University of Strathclyde

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Effect of fertilisers on the availability of potentially toxic elements in soil

by

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DEDICATION

To my dear parents Dr and Mrs Akombor Ande Akombor

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TABLE OF CONTENTS

ABSTRACT

In this study, soil was amended with several commercial fertilizers and effects on the levels and availabilities of potentially toxic elements (PTE) studied through a series of pot, column leaching, and plant uptake experiment. Analytes (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, U and Zn) were quantified in sample digests, extracts and leachates using inductively coupled plasma mass spectrometry.

Commercially-available topsoil was treated with 0, 1, 3 or 5% *w/w* chicken manure (CM), growmore (GM), phostrogen (PG), rockdust (RD) or seaweed (SW). The CM and GM affected the pseudototal (aqua regia-soluble) PTE concentrations more than the other amendments, whilst application of the BCR sequential extraction provided evidence that both materials could affect the distribution of PTE (especially Cu, U and Zn) in the original soil.

Column leaching experiments were performed on an urban soil from West Central Scotland after 2% CM, 5% GM and 2% CM + 5% GM addition. Increased levels of PTE were recovered in leachates of all the amended soils, especially the GM-amended soil, compared with leachates obtained from the control soil. The BCR extraction indicated that Cd, Cr, Fe, Pb, and Zn had been mobilized from the exchangeable phase and that Mn had been transformed from reducible to exchangeable forms.

Uptake of PTE by bean plants grown in 2% CM amended soil, and by radish grown in 2% CM, 0.2% GM or 2% CM + 0.2% GM amended soil, were studied. The PTE levels in control bean plant exceeded those in bean plants grown in CM amended soil, and the same trend was observed for radish, suggesting that CM addition can decrease PTE phytoavailability. Addition of growmore resulted in plants with similar PTE burden to control plants. It was found that EDTA-extraction of soil generally overestimated actual plant uptake of PTE.

1 Introduction

The continuous application of chemical fertilisers and other amendments to soils for food production has raised concerns with respect to possible accumulation of potentially toxic elements (PTE) and effect they may cause to humans and the environment.[1, 2] This is because, apart from supplying the nutrients required by plants to grow, some of the fertilisers may contain PTE such as Cd, Hg, Pb and other trace elements. Examples of fertilisers used as sources of soil nutrients that may contain elevated amounts of PTE include sludges and effluents, composted urban refuses, animal wastes, and wastes from industries specialising in agro and food production. These are increasingly and highly beneficially recycled in agriculture as soil amendments to meet the demand for plant nutrients.[3] In the UK for instance, where livestock and other animal farming have been one of the major forms of agricultural practice for decades, crops make extensive use of animal wastes as amendments. These may contain PTE as a result of use of metals in animal feed additives.[3] The uptake of these metals by plants and other biota can serve as routes into the human food chain, with harmful effects. The potential environmental hazards of fertilizer amendments may depend on the amounts used, the elemental composition of the material, the fraction of constituent elements that could be mobilized in the environment, and the ease with which these elements become assimilated into the biota.[2]

1.1 Fertilisers[3-10]

The intensive use of fertilisers began in the nineteenth century. This was when salt petre and guano were shipped from Chile and Peru to the UK and other parts of Western Europe. The first known fertiliser used in bulk, sodium nitrate (16% nitrogen), which is also known as Chilean nitrate, was found in north Chile as a natural mineral. Its importation into Europe and America started in 1830. By 1843, a fertiliser called single superphosphate (SSP) was produced in the UK, after which many SSP production plants were established throughout Europe. By 1860, the production of potash fertilisers

began in Germany, whilst nitrogen-containing fertilisers from ammonia derived from coal were manufactured in 1890. An advance that was significant in the production of nitrogen fertilisers was the manufacture of synthetic ammonia by the Haber-Bosch method in 1913 in Germany. Urea was first manufactured and used as a fertiliser around 1921. A large number of solid and liquid fertilisers known to contain one or more plant nutrients have now been manufactured and utilized.[7] In general, fertiliser products are made up of nitrogen, phosphorus and potassium compounds in different physical and chemical forms, and their combinations, appropriate to different needs.

The term fertiliser is derived from the Latin word *fertilis*, which means fruit bearing and may be defined as any substance that is added to soil to supply those elements required in nutrition of plants. Specifically, it can be defined as "a mined, refined or manufactured product containing one or more essential plant nutrients in the available or potentially available forms and in commercially valuable amounts without carrying any harmful substance above permissible limits".[7]

Virgin soils usually contain adequate amounts of all the elements required for proper plant nutrition. However, when crops are grown on the same soil year after year, there is a tendency for such soil to become exhausted in one or more specific nutrients, hence the need for fertiliser application.

Types of of fertilisers

In the broadest sense two types of fertilisers are known: inorganic and organic. Inorganic fertilisers are composed of synthetic chemicals and/or naturally-occuring minerals. Examples of inorganic fertilisers are listed in Table 1.1

Table 1.1 Examples of typical inorganic fertilisers and their nutrient compostions[4, 8]

Fertiliser material	N (%)	P (%P_2O_5)	K (%K_2O)	S (%)	Physical state	Formulae
Ammonium nitrate	34	-	-	-	Solid	$NH_4(NO_3)$
Amonium phosphate	11-18	46	-	-	Solid	$NH_4H_2PO_4$
Ammonium phosphate-sulfate	13-16	20-39	-	15	Solid	$NH_4H_2PO_4.(NH_4)_2SO_4$
Calcium ammonium sulfate	18	46	-	-	Solid	$Ca(NH_4)_2SO_4$
Diammonium phosphate	18-21	46-54	-	2	Solid	$(NH_4)_2HPO_4$
Growmore	7	7	7		Solid	-
Magnesium sulfate	-	-	-	13	Solid	$MgSO_4$
Phosphate rock	-	11-27-	-	-	Solid	-
Potassium chloride	-	-	60	-	Solid	KCl
Potassium sulfate	-	-	52	18	Solid	K_2SO_4
Single superphosphate	-	18	-	14	solid	
Triple superphosphate	-	44-53	-	1.5	Solid	
Zinc sulfate	-	-	-	17.8	Solid	$ZnSO_4$

Organic fertilisers (Table 1.2) are derived chiefly from materials of plant and animal origin; they are composed of enriched organic matter. They are materials that occur in nature, usually as by-products of naturally-occurring processes. Organic fertilisers such as manure have been used in agriculture for many years. The chemistry of these substances was not understood by farmers in ancient times but they recognized the benefit of enriching their crops with these materials. Like any other fertiliser, organic fertilisers provide the three major elements required by plants; nitrogen, phosphorus and potassium. These nutrients originate from sources such as bone meal (slow release fertiliser high in phosphorus and calcium); bat guano (contains all the three major macronutrients); poultry manure (waste product from the chicken industry that contain all the three macronutrients); fish emulsion (high in N, P and trace elements etc.) These are used to varying extents in many countries. They are sometimes used in the form in which they are obtained from nature or after being subjected to some form of processing.[7] The kinds of organic fertilisers or manures that are used in a particular area or country are in most cases based on the organic materials that are available and may be generated locally, unlike commercial inorganic fertilisers.

Table 1.2 Some commonly used organic fertilisers and their average nutrient concentrations[11]

Material	N(%)	P (% P_2O_5)	K (% K_2O)
Blood meal	12	1-2	0
Feather meal	12	0	0
Fish meal or powder	6-12	3-7	2-5
Composts	1-3	1-2	1-2
Bone meal	1-6	11-13	0
Sewage sludge	2-6	1-4	0-1
Poultry manure	3-4	1-2	1-2
Processed liquid fish residues	4	2	2
Alfalfa hay	2-3	1	1-2
Kelp	1-1.5	0.5-1	5-10
Meat and bone meal	8	5	1
Seaweed extract	1	2	5
Urea	46	0	0

Classification of fertilisers

There is no standard way of classifying fertilisers. However different researchers [1, 3 5, 7, 9] have attempted their classification as summarized below.

Straight fertilisers. They generally contain or supply only one primary plant nutrient; nitrogen, phosphorus or potassium. Examples of this class of fertilisers include urea and ammonium sulfate, which supply nitrogen; triple superphosphate, which supplies phosphorus; and potassium chloride and potassium sulfate, which supply potassium as the primary nutrient. With respect to secondary nutrients, straight fertilisers include those that contain calcium, magnesium and sulfur. While in the case of micronutrients, iron and iron chelates or their sulfate salts are considered as straight fertilisers.[7]

Compound (or multielement). These fertilisers contain more than one major plant nutrient. These may be subdivided into complex and mixed fertilisers. Complex fertilisers are those that contain two or three primary plant nutrients in a chemical

combination. They are usually produced in solid or granular form, and are homogenous in nature such that each of the granules contain two or more major plant nutrient in a definite proportion.[3] Mixed fertilisers are physical mixtures of straight fertilisers, containing two or three primary plant nutrients, made by thoroughly mixing the ingredients either mechanically or manually. These materials are also called bulk blended fertilisers. Complex fertilisers may further be classified as incomplete or complete fertilisers. Incomplete fertilisers lack one of the major components. Examples of incomplete fertilisers include monoammonium phosphate, diammonium phosphate and ammonium phosphate sulfate. Complete complex fertilisers contain nitrogen, phosphorus and potassium in a chemical combination. Examples of commonly used complete fertilisers are NPK 17:17:17, NPK 19:19:19, and NPK 20:10:10.

Fertilisers may also be either "special-purpose" or "micronutrient" based.[7, 9, 12] Special purpose fertilisers, as the name implies, are formulated to target certain plants requirements or soil defeciencies. They are widely used in the small fruit and nursery industries. Some of these fertilisers are packaged specially for a particular group of foods.[12] The blueberry food is one of these specialty materials and belongs to an old established group, the acid-plant foods.[12] Some of the compounds incorporated into these fertilisers, like ammonium sulfate are utilized simply because they have an acid reaction which helps in reducing the soil pH when it becomes too high. Amendments such as gypsum, lime and potassium-magnesium sulfate are other typical examples. These materials may be used to correct imbalances or deficiencies of calcium, magnesium or potassium, or to elevate pH values in soils. Gypsum for instance can be used specially to improve water infiltration on soils with very poor structure.[12]

Foliar fertilisers are liquids, designed to be sprayed on to the plant.[13, 14] These fertilisers are formulated to supply plant nutrients in small amounts to avoid damage. Gardeners may make use of manures, or seaweed collected from the beach to produce their own forliar fertilisers, suspending the material in water until all of the "goodness'' has been extracted for application. In India, for example, the traditional liquid fertiliser called Panchagavya, a manure tea made by fermenting cow dung in

water, has been shown to have a modest nitogen:phosphorus:potassium contents of 0.03:0:02:0.04 with high iron content of 0.84%.[13] Urea, ammonium sulfate, potassium nitrate, glycine and glutanic acid are other typical materials used in foliar applications to provide plant nutrients in a fast way.[13, 14]

Micronutrient fertilisers. are formulated to address deficiency in nutrients required in very small amounts. The most important of these nutrients, sometimes called trace nutrients, include boron, copper, iron, manganese, molybdenum and zinc. These fertilisers are absorbed after application as cations or metal-chelate ions of copper, iron, manganese, zinc, and as anions in the case of boron (as borates) and molybdenum (molybdates). Excessive application of these fertilisers may lead to crop damage and soil pollution (Figure 1.1).

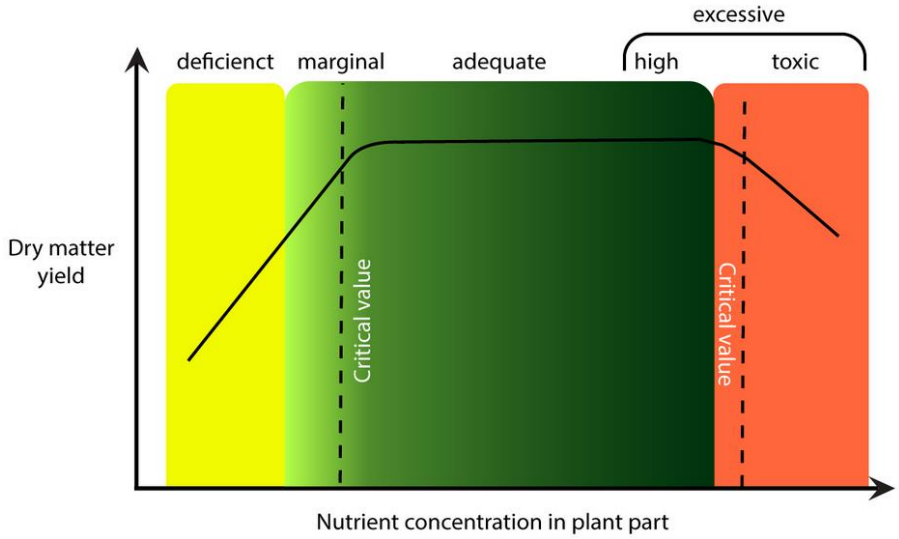


Figure 1.1 Relationship between yield and nutrients concentration to plants[15]

Controlled release fertilisers (CRFs). These are specially designed fertilisers that release active plant nutrients in a gradual or delayed manner according to the needs of plants.[6] They provide enhanced nutrient use efficiency along with enhanced yield. These fertilisers are coated with a natural or semi natural but environmentally friendly macromolecule material that delays fertiliser release.[16, 17] Control release fertilisers have been classified in diverse ways by different researchers. However, the classification reported in Shaviv and Mikkelson[16], and Shaviv[18] as summerised by

Azeem *et al*[6] is most comprehensive (Figure 1.2). They are grouped into three classes:

i. organic compounds: these are further subdived into organic compounds that occur naturally (animal manure, sewage sludge, bone meal etc) and synthetically produced organic-nitrogen, low solubility compounds (generally include condensation products from urea and acetaldehyde). These compounds are further subdivided into biologically decomposing compounds (e.g urea formaldehyde), and chemically decomposing compounds (e.g urea acetaldehyde).

ii. The next category of CRFs include water soluble materials with physical barriers that control the release of nutrients. They are made as granules or coated with a hydrophobic polymer, or as a matrix of active fertiliser nutrients released on continuous basis through a hydrophobic material that impedes fertiliser dissolution. Either the CRFs are coated with organic polymer materials (thermoplastics, resins etc) or with inorganic materials such as sulfur.

iii. The third category includes low solubility inorganic fertilisers. Examples are potassium ammonium phosphate and magnesium ammonium phosphate, and partially acidified phosphate rock.

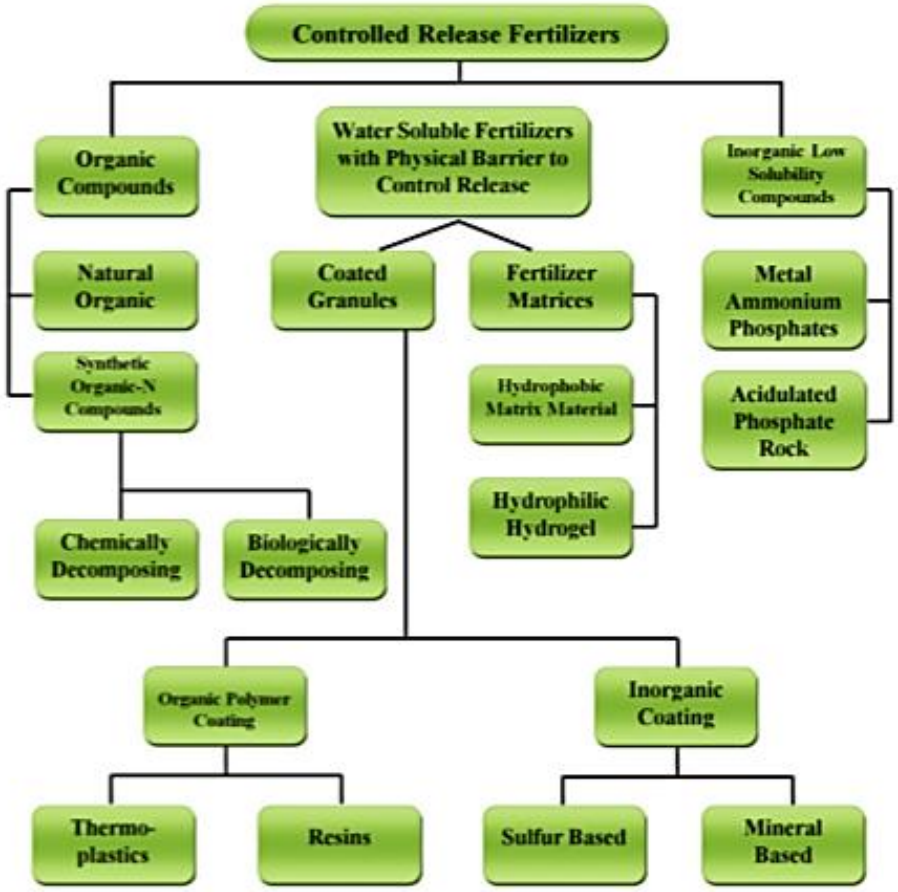


Figure 1.2 Classification of controlled release fertilisers[6]

The mechanism of release of nutrients by CRFs depend on a number of factors which include the nature of the coating material, the type of fertiliser, and agronomic conditions. Different mechanisms are reported in the literature and are still under development.[6] However, Liu and Shaviv as reported in Azeem *et al*[6] have proposed a model for the release of nutrients by coated fertilisers called the multi-stage diffusion model. This model explains that after application of a coated fertiliser, water permeates through the coating and condenses on the fertiliser, which is followed by partial nutrient dissolution (Figure 1.3). After this osmotic pressure builds up within the material and swelling of the granule occurs, which leads to two processes: spontaneous release of nutrients as a result of osmotic pressure surpassing the threshold of the membrane resistance (failure mechanism) or gradual release of nutrients through diffusion (diffusion mechanism) if the membrane holds out against

the osmotic pressure. The first process occurs generally in sulfur-coatings while polymer coatings exhibit the diffusion release mechanism. The mechanism involves nutrient transfer from the fertiliser-polymer interface to the polymer-soil interface controlled by water.

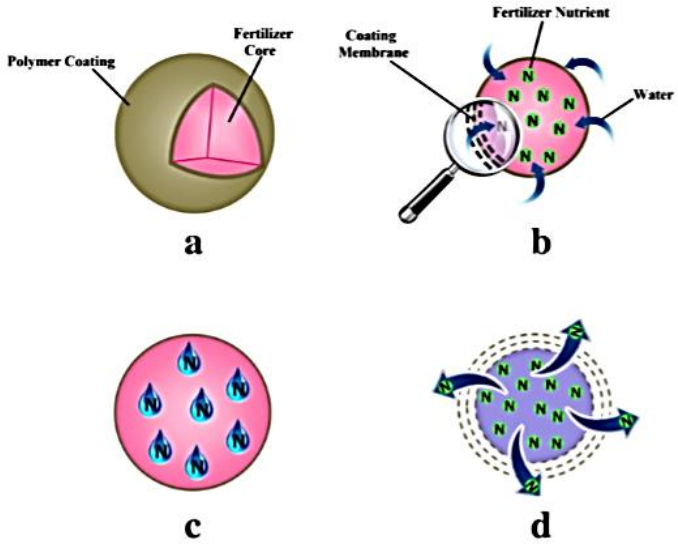


Figure 1.3 Mechanism of controlled release fertilisers[6], (a) Polymer coating with fertiliser (b) Water permeates into the coating and the granule, (c) Dissolution of fertiliser and osmotic presssure builds, (d) Controlled release of nutrients through the swollen coating membrane

1.2 Soil[18-22]

Soil is an important component of the environment containing a variable mixture of minerals, organic matter and water, capable of supporting plant life on the Earth's surface.[19] Bradl[20] described soil as "one of the key elements for all terrestric ecosystems that provides the nutrient-bearing environment for plant life, and is of essential importance for degradation, and transfer of biomass''. It contains air spaces and is generally unconsolidated in nature. Soil is formed slowly through the weathering activity of physical, chemical, and biological processes on parent materials. As the bedrock erodes into smaller particles near the Earth surface, organic matter decays steadily and mixes with inorganic materials which give rise to soil.

Soil exhibits distinctive layers called horizons. Figure 1.4 shows a generalised soil profile with the respective horizons; horizon O is a surface layer dominated by the presence of large amount of organic material and/or decomposed leaf litter. Horizon

A is the next layer below the top surface layer, typically several inches in thickness with maximum biological activity in the soil. Accumulated organic matter is mixed thoroughly in this layer with mineral matter. The next layer is referred to as the B horizon. This layer is also known as the subsoil and it predominantly receives materials such as organic matter, salts and clay particles, dissolved or leached from the top soil. Horizon C is composed of weathered parent rock. This layer is deficient in organic material. Horizon R represents the unweathered rock (not shown in Figure 1.4). The nature of soil can be modified either after or during formation by effects such as flood, erosion and human activity.[21] The soil is a chemically, physically and biologically complex dynamic system, the constituents of which are constantly undergoing change[22]

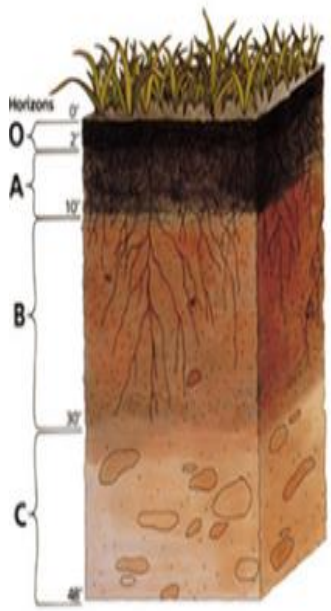


Figure 1.4 A generalised soil profile showing different horizons[23]

1.2.1 Soil constituents

In a typical soil, inorganic constituents constitute over 90% of the solid components present, with and 5% organic materials as coatings on the inorganic particles (with the exception of peat soils which contain about 100% organic matter.)[24, 25] The inorganic contituents of soil are a mixture of both primary and secondary minerals. Typical examples of primary minerals in soils include quartz (SiO_2), and feldspars

which include orthoclase ($KAlSi_3O_8$), microcline ($KAlSi_3O_8$), albite ($NaAlSi_3O_8$) etc. They are referred to as primary minerals because their chemical properties remain unaltered after deposition and crystallisation from molten magma. They usually occur in the sand (2 to 0.02 mm particle diameter) and silt (0.02 to 0.002 mm particle diameter) soil fractions, but can also be found in the clay fraction.[24-26]

Secondary minerals are those that result from the breakdown of primary minerals; either by an alteration in the structure or from reprecipitation of the products of weathering of the primary minerals.[25] Clay minerals (phyllosilicates) are secondary minerals that consist of several assemblages of silica tetrahedral and alumina octahedral sheets.[24-27] When one layer of silica tetrahedral is bound to another layer of alumina octahedral, a 1:1 clay mineral results e.g kaolinite. However where one layer of alumina octahedral is sandwiched between two sheets of silica tetrahedral, a 2:1 clay mineral is obtained; monmorillonite is a typical example. Figure 1.5 shows a general structure for phyllosilicates. Other important secondary minerals include oxides of aluminium, iron and manganese; calcite ($CaCO_3$); and gypsum ($CaSO_4$) etc. They occur as discrete solid phases, and as coatings on clays, though in smaller amounts. They play important roles in soil processes because of their high specific surface areas and reactivity.[24, 25, 27]

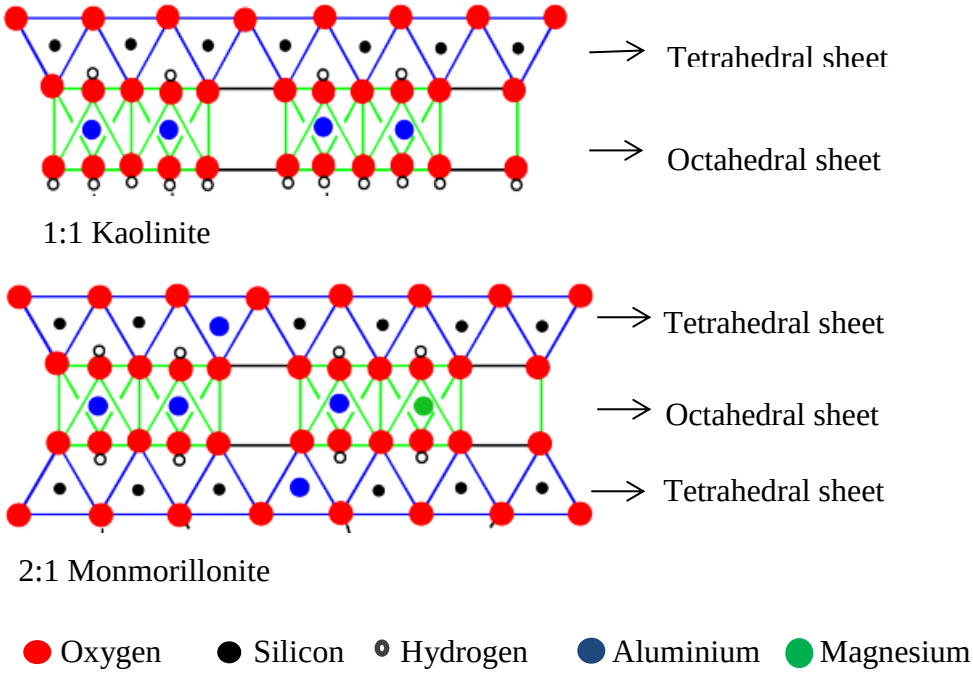


Figure 1.5 Phyllosilicate structure[28]

Soil organic matter (humus) may refer to the total organic compounds in soil excluding undecayed plants and animal tissues, their "partial decomposition" products, and the soil biomass.[29] These have high specific surface area and large amounts of negative surface charge due to ionisation of functional groups such as carboxyl, phenolic, alcoholic and carbonyl groups.[24] As a result they can bind metal ions in soil or serve as a source of plant nutrients. Soil organic matter is composed of humic and non humic substances. The non humic sustances have recognisable physical and chemical properties and consist of carbohydrates, proteins, peptides, amino acids, fats, waxes, and low-molecular weight acids − they are attacked easily by soil microorganisms and persist in the soil for a short time only.[25]

Humic substances are generally referred to as heterogenous organic substances of high molecular weight whose extraction is based on their solubility in acidic or basic medium (Figure 1.6). They are broadly subdivided into humic acids (HA) with high molecular weight (3000 to 1000 000 Da)[29] that are insoluble at low pH values; fulvic acids (FA) with lower molecular weight (500 to 5000 Da)[29] that are soluble at all pH values; and humin (HU) which contains more aromatic compounds and is insoluble at all pH values with lower binding ability.[25, 30] Humic substances, due to the presence of variable functional groups, can chelate PTE. Humic and humin acids are generally immobile (so the cations become fixed) whereas the fulvic acids generally being soluble plays an important function of keeping biologically important metals in solutions.

