (figure 2.13). Two opposite rods are connected to the positive terminal of a variable DC source and the others to the negative terminal. In addition, a variable RF potential, 180 degree out of phase, is applied to each pair. This combination creates an electric field around the rods. Depending on the RF/DC ratio the electric field allows ions of certain m/z range to pass to the detector while the remaining ions strike the rods and form neutral molecules. By changing the RF/DC ratio in a controlled way, the quadrupole can be scanned through the range allowing ions of consecutively higher m/z to pass through. An alternative way is by adjusting the RF/DC voltage to allow ions of a chosen m/z to pass rather than the scanning.

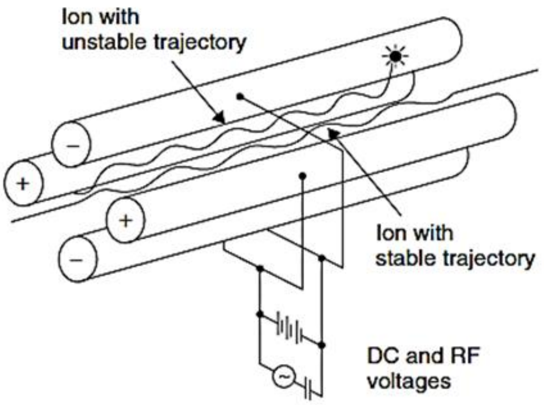


Figure 2.13 Schematic diagram of the quadrupole mass analyser [175]

2.3.4 Detectors

A channel electron multiplier is the most commonly used detector in MS (figure 2.14). Its working principle is similar to PMT, apart from the absence of the dynodes.

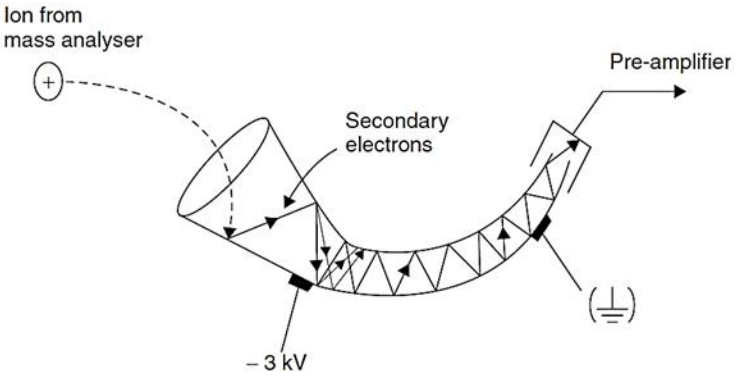


Figure 2.14 Schematic diagram of an electron multiplier tube[180]

The entrance is wide cone shaped and the interior of the tube is coated with lead oxide. The cone is connected to a highly negative potential and held at ground near the collector. The incoming positive ion from the quadrupole is attracted towards the cone surface, on striking the surface one or more electrons (secondary) are removed and are attracted toward the grounded collector. As in the PMT, the multiplication effect continues until all the electrons (up to 10^8) are collected. This pulse of electrons is further amplified and recorded as ion counts per second.

The main advantages of channel electron multiplier are high sensitivity and low random background, but they have a limited lifetime.

2.3.5 Interferences

The following are types of interferences encountered in ICP-MS:

Isobaric interference. This occurs due to the overlap of atomic masses of different elements, i.e. *isobars*. A typical example is ^{58}Ni (67.9 % abundant), which suffers from an isobaric overlap by ^{58}Fe. This type of interference is overcome by choosing an alternative isotope free of interference, in some case at the detriment of the sensitivity, for example in this situation ^{60}Ni (26.2 % abundant) is chosen. In some cases an alternative isotope which gives any good degree of sensitivity is not found. For example, the argon ions in the ICP interfere with ^{40}Ca (99.6 % abundant), and the best available alternative isotope is ^{44}Ca (2.08 % abundant) which does not give a good sensitivity. The use of collision/reaction cell technology (described below) is the best alternative.

Polyatomic interference. This arises from polyatomic ions formed between species in the plasma and species in the matrix or atmosphere, for example, $^{40}Ar^{16}O^+$ interferes with ^{56}Fe (91.66 % abundant); $^{40}Ar^{12}C^+$ interferes with ^{52}Cr (83.76 % abundant) and $^{40}Ar^{14}N^{1}H^+$ interferes with ^{55}Mn (100 % abundant).

Matrix dependent interference. This normally occurs when concentrated sample matrix is used. It manifest as an apparent enhancement or suppression of the analyte signal. This can be overcome by the use of internal standard, or by separating the matrix from the analyte before analysis. The sample can also be diluted to the extent that the interference becomes negligible, provided analyte concentrations remain greater than limits of detection.

2.3.5.1 Interference reduction

Polyatomic interference can be removed by the used of *cold plasma* or *collision/reaction cells.* In cold plasma the ICP is operated at lower power (typically 0.6 kW) and higher central (injector) gas flow (typically 1.1 l/min). Using these conditions have been found to reduce the interference due to Ar^+, ArH^+, ArO^+ and Ar_2^+ by several orders of magnitude[181]. A collision or reaction cell is normally placed between the skimmer cone and the mass analyser. Various reacting gases are used, e.g. hydrogen and ammonia and in some cases an inert gas like helium is used. Interfering species are removed through various processes such as;

Charge transfer:

In this case there is an exchange of charge between the reacting gas (NH_3 in the example below) and the interfering ion (Ar^+ or ArO^+ in the example below) which is neutralised and then not detected.

$$Ar^+ + NH_3 \longrightarrow NH_3^+ + Ar$$

$$ArO^+ + NH_3 \longrightarrow NH_3^+ + Ar + O$$

Proton transfer:

Proton is transferred, thereby neutralising the interfering ion which is then not detected.

$$ArH^+ + H_2 \longrightarrow H_3^+ + Ar$$

Hydrogen atom transfer:

The interference is removed in this case by transfer of hydrogen atom which changes the mass/charge ratio of the interfering ion by one mass unit.

$$Ar^+ + H_2 \longrightarrow ArH^+ + H$$

3 General experimental procedures

In this thesis, the modified BCR sequential extraction protocol was used to fractionate PTE into various operationally defined phases. The same procedure in the later part of the work was miniaturized and applied to samples collected on filters.

3.1 Extraction Procedures

In all the extractions procedures the glassware and storage bottles used were soaked overnight in a 5 % (v/v) HNO_3 solution. A general purpose grade HNO_3 (Sigma-Aldrich, Gillingham, UK) was used. They were then washed thoroughly with distilled water. Prior to analysis all the solutions were stored in polyethylene bottles at 4^oC.

3.1.1 Pseudototal digestion

Pseudototal digestion of the samples was carried out using a microwave digestion system **(**MARS Xpress, obtained from CEM Microwave Technology Ltd., Buckingham, UK).

The samples were digested with *aqua regia* which was prepared by mixing extra pure hydrochloric (HCl) and nitric (HNO_3) acids (Sigma-Aldrich, Gillingham, UK), in a ratio 3:1 (v/v).

A 1.0 g of the sample was weighed and placed into a high pressure vessel and 20 ml of freshly prepared *aqua regia* was added and left to stand in a fume cupboard overnight; this was to allow any vigorous reactions to subside. The pressure vessel containing the digestion mixture was placed into the MARS Xpress microwave digestion system and digested using the program in table 3.1. The vessels and the contents were allowed to cool and the digested samples were filtered (Fisher brand FB 59023) into 100 ml volumetric flasks and were washed several times with distilled water. The filtrates were made up to mark to obtain the analyte in 20 % *aqua regia,* and these were diluted further as required. In this procedure replicate samples (n=3) were digested together with procedural blank.

Table 3.1 MARS Xpress microwave digestion program conditions for extraction of PTE using aqua regia

Power* (> 16 vessels)	1600 watts
Temp	160 °C
Ramp time	10 min
Holding time	20 min

** 4 – 15 vessels 800 watts, < 4 vessels 400 watts*

3.1.2 BCR sequential extraction[182]

3.1.2.1 Apparatus

A G.F.L. 3040 mechanical end over end shaker (G.F.L, Burgwedel, Germany) was used to perform extractions at a speed of 23 rpm with the appropriate extractant solution. The delay between extract addition and shaking was minimal for steps 1 to 3. The extracts were separated from sample residue using a ACL 4237 centrifuge (CAMLAB Ltd., Cambridge, UK), at 3000*g* for 20 minutes.

3.1.2.2 Reagents

Preparation of extraction solutions

Solution A (acetic acid; 0.11 M)
A 0.43 M acetic acid solution was prepared by diluting 25 ml of glacial acetic acid, analytical grade (Sigma-Aldrich, Gillingham, UK), to 1l with distilled water. This solution was then diluted by taking 250 ml and making up to 1l to obtain the final solution.

Solution B (hydroxylammonium hydrochloride; 0.5 M)

About 34.75 g of hydroxylammonium hydrochloride, analytical reagent grade (Fisher Scientific, Loughborough, UK), was dissolved in 400 ml of distilled water. This solution was transferred to a 1 l volumetric flask and a 25 ml aliquot of 2 M nitric acid was added. The flask was then made up to the mark with distilled water. The solution was always prepared on the same day that the extraction was performed.

Solution C (hydrogen peroxide; 8.8 M)

A 30 % hydrogen peroxide solution, analytical grade (VWR, Leicestershire, England, UK), was used as supplied, i.e. acid-stabilised to pH 2-3

Solution D (ammonium acetate; 1.0 M)

77.08 g of ammonium acetate, analytical reagent grade (Fisher Scientific, Loughborough, UK), was dissolved in 800 ml of distilled water. The pH of the solution was adjusted to 2.0 ± 0.1 with nitric acid and made up to 1 l with distilled water.

Aqua regia: as described in section 3.1.1

Blanks: a procedural blank solution was prepared for each step by adding 40.0 ± 0.1 ml of solution A for step 1, 40.0 ± 0.1 ml of solution B for step 2 and 50.0 ± 0.1 ml of solution D for step 3 to the centrifuging tubes without the sample and carried through the complete procedure and analysed at the end of each extraction step.

3.1.2.3 BCR sequential extraction procedure

Step 1

An approximately 1.0 g test portion was accurately weighed into a 50 ml centrifuge tube with 40 ml of solution A and placed on the shaker for 16 h. The sample was then centrifuged to separate the supernatant liquid from the solid residue. The liquid was decanted into a polyethylene bottle and refrigerated at 4 °C before analysis. The residue was washed by adding 20 ml of distilled water and shaken for 15 minutes. After centrifuging the wash supernatant was decanted and discarded.

Step 2

40 ml of solution B (freshly prepared) was added to the washed residue from step 1 in the same centrifuge tube. The sample was then shaken, centrifuged and stored as in step 1.

Step 3

10 ml of solution C was added, slowly in small aliquots to avoid vigorous reaction to the washed residue from step 2. The centrifuge tube was covered loosely with its lid and left to digest for 1 h with occasional manual shaking. The digestion was continued for a further 1 h in a water bath at 85 ± 2 °C, before removal of lids and reduction of volume to less than 3 ml. Another 10 ml of solution C was carefully added and the covered samples heated for a further 1 h at 85 ± 2 °C. After removal of the lids the volume was reduced to less than 1 ml, with care not to take to complete dryness. The resulting solution was then allowed to cool before adding 50 ml of solution D. The centrifuge tube was then shaken and centrifuged, the solution decanted and stored, and the residue washed as in step 1.

Step 4

20 ml of *aqua regia* was used to wash the residue from step 3 into a pressure vessel and this was digested using the procedure described in section 3.1.1

3.2 Measurement of PTE in the samples

In this thesis, the quantitative elemental analysis was performed using two different analytical techniques: in the early part of the work (chapters 4 and 5), ICP-AES was used. This technique was chosen because of its ability to determine a large number of elements at parts per billion levels, it is also rapid with very wide linear dynamic range, and it has the ability for multi element analysis. In the later part of the work (chapters 6 and 7) were small sample masses were used, a more sensitive technique is required, therefore ICP-MS was used.

3.2.1 Inductively Coupled Plasma Atomic Emission Spectrometer

A Perkin Elmer Optima 3000 ICP-AES instrument (Perkin Elmer, Bucks, UK) equipped with radial-view plasma and an AS-91 autosampler was used for the measurements of the analytes' concentrations. The sample was pumped using a peristaltic pump at a constant flow rate of 1.00 ml/min through a cross flow nebuliser into a Scott double pass type spray chamber constructed of Ryton for complete corrosion resistance to most acids (figure 3.1). The spray chamber reduces the pulsations from the peristaltic pump; the large droplets were drained out allowing fine droplets into the plasma.

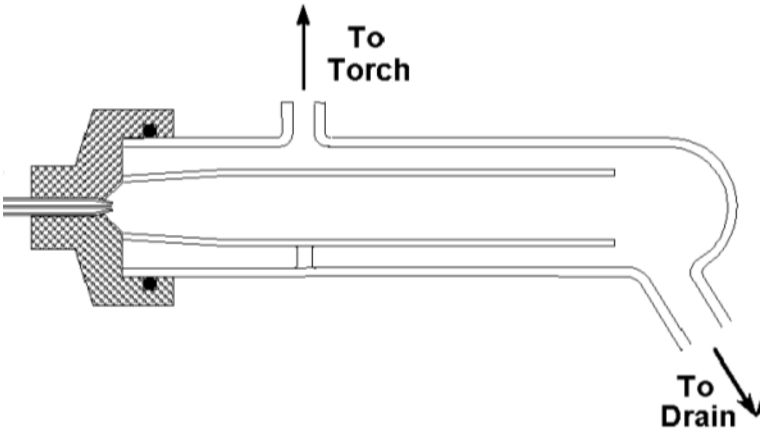


Figure 3.1 ***Schematic diagram of a Scott double pass type spray chamber (after[179])***

A one-piece torch which consists of three concentric quartz tubes sealed together was used. Through these three tubes, argon was passed at rates of 15, 0.5 and 0.8 l/min for plasma generation, auxiliary flow and nebulisation, respectively. An RF power of 1300 watts and frequency 40 MHz was supplied to the ICP torch by a free running RF generator. In the plasma the sample aerosol was desolvated, decomposed, and the atoms exited.

The radiation from the plasma was then passed into the spectrometer which is echelle-based with two detector focal planes and two cross dispersers (figure 3.2). The radiation is first dispersed by a 79 line/mm grating with blaze angle of 63.4°, the dispersed radiation is then cross dispersed by the UV region disperser, which is a 375 line/mm grating with Schmidt correction incorporated into the surface, and reflected onto the UV detector. Some of the radiation dispersed by the first grating is allowed to pass through a central hole on the visible region disperser which is a 60° fused quartz prism and this cross disperses the radiation which is then focused onto the detector for visible region radiation.

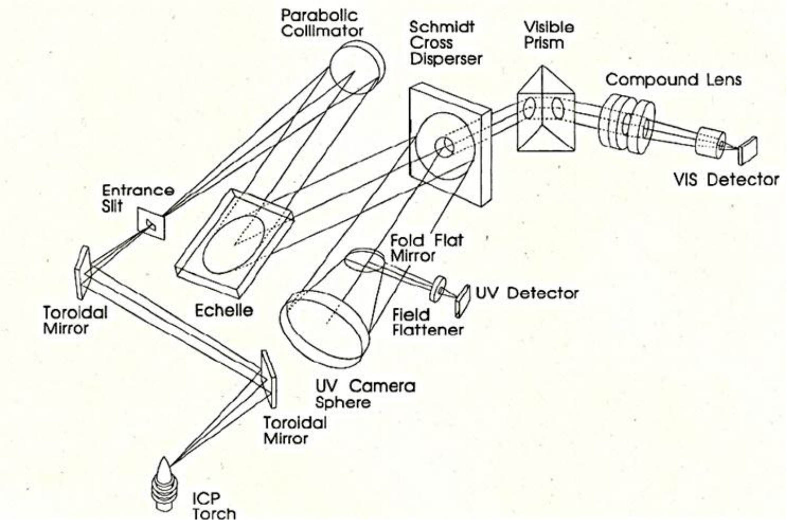


Figure 3.2 Schematic diagram of the optical system[172]

A segmented-array charge-coupled device detector (SCD) detects the radiation across the UV and visible regions (detector for UV 167 – 375 nm and detector for visible 375 – 782 nm). The SCD has 224 addressable subarrays, with over 6000 pixels on a 13 x 18 mm silicon substrate. The entire optical system is in an enclosed unit purged with nitrogen to remove moisture and oxygen and maintained at a temperature of 38 °C, while the detectors are housed in a unit purged with argon to prevent condensation and maintained at a temperature of -40 °C.

The instrument uses WinLab32™ software working on Microsoft® Windows 2000. The software provides all the tools needed to analyse samples, report and archive data.

Calibration

Multi element reagent matched calibration solutions were prepared by serial dilution of 1000 mg/l Spectrosol standard solutions (Merck, Poole, UK) of Cr, Cu, Fe, Mn, Ni, Pb and Zn for the calibration of the ICP-AES. A certified micropipette, 100 - 1000 µl (Jenco (Scientific) Ltd, Leighton Buzzard, UK) was used to accurately measure the required stock solutions for dilution. The following analytical wavelengths were used for this work:

Table 3.2 Emission Wavelengths used for determination of the elements investigated

Element	Wavelength	Viewing height
Cr	205.560 nm	15 mm
	267.716 nm	15 mm
Cu	224.700 nm	15 mm
	324.752 nm	15 mm
Fe	238.204 nm	15 mm
	239.562 nm	15 mm
Mn	257.610 nm	15 mm
	260.568 nm	15 mm
Ni	221.648 nm	15 mm
	232.003 nm	15 mm
Pb	217.00 nm	15 mm
	220.353 nm	15 mm
Zn	206.200 nm	15 mm
	213.857 nm	15 mm

Two wavelengths were chosen for each element to check for any interference. In situation where the two wavelengths were not given consistence values, additional wavelengths were added. For all the calculations the average of the consistent values were used.

3.2.2 Inductively Coupled Plasma Mass Spectrometer

An Agilent 7700x ICP-MS instrument (Agilent Technologies, UK Ltd., Wokingham, Berkshire, UK) with an ASX-500 series autosampler was used to measure the concentrations of the analytes. The sample solution was pumped at a constant rate of 1 ml/min through a concentric nebuliser using a 10-roller peristaltic pump. The peristaltic pump has 3 separate channels for the delivery of the sample and internal standard solution, and for spray chamber drain. A 1 mg/l indium (^{115}In) solution was used as the internal standard. Internal standards are used to correct for instrument drift or matrix effects due to gradient elution. The droplets formed by the nebuliser were then passed into a temperature controlled Scot-type spray chamber. The spray chamber is cooled by the Peltier cooler, a thermoelectric device which enable the temperature inside the chamber to be precisely controlled which gives this instrument a very stable ion signal (figure 3.3). The cooling of the spray chamber also helps in removing water from the sample, thereby reducing the level of polyatomic oxide species formed and this in turn reduces the interference on some analytes.

Figure 3.3 Peltier cooled sample introduction system[183]

The fine aerosol from the spray chamber then passed through the injector tube into a horizontally mounted torch. The torch consists of three concentric quartz tubes through which argon gas passed at rates of 15, 0.9 and 1.01 l/min for plasma gas, auxiliary gas and nebulizer (carrier) gas, respectively. A power of 1550 watts was supplied by a solid state digital drive 27 MHz frequency matching RF generator to the plasma. In the plasma the sample aerosol was desolvated, decomposed and ionised. The ions formed were extracted from the plasma with a sampling depth of 8 mm into the first vacuum stage through a 1 mm diameter orifice, Ni tipped with Cu base sampling cone (figure 3.4 a), the ions then passed into the second vacuum stage through a 0.4 mm diameter orifice Ni skimmer cone (figure 3.4 b).

a b

Figure 3.4 Agilent Ni-tipped with Cu base sampling cone and Ni skimmer cone[183]

For high signal sensitivity, the ion beam emerging from the skimmer cone must be focused before entering the octopole reaction system (ORS), this was achieved using electrostatic plates known as the ion lenses. These ion lenses also prevent the photons and neutral species originating from the plasma from reaching the detector.

The focused beam then passed into the ORS, which was a 12 MHz octopole ion guide contained within a stainless steel vessel and pressurized with a gas, most often He or H_2 (figure 3.5 a). In this work the instrument was operated in He mode, the ions interact with the gas resulting in the reduction of the molecular interference.

Figure 3.5 a) Octopole reaction system and b) Hyperbolic quadrupole[183]

The beam from the ORS then entered into a high frequency (~3 MHz) quadrupole with true hyperbolic rod profile (figure 3.5 b). By varying the RF and DC voltages applied to these four rods, ions of specific mass-to-charge ratio pass through the center of the rods to the detector while others collide with the rods. This voltage variation was done very quickly, scanning a mass range of 2 to 260 amu in 100 milliseconds; this gave a mass spectrum for all elements and their isotopes (Li to U) which was acquired virtually simultaneously. The ion signals were measured with an auto-switching, dual mode discrete dynode electron multiplier detector; this kind of system provides a full 9 orders dynamic range. When an ion enters the electron multiplier, it strikes the first dynode and a shower of electrons is produced. These electrons then hit the next dynode producing more electrons, this amplification continues until a measurable pulse is created. By counting the pulses generated by the detector, the system counts the ions that strike the first dynode.

MassHunter Workstation software was used on the 7700x ICP-MS which was operated on Microsoft® Windows 7 Professional. The software provided general instrument control, from automated startup checks following plasma ignition, through batch and queue method setup and sequencing, to integrated data processing and final report generation.

3.2.2.1 Calibration

Multi element reagent matched calibration solutions were prepared by serial dilution of 10 mg/l Agilent technologies multi element calibration standard 2A solutions

(Agilent technologies UK Ltd., Wokingham, Berkshire, UK). Thermo scientific Finnpepette® micropipette, 10 – 100 and 100 – 1000 µL (Thermo Scientific, Vantaa, Finland) were used to accurately measure the stock solutions for dilution. The following nuclides were used in this work.

Table 3.3 Nuclides measured and their natural abundances

Nuclide	% natural abundance
^{52}Cr	83.8
^{53}Cr	9.50
^{63}Cu	69.2
^{65}Cu	30.8
^{56}Fe	91.8
^{57}Fe	2.10
^{55}Mn	100
^{60}Ni	26.2
^{206}Pb	24.1
^{207}Pb	22.1
^{208}Pb	52.4
^{64}Zn	48.6
^{66}Zn	27.9

Two isotopes were chosen for each element to check for any interference. Manganese is monoisotopic element, therefore only ^{55}Mn was used. Despite the lower percentage abundance of ^{60}Ni (26.22 %), it was preferably used to ^{58}Ni (68.08 %) because ^{58}Ni suffers interference from both Fe and ArO. The presence of Ca compounds in the sample can also lead to interferences from CaO. For all the calculations the average of the consistent values were used.

3.3 Data Handling

3.3.1 Detection limits

Detection limits (DL), is the measure of the minimum concentration of analyte signal which can be distinguished from the background within a specified statistical uncertainty. The instrumental detection limits were calculated using the relationship in Equation 3.1.

$$DL = \frac{(3\, x\, s)}{gradient\ of\ the\ calibration\ slope}$$

Equation 3.1

Where, s, is the standard deviation of the 10 replicate values of the lowest concentration of the standards and is defined according to Equation 3.2.

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

Equation 3.2

Where, x_i is the individual value, n is the number of replicate measurements and, $\bar{x}$ is the mean defined as in Equation 3.3

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

Equation 3.3

The procedural detection limits (DL_{pro}), the minimum analyte concentration that can be determined in the environmental substrate allowing for the method of sample preparation, were calculated from the instrumental detection limits DL. DL_{pro} was calculated using Equation 3.4.

$$DL_{pro} = \frac{DL \times \text{volume of extractant} \times \text{dilution factor}}{\text{mass of sample}}$$

Equation 3.4

3.3.2 Precision

This is the degree of agreement between different measurements carried out in the same way. It can be expressed as a relative standard deviation (RSD) calculated, using Equation 3.5, as a percentage for a number of replicate samples.

$$RSD = \frac{s}{\bar{x}} \times 100\,\%$$

Equation 3.5

The percentage accuracy of an analytical method can be determined by comparing the mean measured analyte concentration ($\bar{x}$) with the certified or indicative analyte concentration (μ) using Equation 3.6.

$$\text{Accuracy} = \frac{\bar{\text{x}} - \mu}{\mu} \times 100\,\%$$

Equation 3.6

The concentration of the elements obtained from the ICP-AES is normally in mg/l, but in soil analyses the concentration is normally expressed in terms of the mass of the analyte per mass of dry soil. This can be converted by using Equation 3.7.

$$\text{Concentration (mg/kg)} = \frac{\text{analyte concentration (mg/L)} \times \text{volume of extract(L) x dilution factor}}{\text{mass of sample (dry weight)in kg}}$$

Equation 3.7

3.3.3 Moisture content

In this work the concentrations of the elements in the samples are expressed on a dry weight basis. To calculate the moisture content of the samples, 1 g of the test sample was dried in a crucible in an oven at 100^{o} C for 24 hours. The loss of mass was found and the moisture content calculated using equation 3.8

$$\%\, moisture \ = \frac{(initial\, weight - oven\, dried\, weight) \times 100}{initial\, weight}$$

Equation 3.8

3.3.4 Statistics[184-186]

3.3.4.1 T-test

This test can be used to determine whether the mean results of two sets of data are the same (within random error) or significantly different. A null hypothesis is set, that is there is no difference between the two sample means, other than the one due to random error. This test is done in two stages: first carrying out F-test to determine whether the variance in the two sets of sample data are similar or not. A t-test is then done using the appropriate equations depending on the result obtained from the F-test. The F- statistic is calculated using equation 3.9:

$$F_{calc} = \frac{s_1^2}{s_2^2}, \text{ where } \ s_1^2 > s_2^2$$

Equation 3.9

Where s_1 and s_2 are the standard deviations of the measurements.

This result is compared with the tabulated F value known as the critical value ($F_{\alpha/2,v1,v2}$), where α is the confidence level and $v1$ and $v2$ are the corresponding number of degree of freedom of the sets of data. If $\boldsymbol{F_{calc}} < F_{\alpha/2,v1,v2}$, the test is passed, the null hypothesis is accepted, and the differences in variance are due to random error; if $\boldsymbol{F_{calc}} > F_{\alpha/2,v1,v2}$, the test is failed, the null hypothesis is rejected, and the differences are significant.

If the F-test **passes**, equation 3.10 is used to calculate the t-statistic:

$$|t_{calc}| = \frac{|\bar{x}_{1-}\ \bar{x}_2\ |}{s_p\sqrt{\frac{1}{n_1}+\frac{1}{n_2}}}$$

Equation 3.10

Where $\bar{x}_1$ and $\bar{x}_2$ are sample means of n_1 and n_2 number of replicates for samples 1 and 2 and s_p is the pooled standard deviation calculated using equation 3.11:

$$s_p = \sqrt{\frac{(n_1-1)s_1^2+(n_2-1)s_2^2}{n_1+n_2-2}}$$

Equation 3.11

This result is compared with the critical value ($t_{\alpha/2,\ \upsilon}$) at, $\upsilon = n_1 + n_2 - 2$, degrees of freedom and the required confidence level. If $t_{calc} < t\alpha_{/2,\ \upsilon}$, the test is passed and hence the null hypothesis accepted and differences in mean values are due to random error. If $t_{calc} > t_{\alpha/2,\ \upsilon}$, the test is failed and hence the null hypothesis rejected and the differences are significant.

If the F- test **fails**, the equation 3.12 is used to calculate the t value:

$$|t_{calc}| = \frac{|\bar{x}_{1-}\ \bar{x}_2\ |}{\sqrt{\frac{s_1^2}{n_1}+\frac{s_2^2}{n_2}}}$$

Equation 3.12

This result is compared as in the previous t-test but the degree of freedom, υ in this case is calculated using equation 3.13

$$\upsilon = \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)^2}{\frac{s_1^4}{n_1^2(n_1-1)} + \frac{s_2^4}{n_2^2(n_2-1)}}$$

Equation 3.13

3.3.4.2 Analysis of variance (ANOVA)

When comparing more than two mean values, the t-test (above) cannot be used; ANOVA is used in this situation. In this kind of situation where more than 2 means are compared, there are two possible sources of variation. The first one can be due to random error in the measurements which are inherent and the second one due to what is known as a *controlled* or *fixed effect factor*. This statistical method of analysis (ANOVA) can be used to separate and estimate the different causes of variation. In this thesis, ANOVA has been used to separate variation caused by changing the sample sizes (mass) on the amounts of analytes extracted in all the 4 steps of the sequential extraction procedure proposed by the European Union Community Bureau for References (BCR) (chapter 5) from random variation. The type of ANOVA used in this thesis, in which only one fixed effect factor is investigated, is known as a *one-way* ANOVA.

Consider a one-way ANOVA with h samples and n replicate measurements, the data can be laid out in general in a matrix with the variable factor in the first column and replicates in a row each (table 3.4).

Table 3.4 General lay out of a one-way ANOVA

					Mean	Variance
Sample 1	x_{11}	x_{12}…..	..x_{1j}…	….x_{1n}	$\bar{x}_1$	s_1^2
Sample 2	x_{21}	x_{22}…….	..x_{2j}…..	….x_{2n}	$\bar{x}_2$	s_2^2
:	:	: …..	…..:	……..:	:	:
Sample i	x_{i1}	x_{i2}…..	..x_{ij}….	…..x_{in}	$\bar{x}_i$	s_i^2
:	:	: …..	…….:	……...:	:	:
Sample h	x_{h1}	x_{h2}…..	..x_{hj}…..	…..x_{hn}	$\bar{x}_h$	s_h^2
				Overall mean	$\bar{x}$	

Where x_{ij} is the *j*th measurement of the *i*th sample, $\bar{x}_i$ and s_i^2 are the mean and variance of the *i*th sample and $\bar{x}$ is the overall mean.

The null hypothesis assumed in this case is that all the samples are drawn from a population with mean μ and variance σ_0^2. On this basis the variance can be estimated in a number of ways, first involving the variation *within* the samples and the other variation *between* the samples.

Within-sample variation

For each sample, the variance is given by equation 3.14:

$$\frac{\sum_{j=1}^{n_i}(x_{ij}-\bar{x}_i)^2}{n-1}$$

Equation 3.14

The overall within-sample variance $\boldsymbol{\sigma_0^2}$ is calculated by taking the average of all the variances of all the samples (see equation 3.15)

$$\sigma_0^2 = \frac{\sum_{i=1}^{h}\sum_{j=1}^{n_i}(x_{ij}-\bar{x}_i)^2}{h(n-1)}$$

Equation 3.15

Between-sample variation

If the samples are drawn from the same population with variance σ_0^2, then their means come from a population with variance σ_0^2/n . Then the overall between-sample variance σ_0^2 is given by equation 3.16.

$$\sigma_0^2 = n\frac{\sum_{i=1}^{h}(\bar{x}_i - \bar{\bar{x}})^2}{h-1}$$

Equation 3.16

To determine whether the variations are significant or due to random variation, an F-test is used. F_{calc} is calculated by dividing the output of equation 3.16 by that of equation 3-15 and this is compared with critical F values ($F_{\alpha, v1, v2}$).

If $F_{calc} < F_{\alpha, v1, v2}$, the F-test passed, and the null hypothesis is accepted; therefore the variation is due to random error only; while if $F_{calc} > F_{\alpha, v1, v2}$, the F-test failed and the null hypothesis is rejected, so variation is significant.

3.3.4.3 The product-moment (or Pearson) correlation coefficient, r

The Pearson correlation coefficient, r is a measure of the strength of a linear association between 2 variables. Consider two variables x and y, the Pearson correlation coefficient for these variables is given by equation 3.17

$$r = \frac{\sum_i\{(x_i - \bar{x})(y_i - \bar{y})\}}{\sqrt{[\sum_i(x_i - \bar{x})^2][\sum_i(y_i - \bar{y})^2]}}$$

Equation 3.17

The Pearson correlation coefficient, r, is dimensionless quantity which can take range of values from – 1 and + 1. A value of 0 indicates no correlation between the two variables (i.e. they are independent) while values of – 1 and + 1 indicate perfect negative and positive association respectively. The closer the r values to unity the stronger the correlation.

3.4 Safety

All work involving apparatus and chemical substances were performed in a safe and controlled manner in accordance with experimental risk assessments. In this study several substances were used which are classified as hazardous to health in relation to the "Control of Substances Hazardous to Health" regulations (Table 3.5). Due to their hazardous chemical properties, substances were handled with gloves in a fume cupboard and other appropriate personal protective equipment worn. When not in use chemicals were sealed and stored in acid or solvent cupboards as appropriate. Disposal of chemicals was undertaken as highlighted in the risk assessments.

Table 3.5 Hazardous substances associated with present work

Substance	**Very Toxic**	**Toxic**	**Harmful**	**Corrosive**	**Irritant**	**Sensitizer**
Acetic acid		yes	yes	yes	yes	
Ammonium acetate			yes		yes	
Hydrochloric acid		yes	yes	yes	yes	
Hydrogen peroxide		yes	yes	yes	yes	
Hydroxyammonium hydrochloride		yes	yes		yes	yes
Nitric acid	yes	yes	yes	yes	yes	
Spectrosol standard solutions			yes		yes	
EDTA			yes	yes	yes	
Glycine						
Agilent multi element calibration standard 2A solutions				yes		

4 Mobility, phytoavailability, and human bioaccessibility studies of PTE in soil from an urban allotment.

4.1 Introduction

As part of its responsibility under the Environmental Protection Act Part IIA, the Inverclyde Council carried out assessment on soil samples from the Wellington Street Allotment in Greenock in July 2005. The preliminary results showed elevated concentrations of some metals including lead and nickel. The tenants of the allotment were instructed to stop growing vegetables for consumption pending further investigations. University of Strathclyde was contacted to conduct further systematic, scientific tests on the soil and vegetables from the allotment. In 2006, soil and vegetable samples were collected from each of the 22 plots on the allotment.

All previous studies of these soil samples focused on the PTE pseudototal content in the samples, no attempt was made to investigate the various forms in which these PTE occur in samples. Therefore in this part of the work, a sequential extraction method was used to investigate the different operationally defined forms of the PTE in the soil samples. Other extraction methods were also used to investigate the plant available and bioaccessible fractions.

4.1.1 Urban allotments sites in the UK

At present there are about 300 000 allotment sites in the UK, [187] the majority of which are located on previously industrial sites, which potentially poses health risk due to the contamination of the allotments. According to an audit report “Finding Scotland’s Allotment, 2007”,[188] there are 211 active allotments sites in Scotland containing about 6300 individual plots which are widely spread over the country. In Scotland, more people are appreciating the social, environmental and health benefit derived from allotment plots which has led to a dramatic increase in interest in allotments thereby causing the demand for plots to outstrip the supply.[188] However in addition to benefits, unfortunately allotments also provide various possible paths by which human beings come into contact with PTE, that is by inhalation, dermal contact, or through the trophic chain.

4.1.2 PTE in urban allotments

Allotments can be sited on locations that have been used for waste disposal from industries and homes. They are often positioned by road and industrial sites and for this reason the soils may contain elevated concentration of PTE. Most of the contamination of the soils due to PTE in urban allotments comes from urban/industrial activities, traffic related and domestic activities, in some cases due to the actions of the allotment tenants. Culbard *et al.*[189] carried out a survey of PTE in over 4000 domestic gardens and allotments and associate house dust in 50 cities and towns in England, Scotland and Wales. They confirmed elevated concentrations of Cu, Pb and Zn in London boroughs when compared with all areas surveyed. In recent analysis of soil from 180 allotments from the city of Wroclaw, Poland,[190] concentration ranges of 12.9 – 595 mg/kg Cu, 12.5 - 659 mg/kg Pb and 38.1 - 2100 mg/kg Zn were found. Hursthouse *et al.*[191] in their pilot study of metal content in a number of parks and allotments in the cities of Glasgow (UK), Torino (Italy) and Sevilla (Spain) from two depths, found that Cu, Pb and Zn have high variability while Cr and Ni are more homogeneous; this indicated that Cu, Pb and Zn must have come from anthropogenic activities while Cr and Ni may have come from geogenic sources. The concentrations of all the PTE studied in their work show a higher level in Glasgow with the exception of Cr and Ni that were found to be higher in Torino which is reflecting the contribution of basic/ultrabasic Alpine geology of the area.[192]

The mobility, availability, and solubility of PTE determine their potential uptake by plants and their transfer within the environment and this in turns depends on the binding to the soil reactive sites which is dominated by sorption, complexation and redox processes.[193] Sequential extraction method is used; examples of the sequential extraction methods have been mentioned in section 1.5.1.

For the estimation of plant-available metals, single selective extraction methods using chelating agent or inorganic compounds can be used to provide an insight into the fraction that could be absorbed by the plant roots. Some of the extractants used for this kind of study were listed in section 1.4.2. A number of studies have shown that extraction methods that give data closely correlated to pot cultivation experiments are generally specific to elements, types of crops, and even types of soils.[108, 194-196]

Ingestion of soil orally either accidently (e.g. hand-to-mouth oral ingestion mostly by children, eating unwashed food stuffs), or deliberately (e.g. by pica or due to geophagia) by humans is another pathway through which they come in contact with PTE. The health risks of ingesting the PTE can be estimated by measuring the *bioavailable fraction* of PTE in the soil. Bioavailability is the fraction of the oral dose that is absorbed into the systemic circulation,[197, 198] and its estimation requires the use of *in vivo* testing using humans and animals. Unfortunately this process is costly, time consuming and ethically challenging. However *in vitro* approaches known as bioaccessibility testing have been developed and are used as a conservative estimate of bioavailabilty.

Bioaccessibility is the fraction of PTE content that is soluble in the gastrointestinal tract and is available for transport across the intestine lumen. Various methods have been developed to estimate the fraction of the total of PTE in soils that would be bioaccessible in the human gastro-intestinal tract after ingestion of contaminated soils.[199, 200] These methods vary in complexity from simple ones that involve shake-flask methods to complex ones which involve use of specialised apparatus that simulate the human digestion with a high degree of accuracy. One of the most widely used method is the physiologically based extraction test, PBET developed by Ruby *et al*.[197] which simulates stomach and intestinal tract conditions. This method has been used in various bioaccessibility studies,[201-203] but unfortunately is tedious and difficult to carry out for large batches of sample.[204] A low reproducibility was also obtained in the simulated intestinal phase in this method.[198] A modified method was later developed which simplified the procedure by taking only the stomach into account: this simplified bioaccessibility extraction test (SBET) has been extensively used.[205-209] In recent times attempt have been made to establish a standardized method that is capable of providing a conservative estimate of bioaccessibility. The Bioaccessibility Research Group of Europe (BARGE) has developed a unified method known as unified BARGE method (UBM), and a recent preliminary study [210] suggested that there is a good correlation between the data from this method and the data from the *in vivo* experiment using swine model for As, Cd and Pb.

4.2 Aim

The aim of this work was to apply a suite of commonly-used extraction methods to provide information on PTE availability to the same set of urban allotment soils and to compare the information obtained for Cr, Cu, Fe, Mn, Ni, Pb and Zn. Specific objectives were to

i. use the modified BCR extraction protocol to assess the potential mobility and availability
ii. to estimate phytoavailablity elements using EDTA extraction, and
iii. to estimate bioaccessibility using the SBET method.

4.3 Experimental

4.3.1 Sample and sampling site

Sampling site

The Wellington Street allotment is located in the industrial town of Greenock on the south side of the River Clyde approximately 25 miles west of Glasgow, Scotland, UK. The allotment was surrounded by different industries in the past (figure 4.1), for example shipbuilding, shipping and related works, sugar refining, breweries, cotton mills and foundries. None of the features highlighted in figure 4.1 was present in a 1856 map obtained from the Inverclyde Council, including the allotment area and it is assumed that the area was a greenfield. All the highlighted features appeared in 1897 map, excluding the allotment area. The allotment area appeared first in the 1912 map and, in this map, the ropeworks and the brewery are no longer present. The allotment, the school and the tunnel are the only remaining features that appear in the map from 1965 to date. It is thought that the railway line was closed down in the 1960s.

Figure 4.1 Ordinance Survey map of site from 1896. Highlighted in different colours are key areas of the map: red is the allotment (present to date), dark green is a ropeworks (not present now), yellow is a brewery (not present), orange is a foundry (not present), bright green is a sugar refinery (not present), blue is a water works (not present), the purple lines mark the railway tunnel (present to date but closed) and pink is a primary school (present to date).

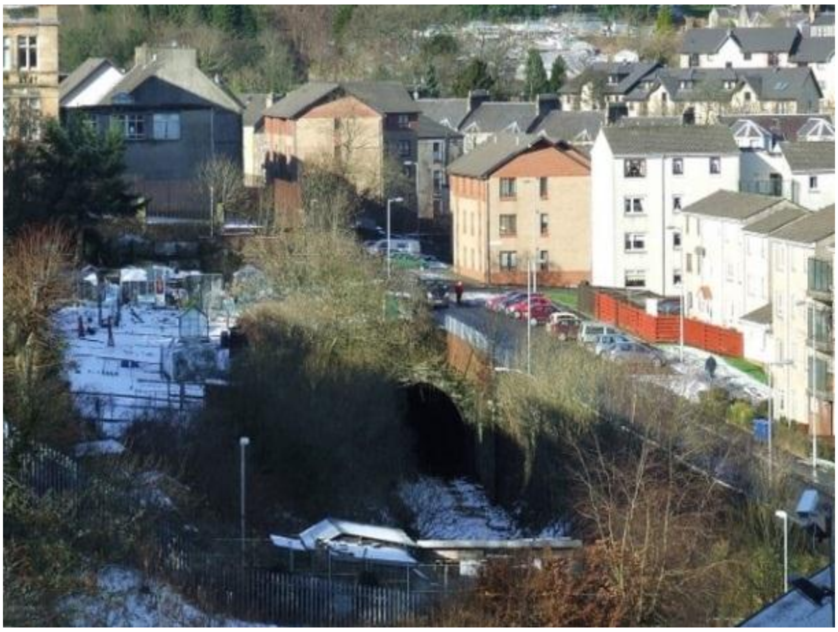
Currently the main industries are small cargo shipping, small scale ship building, cruise liners and electronics manufacture. The allotment site is presently surrounded by residential accommodation and still next to the site is the primary school and a children's playground (figure 4.2 a and 4.3 a), the locations of which raise concern over children possibility coming in contact with contaminated soil. The site is approximately half way up a steep hill which could also have been contaminated by either metals in water runoff from the industries above or possibly airborne metal particles from the shipbuilding areas which were located on the shorefront. The allotment consisted of 22 plots of different sizes and shapes at the time of sampling in 2006, but a recent visit to the allotment in 2012 showed that more plots have been created by sub-dividing the former 22 plots (figure 4.2 b).

a

b

Figure 4.2 Views of the Wellington Street allotment plots

a



b

Figure 4.3 (a) close up of a plot showing evidence of burning, broken brickwork etc. (b) allotments viewed from Dumpster Street and showing the Princes Pier railway line which emerges briefly between tunnels at the allotment site.

Sampling and sample preparations

Soil samples from the 22 plots were collected in 2006 by Ardaya Daza.[211] A clean stainless auger was used to sample from each plot at the depth of 25 cm. Five sub-samples were taken from each plot (four from each corner of the rectangle and one from the centre) and homogenised "on site" forming a bulk sample. Each bulk sample was stored in a plastic sample bag and labelled B1 to B22. The samples were taken to the laboratory, evenly distributed onto polyethylene sheets, placed on plastic trays and dried in a laboratory oven at 27 ^{o}C for five days. During this period, soil aggregates were periodically broken-up to ease drying. The samples were then sieved to 1 mm and then stored in plastic bags.

4.3.2 Characterisation of the soil

Soil pH[212]

Approximately 20 g of soil sample was weighed into a 100 ml beaker and 40 ml of water was added to it. The mixture was shaken vigorously on a magnetic stirrer for 15 minutes and was allowed to stand for 30 minutes. The pH of the supernatant solution was measured using Jenway 3305 pH meter (Jenway Ltd. Felsted, Dunmow, Essex)

Moisture content

The moisture contents of the soil samples were determined as described in section 3.3.3.

Soil organic matter content

Approximately 1 g of the soil sample was accurately weighed into a ceramic crucible and the sample was ashed in a muffle furnace at 550 ^{o}C for 24 hours, this was then cooled in a desiccator and reweighed.

The percentage lost on ignition (LOI) was calculated using equation 4.1

$$\%\ \text{LOI} = \frac{(dry\ weight\ of\ soil - ashed\ weight\ of\ soil)}{dry\ weight\ of\ soil} \text{ x } 100 \qquad \textbf{Equation 4.1}$$

Cation exchange capacity (after[212, 213])

Approximately 5 g of the soil sample was weighed into a 50 ml centrifuge tube. A 25 ml of 1 M ammonium acetate solution (pH 7) was added and the mixture shaken on an end-over-end shaker for 1 hour. The mixture was centrifuged for 10 minutes and the supernatant discarded. The residue was then washed twice with 20 ml of 95 % ethanol. A 25 ml of 1M KCl (pH 2.5) was added to the residue and the mixture was shaken for 30 minutes; this was then filtered into a 100 ml volumetric flask. The soil in the filter was leached with successive small portion of the KCl solution into the flask, and then the filtrate was made up to mark. A 50 ml of the filtrate was transferred into a 250 ml round bottom flask, was diluted with 50 ml distilled water and 5 ml of borate buffer was added to it. The mixture was distilled into a receiving flask containing 10 ml of 0.3 M boric acid and few drops of mixed indicator solution (methyl red and bromocresol green). The distillation was continued until about 50 – 60 ml of the distillate was collected. This was then titrated with 0.01 M HCl.

Equation for the reaction:

$$NH_4OH + HCl \longrightarrow NH_4Cl + H_2O$$

The mole ratio for the reaction is 1:1

$\therefore$ no. of moles of $NH_4^+ = \frac{M \times V}{1000}$ where M is molarity of HCl

V is the volume of HCl used (in cm^3)

No of moles per kg of the soil = $\frac{2 \times M \times V}{5}$

$\therefore$ CEC (centimole charge per kg, $cmol_c/kg$) = $\frac{2\ X\ M \times V\ X\ 100}{5}$ (because NH_4^+ is univalent)

4.3.3 Extraction procedure

4.3.3.1 Pseudototal

The pseudototal contents were determined by microwave assisted digestion of the samples using *aqua regia* as described in section 3.1.1. An in-house reference material, Glasgow URBSOIL reference material (URM) [214] was included in soils digestion batches, in order to assess the accuracy of the digestion. The extracts were analysed using ICP-AES as described in section 3.2.1.

4.3.3.2 Sequential extraction

The PTE were fractionated into different forms using the modified BCR sequential extraction procedure as described by Rauret *et al.*[159] The detail descriptions of the procedure have been discussed in section 3.1.2. To assess the accuracy of the extraction procedure a certified reference material BCR 601 was analysed in parallel with the samples. The extracts were analysed using ICP-AES as described in section 3.2.1.

4.3.3.3 EDTA extraction

A 0.05 M EDTA at pH 7 was used in this extraction. It was prepared using the harmonized procedure recommended by the BCR[147]. Approximately 146.12 ± 0.05 g of EDTA free acid was added to 800 ml of distilled water, with constant stirring, then about 130 ml saturated ammonia solution was added until all the EDTA dissolved. The solution was filtered into a 10 l polyethylene container, diluted with 9.0 ± 0.5 l of water, and the pH was adjusted to 7.00 ± 0.05.

The extraction was carried out by shaking on an end-over-end shaker a mixture of approximately 1 g of each of the soil samples with 10 ml of the 0.05 M EDTA solution for 1 hour at room temperature (20 ± 2 ^{o}C). The mixtures were filtered (Fisher brand QL 100) into 100 ml volumetric flasks and the filtrates were made up to the mark. These solutions were kept in a polyethylene bottles at 4 ^{o}C prior to analysis. Three replicate test portions of each soil sample were extracted, together with a procedural blank. No reference material was available and the results were considered satisfactory when the relative standard deviation was below 10 %.

4.3.3.4 Physiological based extraction test

The oral bioaccessibility of the PTE was estimated using the SBET, [206, 215] which considers only the gastric phase in fasting condition. A 1.00 ± 0.05 g portion of the soil was shaken at 37 ^{o}C for 1 h with 100 ml of a solution of 0.4 M of glycine adjusted to pH 1.5 with HCl. At the end of the extraction 15 ml of the extract was removed using a disposable 20 ml syringe and filtered using a 0.45 µm cellulose acetate disk filter. Three replicate test portions of each soil sample were extracted, together with a procedural blank. No certified reference material is available for these analytes extractable by this physiologically based extraction procedure. The results were considered satisfactory when the relative standard deviation was below 10 %.

4.3.4 Detection limits

The extracts were analysed with respect to matrix-matched standards by ICP-AES. The detection limits found for each different matrix are shown in tables 4.1, 4.2 and 4.3.

Table 4.1 Instrumental (D_L inst.) and procedural (D_L pro.) detection limits for BCR sequential extraction in different matrices by ICP-AES

	Step 1		Step 2		Step 3		Step 4	
Analyte and wavelength (nm)	D_L inst. (mg/l)	D_L pro (mg/kg)	D_L inst. (mg/l)	D_L pro (mg/kg)	D_L inst. (mg/l)	D_L pro (mg/kg)	D_L inst. (mg/l)	D_L pro (mg/kg)
Cr 205.560	0.009	0.368	0.014	0.560	0.013	0.650	0.009	0.850
Cr 267.716	0.356	0.356	0.009	0.364	0.008	0.395	0.009	0.890
Cu 224.700	0.011	0.432	0.017	0.680	0.010	0.480	0.020	2.00
Cu 324.752	0.006	0.228	0.009	0.364	0.007	0.350	0.010	1.02
Fe 238.204	0.036	1.44	0.470	18.8	0.015	0.750	0.025	2.50
Fe 239.562	0.025	1.00	0.455	18.2	0.019	0.950	0.030	3.00
Mn 257.610	0.014	0.560	0.029	1.16	0.010	0.505	0.001	0.130
Mn 260.568	0.013	0.520	0.031	1.24	0.012	0.595	0.001	0.110
Ni 221.648	0.015	0.600	0.021	0.840	0.019	0.950	0.016	1.60
Ni 232.003	0.029	1.16	0.026	1.04	0.021	1.05	0.031	3.10
Pb 217.000	0.157	6.28	0.251	10.0	0.166	8.30	0.234	23.4
Pb 220.353	0.040	1.60	0.045	1.80	0.049	2.45	0.029	2.90
Zn 206.200	0.016	0.640	0.048	1.92	0.029	1.45	0.021	2.10
Zn 213.857	0.017	0.680	0.039	1.56	0.020	1.00	0.018	1.80

Table 4.2 Instrumental (D_L inst.) and procedural (D_L pro.) detection limits for EDTA extraction by ICP-AES

Analyte and wavelength (nm)	D_L inst. (mg/l)	D_L pro (mg/kg)
Cr 205.560	0.003	0.031
Cr 267.716	0.003	0.027
Cu 224.700	0.005	0.052
Cu 324.752	0.003	0.027
Fe 238.204	0.003	0.033
Fe 239.562	0.004	0.037
Mn 260.568	0.001	0.010
Mn 257.610	0.000	0.002
Ni 232.003	0.016	0.164
Ni 221.648	0.014	0.139
Pb 217.000	0.053	0.529
Pb 220.353	0.024	0.238
Zn 213.857	0.002	0.016
Zn 206.200	0.008	0.084

Table 4.3 Instrumental (D_L inst.) and procedural (D_L pro.) detection limits for SBET extraction by ICP-AES

Analyte and wavelength (nm)	D_L inst. (mg/l)	D_L pro (mg/kg)
Cr 205.560	0.006	0.582
Cr 267.716	0.001	0.145
Cu 224.700	0.005	0.451
Cu 324.752	0.001	0.125
Fe 238.204	0.010	1.04
Fe 239.562	0.009	0.941
Mn 260.568	0.001	0.018
Mn 257.610	0.001	0.055
Ni 232.003	0.010	0.950
Ni 221.648	0.006	0.563
Pb 217.000	0.158	15.8
Pb 220.353	0.033	3.33
Zn 213.857	0.001	0.145
Zn 206.200	0.001	0.096

4.4 Results and Discussion

4.4.1 Physical properties

pH

The pH of a soil applies to the "H^+ concentration in the solution present in soil pores which is in dynamic equilibrium with the predominantly negatively charged surfaces of the soil particles".[1] It is the measure of the acidity or alkalinity of the soil. pH is one the most important factors that controls the mobility, adsorption and bioavailability of PTE in soil solution. In general metal cations are most mobile under acidic conditions and the mobility decreases as the pH increases. The pH values for the soils from different plots in the allotment (table 4.4) show that the soils were slightly acidic. The values ranged from 5.6 – 7.0 with an average value of 6.3. The slight variations in the pH may be due to individual activities on the plots by individual tenants such as fertilizer application, liming and burning of materials on the plots (figure 4.3a). The pH values for these soils fell within the normal range of 4 – 8 for soils in a typical temperate environment such as UK.[1]

Table 4.4 Physical properties of the soils from the Wellington street allotment

Sample	pH	Organic content (%)	CEC ($cmol_c$/kg)
B1	6.0	20.6	6.35
B2	6.1	23.1	7.64
B4	6.4	25.3	8.16
B5	6.3	24.2	7.97
B6	5.6	20.1	7.49
B7	6.1	21.4	7.24
B8	6.4	22.8	7.01
B9	6.8	24.8	8.16
B10	6.3	21.3	7.79
B11	6.6	21.7	7.97
B12	5.9	23.1	7.64
B13	6.4	20.5	8.09
B14	6.1	19.0	8.14
B15	5.9	14.0	7.51
B16	6.1	23.1	7.18
B17	6.4	23.2	7.44
B18	6.3	19.6	7.46
B19	6.1	21.7	7.64
B20	6.2	21.9	7.79
B21	7.0	20.6	7.72
B22	6.9	24.0	7.85
Mean	6.3	21.7	7.60

Organic matter content

The organic matter contents in the various plots were similar to one another with the exception of plot B15 where it was somewhat lower than the other plots (table 4.4). The mean organic matter content values for these soils was 21.7 % and this is higher than the average value of < 10 % for cultivated soils.[1]

CEC

The CEC gives the measure of the soil's ability to hold cations by electrostatic attraction; its value depends on the nature of the soil. Humus, the end product of organic matter degradation, typically has the highest CEC because the organic matter colloids have large quantities of negative charge. Humus has CEC 2 to 30 times that of clay depending on the type of the clay. Sand has the lowest CEC because it has no electrical charge. In this present work, due to the limited quantities of the samples, the particle size distribution was not determined. Therefore relationship between the soil texture and the CEC in this study could not be discussed. But a study of urban soils collected from some parks and allotments around Glasgow showed that the soil textures of these samples fell into the 'sand-loamy sand-sandy loam' region of the US department of Agriculture (USDA) classification.[191] The CEC values for the soils from these plots varied slightly (6.35 – 8.16 cmol_c/kg) with an average value of 7.60 cmol_c/kg (table 4.4). Hursthouse *et al.*[191] obtained a higher average value of 17 cmol_c/kg when they studied 43 soils samples from 4 parks and 4 allotments from Glasgow city. CEC values of allotments soils from three medium sized towns of coastal Tuscany, central Italy[216] ranged between 13.1 and 28.1 cmol_c/kg and these values were again higher than the values obtained in this present study. However, a similar low values were obtained for agricultural soils from Thessaly in central Greece.[217]The low values obtained in this study may have some environmental implications. The low CEC means cation retention is low, and this may lead to the leaching of the nutrients required by the plants. The PTE can be leached into the ground water or may be easily available to the environment. The CEC values can be increased by adding organic matter (by adding manure, leaving crop stubbles to rot, *etc*).

4.4.2 Pseudototal content

Quality control

Table 4.5 gives the results of the pseudototal content of the PTE in the in-house reference material, URM, obtained in the two batches of the extraction and their indicative values. In general the recoveries were 100 ± 10 %, with the exception of Cr and Zn in the first batch, which were slightly higher at 117 and 115 %.

Table 4.5 Results of the pseudototal content of URM

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
target values (n=34) **	43.2	111	30600	442	48.8	389	177
values obtained 1st batch (n=3)	50.6	116	32400	445	52.3	349	203
values obtained 2nd batch (n=3)	44.2	114	31400	469	47.8	396	184
% recovery 1st batch	117	105	106	101	107	89.7	115
% recovery 2nd batch	102	102	103	106	98	102	104

**[168]

Chromium

The results pseudototal concentrations of Cr in the soil samples from the plots are showed in figure 4.4 and table 1 in appendix A. The RSD were generally < 10 % (n=3) which show an acceptable precision. The concentrations ranged from 26.3 to 49.4 mg/kg with plots B4 and B22 having the highest concentrations (*ca.* 49 mg/kg). The average concentration of Cr in these plots was 34 mg/kg. This is lower than the average concentration of 44.7 mg/kg of Cr in Scottish soils as determined by the National Soil Inventory of Scotland (NSIS_1), lower than the median concentration of 44.2 mg/kg (urban) as determined by UK Soil and Herbage Survey (UKSHS), but higher than the median background concentration (rural) of 26.2 mg/kg as determined also by the UKSHS.[218] A much higher median concentration of 108 mg/kg was obtained in urban topsoil collected across and round the Glasgow conurbation by the British Geological Survey (BGS) Geochemical Survey of Urban Environments (G-BASE urban) project.[219] The results from this present work was also lower than the mean concentration of 93 mg/kg obtained for soil samples analysed from some parks and allotments in Glasgow[191]. The new UK Contaminated Land Exposure Assessment (CLEA) soil guideline values (SGV) for Cr is still awaited at the time of writing (July 2013), but none of the concentrations in any of the plots exceeded the existing UK CLEA residential and allotments SGV of 130 mg/kg.[220]

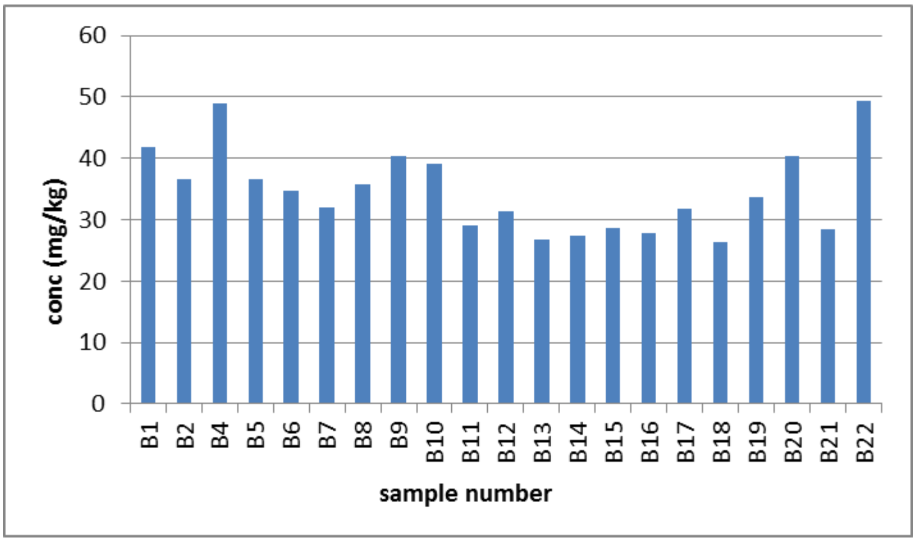


Figure 4.4 Pseudototal concentrations of chromium in the urban allotment soil samples (mg/kg)

Copper

The pseudototal concentrations of Cu in the various plots ranged from 141 to 248 mg/kg (table 2 appendix A) with an average concentration of 193 mg/kg. Generally the RSD were below 10 % (n=3), showing a good precision in the extraction. The mean value obtained in this work was very much higher than the mean concentration of 9.4 mg/kg Cu in Scottish soil as determined by NSIS_1 and the median concentrations of 13.6 and 28.7 mg/kg in rural and urban soils respectively as determined by UKSHS.[218] When compared with the GBASE project[219] median concentrations of 51.4 mg/kg (urban) and 31.0 mg/kg (rural) obtained for Glasgow soils, this study recorded a higher value. The result was also higher than the mean concentration of 27.3 mg/kg obtained for soils from 105 gardens in eastern France,[221] but a similar high concentration of 140 mg/kg was obtained in soils from parks and allotments in Glasgow by Hursthouse *et al.*[191] Currently there is no UK CLEA SGV for Cu, but the average value obtained in this work was slightly higher than the Dutch Soil Intervention Value of 190 mg/kg,[222] and much higher than the value of 100 mg/kg of the German Agricultural Soil Guideline.[223] The high concentration of Cu in these soils is an indication of the influence of some anthropogenic activities around the area; the Cu may have come from the foundry (figure 4.1), electronic manufacturing (an IBM factory is located in this area) or through the individual tenants' activities such as application of Cu based pesticides and fungicides. Although the concentration of Cu in these soils have exceeded some of the reference values, its risk to humans and the environment depends on the form in which it exists and its mobility, this is discussed in the next section.

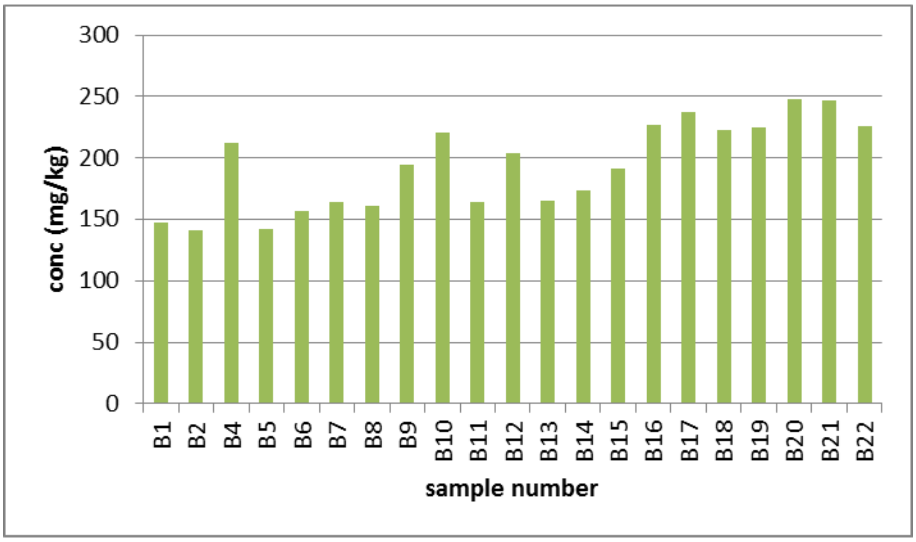


Figure 4.5 Pseudototal concentrations of copper in the urban allotment soil samples (mg/kg)

Iron

The concentrations of Fe in the soils from the plots ranged from 36100 to 48800 mg/kg (table 3 appendix A) with a mean concentration of 40400 mg/kg. The RSD were generally below 10 % (n=3). Figure 4.6 shows a nearly uniform distribution of Fe across the plots, which is an indication of the geogenic nature of the element. A similar concentration of 35300 mg/kg was obtained for soils from allotments in Glasgow.[191]Although in large abundance in soils, in some cases a deficiency of this element to plant is observed and this may be due to an insufficient amount in mobile forms for intake by plants.

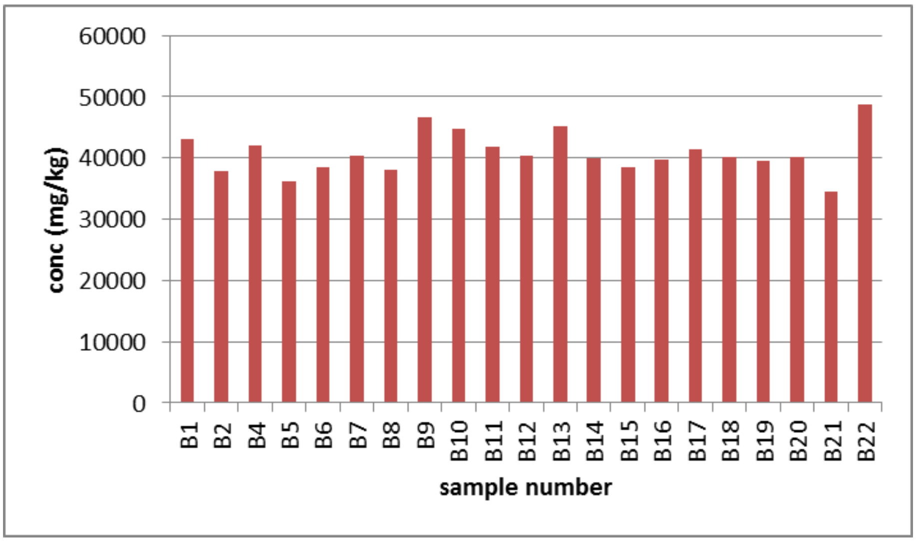


Figure 4.6 Pseudototal concentrations of iron in the urban allotment soil samples (mg/kg)

Manganese

Table 4 in appendix A shows the concentrations of Mn in the soil samples analysed from the Wellington Street allotments which ranged from 556 to 874 mg/kg, with mean concentration of 720 mg/kg. The RSD were generally < 10 % (n=3), which indicated a good precision in the measurements. As in the case of Fe the concentrations were fairly uniformly distributed (figure 4.7) which may indicate that the Mn originated mainly from the parent material instead of from anthropogenic input. The global mean concentration of Mn is 437 mg/kg.[81]The result from the present study was lower than the concentration of 947 mg/kg obtained for urban soils from Sevilla but higher than the concentration of 491 mg/kg obtained for urban soils from Torino.[191]

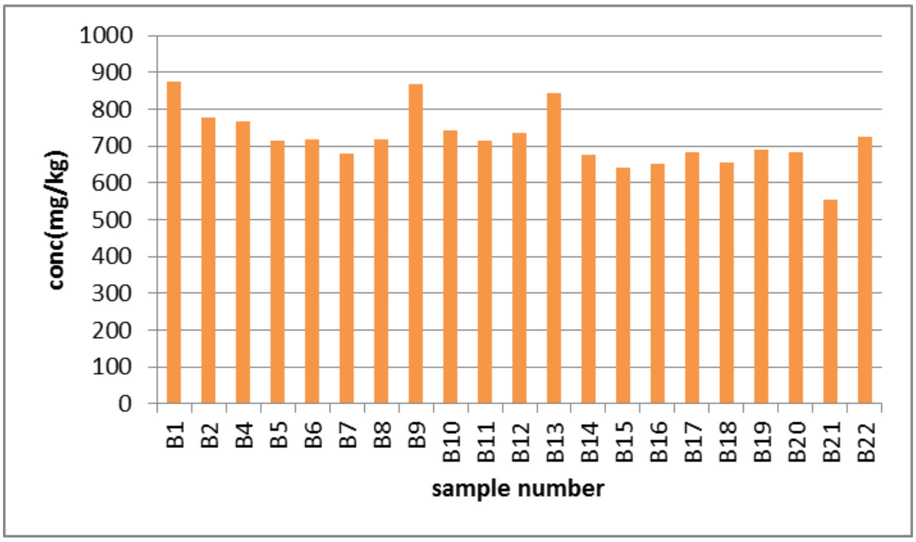


Figure 4.7 Pseudototal concentrations of manganese in the urban allotment soil samples (mg/kg)

Nickel

The concentrations of Ni in these soils ranged between 63 and 128 mg/kg with a mean concentration of 75.2 mg/kg (table 5 appendix A). Plot B20 stands out from the other plots - its concentration was relatively high - and this is likely to be due to localized contamination. The concentrations from this study were higher than the mean concentration in 20.5 mg/kg of Scottish soils as determined by NSIS_1 and median concentration of 11.0 mg/kg (rural) and 27.2 mg/kg (urban) as determined by UKSHS.[218] The current results were also higher than the median concentrations of 33.8 mg/kg (rural) and 46.9 mg/kg (urban) as determined by BGS GBASE project for soil from Glasgow area.[219] The average concentration of Ni in these plots was also higher than the mean concentrations obtained in garden soils from France (19.3 mg/kg) and Germany (14 mg/kg),[221] but a similar relatively higher concentration of 58 mg/kg was obtained from soils from some parks and allotments from Glasgow.[191] Nickel concentrations in all the plots exceeded the former 50 mg/kg UK CLEA SGV for residential gardens and allotments but none exceeded the new Ni SGV of 230 mg/kg.[224] The relatively high concentration of Ni in these soils may come from some human activities taking place in the area presently and in the past, such as the foundry and the ship building industries. Another source may be geogenic, because

Glasgow soils have generally higher concentration of Ni than other UK cities and this may be due to the coal and volcanic bedrock in the area.[219]

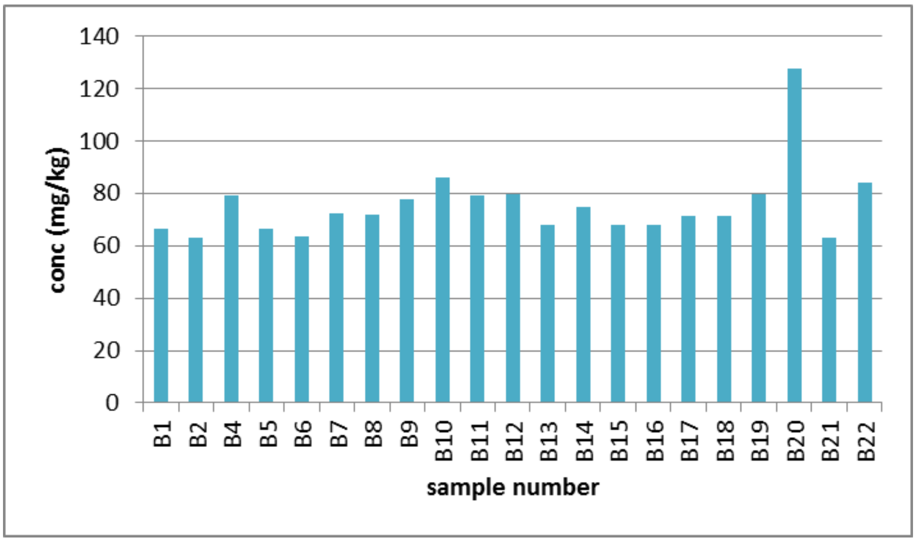


Figure 4.8 Pseudototal concentrations of nickel in the urban allotment soil samples (mg/kg)

Lead

Figure 4.9 and table 6 in appendix A, show variation in concentrations of Pb among the plots, the concentrations varied between 825 and 2160 mg/kg with a mean concentration of 1280 mg/kg. The RSD were generally below 10 % (n=3) with the exception of results of plots B1 (13 %) and B21 (19%). These RSD were just slightly high and this was due to non-agreement between the replicate samples. The results from this work were higher than the mean concentration of Pb of 31.8 mg/kg for Scottish soils reported by NSIS_1 and the median concentration for rural and urban soils of 27.6 and 90.8 mg/kg respectively reported by UKSHS.[218] When compared with the median concentrations of Pb in urban and rural top soils around Glasgow of 127 and 77.5 mg/kg, respectively, the mean concentration obtained here was much higher. Relatively higher concentrations of 654 and 971 mg/kg were reported for Pb in garden soils from London boroughs[189] and in urban allotments from Glasgow, [191] respectively. The mean concentration of Pb in the Wellington Street plots was three times higher than the existing UK CLEA SGV of 450 mg/kg for residential gardens and allotments, [225] although a new value for Pb is awaited. The results obtained in this work showed that the soils from these plots were polluted with Pb, which likely came from the previous and present anthropogenic activities in the area such as from former leaded petrol, paints, pesticides, garden ornaments *etc*. This high level of Pb in these soils is alarming, but its effect on humans and the environment depends on the form in which it exists and its mobility. This can be predicated by the sequential extraction and is discussed in the next section.

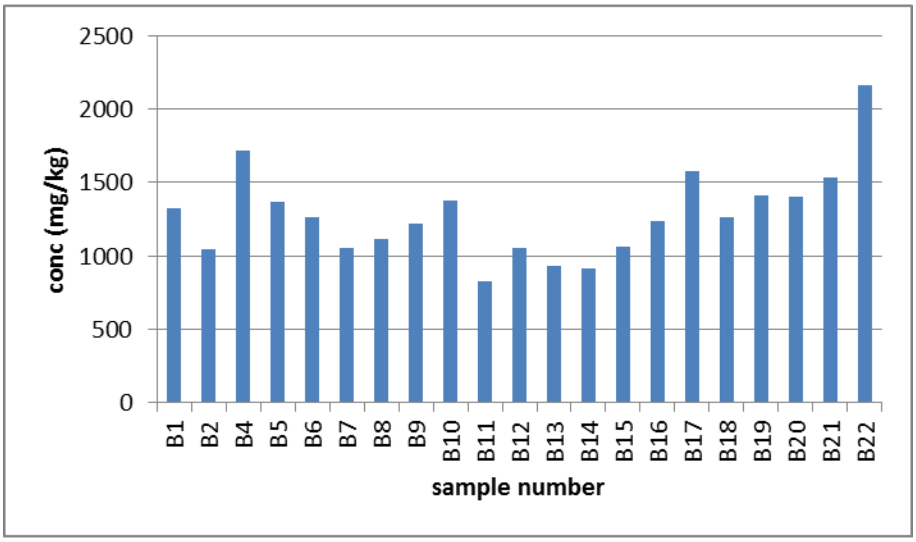


Figure 4.9 Pseudototal concentrations of lead in the urban allotment soil samples (mg/kg)

Zinc

The concentrations of Zn in the soils (table 7 appendix A and figure 4.10) showed a relatively high variability across the plots. This is an indication of the influence of anthropogenic activities. The concentrations varied between 455 and 1230 mg/kg with a mean concentration of 761 mg/kg. This concentration was much higher than the mean concentration of 53.8 mg/kg of the Scottish soils reported by NSIS_1 and 55.5 and 96.3 mg/kg for rural and urban soils respectively as reported by UKSHS.[218] The result obtained in this work was also higher than the concentrations of 105 and 152 mg/kg of Zn in rural and urban soils around Glasgow respectively.[219] When compared with the results obtained from other garden and allotment soils the current results were much higher. Culbard *et al.*[189] reported a mean concentration of 278 mg/kg of Zn in garden soils in England, Scotland and Wales and a value of 424 mg/kg for London boroughs. A mean concentration of 364 mg/kg was reported for soils from parks and allotments around Glasgow city.[191] At present there is no UK CLEA SGV for Zn, but the mean concentration of Zn in this work was higher than the Dutch Soil Intervention Value of 750 mg/kg,[222] and the German Agricultural Soil Guideline of 300 mg/kg.[223] The elevated concentration of Zn in these plots showed an influence of anthropogenic activities in the area, which could be from individual activities on each plots such as fertilizer application, pesticides and manure applications. Other sources may be leaching of galvanized materials around the area and atmospheric deposition from the smelting of non-ferrous metals.

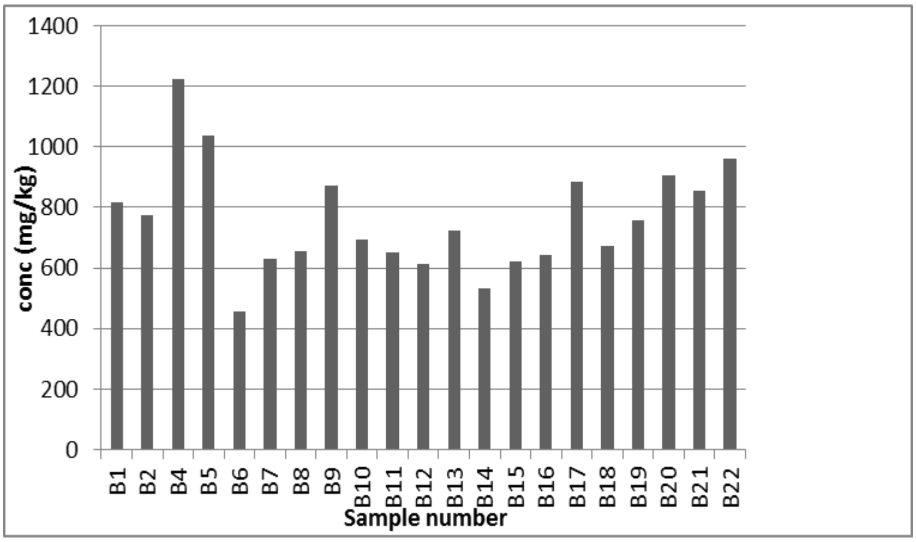


Figure 4.10 Pseudototal concentrations of zinc in the urban allotment soil samples (mg/kg)

4.4.2.1 Correlations between PTE extracted using pseudototal extraction method

A simple correlation method was used to study and assess any relationship between the PTE extracted using the pseudototal extraction method. Correlations were calculated by plotting the concentrations of one PTE in all the samples against the concentrations of all the others and considering the correlation coefficient (r) at 95 % confidence level.

Table 4.6 gives the correlation coefficients calculated for the extracted with *aqua regia*.

Table 4.6 Pearson correlation coefficients (r) between PTE extracted using aqua regia (pseudototal content) (n=3) at 95 % confidence limit

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Cr							
Cu	0.047						
Fe	0.459	0.121					
Mn	0.452	-0.483	0.614				
Ni	0.357	0.479	0.282	-0.023			
Pb	0.680	0.551	0.285	-0.106	0.214		
Zn	0.669	0.264	0.191	0.197	0.237	0.670	

Manganese was found to moderately correlate with Fe, this is an indication they are likely to have similar source. Levels Pb and Cr were found to correlate, while Zn also moderately correlates with Cr and Pb.

4.4.3 Fractionation of the PTE

Quality control

The quality of the analytical sequential extraction data was assessed by analyzing a certified reference material (CRM 601) in parallel with the samples. The results obtained and the indicative values for the CRM 601 are presented in table 4.7. The recoveries obtained for Cr, Cu, Ni Pb and Zn when compared with the indicative values were within ± 20 % (except Cr and Ni for step 3) indicating the good accuracy of the extraction.

In the allotment soil samples, moreover the sum of steps 1 to 4 for most of analytes and for most of the plots compared well with the pseudototal contents from the original sample (within ± 20 %). This indicated that the quality of the analytical extraction data was ensured.

Table 4.7 Results obtained for the analysis of CRM 601 for extractable PTE contents (mg/kg)

Value	Cr	Cu	Ni	Pb	Zn
Step 1					
Indicative	0.35 ± 0.08	10.5 ± 0.8	7.82 ± 0.84	2.28 ± 0.44	260 ± 13
Obtained	0.34 ± 0.11	10.7± 1.1	8.12 ± 0.63	1.98 ± 0.63	254 ± 0.51
Recovery (%)	97	101	103	87	98
Step 2					
Indicative	10.6 ± 0.9	72.8 ± 4.9	10.6 ± 1.2	205 ± 11	266 ± 17
Obtained	9.96 ± 0.5	69.7 ± 9.5	12.1 ± 1.3	227 ±35	268± 20
Recovery (%)	94	96	114	110	101
Step 3					
Indicative	14.4 ± 2.60	78.6 ± 8.9	6.04 ± 1.27	19.7 ± 5.80	106 ± 11
Obtained	10.8 ± 1.51	63.8 ± 3.9	4.13 ± 0.19	17.8 ± 4.77	105 ± 6
Recovery (%)	75	81	68	90	99
Step 4					
Indicative	78.2 ± 6.5	60.4 ± 4.9	50.5 ± 6.1	38.0 ± 8.7	161 ± 14
Obtained	79.3 ± 7.1	54.2 ± 3.1	53.1 ± 3.6	43.1 ± 3.9	165 ± 5
Recovery (%)	101	90	105	113	102

Indicative values are n= 7; obtained values are n= 6

Chromium

Figure 4.11 shows the distributions of Cr in the various steps of the BCR extraction procedure whilst table 1 in appendix A shows the data in which the plot was based. The RSD in step 1 were generally very poor, and this was because the concentrations of Cr in all the plots in this fraction were below or very close to the procedural detection limits (Table 4.1). The RSD for the other steps were generally good with some few exceptions. Chromium was predominately (*ca.* 70 %) in the residual fraction. These results are similar to the results obtained in Glasgow, Ljubljana and Torino soils collected for the EU URBSOIL project.[168] Li and Feng[169] also found that *ca.* 82 % of Cr in urban soil from an industrial district in Weinan, NW China was in the residual fraction. These results indicate that Cr was predominantly in an immobile phase. Environmentally it is unlikely to pose any danger as it will be difficult to be mobilised under ordinary environmental conditions and also the pseudototal concentrations (section 4.4.2) was below the current UK CLEA values. The high percentage of Cr in the residual fraction showed that it was strongly bound to the minerals and is indicative of a predominantly lithogenic origin for Cr at this site. Chromium is known to associate with and preferentially incorporate into the silicate lattice of primary and secondary minerals.[226]

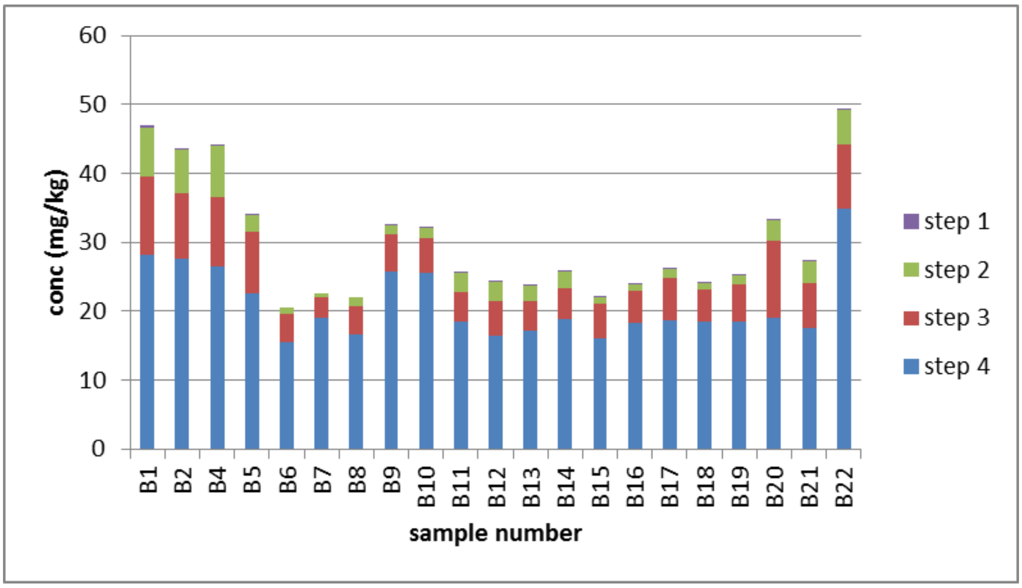


***Figure 4.11** Mean concentration of Cr in the sequential extracts from the plots (n=3)*

Copper

Figure 4.12 shows the distribution of Cu in the soil samples collected from the plots and the data is shown in table 2 in appendix A. The RSD were found to be generally good (< 10 %) with the exception of step 2 for plot B15 (175 %) and step 4 for plot B1 (112 %). The very high RSD in step 2 for sample B15 was not peculiar to Cu but common to all the PTE investigated. A close observation of the raw data showed that the values for the concentrations of the first two replicates for all the PTE were giving negative values, this indicated a common analytical error in that particular step for plot B15. This error may likely come from the extraction or the analyses of these particular replicates, the exact reasons were unclear. The single remaining sensible value was used in the plotting of the sequential extraction chart (figure 4.12) The Cu was found to be mainly in the oxidisable fraction (*ca.* 38.4 %) and residual fraction (*ca.* 34 %). These results were similar to earlier findings of fractionation of PTE in urban and agricultural soils using sequential extraction.[168, 169, 227, 228]The strong association of Cu to organic matter is due to the high formation constant of Cu-organic complex. A scanning electron microscope/energy dispersive x-ray (SEM/EDX) analysis on polluted soils[229] and an x-ray absorption spectroscopic study of a copper-contaminated soil[230] have both confirmed the strong association of Cu with organic matter. Although the pseudototal concentrations of Cu in these plots were higher than most of the values reported in the literature (section 4.4.2), the availability and mobility were low. Roughly 72 % of the total Cu was in the two least mobile fractions (oxidisable and residual) and this makes it difficult to be released into the environment under ordinary conditions, thus indicating a low human health risk from Cu in these plots.

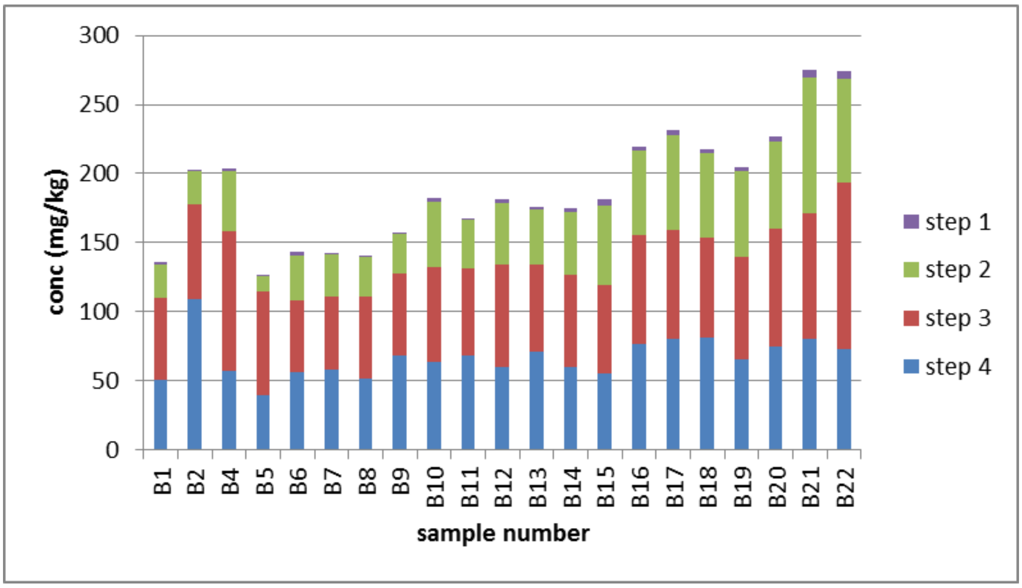


Figure 4.12 Mean concentration of Cu in the sequential extracts from the plots (n=3)

Iron

Figure 4.13 shows the distribution of Fe in various steps of the BCR sequential extraction whilst table 3 in appendix A shows the data in which the plot was based. The RSD were generally good except that of step 2 for plot B15. Iron was predominantly in the residual fraction (*ca*. 85 %). This result was not surprising since its minerals constitute a major structural component of the soil and it is associated with silicate minerals. Similar results have been obtained in studies of urban soils, [168] urban garden soils,[231] and street sediment samples.[31] Iron is one of the most abundant elements in soil, but it may not be available to some crop plant because of its presence in immobile form. Iron deficient soils occur mostly under arid climates and are associated with calcareous, alkaline soils. In humid climatic zones, with predominantly acidic soils, this deficiency is most unlikely to occur.[81]

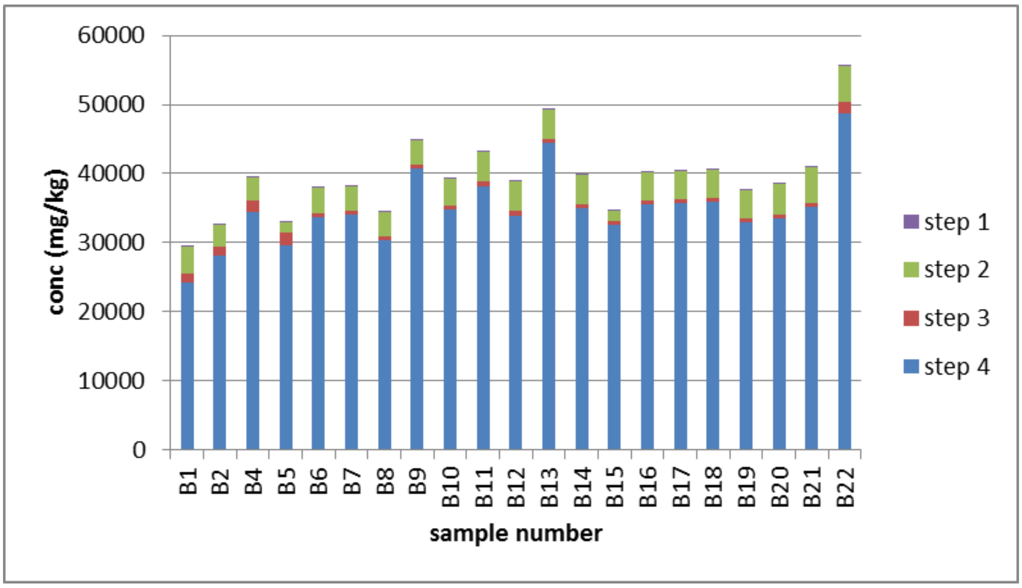


Figure 4.13 Mean concentration of Fe in the sequential extracts from the plots (n=3)

Manganese

Figure 4.14 shows the distribution of Mn in the various steps of the BCR sequential extraction for all the samples from the individual plots and the data is shown in table 4 in appendix A. The RSD were generally good (< 10 %) except that of step 2 for B15 (173%). In these soil samples, Mn was mainly found in two fractions, reducible and residual (*ca.* 40 % each). As in the case of Fe, Mn is one of the most abundant elements in soils. Its presence in the step 2 extract was expected because the reagent used in step 2 (hydroxlammonium chloride) was principally targeting the Mn (also Fe and perhaps Al) oxyhydroxides in the soils. Similar distribution patterns were found for urban soils from Aveiro, Glasgow, Ljubljana, Sevilla and Torino by Davidson *et al.*[168] Tokalioglu et *al.*[231] found 51 % of Mn in step 2 of the BCR sequential extraction for soils collected from three urban vegetable garden in Turkey, while Tokalioglu and Kartal[226] obtained 28 and 27 % of Mn in step 2 and 4 respectively in street sediments and a substantial amount (*ca.* 30 %) in a mobile phase (step 1).

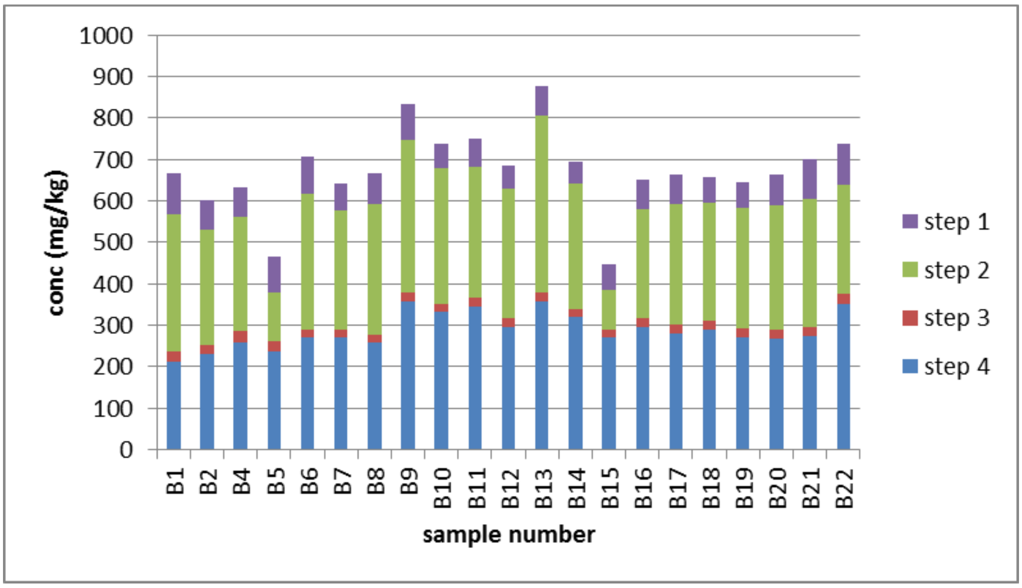


Figure 4.14 Mean concentration of Mn in the sequential extracts from the plots (n=3)

Nickel

The results of sequential extraction for Ni are presented in figure 4.15 and in table 5 in appendix A. The RSD were generally < 10 %, except for step 2 for samples B5 and B15, and for step 1 for B1. As in the case of Cr and Fe, Ni was predominantly in the residual fraction (*ca.* 69 %), which suggested it was likely to be of low mobility and bioavailability. These three elements are generally lithogenic, associated with the silicate matrix,[31] but an appreciable amount of Ni (*ca.* 35 %) was found in a mobile fraction (step 1) in the sample from plot B20 which suggest an anthropogenic input at that particular location. This supports the suggestion in section 4.4.2 that the relatively high concentration of Ni in sample B20 may be due to localized contamination. Various studies[31, 168, 231] have suggested that Ni is predominantly found in the residual fraction in soil. Although the mean pseudototal concentration of Ni in these soils was higher than the concentration of Ni in most of the soils discussed in section 4.4.2 and also exceeded the former UK CLEA SGV, it was of less environmental concern as 69 % was in the residual fraction.

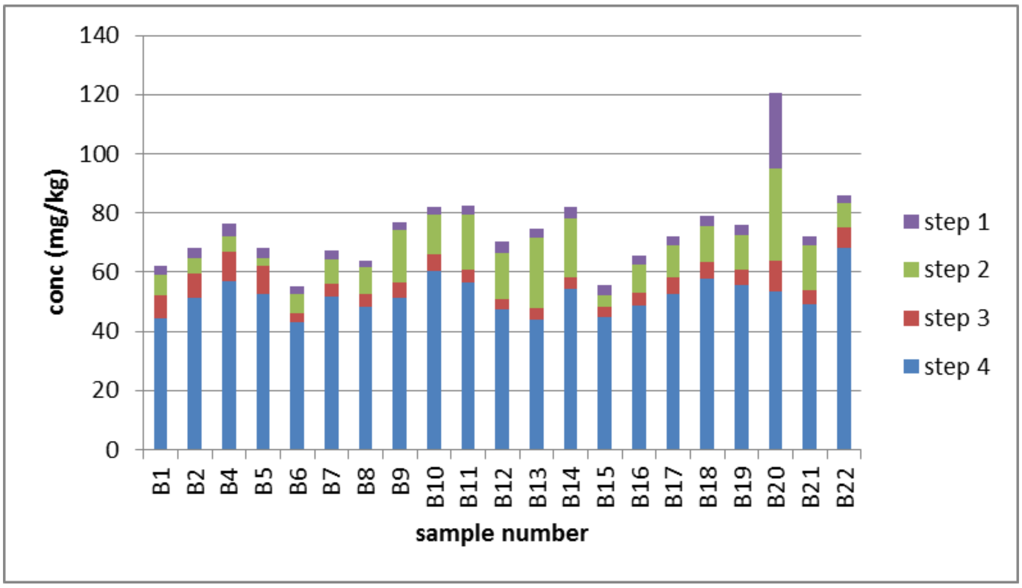


Figure 4.15 Mean concentration of Ni in the sequential extracts from the plots (n=3)

Lead

The results of the distribution of Pb in the various fractions of the BCR sequential extraction are shown in Figure 4.16 and in table 6 in appendix A. The RSD were generally good except for step 2 for sample B15 and step 4 for sample B1. The results of the sequential extraction of Pb in the various plots showed that Pb was predominantly (*ca.* 74 %) in the reducible fraction which indicates that Pb was occurring in a form bound to Fe/Mn oxides. The adsorption of Pb ions on the hydrous (amorphic) oxides of Fe/Mn was considered as a reasonably universal fixation mechanism.[24] Similar results were obtained in some agricultural soils from

China, [227] in which 50 % of the Pb was found in the reducible fraction. Li and Feng[169] found 43 % of Pb in the reducible fraction in urban soils from an industrial area. In their studies of urban soils from five European cities, Davidson *et al.*[168] found Pb to be mainly in reducible form in Aveiro, Glasgow and Torino but in oxidisable and residual forms in Sevilla. In the current study, the very large amount of Pb in the reducible fraction (942 mg/kg) is of great environmental concern as it potentially poses high environmental risk. This amount was twice the existing UK CLEA SGV of 450 mg/kg for residential gardens and allotments and Pb could easily be mobilised in event of reducing conditions becoming established such as due to water logging.