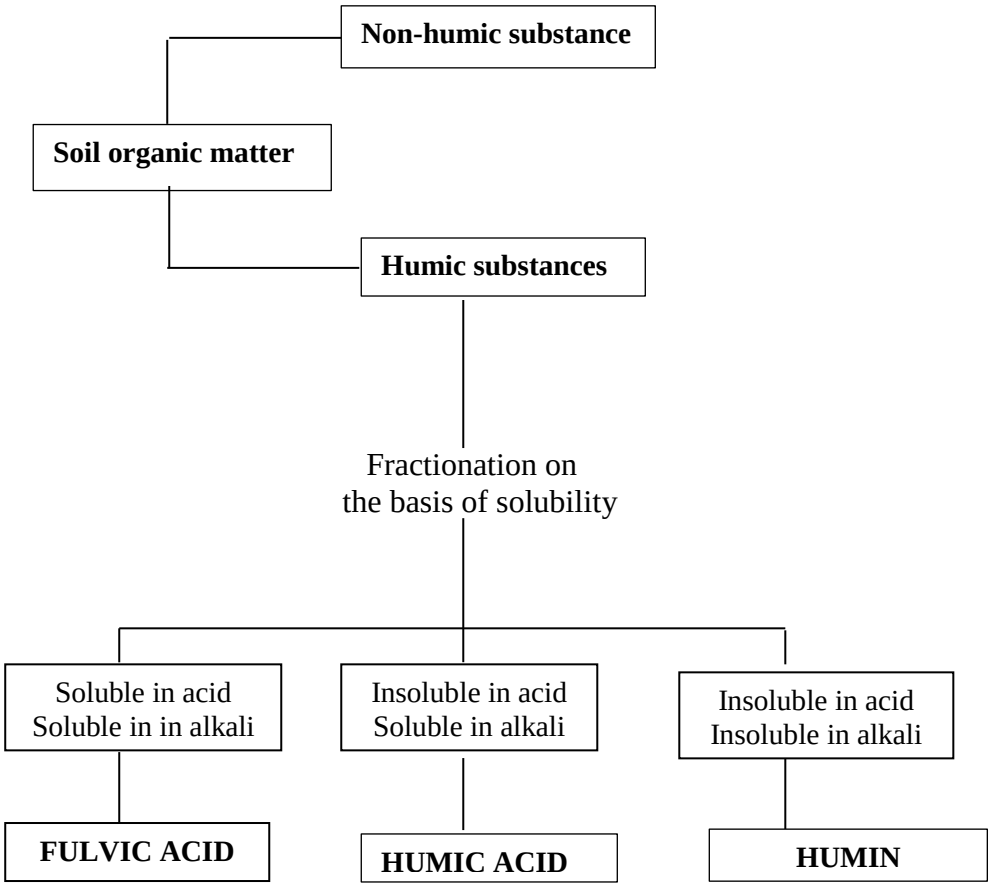


Figure 1.6 Fractionation of soil organic matter components[31]

1.2.2 Sources of potentially toxic elements in soil

Potentially toxic elements occur naturally in the soil environment derived from the original minerals that were subject to weathering and produced the soil.[32] Soil weathering helps to expose trace metals that were deeply and stably buried in the Earth's crust. Levels of these elements are low in nature and therefore toxicity is rare and regarded as trace (<1000 mg kg^{-1}). [33, 34] However, human activity can influence the input of PTE and, by so doing, increase their concentrations in soils. Potentially toxic elements arising from human activity may be referred to as contaminants or pollutants. Contamination can either be extensive or localised. Extensive contamination results from atmospheric deposition. Here, contaminants are transported from point sources to other locations. Inputs of PTE into the atmosphere arise from coal and power generating stations, industrial emissions from smelters,

metallurgical industries, vehicular emissions through exhausts, bonfires, and other industrial and domestic heating systems. The PTE are deposited from the atmosphere onto soil surfaces either by dry deposition or wet deposition. Flood and sediment deposition is another example of extensive contamination. This is where PTE from sources such as mines wastes are transported into rivers, and when flooding occurs, much of the suspended sediment in water overflows on to the land and become deposited on soil. Localised contaminations result from the use of livestock manures, sewage sludges, inorganic fertilisers, pesticides and other agricultural inputs to soil. Livestock manures are a good source of PTE in soil as some of these elements are added to animal feed. For example, Large amounts of Cu (140 mg kg^{-1}) and Zn (800 mg kg^{-1}) were added to feed for piglets in Europe in the past.[35] The use of poultry or livestock manure on farmland may increase the levels of these elements in soil. Sewage sludges as a form of biosolids contain high level of N, P, organic matter and PTE, and are used extensively as fertilisers. Inorganic fertilisers such as micronutrient fertilisers constitute one of the major sources of PTE inputs into agricultural lands in most parts of the world, except for developing countries like Nigeria where little commercial fertilisers are used. Macronutrient fertilisers may also contain PTE. This is because most of the inorganic compounds used in the manufacture of these fertilisers contain substantial quantities of PTE contaminants. Phosphatic fertilisers for instance contain the highest amounts of As, Cd, U, and Zn. Frequently fertilised agricultural soil can therefore accumulate these elements to toxic levels.

1.2.3 Potentially toxic elements, occurrences, uses and their health implications

Potentially toxic elements are a constituent of Man's environment.[36] Their continuous and excessive release into the environment could give rise to health implications. A few of the PTE are essential in trace amounts for plants and for animals. Metals such as Cd and Pb are essential neither for plants nor for animals.[37] The subsequent transfer of these metals from soil to plants is of most increasing concern due to the possible adverse effects that they might have on plants, animals

and human health.[37] The PTE of interest in this study include As, Cd, Cu, Cr, Cu, Fe, Mn, Ni, Pb, U, and Zn.

Arsenic[38-40]

Soil contamination with As is one of the major environmental problem due to its toxic nature.[37] Typical concentrations of As in earth crust range between 0.5 and 2.5 mg kg^{-1}. However, Wanzel[39] reported that the total concentration of As in the soil solid phase ranges between 0.1 and 55 mg kg^{-1}. Arsenic exhibits several oxidation states which include 3–, 0, 3+ and 5+. Its common minerals are arsenopyrite (FeAsS), orpiment (As_2S_3), realgar (AsS), arenolite (As_2O_3) etc. Under aerobic conditions, As^{5+} dominates mainly in the form of arsenate, AsO_4^{3-} in a number of protonation forms which include H_3AsO_4, $H_2AsO_4^-$, and $HAsO_4^{2-}$. Under reducing environments, As^{3+} which exists as arsenite (AsO_3^{3-}) is the dominant species and its protonation forms include H_3AsO_3, H_2AsO_3 and $HAsO_3^{2-}$. The cationic species of As (As^{3+} and As^{5+}) are readily adsorbed on to soil minerals such as iron oxyhydroxides, clay and organic matter in soil and their maximum adsorption occurs at pH 7.0 and pH 4.0 respectively.

Arsenic is widely used in the manufacture of agrochemical such as pesticides. It is utilized in the manufacture of wood preservatives, photoelectric devices, glassware and Pb acid batteries. Arsenic is also used as an anti-corrosion agent and improves tensile strength in alloys of Cu.

Arsenic is a toxic element that affects both humans and animals. Arsenic, As^{3+} and As^{5+} can cause similar toxicological effects but the mobility of As^{3+} in soil is greater than As^{5+}. It is associated with skin problems, cardiovascular diseases, respiratory disorders, and hypertension. In addition, when inorganic As is ingested, it could cause both non-cancer and cancer health effects to the human body.[41]

Cadmium[19, 33, 38, 40, 42]

Cadmium is a potentially important environmental contaminant that occurs naturally in the earth crust. It occurs in soil as a divalent metal species, Cd^{2+} typically at levels ranging from 0.1 to 1.0 mg kg^{-1}. Kabata-Pendias and Pendias[40] reported however that the average concentration of Cd in the earth crust ranges between 0.1 and 0.2 mg kg^{-1} and its abundance is fairly similar in both igneous and sedimentary rocks, while the

global average concentration in soil is estimated to range between 0.06 and 1.1 mg kg^{-1}. Few Cd minerals exist in the environment. They include, greenokite (CdS), otavite, ($CdCO_3$) etc. In addition to the inorganic species, it is reported that Cd in soil solution also occurs in complexes with various organic acids such as amino acids, humic acids, and fulvic acids.

Cadmium is used extensively in the manufacture of Ni-Cd batteries. It serves as a good corrosion resistance material when coated on vessels and other vehicles, particularly in high-stress environments such as marine systems. Other useful applications of Cd are in the area of manufacture of pigments and stabilisers for various plastics.

It is one of the most toxic elements to human health. It is known for combining with sulfhydryl groups and as a result alters the function of several SH-group enzymes that lead to protein denaturation. Similarly, Cd can accumulate in the kidney with detrimental consequences. Exposure to excess Cd may also cause lung and prostate cancer, anaemia, yellow teeth and heart failure.

Chromium[19, 38, 40, 43]

Chromium occurs naturally and predominantly in the earth crust as chromite ($FeCr_2O_4$). Crocoite ($PbCrO_4$) is also a relatively common mineral ore of chromium. Chromium exhibits variable oxidation states. The common ones in soil include Cr^{3+} and Cr^{6+} which are dependent on pH and redox condition. Chromium 3+ forms the dominant species at pH less than 4 and its solubility is reduced at pH values greater than 5 due to adsorption onto soil particles and formation of $Cr(OH)_3$[44] which explain its low mobility and bioavailability in soil. Chromium 6+ exists mainly under aerobic condition as CrO_4^{2-} or $Cr_2O_7^{2-}$ at a pH range of 4-8. Chromium 6+ can be reduced to Cr^{3+} under anaerobic environments. It is more mobile, toxic, and readily adsorbed on soil surfaces such as clay minerals, Fe, Mn, and Al (oxy) hydroxides.[19, 43]

Chromium is used extensively in pigments for paint, cement, rubber, stainless steel and chromate plating, and in other materials. Chromium 3+ serves as a co-factor for certain metabolic activities especially in humans. For example during carbohydrate metabolism.

Inadequate supply of Cr can cause ulceration and skin defects, while constant exposure to Cr may lead to damage to kidney and circulatory tissues. Inhalation of excess amounts of Cr may result in lung, nasal and gastro-intestinal disorder.

Copper[33, 38, 40, 45, 46]

The concentration of Cu in the earth crust ranges from 25 to 75 mg kg^{-1}, which is typical of levels found in igneous rocks.[40] Oorts[45] reported average natural concentration of Cu in the earth crust as 60 mg kg^{-1} and stressed that its abundance in rock materials remains highly variable with basaltic igneous rocks containing 90 mg kg^{-1} and granite rocks 15 mg kg^{-1}. Background average concentration of total Cu in soil in the world ranges between 2 and 50 mg kg^{-1}, although natural levels greater than 100 mg kg^{-1} can also be found in some soils.[45] Copper, which shows strong affinity for sulfur, is found in the following minerals: chalcopyrite ($CuFeS_2$), bornite (Cu_5FeS_4), chalcocite (Cu_2S) and covellite (CuS). Copper occurs in several oxidation sates. The most common forms of Cu are Cu^+ and Cu^{2+}. In soil solutions Cu^+ ions are generally unstable and could be transformed to Cu^{2+}/Cu(s) as precipitate. In soil, Cu is bound to both the inorganic and organic constituents of the soil while, in the soil pore water, it binds to humic and fulvic acids (dissolved organic matter). The general trend for Cu adsorption by soil minerals and organic matter is: Mn oxides > organic matter > Fe oxides > clay minerals.[45]

As a result of its versatile properties, it is used in the manufacture of wire, rod, electrical cables and household materials. Copper finds applications in the manufacture of ammunitions and other industrial materials. It is an essential trace metal that plays a very important role in the growth of plants and animals, and helps in the production of blood hemoglobin in humans. Resistance to diseases and water regulation in plants is another role played by Cu. In agriculture, it is used as an additive for livestock feeds.

Copper becomes toxic at elevated concentrations. High level exposure to Cu may result to biochemical effects to include difficulty in the synthesis of haemoglobin, damage to the kidney and the nervous system.[47]

Iron[40, 48]

The average concentration of Fe in the earth crust is reported to be about 5 %. In soils, the percentage concentration of Fe lies between 0.1 and 10%. Iron is a very chemically reactive element whose geochemistry is quite complex in the terrestrial environment. The ease with which Fe changes its oxidation state in response to physicochemical conditions determines its geochemistry.[40] Iron occurs as Fe^{3+} under aerobic environment (near the surface of the earth crust) but occurs as Fe^{2+} in most cases under reducing conditions (deeper rocks). The major Fe ore minerals are hematite or hydrated Fe oxide (goethite), $Fe_2O_3.xH_2O$ (siderite), $FeCO_3$ (pyrite), FeS_2 and ilmenite ($FeO.TiO_2$).

Iron is employed in the manufacture of various tools, and finds application in the transport and construction industry.

Iron participates extensively in metabolic processes and helps to transport oxygen in the human body. The synthesis of DNA is a well-known function in which Fe is involved.

Lack of Fe in the human body causes anaemia but excessive intake of Fe can lead to liver or lung damage. Iron and steel miners are the most affected as a result of continuous inhalation of Fe oxide fumes which may result in the deposition of Fe particles in the lungs.

Manganese[33, 40, 49]

Manganese is one of the most abundant elements that occur naturally in the lithosphere. It is closely related to Fe in geochemical processes. The concentration of Mn in rocks ranges between 350 and 2000 mg kg^{-1}. However in soils, the level of Mn is reported to have ranged between 10 and 9000 mg kg^{-1} with a global mean concentration of 437 mg kg. Kabata-Pendias and Pendias[33] reported values of 495 and 600 mg kg^{-1} for USA and Finnish soils respectively, explaining further that the variation of Mn contents in soils rarely correlates with soil classification but is highly associated with clay minerals. The most common Mn ore is pyrolusite (MnO_2).

Manganese, being an essential nutrient, participates in several enzyme catalytic processes in the human body. It is required for the formation of healthy cartilage and bone. It is active in the production of of glucose and helps in the healing of wounds.

It is employed in the manufacture of batteries and fertilisers. Exposure to Mn at higher concentrations may lead to neurological complications, pneumonia, respiratory disorder and liver cirrhosis. Deficiency in Mn can result in skeletal and cartilage disorders.

Nickel[38, 40, 43]

The average concentration of Ni is approximately 80 mg kg^{-1} in the earth crust. Nickel shows both chalcophilic and siderophilic tendency and as a result combines readily with Fe. For this reason, Ni-Fe compounds are readily present in the earth crust. It is known to be co-precipitated along with Fe and Mn oxides after weathering and becomes incorporated in goethite, limonite and other Fe minerals. It can be readily adsorbed by organic matter, phosphates, carbonates and silicates – its high affinity for these soil constituents explains its low concentration in soil solutions. Amongst common Ni metallic ores are pentlandite [$(Ni,Fe)_9S_8$], millerite (NiS), nicolite (NiAs) and ullmanite, (NiSbS).

Nickel is used extensively in the production of a variety of metal alloys for aircraft and plating industries. It is also used in the manufacture of permanent magnets and electrical equipment. Its compounds are utilized as dyes in ceramic and glass manufacture, and in batteries containing Ni-Cd compounds. It is employed as a catalyst for fat hydrogenation and for the oxidation of organic compounds.

High concentration of Ni may result in gastric, liver and kidney disorders, and neurological effects. Lungs and nasal cancer have been linked to continued inhalation of insoluble Ni which is retained in the lungs.[40]

Lead[38, 40, 50]

Lead occurs naturally in the environment. However, where high concentrations of Pb are found in the environment these are attributed to human activities. The average abundance of lead in the earth crust is estimated to be 14.8 mg kg^{-1}. In uncontaminated soils globally, concentration is estimated to be 17 mg kg^{-1}. The most important mineral ore of Pb is galena (PbS). Others which are also common include anglesite ($PbSO_4$), cerussite ($PbCO_3$), minium (Pb_3O_4), pyromorphite [$Pb_5(PO_4)_3Cl$] and mimetesite [$Pb_5(AsO_4)_3$]. In soil, Pb chiefly exists in the +2 oxidation state. Under reducing conditions, Pb exists as insoluble PbS usually

precipitated by sulfide generated from the reduction of sulfate. Lead exists as Pb^{2+} ion under aerobic environment but becomes insoluble at increasing pH values (pH > 4) in the soil solution. This is attributed to sorption on organic matter, clay minerals, and oxides.[50] However, in alkaline soils, solubility of Pb may increase with formation of soluble Pb-organic and Pb-hydroxy complexes.

Lead is widely used in batteries and added to petrol as an anti-knocking agent in motor vehicles especially in developing countries like Nigeria. However, the use of leaded petrol has been phased out completely in most of the developed countries like UK between 1998 and 2001. Exposure to Pb can result in a wide range of health implications depending on the level and extent to which it happens, with young children being at higher risk than adults. Chronic exposure and accumulation of Pb may result in short term effects. These include loss of appetite and vomiting. Acute exposure effects may lead to kidney malfunction, hyperactivity and brain damage.

Uranium[51, 52]

Uranium is a trace element that occurs naturally in the environment. The average content of U in the earth crust is 2.8 mg kg^{-1} with most of rocks containing typical values ranging from 1 to 4 mg kg^{-1}.[51] Naturally occurring U ores include; Uraninite, (UO_2); Pitchblende, ($U_2O_5.UO_3$); Carnotite, [$K_2(UO_2)_2(VO_4)_2.2H_2O$]; Autunite [$Ca(UO_2)_2(PO_4)_2 \cdot 10H_2O$], Torbernite [$Cu(UO_2)_2(PO_4)_2 \cdot 10H_2O$] and Tyuyamunite, [$Ca(UO_2)_2(VO_4)_2 \cdot 5\text{-}8H_2O$].[52] Three naturally occurring radioactive isotopes of U are known. They are ^{238}U, ^{235}U and ^{234}U. Uranium-235 only accounts for about 0.72% of the composition of the three isotopes. Exposure to U can lead to wide range of complications. Renal and neurological toxicities, damage to DNA which may result in carcinogenesis in humans, bone and muscular toxicity, and reproductive toxicity are prominent. Other toxicities associated with U exposure include gastro intestinal and dermal complications.

Zinc[38, 40, 53]

Zinc is the 24th most abundant element in the world and occurs naturally in all soils. Its concentration in the Earth crust is estimated to be 70 mg kg^{-1}. In soils, the average typical background concentration of Zn ranges between 10 and 100 mg kg^{-1}.

Common mineral ores of Zn are Sphalerite (ZnS), Zincite (ZnO), Smithsonites ($ZnCO_3$) and Willemite (Zn_2SiO_4). Zinc is highly mobile during weathering processes and its soluble compounds form precipitates readily when in contact with carbonates. It is also adsorbed by organic and inorganic minerals. Zinc may become immobilised especially at neutral and alkaline pH conditions.[33]

It is used expansively during industrial processes such as mining, waste combustion and steel processing. It is employed widely in the manufacture of batteries, pigments in paints, pipes and many household materials. Compounds of Zn to have dental and medical applications[40]. Zinc is essential to plant growth and plays some physiological roles in animals including humans. Deficiency of Zn results to anaemia. Excessive intake or exposure of Zn may cause damage to the alimentary canal. Vomiting, dehydration, abdominal pains, diarrhea, excessive sweating and weakness are other complications associated with Zn exposure.

1.3 Phytoavailability of PTE in soil

It is a well-known fact that the behaviour of PTE in the environment depends on the form in which they occur.[54] As a result total concentration of PTE in soil does not provide reliable information concerning their risk of toxicity to plants or animals. Only a portion of the total concentration becomes available in soil for potential uptake by plants. This phenomenon is referred to as phytoavailability and by definition, may be considered as the fraction of the total concentration of the element present in a specific environmental compartment that, within a defined time period, is either available or can be made available for uptake by plants.[55] The PTE associated with such compartment(s) or phases include weakly adsorbed metals that are retained on the solid surface by electrostatic interaction and/or those released by ion exchange processes.[55] Phytoavailability has been widely estimated in soils by the use of various chemical extractants which include neutral salts, mild acids, organic extractants etc.[56] Besides single extraction procedures, sequential extraction methods have also been used to predict PTE phytoavailability in soils.[57] A number of soil properties/processes are known to control the availability of PTE in the soil, and more importantly, their transfer to plants and accumulation.[58] The properties pH, organic matter content, cation exchange capacity, redox conditions and

adsorption/precipitation are the main parameters that control PTE availability to plants in soils.[59-61] These factors are considered in detail in sections 1.3.1 to 1.3.5.