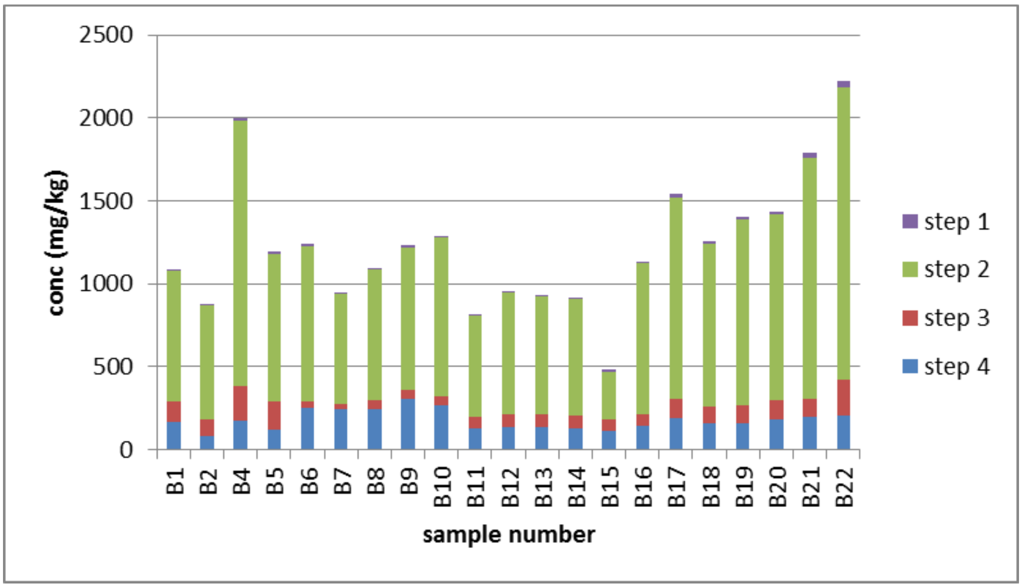


Figure 4.16 Mean concentration of Pb in the sequential extracts from the plots (n=3)

Zinc

The distribution pattern of Zn in the various steps of the BCR sequential extraction is shown in figure 4.17 whilst table 7 in appendix A shows the data in which the plot was based. The RSD were generally < 10 % except for step 2 for sample B15, step 3 for samples B4 and B5 and step 4 for samples B1, B2 and B5. Zinc in these soil samples was found in all four fractions, typically 29 % was found in the first two fractions each, 10 % in the oxidisable fraction and 32 % in the residual fraction. The high proportion of Zn in the exchangeable fraction made Zn the most mobile of all the PTE studied in this work. The presence of Zn in the mobile fraction was an indication that it originated from anthropogenic activities and this may explain the relatively high variability of the pseudototal concentrations of the element among the plots, as described in section 4.4.2. A similar pattern was observed in previous studies.[168, 169, 226]

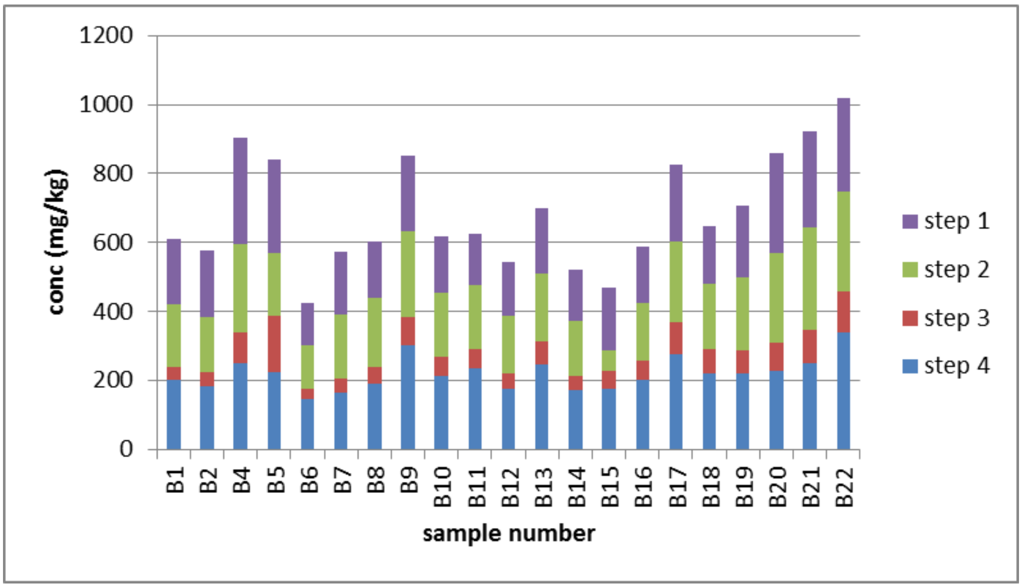


Figure 4.17 Mean concentration of Zn in the sequential extracts from the plots (n=3)

4.4.3.1 Correlations between PTE in various fractions of the BCR sequential extraction

Different PTE interact with various components of soil and various extracting reagents can remove them to different extents. A simple correlation method was used to study and assess any relationships between the PTE extracted from operationally defined phases of the BCR sequential extraction method. Correlations were calculated by plotting the concentrations of one PTE in all the samples against the concentrations of all the others and considering the correlation coefficient (r) at 95 % confidence level.

Table 4.8 gives the correlation coefficients calculated for the PTE extracted in step 1.

Table 4.8 Pearson correlation coefficients (r) between PTE extracted using step 1 of the BCR method (n=3) at 95 % confidence limit (values > 0.7 are shown in bold)

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Cr							
Cu	-0.137						
Fe	0.055	-0.308					
Mn	0.318	0.208	0.480				
Ni	0.097	0.261	-0.138	-0.028			
Pb	0.079	**0.801**	-0.137	0.510	0.035		
Zn	0.302	0.378	-0.046	0.443	0.389	0.610	

Only the levels of Pb and Cu were found to correlate strongly, while Zn moderately correlates with Pb. This could indicate that these elements originate from similar sources; indeed these elements are regarded as “urban metals”.[232]

Table 4.9 shows the correlation coefficients for PTE extracted at step 2.

Table 4.9 Pearson correlation coefficients (r) between PTE extracted using step 2 of the BCR method (n=3) at 95 % confidence limit

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Cr							
Cu	-0.042						
Fe	0.110	**0.768**					
Mn	0.055	0.232	**0.704**				
Ni	-0.188	0.344	0.528	0.556			
Pb	0.369	**0.699**	0.509	0.114	-0.022		
Zn	0.313	0.646	0.610	0.364	0.328	**0.828**	

Iron showed correlation with Cu, both were released to some extent in step 2. Manganese was found to correlate with Fe and this may indicate a common source for these elements e.g. oxyhydroxide minerals. Lead was found to moderately correlate with Cu and strongly with Zn. The correlation between the 3 elements, Cu, Pb and Zn might be due to the anthropogenic influence in the soil.

Table 4.10 shows the correlation coefficients between the PTE extracted in step 3.

Table 4.10 Pearson correlation coefficients (r) between PTE extracted using step 3 of the BCR method (n=3) at 95 % confidence limit

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Cr							
Cu	0.527						
Fe	**0.730**	0.515					
Mn	**0.737**	0.582	**0.876**				
Ni	**0.907**	0.486	**0.704**	**0.758**			
Pb	**0.737**	**0.842**	**0.838**	**0.856**	**0.700**		
Zn	0.411	0.596	0.537	0.655	0.536	**0.706**	

More correlation between the PTE were found in this step. Lead was found to correlate with all the elements, Ni was found to strongly correlate with Cr, Fe and Mn. Manganese also strongly correlates with Cr and Fe while Fe correlated with Cr. Zn weakly correlates with Cu, Fe, Mn, Ni and strongly correlates with Pb. In step 3 the targeted phases are organic matter and sulfide minerals. Organic matter can chelate with most elements from both natural and anthropogenic sources. Therefore, in this step, many correlations between the metals are expected.

The correlation coefficients between the PTE in step 4 are given in table 4.11.

Table 4.11 Pearson correlation coefficients (r) between PTE extracted using step 4 of the BCR method (n=3) at 95 % confidence limit

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Cr							
Cu	0.142						
Fe	0.159	0.203					
Mn	0.039	0.133	**0.876**				
Ni	0.567	0.191	0.442	0.356			
Pb	0.129	-0.223	0.228	0.256	0.112		
Zn	0.543	0.239	0.661	0.472	0.561	0.236	

In step 4 only Mn was found to strongly correlate with Fe and, since elements released in step 4 are relatively immobile, may be indicative of geogenic influence. Manganese was found to be correlated with Fe as in steps 2 and 3. This is further indicating a common source for both elements.

4.4.4 Phytoavailable content of PTE in soils from the allotment

It is difficult to set a general threshold values for either deficiencies or toxicities for PTE to plants because the availability of the PTE for uptake and the uptake rate depend on many factors.[2] These factors include the type of plant species, the stage of growth of the plant, the soil properties (PTE source, loading rate, soil pH, redox potential, texture, organic matter, mineral composition, *etc.*), as well as external factors such as climate and human agricultural practices.[233-236] The deficiencies and the phytotoxicities lead to stunted growth and low yield of the plants. In this section, the EDTA extractable concentrations obtained will be compared to what has been reported in some literatures to ascertain the phytotoxicity of the PTE.

Chromium

The EDTA extractable concentrations of the various PTE in each of the plots are shown in figure 4.18 and table 1 in appendix A. The precision of the results of the Cr EDTA extractable were generally bad (RSD > 10 %, n=3), this was likely due to the closeness of the concentrations to the detection limits (table 4.2). The concentrations varied between 0.08 and 1.37 mg/kg, these values were less than the total concentration values of 75 – 100 mg/kg which were thought to be the soil Cr concentration above which plant toxicity can occur.[237] The low concentration of Cr in this extract may be attributed to the fact that Cr is predominantly associated with the residual fraction in the BCR results (section 4.4.3) and elements in this fraction are usually not readily available for uptake by plants.

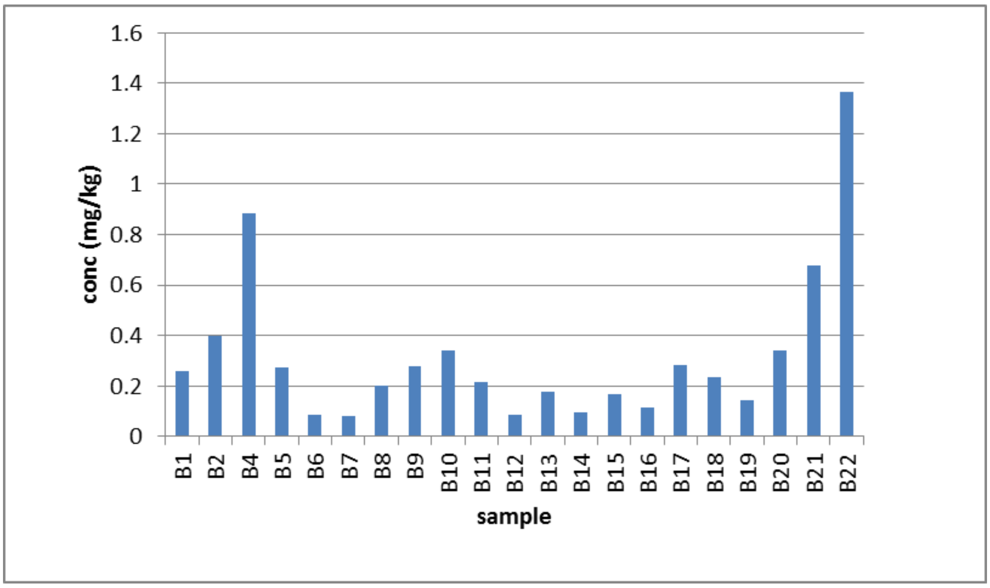


Figure 4.18 Phytoavailable concentrations of chromium in the urban allotment soil samples (mg/kg)

Copper

Figure 4.19 shows the phytoavailable concentrations of Cu in soil samples from the various plots and the data is shown in table 2 in appendix A. For Cu results the RSD were generally < 10 %, (n=3) which indicated good precision. The only exception was for plot B17 (RSD = 16.14 %), but this relatively high RSD was not only peculiar to Cu but also the other PTE for this particular plot. This indicated a common error with the extraction of the sample from this particular plot. The EDTA extractable concentrations of Cu in these plots varied between 24.8 and 114 mg/kg. In a European Union risk assessment report of Cu, [238] the chronic soil toxicity threshold concentration for monocotyledon plants ranged between 18 and 537 mg/kg, while for dicotyledon plants it ranged between 36 and 698 mg/kg. The values obtained from this work fell within the range for the dicotyledon plants, therefore depending on the plant species; the concentrations in some of the plots may be phytotoxic.

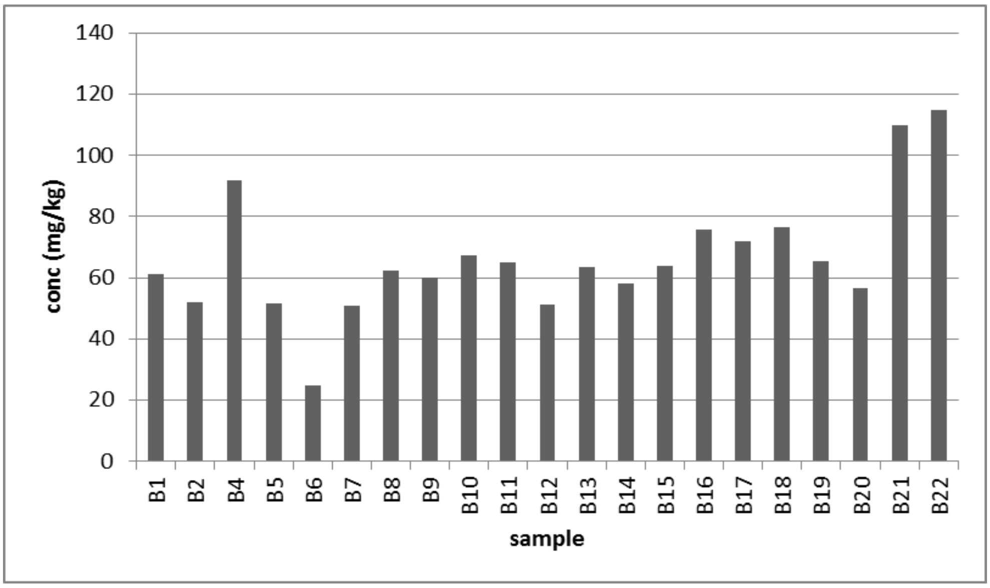


Figure 4.19 Phytoavailable concentrations of copper in the urban allotment soil samples (mg/kg)

Iron

The EDTA extractable concentrations of the various PTE in each of the plots are shown in figure 4.20 and table 3 in appendix A. The RSD were generally good except for sample from plots B10, B12 and B21. In this work a relatively small amount of Fe was extracted by EDTA (241 – 449 mg/kg) which is about 0.84% of the pseudototal concentration. Under normal soil conditions Fe is only slightly mobile;[2] this is supported in this work by the sequential extraction results (section 4.4.3), which shows that 85 % of Fe was in the residual fraction. Because of the general immobility of Fe, phytotoxicity due to Fe is rare, but this is likely to occur in strongly acidic soils and on flooded soils. For example, it was reported[239] that rice seedlings were killed in a paddy soil at a concentration of about 500 mg/kg of Fe^{2+}.

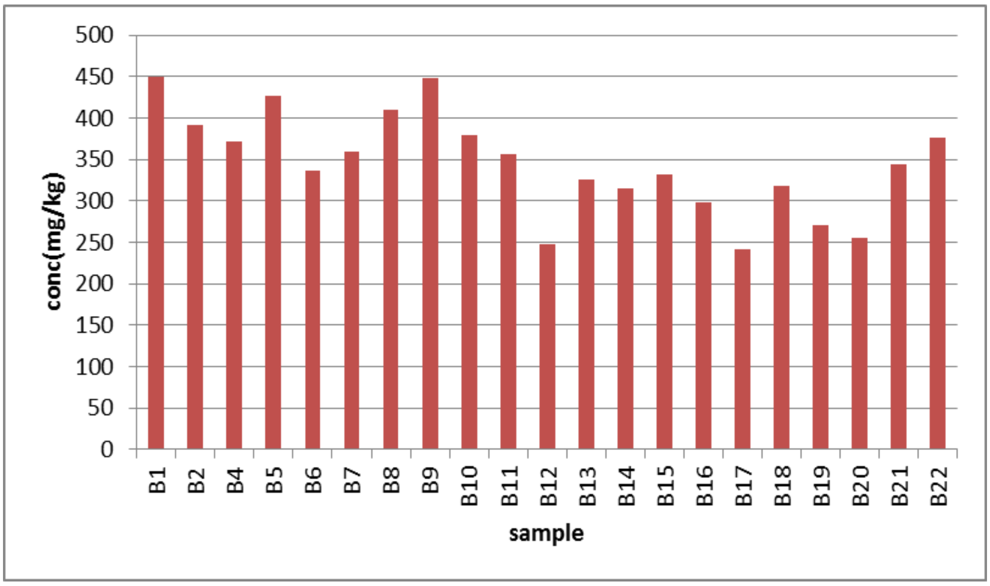


Figure 4.20 Phytoavailable concentrations of iron in the urban allotment soil samples (mg/kg)

Manganese

The results of the phytoavailable concentrations of Mn in various plots are shown Figure 4.21 and table 4 in appendix A. The Mn EDTA extractable concentrations in the samples ranged between 31.3 and 102 mg/kg with average concentration of 50.6 mg/kg. The RSD for the measurements were generally < 10 % except for plots B10, B17 and B21. Just like other PTE, sensitivity to excesses of Mn varies with plant species. Legumes, cereals and potatoes were found to be most sensitive to high Mn concentration in soil.[2] Most plants were found to be affected by Mn at a concentration of about 500 mg/kg, although higher concentrations of 1000 and 10,000 mg/kg were reported[240] for higher resistance plants and hyperaccumulators. Comparing these values with the current results, phytotoxicity due to Mn in the plots in the allotment is unlikely.

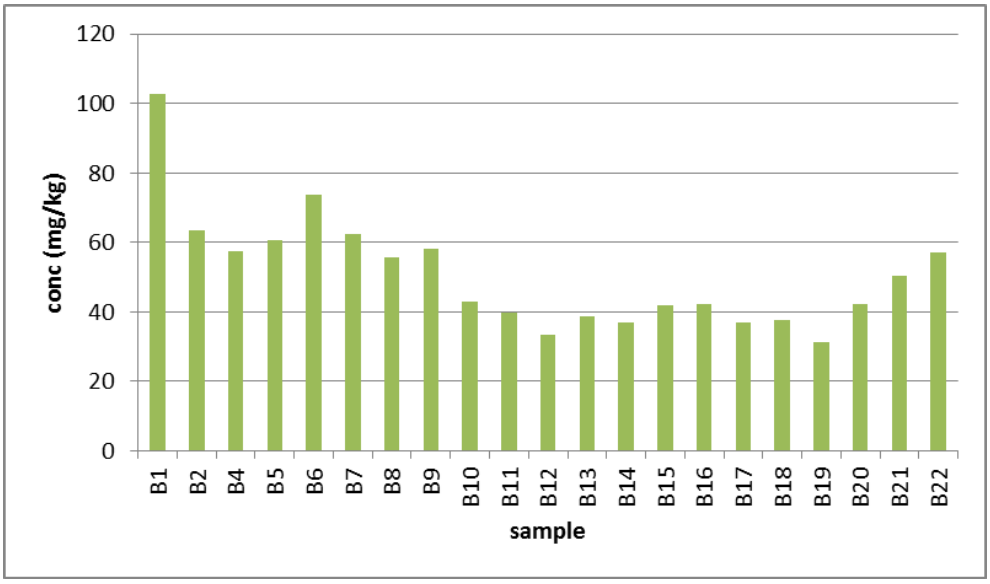


Figure 4.21 Phytoavailable concentrations of manganese in the urban allotment soil samples (mg/kg)

Nickel

Figure 4.22 shows the phytoavailable concentrations of Ni in the soil samples from the various plots from the Wellington street allotment and the data is shown in table 5 in appendix A. The RSD were generally good except for samples from plot B21. The concentrations of Ni extracted were generally low (2.47 – 6.80 mg/kg), just about 6 % of the pseudototal concentration. This may be because of the geogenic nature of Ni, it is mainly associated with silicate minerals which are not likely to be extracted by EDTA. In literatures, concentrations of less than 100 mg/kg were mostly reported [241, 242] for the phytotoxic threshold concentrations of Ni. As with other elements, the responses to Ni toxicity depend on the concentration of Ni in the soil, plant species, growth stage, cultivation conditions and exposure time.[2, 90] In their review, Chen *et al.*[243] gave the critical phytotoxicity level for Ni as > 10 mg/kg for sensitive species, > 50 mg/kg for moderately tolerant species and > 1000 mg/kg for Ni hyperaccumulator plants, such as *Alyssum* and *Thlaspi* species. In general the concentrations of Ni in all the plots obtained in this work were lower than the critical toxicity value of even the sensitive species, therefore it is unlikely to have any toxic effect due to Ni in these plots.

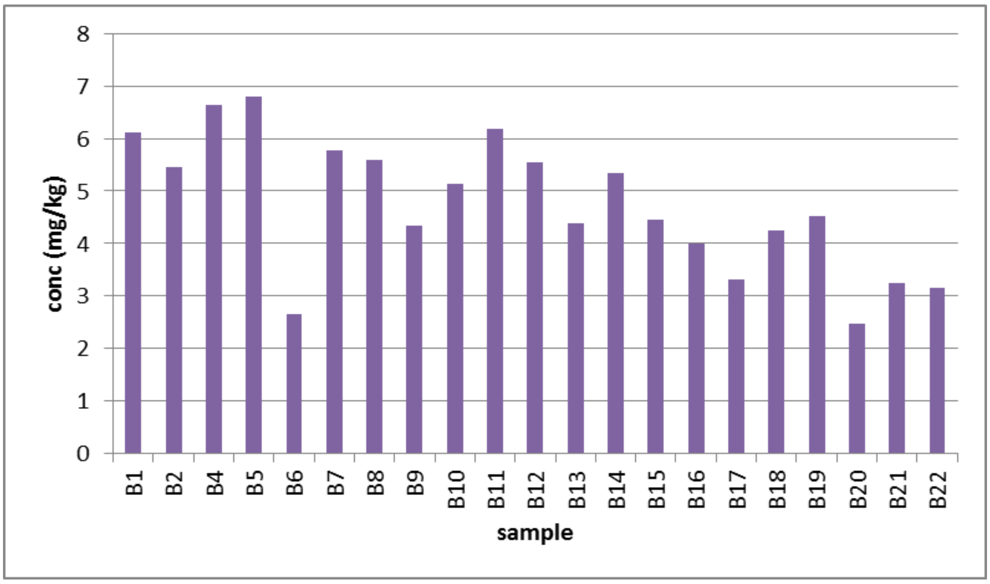


Figure 4.22 Phytoavailable concentrations of nickel in the urban allotment soil samples (mg/kg)

Lead

The concentrations of Pb in soil samples from various plots are shown in figure 4.23 and in table 6 in appendix A. The RSD were generally good except for plots B7, B14, B17 and B19. The plant toxicity levels of Pb in soils are not easy to evaluate, but it is still generally agreed that a soil Pb concentration ranging from 100 to 500 mg/kg is considered as excessive.[2] The EDTA extractable Pb concentrations obtained in this present work ranged between 328 and 1110 mg/kg, these values were relatively high when compared with other PTE under study. Lead in these plots may be phytotoxic to some plants grown on these plots because most of the EDTA extractable Pb concentrations were within or above the concentrations of Pb considered to be excessive from the literature.[2]

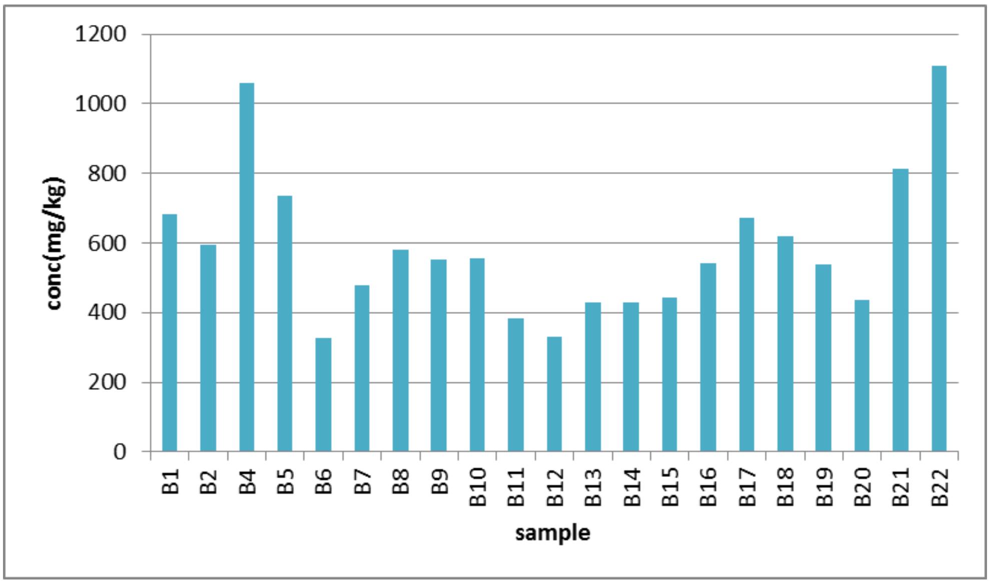


Figure 4.23 Phytoavailable concentrations of lead in the urban allotment soil samples (mg/kg)

Zinc

Figure 4.24 shows the various phytoavailable concentrations of Zn extracted from the soil samples from the different plots and the data is shown in table 7 in appendix A. The concentration of EDTA extractable Zn ranged between 107 and 366 mg/kg, and the RSD were generally < 10 %, (n=3), with the exception of plots B17 and 21 (RSD = 15 %), which indicated good precision in the extraction. For sensitive terrestrial plants, concentration of Zn exceeding 100 mg/kg in soil can be lethal, while higher concentrations of Zn in soil were tolerated by some species known to hyperaccumulate Zn, such as *Thlaspi* species.[81] Comparing the results from this work and the critical values, it is possible that the amounts of Zn in these plots may be toxic to some plant species.

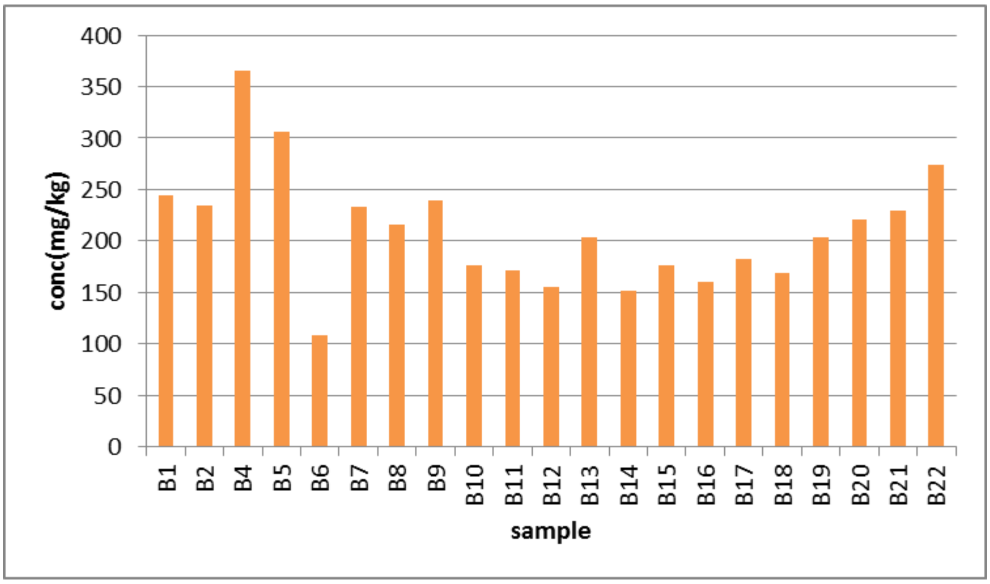


Figure 4.24 Phytoavailable concentrations of zinc in the urban allotment soil samples (mg/kg)

4.4.4.1 Correlations between PTE extracted using EDTA method

The Pearson correlation method was used to study and assess any correlation between the PTE extracted using EDTA. The correlations were calculated as described in section 4.4.3.1.

Table 4.12 shows the correlation coefficients between PTE extracted using the EDTA extraction method.

Table 4.12 Pearson correlation coefficients (r) between PTE extracted using the EDTA extraction method (n=3) at 95 % confidence limit

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Cr							
Cu	**0.788**						
Fe	0.232	0.018					
Mn	0.157	-0.165	**0.714**				
Ni	-0.153	-0.167	0.478	0.214			
Pb	**0.880**	**0.780**	0.345	0.268	0.066		
Zn	0.628	0.429	0.495	0.354	0.405	**0.774**	

Chromium was found to be strongly correlated with Cu, Pb and moderately correlated with Zn, which might indicate that there was possible anthropogenic influence due to Cr, although the concentrations of Cr extracted were very small. Manganese was again found to be strongly correlated with Fe. The level of Zn extracted with EDTA was found to be moderately correlated Cr and strongly with another “urban metal”, Zn.

4.4.5 Oral bioaccessibilty estimated by the Simplified Bioaccessibilty Extraction Test

The estimation of the potential human health risk was evaluated from the amount of the PTE extracted using the SBET extraction procedure which simulates the stomach compartment only. The results of the SBET for the individual plots for all the PTE under study are shown in figures 4.25 – 4.31 and in tables 1 – 7 in appendix A. The RSD were generally < 10 %, (n=3), with the exception of Cr where the RSD were very high, because the concentrations of Cr were either close to or below the procedural limits (table 4.3). Some of the values were negative and were considered zero for the graphical presentation (figure 4.25), as suggested by the Analytical Methods Committee of the Royal Society of Chemistry.[244] Other exceptions were for Cu, Mn and Ni in plot B2, Fe in plot B8 and Pb in B13.

The concentrations of Cu extracted using the SBET ranged between 24.4 and 89.9 mg/kg, Fe ranged between 110 and 194 mg/kg, and Mn between 106 and 225 mg/kg. Ni concentration ranged between 5.66 and 43.0 mg/kg, and the sample from plot B20 stands out again from other plots as seen in the case of pseudototal content Ni (section 4.4.2). The amount of Pb extracted ranged between 329 and 1110 mg/kg, much higher than concentration of the other PTE extracted. The concentration of Zn extracted was between 157 and 366 mg/kg.

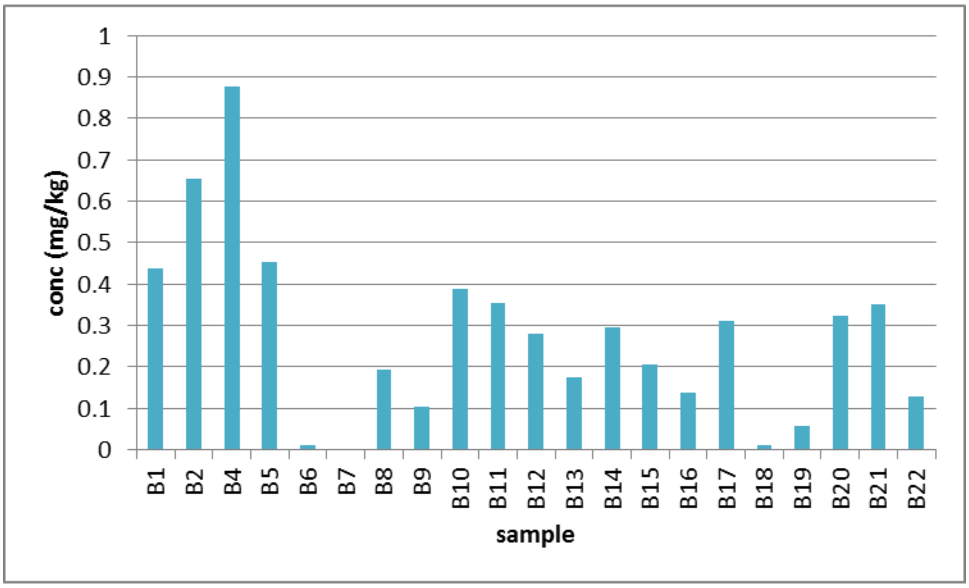


Figure 4.25 Bioaccessible concentrations of chromium in the urban allotment soil samples (mg/kg)

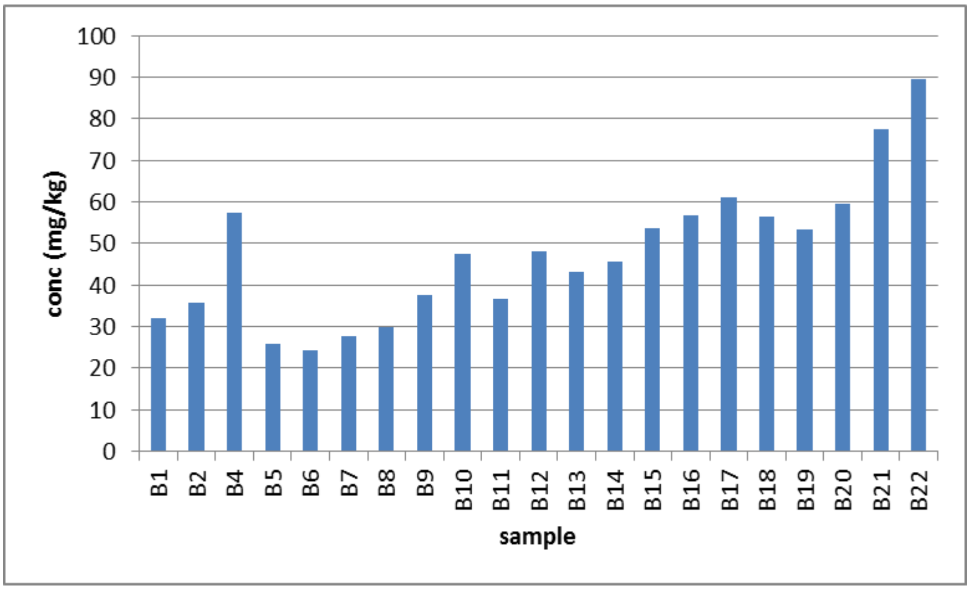


Figure 4.26 Bioaccessible concentrations of copper in the urban allotment soil samples (mg/kg)

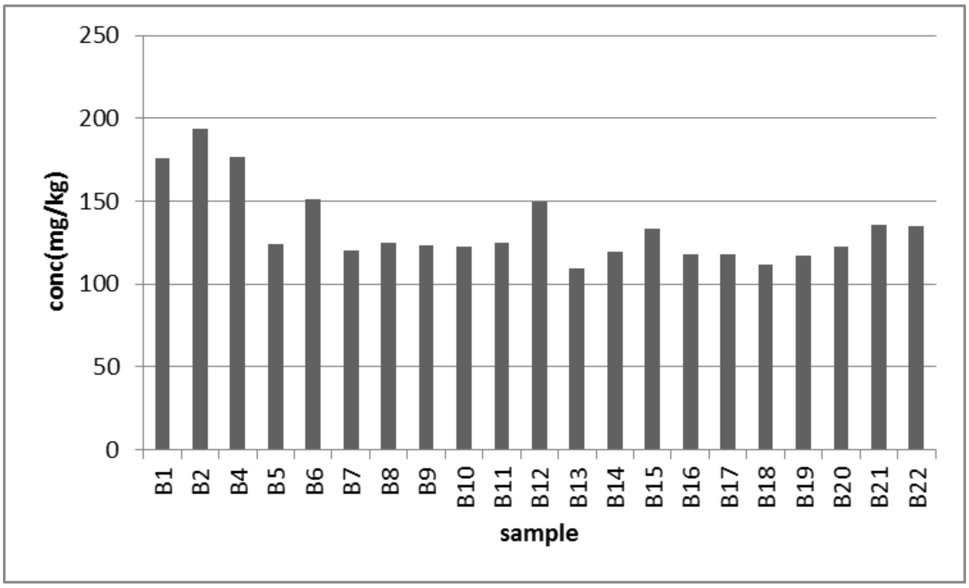


Figure 4.27 Bioaccessible concentrations of iron in the urban allotment soil samples (mg/kg)

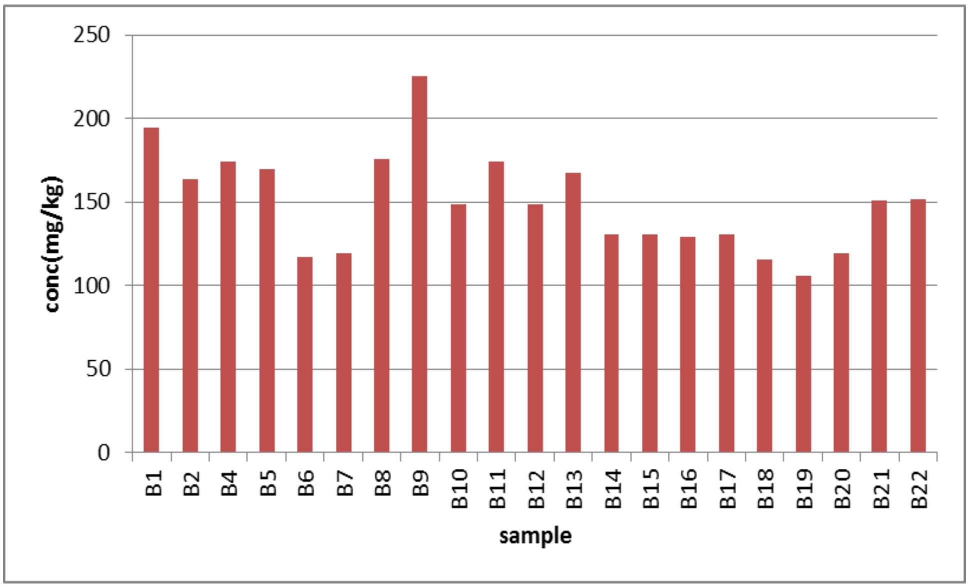


Figure 4.28 Bioaccessible concentrations of manganese in the urban allotment soil samples (mg/kg)

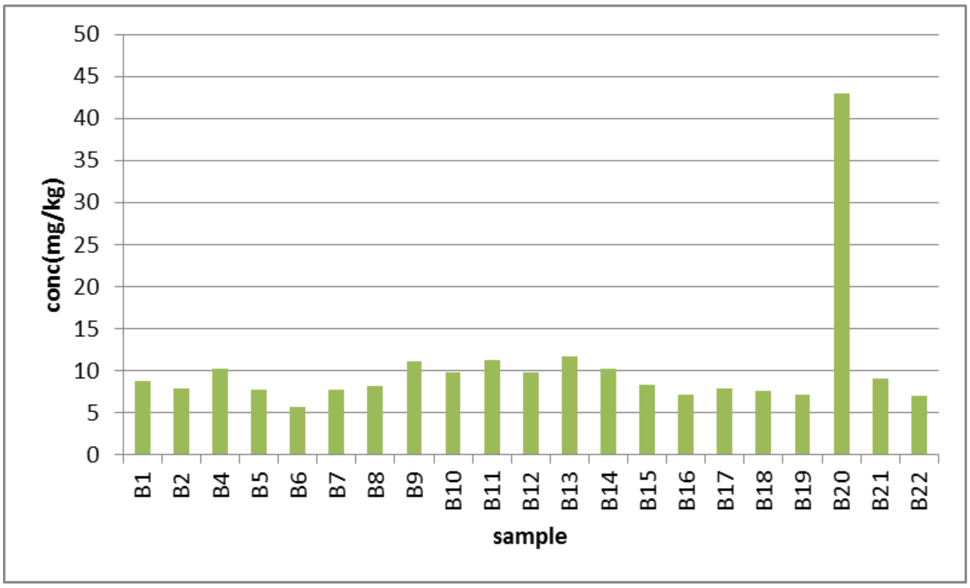


Figure 4.29 Bioaccessible concentrations of nickel in the urban allotment soil samples (mg/kg)

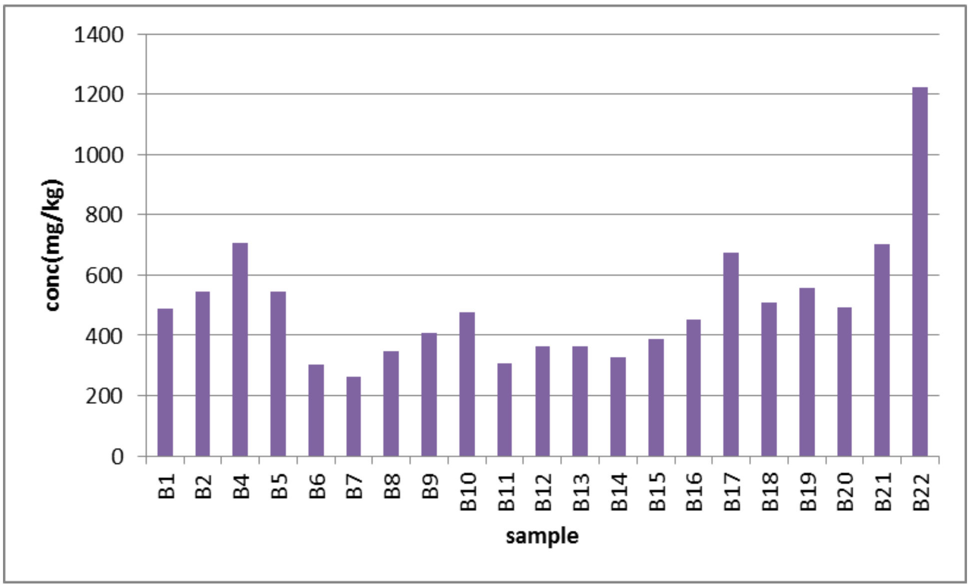


Figure 4.30 Bioaccessible concentrations of lead in the urban allotment soil samples (mg/kg)

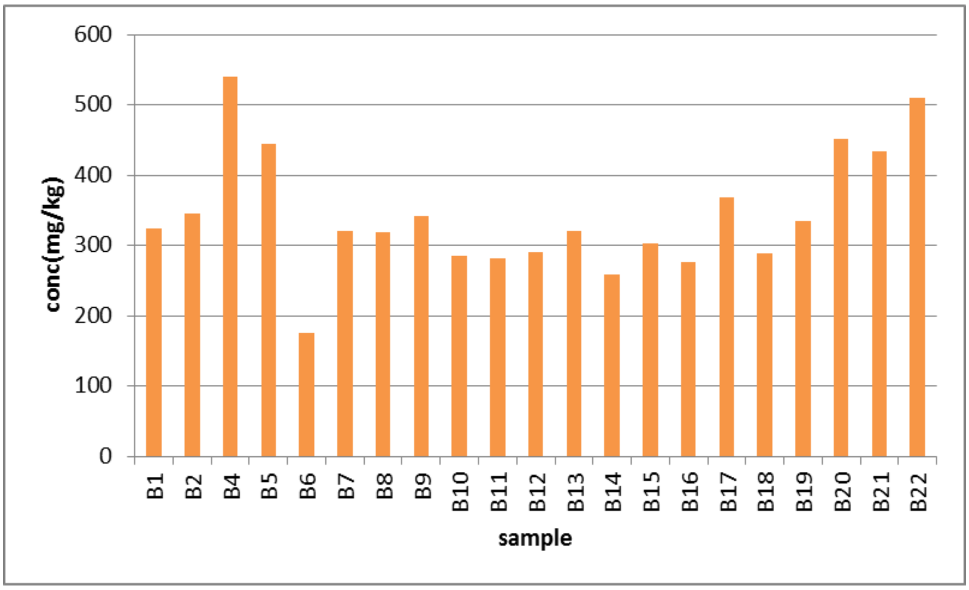


Figure 4.31 Bioaccessible concentrations of zinc in the urban allotment soil samples (mg/kg)

Estimation of potential human health risk from the soil samples

The main objective of bioaccessibility investigation is to assess the potential hazard of PTE to human health when ingested orally from contaminated soils. This is especially important in children who come in contact with the soil when playing in gardens and parks through hand-to-mouth transfer. Risk can be estimated crudely by calculating the amount of the soil that a hypothetical child needs to ingest to reach a toxicologically significant level. This can be calculated using the equation:

Mass of soil required =tolerable daily intake / bioaccessible PTE concentration

Equation 4.2

This estimation is unrealistic because it is assumes that the oral source is the only intake way, however it allows the determination of the relative risk from different PTE and soils. Recommended values for tolerable daily intake vary between sources. Table 4.13 shows the toxicological data for the PTE studied. From the values given, the tolerable daily intakes for a 20 kg child were calculated. The amounts of the soil needed to be ingested to reach the tolerable daily limit were then calculated using equation 4.2 for each PTE.

Table 4.13 Toxicological data for PTE studied

Element	Unit	Ref.	Value	Tolerable daily intake for a 20 kg child (mg/day)
Cr	TDI[a]	DEFRA[245]	3 μg/kg bw	0.06
Cu	TDI[a]	Selinus[246]	3 mg/day	3
Fe	PMTDI[b]	JECFA[247]	0.8 mg/kg bw	16
Mn	LOAEL[c]	Greger[248]	0.06 mg/kg bw/day	1.2
Ni	TDI[a]	DEFRA[249]	12 μg/kg bw/day	0.24
Pb	PTWI[d]	WHO[250]	25 μg/kg bw	0.072
Zn	TDI[a]	Selinus[246]	12 mg/day	12

[a] Tolerable daily intake, [b] Provisional maximum tolerable daily intake,
[c] Lowest observable adverse effect level, [d] Provisional tolerable weekly intake

Table 4.14 gives the average amount of the soil required to be ingested by a 20 kg child to reach the TDI values for the various PTE for each plot. The amounts calculated for Cr showed very wide variation and are probably unrealistic due to the closeness of the concentrations of Cr extracted using the SBET method to the detection limits. With the average daily ingestion of soil in children of between 50 and 200 mg,[199] Cu, Fe, Mn, Ni and Zn in these samples did not represent a significant human health risk, because the amounts required to reach the TDI were much higher than this average daily ingestion values. However, Pb is of concern since the ingestion of, on average, 160 mg of soil per day would reach the TDI value.

Table 4.14 Amounts of soil (g/day) that a 20 kg child would require to ingest to reach TDI values in Table 4.13 assuming soil ingestion is the only oral source of PTE intake

Plot	Cr	Cu	Fe	Mn	Ni	Pb	Zn
B1	137	93.4	90.8	6.18	27.3	0.15	37.0
B2	91.6	83.7	82.6	7.32	30.4	0.13	34.7
B4	68.3	52.4	90.4	6.89	23.5	0.10	22.2
B5	132	116	129	7.08	30.8	0.13	27.1
B6	4870	123	106	10.2	42.4	0.24	68.3
B7	6000	109	133	10.1	30.9	0.27	37.5
B8	311	100	128	6.82	29.2	0.21	37.7
B9	570	79.5	130	5.33	21.5	0.18	35.0
B10	155	63.3	130	8.06	24.6	0.15	42.0
B11	169	81.6	128	6.90	21.4	0.23	42.7
B12	213	62.2	107	8.08	24.6	0.20	41.3
B13	346	69.6	146	7.17	20.4	0.20	37.5
B14	203	65.7	134	9.16	23.4	0.22	46.4
B15	290	56.0	120	9.16	28.7	0.19	39.6
B16	433	52.8	135	9.29	33.7	0.16	43.4
B17	193	49.0	136	9.16	30.6	0.11	32.6
B18	6000	53.0	143	10.4	31.6	0.14	41.7
B19	1030	56.4	137	11.3	33.3	0.13	35.9
B20	186	50.3	130	10.1	5.58	0.15	26.6
B21	171	38.7	118	7.97	26.6	0.10	27.6
B22	463	33.5	118	7.91	34.2	0.06	23.5
Mean	1050	70.9	123	8.31	27.4	0.16	37.1

4.4.5.1 Correlations between PTE extracted using SBET method

The Pearson correlation method was again used to study and assess any correlation between the PTE extracted using the SBET extraction method. The correlations were calculated as described in section 4.4.3.1.

The correlation coefficients calculated for PTE extracted using the SBET method are shown in table 4.15

Table 4.15 Pearson correlation coefficients (r) between PTE extracted using the SBET extraction method (n=3) at 95 % confidence limit

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Cr							
Cu	0.013						
Fe	0.658	-0.134					
Mn	0.404	-0.256	0.322				
Ni	0.117	0.147	-0.125	-0.107			
Pb	0.218	**0.768**	0.171	0.034	-0.058		
Zn	0.506	0.531	0.192	0.234	0.291	**0.746**	

Lead was found to correlate with Cu, while Zn correlated with Pb in this extraction method. As already established, these 3 elements are mainly anthropogenic metals associated with urban settings, what this showed was they all have relatively high bioaccessibilities.

4.4.6 Correlations between extraction methods for various PTE

In sections 4.4.3.1, 4.4.4.1 and 4.4.5.1, Pearson correlation was used to assess any correlation between the PTE in various extraction methods. In this section, Pearson correlation was used to assess any correlation between different extraction methods applied to each PTE studied.

Chromium

The correlation coefficients calculated for the amounts of Cr released by various extraction methods are shown in table 4.16.

Table 4.16 Pearson correlation coefficients (r) between various extraction methods for Cr (n=3) at 95 % confidence limit

	Cr pseudo	Cr step 1	Cr Σ step 1-3	Cr EDTA	Cr SBET
Cr pseudo					
Cr step 1	0.468				
Cr Σ step1-3	0.698	**0.844**			
Cr EDTA	0.681	0.289	0.595		
Cr SBET	0.424	0.621	**0.733**	0.349	

The amounts of Cr extracted in step 1, EDTA and SBET methods were low, mostly lower or very close to the detection limits. The values obtained are not reliable, therefore it is difficult to discuss the correlations between the methods based on these results.