1.3.1 pH

The pH can directly or indirectly alter chemical processes, and eventually determine the behavior of PTE in soil.[59] Many researchers have demonstrated the influence of pH on metal availability in soils. Evans *et al*[62] undertook studies on the effect of pH changes on the concentration of Cd, Co, Cr, Cu, Ni, Pb, V and Zn in soils amended with biosolids (sewage sludge). They found that the solubilities of all the PTE increased in the soil remarkedly as the pH values decreased below pH 5. Pinto *et al*[61] assessed the effect of physicochemical properties in intensive agricultural soils and reported that, when soil pH tends to be acidic, PTE availability is enhanced, probably as a result of replacement of cations on the surface of soil binding sites with hydrogen ions. On the other hand, soil alkalinity is most undesirable for phytoavailability[26] as it decreases the solubility of PTE in soil. The study by Silveira *et al*[63] was in agreement with Donahue[26] who reported that the mobility and subsequently availability of PTE (except As, Mo, and Se) is decreased with increasing pH values due to their precipitation as insoluble hydroxides, carbonates and organic complexes.

1.3.2 Organic matter content

Soil organic matter, according to Stevenson[29] includes the total organic compounds excluding undecayed plants and animal tissues, their ‘‘partial decomposition’’ products, and the biomass. The composition of organic matter has already been discussed in section 1.2.1. It is an important parameter that influences the mobility and availability of PTE in soils. The amount of organic matter present significantly influences PTE availability in soil. Pinto *et al*[64] confirmed that high amounts of organic matter immobilises both anionic and cationic metal species. However dissolved organic matter, which consists of low molecular weight compounds, such as amino acids, sugars and polyphenols, may be linked to increased plant available PTE.[65] Dissolved organic matter may suppress PTE adsorption onto the surfaces of soil by effectively competing for free metal species, forming organo-metallic complexes, or by being adsorbed onto the surfaces in a preferential manner in

competition with the PTE.[66] Dissolved organic matter can be taken up easily by plants together with the bound PTE,[67] and this can increase phytoavailability. The fraction of the SOM to which the PTE is bound is an important factor that drives this process. To this end, Tan[68] explained that the fulvic acid fraction readily forms soluble metal chelates due to its solubility in water, low molecular weight and higher content of functional groups and therefore can serve as a carrier of PTE, thereby enhancing their availability to plants. It may be appropriate to see organic matter as a "double-edged sword'' in that its overall effect on metal availability will depend on the solubility of the organo – metallic complexes formed.

1.3.3 Cation exchange capacity (CEC)

Cation exchange capacity is an important soil property that is related to the ability of soil to retain metals.[64] It is the estimation of negatively charged sites[69]. The surface charges are usually neutralized by the electrostatic attraction of cations. These cations which are held electrostatically on the soil mineral surface can be replaced by other cations from the soil solution.[70] Soils with large amount of negative charge have high CEC with corresponding low mobility of cations, and hence less PTE available. Clay minerals have high negative surface charges in soil and are the major contributor to their CEC especially in mineral soils. Cation exchange capacity is greater in the 2:1 clays such as montmorillonite (80 to 100 cmol kg^{-1})[59] compared to the 1:1 clays such as kaolinite whose CEC values range from 2 to 16 cmol kg^{-1}.[69] Cation exchange capacity is also influenced by the amount of soil organic matter and pH.[71] The capacity of soil to adsorb cations can be determined by measuring the amount of NH_4^+ retained after displacing other cations in the soil. After this the excess NH_4^+ is washed off the soil, and the remaining NH_4^+ is displaced with another salt solution such as KCl (pH 2.5)[70]. The amount of NH_4^+ displaced by KCl give a measure of CEC in the soil.

1.3.4 Redox conditions[72, 73]

Redox is one of the most important parameters that controls metal availability or chemical reactions in soils.[72] Most of the PTE under consideration in this work have more than one oxidation state in the soil environment and are affected by changes arising from reduction-oxidation.[73] Soils which are well-drained are well aerated and

as a result, oxidizing while, waterlogged soils are reducing in nature. A typical range of redox conditions in soil as reported by Mclean and Bledsoe[73] at pH 7 is as follows: oxidised soils (> +400 mV); moderately reduced soils (+400 to +100 mV); reduced soils (+100 to -100 mV) and highly reduced soils (-100 to -300 mV). This results in change in oxidation of trace metals which in turn affect their mobility and phytoavailability in soils. The behaviour of Cr well illustrates the effect of redox conditions on metal availability and mobility; Cr^{6+} is toxic, mobile and available, whereas Cr^{3+} is less toxic, insoluble and adsorbs on surfaces thereby reducing its availability. Arsenic can undergo reduction from AsO_4^{3-} to a more toxic AsO_3^{3-} and could also be converted to a more volatile form and removed from the soil system.[72] Iron 3+ precipitates as a highly adsorptive solid phase, $Fe(OH)_3$, whereas, Fe^{2+} is more soluble and does not retain other PTE.[73] Neal[72] further summarised the effect of redox conditions on availability of PTE in soils as follows: "under reduction conditions, soil pH typically tends towards neutrality, decreasing metal availability of metals in acid soils. That in strongly reduced soils, metal availability may be reduced by precipitation as low solubility sulfide minerals or, in less strongly reducing environments, as carbonates". Yoo and James[74] carried out a study on Zn extractibility and uptake by rice in flooded silt loam soil that was amended with biosolid. They reported that reduced conditions depressed the phytoavailability of Zn and enhanced non-labile phases in all the soils. However, in the unflooded soils, Zn levels in non-exchangeable forms were not affected on addition of biosolid.

1.3.5 Adsorption

According to Stumm in Sparks *et al*[25], adsorption may be referred to as the accumlation of matter or a material at an interface between the solid surface and the bathing solution. However when the retention mechanism is unknown, a general term, sorption is preferably used. Adsorption is an important chemical process in soil that is capable of determining the amount of metals, nutrients and other chemicals retained on soil surfaces, their availability and mobilities.[31]

Different mechanisms are involved during the adsorption of metal ion onto the surface of the soil particles. These are both physical (non specific adsorption) and chemical (specific adsorption) in nature. Non specific adsorption is decribed traditionally in terms of electrostatic interactions where the metal ion forms outer-

sphere complexes with the surface functional groups at a certain distance from the surface.[20, 31, 73] Here, a water molecule is present between the surface functional group and the bound ion or molecule. Cations from the pore water are easily exchanged for those near the surface. Because the bonding is electrostatic in nature, it is very weak and the ions involved in this mechanism can be easily available. The process is usually reversible, and occurs only on surfaces of opposite charge to the adsorbate.

Specific adsorption on the other hand may be defined as surface complexation interactions involving formation of inner-sphere surface complexes of the metal ion and the respective functional groups with no water molecule being present between the ion or molecule and the functional groups to which it is bound. This can result in a stable unit.[20, 25, 31, 73] Specific adsorption brings about strong and irreversible binding of PTE with organic matter and variable charge minerals.[20] As a result, metal ions adsorbed through specific adsorption tend to be less available compared to those bound by cation exchange.

Apart from adsorption, surface precipitaion, which involves growth of a new solid phase may occur and metals precipitate as oxides, hydroxides, sulfides and phosphates onto soils and become non available. Similarly metal ions that are specifically adsorbed onto clay minerals, metal oxides could diffuse into the lattice structures of these minerals and get "stuck" in these spaces which may require total dissolution of the particles to make them available[20]

1.4 Extraction of PTE in environmental solid samples

Soil, an important environmental component is a medium in which contaminants such as PTE are deposited through various natural and anthropogenic activities.[75] Due to the toxic nature of these elements to plants and animals, it is therefore important to explore various approaches employed in the determination of the occurence of PTE in soil. Some of these approaches are shown in Figure 1.7 and are outlined in 1.4.1 to1.4.4.

1.4.1 Total digestion

Total metal content of soil takes into account the geological origins and anthropogenic inputs such as contaminants from industrial processes.[55] The common reagent used in this approach is hydrofluoric (HF) acid. This reagent results in complete dissolution of the solid material, and guarantees the release of all the PTE, including those bound to the silicate. Under normal environmental conditions, release of these metals is unlikely and could exert minimum effects on plants and animals.

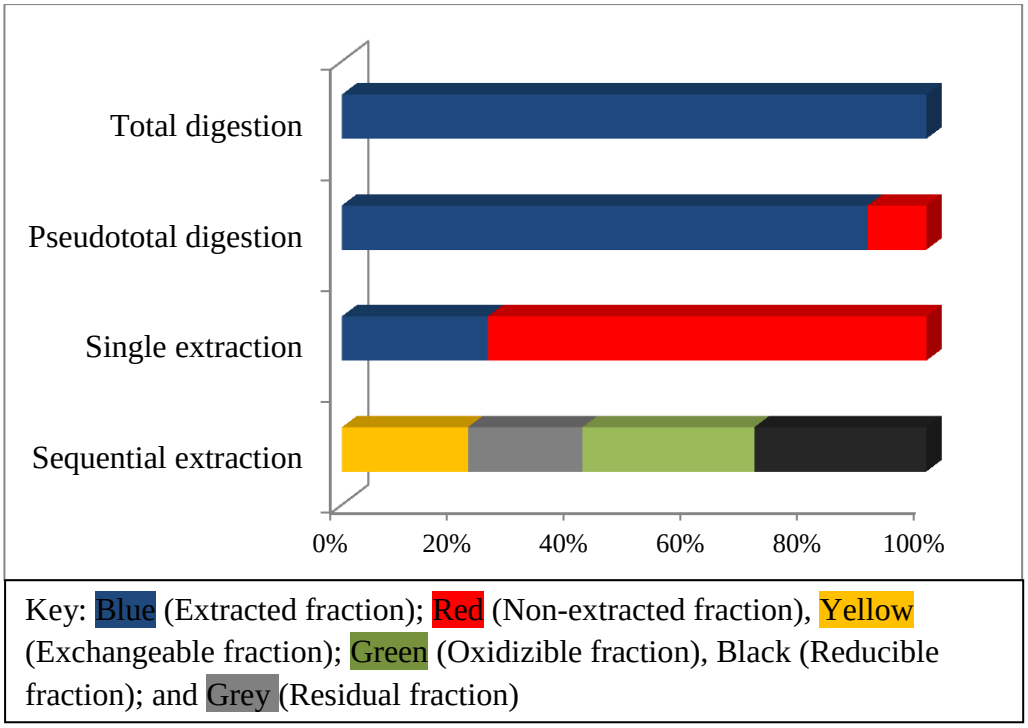


Figure 1.7 Some approaches to the determination of PTE in soil samples (after[76])

1.4.2 Pseudototal digestion

Pseudototal digestion is better approach to assessing risk of PTE to the environment. Pseudototal digestion gives an indication of the maximum potentially soluble or mobile concentration of PTE, usually not bound to silicates. A mixture of hydrochloric and nitric acids in the ratio of 3:1 (*aqua regia*) used for the dissolution of the matrix and release of the bound PTE in solution. Both total and pseudototal approaches may be performed using beakers containing water and heated on a hot

plate in a fumehood.[76] Microwave assisted digestion system (detail discussion can be found in the next chapter) are now available for this purpose – it is fast, and provides a uniform heating throughout the mixture, and minimises loss of material.

1.4.3 Single extraction

The partitioning of PTE among soil phases is important in assessing the possibility of the soil to supply the required micronutrients for plant development and to retain these PTE in soil.[77] Ethylenediaminetetraacetic (EDTA) and is a common reagent that can be used for the purpose of estimating potential plant available PTE in soil . Alvarez *et al*[77] and Marques *et al*[78] have successfully employed EDTA to extract a suite of PTE which include As, Cd, Cu, Mn, Ni, Pb, and Zn. Other reagents have been reported.[79-81] Most of these extractants are not specific and may attack more than one site or partially release the target PTE.[55] Despite the fact that the specificity of these reagents may not always be guaranteed, the concept of single extraction approach remains useful and it is among one of the few methods for predicting mobilisation and uptake of PTE by plants in soil.

1.4.4 Sequential extraction

Sequential extraction involves the use ofa series of less or more aggressive chemicals to liberate PTE from different fractions that are responsible for retention of these elements in soil and other solid substrates. Bacon and Davidson[57] concluded that the treatment starts with mild conditions which involves shaking with water, salt solutions or dilute acetic acid, up to strong mineral acids. These fractions are operationally-defined, and it implies that specific mineral phases may not be attacked.[57] The behaviour of PTE in the environment is dependent on the chemical form in which the PTE occur.[82, 83] The nature in which the PTE are bound to these fractions determines their toxicity, mobility, and availability to plants or animals through the food chain. The first sequential extraction procedure was developed and reported by Tessier *et al*[84] where they used a five-step approach with the following fractions: exchangeable, carbonates, Fe and Mn oxides, oxidisable and residual. Although this scheme was developed specifically for fractionation of PTE in sediments, it has since been extended for PTE fractionation in soils and other

environmental solid samples. In 1987, the Measurement and Testing Programmes formerly BCR (The European Community Bureau of Reference) sponsored several projects that focused on harmonising sequential extraction schemes which were all based on the Tessier's scheme.[85] By 1992, a three-stage sequential extraction protocol using 0.5 M acetic acid (step 1); 0.1 M hydroxylamine hydrochloride acidified with 2 M HNO_3 (step 2); and 8.8 M H_2O_2 (step 3) was developed. The BCR sequential extraction protocol underwent modification as a result of systematic uncertainty sources,[86, 87] such as the type of acid used in adjusting pH, temperature and duration of extraction – hydroxylamine hydrochloride (step 2) concentration was increased to 0.5 M, pH adjusted to 1.5, by adding fixed volume of HNO_3 and centrifugation speed increased from 1500 to 3000g. In addition to these, the residue from the third stage was recommended to be treated with *aqua regia* – which serves as a fourth stage, but in practice, as an internal check where the sum of the steps are compared with the pseudototal concentration of the soil to assess the effectiveness of the sequential extraction. Several pitfalls have beed identified with the use of sequential extraction protocols as summerised by different workers.[55, 57, 85, 88] These include

i) Non-selectivity of reagents as they may be influenced by experimental conditions;
ii) Possibility of labile fractions being transformed during sample preparation and sequential extraction schemes application
iii) Analytical problems arising from low level of PTE to be measured
iv) Incomplete extraction
v) Re-adsorption and redistribution of analytes among phases during extraction process.

These challenges notwithstanding, sequential extraction procedures have provided meaningful information in predicting PTE mobility, bioavailability, uptake by plants in assessing contamination risk.

1.5 Review of related works

1.5.1 Potentially toxic elements in fertilisers

A summary of the actual concentrations (mg kg^{-1}) measured in the various fertilisers reviewed in this study are presented in Table 1.3. The measurement of PTE in fertilisers may be motivated by interest in micronutrients for the growth of plants.[2] Due to increasing concern over soil pollution and disposal of urban, agricultural and industrial wastes, PTE have become one of the major sources of environmental and health problems.[2] The accumulation of PTE in fertilisers, agricultural and other related soils has been extensively researched. Raven and Loeppert[2] evaluated the elemental composition of twenty four varieties of fertilisers of organic origin: corn leaves, cow manure, two composted cattle manure and sewage sludge samples and materials inorganic origin-ranging from nitrogen, potassium and phosphate based fertilisers. The PTE concentrations studied included As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn. Phosphate fertilisers were highest in PTE content followed by sewage sludge and the organic amendments. The potassium and nitrogen based fertilisers contained the least amount of the PTE. The authors claimed that the relatively high concentration of PTE in sewage sludge and phosphate based fertilisers should be a reason to consider these materials as the primary target materials for environmental evaluations.

To determine the level of As, Cd, Cr, Cu, Hg, Ni, Pb and Zn in organic based fertilisers, Nicholson *et al*[89] investigated the metal content of a range of animal manures – poultry, cattle, and pigs – in England and Wales. Concentrations of Cu (80 mg kg^{-1}) and Zn (400 mg kg^{-1}) were reported in poultry manure samples. Arsenic, Cd, Cr, Ni and Pb concentrations were reported to be generally less than 10 mg kg^{-1}. However, relatively high concentration of As (40 mg kg^{-1}) and Cr (70 mg kg^{-1}) were found in two broiler/turkey litter samples. The authors explained that one was obtained from a unit where high concentration of As and Cr were found in two of the bird's feed samples with virtually no indication of high amounts of these metals in feeds from the other source. No explanation was put forward to why the feeds had high concentrations of As and Cr. The elevated concentrations of Cu and Zn compared to other PTE in the samples were attributed to the fact that Cu (mostly in

the form of $CuSO_4$) and Zn (in the form of ZnO) were added to animal diet as supplements.[90]

Table 1.3 A summary of the actual concentrations (mg kg^{-1}) measured in the various fertilisers reviewed in this study

Organic fertiliser materials	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn	Reference
Sewage sludge	9.40	3.30	106	300	24600	430	36.7	86.9	6.19	563	Raven and Loeppert[2]
Sewage sludge	ND	7.20	2940	ND	58200	142	31.2	130	1.79	450	Raven and Loeppert[2]
Corn leaves manure	2.50	0.300	< 0.860	9.40	85.5	276	3.20	0.700	<0.130	192	Raven and Loeppert[2]
Cow manure	6.8	0.700	ND	17.5	ND	172	9.60	7.50	ND	ND	Raven and Loeppert[2]
Compost	5.20	0.400	14.4	ND	6460	357	8.70	5.40	1.65	164	Raven and Loeppert[2]
Poultry manure	4.73	0.740	10.9	80.0	NR	NR	6.25	6.00	NR	400	Nicholson *et al*[89]
Chicken manure	47.0	1.84	81.0	89.0	NR	624	17.5	11.1	NR	417	Cang *et al*[80]
Pig manure	33.0	0.8.00	46.0	399	NR	452	9.51	12.8	NR	506	Cang *et al*[80]
Seaweeds	NR	0.220-0.450	0.800-1.80	4.80-9.40	65.0-616	276-788	0.100-0.700	0.100-1.10	NR	12.9-17.7	Giusti *et al*[91]
seaweeds	7.00-242	0.100-0.400	0.500-4.60	2.00-13.0	171-1030		1.10-4.6	1.60-7.30	NR	38.0-248	Caliceti *et al*[92]
Seaweeds	NR	0.105-0.598	0.054-1.07	NR	NR	NR	NR	0.118-2.11	NR	NR	Morrison *et al*[93]

ND = Not detected; NR = not reported

Table 1.3 Continued…..

Inorganic fertiliser materials	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn	Reference
NPK fertilisers	NR	NR	NR	<1.00-261	<100-261	<1.00-875	NR	2.00-650	NR	NR	Otero *et al*[5]
Diammonium phosphate	NR	9.10	70.8	17.3	438	1830	67.0	24.5	NR	142	Unsal *et al*[94]
Compound fertiliser (NP: 20 :20)	NR	36.0	302	4.90	963	141	201	3.80	NR	141	Unsal *et al*[94]
Urea	<0.400	<0.200	ND	<0.600	ND	0.300	<0.200	<0.400	ND	ND	Raven and Loeppert[2]
Ammonium sulfate	<0.4.00	<0.200	ND	<0.600	ND	0.4.00	<0.200	<0.40	ND	ND	Raven and Loeppert[2]
Potassium chloride	<0.4.00	<0.2.00	<1.05	3.50	1440	5.30	<0.200	1.00	<0.98	8.75	Raven and Loeppert[2]
Monoamonium phosphate	13.7	4.00	16.9	13.2	5050	433	22.2	3.70	5.82	10.3	Raven and Loeppert[2]
Triple superposphate	16.2	6.20	88.9	3.50	17300	298	25.2	13.2	232	61.3	Raven and Loeppert[2]
Rock phosphate	20.5	48.8	140	9.60	47300	6700	50.4	29.2	79.3	382	Raven and Loeppert[2]
Rockdust	NR	0.01	5.61	8.17	20.2	297	10.2	1.90	NR	48.5	Ramezanian *et al*[95]
Rockdust	NR	0.039	12.0	7.30	31.0	375	9.7	2.50	NR	46	Ramezanian *et al*[96]

ND = Not detected; NR = Not reported

In a related study, Cang *et al*[80] investigated PTE pollution in poultry and livestock feeds and manures under intensive farming in Jiangsu Province, China and reported that the concentrations of Cd, Cr, Cu, Pb and Zn in animal manure were also high, stressing that Cu levels in one of the manures (pig) reached as high as 1726 mg kg^{-1}. They confirmed that fortification of animal feeds with metal compounds can result in increase in PTE concentration in animal manures, therefore metal levels in animal feeds must be controlled as soon as possible, and PTE accumulation ability be considered at the same time.

The accumulation of PTE by other organic materials used as fertilisers such as seaweed is also known.[91, 92] In UK, brown seaweeds obtained from sites situated off the coast of the rural part of Northumberland has been reported to contain PTE in the range of Cd (0.22 to 0.45 mg kg^{-1}), Cr (0.80 to 1.80 mg kg^{-1}), Cu (4.80 to 9.40 mg kg^{-1}), Fe (65.0 to 616 mg kg^{-1}), Mn (276 to 778 mg kg^{-1}), Ni (0.10 to 0.70 mg kg^{-1}), Pb (0.10 to 1.10 mg kg^{-1}), and Zn (12.9 to 17.7 mg kg^{-1})[91] These values were lower than those reported by Caliceti *et al*[92] when they investigated the level of metal contamination in seaweeds obtained from Venice lagoon. Morrison *et al*[93] also assessed metal contamination using seaweed *Ascophyllum nodosum* in six locations and reported various concentrations of Cd, Cr, and Pb. Of the mean concentrations of the metals, Pb was the highest with a range of 0.118 to 2.11 mg kg^{-1}. The determination of PTE in various seaweeds can therefore be an effective approach not only to assessing the degree of contamination of the coastal environment but also to provide adequate information regarding suitability for application to soil as a fertiliser.[93, 97]

Otero *et al*[5] gave a comprehensive chemical characterisation of different commercial inorganic fertilisers in Spain. The authors indicated that compound fertilisers employed for foliar application had low concentrations of PTE, whereas those used for basal and top dressing contained the highest amounts of PTE and other elements. They claimed that the high content of metals of environmental concern such as As, Cd and U in the fertilisers could be due to their phosphate content.

The concentrations of Cd, Cu, Mn and Pb in sixteen NPK fertilisers imported into Serbia have also been reported.[98] The content of PTE showed variation in the various samples depending on the ratio of N:P:K and source. An NPK (15:15:15) fertiliser

imported from Romania contained the highest amounts of Cd and Pb. Fertilisers obtained from hungary were predominantly highest in Cu content – a range of 7.1 to 975 mg kg^{-1} of Cu in coloured NPK fertilisers from Hungary, Netherlands and Greece was reported. The authors further reported that Mn in one of the Hungarian NPK products (10:10:20) showed a concentration of (9570 mg kg^{-1}) which exceeded the average concentration of Mn in soil (800 mg kg^{-1}) suggesting the need for regular characterisation of fertiliser products in order to minimise surface and ground water contamination.

In their study on sequential extraction of PTE in some inorganic fertiliser samples – diammonium phosphate, DAP (18% N:46% P as P_2O_5) and a compound fertiliser (20% N:20% P as P_2O_5) – Unsal *et al*[94] reported that both fertilisers contained high concentrations of Cd, Cr, Fe, Mn and Zn with the values of Cd and Mn as high as 36.0 mg kg^{-1} and 1830 mg kg^{-1} in the compound fertiliser and DAP fertiliser respectively. This confirms the need for continuous monitoring of PTE in fertiliser products. Rockdust which is widely available as a by-product of quarrying operations, (typically of 90% particle size less than 0.074 mm size)[99] has been in increasing demand for use as a low cost and locally available fertiliser.[95] Ramezanian *et al*[96] in their work which included the effect of rockdust on soil chemistry and microbial community composition reported low levels of Cd, Cr, Cu, Fe Mn, Ni, Pb and Zn with Mn showing the highest concentration of 345 mg kg^{-1} in the sample. Studies on the measurement of PTE in rockdust are lacking and this is one of the few available in literature.

1.5.2 Potentially toxic elements in soil amended with fertilisers

Soil is an important resource at Man's disposal – it produces food and other materials that are of benefit to human life.[100] It remains the reservoir for all kinds of wastes disposal, agricultural, industrial and animal. Fertilisers and many other agrochemical substances are extensively applied in small or large quantities depending on their purpose on garden, allotment, agricultural and urban soils. As indicated in section 1.5.1 these materials may contain PTE in varying amounts and under extensive and persistent use, the PTE can accumulate in soil.

Fertilisers when added to soils may not only increase or affect the total concentrations of PTE in the soils but can modify their mobility, phytoavailability and distribution in the soil environment.[101] Therefore in addition to assessing the pseudototal PTE concentrations in these materials and soils, interest has grown in estimating their bioavalable fractions since behaviour and potential risks to plants and human health depend on the form in which PTE occur.[57] Han *et al*[100] reported that PTE in soils amended with various organic wastes are redistributed and transferred with time from the available fraction to a more stable form, and the redistribution phenomena depends on the source, amount added, soil properties such as pH and other processes.

Organic fertilisers with low concentration of PTE such as biosolids have been reported to decrease phytoavailability in soils. As soon as organic amendments are added to soil, PTE tend to accumulate, as total removal is small.[102] The bioavailability of PTE to plants in the applied material, particularly manure, may not remain the same over a given period of time but tends to decrease[103] through the formation of bonds with metal oxides. Interactions of PTE with Fe may contribute significantly to reduced plant availability in soil.[104] Organic components in fertilisers such as manure has been reported to have high affinity for metals in soil due to the presence of functional groups that can bind these metals.[79] With increasing pH, the carboxyl, phenolic, and cabonyl functional groups in the material disssociate, and this could result in increased affinity for metal cations leading to reduction in phytoavailability. However, the availability of PTE to plants may be enhanced significantly in soils receiving dissolved organic carbon because of increased amount of soluble metal-organic complex in solution which may result to increase in the concentration of metal ions taken up by plants. Inorganic fertiliserss according to Puschenreiter *et al*[105] have been found to reduce plant availability of PTE in soils. In their study on effects of inorganic and organic fertilisers on the mobility of Cd, Cu, Pb and Zn in contaminated soil, Janoš *et al*[101] also reported that the addition of inorganic fertilisers to soil can effectively reduce plant available PTE even at relatively low doses.

Kidd *et al*[106] conducted a pot experiment to evaluate plant production, PTE fractionation and plant availability in an agricultural soil using sewage sludge, and

reported increased concentrations of EDTA-extractable Cu and Zn on fertiliser addition. Increase in the total metal and acid exchangeable fraction Cu, Mn, Zn and Mn, Zn respectively when compared with the control soil was observed. Similarly, they reported that the reducible Mn and Zn, and the oxidisable Cu and Zn fractions increased in the amended soil. The effects of chicken manure and composted pig manure mixed with rice straw on plant available Cd, Cu, Pb and Zn have been reported.[107] In this study, six different application rates from 0 to 450% represented the normal annual dose used by farmers from peri-urban areas of Hanoi, Ha Tay and Vinh Phuc provinces, Vietnam. The application of both amendments resulted in increased EDTA-extractable Cd, Cu and Zn.

Baldantoni *et al*[108] applied compost over a long term regime to evaluate total and available PTE in two different agricultural soils. Total and plant available (DTPA-extractable) Cd, Cu, Pb and Zn in both soils generally increased in accordance with the compost rates. The soil characterised by a higher clay content and lower organic matter content exhibited a lower increase in plant availability.

Carbonell *et al.*[109] conducted a pot experiment using municipal solid waste (MSW) and NPK ferilisers and reported that the plant available Cu, Pb and Zn fractions in the original soil increased with addition of MSW while the NPK fertiliser increased Cd and Ni levels. In their study to evaluate the effetiveness of fertilisers on PTE stabilisation by chemical and biological methods, Lee *et al*[110] applied four different amendments; zero valent iron, limestone as acid mine drainage treatment sludge, bone mill and bottom ash as organic materials on contaminated sites in the Suseong gold mining area, Chungnam, Province, Korea. They reported that addition of thesematerials, especially limestone and bottom ash, resulted in a significant decrease in the extractability and mobility of the studied PTE, Cd, Pb and Zn.

1.6 Soil guideline values (SGVs) and standards

Soil guideline values/standards according to Environment Agency, are scientifically based generic assessment criteria that can be used to simplify the assessment of human risks arising from long-term contamination in soil. They are values used for identifying areas which are associated with low or high contaminants – they give an indication of the concentration of these contaminants in soil below which the long-

term health risk are likely to be minimal. The guidelines or standards developed over the years by the years by (Table 1.4), the UK's Contaminated Land Exposure Assessment (CLEA) SGVs, which replaced UK's Inter-Departmental Committee on the Redevelopment of Contaminated Land (ICRCL) threshold values, the Dutch Intervention values for soil and Czech legislative soil limits were adopted for use in this study. The UK CLEA SGVs were used for the fact that the soils used in this work were obtained from this part of the world (UK). In addition reference was made to Dutch Intervention values for soil andCzech legislative soil limits because they are amongst some of the European national guidelines used by many other workers.[111]

Table 1.4 Typical concentration ranges, common values and soil guideline values (SGVs; mg kg^{-1})

PTE	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Typical range[32]	0.01-2.0	0.01-2.0	5-1500	2-250	7000-42000	20-10000	2-750	2-300	1-900
Common values[32]	0.1-1	0.1-1	70-100	20-30	-	1000	50	10-30	50
Abundance in Earth's soils[112]	5.00	0.500	200	20.0	38000	850	40	10	50
SGVs									
	43.0*	1.80*	130*	190**	-	-	230*	450*	720**
	24.0***	-	105***	70.0***	-	-	59.0***	71.0***	141***

*SGVs reported by UK CLEA **Dutch soil intervention values ***Czech legislative soil limits; reported in Uprety *et al*[111]

1.7 Conclusions

The concensus knowledge in this field of study has remained that the addition of inorganic or organic fertilisers to soil can alter the lability and distribution of these PTE, and subsequently enhance or limit their availability to plants.[113] Some of the works reported in section 1.5.2 have attested to this fact. However several workers[111, 114-117] applied fertilisers to soil without first assessing the distribution or lability of PTE in the added materials. Rather they only reported their total metal concentrations. It must be noted again that total PTE concentrations are not the best indicators for their availability, and that total concentrations are not sufficient to assessing risk, given that toxicity and mobility depend on the forms in which the PTE occur.

1.8 Aims and scope of the study

The overall aims of this study were to assess the effect of fertilisers on levels, mobilities and PTE availability to plants. In order to achieve this single extraction (EDTA) and sequential extraction (the modified BCR protocol) were used to assess phytoavailability and distribution of the PTE in the fertilisers, and amended soils. The study therefore involved:

i) Amendment of a top commercial garden soil with five fertilisers and extraction using EDTA and BCR protocol.

ii) The assessment of effect of fertilisers on mobilities of PTE in an urban park soil using soil columns.

iii) The investigation of uptake of PTE by two vegetables plants (runner beans and radish) in the urban park soil amended with chicken manure and growmore fertilisers.

Chapter 4 describes an investigation of PTE concentrations, phytoavailability, fractionation in five fertilisers, and a commercial garden top soil treated at various dosages with the materials. The samples were subjected to EDTA and the modified BCR sequential extraction procedure. Student t-test, F-test and analysis of variance (ANOVA) were used to establish whether significant differences occurred among the different treatments. Chapter 5 describes the effect of chicken manure and growmore

fertilisers on mobilities of PTE an urban park soil. In addition, EDTA and BCR sequential extraction of PTE in the unleached and leached amended soils were presented. Chapter 6 describes the uptake of PTE by runner bean plant in the urban park soil amended with chicken manure (experiment 1). Further investigation of uptake of PTE by radish vegetable plant in another set of urban park soil amended with chicken manure or growmore or both fertilisers in Autumn as (experiment 2) was performed.

2 Theory of experimental techniques

2.1 Introduction

The chapter describes the fundamental theory of the main instrumental techniques that were used in this research work; microwave-assisted digestion and inductively coupled plasma-mass spectrometry (ICP-MS) for the determination of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, U and Zn in the environmental sample digests and extracts.

2.2 Microwave-assisted digestion

The determination of PTE in nearly all environmental samples by ICP-MS generally requires the conversion of the sample prior to instrumental analysis into aqueous solution before introduction into the plasma.[118, 119] This involves the application of microwave energy to acid solutions which are directly heated by coupling the digesting solutions to the source of the energy.[119]

2.2.1 Theory of microwave heating

Microwaves are a form of electromagnetic radiation (Figure 2.1)**.** This occurs in the region between infrared and radio frequencies (300 MHz and 300 GHz which corresponds to wavelengths of 1 cm to 1 m).

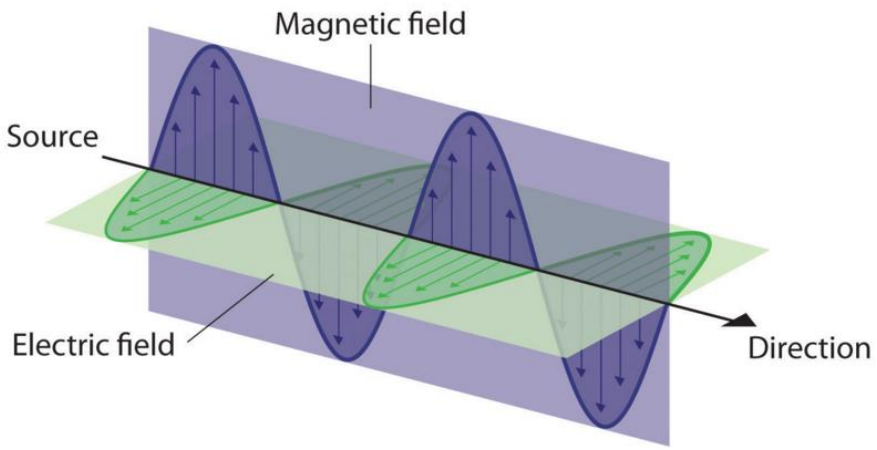
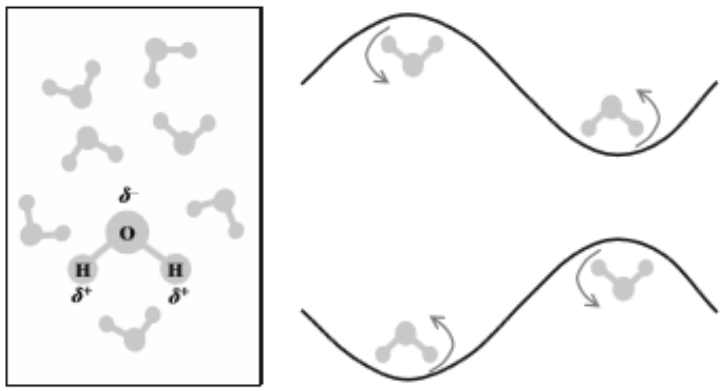


Figure 2.1 Schematic diagram of an electromagnetic wave[120]

Microwaves can be transmitted, absorbed or reflected by some dielectric materials. Most laboratory microwave systems are usually operated at 2.45 GHz. Two basic

phenomena occur when a material absorbs microwave energy during heating; dipole rotation and ionic conduction (or migration).

Dipole rotation. This mechanism is described as the effect that an oscillating electric field, which results from microwaves, causes in induced or permanent dipolar molecules.[121] When the electric field is removed (Figure 2.2A), there occurs a rapid reversal of the molecules (phase lag) to the disordered state (in a relaxation time), leading to dissipation of heat energy in the material. The molecular dipoles align with the electric field (Figure 2.2B). The alignment of molecules and their return to a disordered state for a microwave operating at 2.45Hz is shown to occur 4.9×10^9 times/second and results in fast heating.[121-123]



A B

Figure 2.2 Rrepresentation of the alternating alignment of water molecules under the oscillating electric field induced by microwaves; (A) without influence of electric field (B) under influence of electric field generated by microwaves[121]

Ionic conduction. This process involves the interaction between the oscillating electric field and ions (Figure 2.3). These interactions give rise to movement of charge particles and they oscillate randomly under the influence of the electric field of the microwave radiation. Free flow of the ions is resisted due to the presence of other species or collision with their neighboring molecules or atoms.[121] As a result, heat is generated with a corresponding increase in temperature. As the temperature increases, the flow of ions increases producing a continuous effect. This effect is considered to be a stronger effect compared to the dipole rotation mechanism with respect to heat intensity produced.

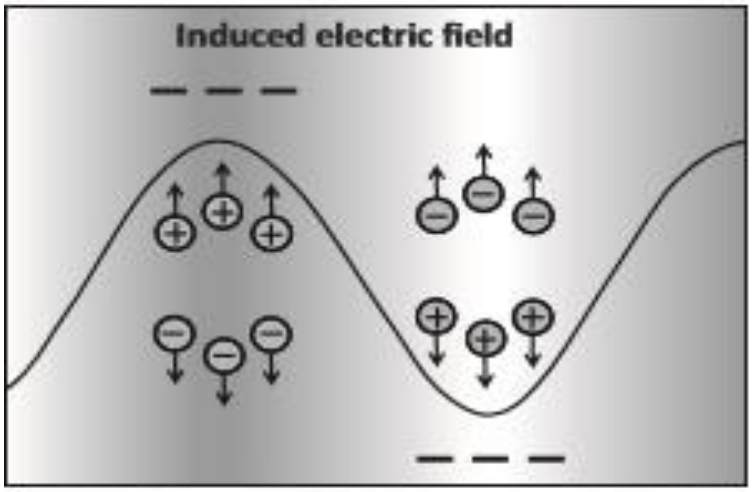


Figure 2.3 Schematic diagram of the ionic conduction process showing positively charged (+) species and negatively charged (-) species under the induced oscillating electric field of microwave radiation passing through the material[121]

Generally, the ability of a material to absorb microwave radiation and subsequently transfer the heat or energy generated to other molecules is defined by Equation 2.1.

$$\tan \delta = \varepsilon'' / \varepsilon' \qquad \textbf{Equation 2.1}$$

Where (tan δ) is the dissipation factor, (ε'') is the dielectric loss (a measure of the efficiency of converting microwave energy into heat) and (ε') is the dielectric constant (a measure of the polarizability of a molecule in an electric field). The higher the dissipation factor, the better the absorption of the microwave radiation and hence energy transfer. For efficient heating, a mixture containing a higher dissipation factor is recommended. On the other hand, vessels with lower values of dissipation factor are more suitable in order to prevent absorption of radiation by the walls of the vessels.

A magnetron is widely used in microwave equipment as the main source of microwave radiation. Figure 2.4 shows the inside of a magnetron. Its principle of operation is based on the motion of electrons under the influence of combined electric and magnetic fields. It is an electron tube which consists of a cylindrical solid copper material (cathode) located at the center perpendicular to the anode.

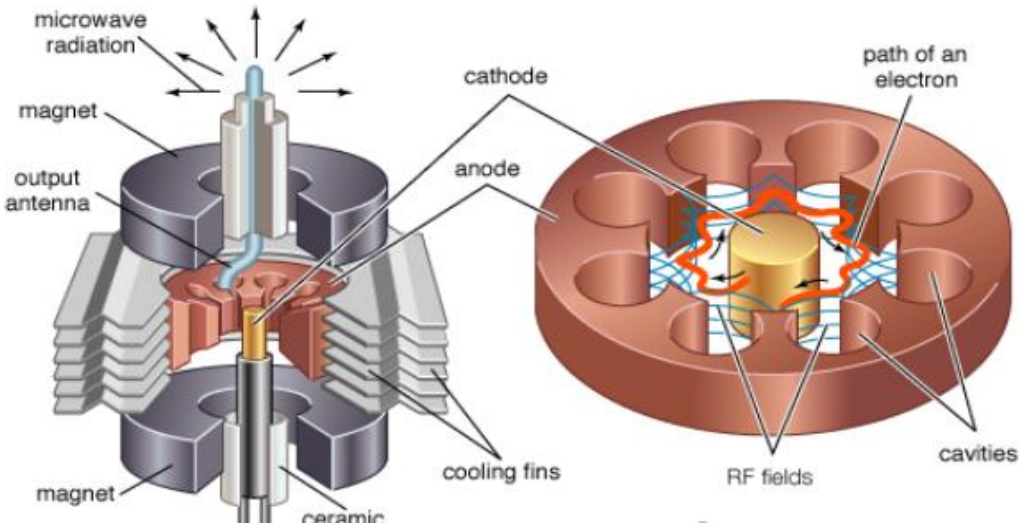
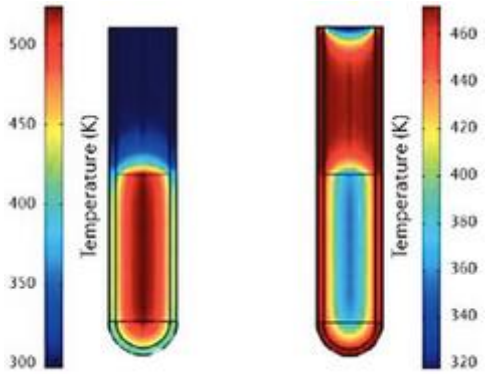


Figure 2.4 A schematic diagram of a magnetron[124]

The anode consists of many cavities. When a high voltage is applied, the cathode gives out electrons as it heats up. These electrons move towards the anode.The presence of permanent strong magnetic field located between the cathode and anode causes the electrons to spiral outward in a circular path instead of moving in a straight line. As the electrons sweep past the resonating cavities, a resonant high-frequency is induced in the cavities. A portion of the field is isolated with the antenna as microwave radiation and harnessed accordingly for heating.

The mechanism of heating using microwave sytems is different from that of conventional heating. In conventional heating, the digestion vessel is heated thermally using an external source and the heat is transferred to the material by thermal convection (see Figure 2.5B). This process is not only slow but the surface of the material tends to be hotter than the inside giving rise to non-uniform distribution of heat in the sample. Microwave assisted digestion offers advantage (s); the material absorbs directly the microwave energy (see Figure 2.5A) through the vessel which results in a more uniform distribution of heat throughout the absorbing material and providing a higher reaction rate. Microwave digestion also requires low consumption of reagents. A number of reagents or acid mixtures are typically used for microwave-assisted digestion technique: HNO_3-HCl (1:3); HNO_3-H_2SO_4; HNO_3-$HClO_4$-HF; HNO_3-$HClO_4$ and HF-HNO_3-HCl.



(A) (B)

Figure 2.5 Heat distribution in digestion vessels, (I) microwave heating and (II) conventional heating[124]

Microwave assisted digestors are generally classified as either closed-vessel or open-vessel systems. Closed-vessel systems are operated under controlled pressure and temperature. However, it is advisable that the pressure generated during sample digestion should be less than the highest permitted for the pressure vessel. The microwave energy is directed into a cavity where the digestion vessels containing the samples are arranged. Figure 2.6 shows a typical closed-vessel microwave digestion system.

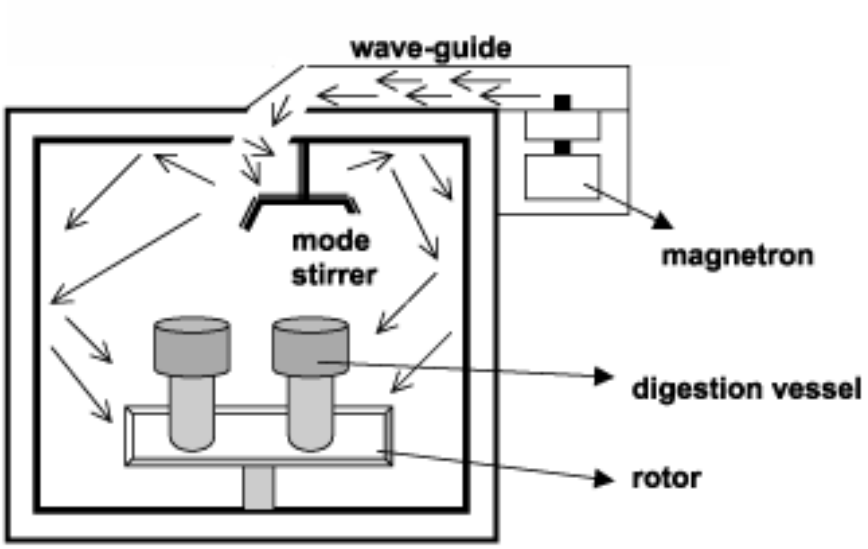


Figure 2.6 Diagram of a closed-vessel microwave assisted digestion system[125]

With the enclosed nature of the system, cross contamination and loss of samples are eliminated compared to the open-vessel microwave digestion systems. In open-vessel

microwave assisted digestion systems, the microwave radiation is usually focused directly where the sample is located in the vessel (Figure 2.7). One major advantage of these systems over the closed system is the absence of pressure "built-up" since gases are expelled from any reaction occuring in the vessel.

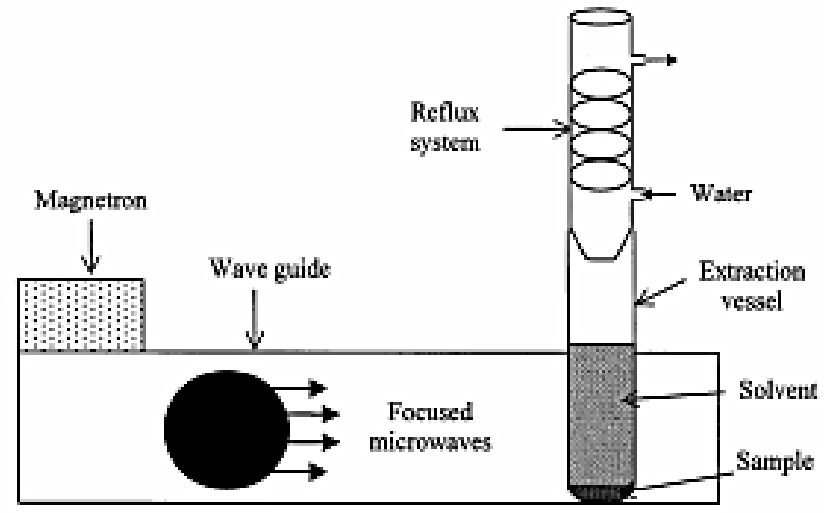


Figure 2.7 Diagram of an open-vessel microwave assisted (focused) digestion system[123]

2.3 Inductively coupled plasma mass spectrometry (ICP-MS)

Inductively coupled plasma mass spectrometry (ICP-MS) is a relatively new analytical technique developed in the 1980's for elemental analysis.[126, 127] Inductively coupled plasma (ICP) which was developed and applied basically as a source of atomic emission in the past decades, is now widely applied in elemental analysis as a source of ions in mass spectrometry.[128] The technique offers a combined simple and fast sample introduction, and short time of analysis. Its high multielement capability and good detection limit explains its wide application in the field of environmental analytical studies.[127] The basic principle of ICP-MS is based on the dissociation of a dilute homogenous sample solution into its constituent atoms or ions in a plasma. The ions generated in the plasma are conveyed into an interface which consists of a sampling cone followed by a skimmer cone.[129] The ions then pass through the interface into the ion optics where neutral species are ejected, allowing the ions to be separated based on their mass/charge (m/z) ratio in the mass analyser, usually a quadrupole, before arrival at the detector.