Copper

Table 4.17 shows the correlation coefficients between the amounts of Cu released by various extraction methods.

Table 4.17 Pearson correlation coefficients (r) between various extraction methods for Cu (n=3) at 95 % confidence limit

	Cu pseudo	Cu step 1	Cu Σ step 1-3	Cu EDTA	Cu SBET
Cu pseudo					
Cu step 1	**0.776**				
Cu Σ step1-3	**0.833**	**0.836**			
Cu EDTA	0.602	0.617	**0.814**		
Cu SBET	**0.833**	**0.884**	**0.938**	**0.849**	

Most of the methods for Cu were found to correlate with each other. This may likely be due to anthropogenic influence. Copper is added into the urban soils mainly through anthropogenic activities, therefore it can easily be removed by these reagents used in the various methods.

Iron

Table 4.18 shows the correlation coefficients between the amounts of Fe released by various extraction methods.

Table 4.18 Pearson correlation coefficients (r) between various extraction methods for Fe (n=3) at 95 % confidence limit

	Fe pseudo	Fe step 1	Fe Σ step 1-3	Fe EDTA	Fe SBET
Fe pseudo					
Fe step 1	-0.202				
Fe Σ step1-3	0.398	-0.259			
Fe EDTA	0.182	0.556	-0.112		
Fe SBET	-0.104	0.360	0.118	0.340	

In contrast to Cu, the amounts of Fe extracted by these methods were not strongly correlated. This may be an indication of the geogenic nature of Fe, it is mostly incorporated into the silicate lattice of the minerals in soil and this makes it difficult to be released by most of these reagents. Therefore Fe in these soils was poorly extracted by any of these methods (except for pseudototal method).

Manganese

The correlation coefficients calculated for the amounts of Mn released by various extraction methods are shown in table 4.19.

Table 4.19 Pearson correlation coefficients (r) between various extraction methods for Mn (n=3) at 95 % confidence limit

	Mn pseudo	Mn step 1	Mn Σ step 1-3	Mn EDTA	Mn SBET
Mn pseudo					
Mn step 1	0.223				
Mn Σ step1-3	0.497	0.260			
Mn EDTA	0.465	0.687	0.169		
Mn SBET	**0.703**	0.445	0.330	0.440	

In case of Mn, the amounts extracted by EDTA were found to moderately correlate with the amount extracted in step 1. The amounts extracted by the SBET methods were found to strongly correlate with amounts extracted with *aqua regia* in the pseudototal method. Almost none of the methods correlated with one other, as in the case of Fe, this is further indicting geogenic influence of Mn. Some reasonable amounts of Mn were extracted in step 1, EDTA, and SBET methods.

Nickel

The correlation coefficients calculated for the amounts of Ni released by various extraction methods are shown in table 4.20.

Table 4.20 Pearson correlation coefficients (r) between various extraction methods for Ni (n=3) at 95 % confidence limit

	Ni pseudo	Ni step 1	Ni Σ step 1-3	Ni EDTA	Ni SBET
Ni pseudo					
Ni step 1	**0.864**				
Ni Σ step1-3	**0.853**	**0.899**			
Ni EDTA	-0.278	-0.370	-0.341		
Ni SBET	**0.883**	**0.977**	**0.950**	-0.321	

The amount of Ni extracted in the step 1 of the BCR sequential method was found to correlate with pseudototal concentration. The sum of amounts extracted in steps 1 to 3 of the BCR procedure was found to correlate with the amount extracted by the pseudototal method and in the step 1. The Ni extracted by the SBET method correlated with the Ni extracted by pseudototal method and the amounts extracted in step 1 and the sum of the amounts in steps 1 to 3 of the BCR sequential extraction. The correlation seems to indicate anthropogenic influence but the sequential extraction results for Ni in section 4.4.3 suggested otherwise. However plot B20 which has a relatively high concentration of Ni and which was thought to be due localized contamination may have influence this present result (correlation).

Lead

Table 4.21 shows the correlation coefficients between the amounts of Pb released by various extraction methods.

Table 4.21 Pearson correlation coefficients (r) between various extraction methods for Pb (n=3) at 95 % confidence limit

	Pb pseudo	Pb step 1	Pb Σ step 1-3	Pb EDTA	Pb SBET
Pb pseudo					
Pb step 1	**0.885**				
Pb Σ step1-3	**0.917**	**0.844**			
Pb EDTA	**0.833**	**0.725**	**0.824**		
Pb SBET	**0.907**	**0.924**	**0.862**	**0.868**	

The amounts of Pb extracted in all the various extraction methods were found to strongly correlate with each other. As in the case of Cu, this may indicate anthropogenic influence.

Zinc

Table 4.22 shows the correlation coefficients between the amounts of Zn released by various extraction methods.

Table 4.22 Pearson correlation coefficients (r) between various extraction methods for Zn (n=3) at 95 % confidence limit

	Zn pseudo	Zn step 1	Zn Σ step 1-3	Zn EDTA	Zn SBET
Zn pseudo					
Zn step 1	**0.921**				
Zn Σ step1-3	**0.878**	**0.940**			
Zn EDTA	**0.898**	**0.817**	**0.735**		
Zn SBET	**0.928**	**0.962**	**0.915**	**0.872**	

As was in the case of other “urban metals” (Cu and Pb), the amount of Zn extracted in all the methods were found correlate with one another and this further demonstrates an anthropogenic influence.

4.4.7 Principal Component Analysis (PCA)

In order to obtained more information from the data collected, principal component analysis was applied to the whole data set to obtain any possible relationships between the samples, analytes, and extraction methods.

The data were imported into Matlab, version 2012a (Mathsworks Inc. Natick, MA) for analysis using the PLS_Toolbox, version 6.7.1 (Eigenvector Research, Manson, WA). The data were auto scaled prior to analysis, which meant that the mean and standard deviation of each column (variable) was 0 and 1, respectively.

Eigen value plot

To determine the number of principle components (PC) that adequately described the data, eigenvalues were needed. The eigenvalue represents the variance in the data that is described by its associated PC. Figure 4.32 shows the eigenvalues plot for the data, 90.1 % of the variation in the data was described in PCs 1 and 2, while PCs 1 to 4 accounted for 96.7 % of variances for all of the data. The 90.1 % was enough to reasonably describe the variations so PCs 1 and 2 were chosen to describe the data.

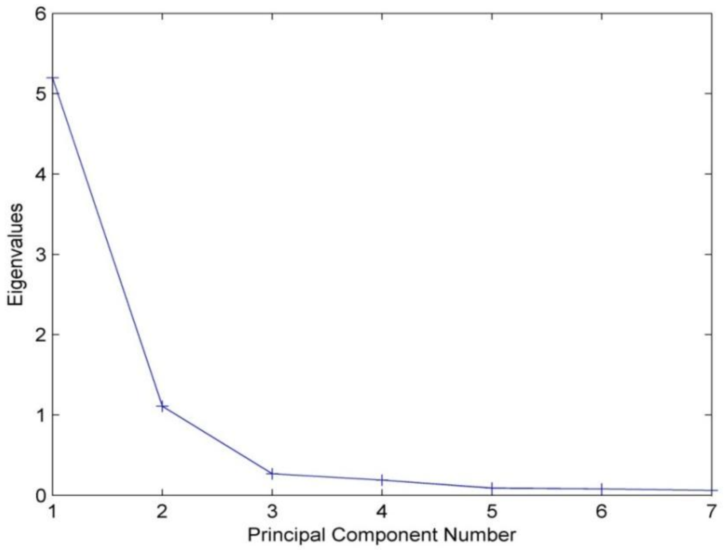


Figure 4.32 Principal Component Eigenvalue plot

PC loadings

The PC loadings described the relationship between individual measurement variables (in this case the analytes). Figures 4.33 and 4.34 show the loading plots of PCs 1 and 2 respectively.

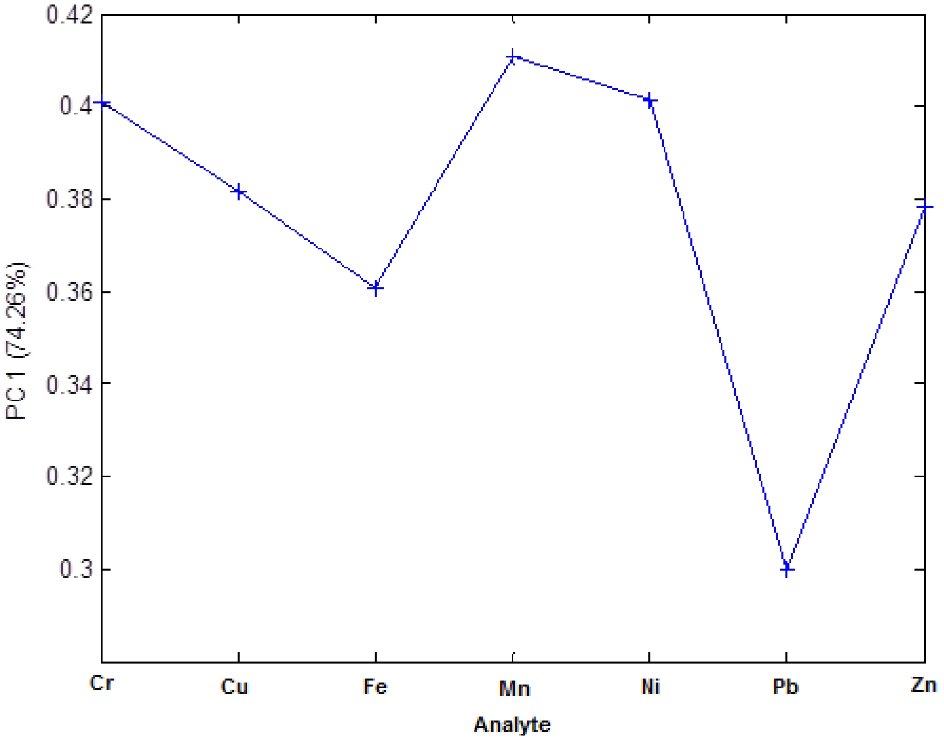


Figure 4.33 Loading plot for PC1

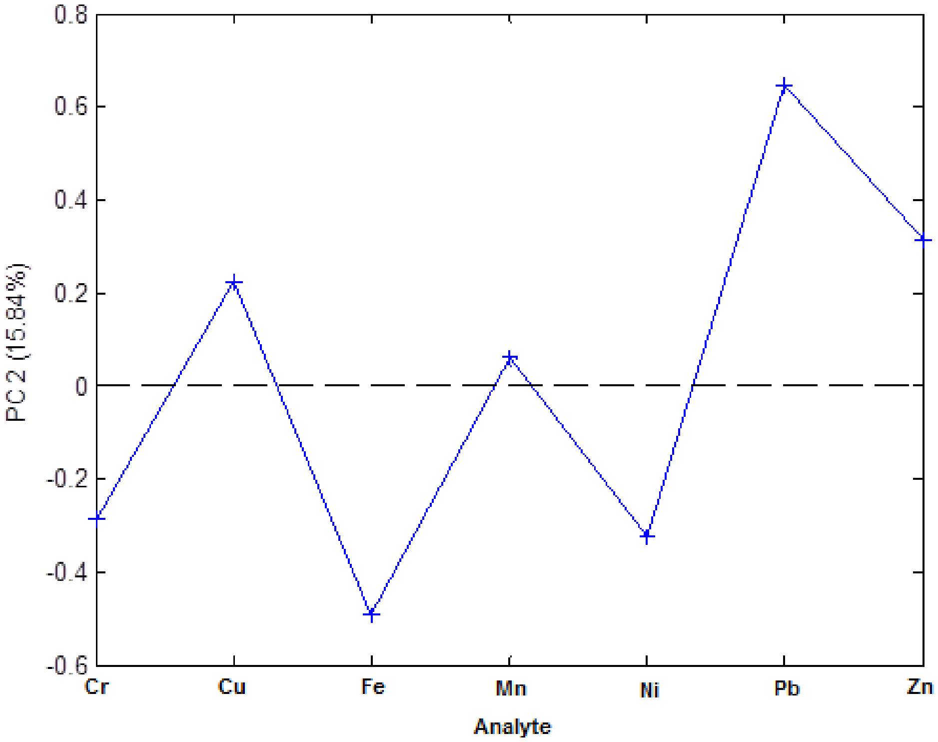


Figure 4.34 Loading plot for PC2

The first PC explained 74.3 % of the variation in the data. The loadings on PC1 for the analytes were between 0.3 and 0.4, this meant that PC1 was associated with all the analytes. The second PC explained 15.8 % of the variation in the data. The PC2 loading indicated that PC2 was mainly associated with Pb, and also correlated with Zn and it was anti-correlated with Cr, Fe and Ni. There was also some association with Cu but none with Mn. Although only a small percentage of the variation in the data was described in PC2, this component had grouped the elements likely to be mainly of lithogenic origin, i.e. Cr, Fe, and Ni and Cu, Pb and Zn the "urban metals",[232] mainly of anthropogenic origins.

Score loadings

Figures 4.35 and 4.36 show the score plots for PC1 and PC2 respectively, the data is colour-coded according to type of extraction applied, for the example, entries 1 to 8 present plot 1, with entry 1 representing the EDTA extractable PTE, entry 2 representing the SBET extractable PTE, entries 3, 4, 5, 6, 7, representing pseoudototal, exchangeable, reducible, oxidisable, residual fractions respectively and entry 8 representing the sum of steps 1 to 3. Entry 9 to 16 represent plot 2 and so on.

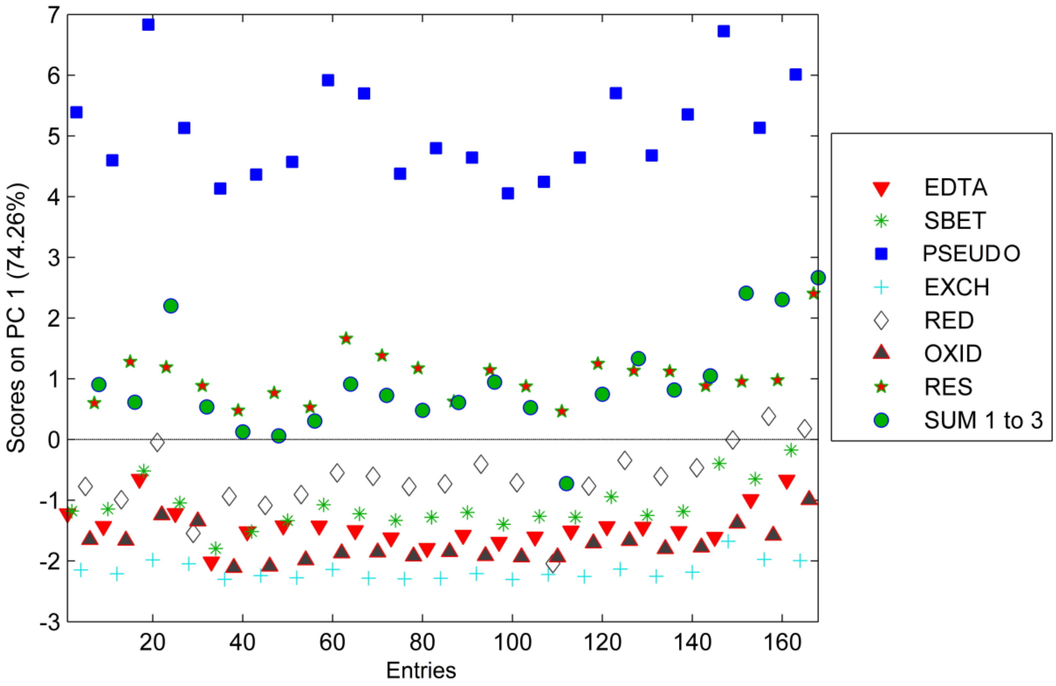


Figure 4.35 Score plot for PC1

Since PC1 was associated with all analytes, then a high PC1 score would mean that the sample was high in all the analytes. Conversely, the most negative PC1 score meant that the sample was low in all the analytes. Therefore from figure 4.35, the highest concentration of all the analytes is found in the pseudototal fraction across all the plots, this was obvious because the pseudototal fraction represents the maximum potentially soluble or mobile contents of metals[105]. It usually consists of all the metals bound to the soil solid phase with the exception of the silicate bound ones. The lowest concentration of all the analytes was found in the exchangeable fraction.

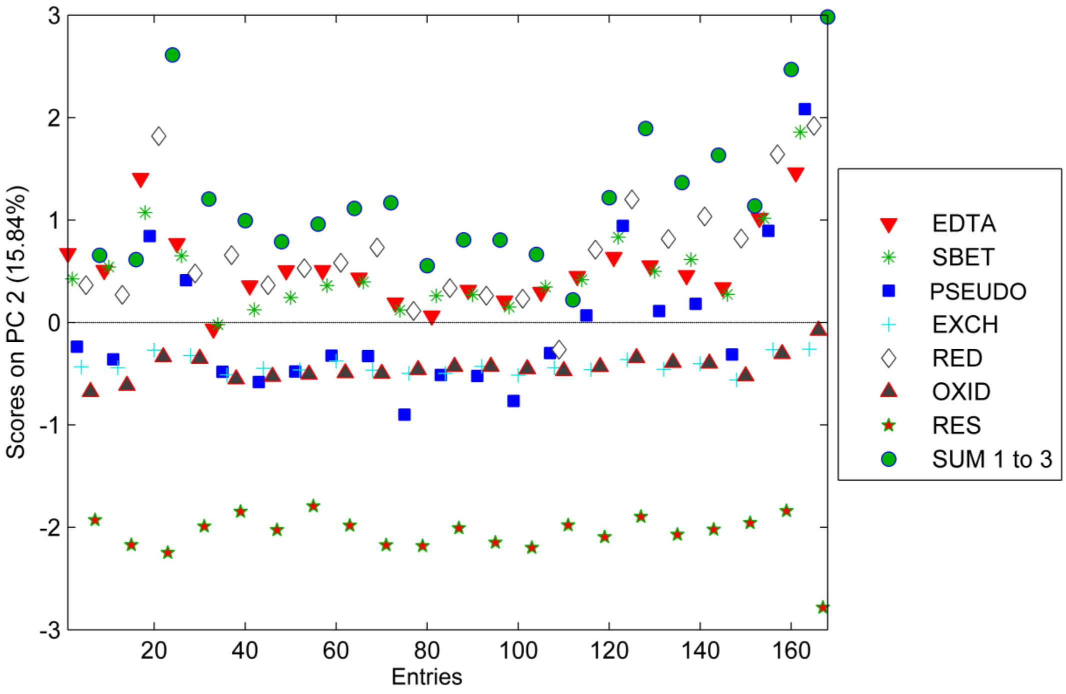


Figure 4.36 Score plot for PC2

The PC2 score (figure 4.36) suggested that, the residual fraction has the most negative PC2. This suggested that the residual fraction should be lowest in Pb and Zn, but highest in Cr, Fe and Ni. The lithogenic metals (Cr, Fe and Ni) are generally encompassed within silicate minerals in soils, and are predominantly found in the residual fraction of the sequential extraction scheme.

Rotated component matrix (Varimax rotation method)

To get clearer information from the PCA performed above, the two PC loadings were then rotated using the varimax algorithm. This resulted in the redistribution of the information between the PCs, which was simpler to interpret. The eigenvalues and scores were recalculated for the new loadings.

Varimax eigenvalues plot

Figure 4.37 is the eigenvalue plot after rotating the PC to maximise the total simplicity. The first two PCs still described 90.1 % of the variation in the data as in the original PCA model, but now PC1 described 52.1 % of the variation in the data instead of 74.3 % and PC2 described 38.0 % of the variation instead of 15.8 %.

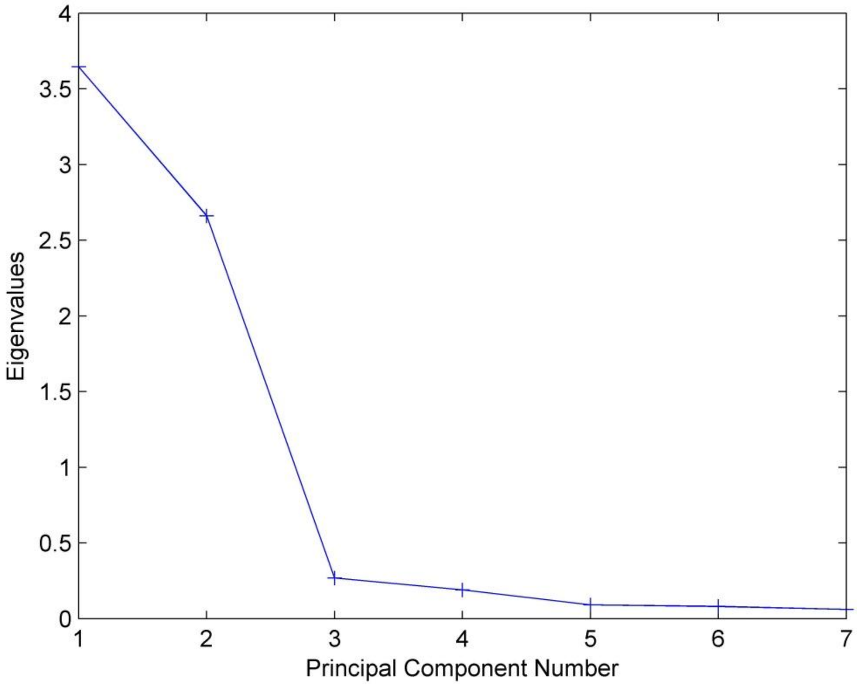


Figure 4.37 Varimax eigenvalue plot

Varimax loadings

Figure 4.38 shows the varimax loading plot. It was clear from this plot that PC1 was now mainly associated with Cr, Fe and Ni, which are mainly lithogenic in nature. It was also associated but to lesser extent with Mn. It has little association with Cu, Pb and Zn (urban metals).

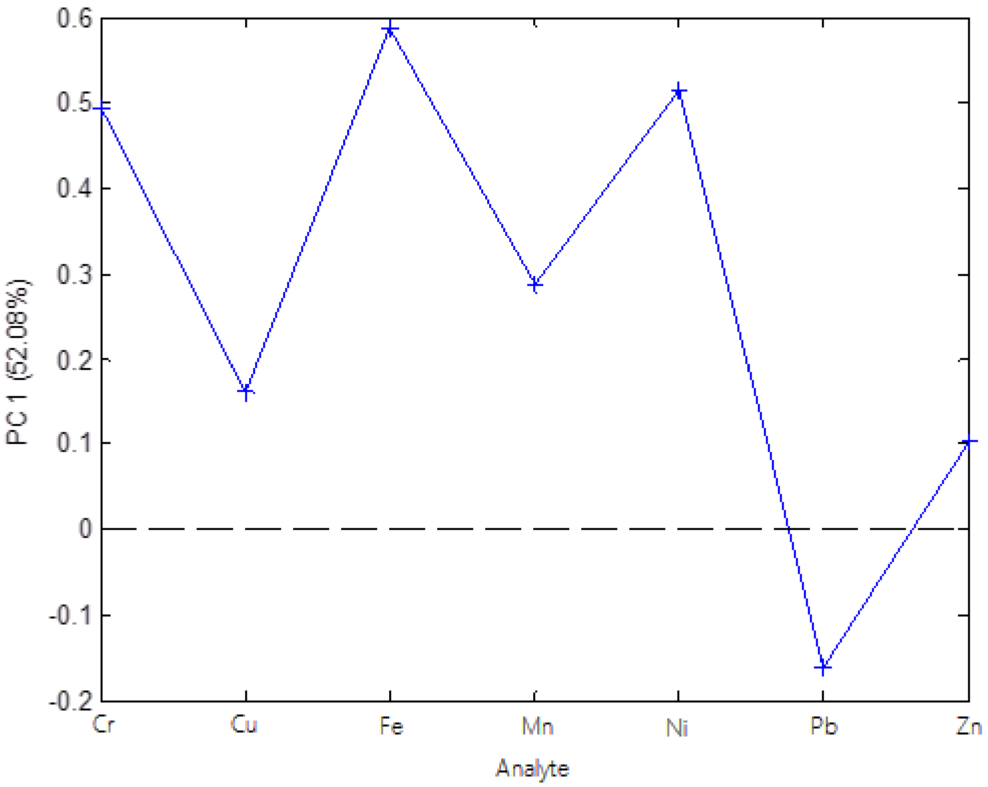


Figure 4.38 Varimax loading plot for PC1

Figure 4.39 shows the varimax loading plot for PC2. It suggested that PC2 was more associated with the variables that PC1 was not associated with. So, for example, it was strongly associated with Pb and Zn, and had some associated also with Cu and Mn but had no association with Cr and Ni. This gave a more distinct grouping between metals predominantly of lithogenic origin on the one hand and those whose distribution is likely to be dominated by anthropogenic input, on the other.

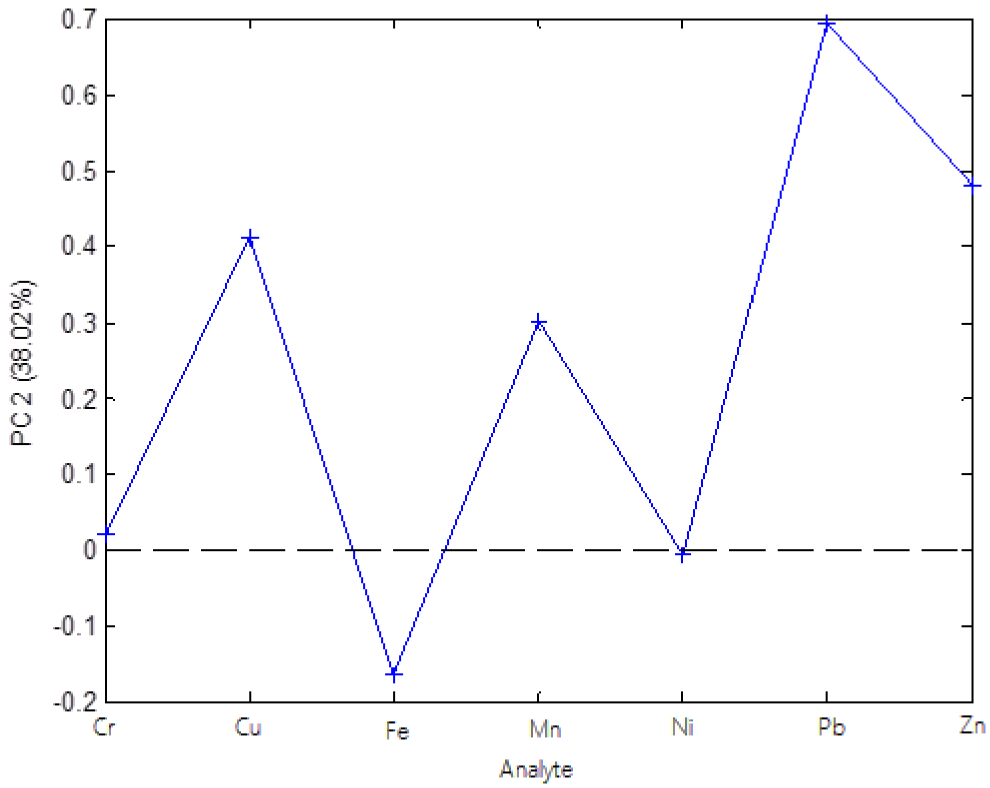


Figure 4.39 Varimax loading plot for PC2

Varimax scores

Figures 4.40 and 4.41 show the varimax scores plots for PC1 and PC2 respectively. The data is colour-coded according to type of extraction applied, for the example, entries 1 to 8 present plot 1, with entry 1 representing the EDTA extractable PTE, entry 2 representing the SBET extractable PTE, entries 3, 4, 5, 6, 7, representing pseoudototal, exchangeable, reducible, oxidisable, residual fractions respectively and entry 8 representing the sum of steps 1 to 3. Entry 9 to 16 represent plot 2 and so on.

The PC1 score was now a measure of predominantly the Cr, Fe and Ni (and to some extent Mn) content. The pseudototal concentration have the highest PC1 score, this was because the pseudototal fraction represents the maximum amount of the element that can be released. The residual fraction was next then the sum of steps 1 to 3 fraction. This trend is expected as Cr, Fe and Ni are mainly lithogenic in nature, they should have high concentrations in the residual fraction as they associated with silicate minerals. The EDTA and exchangeable fractions contained similarly low amounts of these analytes. The SBET fraction contained slightly higher concentrations (but still low amounts) of these analytes than the EDTA and exchangeable fractions. It was likely due to the relative acidity of the media used in the EDTA and SBET extractions. In the SBET method, the extraction reagent, glycine was in a highly acidic medium (pH = 1.5), and it was likely to extract more PTE than the EDTA which was in a neutral medium.

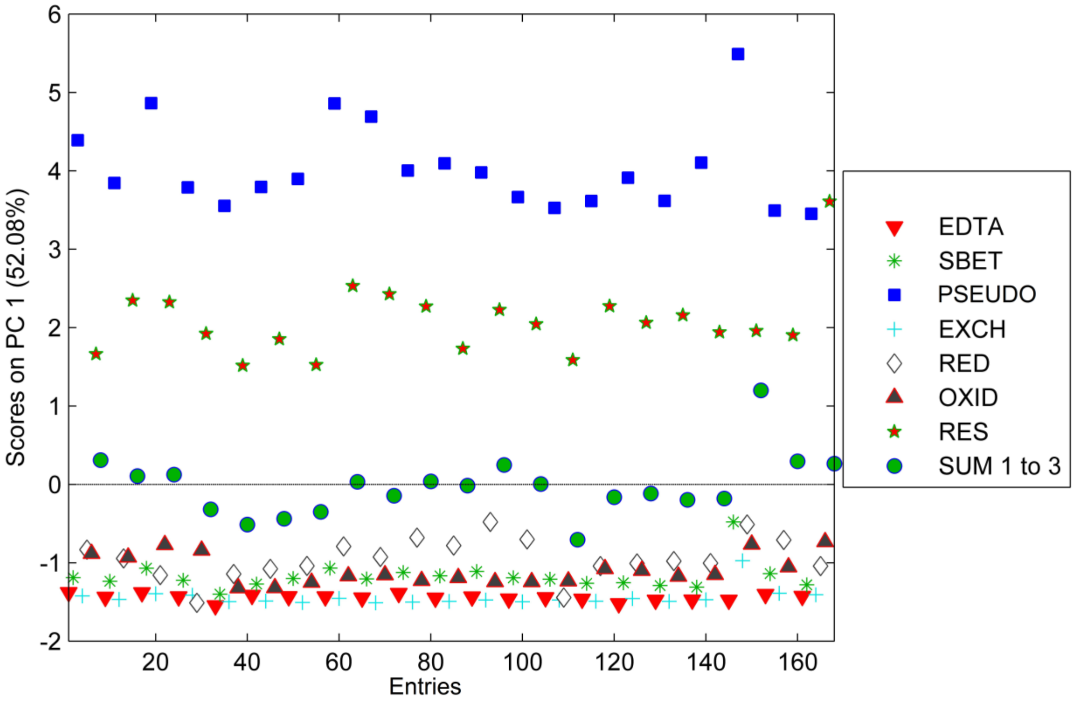


Figure 4.40   Varimax scores plot for PC1

The varimax PC2 loadings show that samples with the highest concentrations of Pb and Zn (and to some extent Cu and Mn) have the highest PC2 scores. The varimax PC2 scores shows that the pseudo-total fraction again has the highest score value for all plots, with samples from the sum of steps 1 to 3 fraction containing the next highest amount of these analytes. The sum of the steps 1 to 3 gives the easily labile fractions under ordinary environmental conditions. The association of these elements Cu, Pb, and Zn with these fractions indicates the likelihood they are from anthropogenic inputs.

Samples corresponding to the exchangeable fraction contain the least amount of the analytes, with the oxidisable fraction also containing low amounts of the analytes associated with PC2. The concentration of Pb and Zn (and to some extent Cu and

Mn) in the EDTA and SBET fractions is comparable (and low) for each individual plot.

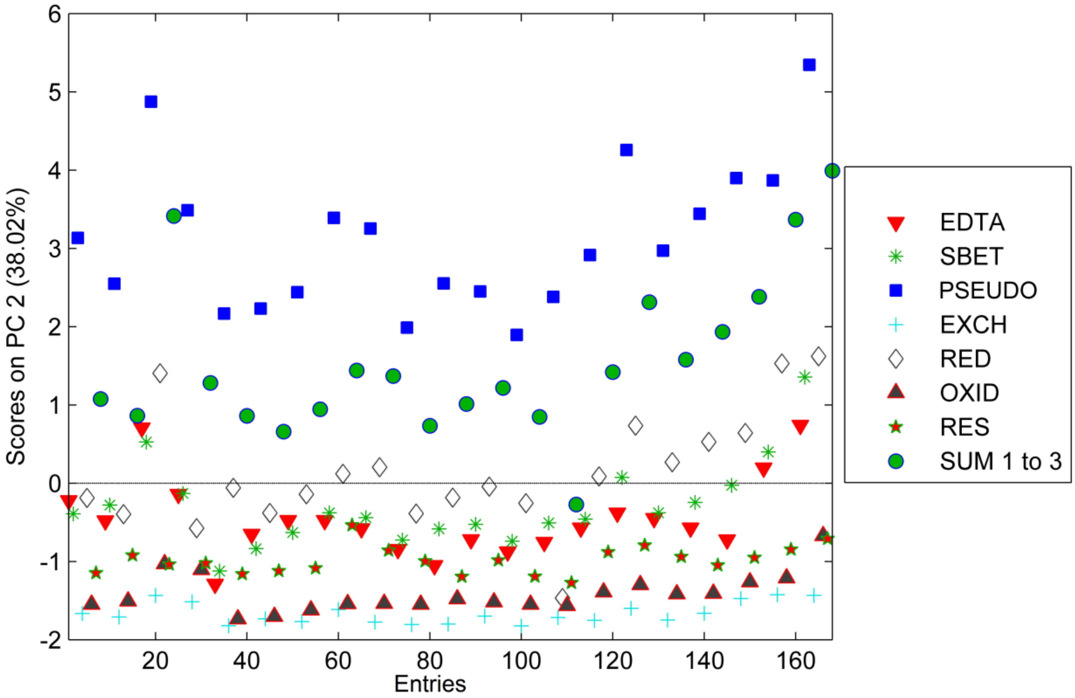


Figure 4.41 Varimax scores plot for PC2

4.5 Conclusions

In the work in this chapter, various extraction methods were successfully applied to soil from a typically urban allotment. Information about the potential availability, mobility and possible human health risk were revealed.

The concentrations of Cr in the plots were lower than the typical values for Scottish, and UK soils. The average concentration of Cr was also lower than the existing SGV. The concentrations of Cu, Ni, Pb and Zn were higher than the values obtained for Scottish and UK soils and even for some soils from allotments in Glasgow. The amounts of Cu, Pb and Zn exceeded the SGV, which indicated elevated levels of these elements in the allotment and this confirmed previous findings that the allotment was likely polluted with these elements. Although the average concentration of Ni in the soils was higher than reported Ni concentrations in typical Scottish soils, it was lower than the new SGV. Iron and Mn were relatively uniformly distributed which indicated that they were likely to come mainly from parent rock materials rather than anthropogenic sources.

The sequential extraction results indicated that Cr, Fe and Ni were predominantly in the residual fraction; this made them the least mobilise among the PTE studied. Environmentally they are of less impact as they would not be readily available to the environment. Therefore they pose less danger. Copper was mainly found in the oxidisable and residual fractions. The presence of Cu in the oxidisable fraction further showed the affinity of Cu for organic matter in soils. In these plots Cu is likely to pose less environmental problem despite the elevated concentration because it was mainly found in the two less labile fractions (steps 3 and 4). The high concentration of Pb in the reducible fraction is of great concern especially in reducible conditions (such as water logging). Lead is likely to be mobilised under such condition and the amount of Pb in this fraction alone was twice the existing UK CLEA SGV. Zinc was found in all the fractions with a substantial amount released in step 1 which made it the most mobile of the PTE under study, whilst Mn was mainly found in steps 2 and 4.

Although it is difficult to set threshold values for phytotoxicity and deficiencies to plants for the PTE, the results of the EDTA extractable concentrations gave an idea of the relative availability and phytotoxicity of some of the PTE. Copper, Pb and Zn in the soils from this allotment were likely to be phytotoxic to some plants species whilst Cr, Fe, Mn and Ni were likely not to cause any toxic effect to plants.

The human bioaccesiblity studies indicated that in this allotment only Pb is likely to pose a danger to a 20 kg child with a typical soil intake of 50 to 200 mg per day, while other PTE posed no danger as the amounts needed to reach TDI were higher than a typical soil intake by the 20 kg child.

Correlations between PTE were found in different operationally defined fractions of the BCR sequential extraction procedure and in various extraction methods. The correlations differ from one step to another, but in general, in most of the steps and the extraction methods, Mn was normally found to be correlated with Fe. This is likely indicating common source of these elements, e.g. oxyhyroxide minerals. The "urban metals" were found to be correlated with one another in most cases, this further indicated that Cu, Pb and Zn in this soil were likely to come from anthropogenic activities.

The correlation results between the various extraction methods used indicated that for the "urban metals" all the methods were found to correlate with one another. This may likely be due to anthropogenic influence. These metals are added into the urban soils mainly through anthropogenic activities, therefore they can easily be removed by the reagents used in the various methods. This is in contrast to Fe where the amounts of Fe extracted by these methods were not strongly correlated. This may be an indication of the geogenic nature of Fe, it is mostly incorporated into the silicate lattice of the minerals in soil and this makes it difficult to be released by most of these reagents. For Mn, the amounts extracted by EDTA were found to moderately correlate with the amount in step 1 whilst the amounts extracted by the SBET method were found to be strongly correlated with pseudototal content.

When PCA was applied to the whole data, the varimax loading plot showed that PC1 was mainly associated with Cr, Fe and Ni, which are mainly lithogenic in nature and has little association with Cu, Pb and Zn. The varimax loading plot for PC2 showed that PC2 was more associated with the variables that PC1 was not, that is, it was strongly associated with Pb and Zn, and had some associated also with Cu and Mn but had no association with Cr and Ni. The varimax scores for PC1 showed a close association between EDTA extraction and exchangeable fraction of the BCR sequential extraction method. The varimax scores for PC2 showed a close association between the EDTA and SBET methods.

In general PCA did not give more information than the simple correlation coefficient approach as it was thought.

5 Miniaturisation of the modified BCR sequential extraction method

5.1 Introduction

Sequential extraction methods have been applied to varieties of environmental substrates (section 1.5.1) to predict the mobility, availability and toxicity of PTE. Most of these samples were collected in large amounts and the BCR sequential extraction protocol was easily applied (the BCR protocol specifies the use of 1 g test portions). However, airborne particulate matter is usually collected in a few milligrams, as they are present only in small amounts in the environment; therefore the standard BCR protocol cannot be used. There is the need to fractionate PTE in, especially, urban airborne particulate matter in order to evaluate their environmental and human health impacts. Here, sample mass is the major limiting factor in the application of the BCR protocol. Hence there is a need to miniaturise the procedure with the aim to apply it to samples collected in milligrams quantities.

In this section, an urban soil sample was used and it was chosen based on the following criteria: urban soils are similar in nature to urban PM, the urban soil used in this work was well characterised for both pseudototal PTE content and the BCR sequential extraction PTE content for all the various steps. The bulk sample also has particle size < 2 mm, this made it easier to fractionate the sample into various particle sizes which were used to study of the effect particle size on the pseuodototal content.

5.2 Aim

The main aim of this work was to miniaturise the BCR procedure with the aim of applying it to road side dust and airborne particles, which may be available in only milligrams quantities. The specific objectives were

- To investigate the relationship between sample particle size and pseudototal PTE contents, using an urban soil as test substrate since that was available in large quantities and is relatively similar in composition to urban particulate.

- To investigate the effect of varying sample mass on the pseudototal content of the PTE, using samples containing particles of two different sizes.
- To investigate the effect of varying sample mass on the fractionating pattern and content of the PTE in the various fractions using the modified BCR sequential extraction method.

5.3 Experimental

5.3.1 Sample

The soil sample used in the investigations was obtained from the city of Glasgow during the EU URBSOIL project (Contract EVK4-CT-2001-00053), and was used in that work as a secondary urban soil reference material (URM).[214]The soil is sandy loam and the indicative values of pseudototal digestion and sequential extraction for this soil are shown in table 5.1.

Table 5.1 Indicative values for pseudototal content of PTE and BCR sequential extraction metal content (mg/kg) in URM (n = 34).[251]

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Step 1	0.64	9.19	160	123	2.05	25.1	15.4
Step 2	3.14	28.3	6740	134	3.03	235	26.3
Step 3	9.72	33.2	1190	25.3	4.7	109	27.2
Step 4	27.8	25.8	21400	147	40.2	73.3	118
Pseudototal	43.2	111	30600	442	48.8	389	177

5.3.2 Sieving

The bulk soil sample was separated into the following particle size fractions: < 45, 45 – 63, 63 - 90, 90 – 150, 150 – 250, 250 – 500 and 500 -1000 µm in 10 cm diameter stainless steel-pan sieves (Endecotts Ltd, UK) using a mechanical shaker. The sieves were stacked in a descending mesh size order (from top to bottom) so that the smaller particles were able to pass through each mesh until they reached a sieve where they were too big and were thus retained in that particular size fraction.

5.3.3 Investigation of the effect of sample particle size on the pseudototal PTE content

To study the relationship between particle size and the PTE content, 1 g each of the various particle size fractions (section 5.3.2) were digested for determination of the pseudototal content as described in section 3.1.1.

5.3.4 Investigation of the effect of varying the sample mass (miniaturisation)

The effects of varying the sample mass on the pseudototal content and on the BCR fractionation were studied using two particle sizes, the coarsest (500-1000 µm) and the finest (< 45 µm). The main purpose of this investigation was to find out if changing the sample masses will have any significant effect on the amount of PTE extracted using the pseudototal digestion method and also on the amount of PTE extracted in the various fractions of the BCR sequential extraction procedure. To also find out if these changes will have any effect on the fractionation pattern of the BCR sequential extraction procedure.

Pseudototal content

1, 0.5, 0.25, 0.125, and 0.0625 g of each of the two particle size samples were digested with *aqua regia* for determination of pseudototal content as described in section 3.1.1. For each of the different sample masses the volumes of *aqua regia* used were adjusted to keep the sample to reagent ratio constant, this was done because changing this ratio is known to have significant effect on the amount of PTE extracted using these procedures. Also the operational conditions of the microwave digestion system were adjusted. A power of 800 watts and ramping time of 20 mins were used for mass < 0.25 g (*cf.* 1600 watts and 10 mins, the default settings), while the temperature and the holding time were left unadjusted as 160 ^{o}C and 20 minutes respectively. This was done to avoid applying excess energy to a small sample mass which may cause the acid to vent, thereby losing some of the analytes and also causing damage to the microwave digestor.

BCR sequential extraction

This investigation was carried out in two parts: these will be referred to as Set 1 and Set 2 hereafter.

Set 1

1, 0.5 and 0.25 g of the two particle size samples were extracted using the BCR procedure as described in section 3.1.2 in 50 ml centrifuge tubes.

Set 2

0.25, 0.125 and 0.0625 g of the two particle size samples were also extracted using the BCR procedure as described in section 3.1.2, but in 15 ml centrifuge tubes.

In both sets the volumes of the reagents in all the steps were adjusted to keep the sample to reagent ratio constant, but all other conditions remain the same as in the modified BCR protocol.

5.3.5 Analysis

The extractions were performed in triplicates to check the precision of the extraction. Appropriate procedural blanks were also included to check for any contamination from the reagents.

The extracts were analysed using a Perkin Elmer Optima 3000 ICP-AES instrument (Perkin Elmer, Bucks, UK) as described in section 3.2.1.

5.4 Results and Discussion

5.4.1 Effect of sample particle size on the pseudototal PTE content

Figure 5.1 shows how the concentrations of the PTE in the soil varied with particle size.

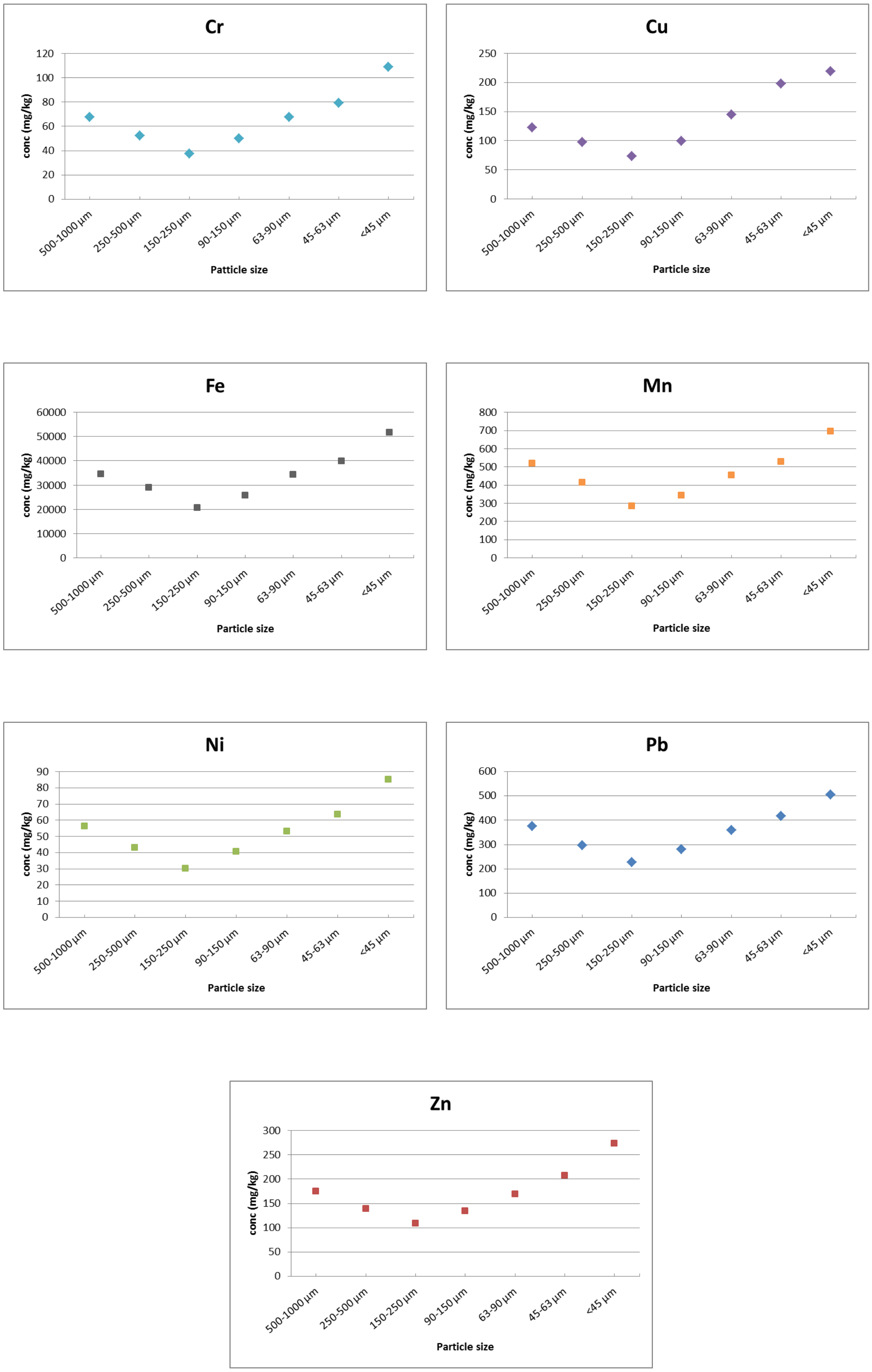


Figure 5.1 Variation of the PTE concentration with sample particle size

All the PTE studied in this soil sample showed a very similar trend. The finest fraction (< 45 µm) contained the highest amount of the PTE. Similar results which show the preferential partitioning of PTE to fine fractions in soil have been obtained elsewhere.[207, 252-254]This may be due to a larger surface area per unit mass of the finer particles which increases the reactive/adsorption sites of this fraction. Some authors[255, 256] also show that finer fractions tend to have higher concentrations of PTE than the coarse fraction because they contain higher clay minerals and organic content and these promote PTE accumulation essentially by co-precipitation, occlusion, adsorption and complexation. It therefore was expected that, as the sample particle size decreases, the concentration of PTE should increase. This was observed for particle size 150 – 250 µm to < 45 µm for all the analytes (figure 5.1). However relatively higher concentrations were observed for the two coarsest fractions (250 - 500 and 500 – 1000 µm), which indicate that the PTE can accumulate not only in the finer fractions but also in the coarse fraction. This may be due to several reasons including addition of PTE as extraneous large particles of anthropogenic source, or aggregation of fine particles of iron oxides, organic matter and carbonates[257] with which the PTE could be associated.

The presence of PTE-rich fine particles in soils in urban environments is of great health and environmental concern. These particles can be easily dispersed into the atmosphere by the action of wind, traffic and other mechanical actions and this contributes to the load of atmospheric particulate matter such as PM_{10}.[258] The PTE in the soil are transported to various segments of the environment through this dispersion of fine particles, thereby posing risk to human health and the ecosystem.[259, 260] Hand-to-mouth ingestion, dermal contact and inhalation are the main methods by which PTE are transferred to humans. Yamamoto *et al.*[261] have shown that finer particles tend to adhere more efficiently to human hands, so there is a greater risk of the transfer of PTE associated with these particles to humans.

5.4.2 Effect of varying the sample mass on the BCR extraction

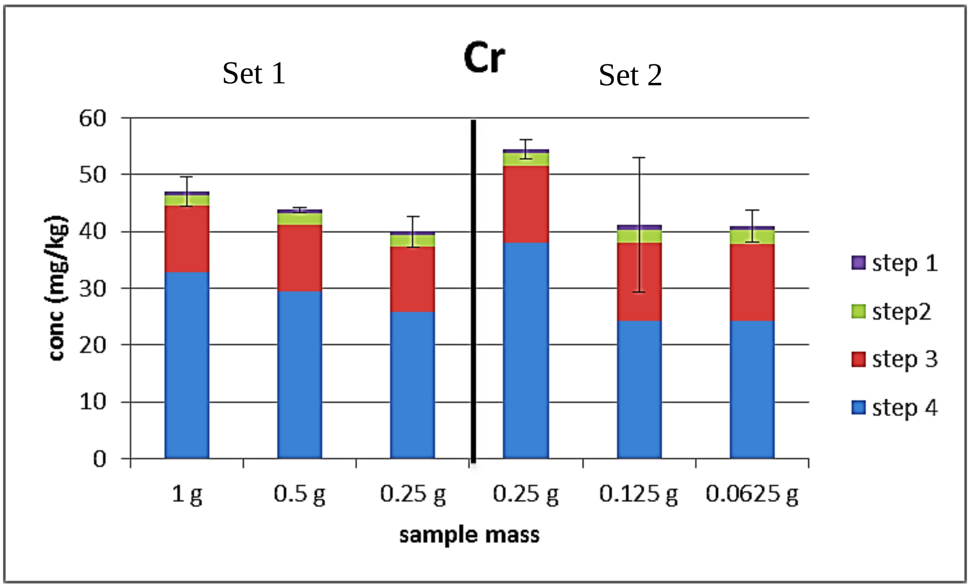
Figures 5.2 – 5.8 show the various distributions of the PTE in the soil sample for various sample masses for the two particle sizes, 500 - 1000 and < 45 µm. The results are presented for Set 1 and Set 2. Set 1 as described in the experimental section was the BCR extraction for 1 g, 0.5 and 0.25 g in 50 ml centrifuge tubes. Set 2 was the BCR extraction for sample masses 0.25, 0.125 and 0.0625 g in 15 ml centrifuge tubes. As the sample mass was reduced to 0.125 g, it became apparent that manipulating a small mass in a large centrifuge tube was difficult and a lot of error could be introduced through loss of material during extraction; this was what necessitated the Set 2 experiment. Extraction of the 0.25 g sample mass was repeated in Set 2 of the extraction in order to check if changing the size of the centrifuge tube had any influence on the amount of PTE extracted in the various fractions of the BCR.

A one-way ANOVA (section 3.3.4.2) was used to test whether the variation observed in the mean concentrations of PTE isolated in the steps of the BCR extraction were due to random error or due to the controlled factor, in this case the sample mass. This test was conducted on the two sets separately. A t-test (section 3.3.4.1) was also used to test whether the mean concentrations obtained in the BCR extraction for 0.25 g sample size in Set 1 and 2 were the same (within random error) or differed significantly. The calculated F results from the ANOVA test and calculated t from the t-test at 95 % confident level are presented in tables 5.2 – 5.8. No ANOVA and t-test results for steps 3 and 4 for < 45 µm in Set 2 are given, because in the course of the extractions, during the step 3, two out of the three replicates were lost. This procedure could not be repeated because of limited amount of sample. However for the sake of illustration of the pattern of the distributions (figures 5.2 – 5.8) the values obtained for the one remaining replicate were used.

Chromium

For both sets 1 and 2, irrespective of the sample masses and particles sizes, the distribution pattern of Cr in the soil sample remained the same, i.e. residual > oxidisable > reducible > exchangeable (figure 5.2). Similar patterns have been observed elsewhere[168, 169] in which the residual fraction was shown to be the major fraction. Recoveries (the sum of the metals extracted in steps 1 to 4, relative to the *aqua regia* extractable content in the original sample) for all the sample masses were generally > 85 %. The only exception was the low recovery (48 %) obtained when samples of 0.0625 g mass were used for the < 45 µm particle size (tables 1 and 2 in appendix B). This may be due to the difficulties in handling such a small amount of a fine sample. Some of sample may have been lost during the extraction especially when transferring the residue from step 3 from the centrifuge tubes to the microwave digestion vessels. When the pseudototal content and the concentrations in each fractions for 1 g sample mass were compared with the indicative values in previous studies[251] (table 5.1) agreements were generally found to be acceptable *i.e.* the results within ± 20 % of the indicative values. This is taking into consideration that the indicative values are for the whole soil not a specific size fraction as in the present case. An exception occurred in step 2, where Cr was found to be under extracted. The overall uncertainties on fractions were relatively small, except for sample size 0.125 g for both particle sizes.

500 – 1000 µm



< 45 µm

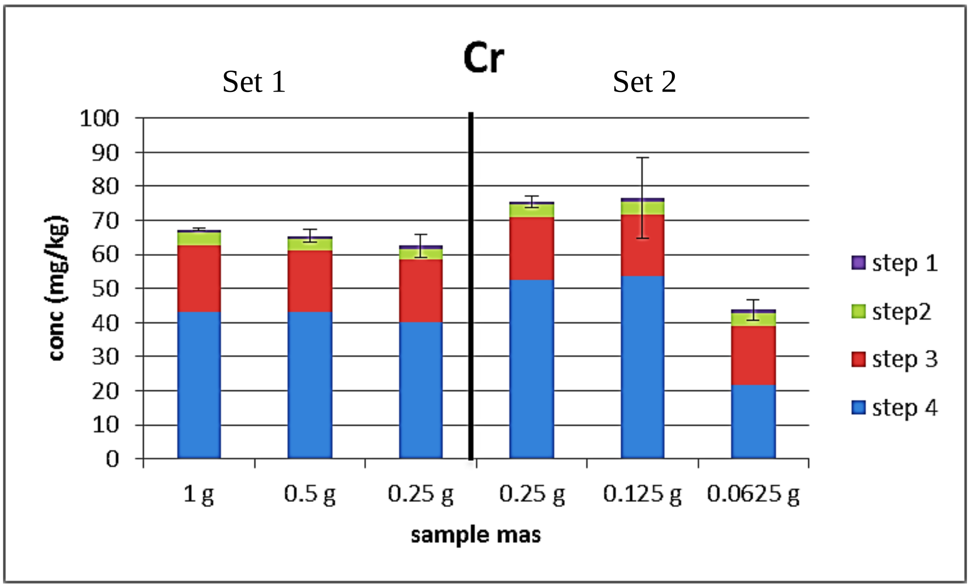


Figure 5.2 Chromium BCR SE distributions for different sample masses

The one-way ANOVA results (table 5.2) show that for particle size 500 -1000 µm, the values of calculated F (F*cal*) for all the steps for both sets were less than the critical F values (F*cri*), which imply that there was no significant difference in the mean concentrations of Cr extracted when the sample masses were varied. Although the ANOVA calculated failed in step 4 for Set 1, the calculated value of F just exceeded the critical value at 95 % confidence level. Overall, the data indicated that the concentrations of Cr extracted from different sample mass (mg/kg) were analytically similar. Similar ANOVA results were obtained for particle size < 45 µm, which show no significance difference in the mean concentrations of Cr extracted for all steps except for step 2.

Results of t-tests (table 5.2) show that calculated t values for all the steps were greater than the critical values at 95 % confidence level except for step 1, this imply that there was a significant difference between the mean values of Cr extracted using 0.25 g sample mass in Set 1 and 2 for all the steps except for step 1. This indicated that changing the size of the centrifuge tube had an influence on the amount of Cr extracted. For all the steps, the amount of Cr extracted from the 0.25 g sample in Set 2 was higher than in Set 1. This was likely due to closer interaction between the sample and the reagent when a smaller centrifuge tube was used in Set 2. The larger vessel has a large void, so, as the soil and the extracting solution were mixed during the shaking there was a potential for the liquid to be removed from the particles. In contrast the smaller vessel has a much smaller void meaning closer liquid-particle contact throughout the shaking process.

Table 5.2 Calculated F-values obtained from one-way ANOVA test and calculated t-values obtained from t-test for Cr for various steps of the BCR extraction

Step	500 – 100 µm			< 45 µm		
	ANOVA Set 1	ANOVA Set 2	t-test	ANOVA Set 1	ANOVA Set 2	t-test
1	0.275	0.654	1.07	1.22	1.35	3.71
2	2.51	0.849	5.63	49.7	0.407	11.0
3	0.847	0.223	12.0	3.99	nd	nd
4	7.73	3.73	6.75	1.76	nd	nd

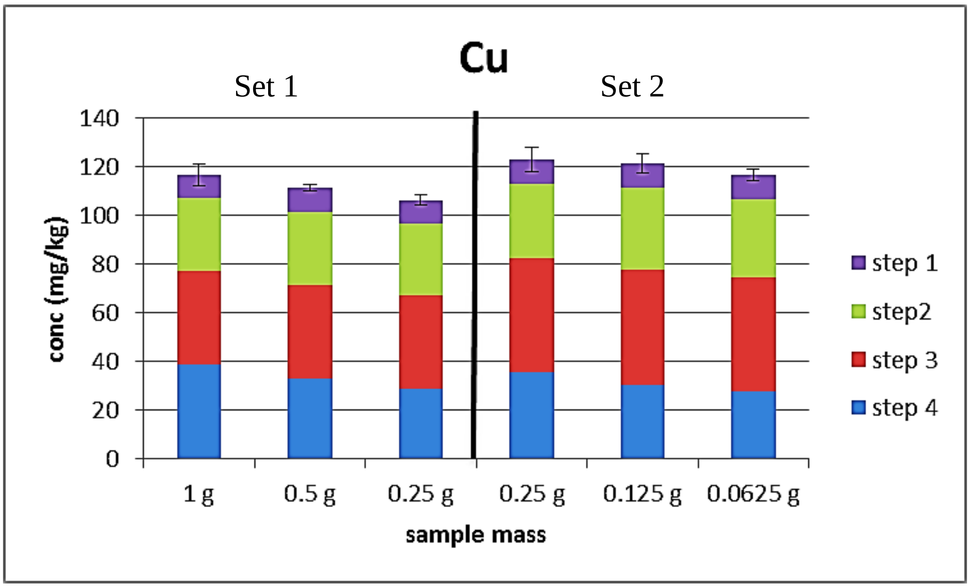
Critical value of F for ANOVA was 5.143; critical value of t-test was 2.776

nd - not determined due to accidental loss of sample

Copper

The recoveries (the sum of the metals extracted in steps 1 to 4, relative to the *aqua regia* extractable content in the original sample) for all the sample masses and particle sizes were generally good (± 10 %) as shown in tables 1 and 2 in appendix B. The recoveries obtained by comparing the pseudototal concentration and the concentrations of Cu in various fractions of the 1 g sample mass with the indicative values from previous studies[251] were found to be within ± 20 % with exception of step 4 (150 %) where Cu was over extracted. Irrespective of the sample masses and particle sizes the oxidisable fraction was the major fraction. This is similar to some previous findings,[169, 227] and may be due to the association of Cu with organic matter. The same fractionating patterns were observed for the 500 – 1000 µm particles in both sets; oxidisable > residual > reducible > exchangeable (figure 5.3). For < 45 µm there was slight change in the pattern, with reducible > residual. This may be because the finer fractions have higher content of secondary minerals (clay minerals, Fe, Mn and Al oxides).[262] The overall uncertainties on the fractions for all the sample masses and particle sizes were generally small.

500 – 1000 µm



< 45 µm

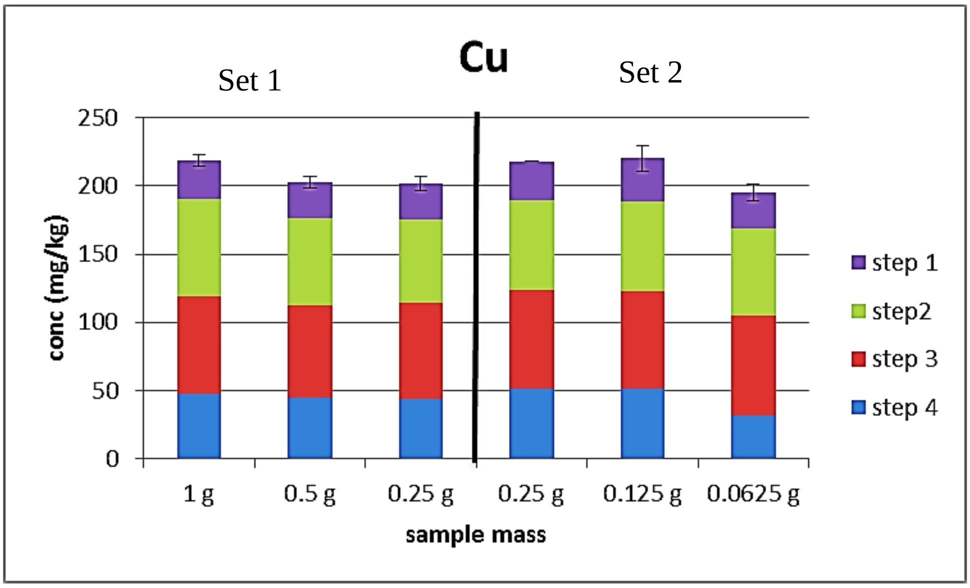


Figure 5.3 Copper BCR SE distributions for different sample masses

The ANOVA results (table 5.3) show no significant differences at 95 % confidence level between the mean values for all the steps in each set for the 500 – 1000 µm particle size, with the exception of the step 4 of Set 1. The results also show no significant differences in the mean values for all the steps for the < 45 µm particle size, except for step 2.

The t-test results (table 5.3) also show no significant differences in the mean concentration of Cu in 0.25 g sample of Set 1 and Set 2, except for step 3 in the 500 - 1000 µm sample. This implies that in case of Cu changing the size of the centrifuge tubes had no influence in the amount of Cu extracted.

Table 5.3 Calculated F-values obtained from one-way ANOVA test and calculated t-values obtained from t-test for Cu for various steps of the BCR extraction

Step	500 – 100 µm			< 45 µm		
	ANOVA Set 1	ANOVA Set 2	t-test	ANOVA Set 1	ANOVA Set 2	t-test
1	1.01	1.06	0.323	3.40	1.64	3.33
2	0.975	2.07	2.72	9.12	0.699	2.57
3	3.21	0.42	27.5	1.71	nd	nd
4	10.2	4.26	2.30	3.48	nd	nd

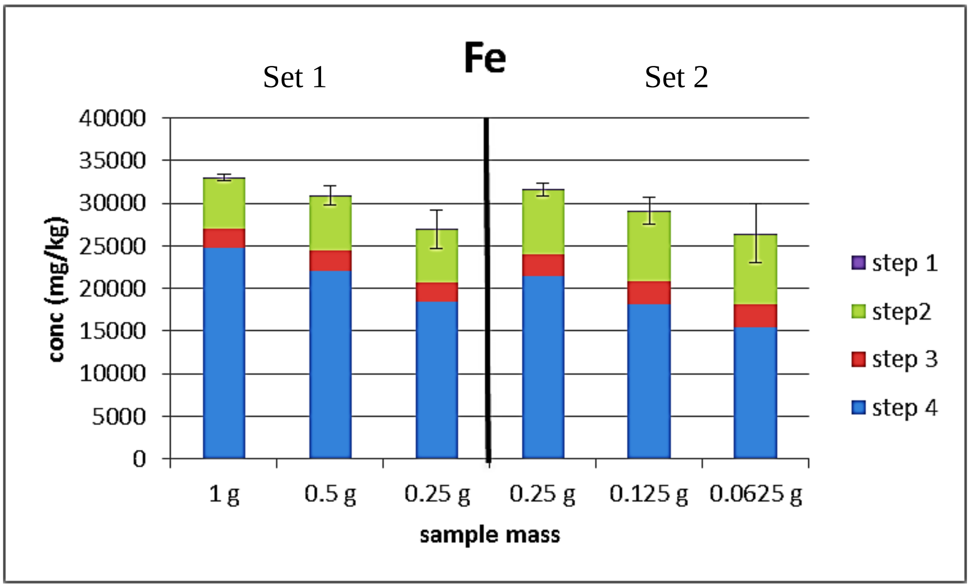
Critical value of F for ANOVA was 5.143; critical value of t-test was 2.776

nd - not determined due to accidental loss of sample

Iron

The residual fraction was the predominant fraction in all cases, irrespective of the sample mass and particle size. Also the fractionation patterns were the same in all the cases, i.e. residual > reducible > oxidisable > exchangeable (figure 5.4). Similar patterns have been obtained for Fe in some previous works.[31, 168] The recoveries (the sum of the metals extracted in steps 1 to 4, relative to the *aqua regia* extractable content in the original sample) were generally > 80 %, as shown in tables 1 and 2 in appendix B. The uncertainties on the fractions for particle size 500 -1000 µm were relatively large and increase as the sample masses decrease, this was what was expected. However for < 45 µm, the uncertainties were small.

500 – 1000 µm



< 45 µm

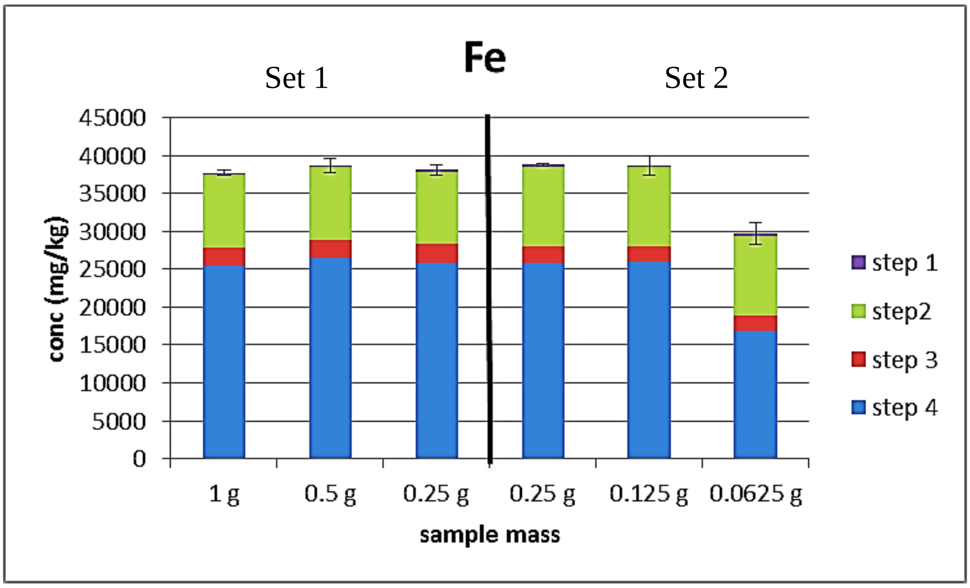


Figure 5.4 Iron BCR SE distributions for different sample masses

The ANOVA results (table 5.4) indicate that there were no significant differences in the mean concentrations of Fe in all the steps for both the sets except for step 4, this exception may have to do with the difficulties encountered in manipulation at that step. In general the results showed that varying sample mass has no influence in the amount of extractable Fe in this sample.

The t-test between 0.25 g sample masses in the two sets showed significant differences in the mean values for all steps with the exception of step 4. This highlighted the influence of the centrifuge tubes size. As was the case in the previous discussions the finer samples (< 45 µm) contained higher amounts of the element in the fractions than the corresponding fractions in the 500 -1000 µm particle size.

Table 5.4 Calculated F-values obtained from one-way ANOVA test and calculated t-values obtained from t-test for Fe for various steps of the BCR extraction

Step	500 – 100 µm			< 45 µm		
	ANOVA Set 1	ANOVA Set 2	t-test	ANOVA Set 1	ANOVA Set 2	t-test
1	0.617	4.15	3.39	1.54	2.36	24.2
2	2.611	1.72	5.43	4.77	0.128	15.4
3	0.432	1.44	21.9	3.27	nd	nd
4	14.2	6.05	2.22	1.77	nd	nd

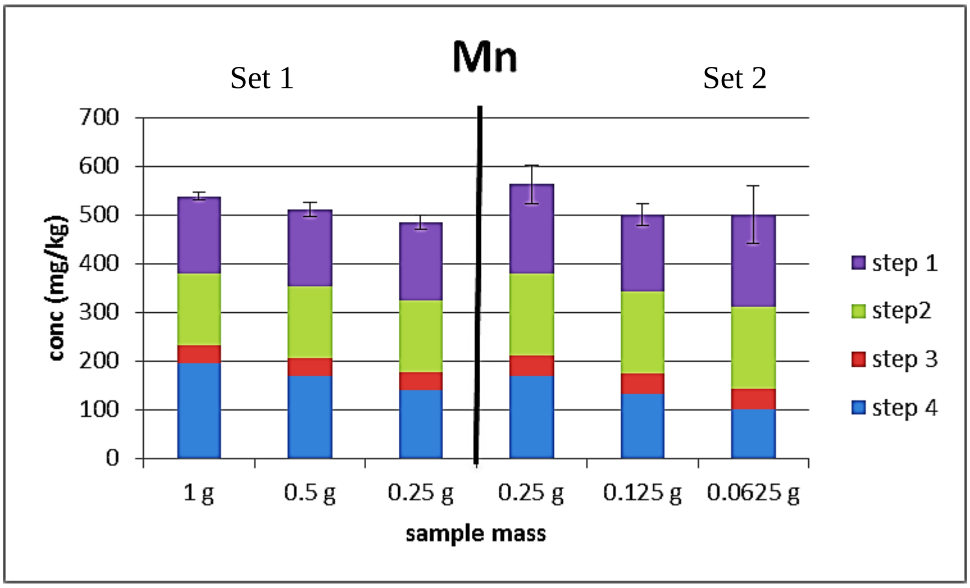
Critical value of F for ANOVA was 5.143; critical value of t-test was 2.776

nd - not determined due to accidental loss of sample

Manganese

Figure 5.5 shows that the dominant fractions in all the sample mass and particle sizes are exchangeable, reducible and residual, while the least is the oxidisable fraction. The recoveries (the sum of the metals extracted in steps 1 to 4, relative to the *aqua regia* extractable content in the original sample) in all the cases are between 82 and 107 %, as shown in tables 1 and 2 in appendix B. The overall uncertainties on the fractions were large for set 2 of the 500 -1000 µm whilst for the rest they were small.

500 – 1000 µm



< 45 µm

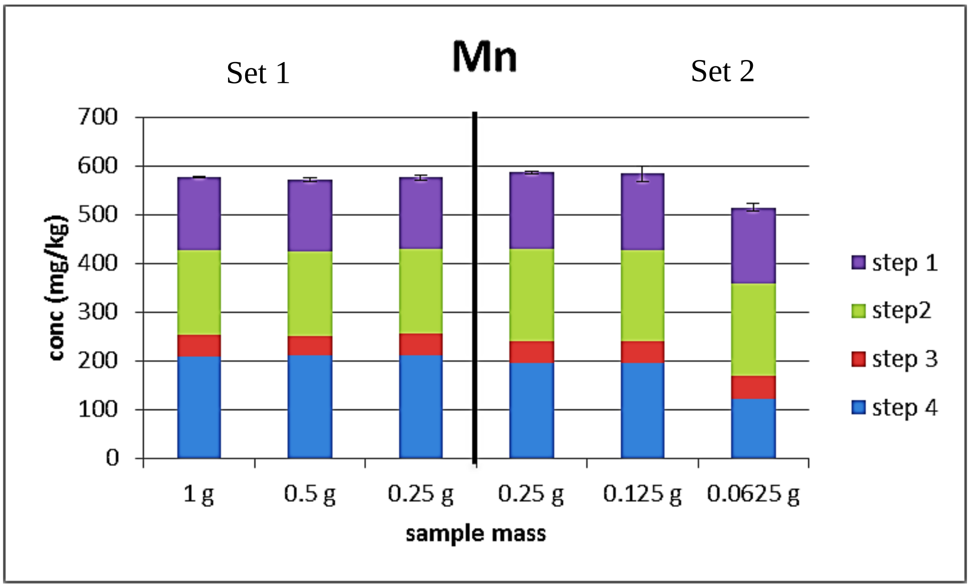


Figure 5.5 Manganese BCR SE distributions for different sample masses

The ANOVA results for the 500 – 1000 µm (table 5.5) showed that there were no significant differences between Mn concentrations in various sample masses for all the steps, except once again the step 4. For < 45µm particles, fractions 1 and 3 failed the ANOVA test while fractions 2 and 4 for Set 1 and fractions 1and 2 for Set 2 all passed the test.

The t-test showed significant difference in amount extracted in step 2 and 3 when changing vessel size, but no significant difference in steps 1 and 4. From these trends, it is difficult to make any general statement for Mn.

Table 5.5 Calculated F-values obtained from one-way ANOVA test and calculated t-values obtained from t-test for Mn for various steps of the BCR extraction

Step	500 – 100 µm			< 45 µm		
	ANOVA Set 1	ANOVA Set 2	t-test	ANOVA Set 1	ANOVA Set 2	t-test
1	1.62	0.642	1.34	17.6	0.188	7.94
2	1.57	0.402	59.2	4.64	0.109	9.65
3	1.00	0.504	10.1	6.38	nd	nd
4	14.1	8.78	1.69	0.109	nd	nd

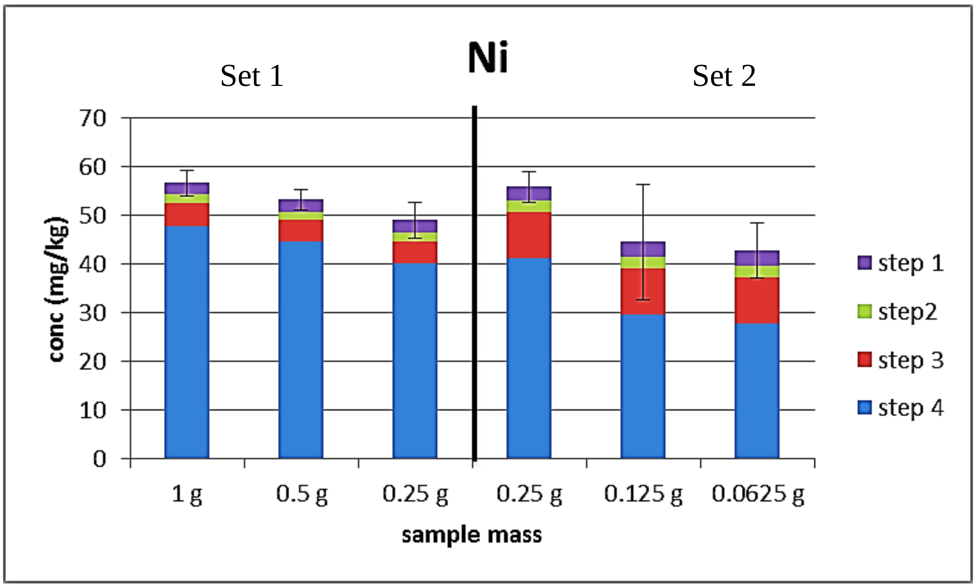
Critical value of F for ANOVA was 5.143; critical value of t-test was 2.776

nd - not determined due to accidental loss of sample

Nickel

Nickel, like iron, was predominantly found in the residual fraction. Both elements are typically lithogenic and associated with the silicate matrix.[31] Some previous studies[168, 231] have shown Ni to be predominantly in the residual fraction. The fractionating pattern (figure 5.6) is residual > oxidisable > reducible ≈ exchangeable. This pattern in general was not affected by sample mass in both particle sizes. The recoveries (the sum of the metals extracted in steps 1 to 4, relative to the *aqua regia* extractable content in the original sample) as shown in tables 1 and 2 in appendix B, were between 81 and 96 %, with the exceptions of 0.125 g sample mass for 500 - 1000 µm (64 %) and 0.0625 g sample mass for both 500 – 1000 µm and < 45 µm particle sizes (62 and 69 % respectively). These low recoveries may be attributed to loss of the material from relatively small samples. The overall uncertainties on the fractions were relative large for 0.125 g sample mass for both particle sizes.

500 – 1000 µm



< 45 µm

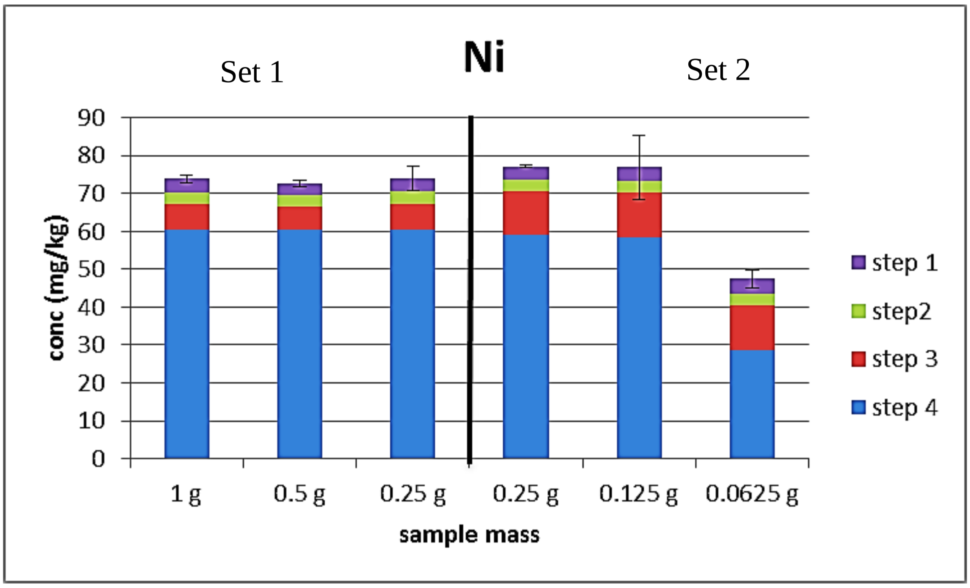


Figure 5.6 Nickel BCR SE distributions for different sample masses

The ANOVA test results (table 5.6) show that, the test passed at 95 % confidence level for all steps irrespective of the particle sizes. These indicated that there were no significant differences between the mean concentrations of Ni in all the steps when the sample masses were varied.

The t-test between the 0.25 g samples in the two sets failed at 95 % confidence level except for step 4 which imply that there were significant differences in the mean concentration for all the steps, except the step 4. This is once again showing the influence of changing the size of the centrifuge tubes.

Table 5.6 Calculated F-values obtained from one-way ANOVA test and calculated t-values obtained from t-test for Ni for various steps of the BCR extraction

Step	500 – 100 µm			< 45 µm		
	ANOVA Set 1	ANOVA Set 2	t-test	ANOVA Set 1	ANOVA Set 2	t-test
1	2.56	4.86	2.91	0.797	2.27	0.075
2	0.330	0.798	9.36	4.11	0.75	0.642
3	1.42	0.006	16.4	0.660	nd	nd
4	5.12	2.63	0.372	0.001	nd	nd

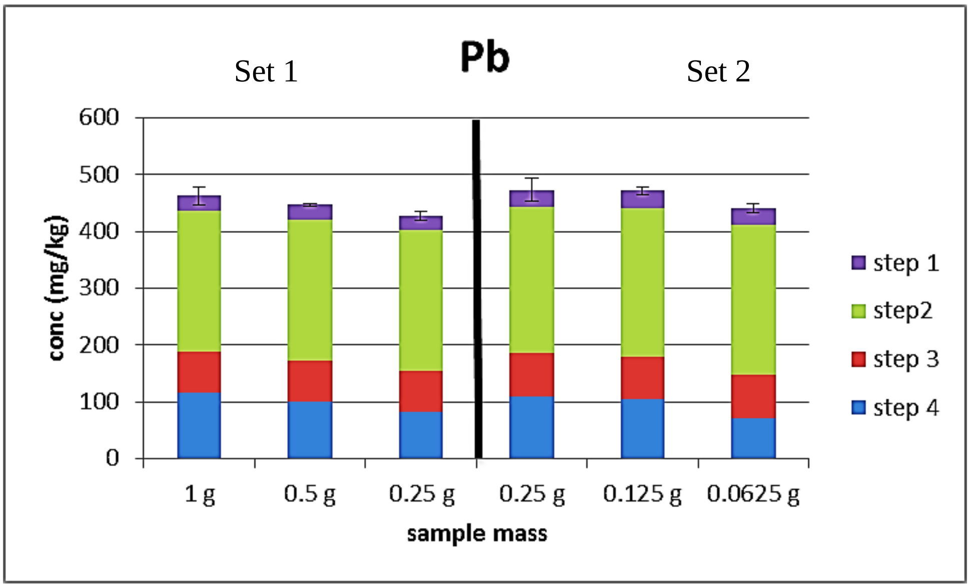
Critical value of F for ANOVA was 5.143; critical value of t-test was 2.776

nd - not determined due to accidental loss of sample

Lead

The predominant fraction in this case was the reducible fraction and similar results have been reported by previous authors.[169, 227] The fractionating pattern-reducible > residual > oxidisable > exchangeable-was unaffected by the change of the sample masses. The recoveries (the sum of the metals extracted in steps 1 to 4, relative to the *aqua regia* extractable content in the original sample) as shown in tables 1 and 2 in appendix B, were between 89 and 106 %, indicating a good quality extraction. Also comparing the amount extracted in each step with indicative values from previous studies,[251] the recoveries for step 1 and 2 (104 and 105 % respectively) were very good, while Pb was under- extracted in step 3 (66 %) but the “missing” Pb was latter extracted in step 4 (160 %). The overall uncertainties on the fractions were all small.

500 – 1000 µm



< 45 µm

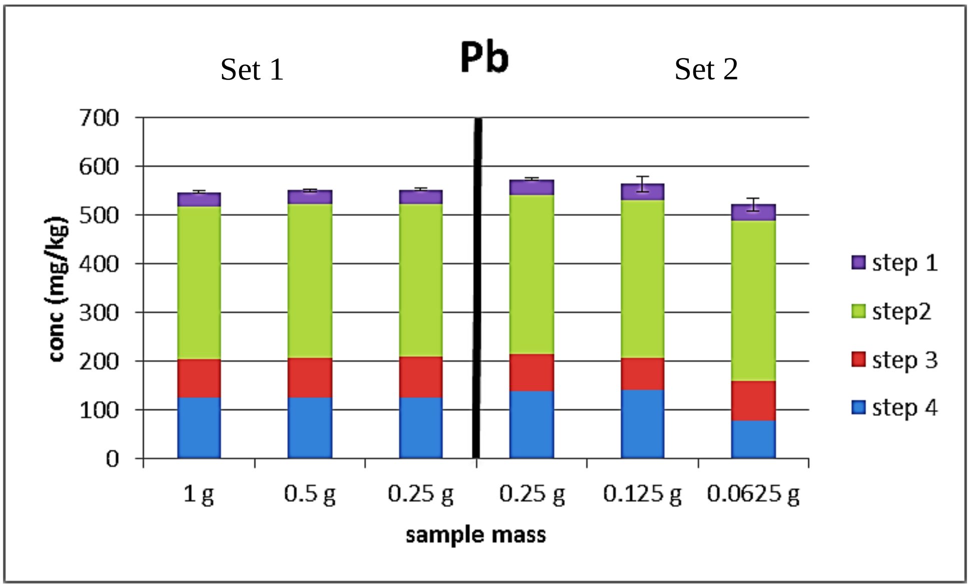


Figure 5.7 Lead BCR SE distributions for different sample masses

The ANOVA results (table 5.7) for all the particle sizes showed that no significant difference in mean concentrations of Pb in all the steps except step 4 for 500 - 1000 µm particle sizes when the sample masses were varied for both particle size fractions.

The t-test results showed significant differences in the mean concentration of Pb between the 0.25 g sample mass for Set 1 and Set 2. Again, more Pb was isolated when the extraction was performed in a smaller vessel.

Table 5.7 Calculated F-values obtained from one-way ANOVA test and calculated t-values obtained from t-test for Pb for various steps of the BCR extraction

Step	500 – 100 µm			< 45 µm		
	ANOVA Set 1	ANOVA Set 2	t-test	ANOVA Set 1	ANOVA Set 2	t-test
1	0.008	1.04	4.25	0.779	1.30	9.40
2	0.160	0.977	4.93	0.607	0.618	7.54
3	0.278	0.428	5.82	3.86	nd	nd
4	8.97	7.60	2.04	0.442	nd	nd

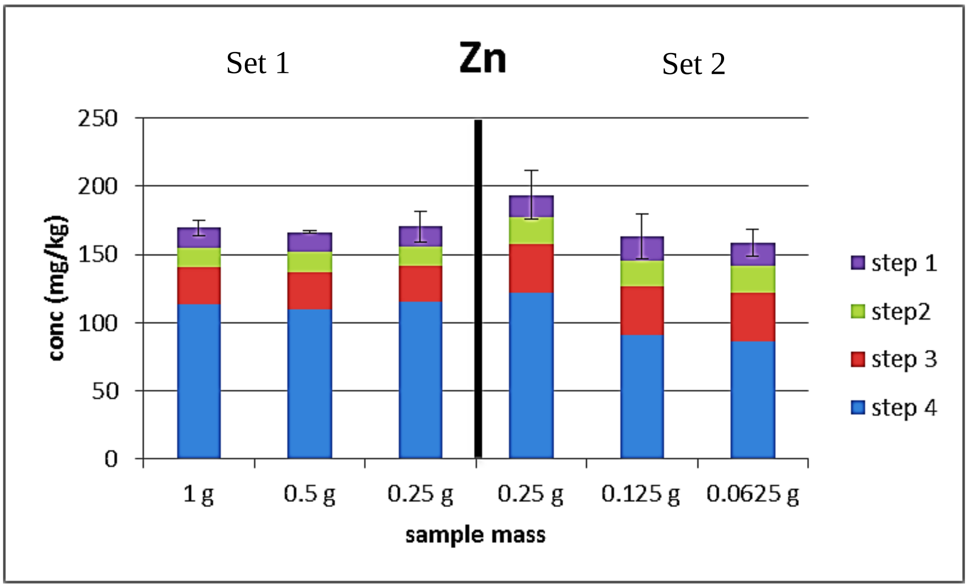
Critical value of F for ANOVA was 5.143; critical value of t-test was 2.776

nd - not determined due to accidental loss of sample

Zinc

The fractionating pattern of Zn in the sample was residual > oxidisable > reducible ≈ exchangeable. This pattern remained the same when the sample masses were varied irrespective of the particle size. The recoveries (the sum of the metals extracted in steps 1 to 4, relative to the *aqua regia* extractable content in the original sample) for all the sample mass and particle size were between 75 and 106 % as shown in tables 1 and 2 in appendix B. The overall uncertainties on the fractions were relatively large for sample mass 0.25 g in both particle sizes.

500 – 1000 µm



< 45 µm

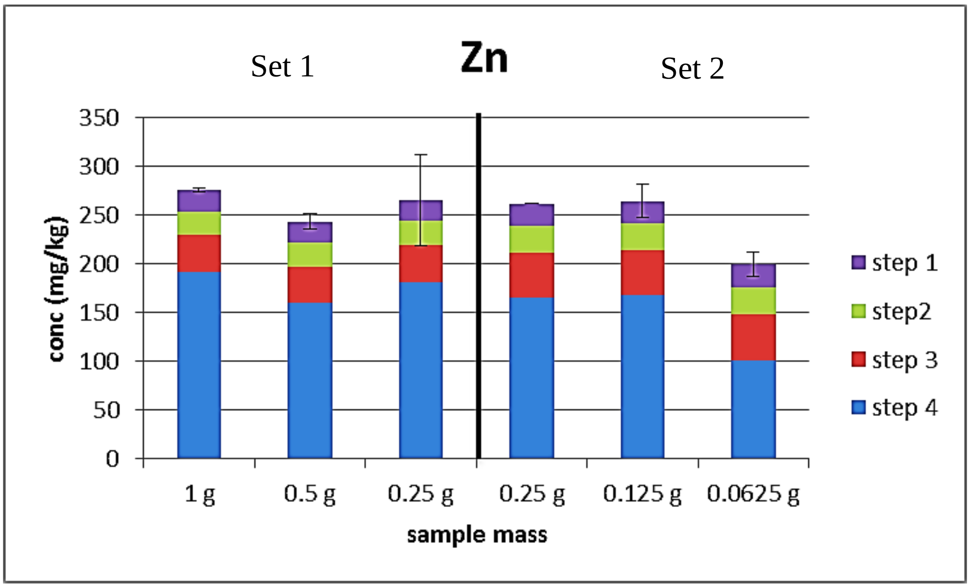


Figure 5.8 ***Zinc BCR SE distributions for different sample masses***

The ANOVA results (table 5.8) showed no significant differences in the mean concentrations of Zn in all the steps and for all the sample mass and particle sizes. The t-test comparing the amount of Zn extracted from 0.25 g sample mass in the two sets failed at 95 % confidence level for all the steps, once again indicating the influence of the size of centrifuge tubes.

Table 5.8 Calculated F-values obtained from one-way ANOVA test and calculated t-values obtained from t-test for Zn for various steps of the BCR extraction

Step	500 – 100 µm			< 45 µm		
	ANOVA Set 1	ANOVA Set 2	t-test	ANOVA Set 1	ANOVA Set 2	t-test
1	0.426	4.34	5.40	4.96	0.016	11.134
2	0.387	0.182	29.8	1.51	0.797	11.704
3	0.571	0.104	13.4	2.33	nd	nd
4	0.411	4.90	0.544	1.06	nd	nd

Critical value of F for ANOVA was 5.143; critical value of t-test was 2.776

nd - not determined due to accidental loss of sample

5.5 Conclusions

The results of investigation of the effect of particle size on the pseudototal content of PTE in the soil sample analysed showed that, for the finer sample fractions, there was an increase in the pseudototal PTE content with decease in the particle size. This was likely due to the increase in the surface area as the particle size decreases. The large surface area per mass provides more adsorption sites on the sample. A high content of the PTE was also observed in the coarsest fraction which is attributed to addition of PTE as extraneous particles of anthropogenic source or aggregation of fine particles.

The results of the BCR sequential extractions showed that in general, the fractionating patterns were not affected by changing sample mass and the particle size. In general the amount of the PTE extracted in most of the steps with the exception of step 4 in most cases was not affected by varying the sample mass. However significant differences were found when the extraction vessel size was changed. Increase in the amounts of the PTE extracted in most of the steps and for most of the sample masses were observed when the smaller centrifuge tubes were used. This was likely due to the closer interactions between the samples and the reagents. This clearly demonstrated the operational nature of sequential extraction methods, and the critical need to apply standard protocols exactly as prescribed to avoid variability in results obtained.

Although the fractionation pattern did not change markedly with the particle size, the amounts of the PTE extracted in the < 45 µm in all the steps for the various sample masses were always greater than the corresponding steps and sample masses of the 500 -1000 µm particle size. This is likely due to larger surface area in the finer fraction where there was more PTE sorbed.

The overall uncertainties on all the four steps of the BCR results in the various sample masses were generally small. It was thought that as the sample masses become smaller, the uncertainties might become larger but this was not observed. The likely reason may be that the minimum weight of the soil that will be a representative of the whole soil sample had not been reached.

This study highlighted the difficulties involved in performing quantitatively step 4 of the BCR extraction, which involves transferring the residue from step 3 into the microwave digestion vessels. In doing so considerable amounts of the sample were sometimes lost. This was particularly noticeable when dealing with a small sample masses. Another challenge in application of BCR extraction to airborne particle samples is the presence of a filter and this is studied in the next chapter (chapter 6).

6 Fractionation studies on particulate matter collected on tapered element oscillating microbalance filter dynamics measurement system filters using miniaturised BCR extraction procedure.

6.1 Introduction

Particulate matter (PM) is released into the atmosphere through both natural and anthropogenic activities. Depending on their sizes, particles can stay for a long time in the atmosphere and can travel long distances. For example, the residence time of PM_{10} in the atmosphere is from minutes to hours and its travel distance varies from less than 1 km to 10 km. The residence time for $PM_{2.5}$ is from days to weeks and travel distance ranges from hundreds to thousands of km. In urban areas most of this particulate matter is released through anthropogenic activities and there is an increase concern about the hazardous effect of these pollutants in the atmosphere on humans and other living organisms.[263, 264]

Usually analytical PTE measurements in atmospheric PM involve the determination of total PTE concentrations, which gives valuable information about the overall pollution levels. However the determination of total PTE concentration in atmospheric PM is a poor indicator of PTE bioavailability, mobility and toxicity, because these depend on the chemical association of the PTE with different components of the solid phase. In this context, sequential extraction methods are suitable tools for assessing the mobility, bioavailability and toxicity. The fractionation of PTE in atmospheric PM has, until recently received little attention because of the difficulties in measuring even the total concentration of most PTE in atmospheric PM. Few attempts were made to apply some sequential extraction procedures to PM (table 6.1) but no attempt was made to miniaturise and apply the modified BCR sequential extraction method to PM collected on filters. Even the “modified BCR” used by Sysalova and Szakova[265] was actually the original BCR used but using different sample mass to reagent ratio. Therefore in this work, the miniaturised modified BCR sequential developed in chapter 5 was applied to samples on FDMS filter. This is with the view to develop a method of fractionating PTE in atmospheric PM collected on filters.

6.1.1 Sampling of particulate matter

The common method for measuring the amount of PM in the atmosphere is by gravimetric mass. This involves drawing air through a filter, particles are collected on the filter and are weighted. From the volume of air drawn, the concentration of the PM in the air can be expressed in terms of mass of the PM per cubic meters (m^3) of air. Depending on the amounts of PM, this can be expressed in ng/m^3, $\mu g/m^3$, or mg/m^3. As larger particles are not thought to be significant in terms of human health, they are often removed using impactors and cyclones. This is what forms the basis for official environmental monitoring and compliance standards such as PM_{10} and $PM_{2.5}$, where the total mass of the particles with aerodynamic diameters less than 10 and 2.5 µm respectively is measured.

Both high- and low-volume samplers are used for the sampling of PM, but high-volume samplers are more frequently used as they allow the collection of larger amounts of PM. A high-volume sampler draws air through a large-diameter membrane filter (20 – 25 cm), typically at 1 - 2 m^3/min. The filters are generally changed every 24 or 48 hours and weighed. This method gives an average concentration of the PM during the sampling period. Nowadays a real-time measurement of the concentration of PM is possible. One of the methods used is the tapered element oscillating microbalance (TEOM). In the UK, the TEOM is now widely used in most of the ambient air-quality monitoring stations for measuring the PM concentration in air. In this present work, filters from this system were used; the working principle of this method is explained in the next section of this chapter.

Other types of PM samplers are the personal samplers. In these samplers a filter-holder is clipped to the lapel, with the pump around the waist with a pumping rate typically 2 l/min through a 25 mm diameter filter.

Tapered Element Oscillating Microbalance (TEOM)

Figure 6.1 shows a schematic diagram of a TEOM. The instrument can be fitted with a suitable size-selective sampling inlet to monitor total suspended particulate, PM_{10} or $PM_{2.5}$ concentrations.

Air is sampled into the TEOM at a rate of 16.7 l/min, and is split isokinetically (constant rate) into two streams flowing at rates of 3 l/min and 13.7 l/min. The air at 3 l/min is directed into the mass transducer (tapered element) in the sensor unit, while the remaining air goes to exhaust. The sensor unit consists of a Teflon-coated glass fibre filter cartridge mounted at the tip of a tapered glass tube. This tube is fixed at the base while the tip is allowed to vibrate at its natural frequency. As the particles are accumulating on the filter, the oscillation frequency of the tube changes due to the increase in weight of the particles. The mass transducer in this instrument is a piezoelectric transducer which utilizes the piezoelectric properties of certain crystals such as quartz. When this material is subjected to force or stress (in this case change in mass on the TEOM), it generates an electric potential proportional to the magnitude of the force. The sensor unit computes the oscillation frequency into a particulate concentration by dividing the mass rate by the flow rate.