2.3.1 Instrumentation for ICP-MS

A typical ICP-MS instrument (Figure 2.8) consists of four major components; inductively coupled plasma (ICP), sampling interface, ion focusing and mass analyser, and detector which are critical to the operation of the instrument.

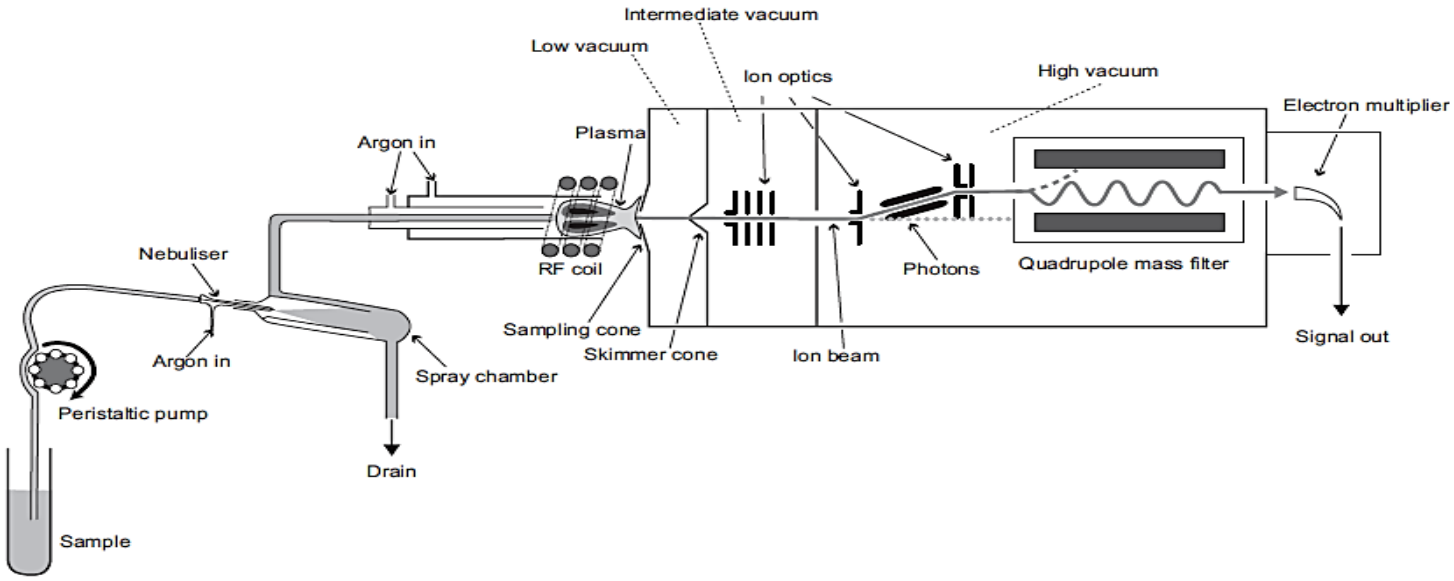


Figure 2.8 Schematic diagram showing the major component of an ICP-MS instrument[126]

2.3.2 Inductively coupled plasma (ICP)

In ICP-MS, the inductively coupled plasma (Figure 2.9) constitutes the source of ionisation. It provides a high temperature in the range of 6000 to 10 000K.[129] It is the co-existence of positive ions, electrons and neutral species of an inert gas (argon) in a confined space.[129] The argon gas is passed into a specially constructed quartz glass ‘’torch’’ which is an assembly of three concentric glass tubes namely the outer, intermediate and inner gas tubes. A water-cooled induction copper coil is placed arround the top of the torch and is connected to a radio frequency generator operated at 27 or 50 MHz producing 1- 2 kW energy.[129]

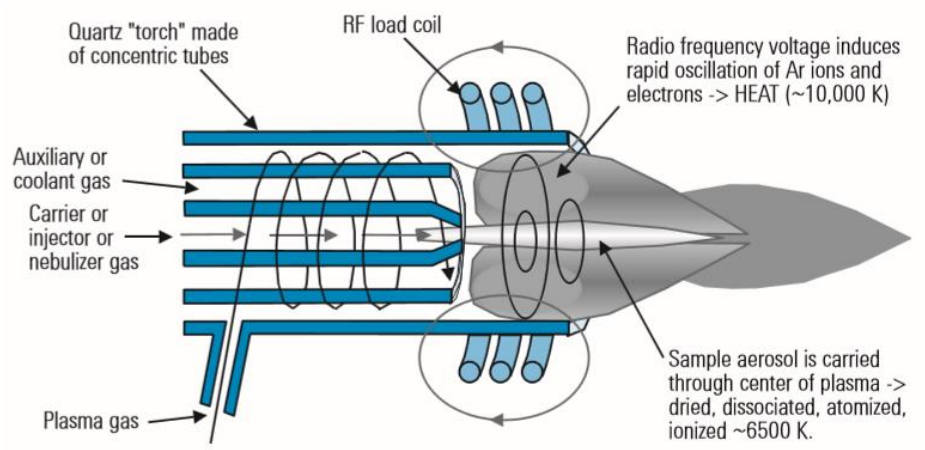


Figure 2.9 Schematic representation of an inductively coupled plasma torch[130]

An oscillating electric and magnetic field is induced around the top of the torch as a result of the power input whose lines of force are axially positioned inside the plasma torch. A spark from a Tesla coil initiates the ionisation of the argon carrier gas. The ions and electrons generated interact with the magnetic field which result to their rapid flow in a circular manner thereby colliding with more argon atoms, stripping them of electrons and causing further ionisation. This process occurs on a continuous basis giving off excess energy as heat and radiation in form of plasma. This plasma can be seen protruding from the top of the torch giving a white luminous bullet-shape plume which is hot and energised enough to atomise and ionise efficiently sample solution introduced as a spray of droplet through a nebuliser and spray chamber.[129]

Three operationally defined zones have been identified in the plasma[131, 132] as shown in Figure 2.10. The inductive region which is located at the base of the plasma allows for the passage of the sample, in the form of an aerosol by the argon carrier gas. The transfer of the inductive energy takes place here. After this region is the preheating zone. This zone allows predominantly for desolvation, vapourisation and breakdown of the sample into component molecules. Then atomisation, dissociation and ionisation take place in the initial radiation zone and the normal analytical zone.

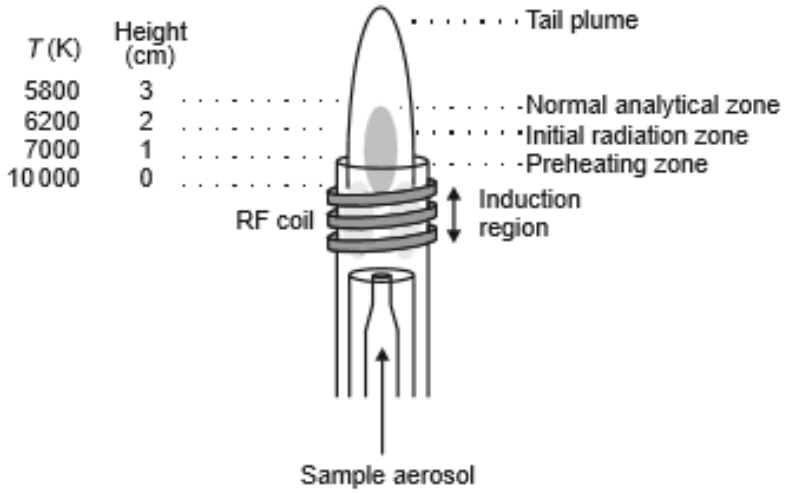


Figure 2.10 Various zones of the inductively coupled plasma[133]

2.3.3 Sample introduction

Sample can be introduced into the ICP as a solid, liquid vapour or gas molecules. Several methods such as nebulisation (use of nebulisers), hydride generation, electrothermal vapourisation, cold-vapour generation, laser ablation and chromatographic techniques have been developed for this purpose.[134, 135, 136] In this research, samples were introduced as liquids and for this reason, emphasis is laid on nebulisation, the most common method of liquid sample introduction. Generally, the liquid sample is first introduced by the use of a peristaltic pump at a constant flow rate of 1 mL min^{-1} into the nebuliser system.

Nebulisers

Nebulisers are devices that convert liquid samples by the action of carrier gas into aerosols which are subsequently transported to the plasma. The different types of nebulisers that are commonly used include; pneumatic concentric nebuliser, cross-flow nebuliser and the Babington nebuliser shown in Figure 2.11.

Pneumatic concentric nebuliser consists of a concentric glass tube through which a capillary tube is fitted. By the action of the argon gas moving at a high speed, the liquid sample is drawn from the capillary tube and introduced through the orifice at the gas exit. The gas exit is located between the outside of the capillary tube and the inside of the concentric glass tube. The interaction between the carrier gas and the liquid is capable of breaking the liquid samples into aerosols. Concentric nebulisers show high sensitivity and stability. However, due to the small gas orifice, they are prone to clogging especially when the sample contains high salt content. The cross-flow nebuliser was designed to reduce the problem of clogging associated with the concentric nebuliser. In this nebuliser, the tips of two capillary tubes are placed perpendicularly to each other, but not quite in contact. The argon gas flows through one of the capillary tubes while the sample is introduced in the other. The high speed carrier gas breaks the sample solution into aerosol. Lower sensitivity and possibility of misalignment of the capillary tubes are the disadvantages of cross-flow nebuliser. The Babington nebuliser has been designed specifically for the nebulisation of viscous samples that contain high contents of dissolved solids including slurries.[137] This nebuliser allows a film of the liquid sample to flow over a smooth surface with a small gas exit. A high speed carrier gas emerging from the small orifice breaks the liquid sample into coarse aerosol. The most striking feature of a Babington nebuliser is that the sample moves freely over the tiny hole unlike in the other nebulisers where the the sample passes through the capillary, which gives rise to its ability to nebulise sample solutions with high amount of dissolved solids.[133, 137, 138]

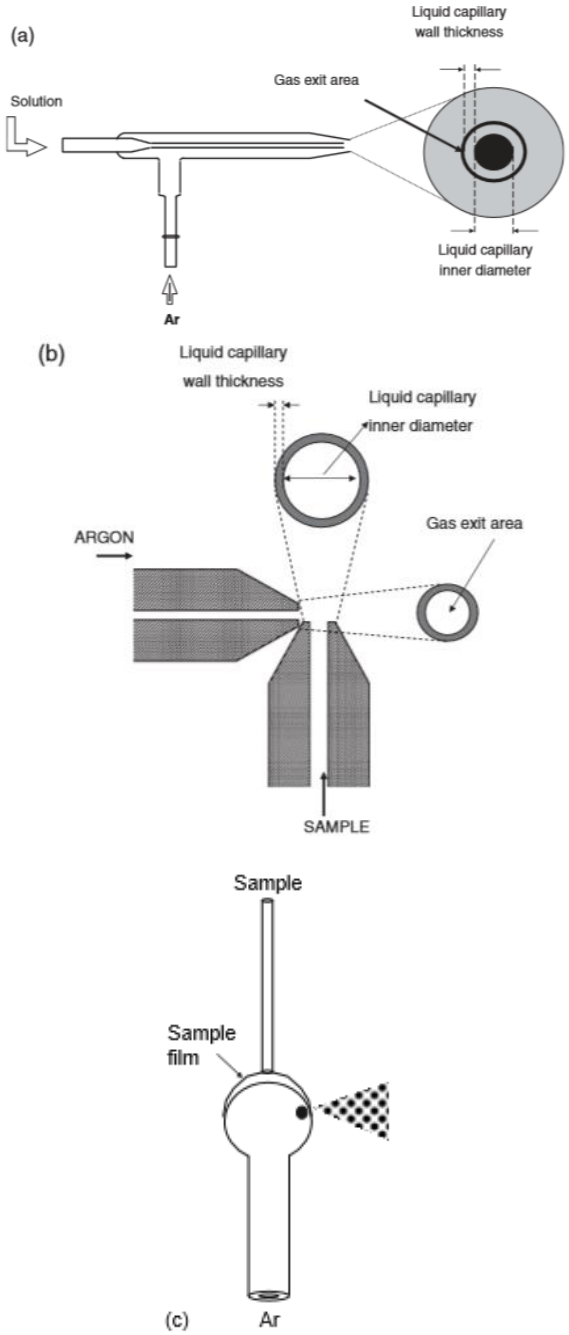


Figure 2.12 Schematic diagram of different pneumatic nebuliser (a) the concentric nebuliser (b) cross-flow nebuliser (c) the Babington nebuliser[133, 139]

Spray Chambers

Spray chambers play a significant role during the introduction of liquid sample into the ICP. They function to eliminate any larger droplets from the coarse aerosol and further reduce the aerosol to the required size before passing into the ICP. It has been

determined that desolvation and ionisation/excitation of these droplets occurs efficiently when diameters are ca. 10 µm.[129, 140] Spray chambers are usually placed between the nebulisers and the torch. Several designs are known and they include: the Scott double-pass type, the cyclonic and single (or cylindrical) type spray chamber. The most common of these is the Scott double-pass spray chamber (Figure 2.13). The temperature of the spray chamber can significantly affect its efficiency by influencing the percentage of vapour entering the plasma. The spray chamber should therefore be operated at a temperature lower than the room temperature in order to condense most of the water vapour.

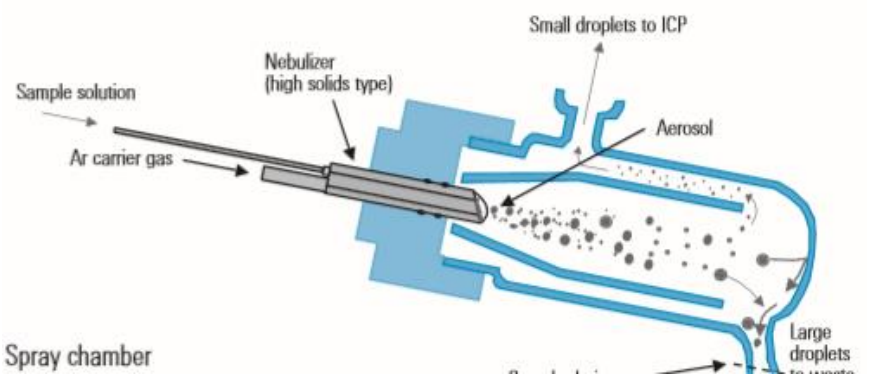


Figure 2.13 Schematic diagram of a Scott double-pass spray chamber[130]

Excitation processes

As the aerosol droplets are swept into the central channel of the plasma from the exit tube of the spray chamber, the analyte atoms (M) undergo several processes to become ionised (M^+). The mechanisms of these processes are decribed below.

Electron collisions: if the concentration of electrons is high and they have high kinetic energy.

$$M + e^- \leftrightharpoons M^+ + 2e^-$$

$$E_{e\text{-}1} > E_{e\text{-}2}$$

Electron excitation: the analyte atom on collision with the electrons may be elevated to a metastable state (M_m^*) through absorption of /some of the kinetic energy of the electron.

$$M + e^- \leftrightharpoons M_m^* + e^-$$

Molecular collision: these occur if the concentration of molecules (XY) is high. The energy tranferred is from excited vibrational and rotational levels in the molecule.

$$M + XY^* \leftrightharpoons M^* + XY$$

A collision may occur between a newly formed analyte cation and an electron leading to recombination, and subsequent formation of a metable stable excited atom giving out energy (hv), equivalent to the kinetic energy of the electron.

$$M^+ + e^- \leftrightharpoons M_m^* + hv$$

Collision with metastable argon species (Ar_m^*): a metastable argon species can transfer its energy on collision with an analyte atom. This energy is sufficient to ionise the analyte with release of an electron. The argon returns to ground state. This process is known as Penning ionisation.

$$M + Ar_m^* \leftrightharpoons M^+ + Ar + e^-$$

2.3.4 The ICP-MS Interface

Interface

The interface (Figure 2.14) is located between the ICP torch and the MS and allows for their coupling. The interface transfers the ions produced from the plasma under high atmospheric pressure (760 Torr) to the mass spectrometer analyser region at a lower pressure, approximately 10^{-6} Torr.[129, 141, 142] The interface typically consists of a water-cooled outer sampling cone made of nickel with 1.0 mm orifice. It samples ions 10 mm from the ICP load coil, which creates a pressure differential. A second cone (skimmer) also made of nickel with 0.75 mm orifice is placed just behind the sampling cone and allows the central portion of the expanding supersonic jet of the plasma and ions to pass through. The pressure behind the skimmer cone is maintained at 10^{-4} - 10^{-5} torr.[126, 129] The ions then pass into the ion optics and mass analyser.

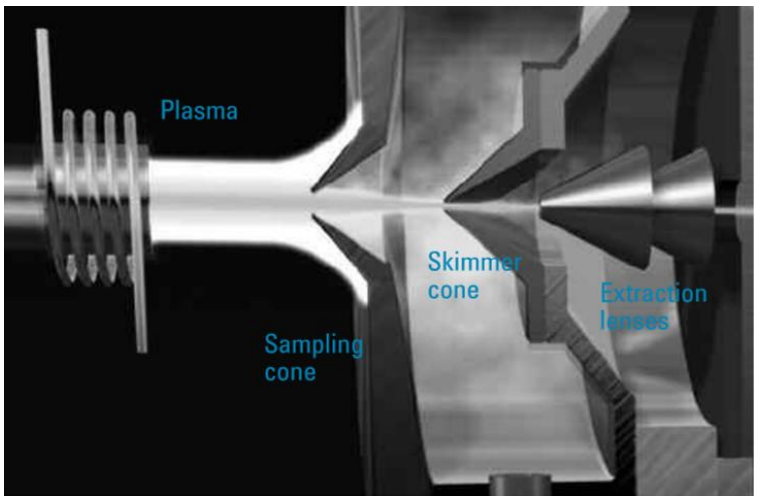


Figure 2.14 Schematic diagram of interface in ICP-MS[130]

2.3.5 Ion focusing optics

These are series of electrostatic lenses (Figure 2.15) made of metal plates or rings with specific voltage. They are either located in the high vacuum environment or just behind the skimmer cone of the sampling interface to extract ions from this region and transport them specifically to the mass analyser. The system helps also to remove photons and other species such as neutral atoms or particulate materials from the ion beam that could arrive at the detector alongside the positively charged ions. These species can cause instability in signal and result to additional background noise.

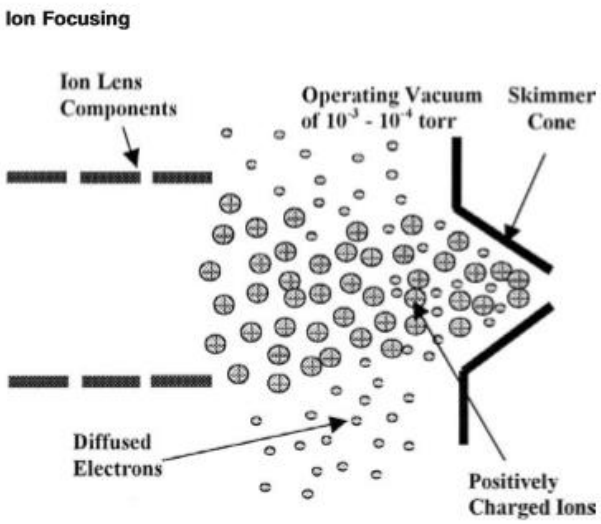


Figure 2.15 Schematic diagram showing the Ion lens component[142]

2.3.6 Mass analysers

The mass spectrometer serves as a filter that distinguishes between different ions according to their *m/z*. The most commonly used mass spectrometer in ICP-MS is the quadrupole mass filter (Figure 2.16). The quadrupole consists of four identical metal rods placed parallel to and equidistant from a central axis or ion beam (Figure 2.16). Each rod is connected electrically to the one opposite and, when voltage is applied, the ions entering the quadrupole move towards the central point and oscillate. When appropriate RF and DC voltages are selected, only ions of a particular *m/z* ratio proceed and emerge at the other end for detection. Ions whose oscillatory paths are too large collide with the rods and are lost. Other mass analyzers available for ICP-MS are magnetic sector (high resolution) and time of flight (TOF). A disdisadvantage of a quadrupole is its low ability to resolve ions with similar m/z ratio, compared with other types of analyser. However, it is much cheaper and the single mass unit resolution typically available is adequate for most applications.

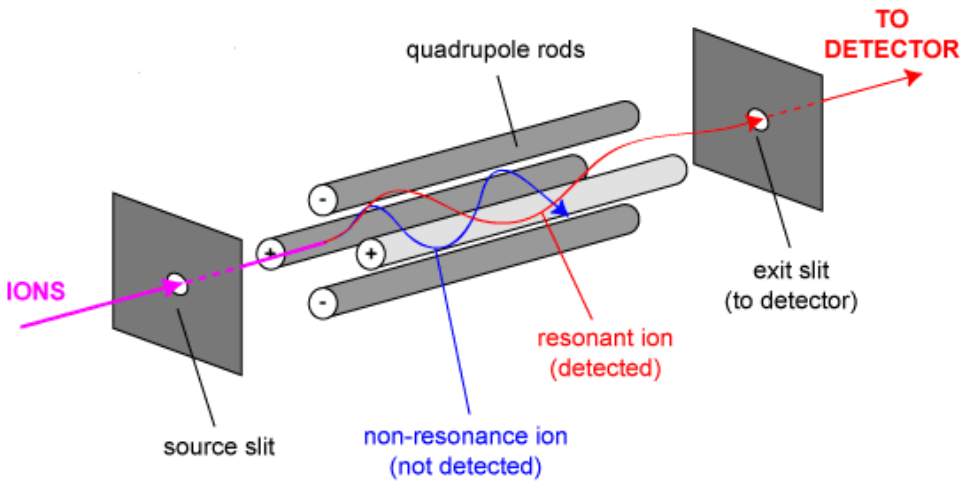


Figure 2.16 Schematic diagram of the quadrupole mass filter[143]

2.3.7 Detectors

It is only *m/z* selected ions that emerge from the quadrupole and each of the ions is converted to an electrical signal or pulse which can be registered by the detector. The most common type of detector employed in ICP-MS is the electron multiplier

(Figure 2.17). The device consists of an open tube with a wide cone entrance.[144] The inside of the tube is coated with PbO as a semiconducting material. A high negative potential of about -3 kV is associated with the entrance of the cone.[129] When positive ion emerges from the mass analyser, it strikes the surface of the cone, gets deflected to the first dynode (held at high negative voltage). This results to emission of several electrons from the surface of the dynode, which are repelled from the high negative voltage at the front and strikes the next dynode. Each electron which strikes the second dynode results to release of several other electrons from that surface and process of electron multiplication continues until they reach the final dynode as cascade. By this time the multiplication factor builts up a pulse which is large enough to be measured reliably as an ion "count".[145] The detector operates in dual mode, which allows for measurement of higher count rates. These dual mode detectors use pulse-counting at lower count rates (typically 0 to 10^4 counts per seconds - CPS) and then, at high count rates (typically 10^4 to 10^9 CPS), switch to analog mode, in which the current produced by stream of electrons is measured, rather than the pulse that derives from each individual ion impact.[130]

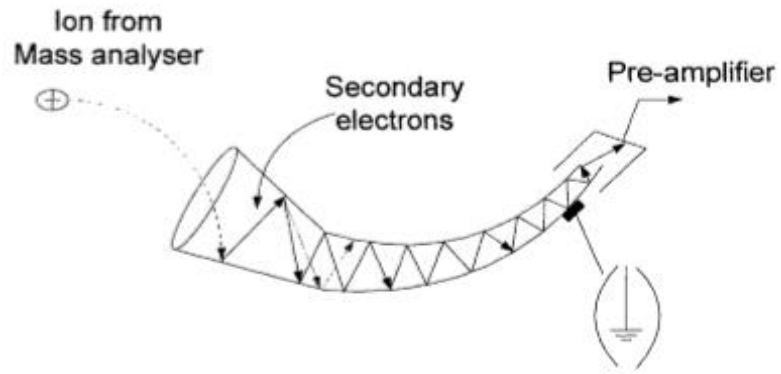


Figure 2.17 A schematic represention of an electron multiplier[129]

2.3.8 Interferences in ICP-MS

Interferences in ICP-MS are broadly classified as physical, isobaric and molecular.

Physical (matrix) interference. These are physical processes associated with sample nebulisation (viscosity effects), transportation into the plasma, and ion

transmission through the interface.[146] They can result in differences between instrument responses for the sample and the calibration solutions. Dissolved solids (e.g NaCl) in the sample may build up on the extraction cones at the ICP-MS interface which may result in a reduction in the size of the orifices which makes ion transmission less effective.[129, 133, 146] To minimise this effect, matrix matching is usually required, although use of internal standards can be an alternative.

Isobaric interference. When the ions in the ICP tail plume are extracted into the mass spectrometer, reactions between components in the sample and the argon plasma gas can occur producing ionic species that can overlap with the *m/z* value of the isotope of an analyte. This makes accurate quantification of some analytes difficult as the peak of the interfering ion overlaps with the peak of the analyte ion. Typically, ^{58}Ni interferes with ^{58}Fe and ^{40}Ar interferes with ^{40}Ca. Selection of an alternative analyte isotope can prevent this problem from occuring.

Molecular interference. Molecular interferences result due to formation of polyatomic species and doubly charged species. Polyatomic interferences occur when the isotope of interest interacts with a component of its aqueous solution, the plasma gas, or reagents employed during sample preparation. Typically, $^{40}Ar^{35}Cl^{+}$ interferes with ^{75}As; $^{40}Ar^{1}H^{+}$ interferes with ^{41}K; $^{40}Ar^{16}O^{+}$ interferes with ^{56}Fe; $^{40}Ar^{15}N^{+}$ interferes with ^{55}Mn etc. Further details are provided in Dean[129] and Thomas.[142] The formation of doubly charged species is an extension of polyatomic interference. These result in spectral interference at half the *m/z* of the singly charged ions: for example ^{138}Ba interferes with ^{69}Ga and ^{208}Pb interferes with ^{104}Ru.[92] Other species of concern include Ce, La, Sr and Th.[129, 140]

2.3.8.1 Minimising molecular (polyatomic) interferences

The use of *cold plasma* or *collision/reaction cells* have been reported[129, 140] as effective methods of minimising these interferences. Cold plasma involves the operating the ICP at lower power (0.6 kW) and higher central gasflow rate (1.1 L min^{-1}) conditions. These condition have been proved effective in reducing interference that result from Ar^{+}, ArH^{+}, ArO^{+} and Ar_2 species.[147] Extensive use has been made of collision/reaction cells recently in ICP-MS. These are placed behind the sample and skimmer cone and before the mass analyser.

The reaction cell uses a reactive gas (H_2, NH_3 and N_2O) to convert the interfering molecular ions to a different species. The processes are summerised below.

Charge exchange: this allows for the removal of the argon plasma gas ion interference and the resultant formation of unchanged argon plasma gas detected at m/z different from the analyte.

$$Ar^+ + NH_3 \rightarrow NH_3^+ + Ar$$

Proton transfer: invoves the neutralisation of the interfering species which results in a neutral argon plasma gas.

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

Hydrogen atom transfer: this results to an increase in the mass/charge ratio by one thereby making it impossible for the interfering species to be detected.

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

Collision cell uses helium gas which diminishes the concentration of molecular ion through collisional dissociation. Today, modern instruments use a combined collision/reaction cells.

3 General experimental procedures

3.1 Introduction

This chapter outlines the general experimental procedures used in this study. Glassware, plastic containers and storage bottles used were always soaked overnight in 5% HNO_3 (a general purpose grade supplied by Sigma Aldrich, Gillingham, UK), to remove any metal which may have adhered to the surface of the glassware and and rinsed with distilled or deionised water (ultra-pure; 18.2 MΩ .cm; 25 °C), and kept dry before each experiment was performed.

3.2 Pseudototal digestion

A microwave assisted digestion system (MARS Xpress^{TM}, CEM Microwave Technology Ltd., Buckingham, UK) was used to carry out sample digestion. *Aqua regia* was used as the digestion reagent, prepared by mixing concentrated HCl and HNO_3 acids for trace element analysis (obtained from Aldrich, Gillingham, UK) in the ratio of 3:1(*v/v*). A 1.0 g test portion of each sample (n = 3) was weighed into high pressure digestion tubes and 20 mL of freshly prepared *aqua regia* was added to the tubes containing the samples. They were placed in a fume cupboard and allowed to stand overnight. This was to allow for gaseous species arising from any vigorous reaction in the tubes to be given off, which may have increased the pressure during digestion. The digestion tubes containing the sample mixed with *aqua regia* were transferred into the MARS Xpress^{TM} microwave assisted digestion system and digested based on the conditions shown in Table 3.1. At the end of the digestion, the vessels and the contents were allowed to cool, and filtered through Fisher Brand FB 59023 filter papers into 100 mL standard volumetric flasks. The sample residues were washed with deionized water (ultra-pure; 18.2 MΩ .cm; 25 °C), after which the filtrates were made up to mark with deionised water to give 20% *aqua regia* solution. Exactly 1 mL of the solution was diluted in a 10 mL standard volumetric flask with deionised water to obtain a 2% solution suitable for introduction in the ICP-MS. Procedural blanks were digested alongside the samples. Digests were then transferred into 10 mL transport tubes and stored in a fridge prior to analysis.

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

Power* (W) (>16 tubes)	1600
Temperature (°C)	160
Ramp time (min)	20
Holding time (min)	20

*4 to 15 tubes; 800 W; < 4 tubes; 400 W

3.3 The EDTA extraction[148]

3.3.1 Apparatus

i. Centrifuge tubes (50 mL, Fisherbrand-Fisher Scientific, UK)
ii. End-over-end mechanical shaker (GFL® 3040, Gasellschaft für Labortechnik mbH, Burgwedel, Germany).
iii. An ACL 4237 centrifuge (CAMLAB Limited, Cambridge, UK).

3.3.2 Extraction procedure

Extraction of samples was carried out with 0.05 M EDTA at pH 7. Approximately 14.612 ± 0.05 g of EDTA was added to 80 mL of deionised water in a 1 L beaker and 13 mL of ammonia solution was added gradually until complete dissolution of the EDTA was achieved. About 800 mL of deionised water was added and the pH adjusted accordingly with few drops of concentrated HCl in a fume cupboard. The solution was made up to 1 L with additional deionised water.

Approximately 1.0 g of each sample was weighed into a 50 mL centrifuge tubes and 10 mL of 0.05 M EDTA solution was added and shaken on an end-over-end mechanical shaker at 23 rpm for 1 hour at room temperature. The mixture was then centrifuged for 10 min at 3000 rpm and the supernatant decanted, filtered through (Fisher brand QL 100) and stored in 10 mL transport tubes prior to analysis. Procedural blanks were included during the extraction.

3.4 The BCR sequential extraction[149, 150]

3.4.1 Apparatus

i Centrifuge tubes (50 mL, Fisherbrand-Fisher Scientific, UK)

ii End-over-end mechanical shaker (GFL® 3040, Gasellschaft für Labortechnik mbH, Burgwedel, Germany).

iii An ACL 4237 centrifuge (CAMLAB Limited, Cambridge, UK).

3.4.2 Extraction reagents

All reagents used were of analytical grade.

(**Acetic acid, 0.11 M**)

A 0.43 M acetic acid was prepared by addition of approximately 25 mL of glacial acetic acid (Sigma-Aldrich, Gillingham, UK) to 500 mL of distilled water in a 1 L standard volumetric flask and made up to volume with distilled water. Exactly 250 mL of the solution was diluted to 1 L with distilled water to obtain the final solution of 0.11 M acetic acid.

(**Hydroxylammonium hydrochloride, 0.5 M**)

Approximately 34.75 g of hydroxylammonium hydrochloride (Fisher Scientific, Loughborough, UK) was weighed and dissolved in 400 mL of distilled water and transferred into a 1 L standard volumetric flask, after that 25 mL of freshly prepared 2 M HNO_3 was added and made up to volume with distilled water. The solution was always prepared on the same day the extraction was to be carried out.

(**Hydrogen peroxide, 8.8 M**)

Hydrogen peroxide solution was used as supplied by VWR, Leicestershire, UK (30 %; acid-stabilized to pH 2-3.)

(**Ammonium acetate, 1.0 M**)

Approximately 77.08 g of ammonium acetate (Fisher Scientific, Loughborough, UK) was dissolved in about 900 mL of distilled water. The pH of the solution was adjusted to 2.0 ± 0.1 with concentrated HNO_3 and made up to 1 L with distilled water.

Aqua regia

Aqua regia was prepared as described in section 3.2

In addition to the preparation of solutions used in the extraction procedure, procedural blank solutions were prepared by adding appropriate volumes of the solutions to the extraction tubes without sample (s). They were carried through the complete procedure and analysed at the end of each extraction step.

3.4.3 Extraction procedure

Three extraction steps are described in detail below.

Step 1 (Exchangeable fraction)

One gram of sample was weighed into a 50 mL centrifuge tube and 40 mL of 0.11 M acetic acid was added. The mixture was shaken for 16 h at room temperature using an end-over-end shaker. The extracts were separated from the residue by centrifuging at 3000 g for 20 min. The supernatant was decanted into polyethylene sample tubes and stored in a refrigerator at about 4 $^{\circ}$C pending analysis. The residue was washed by adding 20 mL of distilled water and shaken for 15 minutes. The mixture was centrifuged and the supernatant separated from the sample was discarded.

Step 2 (Reducible fraction)

To the residue from step 1, 40 mL of 0.5 M hydroxylamine hydrochloride solution was added and placed on an end-over-end shaker for 16 h at room temperature. The mixture was then centrifuged, supernatant decanted, washed and stored in a similar way as in step 1.

Step 3 (Oxidisable fraction)

Exactly 10 mL of 30% 8.8 M H_2O_2 as supplied was added slowly to the residue from step 2. The centrifuge tubes were loosely covered and allowed to digest with occasional manual shaking for 1 h at room temperature. The digestion was continued in a water bath at 85 ± 2 °C for 1 h, after which the cover of the centrifuge tubes were completely removed and the volume reduced to less than 3 mL by heating the tubes uncovered. A second portion of 10 mL of H_2O_2 was added. The loosely covered tubes were heated again at 85 ± 2 °C and their contents digested for another 1 hr. The cover was removed, and the volume reduced to about 1 mL with care not to take to complete dryness. Exactly 50 mL of 1.0 M ammonium acetate solution was added to the moist residue after it was allowed to cool. The mixture was then shaken on the end-over-end shaker, centrifuged, washed and the supernatant decanted and stored as in step 1.

Step 4 (Residual fraction).

The residue from step three was transferred into a microwave digestion tube and digested with *aqua regia* as described in section 3.2

3.5 PTE measurement in the samples

In this work, digests and extracts were all analysed by ICP-MS (Model 7700x, Agilent Technologies, UK) fitted with an ASX-500 series autosampler. The sample solution was pumped at a flow rate of approximately 0.1 mL min^{-1} through a concentric nebuliser by a 10-roller peristaltic pump which consisted of three separate tubes that delivered the sample, the internal standard (^{115}In), and allowed the spray chamber to be drained. The sample aerosols formed by the nebuliser were passed into a Scott-type double-pass spray chamber at a controlled temperature of 2 °C. This temperature was achieved through the introduction of a Peltier cooler (Figure 3.1). The cooling prevents the vaporisation of water droplets from the sample which could increase the formation of polyatomic oxide species and interfere with some of the analytes.

Figure 3.1 A Peltier cooled sample introduction system[151]

The fine aerosol droplets were then channeled through an injector with an internal diameter of 2.5 mm into the torch. The torch consists of three concentric quartz tubes and allowed for the passage of argon gas at the rates of 15, 0.9 and 1.05 L $minj^{-1}$ for plasma gas, auxiliary gas, and carrier gas respectively. The plasma was fed with a power of 1550 W by a maintenance-free solid state digital drive 27 MHz matching RF generator. The ions in the plasma were extracted at a sampling depth of 8 mm through a 1 mm diameter orifice, Ni-tipped with Cu base sampling cone (Figure 3.2) into the first vacuum stage. After which they moved into the second vacuum stage through a 0.4 mm diameter orifice, Ni skimmer cone (Figure 3.2). The small skimmer orifice has the capacity of reducing matrix contamination of the high vacuum region.

A B

Figure 3.2 Agilent sampling cone (A) and skimmer cone (B)[15]

The ions emerging from the skimmer cone were focused for high sensitivity by the extraction lenses and transmitted into the octopole reaction system (ORS) a thermally

stabilized cell with 12 MHz octopole ion guide contained in a stainless steel vessel and pressurized with He gas (Figure 3.3).

Figure 3.4 Octopole reaction system[151]

The ion beam which emerged from the ORS was transmitted into a true hyperbolic quadrupole operated at a high frequency (3 MHz).

Figure 3.5 Hyperbolic quadrupole[151]

When the RF and DC voltages applied to the pair of the opposite rods were varied, ions of specific m/z ratio passed through the middle of the parallel rods on to the detector while ions whose oscillatory paths were too large collided with the rods. The variation in voltage was carried out as quickly as possible in order to achieve scanning of masses from 2 to 260 amu in 100 milliseconds. The mass spectra for all the elements including their isotopes (Li to U) were obtained in fast sequential mode. The ion signals were measured by an auto-switching, dual mode discrete dynode electron multiplier detector with a full 9 orders dynamic range. When the ions emerged from the quadrupole, they struck the first dynode and electrons were sputtered. The released electrons accelerated towards the second dynode and became multiplied in a cascade by a series of further dynodes arranged in order of increasing positive potential from the cathode culminating at the anode. A cluster of these electrons finally reached the anode. The dual mode detector then detected the signal either in a pulse-counting mode or analog mode depending on the intensity of the

signal. Where high signal amplification occurred, the pulse-counting mode was used while the analog mode was used in cases where less amplification was required. The resulting data was represented as counts per second (CPS) for every m/z ratios and fitted into a calibration curve obtained from CPS values of standard solutions measured earlier. Software called Agilent ICP-MS MassHunter workstation was used on the instrument and was operated on Microsoft® Windows 7 professional, which allowed for instrument control, method and sequence set up, data acquisition and data analysis.

3.5.1 Calibration of the instrument

Apart from Fe for which a single element standard was used due to the analyte's consistent high concentration in the samples, multi-element standards supplied by Qmx Laboratories, Thaxted, UK were used in this work.

Mixed calibration standards solutions were prepared diluting 1000 mg L^{-1} and 10 mg L^{-1} of the Fe and multi-element standards, respectively, by measuring accurately the corresponding amount of the stock solutions with Thermo Scientific Finnpepette® pipettes, 10 to 100 and 100 to 1000 µL and 5 mL (Thermo Scientific, Vantaa, Finland). All standard solutions were prepared in 2% *aqua regia* or as appropriate to match the sample reagent. Indium-115 (1000 µg l^{-1}) was used as the internal standard, prepared by measuring 50 µL of its stock solution (1000 mg l^{-1}) in a 50 mL standard volumetric flask and made up to mark with deionised water. External calibration was employed throughout the course of the study. The following nuclides were measured in the sample solutions; ^{75}As, ^{111}Cd, ^{53}Cr, ^{63}Cu, ^{57}Fe, ^{55}Mn, ^{60}Ni, ^{208}Pb, ^{238}U and ^{66}Zn.

Table 3.2 Typical volumes of stock standard taken and final concentration of each standard solution (except In^{115}) used for calibration

	Iron		Other elements	
Standard	Volume of stock (µl)	Concentration (µg L^{-1})	Volume of stock (µl)	Concentration (µg L^{-1})
1	0	0	0	0
2	25	1000	25	10
3	250	10000	250	100
4	2500	100000	1250	500
5	5000	200000	2500	1000

3.6 pH measurement[152]

3.6.1 Equipment

i. Centrifuge tubes (50 mL, Fisherbrand, Fisher Scientific Ltd., Loughborugh UK)

ii Calibrated pH meter [(Metller Toledo (SevenGO™) pH meter, Schwerzenbach, Switzerland] (Metller Toledo, Schwerzenbach, Switzerland)

iii End-over-end mechanical shaker (GFL® 3040, Gasellschaft für Labortechnik mbH, Burgwedel, Germany).

3.6.2 Analytical method

About 5 g of each air-dried, sieved sample (< 2 mm) was weighed into the 50 mL centrifuge tubes, after which 25 mL of deionised water was added. The centrifuge tubes were then closed firmly and placed on the end-over-end mechanical shaker for 1 h, then removed. They were allowed to stand for 2 h. The pH was then measured in the suspensions after calibrating the pH meter with the buffer solutions (Metller Toledo, GmbH, Switzerland).

3.7 Moisture content and loss on ignition[153, 154]

3.7.1 Equipment

i. An analytical weighing balance (AE 200, Mettler, Leicester, UK)
ii. Desiccator containing silica gel
iii. Oven (Memmert GMbH and Co. KG, Camlab Ltd., Cambridge, UK)
iv. Muffle furnace (Box Furnace, Elite Thermal Systems Ltd., Market Harborough, UK)

3.7.2 Analytical method

Moisture content was determined in order to express the PTE concentrations as dry matter. The British standard method was employed. Approximately 1.0 g of each sample (< 2 mm) was weighed into dry and pre-weighed crucible and placed in the oven, at 110 ^{0}C for 24 hours. This was then removed and placed in the desiccator and allowed to cool for some hours. The crucible containing the sample was then weighed accurately and loss in weight was determined. Percentage moisture content was estimated using equation 3.1

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

Equation 3.1

The residues from the determination of moisture content were used to estimate the organic matter content of the samples by loss of ignition. The residue was placed in the muffle furnace ramped at 10 °C per minutes and held at 550 °C for 8 h and allowed to cool to about 110 °C. The crucibles containing the residues were transferred into the desiccator and allowed to cool for a few hours. They were weighed thereafter and the difference in mass obtained before and after ignition was used to estimate the % organic matter content using the following equation 3.2

$$\%\, LOI = \frac{(oven\ dried\ weight - weight\ after\ combustion) \times 100}{oven\ dried\ weight}$$

Equation 3.2

3.8 Particle size determination[155]

3.8.1 Equipment

i. An analytical weighing balance (AE 200, Mettler, Leicester, UK)

ii. Stainless steel sieve [(2 mm mesh size), Fisherbrand, Fisher Scientific, Ltd., Loughborough, UK]

iii. Borosilicate glass cylinders [(I L), Fisherbrand, Fisher Scientific, Loughborough, UK]

iv. Thermometer (THL-210-110E, A. Gallenkamp and Co. Ltd., London, UK)

v. Hydrometer (ASTM E100 152H, S. Brannan and Sons Ltd., Cumbria, UK)

3.8.2 Reagent

Sodium hexametaphosphate was used as a dispersing agent, (Fisher Scientific Loughborough, UK)

Five percent of the salt was prepared by dissolving 12.5 g in distilled water and transferring to 250 mL standard volumetric flask. The solution was made up to mark with distilled water.

3.8.3 Analytical method

Approximately 50 g of air dried soil sample, passed through a 2 mm mesh size sieve, was weighed and delivered into a 400 mL beaker. Exactly 50 mL of sodium hexametaphosphate solution (5%) was added alongside 100 mL of distilled water and mixed thoroughly with a stirring rod for 30 mins. The soil suspension was then transferred quantitatively into a 1 L-measuring cylinder and made up to mark with distilled water. The top of the cylinder was inverted several times for 30 mins until a uniform suspension was obtained and placed on a bench top. The time was noted, and immediately, the hydrometer was gently lowered into the suspension until it began to float. Hydrometer reading was recorded at 40 sec after the cylinder was set down. The temperature of the suspension was also measured with the thermometer. The suspension was then allowed to stand for 3 h. A second set of hydrometer and temperature readings were taken thereafter.

Blank sample. Blank sample was prepared by mixing 100 mL of 5% dispersing solution and 880 mL of distilled water in a I L measuring cylinder.

Calculations. Results were corrected to a temperature of 68 °F (20 °C); for every 1 °F above 68 °F, 0.2 units were added to the hydrometer reading of the sample. Similarly, 0.2 units were subtracted for every 1 °F below 68 °F from the hydrometer reading of the sample. The density of the blank at each reading was subtracted from the corresponding density of the sample. The percentages of clay, silt and sand were estimated using equations 3.3, 3.4 and 3.5 respectively.

$$Percentage\ clay\ (\%) = corrected\ hydrometer\ reading\ at\ 3\ h\ \times \frac{100}{weight\ of\ sample}$$

Equation 3.3

$$Percentage\ silt\ (\%) = corrected\ hydrometer\ reading\ at\ 40\ \text{sec} \times \frac{100}{weight\ of\ sample}$$

Equation 3.4

$$Percentage\ sand\ (\%) = 100 - (\%\ silt + \%\ clay)$$

Equation 3.5

3.9 Data handling

The analyte concentrations obtained from the ICP-MS were expressed in µg L^{-1} of solution. These values were converted to mg $kg^{-1\ (\text{dry weight})}$ of sample used in this work. Equation 3.6 shows the relationship used to achieve this conversion.

$$Concentration\ (mg\ kg^{-1}) = \frac{analyte\ concentration(mg\ L^{-1}) \times volume \times dilution\ factor}{mass\ of\ sample\ (dry\ weight) in\ kg}$$

Equation 3.6

3.9.1 Detection limits

The detection limits (DL) of all the PTE measured were determined. It is the measure of the absolute concentration of analyte that can be detected with statistical confidence.

The obtained values from equation 3.6 are used in calculating the procedural detection limits (DL_{pro}) , referred to as the lowest concentration of analyte that can be determined in an environmental sample which allows for the method of sample preparation, were according to equation 3.7

$$DL_{pro} = \frac{DL \times volume\ of\ extractant \times dilution\ factor}{hypothetical\ dry\ weight\ of\ sample}$$

Equation 3.7

3.9.2 Precision

This is the degree of agreement of series of measurements of the same quantity carried out in a similar manner. It can be expressed as a percentage relative standard deviation (RSD).

3.9.3 Accuracy

Accuracy defines the closeness of a measured mean concentration ($\bar{x}$) of an analyte with a target value (μ) expressed as a percentage.

3.10 Statistics[156-158]

3.10.1 The t-test

The t-test is one of the statistical tools used in determining whether the mean results of two sets of measurement are significantly different or not. A null hypothesis (H_0) is assumed i.e there are no significant differences between the two mean values other than that which can be attributed to random variation. Before the t-test is applied, an F-test which determines whether there is a difference between the two sample variances is first determined.

Once the calculated F value is obtained, it is compared to a critical value. If the calculated statistic is less than the critical value the test is said to have passed then the null hypothesis is accepted signifying that the differences between the sample variances are due to random error. However, if the calculated statistic is higher than the critical value, the test is said to have failed and the null hypothesis rejected. Here, the differences between the sample variances are statistically significant and due to both random and systematic errors.

Again, if the t_{calc} is less than the critical value at this number of degrees of freedom and the required confidence level, the null hypothesis is accepted. This means that the differences in the mean values are due to random error. If t_{calc} is more than the critical value, the null hypothesis is rejected, signifying that the differences are significant and possibly due to systematic error. The t_{calc} is again compared to the critical value and appropriate conclusions are then made.