The sensor unit temperature is maintained at 50 ^{o}C which minimises the water collection on the sample filter and also reduces thermal expansion of the tapered element which may affect the oscillation frequency. During this heating semi-volatile substances such as ammonium nitrate and organic aerosols are driven off and this is a widely accepted disadvantage of the approach.[266-269]

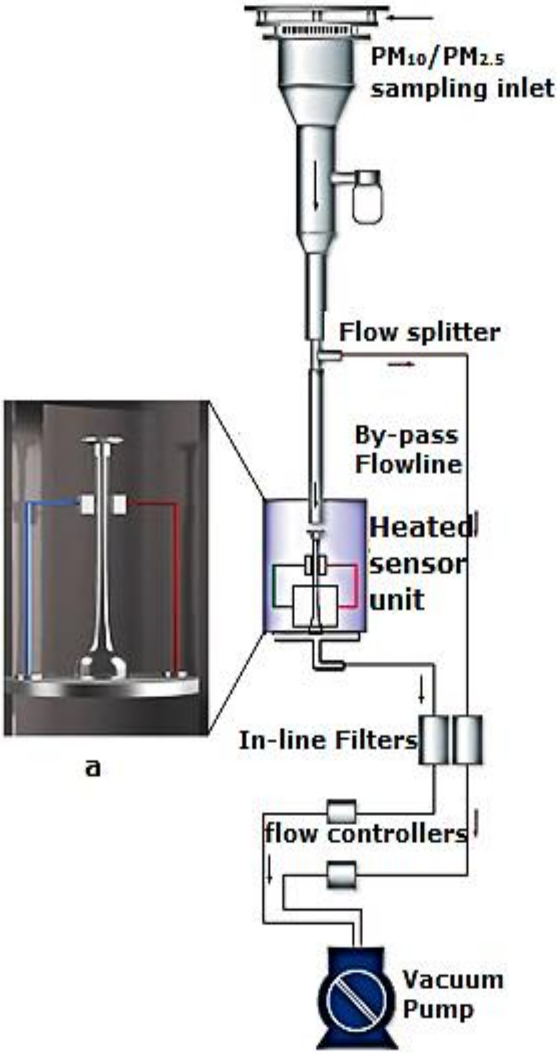


Figure 6.1 Flow/component schematic of a TEOM with the tapered element shown in (a) (after[270])

The Filter Dyanamics Measurement System (FDMS)

To allow the measurement of both non-volatile and volatile components of PM, which is not possible with the TEOM described above, a filter dyamanics measurement system (FDMS) unit is added to the TEOM. The FDMS uses a switching valve to switch between a 'base' measurement and a 'reference' measurement every six mins. Figure 6.2 shows a schematic diagram of TEOM FDMS, showing the main flow (either base flow or reference flow), the bypass flow, and the purge flow.

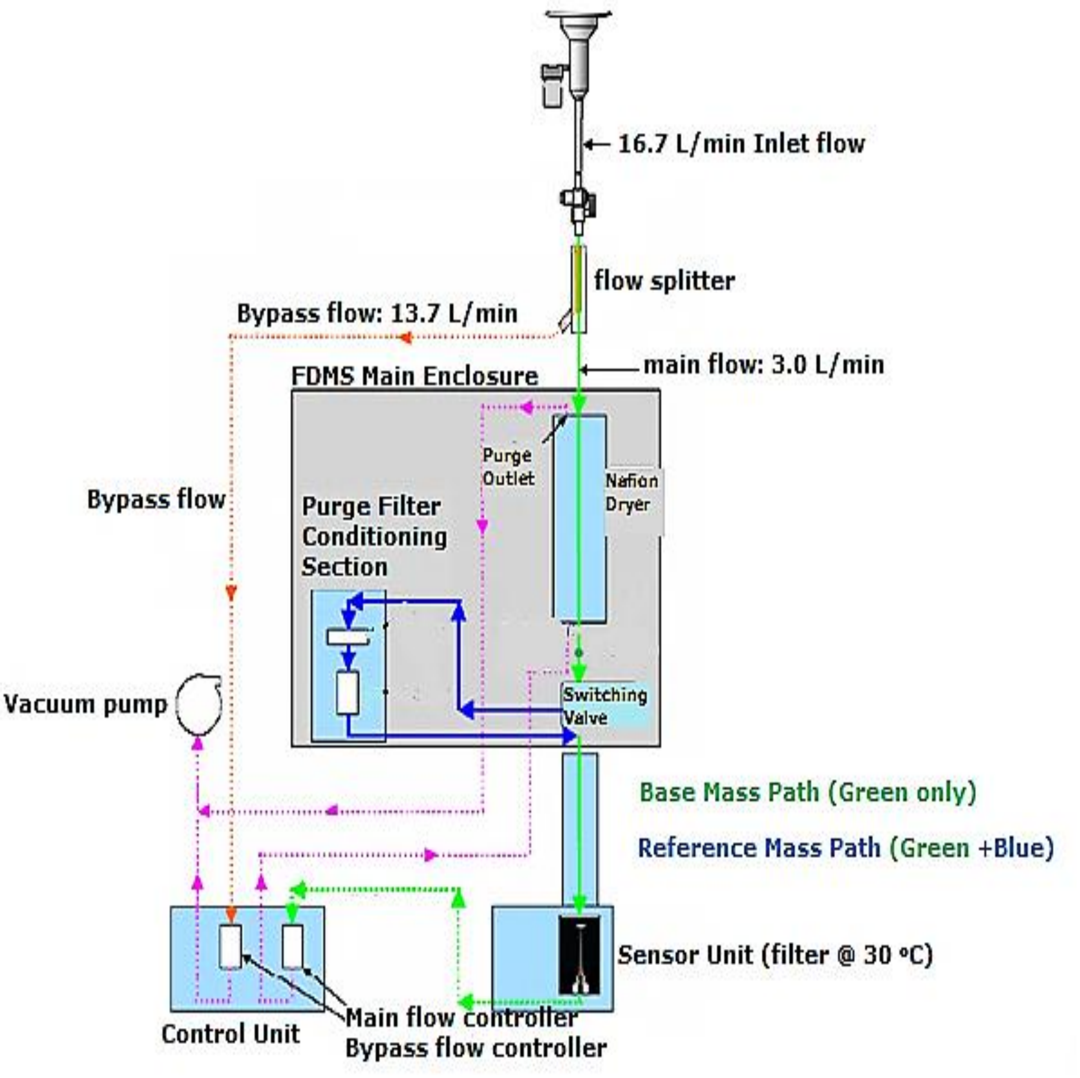


Figure 6.2 Schematic diagram of TEOM FDMS[271]

The air is drawn at the rate of 16.7 l/min and split as in the TEOM into two air samples at the rate of 3.0 l/min as main flow and 13.7 l/min as bypass flow. The main flow stream is then passed into a Nafion dryer which lowers the sample relative humidity thereby minimising the positive mass artefact associated with water sorption onto the collection filter. The dryer also makes it possible for the mass transducer (tapered element) to operate at 30 ^{o}C instead of the 50 ^{o}C used in the TEOM instrument. The dry air then passed into the switching valve that, every 6 minutes, alternately directs the air to the mass transducer (the base cycle) or to alternate flow path (reference cycle). The reference flow path contains a 47 mm filter cassette with a TX-40 filter (Teflon-coated borosilicate) which is kept at 4 ^{o}C. This low temperature causes the volatile PM components to condense on the filter resulting in “clean” air free of both volatile and non-volatile PM components. This clean air is then passed into the mass transducer in the sensor unit for measurement. During the reference cycle, any volatile particulates that were collected during the base measurement cycle can evaporate from the sensor filter. This means that the reference cycle average is usually a negative value, as material has been lost from the filter. The FDMS uses the average base and reference concentrations to calculate an overall mass concentration for the 12 minutes cycle, according to equation 6.1.

Mass Concentration = Base Concentration - Reference Concentration.

Equation 6.1

6.1.2 Determination of PTE in particulate matter on filters

The loaded filters from the TEOM FDMS can be analysed for PTE. Recent epidemiological studies have shown numerous health effects due to the presence of PTE in PM.[272-274] As with soils, the total PTE concentrations in the PM are useful but will not give information on analyte mobility, bioavailability and toxicity, because these depend on the form in which an element exists in an environmental substrate.[19] Sequential extractions are suitable tools for assessing the mobility of elements, because they provide additional information about the solubility of the elements in specific reagents, which can be nominally associated to specific matrix components.[275] As discussed in earlier chapters, sequential extractions have been widely and successfully applied to sediments and soils. Among sequential extraction schemes, the procedures by Tessier[117] and BCR[147] have been the most popular. Various attempts have been made to apply these procedures or other sequential extraction schemes to atmospheric PM. Other methods developed and applied to airborne PM include those developed by Chester *et al.*[276] This method is a 3-stage sequential extraction designed to extract the following phases; (i) loosely-held environmentally mobile fractions, (ii) bound to carbonates and oxides, and (iii) organic and refractory-associated fractions. The extractants used were: (i) 1M NH_4OAc, (ii) 1M $NH_2OH.HCl$ and 25 % HOAc, and (iii) HNO_3 and HF respectively. Another method used was developed by Zatka *et al*.[277] The method was developed initially for the fractionation of Ni in dust from Ni-producing factories and workplaces. It exploits the differences in chemical properties of the various Ni phases found in the dusts. It is a 4-step procedure utilising ammonium citrate (soluble Ni), hydrogen peroxide-ammonium citrate (sulfidic Ni), bromine-methanol (metallic Ni) and nitric and perchloric acids (oxidic Ni). Table 6.1 shows examples of the sequential extraction methods applied to airborne PM mainly using the developed methods mentioned above or their modified forms.

Table 6.1 Example of sequential extraction methods applied to airborne PM

Author	Method used/modified	Steps	Sample	Element
Fraser and Lum[278]	Tessier	4	Incinerated sludge ash	Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, P, Pb and Zn
Hong *et al*[279]	Tessier	4	Fly ash	Cr, Cu, Pb and Zn
Hansen *et al*[280]	Modified Tessier	6	Fly ash	Cd
Hlavay *et al*[281]	Chester	3	Aerosol	Al, As, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, Pb and V
Bikes *et al*[282]	Chester	3	Atmospheric aerosol	Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn
Dabek-Zlotorzynska *et al*[283]	Chester	3	SRM 1648	Cd, Cu, Fe, Mn and Zn

Kocak *et al*[284]	Chester	3	Aerosol	Al, Cd, Cu, Fe, Mn, Pb and Zn
Andersen *et al*[285]	Modified Zatka	2	Dust	Co, Cu, Fe and Ni
Vincent *et al*[286]	Zatka	4	Aerosol	Ni
Dabek-Zlotorzynska *et al*[287]	BCR	3	SRM 1648	Cd, Cu, Fe, Mn and Zn
Dabek-Zlotorzynska *et al*[288]	BCR	3	Urban ambient air $PM_{2.5}$	Cd, Cu, Fe, Mn and Zn
Smeda and Zyrnicki[289]	Modified BCR	4	Fly ash	Al, Ba, Ca, Cr, Cu, Fe, Mg, Mn, Ni, Sr, V and Zn
Sysalova and Szakova[290]	Modified BCR	3	Homogenised urban dust	As, Cd, Cr, Mn, Ni, Pb, and Zn
Sysalova and Szakova[265]	Modified BCR	3	Urban dust samples and simulated air filters	As, Cd,Cr, Mn, Ni, and Pb
Horvath et al[291]	Modified BCR	3	Gravitational dusts	Cd, Cr, Cu, Ni, Pb and Zn
Fujiwara *et al*[292]	Others	4	Aerosol	Al, As, Cr, Cu, Fe, Mn, Ni, Pb, Ti, V and Zn

The BCR sequential extraction scheme was developed to harmonise and improve the extractants and procedures for sequential extraction of PTE in soils and sediments. Although the BCR protocol has been widely used for soils and sediments, less attention has been paid to fractionation studies on PM using the BCR scheme. Various attempts have been made to modify the BCR extraction for application to PM (table 6.1) but so far no report has been made on the use of a specially miniaturised BCR procedure to fractionate PTE in filters loaded with atmospheric PM obtained from TEOM FDMS instruments, which are used in most air quality monitoring stations in UK.

6.2 Aim

The main aim of the work in this chapter was to apply the small scale BCR sequential extraction procedure developed in chapter 5 to TEOM FDMS filters to fractionate PTE in the PM collected on them. A CRM 701 was chosen as test substrate because it has certified for the modified BCR sequential extraction so that recoveries can be calculated from these values. The specific objectives were

- To investigate the levels of PTE in various un-used FDMS filters so as to establish probable blank levels and any variation therein
- To determine the pseudototal concentrations of Cr, Cu, Fe, Mn, Ni, Pb and Zn in loaded TEOM FDMS filters collected from air quality monitoring stations under the Glasgow City Council
- To study the homogeneity of metal-bearing PM on loaded FDMS filters by cutting some filters into segments and measuring the repeatability of extractions performed on these segments
- To perform the sequential extraction using the miniaturised BCR procedure on blank and loaded FDMS filters

6.3 Experimental

6.3.1 Sample and sample preparation

The samples used in this work were loaded FDMS filters collected from some TEOM FDMS instruments from certain air quality monitoring stations by staff of Glasgow City Council, Scotland, UK. The FDMS filters used to determine the levels of PTE in the blank filters were randomly selected from two different packets with the aim of studying if there were any variations within filters of the same packet and between filters from different packets. The FDMS filters used were 47 mm diameter PTFE-coated borosilicate glass fibre filter. Figure 6.3 shows heavily loaded TEOM and FDMS filters.

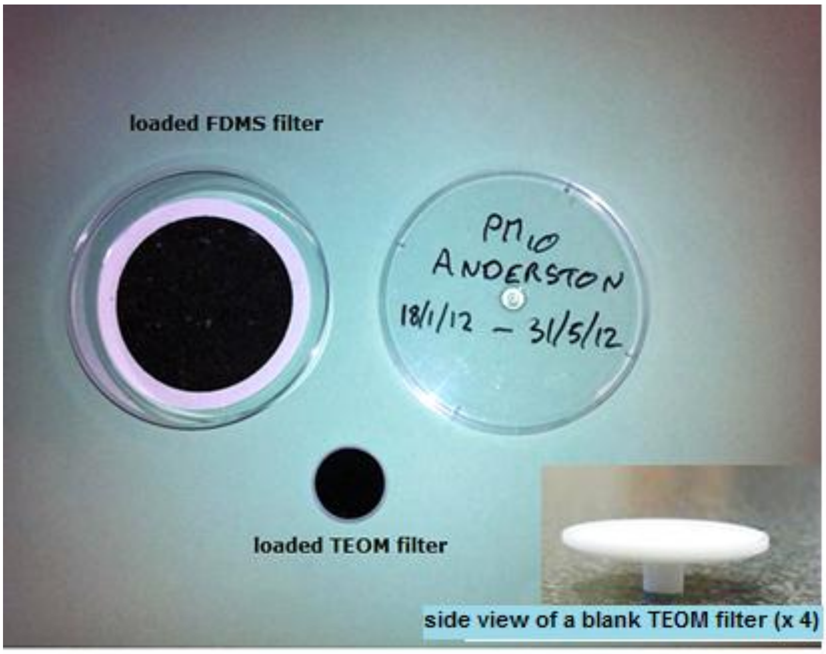


Figure 6.3 TEOM and FDMS filters loaded with PM

The following samples were used in this chapter,

- Blank Pallflex® TX40 47 mm FDMS filters (Air monitors Ltd. Gloucestershire, UK)
- Two loaded PM_{10} FDMS filters from Glasgow Abercromby Street (exposed from 29th November 2011 to 16th January 2012 and from 20th March 2012 to 30th May 2012) supplied by Glasgow City Council
- Loaded PM_{10} FDMS filter from Glasgow Anderston (exposed from 18th January 2012 to 31st May 2012) supplied by Glasgow City Council
- Loaded $PM_{2.5}$ FDMS filter from Glasgow Kerbside (exposed from 22nd March 2012 to 30th May 2012) supplied by Glasgow City Council
- Loaded FDMS filter from Glasgow Broomhill (no exposure dates available) supplied by Glasgow City Council
- Loaded FDMS filter from Glasgow Byres Road. (no exposure dates available) supplied by Glasgow City Council
- Certified reference material BCR 701, which was loaded onto filters to serve as a test substrate of known composition. The certified values for BCR sequential extraction content of steps 1-3 and indicative values for *aqua regia* soluble contents of step 4 and direct analysis of Cd, Cr, Cu, Ni, Pb and Zn are given in table 6.2

Table 6.2 Certified and indicative values for BCR sequential extraction metal content (mg kg^{-1} dry mass) in CRM 701[160]

		Cd	Cr	Cu	Ni	Pb	Zn
Step 1	Certified Value [a]	7.3	2.26	49.3	15.4	3.18	205
	Uncertainty [b]	0.4	0.16	1.7	0.9	0.21	6
	P	14	14	14	13	14	14
Step 2	Certified Value [a]	3.77	45.7	124	26.6	126	114
	Uncertainty [b]	0.28	2.0	3	1.3	3	5
	P	14	12	14	12	11	12
Step 3	Certified Value [a]	0.27	143	55.2	15.3	9.3	45.7
	Uncertainty [b]	0.06	7	4	0.9	2.0	4
	P	13	12	13	13	12	11
Step 4	Indicative Value [a]	0.13	62.5	38.5	41.4	11.0	95
	Uncertainty [b]	0.08	7.4	11.2	4.0	5.2	13
	P	9	13	14	11	14	13
Pseudototal	Indicative Value [a]	11.7	272	275	103	143	454
	Uncertainty [b]	1.0	20	13	4	6	19
	P	14	14	13	12	14	12

[a] ***mean value of p sets of data obtained from different laboratories***

[b] ***standard deviation***

6.3.2 Investigating the blank level of PTE in FDMS filters

Six blank FDMS filters each were selected randomly from two different packets of 25 filters each (Air monitors Ltd. Gloucestershire, UK). The filters were digested with 5 ml *aqua regia* for determination of pseudototal content as described in section 3.1.1. The operational conditions of the microwave digestion system were a power of 800 watts and ramping time of 20 minutes (*cf.* 1600 watts and 10 minutes, the default settings), while the temperature and the holding time were left as 160 °C and 20 minutes respectively. This was done to avoid applying excess energy to a small sample mass which may cause the acid to vent, thereby losing some of the analytes and also causing damage to the microwave digestor. The digests were filtered, made up to 10 ml, and then further diluted 200 fold for analysis by ICP-MS.

6.3.3 Determination of pseudototal concentration of PTE in FDMS filter

Four loaded FDMS filters, two from Glasgow Abercromby Street, one from Glasgow Anderston and one from Glasgow Kerbside (located at the start of Hope Street) air quality monitoring stations and a blank FDMS filter were digested with 5 ml *aqua regia* as described in 6.3.2.

6.3.4 Investigating the repeatability of the extraction of PTE in loaded filters

Two loaded FDMS from Glasgow Broomhill and Glasgow Byres Road monitoring stations were each cut accurately into quarters as shown in figure 6.4 using a silica knife. Each of the quarters was digested with *aqua regia* as described in 6.3.2. A blank filter was also digested at the same time.

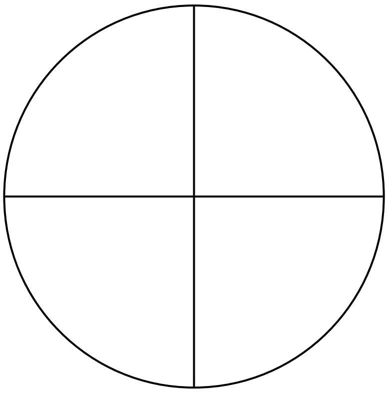


Figure 6.4 Illustration of cutting the filters into quarters

6.3.5 Fractionation of PTE from PM on FDMS filters using sequential extraction

Five blank FDMS filters and two FDMS filters loaded with about 0.125 g of CRM 701 (by smearing on blank filters as evenly as possible) were taken through the miniaturised BCR sequential extraction procedures as developed in chapter 5. The volumes of the reagents were reduced 8 fold to keep the solid to reagent ratio the same as in the conventional BCR protocol. The extractions were carried out in 15 ml centrifuge tubes.

6.3.6 Analysis

All the extracts were stored in plastic transportation tubes at 4 ^{o}C prior to analysis. The extracts were analysed using an Agilent 7700x ICP-MS instrument (Agilent Technologies, Inc., USA) against reagent-matched standards as described in section 3.2.2.

6.4 Results and Discussion

6.4.1 Blank levels of PTE in FDMS filters

The FDMS filters supplied were initially assumed to be low in metals content, so fit for determination of PTE in PM collected on these filters. But when analysing small sample of PM (usually few milligrams) for PTE, low and stable blank values for the filters are important. Some previous studies[293, 294] have showed relatively high blank values for some elements in some filters studied. In fact, it has been reported that the element contents of different filter materials varied considerably and were highly unpredictable even at different batches from the same source.[294] For these reasons it was decided to test the levels of PTE in blank filters and also to test for any possible variations between the concentrations found in blank filters from the same batch (packet) and from different packets.

Results of this experiment are presented in table 6.3. Statistical methods were used to test for any variations in the levels of the PTE in FDMS filters from the two packets. Coefficients of variations were calculated for concentrations of PTE between the individual filters from the same packet to establish any variation within filters of the same packet.

Table 6.3 Concentrations of PTE in blanks FDMS filters (µg/filter) from two different packets

Packet 1

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Filter 1	3.65	1.13	73.3	2.54	0.55	0.76	1150
Filter 2	3.59	1.09	71.5	2.52	0.51	0.75	1130
Filter 3	3.40	0.94	71.7	2.39	0.44	0.69	1130
Filter 4	3.58	1.10	72.7	2.49	0.54	0.73	1160
Filter 5	3.42	1.20	69.3	2.34	0.48	0.83	1120
Filter 6	3.64	1.09	72.7	2.39	0.52	0.73	1150
Mean	3.55	1.09	71.9	2.45	0.51	0.75	1140
sd	0.11	0.08	1.43	0.08	0.04	0.05	16.0
RSD	3.15	7.68	1.99	3.38	8.16	6.19	1.40

Packet 2

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Filter 1	3.27	3.31	6.84	1.70	0.67	0.83	1040
Filter 2	3.31	2.55	6.79	1.66	0.67	0.74	1120
Filter 3	2.79	2.14	5.81	1.54	0.71	0.64	949
Filter 4	2.99	2.44	6.21	1.60	0.76	0.66	999
Filter 5	3.37	2.62	6.94	1.76	0.61	0.74	1070
Filter 6	3.54	2.39	7.31	1.85	0.57	0.74	1160
Mean	3.21	2.57	6.65	1.68	0.66	0.73	1060
sd	0.27	0.40	0.55	0.11	0.07	0.07	77.8
RSD	8.52	15.4	8.21	6.63	9.87	9.16	7.36

The results of the analysis of the blank filters showed low amounts of the PTE in these filters from both packets, except for Zn the concentration of which was relatively high in both packets and Fe which was higher in the first packet than in the second. The high concentration of Zn could be attributed to the use of Zn as a binding material in the manufacturing of the filters, while high Fe in batch 1 may be due to contamination during the handling of the filters. The coefficients of variations for each of the sets of data for all the PTE were < 10 % in both batches. This implied that the concentrations of all the PTE in the filters from same packet were similar. To test for any variation in concentrations of PTE in filters from different packets, t-tests were conducted at 95 % confidence level between the mean concentrations of each of the PTE in the filters from the two different packets, the results are shown in table 6.4.

Table 6.4 t-test results at 95 % confidence level for the mean PTE concentrations in blank FDMS filters from two different packets

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
t_{cal}	2.78^{*}	8.96^{*}	104^{**}	13.4^{**}	5.01^{**}	0.622^{**}	2.60^{*}
$t_{critical}$	2.23	2.23	2.45	2.26	2.31	2.26	2.23

*sample assuming equal variances ** sample assuming unequal variance

The results of the tests showed that for all the PTE with the exception of Pb, the calculated t values were greater than the critical t values. Statistically this implied that there was significant difference in the concentrations of the PTE in the filters from the different packets. The implication of this is that it cannot be assumed that the concentrations of the PTE in blank filters are constant, even if they were obtained from the same manufacturer. Although the blanks levels of the PTE were low, the amounts of the PTE collected per filter may also be low, so there is a need to always analyse a blank filter to correct for the blank. Due to the significant difference in the concentrations between the packets, blanks from the same packet should be analysed together with the loaded filters whenever possible. More appropriately, a procedural blank should be analysed i.e. a blank filter that has been fixed to the instrument and gone through the sampling procedure except that the air pump is not turn on.

Unfortunately, since no procedural blanks were exposed alongside the FDMS samples discussed in the next sections-all of which were obtained from Glasgow city council-, all that could be done was to analyse an unused blank FDMS filter in parallel with the sample. The concentrations of the PTE in the blanks were then subtracted from that of the PTE on the exposed filters in an attempt to approximately correct for blank levels.

6.4.2 Determination of pseudototal concentration of PTE in FDMS filter

Table 6.5 shows the pseudototal concentrations of Cr, Cu, Fe, Mn, Ni, Pb and Zn in FDMS filters collected from Glasgow Abercromby Street, Glasgow Anderston and Glasgow Kerbside air quality monitoring stations, together with concentrations of some PTE from other part of the world and also the WHO air quality guideline values for Europe. The results for the two samples collected from Glasgow Abercromby Street at different times were comparable and generally low with exception of Zn. The concentrations of Cr in the two samples were not reported because the blank values were greater than the values obtained for the samples, therefore leading to negative values after the blank corrections. The concentrations of the PTE in the samples from other two sites were also relatively low. The concentrations of the PTE in the atmospheric PM from the present study were much lower than in highly polluted areas such as Lucknow City[295], Islamabad[296], Yokohama[297] and Paris.[298] The concentrations of Mn, Ni and Pb were all lower than the WHO standard values while Cr from Glasgow kerbside was slightly higher than the value set by WHO.[299]

Table 6.5 Concentration of PTE in FDMS filters from Glasgow, UK (ng/ m^3) and their comparison with other urban areas around the world and WHO guideline values

Sample	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Glasgow Abercromry Street	NR	2.10	7.22	0.210	0.016	0.635	385
Glasgow Abercromry Street	NR	1.74	6.88	0.400	0.143	0.576	360
Glasgow Anderston	0.138	1.98	6.07	0.590	0.437	0.836	68.5
Glasgow Kerbside	0.289	1.07	2.68	0.340	1.86	0.246	210
Edinburgh[300]	1.60	4.93	183	2.94	3.43	14.1	13.3
Lucknow City, India[295]	6.92	128	1030	19.8	36.8	74.6	49.6
Islamabad, Pakistan[296]	7.18	38.1	1340	38.8		63.5	3330
Yokohama, Japan[297]	4.1	47.7		32.5		27.2	130
Paris, France[298]	4.12	18.4	483	6.95		15.4	45.8
WHO[299]	0.25			150	25	500	

NR- not reported

6.4.3 Repeatability of the extraction of PTE from exposed filters

In order to improve the reliability of data obtained, several portions (replicates) of a sample are commonly taken through an entire analytical procedure. At the beginning of work in this chapter, it was thought that each exposed filter should be cut into segments to obtain replicates of the samples. This procedure (cutting of the filter) was applied by some authors[292, 295, 301, 302] in the determination of PTE in PM from filters. This procedure can be valid if the deposition of the PM on the filters is uniform, and little has been done to verify if this is correct. Therefore the main aim of this section was to investigate the homogeneity of deposition of PM on filter samples from some air quality monitoring stations. This homogeneity investigation will help to establish if a segment of filter can be representative of the whole filter. Table 6.6 shows the amount of the PTE in each segment for two FDMS filters from different locations in Glasgow that had been quartered as described in 6.3.4. Note that each value represents the amount on the whole filter (i.e. the result for each individual quarter was multiplied by a factor of 4). The amounts of the PTE were expressed in terms of the mass per filter. This was because the exposure durations of the filters used in this section were not available, therefore the volume of air sampled could not be calculated. Also the purpose was not to obtain concentrations per m^3 of air but to investigate the homogeneity of deposition.

Table 6.6 Amount of the PTE (µg/filter) on loaded FDMS filters

Sample	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Broomhil 1	2.31	2.01	89.2	1.18	0.752	0.683	833
Broomhil 2	2.38	1.47	96.1	1.29	0.594	0.664	600
Broomhil 3	2.44	1.22	85.9	1.18	0.732	0.647	942
Broomhil 4	2.63	1.36	112	1.55	0.631	0.648	986
Mean	2.44	1.51	95.8	1.30	0.677	0.660	840
sd	0.141	0.345	11.6	0.175	0.077	0.017	173
RSD	5.78	22.8	12.1	13.5	11.3	2.54	20.6

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Byres Road 1	3.64	2.25	136	1.93	0.439	1.79	856
Byres Road 2	3.59	2.22	134	1.90	0.420	1.80	802
Byres Road 3	3.35	2.19	131	1.78	0.419	1.69	829
Byres Road 4	3.58	2.18	129	1.89	0.430	1.73	934
Mean	3.54	2.21	133	1.87	0.427	1.75	855
sd	0.131	0.031	3.20	0.066	0.009	0.051	57.2
RSD	3.71	1.39	2.42	3.53	2.15	2.89	6.68

The results obtained from each segments for each filter were statistically compared by calculating the coefficient of variations (RSD) to see how close the results were to each other. A high RSD usually indicates wider the variation between replicate measurements. Therefore in this case the higher the RSD then the greater the variations between the concentrations PTE in the different segments and this will imply the likelihood of non-uniform distribution of the PTE on the filter. For Glasgow Broomhill filter, only the coefficient of variations of Cr and Pb were < 10 % which indicated close agreement between their concentrations in the individual segments of the filter. The coefficient of variations for Cu, Fe, Mn, Ni and Zn were all > 10 %, which showed that, there were wider variations in the concentrations of these elements in the individual segments. These results of the analysis showed that there was likelihood that the PM on this filter was not uniformly distributed. However for the Glasgow Byres Road filter, the coefficient of variations for all the PTE were < 10 %, which showed a close agreement between the concentrations of each PTE in the different segment of the filter cut. This was an indication of uniform distribution of the PM on this filter. The results from these filters showed that some filters may have a non-uniform distribution of PM on the filters. Therefore the assumption[301] that there was a uniform distribution of PM on loaded filters from the samplers may be questionable.

6.4.4 Sequential extraction on a FDMS filter

Tables 6.7 – 6.10 show the concentrations of the PTE in various steps of the miniaturised BCR sequential procedure applied to blank FDMS filters, together with simulated loaded FDMS filters. The simulated FDMS loaded filters were loaded with about 0.125 g of CRM 701. To study the efficiency of the extraction of the PTE from the filters, the various values obtained for different steps were compared with certified and indicative values of the BCR 701[160] (table 6.2).

Step 1

Results for the step 1 showed that for the blank filters all the PTE with exception of Zn were below the detection limits. The amount of Zn in the blank filter was much less than the concentration of the Zn in the amount of CRM 701 added, so no need to blank correct. When the results obtained from the simulated filters were compared with certified values (table 6.2), the % recoveries were found to be > 70 %. Considering the presence of the filter and the small quantity of the CRM added to the filter, the efficiency of the extraction may be considered satisfactory.

Table 6.7 PTE concentrations in step 1 (mg/kg)* of the miniaturised BCR sequential extraction procedure using blank FDMS filters and simulated loaded filters

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Blank filter 1	< dl	< dl	< dl	< dl	< dl	< dl	3.15
Blank filter 2	< dl	< dl	< dl	< dl	< dl	< dl	3.27
Blank filter 3	< dl	< dl	< dl	< dl	< dl	< dl	3.94
Blank filter 4	< dl	< dl	< dl	< dl	< dl	< dl	2.72
Blank filter 5	< dl	< dl	< dl	< dl	< dl	< dl	2.79
filter+ CRM 701	1.88	38.0	51.3	150	11.0	2.54	167
filter+ CRM 701	1.93	39.8	50.9	151	11.6	2.56	178
certified values	2.26	49.3	Na	na	15.4	3.18	205
% recovery 1	83.3	77.1	Na	na	71.5	79.8	81.7
% recovery 2	85.4	80.7	Na	na	75.1	80.6	87.0

***** *Concentrations for blank filters were expressed as mg/filter, while those for loaded filters were expressed as mg/kg of the CRM*

Step 2

The results for step 2 of the BCR sequential extraction showed that Cr, Cu, Mn and Ni were below the detection limits in the blanks, while Fe, Pb and Zn were detected but at very low concentrations when compared to the amounts extracted from the CRM 701 loaded filters. When the values obtained from the extraction of the simulated filters were compared with the certified values (table 6.2), the recoveries were generally found to be > 80 %.

Table 6.8 PTE concentrations in step 2 (mg/kg) of the miniaturised BCR sequential extraction procedure using blank FDMS filters and simulated loaded filters

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Blank filter 1	< dl	< dl	0.274	< dl	< dl	0.005	1.30
Blank filter 2	< dl	< dl	0.182	< dl	< dl	0.005	1.90
Blank filter 3	< dl	< dl	0.301	< dl	< dl	0.013	2.80
Blank filter 4	< dl	< dl	0.331	< dl	< dl	0.005	1.16
Blank filter 5	< dl	< dl	0.174	< dl	< dl	0.008	1.07
filter+ CRM 701	36.6	99.2	6160	124	23.2	105	116
filter+ CRM 701	37.3	104	6330	127	23.6	106	120
certified values	45.7	124	na	na	26.6	126	114
% recovery 1	80.1	80.0	na	na	87.4	82.9	102
% recovery 2	81.6	83.6	na	na	88.7	83.8	105

Step 3

The results of the step 3 of the sequential extraction showed that all the PTE were detected in the blank filters but at much lower concentrations than the amount extracted from the simulated filter, except for Zn. In case of Zn, since the blank values were high, the concentrations of Zn in the simulated filters were corrected for the blank. Therefore the results of simulated filter (filter + CRM) shown in table 6.9 are the blank corrected values. The percentage recoveries for the PTE, when compared with the certified values (table 6.2) were generally > 80 %, but those of Zn were a little high (*ca.* 143 %). This is likely to be due to the extraction of the Zn from the blank filters. A partial dissolution of the filter was noticed during the extraction in this step. It is likely that the hydrogen peroxide used in step 3 was attacking the filter.

Table 6.9 PTE concentrations in step 3 (mg/kg) of the miniaturised BCR sequential extraction procedure using blank FDMS filters and simulated loaded filters

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Blank filter 1	0.667	0.075	14.4	0.328	0.182	0.131	339
Blank filter 2	0.540	0.106	11.3	0.256	0.136	0.106	230
Blank filter 3	1.14	0.115	16.9	0.396	0.413	0.139	356
Blank filter 4	0.408	0.038	8.16	0.183	0.074	0.075	155
Blank filter 5	1.55	0.037	11.8	0.298	0.743	0.072	137
filter+ CRM 701	127	74.1	3540	59.5	21.5	11.0	64.7
filter+ CRM 701	92.7	54.1	2160	39.1	15.5	7.55	66.3
certified values	143	55.2	na	na	15.3	9.30	45.7
% recovery 1	88.6	134	na	na	141	118	142
% recovery 2	64.8	98.1	na	na	101	81.1	145

Step 4

All the PTE were detected in the blank but at lower concentrations than in the simulated samples again with the exception of Zn. As was in step 3, the concentrations of Zn in the simulated filters were corrected for blank. The recoveries for Cu, Ni, Pb and Zn were between 73 to 137 %, while relatively high recoveries for Cr were obtained. A further partial dissolution of the filter was also observed in this step.

Table 6.10 PTE concentrations in step 4 (mg/kg) of the miniaturised BCR sequential extraction procedure using blank FDMS filters and simulated loaded filters

	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Blank filter 1	2.60	0.939	54.0	2.05	0.414	0.530	770
Blank filter 2	3.00	1.08	62.5	2.23	0.483	0.580	950
Blank filter 3	2.52	1.27	53.6	2.05	0.404	0.490	699
Blank filter 4	3.11	1.02	65.7	2.33	0.426	0.595	1030
Blank filter 5	3.14	1.20	66.2	2.28	0.448	0.635	1090
filter+ CRM 701	121	28.1	21200	287	36.6	15.0	77.3
filter+ CRM 701	101	27.0	18800	278	35.3	13.5	77.9
indicative values	62.5	38.5	Na	na	41.4	11.0	95.0
% recovery 1	194	73.1	Na	na	88.4	137	81.4
% recovery 2	161	70.1	Na	na	85.3	123	82.0

6.5 Conclusions

The investigation of blank levels of the FDMS filters showed that the levels of the PTE in the blank filters were low and similar within filters of the same packet, but there were significant differences in the concentrations of the PTE between filters from different packet. Although the amount PTE in the blanks were low, the concentrations of the PTE in the PM collected on the filters were usually also very low. Therefore the contribution arising from the filters may constitute a major source of error in the analysis. Ideally a procedural filter blank from the same batch as the exposed filters is necessary in order to obtain reliable blanks values for appropriate corrections. Relatively high amounts of Zn were found in all the blank filters and this was likely coming from binding materials in the filters.

A microwave assisted digestion method was successfully used to determine the pseudototal concentrations of PTE in PM collected on FDMS filters from some air quality monitoring stations in the Glasgow city area. The results were much lower than the results from some other urban areas around the world. The concentrations of the PTE in the samples were found to be lower than the WHO air quality guideline values for Europe.

The repeatability study also showed that the PM collected on filters may not always be uniformly distributed, therefore taking a part of the filter to be a representative of the whole filter may be erroneous.

The miniaturised BCR sequential extraction was successfully applied to solid particulates on FDMS filter. Relatively good recoveries obtained for all the steps showed that it is possible to use the miniaturised BCR to fractionate PTE in urban PM collected on FDMS filters. Among the challenges of applying this procedure to extract the PTE were the high levels of Zn extracted in steps 3 and 4 from the blank filters. The problem of manipulating the step 4, that is difficulties in transferring the residue from step 3 from the centrifuge tubes to microwave digestion vessels, is yet other challenge of applying this procedure. Desirable would be a PTE fractionation approach that could be applied to FDMS filters directly, with minimal manual handling. The development of such an approach is described in chapter 7.

7 Modification of the chemometric identification of substrates and element distribution extraction protocol to determine solid phase distribution of PTE in airborne urban particulate matter.

7.1 Introduction

Sequential extraction methods suffer a number of limitations which are listed in section 1.5.1.3, one of which is the lack of specificity of the reagents to their target phases[303, 304]. Another setback to the traditional sequential extraction methods is the re-sorption of the released elements on the sample under investigation during the process of the leaching thereby compromising the interpretation of the element distribution in the sample; this was demonstrated by Ariza *et al*[305], Gomez-Aiza *et al*[306] and Ho and Evans[307]. In an attempt to address these problems, Cave and Wragg[304] proposed an extraction method which uses a non-specific reagent. Various nitric acid concentrations, different sample to extractant ratios and reaction times were used, the resulting solutions were analysed for both major and trace elements. They applied a chemometric self modeling mixture resolution (SMMR) procedure to the data generated to determine the number of components present in the sample. They based their approach on the assumption that the soil sample consists of discrete physiochemical components, each having its own composition. They also assumed that, extracting using increasing concentrations of acid will release into solution different proportions of these components. This chemometric procedure was later refined by Cave *et al*[308] and called “chemometric identification of substrates and element distribution” (CISED). The method has the following merits:

i. The extracts provide a simple matrix readily compatible with analytical techniques commonly used (ICP-AES/MS)
ii. It is simple and speedy to carryout
iii. Re-absorption of the leached element is minimized due to short contact time between the sample and the reagent

iv. The results are not operationally defined and reflect the true physicochemical make of the solid under investigation

v. The method is the same for all elements being studied and the data processing helps to remove between-laboratory variability due to differences in the way extractions are carried out.

Despite these merits, the method needs expertise in chemometric and in geochemistry for components assignment. Information on the mineralogy of the soil or the results of the sequential extraction methods if available can help in the correct interpretation of the CISED data.

Table 7.1 shows some previous works which use the mixture resolution procedure.

Table 7.1 Previous works using the mixture resolution procedure

Sample	Study	Reference
River sediment	Element distribution and sediment formation processes	[309]
Standard reference material by National Institute of Standard and Technology, NIST SRM 2711 and a river sediment	Effect of addition of humic acids on the element distribution	[310]
NIST SRM 2710	Evaluation of correlation between the components identified in the sample and existing data on Tessier's fractionation	[304, 308, 311]
Mine-impacted soils and soils naturally enriched in As	Relationship between the soil physico-chemical components and the bioaccessible As	[312, 313]

7.1.1 General CISED Extraction protocol[314]

The extraction setup consists of a centrifuging tube fitted with a filter inserts (figure 7.1). The inner tube contains the sample and extractant and the external tube collects the extract after the centrifuging. The extract is removed easily for analysis by removing the inner tube and the next aliquot is added to the inner tube. The extractants used contain increasing concentrations of mineral acid (table 7.2), and for extractants 7 -14 a specific amount of H_2O_2 is added to re-dissolve any precipitated organic matter.

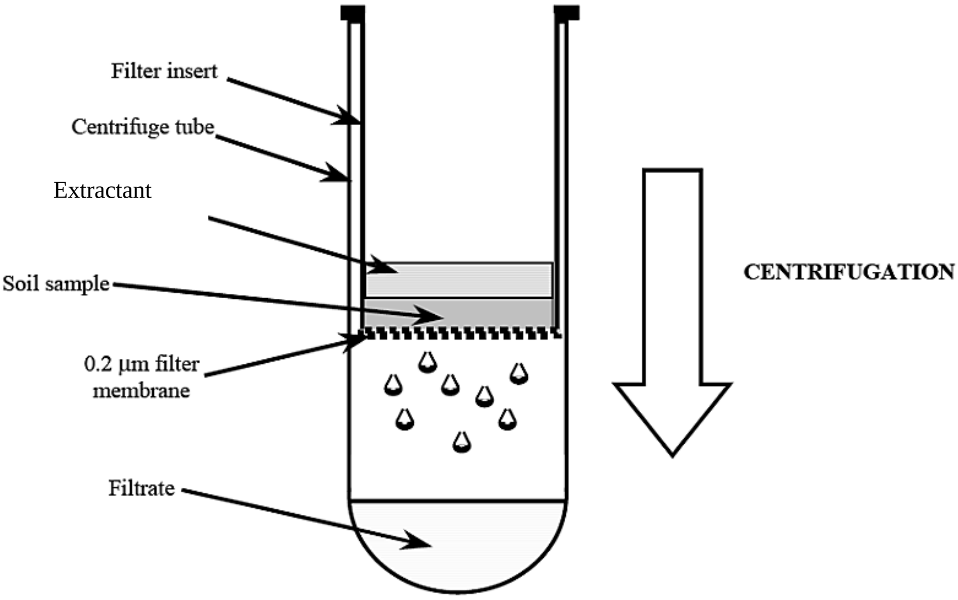


Figure 7.1 Diagram of centrifuge tube arrangement used for the CISED method (after[308])

Procedure for standard CISED protocol

1. Weigh about 2 g of the sample into the inner tube (filter insert).
2. Add 10 ml of the extractant into the inner tube.
3. Centrifuge the contents at 1034 *g* for 10 min
4. Collect the filtrate and keep at 4 ^{0}C prior to analysis.
5. Repeat steps 2-4 using the remaining extractants (table 7.2).

Table 7.2 Extractants used in the CISED method

Extraction Number	Amount and Molarity extractant
1	10 ml deionised water
2	10 ml deionised water
3	10 ml 0.01M *aqua regia*
4	10 ml 0.01M *aqua regia*
5	10 ml 0.05M *aqua regia*
6	10 ml 0.05M *aqua regia*
7	9.75 ml 0.1M *aqua regia* + 0.25 ml H_2O_2
8	9.75 ml 0.1M *aqua regia* + 0.25 ml H_2O_2
9	9.50 ml 0.5M *aqua regia* + 0.50 ml H_2O_2
10	9.50 ml 0.5M *aqua regia* + 0.50 ml H_2O_2
11	9.25 ml 1.0M *aqua regia* + 0.75 ml H_2O_2
12	9.25 ml 1.0M *aqua regia* + 0.75 ml H_2O_2
13	9.00 ml 5.0M *aqua regia* + 1.0 ml H_2O_2
14	9.00 ml 5.0M *aqua regia*+ 1.0 ml H_2O_2

The 14 extracts collected are analysed for both major and trace elements using a suitable analytical technique, usually ICP-AES or ICP-MS. The concentrations are then converted into mg/kg. Software written in the MATLAB programming language (Mathworks, Inc., Natick, MA, USA) is used for the data processing. The chemometric data processing has been described by Cave and Wragg[304] and Cave *et al.*[308] This processing resolves chemical extraction data into components, defining the number of components and their composition in the sample. It also allows the calculation of the quantitative distribution of the elements in the identified components.

The output of this data processing generates:

i. a series of profile plots or ‘extractogram’ of each of the identified components showing where in the extraction the component is removed;

ii. a bar chart showing the percentage elemental composition of each component; the components are named using the elements having percentage composition > 10 % therein (in decreasing order of their contribution);
iii. the amount of the elements in each component i.e. the distribution, and
iv. Scatterplots showing the actual extracted element concentrations compared to the modelled extracted element concentrations or each element.

7.2 Aim

The aim of the work in this chapter was to modify the CISED extraction procedure (by miniaturising and altering the apparatus used) and compare the results obtained from using the normal CISED procedure and the results obtained when the sample was presented on 47 mm FDMS filters.

7.3 Experimental

7.3.1 Sample

Certified reference material BCR 701 was used as a test substrate. This material was chosen because fractionating patterns for the various PTE as well as the amount of each element in the various fractions of modified BCR sequential extractions are well established. Therefore the mobility and to some extent the amount of the PTE as determined by the CISED procedure can be compared with ones as determined by the modified BCR sequential extraction. The sample description, the certified values for BCR sequential extraction content of steps 1-3 and indicative values for *aqua regia* soluble contents of step 4 and direct analysis of Cd, Cr, Cu, Ni, Pb and Zn are given in table 6.2.

7.3.2 CISED extraction

The CISED extraction method described in section 7.1.1 was modified and carried out using two approaches, which will be referred as Method 1 and Method 2 throughout this chapter. The mineral acid used in this extraction was *aqua regia.*

i. Method 1

In this approach, Whatman® “Vectaspin 20” polypropylene centrifuging tubes fitted with filter insert (Fisher Scientific, Leicestershire, UK) were used for the CISED extraction. The filter (pore size 0.45 µm) was made from pigment free polypropylene to eliminate sample contamination. The CISED extraction described in section 7.1.1 was performed on approximately 0.1 g of the sample, which was weighed accurately and using 3 ml aliquots of the extractants as described in table 7.2. The extraction was performed in duplicate, together with a blank extraction. All the extracts were analysed for Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Si, Sr, Ti, V and Zn using ICP-MS.

ii. Method 2

In this approach, the CISED extraction was performed on a FDMS filter. Approximately 0.1 g of the sample was weighed accurately and placed on to the FDMS filter. The sample was spread as evenly as possible all over the filter. The filter containing the sample was placed and held firmly using a 47 mm in-line polycarbonate filter holder (Pall Life Sciences, Portsmouth, UK) (figure 7.2). The CISED extraction described in section 7.1.1 was performed by pipetting 3 ml of the extractant (as described in table 7.2) into the sample through the inlet vent on the top of the holder. The mixture was allowed a contact time of 10 minutes with gentle shaking. The solution was then forced through the filter using a 60 ml plastic hypodermic syringe. The filtrate was collected through the outlet at the bottom of the holder. The extraction was performed in duplicate together with a blank extraction. All the extracts were analysed for Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Si, Sr, Ti, V and Zn using ICP-MS.