3.10.2 Analysis of variance (ANOVA)

The use of the t-test becomes inadequate when there are more than two means to compare. Analysis of variance becomes more appropriate. Two possible errors; random error which remains constant and can never be eliminated, and error due to what is called controlled or fixed effect factors, are associated with this analysis. Analysis of variance relies on two basic understanding. The first is how the variances of different components can be combined to give an overall observed variance. The second is that a difference in the means can lead to a spread for the results of combined data that can be detected in terms of increased variance. One-way ANOVA is predominantly used to look at the effect of one possible factor on a data set, which fits into this study e.g. for assessing the effect of varying the amount of fertiliser amendment on PTE concentration in Chapter 4. The layout of one-way ANOVA with the fertiliser treatments and replicate measurements are shown in Table 3.3.

Table 3.3 Layout for One-way ANOVA

Sample						Mean	Variance
Treat. 1	x_{11}	x_{12}	.....	x_{1j}	.....x_{1n}	$\bar{x}_1$	s_1^2
Treat. 2	x_{21}	x_{22}	.....	x_{2j}	.....x_{2n}	$\bar{x}_2$	s_2^2
:	:	:	.....	:	.....:	:	:
:	:	:	.....	:	.....:	:	:
Treat. 3	X_{31}	X_{32}	.....	X_{3j}	.....x_{3n}	$\bar{x}_i$	s_i^2
:	:	:	.....	:	.....:	:	:
:	:	:	.....	:	.....:	:	:
Treat. 4	X_{41}	X_{42}	.....	X_{4j}	.....x_{4n}	$\bar{x}_h$	s_h^2
					Grand mean	$\bar{x}$	

Treat. 1, 2, 3, and 4 are the various dosages of fertiliser amendments added to soil.

Where x_{ij} is the j^{th} measurement of the i^{th} sample; h is the number of levels (fertiliser treatments), n_i is the number of replicates for each treatment, $\bar{x}_i$ and s_i^2 are the means and variance of the ith sample, $\bar{x}$ is the grand mean.

The null hypothesis (H_0) adopted in ANOVA is that all the samples come from a common population with a mean μ and variance σ_0^2. For this reason the variance in the data can be computed in a number of ways; *within*-sample variation and *between* sample-variation. Irrespective of how the variances are calculated, if there are no significant differences, which means the null hypothesis is true; the two variances estimated should be equal. However, it is worth noting that random variation can never be absent in any experimental work, therefore the two variations will never be exactly the same. In order to determine whether the variances are statistically similar, an F-test is then used.

Within-sample variation

For each level, the variance is generally calculated according to equation 3.8

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

Equation 3.8

The overall estimate of the within-sample variance is arrived at by taking the average of the variances of all the samples as shown in equation 3.9.

$$\sigma_o^2 = \frac{\sum_{i=1}^{h}\sum_{j=1}^{ni}\left(x_{ij} - \bar{x}_i\right)^2}{h(n-1)}$$

Equation 3.9

Between-sample variation

If the measurements are obtained from the same population whose variance is σ_o^2, then the means come with a population with the variance $\frac{\sigma_o^2}{n}$. Therefore, the overall between-sample variance is given by equation 3.20

$$\sigma_o^2 = n_i \frac{\sum_{i=1}^{h}(\bar{x}_1 - \bar{x})^2}{h-1}$$

Equation 3.10

The F-test is then used to determine whether differences are due to random error or if they include systematic error by dividing the value obtained from equation 3.20 by the value from equation 3.9. If F_{calc} is < F_{crit} at $\upsilon = h - 1$ and $\upsilon = h(n-1)$, then the null hypothesis is accepted implying that the F-test has passed and any differences between the mean values of each sample are due to random error only.

If F_{calc} is > F_{crit} at the corresponding degrees of freedom, then the null hypothesis is rejected. Hence the F-test has failed and the differences are significant and include both random and systematic errors. All statistical analyses were computed using Microsoft Excel for Windows 8, 2010.

4 Pseudototal concentration and fractionation of PTE in a commercial top soil treated with fertilisers

4.1 Introduction

As discussed in detail in Chapter 1, it has previously been reported that the application of fertiliser amendments to soil does not only alter the pseudototal PTE content but can also affect their fractionation/distribution pattern, meaning they may become a potential threat to plants and animals.

In this chapter, two experiments are reported:

i) a preliminary experiment which involved treatment of a soil with chicken manure amendment only as an initial study to establish the experimental procedure.

ii) an experiment which involved treatment of soil with five fertiliser amendments, including the chicken manure.

4.2 Sampling

Table 4.1 shows the samples used and their description. The wide and extensive use of these materials by gardeners in the UK, their easy availability and the fact that they are rarely studied, were the reasons that prompted their study in this thesis. The samples were all transported to the laboratory and air dried at room temperature for a period of two weeks. Large objects (sticks, stones broken bottles) were carefully removed in the case of the soil. The air dried soil, chicken manure, growmore and rockdust were gently ground in a ceramic mortar using a pestle made of porcelain and passed through a 2 mm sieve. The remaining samples were not subjected to grinding as they were in powdered form. All the samples were subjected to coning and quartering in order to obtain representative samples for analysis.

Table 4.1 Samples and their description

Sample	%N	P (%P_2O_5)	K (%K_2O)	Physical state	Supplier
Soil	NR	NR	NR	Solid	B & Q, Glasgow UK.
Chicken manure	4.5	3.5	2.5	Solid-pelleted	,,
Growmore	7.0	7.0	7.0	Granulated	,,
Phostrogen	14	10	27	Powdered	,,
Rockdust	NR	NR	NR	Coarse	,,
Seaweed	1.4	0.32	4.0	powdered	Böd Ayre Seaweed Products Limited Shetland, UK

NR = not reported on packaging; n = 3

4.3 Experiment 1

As mentioned briefly in Section 4.1, this experiment was carried out specifically to investigate the effect of pelleted chicken manure (Figure 4.1) on levels of PTE in the soil and also to establish protocols for a larger investigation. Arsenic was not studied in experiment 1, but was incorporated in experiment 2.

The procedure involved measurement of different 50 g portions of the soil (moistened with water thereafter) into plastic containers where they were thoroughly mixed with 2.5, 5.0, 12.5 or 25 g of chicken manure resulting to 52.5, 55.0, 72.5 or 75.0 g of mixture. This was done in order to assess the effect of adding too much of the fertiliser on the levels of the PTE in a fixed mass of soil. These were then transferred into separate 5 x 4 cm plastic flower pots. A control was prepared without any addition of the fertiliser. Each treatment was studied i.e three flower pots were filled with each mixture. These were allowed to stand in the laboratory at room temperature (approximately 22°C) for a period of 4 weeks, with addition of 5 to 10 mL of distilled water on a daily basis. A 4-week period was chosen in order to allow the chicken manure reasonable time to break down.

Figure 4.1 Chicken manure pellets used in the experiment

At the end of the 4 week period, the amended samples were transferred to different polyethylene sheets and allowed to dry at room temperature. One gram of sample was taken from each portion after coning and quartering to ensure it was representative and digested as described in section 3.2 under similar conditions as shown in Table 3.1. Pseudototal concentrations of PTE (Cd, Cr, Cu, Fe, Mn, Ni, Pb, U and Zn) were determined in the soil and chicken manure before use and in the amended soils as stated in section 3.2.

4.4 Results and discussions

4.4.1 Detection limits

The detection limits of the instrument ($DL_{inst.}$) and the procedure ($DL_{pro.}$) for the PTE measured as obtained using equations 3.8 and 3.10 in section 3.9.1 are shown in Table 4.2. Generally the detection limits were lower than the concentrations measured in the various samples as expected.

Table 4.2 Instrument detection limits (µg L^{-1}) and procedural detection limits (µg kg^{-1}, dw) for PTE measured in the samples

PTE	$DL_{inst.}$	$DL_{pro.}$
Cd	0.00816	0.816
Cr	0.136	13.6
Cu	0.0414	4.14
Fe	0.135	13.5
Mn	0.0496	4.96
Ni	0.0782	7.82
Pb	0.0308	3.08
U	0.00193	0.193
Zn	0.0895	8.95

4.4.2 Pseudototal PTE concentration in the soil

Quality control

Table 4.3 shows the results of the pseudototal concentration of the PTE in a certified reference material, BCR®-143R obtained during the extraction process and their indicative values. In general the recoveries were 100 ± 10% with the exception of Cr which was higher at 122%. This indicates the analytical method for determination of pseudototal PTE was under control.

Table 4.3 Results of the pseudototal concentration (mg kg^{-1}) of BCR®-143R (Sewage Sludge Amended Soil)

	Cd	Cr	Cu	Mn	Ni	Pb	Zn
Target values (n ≥ 6)	72.0 ± 1.8	426 ± 12	131 ± 2	858 ± 11	296 ± 4	174 ± 5	1060 ± 16
Obtained values (n = 3)	68.6 ± 2.4	520 ± 22	125 ± 4	934 ± 33	290 ± 9	168 ± 5	1030 ± 35
% recovery	95	122	95	109	98	97	97

The pseudototal concentrations of Cd, Cr, Cu, Fe, Mn, Ni, Pb, U and Zn in the commercial top soil (before amendment), typical concentration ranges, common values, and soil guideline values (SGV) for PTE in agricultural and allotment garden soils are shown in Table 4.4 and appendix A. An RSD of less than 8 % (n = 3) for all the trace metals was observed (Appendix A), suggesting good precision, except for Cd which gave an RSD value greater than 39 % . This may be attributed to the difference in the concentration of one of the replicate samples that gave a higher signal compared with the other two replicates (Appendix A), due to measurement close to LOD. In general the PTE concentrations measured were within typical ranges for agricultural and allotment garden soils, and below SGV (Table 4.4) as would be expected for a soil being sold commercially.

Table 4.4 Pseudototal concentration of PTE in the soil (mean ± SD, n = 3; mg kg^{-1})

PTE	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Soil	3.93 ± 0.2	0.106 ± 0.04	43.5 ± 2.0	10.6 ± 0.7	19900 ± 970	348 ± 18	31.2 ± 1.6	6.43 ± 0.30	0.63 ± 0.03	37.1 ± 2.8

4.4.3 Pseudototal PTE concentration in the chicken manure

The results of the pseudototal content of PTE in the amendment and recommended levels for PTE in fertilisers adopted by different countries are shown in Table 4.5 and 4.6 respectively. Results for individual test portions are in appendix A.

Table 4.5 Pseudototal PTE concentration (mg kg^{-1}) in the fertiliser (mean ± S.D, n = 3)

	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
CM	0.280 ± 0.01	4.90 ± 0.4	92.8 ± 4.7	1360 ± 5	506 ± 21	4.8 ± 0.2	1.67 ± 0.1	1.21 ± 0.03	500 ± 17

CM = chicken manure

Table 4.6 Maximum recommended levels for PTE (mg kg^{-1}) in fertilisers adopted by different countries

PTE	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Canada[5]	75.0	20.0	-	-	-	-	180	500	-	1850
China[159]	50.0	8.00	500	-	-	-	-	100	-	-
Czech[111]	10*	2*	100*	100*	-	-	50*	100*	-	400*
EU[160]	-	1.5*	-	200*	-	-	50*	120*	-	600*

*applies to organic fertilisers only

As shown in Appendix A, the RSD of Cd measurement in chicken manure was less than 4% (n = 3). The mean concentration of Cd in the fertiliser was 0.280 mg kg^{-1}. This value is far less than the recommended Czech and EU maximum level of 2 and 1.5 mg kg^{-1} for Cd in organic fertilisers.

The RSD of Cr concentration in the fertiliser was less than 9% (n = 3). The mean concentration of Cr in the material was 4.90 mg kg^{-1}. Nicholson *et al.*[89] have reported a similar concentration (4.57 mg kg^{-1}) in chicken manure in England and Wales. The concentration obtained in this study is far less than the 100 mg kg^{-1} recommended by Czech for organic fertilisers.

The RSD of Cu measurement in the fertiliser was less than 6% (n = 3), showing good precision during the extraction process. The mean concentration of Cu in the material was 92.8 mg kg^{-1}. This value was close to the Czech recommended maximum level of 100 mg kg^{-1} for organic fertilisers but lower than the 200 mg kg^{-1} Cu proposed by the EU for organic fertilisers. It is well known that Cu is an essential nutrient for plants, and its high concentration in chicken manure may be evident - copper is used as a supplement in animal feed.[161] The concentration of Cu reported in this work is relatively close to the mean concentration of 81.8 mg kg^{-1} obtained from 18 chicken manure samples in Beijing and Fuxin, China by Xiong *et al.*[162]

The RSD of Fe concentration in the chicken manure was 0.3% (n = 3). The mean concentration of Fe in the fertiliser material was 1360 mg kg^{-1}. Iron may be added to animal diet, which can result in a fairly high level in the waste material.

A good precision was achieved for Mn measurement in the fertiliser samples as shown by the RSD value of less than 5% (n = 3). The mean concentration of Mn in chicken manure was relatively high (506 mg kg^{-1}). Manganese can be added to animal feed as a supplement therefore its high level in the fertiliser was expected. The concentration obtained in this study was lower than the 624 mg kg^{-1} obtained by Cang *et al.*[80] when they investigated PTE pollution in poultry feeds and manures under intensive farming activity in Jiangsu Province in China. The higher result reported by Cang *et al.*[80] might be due to the sources from which the materials were obtained.

The RSD of Ni measurement in the chicken manure showed good precision as the value obtained was less than 4% (n =3). The mean concentration of Ni obtained for chicken manure in this study was 4.8 mg kg^{-1}. Cang *et al.*[80] and Liu *et al.*[163] have reported higher concentrations of 17.5 and 13.6 mg kg^{-1} in chicken manure respectively in their separate studies. However, the value obtained in this study was

lower than both the Czech and EU maximum recommended level (50 mg kg^{-1}) of Ni in organic fertilisers.

The precision of Ni measurement was good as typified by RSD value of less than 6% (n = 3). The mean concentration of Pb in the fertiliser was as low as 1.67 mg kg^{-1}. Zhang *et al.* [90] have reported higher values of Pb in chicken manure ranging from 2.5 to 4.89 mg kg^{-1}. Although Pb is one of the PTE of environmental concern, the concentration obtained in this study was far less than the maximum recommended level of Pb in organic fertilisers set by Czech (100 mg kg^{-1}) and EU (120 mg kg^{-1}) respectively.

The RSD of U measurement in the fertiliser was less than 3% (n = 3) which suggested good precision. Although, the concentration of U obtained in this study appeared to be low, there has been no legislation for U content in fertilisers and this may be a source of concern since some types of phosphate fertiliser can contain U.

The RSD of Zn concentration in the material was less than 4% (n = 3), indicating good precision during the analysis. The high concentration of Zn (500 mg kg^{-1}) measured in the sample was expected as it is commonly added to animal feeds for different purposes. The value obtained in this study was higher than the mean concentration of 384 mg kg^{-1} reported by Zhang *et al.*[90] and 400 mg kg^{-1} set as the maximum recommended concentration for organic fertilisers by Czech. However, the concentration was lower than the EU value of 600 mg kg^{-1} for this category of fertilisers.

4.4.4 Comparison of pseudototal content of PTE in the original soil (OS) and control (W0) soil samples, and PTE concentration in the chicken manure amended soil (W1 to W4)

The chicken manure amended soil samples were analysed after the 4-week period in order to assess the effect of the amendment on the PTE total content in the soil. Table 4.7 shows the results for the amended soil, where W0, W1, W2, W3 or W4 corresponds to 0, 2.5, 5.0, 12.5, or 25 g of chicken manure mixed with a fixed mass (50 g) of soil.

Table 4.7 **Pseudototal PTE concentration (mg kg^{-1}) in chicken manure amended soil** (Mean ± S.D, n = 3)

PTE		Treatment				
	OS	W0	W1	W2	W3	W4
Cd	0.106 ± 0.04	0.170 ± 0.08	0.110 ± 0.02	0.170 ± 0.09	0.220 ± 0.1	0.270 ± 0.01
Cr	43.5 ± 2.0	48.7 ± 1.0	49.1 ± 1.1	45.3 ± 0.6	42.0 ± 1.75	34.5 ± 2.3
Cu	10.6 ± 0.7	11.8 ± 4.5	14.3 ± 1.4	19.4 ± 1.8	29.4 ± 3.1	55.3 ± 11.7
Fe	19900 ± 970	21600 ± 540	21500 ± 294	20100 ± 432	18400 ± 392	14500 ± 1308
Mn	348 ± 18	399 ± 2.0	414 ± 4	396 ± 17	438 ± 42	499 ± 25
Ni	31.2 ± 1.6	35.5 ± 0.4	35.5 ± 0.6	34 ± 0.2	31.1 ± 0.40	25.1 ± 2.0
Pb	6.43 ± 0.30	7.31 ± 0.05	7.30 ± 0.2	6.9 ± 0.08	6.70 ± 0.1	5.96 ± 0.01
U	0.63 ± 0.03	0.740 ± 0.07	0.680 ± 0.01	0.680 ± 0.01	0.860 ± 0.1	1.02 ± 0.06
Zn	37.1 ± 2.8	40.3 ± 2.1	64.5 ± 2.1	89.0 ± 2.6	150 ± 15	260 ± 7

Where W0 (control soil), W1, W2 , W3 or W4 treatments corresponding to 0, 2.5, 5.0, 12.5 or 25 g of chicken manure mixed with 50 g of soil.

Comparison was first made between the pseudototal PTE concentration of the control sample (W0) which was subjected to the same temperature and watering regime as the treated samples for 30 days and the pseudototal PTE concentration of the original soil (OS) shown in Table 4.4, using the F-test and t-test at 95% confidence interval (Table 4.8A, Appendix B and Table 4.8B, Appendix B1 respectively). This was to determine primarily whether addition of water to the soil affected the PTE status since there was potential concern that losses could occur due to leaching, although the amount of water added each day was small. The F-test revealed there was no significant difference between the variances of pseudototal PTE concentrations of W0 and OS samples ($P < 0.05$), except Cu and Pb where the variances were different. The t-test analysis indicated there was no significant difference between the means of the two samples, except Cr, Mn and Ni. However the watering regime did not lead to significant loss of analyte due to leaching.

Table 4.8A The F-test analysis for OS and W0

Statistic	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Fcalc.	4.54	2.74	44.2	3.22	7.24	18.1	594	3.99	1.76
Fcrit	19.0	19.0	19.0	19	19.0	19	19	19	19
Remark	P	P	F	P	P	P	F	P	P

P = pass F = Fail

Table 4.8B The t-test analysis for OS and W0

Statistic	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Tcalc	1.22	4.31	0.459	2.52	3.88	4.68	1.21	2.57	1.56
Tcrit	2.78	2.78	2.78	2.78	3.18	2.78	4.30	2.78	2.78
Remark	P	F	P	P	F	F	P	P	P

P = pass F = Fail

4.4.5 Effect of chicken manure on PTE levels in the soil samples

Cadmium

The concentrations of Cd in the original soil, control soil, chicken manure amended soil and chicken manure are shown in Figure 4.2. The RSD values were greater than 16% (n = 3) as presented in (Appendix C). As expected, given that the manure is richer in Cd than the soil, Cd levels increased as the amount of fertiliser added increased. Statistical analysis, (Appendix D) however suggested there were no significant differences in Cd levels amongst W0 to W4 samples ($p < 0.05$). This is probably because the uncertainty in concentration values was sometimes high resulting in large error bars (Figure 4.2) because measurement was carried out close to LOD.

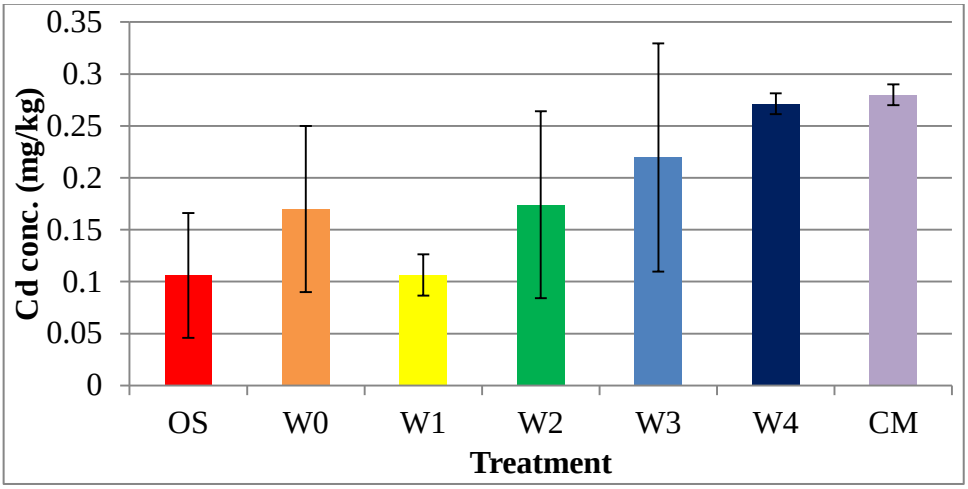


Figure 4.2 The concentration of Cd in the original soil (OS), Control soil (W0), chicken manure amended soil (W1 to W4) and chicken manure (CM) samples (n = 3)

Chromiun

Figure 4.3 shows the levels of Cr in the original soil, control soil, chicken manure amended soil and chicken manure. The RSD values were less than 7% (n = 3). As can be seen, the fertiliser contained a lower amount of the element compared to the soil. The concentration of Cr decreased as the amount of the material added increased, due to dilution of the soil with less contaminated material. The ANOVA analysis revealed the added fertiliser significantly affected the concentration of Cr amongst W0 to W4 samples ($P < 0.05$).

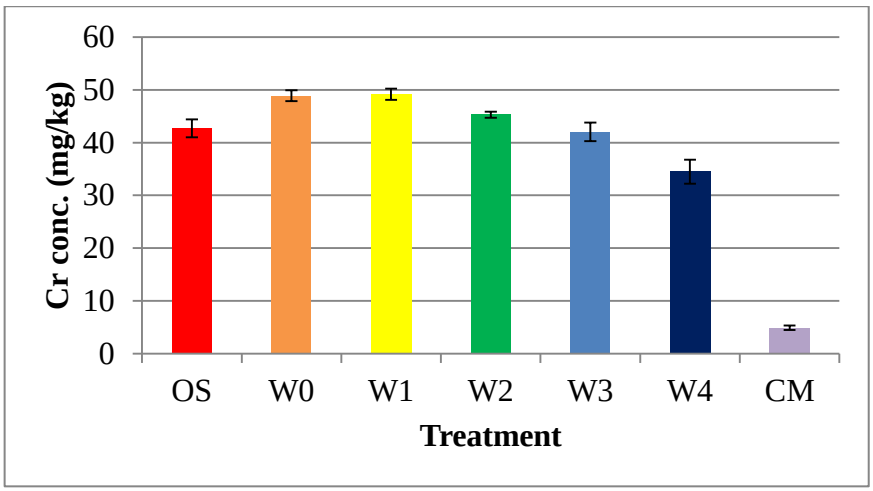


Figure 4.3 The concentration of Cr in the original soil (OS), control soil (W0), chicken manure amended soil (W1 to W4) and chicken manure (CM) samples (n = 3)

Copper

Copper levels in the original soil, control soil, chicken manure amended soil and chicken manure are presented in Figure 4.4 The RSD were generally less than 10% (n = 3). The addition of chicken manure significantly ($P < 0.05$), influenced the concentration of Cu in soil, as the *aqua regia* extractable Cu in W1 to W4 increased steadily. This effect was possible because the fertiliser contained higher amount of Cu than in the soil. Khai *et al*[107] reported an increase in pseudototaltotal metal concentration of an agricultural soil amended with chicken manure. They also suggested that the increase could be due to the high concentration of Cu in the fertiliser.