Figure 7.2 47 mm in-line polycarbonate filter holder

7.3.3 Detection limits

The solutions were analysed with standards prepared in 0.05 M *aqua regia* using ICP-MS. The detection limits found for *aqua regia* are shown in table 7.3

Table 7.3 ***Instrumental (DL_{inst}./ µg/l) and procedural ($DL_{pro.}$ / mg/kg) detection limits in 0.05 M aqua regia by ICP-MS analysis***

Nuclide	DL_{inst}	DL_{pro}
^{27}Al	1.780	0.053
^{137}Ba	0.055	0.002
^{40}Ca	3.500	0.105
^{52}Cr	0.072	0.002
^{53}Cr	0.010	0.000
^{63}Cu	0.232	0.007
^{65}Cu	0.168	0.005
^{56}Fe	0.984	0.030
^{57}Fe	9.940	0.298
^{39}K	2.538	0.076
^{24}Mg	1.122	0.034
^{55}Mn	0.191	0.006
^{23}Na	2.469	0.074
^{60}Ni	0.036	0.001
^{31}P	4.870	0.146
^{206}Pb	0.073	0.002
^{207}Pb	0.141	0.004
^{208}Pb	0.030	0.001
^{28}Si	6.349	0.191
^{29}Si	25.52	0.766
^{88}Sr	0.046	0.001
^{45}Ti	0.336	0.010
^{51}V	0.033	0.000
^{64}Zn	0.733	0.022
^{66}Zn	0.881	0.026

7.4 Data processing

Various combinations of the data from each of methods 1 and 2 were processed in order to assess which combination gave a profile with minimum uncertainties and clear extraction windows. For data from Method 1 averaging the two replicates after blank correcting them was found to give the best reasonable profiles. The layout of the data is shown in table 7.4, the values in each column is the average concentrations of the two replicates after each was blank corrected. This gave a matrix of 18 elements by 14 extracts. Unfortunately this approach was not optimal for Method 2. For data from Method 2 combining the two replicates and the blank to give a matrix of 18 elements by 42 extracts was the best combination. Table 7.5 shows the layout for this data processing. The values for the 14 extracts for the first replicate were laid followed by that of the second replicate then that of the blank. Modified version of the data processing method described by Cave *et al*[308] was used. The modifications and their validation are fully described by Cave[315], and the purpose of these modifications is to enable the measurements of uncertainty on both the composition of the resolved components and the amounts of each component present in the extraction profile.

Table 7.4 Data layout for Method 1

extraction no	Al	Ba	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	Si	Sr	Ti	V	Zn
1	52.05	15.27	377.10	0.30	11.25	5.36	112.17	270.74	88.76	103.00	4.88	303.61	0.38	49.90	20.34	0.40	0.64	83.25
2	140.52	19.44	286.71	0.39	25.23	11.95	98.01	179.73	55.73	44.52	4.23	245.55	2.91	57.96	16.80	0.43	0.55	72.16
3	327.83	5.86	167.90	0.93	37.56	34.47	4.39	130.67	31.05	33.23	3.35	212.08	12.36	78.32	10.94	0.58	0.53	31.37
4	248.91	12.72	67.51	1.02	22.36	36.77	17.57	104.09	14.82	8.05	1.45	121.90	12.35	83.85	4.29	0.66	0.41	15.09
5	302.51	14.27	48.03	2.00	20.96	187.52	25.04	86.72	11.74	20.59	1.92	160.24	25.45	101.09	2.32	2.36	0.50	8.61
6	242.02	13.76	32.95	2.06	13.01	216.00	45.62	71.25	8.53	9.72	1.58	137.23	17.51	102.80	1.45	2.37	0.47	8.41
7	312.01	12.29	28.83	4.63	17.10	437.00	67.49	85.91	12.20	6.93	2.31	165.61	18.09	151.52	1.22	17.21	1.18	10.80
8	253.29	8.33	17.36	4.57	12.42	253.61	53.85	80.24	7.24	2.42	1.70	127.96	11.35	149.79	0.76	13.81	0.99	8.41
9	508.59	9.49	18.47	14.56	20.05	1272.95	103.10	133.62	13.04	6.36	4.48	255.59	15.98	271.87	0.84	26.14	1.79	13.20
10	435.29	6.33	11.82	10.14	11.99	1074.85	92.92	138.74	12.03	5.71	2.88	164.28	9.13	279.03	0.60	23.98	1.60	10.78
11	641.24	8.10	17.06	18.94	12.06	1749.04	133.83	311.37	22.23	18.27	4.71	183.72	8.62	384.27	0.83	37.10	2.31	16.50
12	301.71	4.33	7.18	10.85	5.51	896.77	87.15	142.65	11.49	4.34	2.17	98.21	3.98	208.31	0.38	19.89	1.22	7.28
13	434.78	3.62	6.27	52.33	7.40	1934.81	102.04	145.29	25.14	4.22	3.85	186.88	4.64	248.13	0.44	30.62	1.68	9.87
14	217.42	1.94	4.03	24.55	5.87	909.39	51.44	107.35	16.55	5.16	3.84	37.86	2.52	138.88	0.28	17.40	0.67	10.68

Table 7.5 Data layout for Method 2

samples	Al	Ba	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	Si	Sr	Ti	V	Zn
1 rep1	74.1	19.2	369.1	0.4	13.5	8.4	122.6	277.5	84.2	165.7	4.6	321.3	0.6	61.4	20.1	0.4	0.6	84.0
2 rep1	98.5	14.8	237.1	0.4	19.1	9.8	84.7	162.3	48.1	69.6	3.3	194.5	2.3	51.2	13.6	0.3	0.4	57.8
3 rep1	379.8	31.0	206.7	1.3	39.9	38.8	79.1	157.9	36.7	40.7	3.9	247.8	11.8	91.2	13.4	0.5	0.6	59.0
4 rep1	250.2	20.7	74.6	1.0	22.7	39.9	50.5	103.8	15.3	20.9	1.9	134.6	11.6	76.8	4.9	0.5	0.4	22.0
5 rep1	314.2	21.7	51.2	2.0	21.5	191.1	59.0	86.6	12.2	13.9	2.0	170.1	25.7	106.2	2.6	2.0	0.5	14.6
6 rep1	245.8	15.8	34.8	2.0	13.0	217.5	57.7	75.4	9.1	10.5	1.6	144.5	17.4	103.2	1.6	2.1	0.5	9.8
7 rep1	277.7	12.6	26.9	4.1	15.5	389.5	74.6	79.0	12.3	9.2	2.1	159.8	16.9	128.0	1.1	17.4	1.1	11.0
8 rep1	236.6	8.8	18.1	4.3	11.8	406.4	61.0	72.9	7.4	7.7	1.6	129.2	11.0	134.9	0.8	13.4	0.9	8.4
9 rep1	520.7	9.7	19.8	14.8	21.1	1258.7	111.8	119.2	13.1	9.6	4.4	262.9	16.5	273.9	0.9	26.0	1.8	13.4
10 rep1	429.6	6.3	12.2	9.8	12.0	1034.1	99.3	124.1	11.4	10.1	2.7	165.6	9.1	277.2	0.6	23.3	1.6	10.4
11 rep1	403.5	5.0	9.1	11.0	8.8	1100.5	103.5	115.2	13.0	9.4	2.4	146.9	6.6	253.4	0.5	22.3	1.6	9.5
12 rep1	347.0	4.8	7.1	11.2	6.1	993.4	103.9	118.3	13.3	8.4	2.3	105.2	4.6	235.1	0.4	21.3	1.4	8.1
13 rep1	575.2	4.3	8.1	58.9	9.5	1911.2	124.5	176.3	30.9	9.9	5.5	216.1	5.9	318.0	0.6	38.4	2.2	12.7
14 rep1	91.8	1.0	1.8	26.6	7.4	1135.5	18.3	47.8	20.4	3.4	5.2	9.3	3.1	38.8	0.1	6.9	0.2	15.0
1 rep2	39.1	17.2	387.3	0.3	9.0	4.3	130.6	272.3	93.4	161.2	5.2	287.1	0.1	45.1	20.7	0.5	0.7	91.9
2 rep2	189.8	26.8	337.7	0.7	31.4	14.7	129.2	201.8	63.4	90.7	5.2	297.6	3.5	67.6	20.1	0.6	0.7	91.6
3 rep2	328.0	28.0	133.7	1.1	35.2	34.0	87.2	119.5	25.5	41.9	2.9	177.6	13.0	73.7	8.8	0.9	0.5	39.7
4 rep2	273.7	20.4	62.4	1.1	22.0	38.0	60.8	112.6	14.3	26.8	2.0	110.1	13.1	98.5	3.7	1.0	0.4	19.6
5 rep2	315.5	20.5	47.7	2.0	20.5	191.2	66.4	102.2	11.3	25.4	1.9	152.6	25.3	114.1	2.2	3.0	0.5	14.9
6 rep2	251.2	15.3	32.3	2.1	13.0	217.5	63.3	72.0	8.0	13.9	1.6	131.3	17.7	111.2	1.4	2.9	0.4	10.1
7 rep2	360.9	15.5	33.7	5.2	18.7	488.0	94.2	113.5	12.2	23.0	2.6	173.8	19.3	191.9	1.4	18.8	1.3	14.2
8 rep2	281.0	9.3	18.2	4.9	13.0	471.1	66.1	96.7	7.6	12.5	1.9	128.3	11.7	174.7	0.8	14.9	1.0	9.6
9 rep2	506.6	10.3	18.2	14.3	19.0	1289.4	107.0	153.0	13.0	17.8	4.6	251.3	15.5	281.7	0.8	26.8	1.8	13.6
10 rep2	451.4	7.3	12.9	10.4	11.9	1118.7	98.5	160.2	12.7	15.8	3.1	167.6	9.2	296.6	0.6	25.2	1.6	11.7
11 rep2	883.3	11.9	25.8	26.9	15.4	2400.4	171.0	513.1	31.5	33.4	7.0	222.4	10.7	531.5	1.2	52.2	3.1	24.4
12 rep2	266.8	4.5	8.4	10.5	5.0	803.1	78.0	171.2	9.7	11.8	2.1	92.8	3.4	195.8	0.4	18.8	1.1	7.2
13 rep2	301.5	3.0	4.9	45.8	5.3	1980.1	85.0	114.6	19.4	7.9	3.1	159.2	3.4	195.1	0.3	22.9	1.3	7.0
14 rep2	347.8	3.3	7.1	22.8	4.3	693.8	88.6	172.4	12.9	9.5	2.6	68.1	2.0	244.9	0.5	28.1	1.1	7.4
Blank1	4.6	3.0	1.1	0.0	0.0	1.0	14.5	4.2	0.0	60.5	0.0	0.6	0.0	3.3	0.1	0.0	0.0	4.7
Blank2	3.6	1.4	0.7	0.0	0.0	0.3	9.0	2.4	0.0	35.7	0.0	0.5	0.0	1.5	0.0	0.0	0.0	2.5
Blank3	26.1	23.7	2.3	0.0	0.0	2.0	78.9	8.1	0.0	74.7	0.0	0.6	0.0	4.2	0.1	0.1	0.0	18.0
Blank4	13.0	7.8	1.0	0.0	0.0	2.2	38.1	4.1	0.0	32.0	0.5	0.5	0.0	3.8	0.0	0.1	0.0	5.7
Blank5	12.3	6.8	1.4	0.0	0.0	3.6	37.7	7.7	0.0	40.3	0.0	1.1	0.0	9.1	0.1	0.1	0.0	6.1
Blank6	6.5	1.8	0.6	0.0	0.0	1.5	14.9	2.5	0.0	13.9	0.0	0.7	0.0	4.4	0.0	0.1	0.0	1.5
Blank7	7.3	1.8	1.5	0.0	0.0	1.8	17.0	10.4	0.0	13.4	0.0	1.2	0.0	8.5	0.0	0.9	0.0	1.8
Blank8	5.5	0.7	0.8	0.0	0.0	185.5	9.7	4.6	0.3	8.4	0.1	0.8	0.0	5.0	0.0	0.4	0.0	0.6
Blank9	5.1	0.5	0.5	0.0	0.0	1.1	6.3	2.5	0.0	7.4	0.0	1.5	0.0	5.9	0.0	0.3	0.0	0.3
Blank10	5.2	0.5	0.7	0.0	0.0	1.6	6.0	3.4	0.0	7.3	0.0	2.3	0.0	7.9	0.0	0.2	0.0	0.3
Blank11	2.2	0.3	0.4	0.0	0.0	1.4	3.4	2.8	0.0	3.1	0.0	0.9	0.0	8.2	0.0	0.2	0.0	0.4
Blank12	5.2	0.3	0.5	0.0	0.0	1.5	3.8	2.1	0.0	5.8	0.0	0.8	0.0	7.2	0.0	0.1	0.0	0.4
Blank13	3.6	0.0	0.2	0.0	0.0	10.8	2.7	0.2	0.0	4.7	0.4	0.8	0.0	8.4	0.0	0.0	0.1	0.0
Blank14	2.4	0.2	0.4	0.1	0.0	5.3	2.0	2.7	0.1	1.3	0.0	0.9	0.0	3.0	0.0	0.1	0.0	0.5

7.5 Results and Discussions

7.5.1 Identification of the components

Method 1

The chemometric data processing of results of the Method 1 produced 4 distinct components. Figures 7.3 - 7.6 show the extraction profile plots of these components and their compositions. In the extractogram, the extraction profile is shown by the red line which gives the amount of that component (in mg/kg) being extracted at different acid concentration with plausible uncertainty estimates (represented by the blue shading around the profile plot). On the right hand side of the extractogram is a histogram of the element composition which gives the percentage composition of each element in the component, with associate error bars. Using the extraction profile and composition of each component, tentative geochemical assignments are summarized in table 7.6.

Table 7.6 Tentative assignment of the components in CRM 701 using Method 1

Component name	Tentative assignment
Fe-Al-Si	Iron alumino-silicate
Ca-P-Mg	Carbonate
Al-P-Si	Aluminum oxide
Mg-Ca	Carbonate (noisy)

Fe-Al-Si

This component was extracted over the range 0.05 - 5 M and does not have a clear extraction window. Its composition was dominated by Fe (*ca.* 55 %), Al (*ca.* 17 %) and Si (*ca.* 11 %) and to a lesser extent P (*ca.* 7 %), Mg (*ca.* 4 %), K (*ca.* 4 %) and Ti (*ca.* 1 %). It was tentatively assigned as iron alumino-silicate. The drainage basin of Lake Orta from where CRM 701 was collected consists mainly of gneiss, mica schist and granite[316]. This component may likely come from some of the minerals present in the drainage, e.g. feldspar.

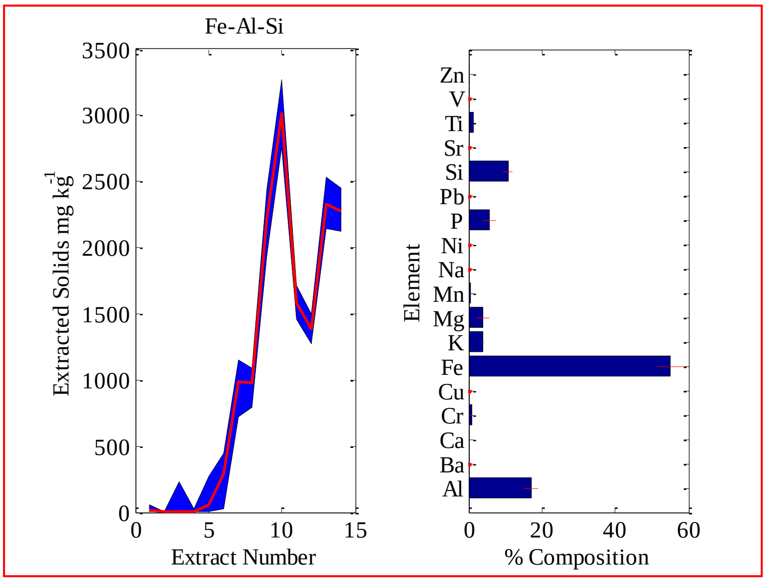


Figure 7.3 Extractogram of Fe-Al-Si component extracted in CRM 701 using Method 1

Ca-P-Mg

This component has a clear window of extraction and was liberated by low acid concentration, (0.01 M) and it contains a high proportion of Ca (*ca.* 32 %). It is therefore probably carbonate. The presence of calcareous schists[316] on the water shed of Lake Orta may be the possible source of this component. Another likely source may come from the liming of the lake which took place between 1989 and 1990[317] in which a total of 14,500 t of limestone was used. The component also contains P (*ca.* 23 %), Mg (*ca.* 15 %), Zn (*ca.* 8 %), Mn (*ca.* 6 %), Al (*ca.* 4 %), Si and Sr (*ca.* 2 %), Ba and Cu (*ca.* 1 %) and < 1 % K.

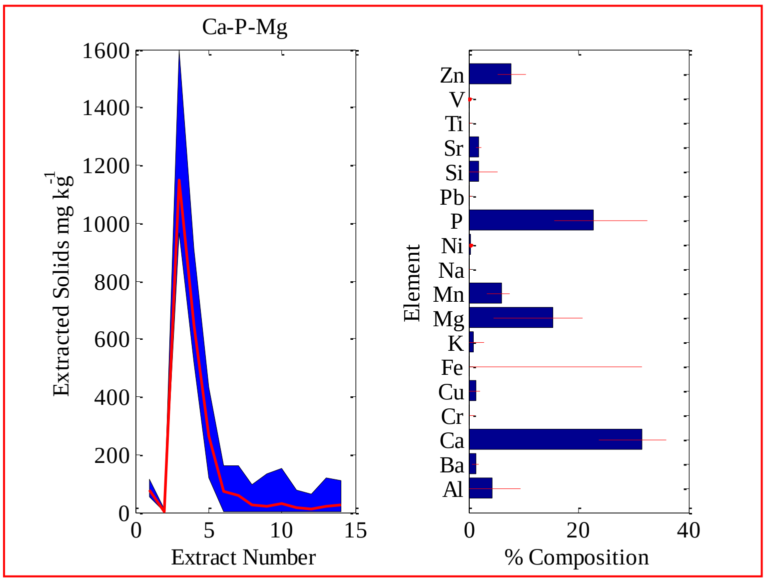


Figure 7.4 Extractogram of Ca-P-Mg component extracted in CRM 701 using Method 1

Al-P-Si

This component appears in extracts 4 - 10 (0.01 - 0.5 M *aqua regia*) and is made up of predominantly Al (*ca.* 41 %), P (*ca.* 22 %) and Si (*ca.* 11 %). This component could be identified as aluminum oxide and may likely come from the minerals in mica schist or gneiss found in the drainage basin of the lake[316]. CRM 701 was reported to contain 14.3 % and 15.6 % Al_2O_3 by Guevara-Riba *et al*[318] and Sahuquillo *et al*[319] respectively.

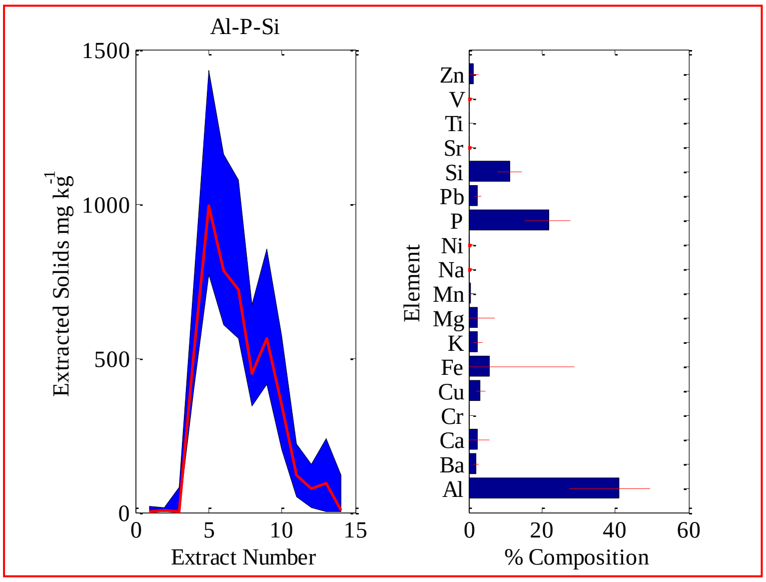


Figure 7.5 Extractogram of Al-P-Si component extracted in CRM 701 using Method 1

Mg-Ca

This is another component likely to be carbonate; it is composed of mainly Mg (*ca.* 41 %) and Ca (*ca.* 26 %). Other elements contributing to this component at < 10 % are; K, Mn, Si, Na and Al. The extraction profile is not well defined and there is high uncertainty (i.e. the extractogram is “noisy”).

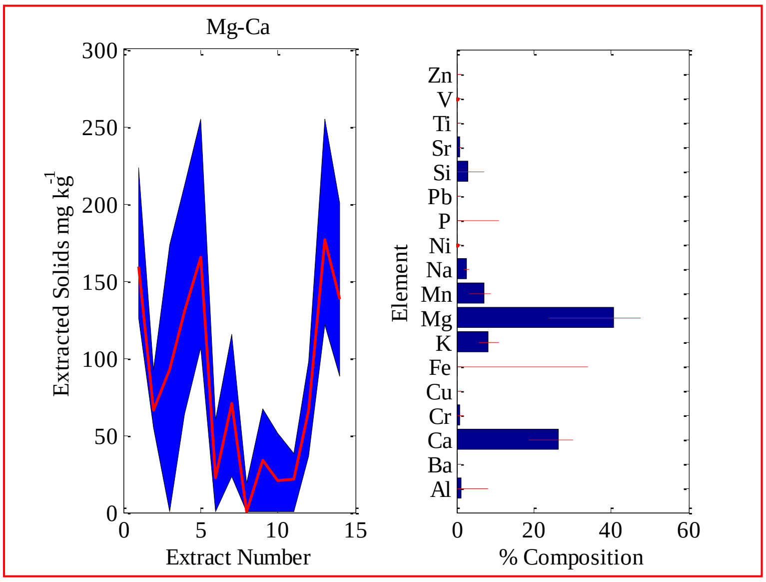


Figure 7.6 Extractogram of Mg-Ca component extracted in CRM 701 using Method 1

Method 2

The chemometric data processing of results using Method 2 produced 11 distinct components. The extraction profile plot and the composition of each component are shown in figures 7.7 - 7.17. The extractograms in this method had some similarities to the extractograms explained in Method 1. The major difference between the two is that the extraction profile in this method contains 42 extraction numbers: 1 - 14 represents sample replicate 1; 15 - 28 represents replicate 2; while 29 - 42 represent the contribution from the blank extraction. Using the extraction profiles and compositions, tentative geochemical assignments were given to each component and are summarized in table 7.7

Table 7.7 Tentative assignment of the components in CRM 701 using Method 2

Component name	Tentative assignment
Ca-P-Mg	Carbonate
Fe-Al-Si	Iron alumino-silicate
Al-P	Aluminum phosphate
Fe	Crystalline iron oxide
K-Ba	Filter component
Al-Si	Aluminosilicate (noisy)
Na	Filter component
Fe-Si	Iron alumino-silicate (noisy)
Si-Mg-Al	Clay
Fe	Crystalline iron oxide
K-Si-Al-Na	Clay (noisy)

Ca-P-Mg

This component is similar to the component identified as carbonate in Method 1, both in terms of the strength of the acid that extracted the component and composition of component. It was extracted by low acid concentration and made of Ca (*ca.* 33 %), P (*ca.* 25 %) and Mg (*ca.* 20 %). Other elements in the component are: Mn (*ca.* 8 %), Zn (*ca.* 7 %), Sr (*ca.* 2 %) and Cu (*ca.* 1 %). The presence of this component in the deionized water extract may also suggest the likelihood of the component to be identified as exchangeable, but the high proportion of Ca suggests it to be carbonate as identified in Method 1.

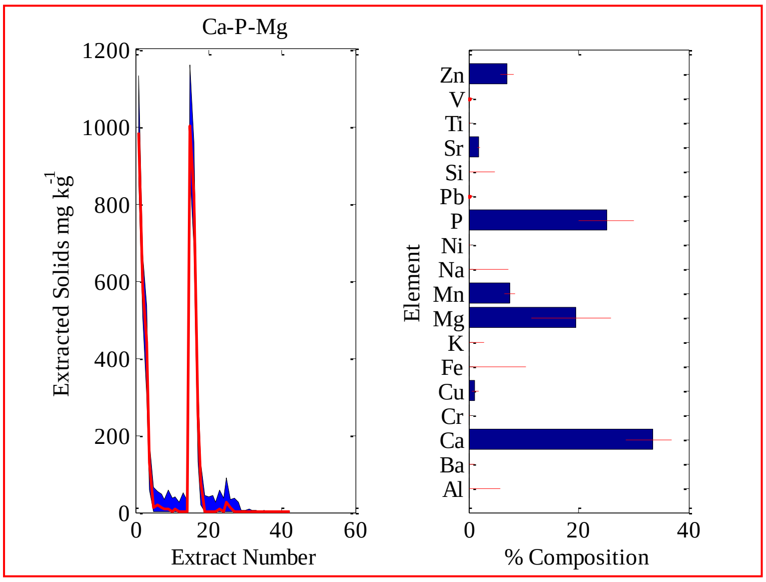


Figure 7.7 Extractogram of Ca-P-Mg component extracted in CRM 701 using Method 2

Fe-Al-Si

This component is similar to the component identified as iron alumino-silicate in Method 1. It was extracted over the range of 0.05 – 5 M *aqua regia*, and consists of mainly Fe (*ca.* 46 %), Al (*ca.* 19 %) and Si (*ca.* 14 %). Other elements in this component are: P (*ca.* 8 %), Mg (*ca.* 6 %), K (*ca.* 3 %) and Ti (*ca.* 2 %).

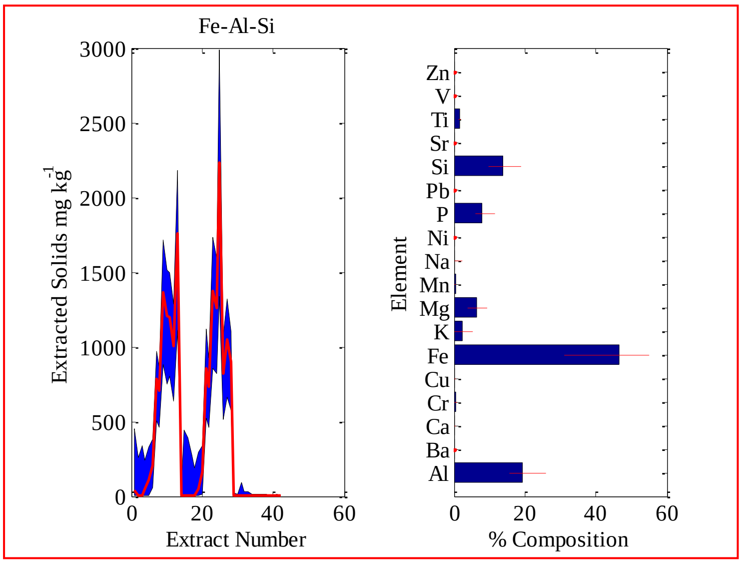


Figure 7.8 Extractogram of Fe-Al-Si component extracted in CRM 701 using Method 2

Al-P

This component was identified as aluminum phosphate; it was extracted over the range of acid concentration of 0.01 - 0.5 M and consists mainly of Al (*ca.* 40 %) and P (*ca.* 29 %) and to a lesser composition of < 10 % for Ca, Pb, Cu, Mg, Fe, Mn and Ba. This component is similar to the aluminum oxide component in Method 1.

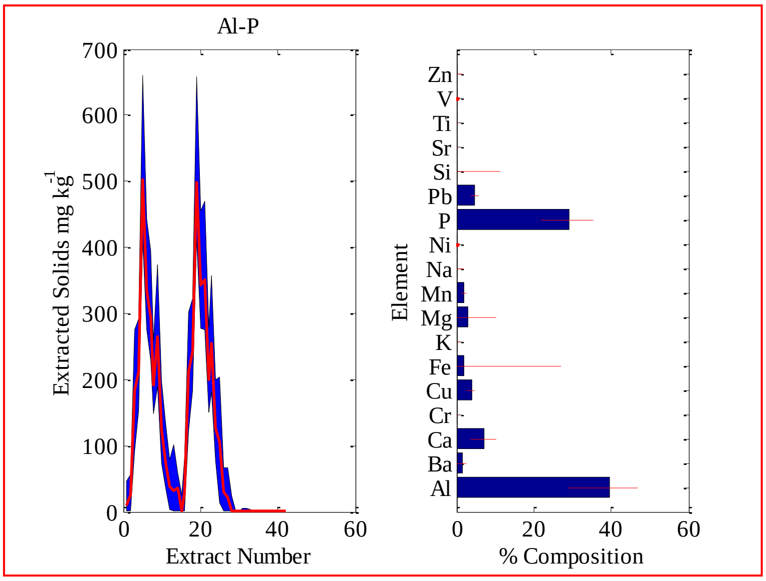


Figure 7.9 Extractogram of Al-P component extracted in CRM 701 using Method 2

Fe

In this component there was clear window of extraction, it was extracted at high acid strength, 5 M *aqua regia* and predominantly consists of Fe (*ca.* 75 %). The elements that are less than 10 % are: Al, Mg, Cr and Mn. This component was identified as crystalline iron oxide and was not identified in Method 1.

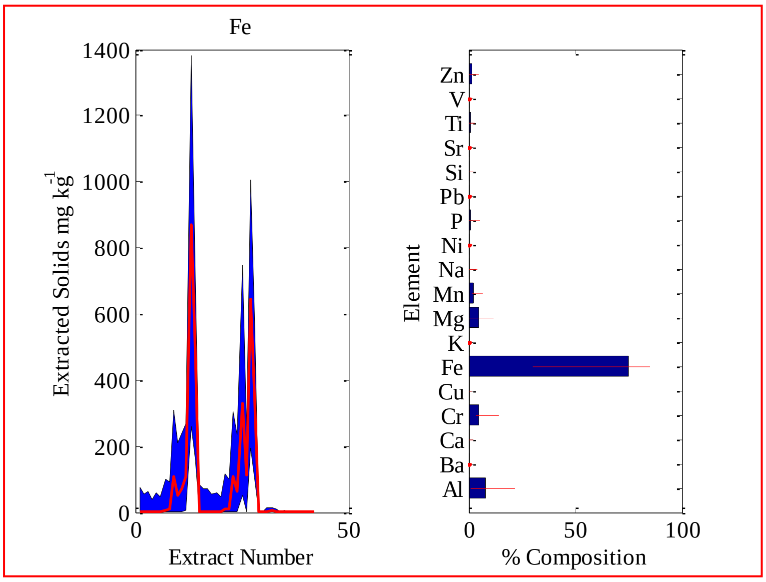


Figure 7.10 Extractogram of Fe component extracted in CRM 701 using Method 2

K-Ba

This component is likely to originate from the filter; the extraction profile has shown that the component was extracted from the blank sample which is the filter only. It was extracted at low acid strength, 0.01 M and consists of K (*ca.* 50 %), Ba (*ca.* 16 %), Al and Zn (*ca.* 10 %) and Mg (*ca.* 7 %). Semi quantitative analysis of the filter shows high levels of both K and Ba in the blank filter (appendix C).

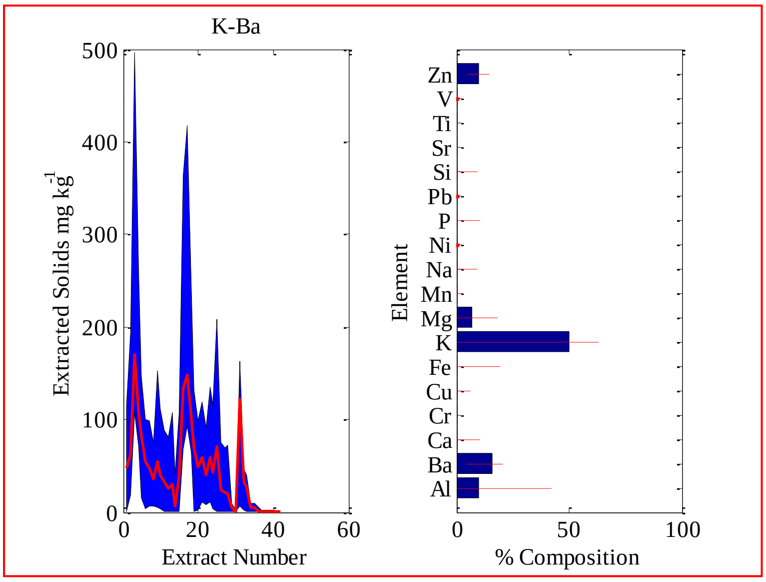


Figure 7.11 Extractogram of K-Ba components extracted in CRM 701 using Method 2

Al-Si

This is a "noisy" component as indicated by the large error bar on the element composition and there is no clear extraction window. It consists mainly of Al (*ca.* 38 %), Si (*ca.* 26 %), P (*ca.* 7 %), Na (*ca.* 6 %), Ca and Cu (*ca.* 4 %) and Mg (*ca.* 3 %). It is likely to be alumino silicate.

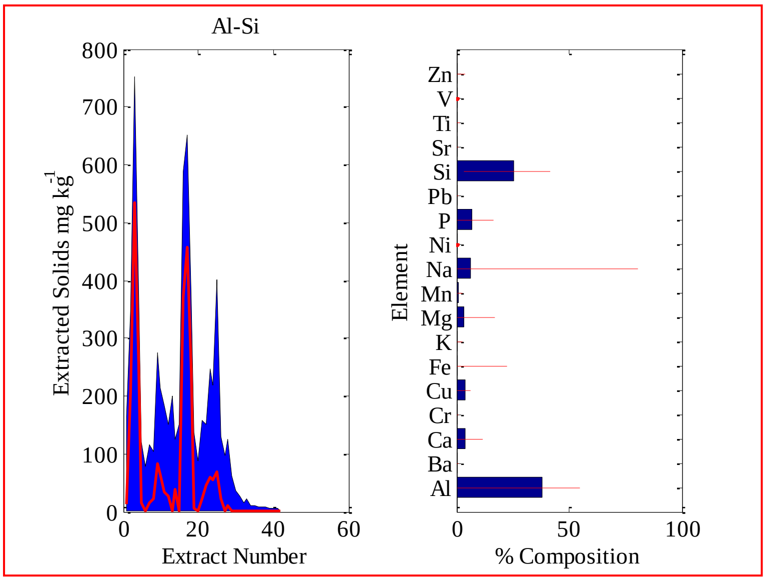


Figure 7.12 Extractogram of Al-Si component extracted in CRM 701 using Method 2

Na

This component is likely from the filter, the extractogram shows that the Na was extracted from the filter blank. The component found in the sample is a contribution from the filter and there is high uncertainty in the measurements in the two replicate samples (extraction numbers 1 - 28) while the extraction window in the blank filter (extraction numbers 29 - 42) is clearer and the uncertainty lower. The high concentration of Na in the semi-quantitative analysis (appendix C) supports this argument. The component was extracted by deionized water and dominated by the presence of Na (*ca.* 78 %), with < 5 % K, Zn and Ba.

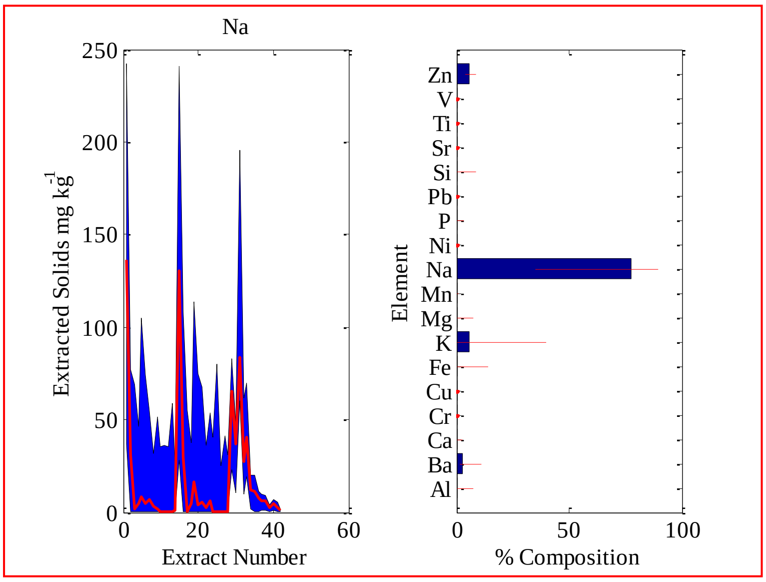


Figure 7.13 Extractogram of Na component extracted in CRM 701 using Method 2

Fe-Si

In this component the extraction window was undefined and “noisy”. It was extracted over all the range of acid strength; it consists mainly of Fe (*ca.* 53 %) and Si (*ca.* 31 %) and to lesser extent Na (*ca.* 5 %) and Al (*ca.* 2 %), it is likely to be iron alumino-silicate.

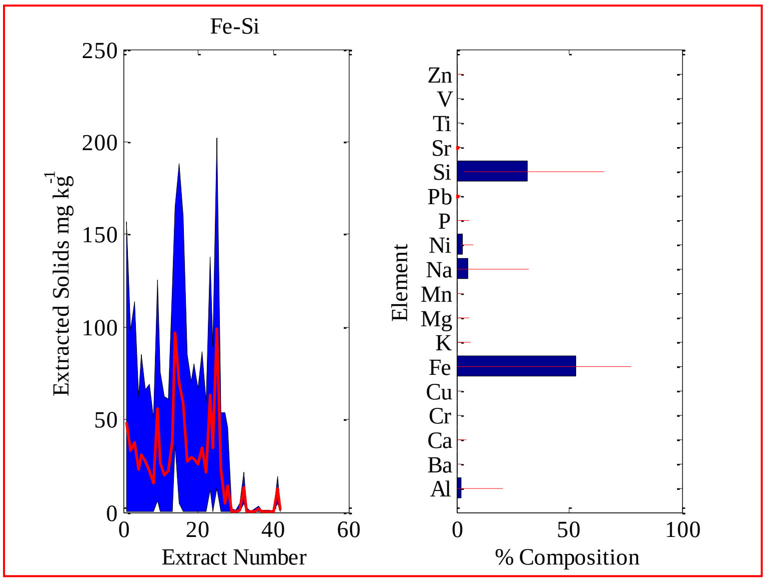


Figure 7.14 Extractogram of Fe-Si components extracted in CRM 701 using Method 2

Si-Mg-Al

The extraction window in this component was not clearly defined and consists mainly of Si (*ca.* 40 %), Mg (*ca.* 25 %) and Al (*ca.* 14 %). The composition shows it may be a clay component.

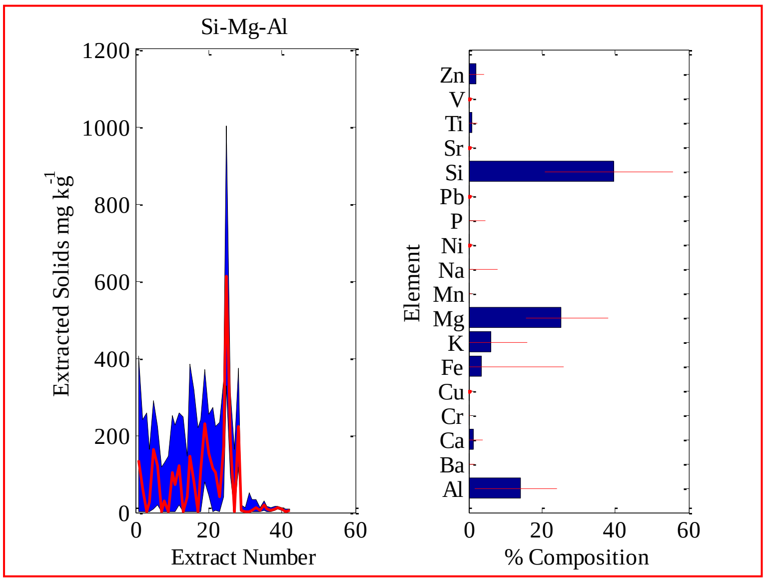


Figure 7.15 Extractogram of Si-Mg-Al component extracted in CRM 701 using Method 2

Fe

This is another component likely to be crystalline iron oxide; it was extracted at higher acid strength (0.5 - 5 M) and consists predominantly of Fe (*ca.* 93 %). This is a much purer form compared to the other crystalline iron oxide identified from figure 7.10 using the same method. The other form from Method 1 contained other elements such as Al, Cr, Mg and Mn and of iron.

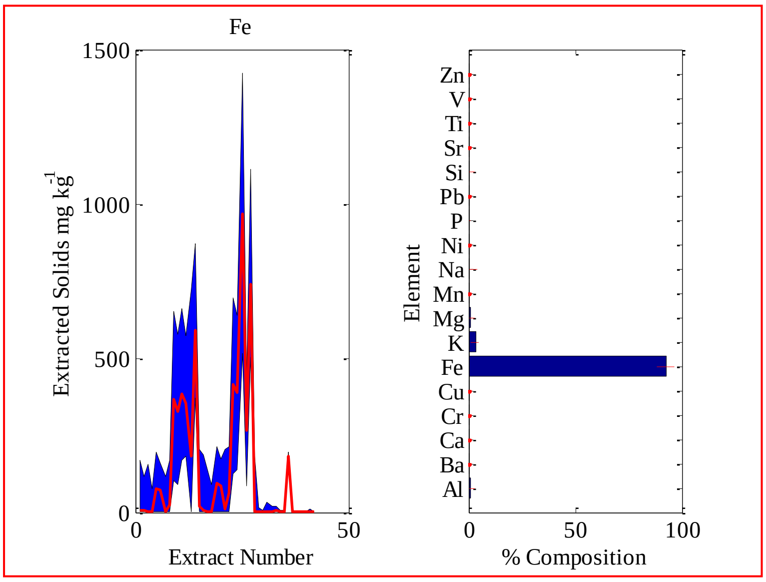


Figure 7.16 Extractogram of Fe component extracted in CRM 701 using Method 2

K-Si-Al-Na

The component is likely to be K-Si-Al, because the Na is likely to come from the filter component as seen in the profile plot. This component consists of K (*ca.* 38 %), Si (*ca.* 19 %), Al (*ca.* 12 %), Na (*ca.* 11 %), P (*ca.* 7 %), Ca (*ca.* 3 %) and Ba (*ca.* 2 %). This component is probably a clay component and it is also “noisy”.

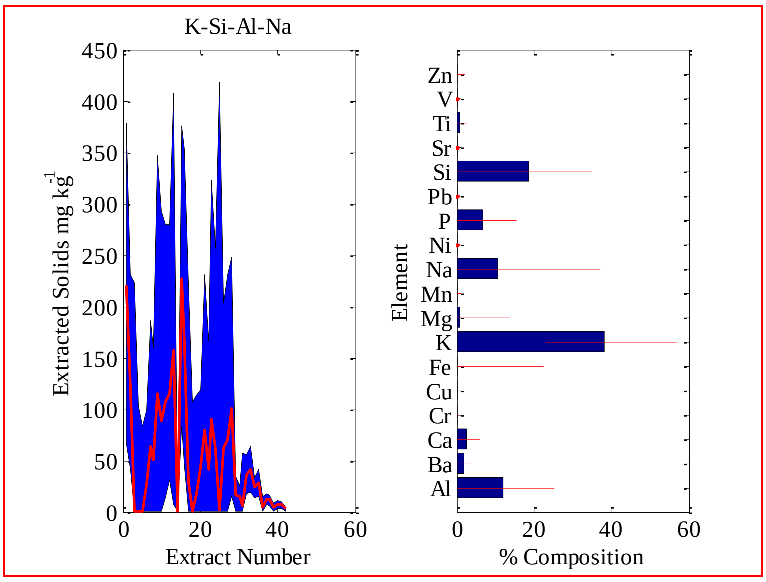


Figure 7.17 Extractogram of K-Si-Al-Na component extracted in CRM 701 using Method 2

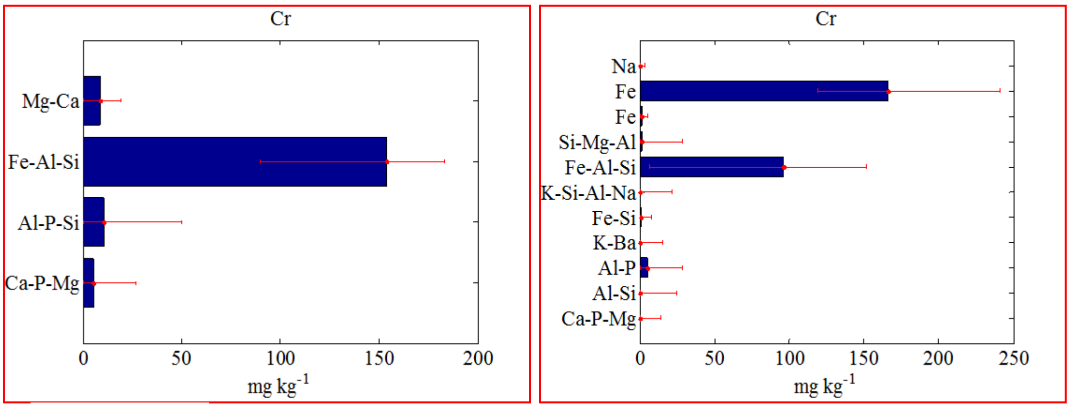
More components were identified in Method 2 (11 components) when compared with Method 1 (4 components), this is likely due to the different ways in which the data were processed. In Method 1 the concentrations of the two replicates were corrected with the blank and their mean results were used, while in Method 2, the blank values and the two replicates were processed together. This difference in the data processing gave rise to two components (K-Ba and Na) that are believed to come from the filter in the second method. Three other components identified in Method 2 (Al-Si, Fe-Si and K-Si-Al-Na), but not found in Method 1, have high uncertainties in the measurement of the compositions of these components. The mineralogy studies of CRM 701 by using methods such as X-ray diffraction methods would help in confirming the various components of the sample. Therefore there is the need to carry out these studies to help support the tentative assignment given by the CISED method.

7.5.2 Distribution of PTE in various identified components

In this section only the PTEs discussed in the previous chapters (Cr, Cu, Fe, Mn, Ni, Pb and Zn) will be considered and their distributions in the identified components discussed in details. Figures 7.18 to 7.24 show the distribution of the PTE in various identified components from Methods 1 and 2, these histograms give the amount (mg/kg) of the PTE is each component. Because of way in which the data in Method 2 was processed, the amount from this method contains a contribution from replicates 1 and 2, and so the overall amount of the PTE reported in Method 2 will appear to be approximately twice the amount from Method 1.

7.5.2.1 Chromium

Chromium was found predominantly in the iron alumino-silicate (*ca.* 154 mg/kg) component from Method 1 and in iron oxide (*ca.* 166 mg/kg) and iron alumino-silicate (*ca.* 96 mg/kg) from Method 2. The association of Cr with aluminum and iron oxides is known.[86, 320] The closeness of the ionic radii of $Cr^{3+,}$ Al^{3+} and Fe^{3+} make their geochemical properties similar. Chromium substitutes for Fe and Al in chromite which is the primary geological source of Cr. These two components in which Cr was found were extracted at a high acid strength; therefore the presence of Cr in these components indicates that it is in immobile forms. There is agreement in terms of mobility between the present finding and the results of certification of BCR 701, [160] in which Cr was found predominantly in the step 3 and the amount in step 3 (143 mg/kg) was close to what was found in these components from the two methods. The amount of Cr extracted in Method 1 (*ca.* 154 mg/kg) was slightly higher than the Cr found in similar component in Method 2 (*ca.* 96 mg/kg), this may be due to the additional component (crystalline iron oxide) identified in Method 2 which contains approximately 166 mg/kg of Cr.



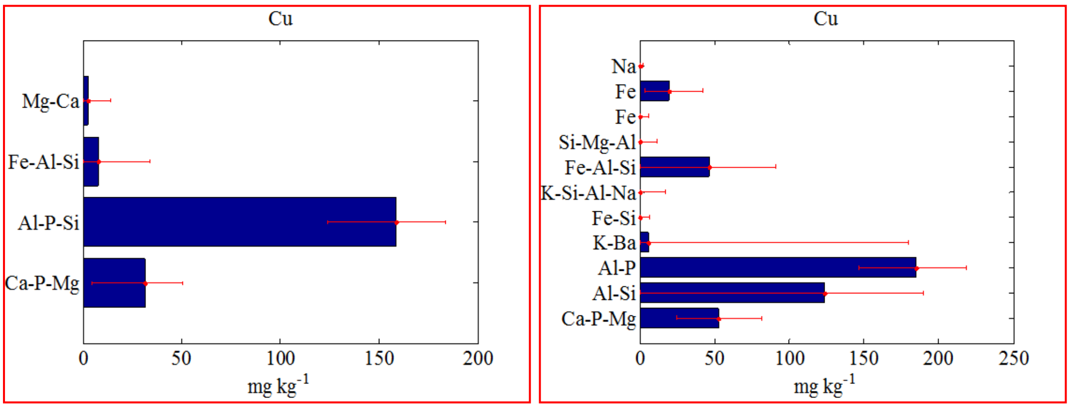
Method 1

Method 2

Figure 7.18 Distribution of Cr in the identified components from Methods 1 and 2

7.5.2.2 Copper

In both methods, copper was associated with the aluminum oxide component and to a lesser extent with the carbonate component, this may reflect the affinity of Al- and Fe-hydroxides, carbonate and phosphate to bind to soil Cu[321]. Copper also has a high affinity for sulfur and hence its main minerals are chalpyrite, $CuFeS_2$; bornite, Cu_5FeS_4; chalcocite, Cu_2S; and covellite, CuS. Unfortunately, due to some analytical problems encountered during the analysis of the extracts, sulfur was not determined in this work. Hence no component containing sulfur was identified and potential associations with this type of component cannot be discussed. The aluminum oxide component was extracted over the acid range strength 0.01- 0.5 M. The copper in this component was in a moderately in mobile form. The BCR sequential extraction of BCR 701[160] shows that Cu is predominantly in the step 2 (124 mg/kg) which is also a relatively mobile phase.

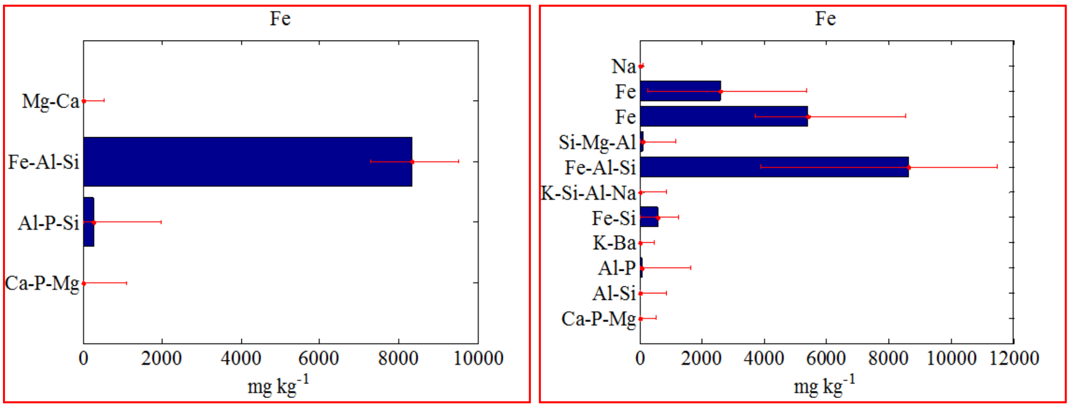


Method 1 **Method 2**

Figure 7.19 Distribution of Cu in the identified components from Methods 1 and 2

7.5.2.3 Iron

Iron was predominantly found in iron aluminum-silicate component in Method 1 (*ca.* 8330 mg/kg) and found in highest concentration in the same component in Method 2 (*ca.* 8622 mg/kg). In Method 2, iron was additionally found in two crystalline iron oxide components which were not identified in Method 1. Iron is generally lithogenic and normally found associated with the silicate matrix and this makes it difficult to be release in to the environment. The iron aluminum-silicate and the crystalline iron oxide components were extracted at higher acid concentrations, so the iron found in these components will be relatively immobile. Using the BCR sequential extraction method, Kubova *et al*[322] obtained a similar result when they found iron predominantly in the residual fraction in BCR 701.

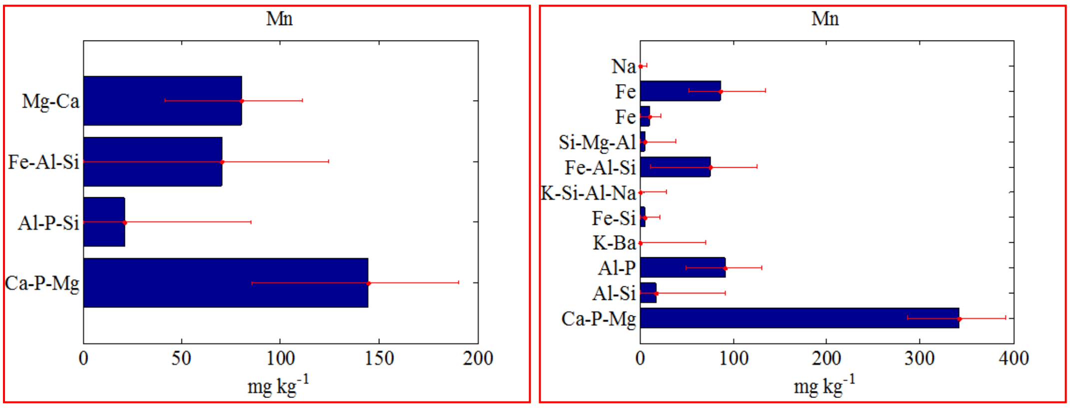


Method 1 **Method 2**

Figure 7.20 Distribution of Fe in the identified components from Methods 1 and 2

7.5.2.4 Manganese

Manganese was found in both methods predominantly in the carbonate component, Ca-P-Mg, and was also found in the “noisy” carbonate component, Mg-Ca in Method 1. To a lesser extent it was found in both methods in the iron alumino-silicate component. The occurrence of Mn in these components may be explained from the fact that geochemically Mn is known to associate with ferromagnesian silicate minerals such as biotite and chlorite; due to similarities of the ionic radii, Mn^{2+} normally substitutes for Fe^{2+} or Mg^{2+} in these minerals. The presence of Mn in these components indicates that it was present in both mobile and relatively immobile forms as the carbonate was extracted by 0.01 M *aqua regia* while the iron-alumino-silicate was extracted by higher acid strength. The sequential extraction of the BCR 701[322] shows a similar mobility trend; Mn was found in both mobile phases, step 1 (170 mg/kg) and relatively immobile phase, step 4 (299 mg/kg).

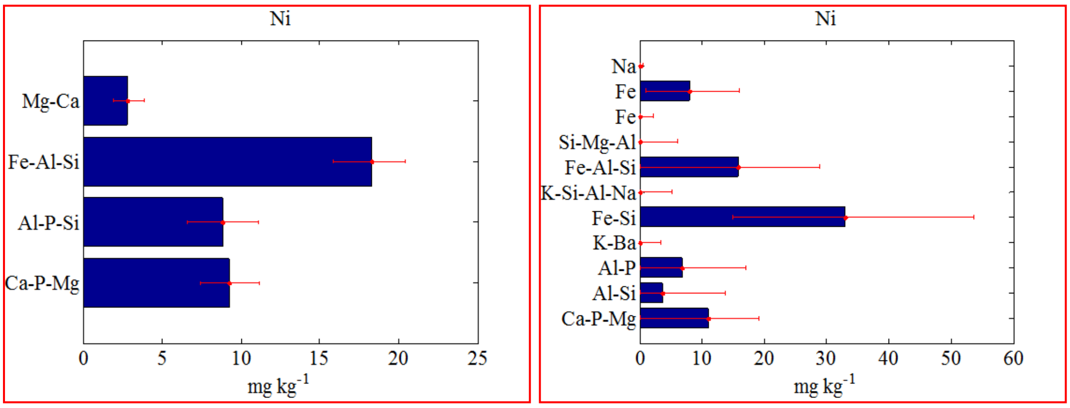


Method 1 **Method 2**

Figure 7.21 Distribution of Mn in the identified components from Methods 1 and 2

7.5.2.5 Nickel

Nickel was predominantly in iron alumino-silicate component in both methods. Nearly the same amount of nickel (*ca.* 18 and 16 mg/kg) was found in this component in Method 1 and 2 respectively, although in Method 2 an additional 33 mg/kg was extracted in the “noisy” iron alumino-silicate component (Fe-Si). Geochemically nickel is known to be siderophilic and has great affinity for sulfur which leads to the formation of many different sulfides, arsenides and antimonides in nature, e.g. millerite (NiS), niccolite (NiAs) and breithauptile (NiSb). It can also be associated with carbonates, phosphates and silicates[237] As mention in section 7.5.1.2, sulfur was not measured so it is not possible to assess any association of Ni with any component containing sulfur. The presence of Ni in the iron alumino-silicate component reveals that it is in an immobile form. Its presence to a lesser extent in the carbonate component in both methods reveals that a small amount it is also in a mobile form. This is consistent with information obtained from the results of the certification of BCR 701[160] in which nickel was found predominantly in step 4 (41.4 mg/kg) which an immobile phase and in step 2 (26.6 mg/kg) which is a relatively mobile phase.

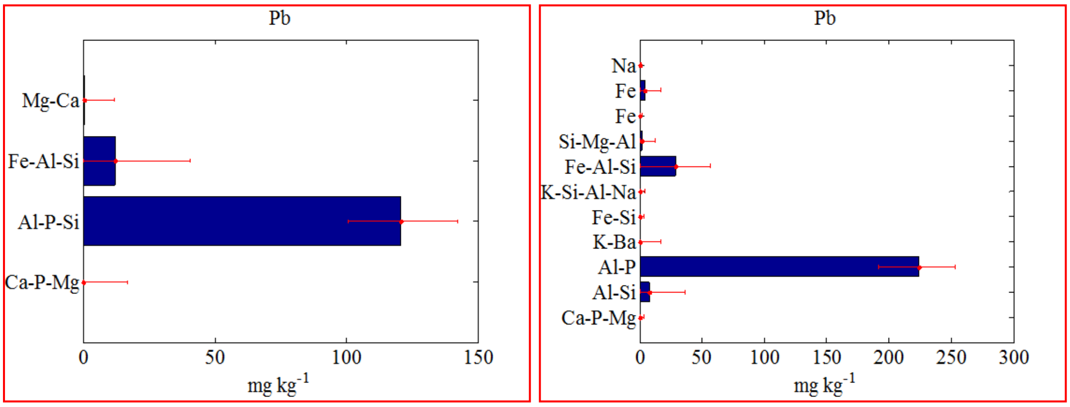


Method 1 **Method 2**

Figure 7.22 Distribution of Ni in the identified components from Methods 1 and 2

7.5.2.6 Lead

Lead was predominantly associated with the aluminum oxide component in both methods which is a relatively mobile phase. In the sequential extraction for the certification of BCR 701[160], Pb was predominantly found in the step 2 (126 mg/kg) and this is also a relatively mobile fraction which can release the Pb in a reducing condition. The amounts of the Pb in the aluminum oxide component (120 mg/kg) in Method 1 and (230 mg/kg) in Method 2 are comparable to the amount extracted in the step 2 of the BCR sequential extraction method. Remembering that the amounts of PTE extracted in Method 2 were approximately twice the amounts extracted in Method 1.

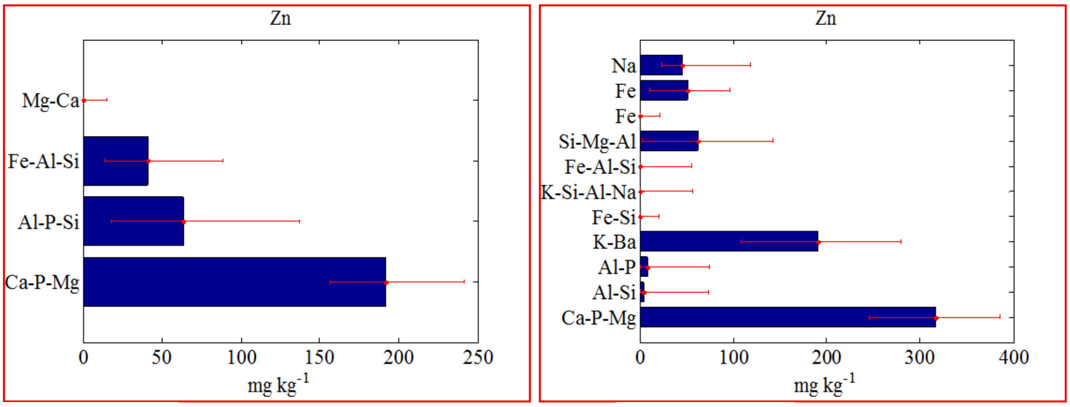


Method 1 **Method 2**

Figure 7.23 Distribution of Pb in the identified components from Methods 1 and 2

7.5.2.7 Zinc

In both methods, zinc was found in the most mobile component, the carbonate. The same pattern was found in the certification of BCR 701 using sequential extraction in which about 70 % of zinc was found the most mobile fractions (step 1 and 2). In method 2, Zn was also found in the K-Ba component which was identified as a filter component. The association of Zn with this component may reflect the high Zn blank concentration noted in chapter 6. The semi quantitative test of the blank FDMS filter (appendix C) indicated relatively high zinc content in the filter.



Method 1 **Method 2**

Figure 7.24 Distribution of Zn in the identified components from Methods 1 and 2

7.6 Conclusions

A miniaturised form of the CISED extraction protocol (Method 1) was developed and used. The data analysis revealed four components; iron alumino-silicate, aluminum oxide and 2 carbonate components one of which has high uncertainty. In Method 2, the miniaturised protocol was used on the test sample placed on an FDMS filter and extracted from the filter in a polycarbonate in-line filter holder. The data analysis revealed 11 components; a carbonate, 2 iron alumino-silicate (one of which was “noisy”), aluminum oxide, 2 crystalline iron oxide, a “noisy” aluminum silicate, 2 filter components and 2 clay components (one of which has a high uncertainty). The differences in the number of components is likely due firstly to the way the data were treated in the two methods and secondly the contribution of the FDMS filter, which is known to have high blank values for some elements, e.g. Na, K, Ba and Zn. The results of the data analysis show similarities between the two methods. All the components identified in Methods 1 were found in Method 2, and the additional components in Method 2 were either noisy (high uncertainties) or components that likely represent contribution from the filter. The components firmly identified reflect the geology of the drainage basin.

The distribution patterns of all the PTE in various components in both methods show a close agreement. In both methods Cr, Fe, and Ni are predominantly in the iron alumino-silicate which is an immobile phase. These results agree with those obtained from the BCR sequential extraction of the same test sample, in which is Cr, Fe and Ni are predominantly in steps 3 or 4. Copper was found in an aluminum oxide component in both methods, while Mn and Zn were associated with the most mobile component (carbonate). This also agrees with the finding of the sequential extraction method in which they were found mostly in step 1 of the BCR sequential extraction.

These findings indicate that, it is possible to use the CISED protocol to determine the components present in samples collected in small amounts such as airborne particulates collected on filters. However there is the need to carry out mineralogy studies using method such as X-ray diffraction and Scanning electron microscopy to give further backing to the tentative assignment of the components identified in samples. It has been shown that it is possible to use the method developed to predict the mobility of PTE in particulate matter collected on FDMS filters and, thereby, the possible environmental impacts of the PTE to the environment. The method was faster than sequential extraction method and could be carried out in about five hours rather than 4 days (excluding analysis time). The matrix (*aqua regia*) used in the developed method is simpler and easier to analyse than some of the matrix used in the sequential extraction, e.g. 1 M ammonium acetate used in step 3 of the BCR.

8 Conclusions and further work

In the first part of this work (chapter 4), four different extraction methods were used for determination of Cr, Cu, Fe, Mn Ni, Pb and Zn in 22 samples collected from urban allotments. The pseudototal concentrations of the Cu, Ni, Pb and Zn as determined by *aqua regia* digestion were found to be higher than the typical values obtained for Scottish and UK soils, while Cr concentrations were lower than these values. The levels of Cu, Pb and Zn exceeded some reference soil guideline values which indicate that these soils were contaminated with these elements, confirming earlier studies. The concentrations of Cr and Ni were both lower than the UK CLEA soil guideline values. Both Fe and Mn were uniformly distributed across the plots-whereas other analytes were not-which indicated that they have originated mainly from the parent rocks rather than from anthropogenic activities.

The fractionation studies of these soils using the modified BCR sequential extraction procedure showed that Cr, Fe and Ni were mainly found in the residual fraction and relatively immobile. Therefore they are unlikely to be released from the soil or pose an environmental risk. However the presence of Pb in large amounts in the reducible fraction (942 mg/kg) was of environmental concern, as it could easily be mobilised in reducing conditions such as water logging thereby becoming more bioavailable. Copper was found mainly in the oxidisable and residual fractions, Mn in reducible and residual fractions while Zn was found in all the 4 fractions.

When the phytoavailable concentrations, as determined by the EDTA extraction of the soils, were compared with some known phytotoxic concentrations, Cu, Pb and Zn were found to be likely phytotoxic to some plants, while Cr, Fe, Mn and Ni were likely not to be phytotoxic. In terms of human bioaccesibility, only Pb was found to pose danger if soil were ingested orally by a 20 kg child, because only 160 mg would be needed to reach the tolerable daily intake limit.

Correlations were found between the concentration of PTE in different operationally defined fractions of the BCR sequential extraction procedure and in the other extracts. The correlations between the PTE differ from one step to another and also between the extraction methods, but in general, in most of the steps and the extraction methods, Mn concentration was found to correlate with Fe concentration.

This was an indication that they may have originated from a common source, most likely dominantly geogenic in nature. The “urban metals” Cu, Pb and Zn were found to be correlating with one another in most cases, this further indicated that these analytes in this soil were likely to come mainly from anthropogenic activities.

When PCA was applied to the whole data set, the varimax loading plot showed that PC1 was mainly associated with Cr, Fe and Ni, which are mainly lithogenic in nature and had little association with Cu, Pb and Zn. The varimax loading plot for PC2 showed that PC2 was more associated with the variables that PC1 was not associated with, that is, it was strongly associated with Pb and Zn, and had some associated also with Cu and Mn but had no association with Cr and Ni. The varimax scores for PC1 showed a close association between the results of the EDTA extraction method and the exchangeable fraction of the BCR sequential extraction method. The varimax scores for PC2 showed a close association between the EDTA and SBET methods. Suggesting that, where EDTA extractable PTE concentrations are already known, this may also provide an indication of human bioaccessiblity.

In the future, there is need to sample again from these allotments as the samples used in this work were collected about seven years ago and new plots have been created from the previous 22 plots. In addition to using the extraction methods used in this work, it is suggested that other bioaccessibility methods (PBET and UBM) and single extraction methods used in assessing the plant-available elements such as 0.01 M $CaCl_2$, 0.1 M $Ca(NO_3)_2$, 0.005 M DTPA, and 0.01 M HNO_3 should be used with view of finding any possible correlations between the methods. The non-specific reagent extraction method, CISED can also be used to determine the PTE distributions in these soils in order to further understand their mobility and availability in the environment and also to identify the possible geochemical components of the soils. Besides the PTE studied in this thesis additional PTE such as, As, Cd, Se etc. should be added in future studies. These PTE are commonly added to soils through the use of fertilisers and pesticides. There is need to sample the vegetables grown on these plots and determine the levels of these PTE in them and find if there are any correlations between the amounts in the soils and in the plants.

In the next part of this work, the modified BCR sequential extraction was miniaturised from the usual 1 g sample mass to 0.125 g. It was found that, in general, changing the sample masses (1, 0.5, 0.25, 0.125 and 0.0625 g) had no effect on the distribution patterns of all the PTE among the various steps irrespective of the particle size (500 – 1000 and < 45 µm). This was with the exception of Cu, where there was slight change in the distribution pattern with the reducible fraction > residual fraction in < 45 µm *cf.* residual fraction > reducible fraction. The recoveries both in terms of the sum of the PTE extracted in steps 1 to 4, relative to the *aqua regia* extractable content in the original sample, and those obtained by comparing the pseudototal concentration and the concentrations of PTE in various fractions of the 1g sample mass with the indicative values from previous studies for all the sample masses and particle sizes, were found to be generally good. However the recoveries in some cases, especially steps 4, were poor and this highlighted the difficulties in carrying out this particular step for small samples.

An ANOVA test indicated that, in general, there were no significant differences in the mean concentrations of the PTE released when the sample masses were varied using the same centrifuge tubes. Significant differences in the mean concentrations of the PTE (except Cu) extracted in various steps were observed when 0.25 g sample masses were used in different centrifuge tube sizes. This showed the influence of changing the size of the centrifuge tubes, and highlights the operational nature of the sequential extraction. In general more PTE were extracted from the sample when smaller vessels were used, and this was may be due to closer interaction between the samples and the extracting reagents. Also the amounts of the PTE extracted for all sample masses and in all the steps from the finer fraction (> 45 µm) were always generally higher than from the coarse fraction (500 -1000 µm), which were likely due to the influence of the particle surface area. The overall uncertainties on all the four steps of the BCR results in the various sample masses were generally small. It was thought that as the sample masses become smaller, the uncertainties might become larger but this was not observed. The likely reason may be that the minimum weight of the soil that will be a representative of the whole soil sample had not been reached.

The levels of the PTE in blank FDMS filters were generally very low with the exception of Zn which is thought to be used as binding material in the filters. Statistically it was showed that the concentrations of the PTE in the different blank filters within the same package were similar, while significant differences were found between the mean values of the individual PTE in filters from two different packs. The pseudototal concentrations of PTE in PM collected from the same air sampling station (Glasgow Abercromy Street) exposed at different times were found to be similar. Generally the amount of PTE in the atmospheric PM from the Glasgow filters studies were much lower than the results from some other urban areas around the world and also lower than the WHO air quality guideline values for Europe. The results of the pseudototal concentrations of the PTE in different segments cut from exposed FDMS filters showed that the PM might not to be uniformly distributed. Therefore care must be taken when assuming a segment cut from a filter will be representative of the whole filter.

In the sequential extractions of BCR CRM 701-loaded FDMS filters using the miniaturised BRC procedure, it was found that, for steps 1 and 2, all the PTE in the blank filters were below the detection limits while in steps 3 and 4 low concentrations were detected in the blank filters. For these simulated loaded filters, percentage recoveries were obtained by comparing the amount of the PTE extracted from the filters and the certified values for individual steps, these were found to be generally within ± 30 % with the exception of Zn in steps 3 and Cr in step 4.

A non-specific reagent sequential extraction method utilising chemometric data processing, CISED was miniaturised and applied to samples of small mass on FDMS filters. The results of the miniaturised traditional CISED (Method 1) indicated 4 distinct components, while the modified CISED for material supported on the FDMS filters (Method 2) showed 11 distinct components. The differences in number of components between the two methods were due to the different ways in which the data from the two methods were treated and also the contributions from the filter. Similarities were found between the two methods, all the components identified in Method 1 were found in Method 2 with additional components from Method 2 which were either “noisy” (high uncertainties) or contributions from the filters. The components firmly identified reflect the geology of the drainage basin from where samples used to prepare CRM 701 were collected.

The distribution patterns of all the PTE in various components in both methods showed a close agreement. The mobility of the PTE as predicted by the CISED were in good agreements with fractionation patterns obtained using the modified BCR sequential extraction protocol. The distribution patterns in both CIESD methods suggested that Cr, Fe and Ni were predominantly in the iron aluminum silicate component. This component is an immobile phase and agreed with the results of the BCR sequential extraction methods which showed that Cr, Fe and Ni were mostly found in steps 3 and 4.

One of the major challenges in the development any analytical method involving PM collected on filters is lack of commercially available urban PM CRM on filters, like the BCR CRM 128 “flyash on artificial filters”. In chapters 6 and 7 of this thesis, simulated filters were used to perform miniaturised BCR and CISED extraction respectively. These filters were prepared by smearing CRM 701 on blank filters and this was done manually. The consequences of this were lack of uniformity in the distribution of the CRM on the filters and also the CRM hardly stick to the filters. There is the need to improve on the preparation of these filters. This can be done by adapting the method developed by Wang *et al*[323] in preparing simulated filters using sampling chamber. There is also the need to carry out mineralogy studies on the sample of the BCR 701 using methods such as X-ray diffraction and Scanning electron microscopy to give further backing to the tentative assignment of the components identified in samples when the miniaturised CISED was used.

These methods developed need to be applied to real samples to test the effectiveness of the method. They could also be tested, on BCR CRM 128 “fly ash on artificial filters”. This CRM is physically similar to urban PM (small sample mass and particle size) but has a considerably different chemical composition for many elements. The CRM also has no certified values for the BCR sequential extraction but a mass balance method could be used to check the effectiveness of the extraction.

The miniaturised BCR developed for the PM gives information on the mobility and availability of the PTE in the PM but information on the bioaccessibility cannot be obtained from this method. Miniaturisation of one of the methods used to assess human bioaccessibility (SBET, PBET or UBM) can be investigated with the view of applying it to PM collected on FDMS. The method can be developed using a CRM which has a closer matrix, e.g. NIST SRM 1648, which is an urban PM.

There the need to apply the miniaturised BCR or CISED method to other samples of small mass collected on filters eg aquatic suspended particles.

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Appendix A

Table 1 mean concentration of Cr in sequential extracts and pseudototal content (mg/kg) of the allotment samples

	STEP 1		STEP2		STEP3		STEP4		PSEUDOTOTAL	
SAMPLE	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD
B1	0.473	47.9	7.03	1.69	11.3	9.46	28.2	49.4	41.9	3.47
B2	0.308	65.6	6.33	1.17	9.38	1.45	27.7	27.2	36.6	2.12
B4	0.267	258	7.40	8.09	10.0	6.02	26.6	7.61	49.0	10.3
B5	0.100	87.6	2.45	27.2	8.94	5.37	22.5	0.56	36.6	4.83
B6	0.000	-94.2	1.07	5.47	3.96	1.24	15.6	5.92	34.8	9.90
B7	0.000	-51.1	0.71	11.3	2.98	11.2	19.0	20.8	31.9	2.86
B8	0.000	-51.7	1.20	3.28	4.12	8.15	16.7	4.93	35.8	7.10
B9	0.147	143	1.31	5.03	5.32	2.59	25.8	15.1	40.3	4.69
B10	0.034	2.4	1.36	7.00	5.12	3.66	25.5	9.56	39.0	10.6
B11	0.082	54.9	2.81	15.6	4.40	2.00	18.4	9.53	29.0	7.53
B12	0.046	17.2	2.82	8.44	5.04	3.97	16.5	3.89	31.4	5.30
B13	0.009	390	2.20	1.75	4.29	4.80	17.2	2.65	26.8	12.0
B14	0.196	114	2.43	2.86	4.34	6.05	18.9	12.7	27.3	7.44
B15	0.075	86.3	0.84	198	5.06	0.68	16.1	10.5	28.7	11.3
B16	0.092	18.3	0.96	3.12	4.73	2.15	18.2	6.90	27.9	7.34
B17	0.076	20.9	1.38	3.72	6.00	1.31	18.8	7.33	31.7	5.92
B18	0.052	62.4	0.92	16.6	4.65	2.34	18.4	7.08	26.3	2.44
B19	0.068	98.0	1.28	6.47	5.34	10.1	18.6	0.33	33.6	11.8
B20	0.150	17.4	2.83	12.4	11.3	4.35	19.0	1.95	40.4	6.21
B21	0.106	30.9	3.14	4.20	6.46	1.55	17.6	7.19	28.5	12.8
B22	0.138	3.2	5.06	9.75	9.27	9.88	34.9	7.57	49.4	0.54

Table 2 mean concentration of Cu in sequential extracts and pseudototal content (mg/kg) of the allotment samples

	STEP 1		STEP2		STEP3		STEP4		PSEUDOTOTAL	
SAMPLE	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD
B1	1.66	2.50	24.3	1.74	59.3	9.97	51.0	113	147	0.67
B2	1.58	4.43	24.0	4.03	68.8	23.7	109	16.0	142	3.66
B4	2.76	11.1	43.1	9.11	101	2.20	57.3	6.33	212	2.44
B5	1.35	6.01	11.4	24.7	74.5	5.25	39.8	8.67	142	3.25
B6	3.27	0.86	32.4	1.53	51.8	5.97	56.1	4.88	157	2.96
B7	1.46	3.46	30.1	0.32	53.4	0.77	57.7	8.84	164	2.24
B8	1.37	2.33	28.6	1.06	59.7	1.11	51.4	2.37	161	3.77
B9	1.75	7.24	28.3	2.14	59.6	0.41	68.1	1.49	194	3.22
B10	2.51	2.03	47.9	1.57	68.5	2.51	63.5	2.45	221	3.67
B11	1.62	5.53	34.7	1.47	63.3	4.26	68.1	2.02	164	3.92
B12	2.66	10.2	44.3	1.32	74.4	2.76	59.9	4.09	204	3.46
B13	2.65	5.27	39.4	1.43	62.9	4.43	71.3	6.76	165	5.56
B14	2.60	5.69	45.3	3.22	66.9	4.24	60.0	4.46	174	1.81
B15	4.04	1.95	57.5		63.8	3.50	55.7	2.00	191	9.38
B16	3.60	2.56	60.5	2.03	79.4	14.4	76.4	1.15	227	0.06
B17	3.84	11.1	69.0	1.77	78.3	2.15	80.5	6.01	237	1.57
B18	3.37	0.91	60.8	3.75	73.0	2.11	80.8	1.02	222	0.52
B19	3.25	2.03	62.1	0.85	74.3	4.94	65.0	3.12	225	5.38
B20	4.27	6.74	62.5	5.17	86.0	8.50	74.4	4.26	248	2.46
B21	5.57	4.21	97.8	1.45	90.9	3.79	80.4	7.18	247	11.2
B22	5.42	3.13	75.0	6.58	121	13.0	72.4	4.28	226	0.30
URM 1	7.78	1.60	11.43	13.32	33.03	8.40	31.84	1.03	116.40	0.38

Table 3 mean concentration of Fe in sequential extracts and pseudototal content (mg/kg) of the allotment samples

	STEP 1		STEP2		STEP3		STEP4		PSEUDOTOTAL	
SAMPLE	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD
B1	5.46	1.25	3894	4.79	1340	14.8	24183	18.8	43012	1.86
B2	5.88	6.88	3184	3.97	1293	4.92	28106	9.10	37888	0.64
B4	4.74	15.2	3409	25.6	1672	10.2	34401	4.41	41998	0.40
B5	6.31	13.3	1443	31.3	1872	2.49	29664	7.19	36192	2.52
B6	8.30	2.24	3746	1.94	459	6.21	33702	7.42	38451	1.89
B7	6.44	2.16	3566	1.12	507	1.74	34025	2.16	40384	0.97
B8	6.36	4.85	3532	0.47	524	1.11	30362	1.77	38153	3.88
B9	5.57	4.27	3558	1.33	521	1.77	40694	2.18	46564	1.54
B10	4.62	1.58	3891	0.55	521	3.30	34889	1.60	44826	3.03
B11	3.74	5.92	4362	3.16	749	8.28	38100	3.63	41910	6.89
B12	3.05	15.4	4209	3.24	732	1.20	33850	2.63	40282	4.37
B13	2.56	5.76	4261	1.90	559	8.20	44355	7.58	45131	2.83
B14	3.55	7.66	4273	3.98	644	5.48	34951	4.18	39869	1.03
B15	4.31	3.31	1448	174	552	7.74	32641	1.19	38465	2.46
B16	4.54	2.29	4158	4.74	588	13.2	35507	1.70	39785	1.15
B17	3.67	9.07	4160	1.61	588	10.7	35644	1.77	41459	7.93
B18	3.08	3.28	4095	5.54	625	7.02	35913	4.37	40086	6.03
B19	3.94	1.35	4049	5.46	586	4.99	32946	1.68	39487	3.62
B20	4.21	1.16	4362	0.51	613	9.09	33470	3.30	40253	0.84
B21	4.30	2.64	5195	0.82	512	6.72	35157	2.65	34570	5.65
B22	4.96	4.74	5204	3.61	1688	6.34	48685	4.96	48767	1.04

Table 4 mean concentration of Mn in sequential extracts and pseudototal content (mg/kg) of the allotment samples

	STEP 1		STEP2		STEP3		STEP4		PSEUDOTOTAL	
SAMPLE	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD
B1	97.3	2.55	332	2.27	24.6	9.82	212	23.9	874	4.08
B2	70.4	1.69	278	5.53	22.4	4.27	231	7.29	778	3.45
B4	70.5	4.72	276	29.2	27.6	8.19	259	5.05	769	3.37
B5	85.3	2.45	117	34.7	27.4	3.77	235	11.2	713	0.92
B6	87.9	2.09	328	2.64	18.1	4.40	271	7.07	718	4.68
B7	66.7	2.95	287	4.32	19.3	4.02	271	2.82	679	1.77
B8	74.7	1.50	317	6.52	18.3	2.53	258	1.18	718	10.1
B9	87.7	2.30	367	2.62	20.3	4.97	358	3.51	867	7.32
B10	59.0	3.89	328	2.20	19.9	3.97	332	2.48	743	3.45
B11	69.2	3.55	316	6.29	21.8	10.4	345	2.65	716	1.86
B12	54.9	5.32	314	0.92	19.9	5.99	296	4.57	735	3.48
B13	73.3	3.31	425	2.97	23.7	3.04	356	3.69	845	5.77
B14	53.3	2.27	303	2.69	18.5	2.40	320	5.56	678	2.24
B15	63.8	2.79	95	174	17.5	2.79	272	4.84	640	2.87
B16	70.2	3.32	264	5.35	20.6	1.87	296	2.18	653	1.63
B17	71.9	3.21	289	1.54	20.8	4.34	281	3.76	683	2.83
B18	61.0	2.12	285	4.38	20.8	4.71	290	5.37	654	1.68
B19	61.3	1.30	291	0.99	20.1	1.65	271	3.36	689	6.61
B20	74.6	1.84	297	3.11	22.3	4.88	268	2.45	682	1.73
B21	95.0	8.40	308	2.87	20.9	3.48	275	9.73	556	6.67
B22	98.1	6.36	263	2.78	26.3	5.87	350	5.69	724	0.78

Table 5 mean concentration of Ni in sequential extracts and pseudototal content (mg/kg) of the allotment samples

	STEP 1		STEP2		STEP3		STEP4		PSEUDOTOTAL	
SAMPLE	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD
B1	3.28	0.65	6.61	6.29	7.92	10.6	44.5	25.8	66.3	5.85
B2	3.17	4.33	5.37	5.02	8.02	0.36	51.5	5.32	62.8	0.15
B4	4.06	8.25	5.47	8.65	9.69	7.98	57.1	7.74	79.2	5.23
B5	3.51	2.12	2.56	82.4	9.32	2.04	52.7	14.1	66.2	12.7
B6	2.62	4.56	6.24	2.59	2.90	10.3	43.3	1.70	63.5	7.26
B7	3.03	4.50	7.94	3.76	4.25	6.42	52.0	10.5	72.2	2.32
B8	2.11	8.21	8.89	3.09	4.56	7.63	48.2	0.90	71.6	3.40
B9	2.36	7.42	17.8	2.62	5.38	3.87	51.2	10.6	77.5	1.17
B10	2.81	1.64	13.2	1.93	5.91	1.72	60.2	4.67	85.8	8.46
B11	2.91	2.59	18.9	1.31	4.10	7.85	56.7	9.65	79.3	7.43
B12	4.07	4.63	15.2	4.45	3.79	5.14	47.3	5.75	79.6	5.34
B13	3.13	2.17	23.8	5.07	3.77	3.21	44.1	4.40	68.0	8.91
B14	3.85	4.82	20.1	2.11	3.80	4.42	54.4	9.20	74.7	6.42
B15	3.52	2.41	4.03	271	3.20	4.40	44.9	9.13	67.7	11.0
B16	2.88	3.24	9.70	3.47	4.30	6.16	48.7	0.01	67.9	3.17
B17	3.19	9.10	11.0	1.56	5.47	1.10	52.6	5.22	71.2	3.15
B18	3.42	14.4	12.3	10.0	5.37	4.17	57.9	3.54	71.1	2.55
B19	3.49	1.89	11.6	1.72	5.24	5.29	55.6	0.92	79.6	8.78
B20	25.5	3.89	31.1	3.41	10.3	3.78	53.7	5.86	128	3.11
B21	3.35	6.55	14.8	2.79	5.06	8.31	49.0	1.30	62.9	14.9
B22	2.64	2.71	8.06	12.8	6.99	4.04	68.4	7.20	83.8	1.29

Table 6 mean concentration of Pb in sequential extracts and pseudototal content (mg/kg) of the allotment samples

	STEP 1		STEP2		STEP3		STEP4		PSEUDOTOTAL	
SAMPLE	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD
B1	10.9	2.91	788	4.26	117	7.28	171	66.5	1324	19.8
B2	9.20	4.61	689	1.37	101	2.31	80.1	1.76	1049	6.61
B4	15.5	11.4	1601	9.66	205	11.2	177	4.36	1713	7.80
B5	10.9	8.06	890	14.2	171	5.34	120	6.86	1371	10.6
B6	11.1	4.15	935	0.44	41.5	1.57	251	3.30	1264	5.09
B7	4.27	14.9	662	6.62	36.3	5.91	242	7.61	1058	8.65
B8	5.97	3.27	793	4.11	50.3	14.1	246	2.62	1119	3.90
B9	11.8	8.80	861	8.01	53.6	11.2	307	7.26	1219	4.20
B10	11.5	5.73	959	0.69	54.7	9.06	265	4.59	1374	4.72
B11	5.48	8.51	610	2.17	71.2	1.09	128	6.00	825	1.70
B12	7.17	10.5	736	0.89	80.8	3.65	134	4.18	1057	3.15
B13	10.7	5.07	708	6.67	79.0	8.11	138	4.33	935	5.33
B14	6.35	1.65	706	3.06	77.5	11.2	126	13.4	915	2.91
B15	10.6	2.80	289	173	69.4	6.25	114	3.41	1063	3.47
B16	12.6	6.04	906	2.06	73.9	13.3	142	13.2	1236	3.45
B17	21.9	12.7	1210	2.67	118	2.11	190	7.06	1579	3.71
B18	14.7	1.35	981	1.69	98.8	3.08	163	4.64	1262	1.39
B19	16.1	1.68	1124	8.59	103	2.32	162	3.54	1412	7.11
B20	14.2	8.36	1124	7.97	114	8.14	182	7.73	1407	6.04
B21	25.3	10.9	1453	1.35	113	5.97	195	5.89	1530	13.2
B22	35.3	6.62	1763	3.34	215	6.31	207	5.24	2164	4.53

Table 7 mean concentration of Zn in sequential extracts and pseudototal content (mg/kg) of the allotment samples

	STEP 1		STEP2		STEP3		STEP4		PSEUDOTOTAL	
SAMPLE	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD
B1	192	1.97	181	3.55	37.7	31.2	202	23.3	815	1.77
B2	189	0.73	162	2.03	38.2	2.85	186	12.0	774	3.59
B4	309	1.20	257	11.0	87.8	30.8	251	3.92	1225	4.20
B5	270	2.24	183	8.32	162	89.8	226	17.8	1035	3.74
B6	121	1.51	128	1.75	27.6	6.85	147	2.22	455	5.45
B7	185	1.52	184	2.96	40.5	6.86	166	4.43	628	2.03
B8	163	0.65	198	1.03	49.1	5.10	192	5.47	657	1.31
B9	221	3.02	247	2.55	82.9	9.68	302	5.67	872	3.69
B10	163	1.20	186	0.52	55.5	1.99	215	1.85	695	4.81
B11	148	0.95	184	3.54	56.9	2.47	236	3.08	650	2.31
B12	156	6.00	168	1.51	42.3	2.73	178	7.71	613	3.06
B13	189	1.37	199	2.74	65.2	1.69	247	5.12	725	3.83
B14	146	1.69	159	2.57	42.5	0.25	172	4.80	533	1.93
B15	182	2.65	61.3	174	49.9	2.41	177	1.71	621	4.66
B16	162	1.78	166	5.39	57.9	4.03	202	0.82	644	3.05
B17	224	3.75	231	4.40	93.7	2.05	278	3.36	886	2.50
B18	168	2.06	189	3.43	69.1	2.24	223	2.87	671	1.11
B19	207	0.57	210	2.27	66.1	2.53	222	5.27	758	5.86
B20	287	2.02	260	5.23	83.7	3.82	227	6.73	905	2.89
B21	279	4.97	298	5.66	94.5	4.77	252	4.78	856	9.97
B22	271	1.78	292	3.26	115	5.45	342	0.11	962	0.90

Appendix B

Table 1 mean concentrations of the PTE in the sequential extracts and % recoveries (Σ steps 1,2,3,4, vs pseudototal contents) for sample masses 1 - 0.25 g and particle sizes 500 – 1000 and < 45 µm.

	step 1		step 2		step3		step 4		pseudototal		Σ steps 1,2,3,4		recovery %	
	500-1000 µm	<45 µm	500-1000 µm	<45 µm	500-1000 µm	<45 µm	500-1000 µm	<45 µm	500-1000 µm	<45 µm	500-1000 µm	<45 µm	500-1000 µm	<45 µm
	Cr		Cr		Cr		Cr		Cr		Cr		Cr	
sample size	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean				
1 g	0.63	1.05	1.90	3.52	11.8	19.5	32.8	43.7	49.4	71.7	47.1	67.8	95.2	94.5
0.5 g	0.64	0.97	2.06	3.39	11.8	18.1	29.4	42.9	44.6	72.3	43.8	65.4	98.3	90.5
0.25 g	0.65	0.93	1.95	3.22	11.6	18.2	25.8	40.2	44.4	71.5	40.0	62.5	89.9	87.4
	Cu		Cu		Cu		Cu		Cu		Cu		Cu	
1 g	9.76	28.4	30.0	70.8	39.1	72.1	38.5	47.1	117	226	117	218	100	96.8
0.5 g	9.82	26.5	30.2	63.6	39.0	67.3	32.7	45.2	117	217	112	203	95.2	93.3
0.25 g	9.87	26.5	29.5	61.7	37.4	69.9	28.6	44.0	117	214	105	202	90.2	94.3
	Fe		Fe		Fe		Fe		Fe		Fe		Fe	
1 g	130	213	5814	9657	2362	2352	24728	25525	32722	42762	33035	37747	101	88.3
0.5 g	130	215	6272	9515	2375	2390	22106	26544	32777	42626	30883	38664	94.2	90.7
0.25 g	131	213	6074	9533	2346	2469	18401	25882	32271	42170	26951	38097	83.5	90.3
	Mn		Mn		Mn		Mn		Mn		Mn		Mn	
1 g	160	150	145	175	39.0	44.0	196	210	502	623	540	579	107	92.9
0.5 g	157	148	148	172	37.9	41.5	169	211	506	598	512	573	101	95.8
0.25 g	162	145	147	174	37.9	46.5	140	211	507	576	487	577	95.9	100
	Ni		Ni		Ni		Ni		Ni		Ni		Ni	
1 g	2.45	3.46	1.73	3.37	4.17	6.69	47.8	60.4	59.7	82.5	56.2	73.9	94.1	89.5
0.5 g	2.55	3.11	1.69	3.08	4.47	6.06	44.5	60.4	58.3	78.8	53.3	72.6	91.4	92.2
0.25 g	2.64	3.41	1.73	3.13	4.40	7.00	40.3	60.3	59.3	76.9	49.1	73.9	82.7	96.1
	Pb		Pb		Pb		Pb		Pb		Pb		Pb	
1 g	26.0	29.1	248	314	72.0	79.7	117	125	440	570	463	548	105	96.1
0.5 g	26.0	29.0	248	314	71.3	81.2	101	127	441	551	447	551	101	99.8
0.25 g	25.9	29.5	248	315	71.6	82.5	83.2	126	441	533	429	553	97.1	104
	Zn		Zn		Zn		Zn		Zn		Zn		Zn	
1 g	14.4	22.7	14.6	24.2	26.8	38.0	114	191	174	280	169	276	97.6	98.6
0.5 g	14.6	22.0	14.8	24.0	27.0	37.9	110	160	173	275	166	243	95.8	88.5
0.25 g	14.2	21.7	14.6	24.0	26.3	38.5	115	181	173	249	170	265	98.3	107

Table 2 mean concentrations of the PTE in the sequential extracts and % recoveries (Σ steps 1,2,3,4, vs pseudototal contents) for sample masses 0.25 – 0.0625 g and particle sizes 500 – 1000 and < 45 µm.

	step 1		step 2		step3		step 4		pseudototal		Σ steps 1,2,3,4		recovery %	
	500-1000 µm	<45 µm	500-1000 µm	<45 µm	500-1000 µm	<45 µm	500-1000 µm	<45 µm	500-1000 µm	<45 µm	500-1000 µm	<45 µm	500-1000 µm	<45 µm
	Cr		Cr		Cr		Cr		Cr		Cr		Cr	
sample size	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean				
0.25 g	0.669	1.081	2.32	3.68	13.5	18.3	38.0	60.8	61.5	89.9	54.5	83.9	88.6	93.4
0.125 g	0.708	1.040	2.40	3.67	13.6	18.3	24.4	53.6	55.1	91.1	41.1	76.6	74.6	84.0
0.0625 g	0.678	1.017	2.49	3.89	13.5	17.1	24.3	21.7	41.6	90.3	41.0	43.7	98.5	48.4
	Cu		Cu		Cu		Cu		Cu		Cu		Cu	
0.25 g	9.91	28.8	30.7	65.7	47.1	66.9	35.4	61.9	124	234	123	223	99.6	95.4
0.125 g	10.1	31.6	34.0	65.4	47.0	71.9	30.5	51.1	124	223	122	220	97.9	98.7
0.0625 g	9.71	26.7	32.3	63.7	46.5	72.9	27.7	31.9	123	184	116	195	94.4	106
	Fe		Fe		Fe		Fe		Fe		Fe		Fe	
0.25 g	151	246	7375	10574	2613	2074	21464	30209	34275	45622	31603	43103	92.2	94.5
0.125 g	164	251	8194	10453	2642	2080	18142	25917	35439	44735	29142	38701	82.2	86.5
0.0625 g	161	247	8201	10494	2712	2181	15410	16785	28304	35733	26484	29708	93.6	83.1
	Mn		mean		Mn		Mn		Mn		Mn		Mn	
0.25 g	183	156	168	189	42.5	41.3	171	230	608	661	564	616	92.7	93.3
0.125 g	157	157	168	187	42.1	44.4	134	197	609	622	502	586	82.4	94.1
0.0625 g	191	155	168	189	42.0	47.6	101	123	608	502	503	515	82.7	103
	Ni		Ni		Ni		Ni		Ni		Ni		Ni	
0.25 g	2.84	3.44	2.23	3.05	9.49	10.3	41.3	63.7	69.0	84.4	55.9	80.4	80.9	95.2
0.125 g	3.04	3.59	2.38	2.91	9.46	11.9	29.6	58.5	69.5	93.0	44.5	76.9	64.1	82.6
0.0625 g	3.19	3.86	2.32	3.08	9.48	11.7	27.8	28.8	69.0	68.2	42.7	47.5	62.0	69.6
	Pb		Pb		Pb		Pb		Pb		Pb		Pb	
0.25 g	29.1	31.8	259	326	76.8	64.0	109	166	493	646	474	588	96.0	91.0
0.125 g	31.5	33.0	260	323	75.8	67.1	105	141	495	613	472	564	95.5	92.0
0.0625 g	30.6	32.7	263	330	76.9	80.7	71.8	78.9	495	492	442	522	89.2	106
	Zn		Zn		Zn		Zn		Zn		Zn		Zn	
0.25 g	16.7	23.4	19.8	26.9	35.3	39.3	122	201	212	336	194	291	91.4	86.5
0.125 g	17.1	23.4	19.6	26.7	35.4	46.5	90.7	168	210	307	163	265	77.4	86.3
0.0625 g	17.7	23.5	19.6	27.6	35.1	47.8	86.4	100	211	239	159	199	75.3	83.3

Appendix C

Table Results of semi quantitative analysis of FDMS filter (mg/kg)

7 Li	9 Be	11 B [1]	12 C	23 Na	24 Mg	27 Al	28 Si	31 P	34 S	35 Cl	39 K	42 Ca	45 Sc	47 Ti	51 V	52 Cr	55 Mn	56 Fe
0.385	nd	355	5.05	923	326	1021	49.9	5.00	44.4	11331	295	413	nd	54.5	1.04	1.74	0.666	29.0
59 Co	60 Ni	63 Cu	66 Zn	69 Ga	72 Ge	75 As	78 Se	79 Br	85 Rb	88 Sr	89 Y	90 Zr	93 Nb	95 Mo	101 Ru	103 Rh	105 Pd	107 Ag
0.046	0.237	0.533	549	112	nd	0.216	nd	0.152	0.260	20.1	0.223	2.57	0.071	0.075	nd	nd	0.140	nd
111 Cd	115 In	118 Sn	121 Sb	125 Te	127 I	133 Cs	137 Ba	139 La	140 Ce	141 Pr	146 Nd	147 Sm	153 Eu	157 Gd	159 Tb	163 Dy	165 Ho	166 Er
0.025	nd	0.225	0.021	nd	0.003	0.004	499	0.410	0.793	0.066	0.239	0.047	0.021	0.040	nd	0.031	0.005	0.018
169 Tm	172 Yb	175 Lu	178 Hf	181 Ta	182 W	185 Re	189 Os	193 Ir	195 Pt	197 Au	202 Hg	205 Tl	208 Pb	209 Bi	232 Th	238 U		
0.002	0.017	nd	0.055	nd	0.010	nd	nd	0.087	0.006	0.038	nd	nd	0.278	nd	0.209	0.051		