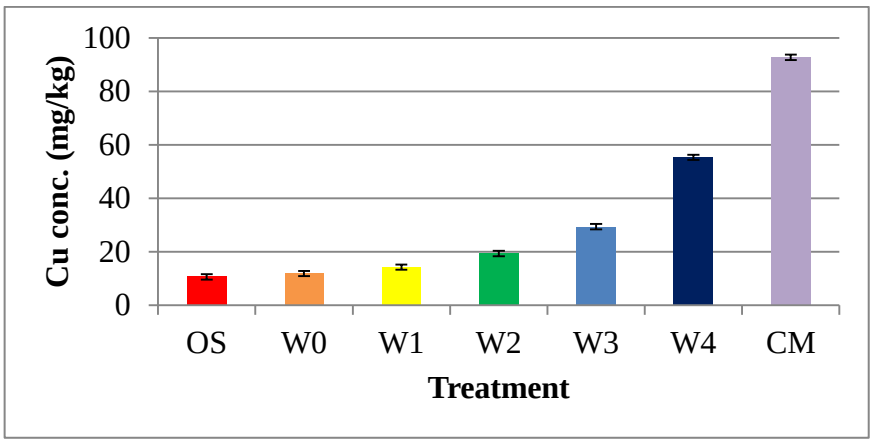


Figure 4.4 The concentration of Cu in the original soil (OS), control soil (W0), chicken manure amended soil (W1 to W4) and chicken manure (CM) samples (n = 3)

Iron

The concentration of Fe in the original soil, control soil, chicken manure amended soil and chicken manure are shown in Figure 4.5. The RSD of Fe concentration were less than 10% (n = 3) suggesting good precision of the analysis. The level of Fe in the added material, as can be seen was much lower than in the soil. This resulted in a decrease in the concentration of Fe in soil as the chicken manure was added. The statistical result also revealed significant differences (P <0.05) among the treatments (W0 to W4).

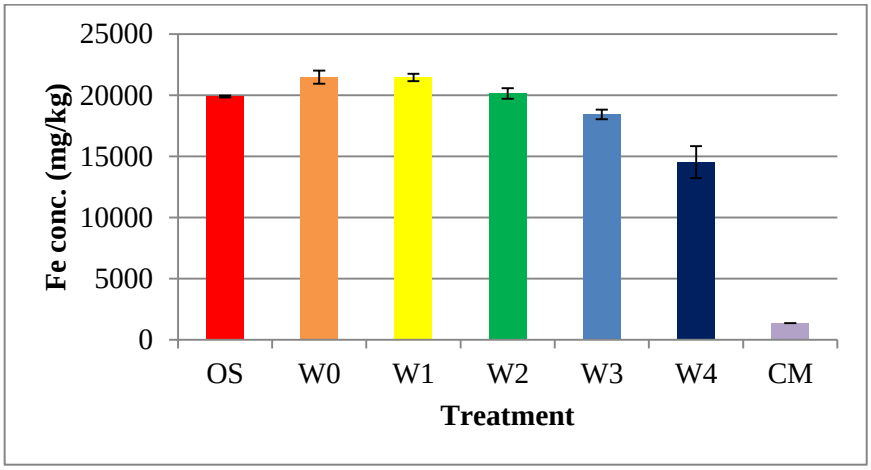


Figure 4.5 The concentration of Fe in the original soil (OS), control soil (W0), chicken manure amended soil (W1 to W4) and chicken manure (CM) samples (n = 3)

Manganese

The concentration of Mn in the original soil, control soil, chicken manure amended soil and chicken manure is shown in Figure 4.6. The RSD of Mn measurement were less than 10% (n = 3). The chicken manure being slightly richer in Mn than the soil, slight increase in the levels of Mn in the soils was observed as the amount of manure added increased. The statistical analysis indicated there were significant differences (P <0.05) in Mn concentration amongst the treatments, W0 to W4.

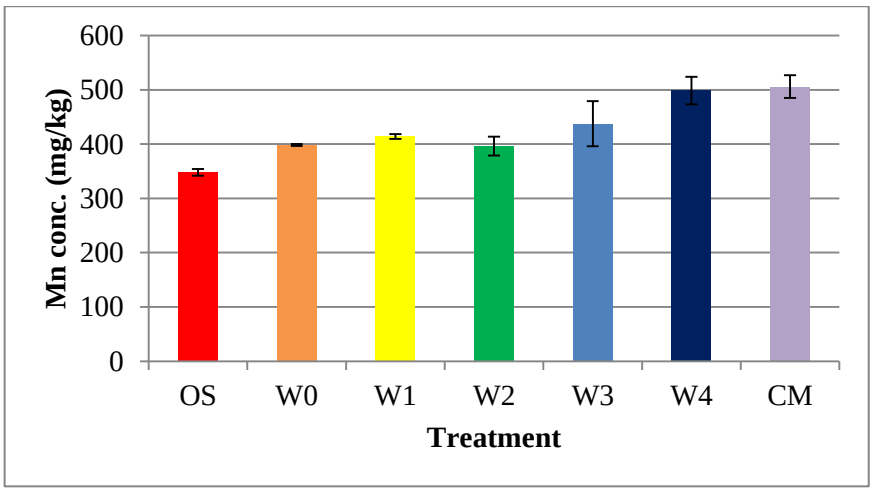


Figure 4.6 The concentration of Mn in the original soil (OS), control soil (W0), chicken manure amended soil (W1 to W4) and chicken manure (CM) samples (n = 3)

Nickel

Figure 4.7 shows the concentration of Ni in the original soil, control soil, chicken manure amended soil and chicken manure. The RSD of Ni measurement in the amended soil were all less than 9% (n = 3) which indicated that the precision of the analysis was good as typified in the corresponding small error bars (Figure 4.7). A lower level of Ni was found in the manure than in the soil. As a result, a decrease in the concentration of Ni was observed as chicken manure was added. The result of the statistical analysis as contained showed that significant differences ($P < 0.05$) existed in the concentration of Ni amongst the treated samples (W0 to W4).

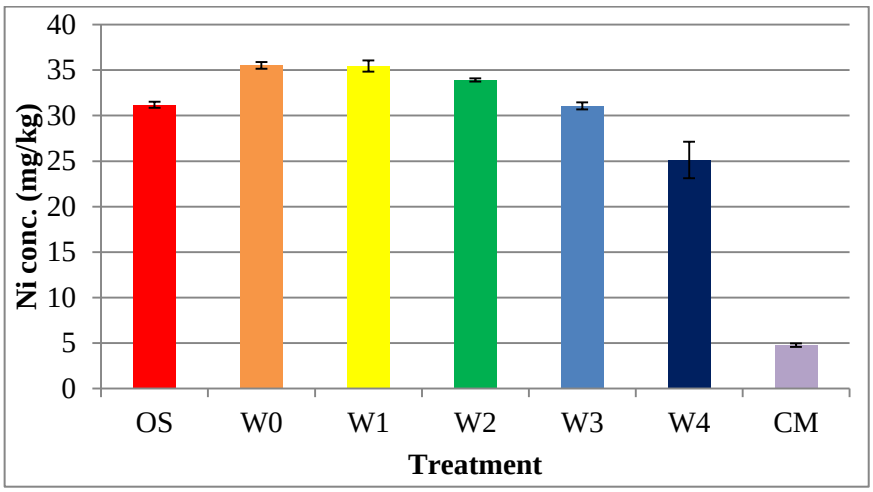


Figure 4.7 The concentration of Ni in the original soil (OS), control soil (W0), chicken manure amended soil (W1 to W4) and chicken manure (CM) samples (n = 3)

Lead

The concentration of Pb in the original soil, control soil, chicken manure amended soil and chicken manure are presented in Figure 4.8. The RSD values of Pb in the various treatments were less than 3% (n = 3). The concentration of Pb in the manure was much lower compared to the soil, but the addition of the material did not show any clear trend or changes in the levels of Pb in all the amended soil samples. This was confirmed by the statistical analysis which indicated that there were no significant differences ($P < 0.05$) in the concentrations of Pb amongst the treatments.

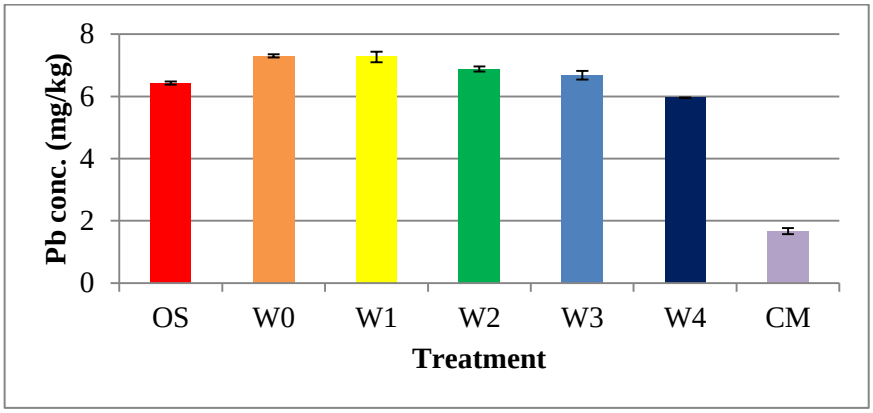


Figure 4.8 The concentration of Pb in the original soil (OS), control soil (W0), chicken manure amended soil (W1 to W4) and chicken manure (CM) samples (n = 3)

Uranium

Levels of U in the original soil, control soil, chicken manure amended soil and chicken manure are shown in the Figure 4.8. The RSD concentration of U in the samples were less than 9% (n = 3). Considering the fact that the fertiliser contained higher level of U than in the soil sample, Uranium concentrations increased in a corresponding manner as the material added increased. The ANOVA statistic revealed that the chicken manure significantly ($P < 0.05$) affected the concentrations of U in the soil.

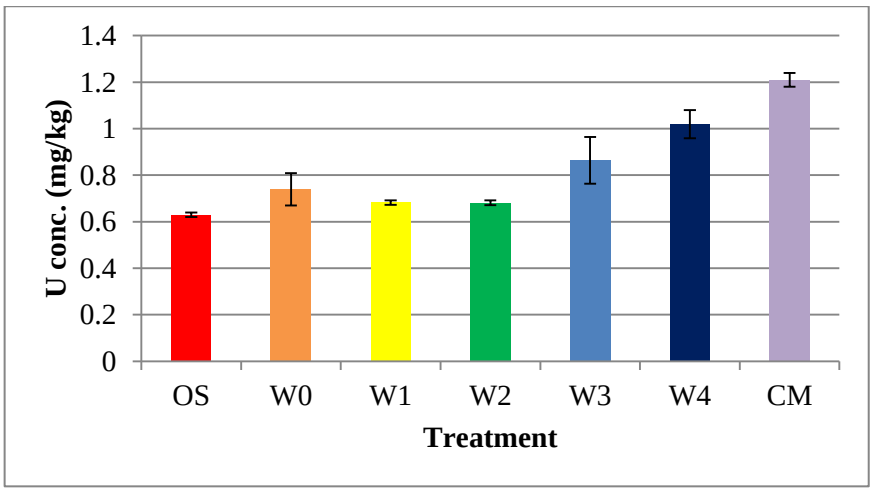


Figure 4.9 The concentration of U in the original soil (OS), control soil (W0), chicken manure amended soil (W1 to W4) and chicken manure (CM) samples (n = 3)

Zinc

The concentration of Zn in the original soil, control soil, chicken manure amended soil and chicken manure are shown in Figure 4.10. The precision of the analysis was found to be good with RSD values of less than 10% (n = 3). Higher concentration of Zn was measured in the chicken manure compared to the amount in the soil. As a result, Zn levels increased with increase in the added material. Significant differences were observed amongst the treated soils. This further confirmed that addition of manure can cause Zn to accumulate over a period of time. The study carried out by Khai *et al*[107] observed a similar trend where the concentration of Zn in an agricultural soil significantly increased after chicken manure application.

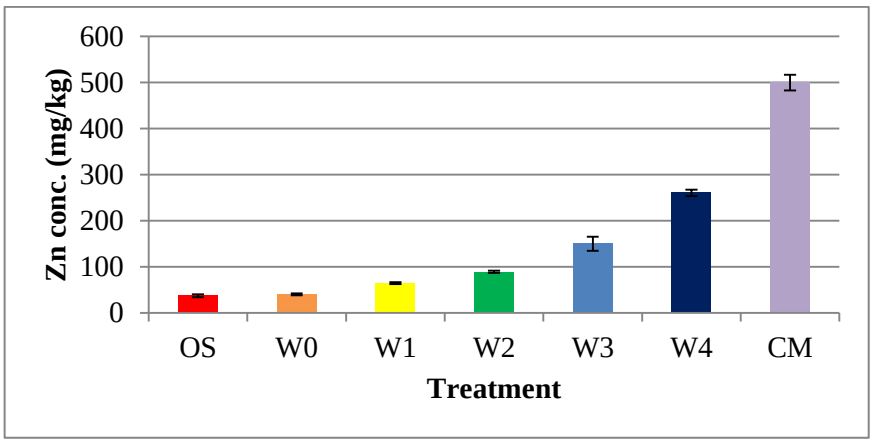


Figure 4.10 The concentration of Zn in the original soil (OS), control soil (W0), chicken manure amended soil (W1 to W4) and chicken manure (CM) samples (n = 3)

4.4.6 Mass balance studies

With the knowledge of the analyte concentrations in both soil and fertiliser, within the limits of measurement uncertainty, calculation of theoretical analyte concentrations in the amended soils is possible, for comparison with found values. Together with comparison between PTE levels in OS and W0 allowed to stand for 30 days, this gives an important indication whether the experimental procedure is robust enough for use with additional fertilisers.

The theoretical analyte concentration (Tc) was calculated as follows:

$$Tc = \frac{\text{Mass of soil (g)} \times \text{Amount(mg)PTE in soil before mixing}}{1000} + \frac{\text{Mass of amendment} \times \text{Amount (mg)PTE in Amendment before mixing}}{1000} \times \frac{1000}{\text{Mass of Mixture (g)}}$$

Average percentage recoveries of Cd in the treatments (W1 to W4) ranged from 97 to 164% and are presented in Table 4.9.

Table 4.9 Results of mass balance studies

	Dosage	Conc. in top-soil before mixing (mg kg^{-1})	Conc. in chicken manure before mixing (mg kg^{-1})	Expected conc. (mg kg^{-1})	Conc. found (mg kg^{-1})	Recov. (%)
Cd						
	W1	0.106	0.280	0.114	0.110	96.5
	W2	0.106	0.280	0.122	0.170	139
	W3	0.106	0.280	0.141	0.220	156
	W4	0.106	0.280	0.164	0.270	164
Cr						
	W1	43.5	4.90	41.7	49.2	118
	W2	43.5	4.90	40.0	45.3	113
	W3	43.5	4.90	35.8	42	117
	W4	43.5	4.90	30.6	34.5	113
Cu						
	W1	10.6	92.8	14.5	14.3	98.6
	W2	10.6	92.8	18.1	19.4	107
	W3	10.6	92.8	27.0	29.4	109
	W4	10.6	92.8	38.0	55.3	146
Fe						
	W1	19900	1360	19020	21500	113
	W2	19900	1360	18200	20100	110
	W3	19900	1360	16200	18400	114
	W4	19900	1360	13700	14500	106

Table 4.9 Results of mass balance studies continued.....

	Dosage	Top-soil (mg kg^{-1}) before mix.	Chicken manure before mix. (mg kg^{-1})	Expected (mg kg^{-1})	Obtained (mg kg^{-1})	Recov. (%)
Mn						
	W1	348	506	356	414	116
	W2	348	506	362	396	109
	W3	348	506	380	437	115
	W4	348	506	401	498	124
Ni						
	W1	31.2	4.80	30	35.5	118
	W2	31.2	4.80	28.8	33.9	118
	W3	31.2	4.80	25.9	31.1	120
	W4	31.2	4.80	21.6	25.1	116
Pb						
	W1	6.43	1.67	6.20	7.21	116
	W2	6.43	1.67	7.36	6.88	93.5
	W3	6.43	1.67	5.48	6.68	121
	W4	6.43	1.67	4.84	5.96	123
U						
	W1	0.63	1.21	0.660	0.680	103
	W2	0.63	1.21	0.683	0.680	100
	W3	0.63	1.21	0.744	0.860	116
	W4	0.63	1.21	0.823	1.02	130
Zn						
	W1	37.1	500	59.1	64.5	109
	W2	37.1	500	79.2	89	112
	W3	37.1	500	130	150	115
	W4	37.1	500	191	260	136

The high percentage recovery could be due to possible contamination of the soil from the flower pots. However a major factor is likely to be variation in the results leading to high RSD values due to proximity to detection limit.

Chromium showed relatively lower percentage recoveries than Cd in the amended soil samples (W1 to W4) and ranged between 113 and 118 percent (Table 4.9). The RSDs of Cr was generally low and higher concentration values were found compared to the theoretical value (Table 4.9).

The percentage recovery for Cu ranged from 98.6 to 147% in the amended soil samples (W1 to W4) as shown in Table 4.9. There was close agreement between the amount of Cu obtained in W1, W2 and W3 and the theoretical value which indicated good recovery. The high percentage recovery of 146 % was observed in W4 which can be attributed to variation in the replicate samples leading to high RSD value

The recovery of Fe in the samples (W1 to W4) ranged from 106 to 114 (Table 4.9). This indicated that the mass balance between the theoretical values and the obtained values could be acceptable (although the obtained values were consistently higher than the theoretical values and the RSD values were good).

Manganese indicated a good recovery in W2 (106%) All treatments indicated acceptable but higher recoveries were obtained for W1-W3 (Table 4.8) suggesting addition of analyte from a yet to be known source.

A similar trend was observed with Ni where high recoveries were obtained in the amended soils (W1 to W4). These ranged from (116 to 120%) as shown in Table 4.9.

The percentage recovery for Pb in the amended soil samples ranged between 94 and 123% Treatments W3 and W4 showed the highest values (121 and 123% respectively).

The percentage recovery of U in the amended soil samples (W1 to W4) was generally good (Table 4.9). The obtained values were comparable with the theoretical values especially in W1 and W2. Exceptions were observed in W3 and W4 where the obtained values showed higher values of 116 and 130% (Table 4.9) respectively.

The percentage recovery for Zn in the samples (W1 to W4) was relatively high and ranged from 109 to 136% (Table 4.9). The obtained values of Zn showed a closer agreement with the theoretical values in W0-W3 indicating an acceptable mass balance result. Although, Zn in W4 was less than 3 %, the recovery was high (136%) suggesting a possible contamination, for example, from the flower pots used in the amendment studies.

Taken overall, the mass balance study provided evidence that addition of analytes occurred during the 30-day incubation period. This is most probably as a result of the water used to maintain field moisture conditions or could be could be from the flower pots used during the experiment. Future experiments were therefore modified such that each individual flower pot was thoroughly washed before use.

Table 4.10 Concentration (ug/L) PTE in distilled water (W) used for watering regime

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
W1	0.02	0.02	0.01	<0.00	1.17	0.02	0.01	0.02	0.01	<0.00
W2	0.002	<0.00	0.001	<0.00	1.33	0.02	0.001	0.02	0.001	<0.00
W3	<0.00	<0.00	<0.00	<0.00	1.02	0.00006	<0.00	0.01	<0.00	<0.00
W4	0.001	<0.00	<0.00	<0.00	1.74	0.02	0.00	0.01	<0.00	<0.00
W5	<0.00	<0.00	<0.00	<0.00	0.47	<0.00	0.001	0.02	<0.00	<0.00

W1 to W5 are replicate sample

Considering the low amount of the analytes found in the distilled water used for the watering regime, it may be assumed however that any addition to the treated soil samples would be negligible. This is because the total volume of distilled water used was about 200 to 400 mL and calculating the amount added based on this volume would indeed be negligible.

4.5 Experiment 2

As stated briefly in section 4.1, in experiment 2, additional fertiliser amendments (growmore, phostrogen, rockdust, seaweed and chicken manure) were used in assessing their effect on PTE levels and fractionation. A few modifications were made to the protocol used in experiment 1:

Equilibration time period of 40 days was used after amendment – this period was adopted in order to allow for a longer contact time between the soil and fertilisers added.

A uniform mass of soil-amendment mixture - 100g - was prepared for each experiment in order to obtain a more uniform mixture and allow for the application of a fixed amount of water after amendment. Lower dosages (1, 3 and 5%) of fertiliser were used because a number of literature studies had adopted similar levels[101, 110, 164], and considering the fact that much higher level of treatment could harm plants growing in the soil, when applied to soil, in later pot experiments.

Saucers were placed under each flower pot in order to minimize loss of analyte through leaching during watering, and where it occurred, the saucers were thoroughly rinsed, and the resultant solution poured back into the pot.

Portions of the soil sample (Table 4.1), were thoroughly mixed with each of the fertilisers: chicken manure, growmore, phostrogen, rock dust and seaweed (Table 4.1) in separate plastic containers and transferred into flower pots which were placed on saucers, in triplicate – the fertiliserss were applied at three dosage rates (1, 3 and 5% *m/m*) and allowed to stand for 40 days at room temperature (approximately 22 °C), with periodic addition of distilled water (10 mL/day) to keep the soil continuously moist. At the end of the experiment, samples were taken from each pot, air dried and homogenized for extraction.

4.6 Results and discussions

4.6.1 Physicochemical properties

The mean pH and organic matter (OM) content for the soil, fertilisers and the amended soil samples are presented in Table 4.11 with full data provided in Appendix E1 and E2. As can be seen, the pH of the soil was slightly acidic. The soil sample showed an intermediate level of organic matter (14.9%), fairly typical of well managed soils (5-15%)[70]. The particle size distribution of the soil indicated 80% sand, 16% silt and 4% clay which show that the soil was a *loamy sand*.

The pH of the added fertiliserss ranged from 3.90 ± 0.1 to 9.90 ± 0.1 with rockdust having the highest value (9.90). This value was higher than the value of 9.1 obtained by Ramezanian *et al.*[95] The pH of chicken manure was relatively high (7.70) compared to the other fertilisers except rockdust. A similar value of 7.95 has recently been reported by Kafle and Chen.[165] Seaweed, growmore and phostrogen showed the lowest pH values.

Chicken manure and seaweed contained the highest OM contents of 59.0% and 62.0% respectively. The OM levels in these materials were expected as they are organic in nature. Growmore and phostrogen samples were not heated to 550 °C for fear of explosion at such a high temperature as they are nitrogen containing fertilisers, but are not expected to contain appreciable concentrations of organic matter in their formulations.

With respect to the control soil, the addition of all the fertilisers did not in any significant way alter the pH values in the amended soil except perhap for growmore and seaweed. This could be attributed to the buffering nature of soil and the small amounts of material added. However, the addition of 5% chicken manure and seaweed resulted in increased OM content of the soil as would be expected given the organic nature of these materials (Table 4.11).

Table 4.11 Some properties of the fertilisers, soil and amended soil samples (Mean ± SD, n = 3)

	Dosage (%)	pH	OM (%)
CM	NA	7.70 ± 0.1	59.0 ± 2.1
GM	NA	3.90 ± 0.1	**
PG	NA	4.10 ± 0.1	**
RD	NA	9.93 ± 0.1	0.80 ± 0.1
SW	NA	5.20 ± 0.1	62.0 ± 0.1
Control	0	6.70 ± 0.1	14.9 ± 2.0
CMAS	1	6.80 ± 0.1	15.4 ± 1.2
	3	6.80 ± 0.1	15.8 ± 1.5
	5	6.90 ± 0.1	16.9 ± 1.6
GMAS	1	6.00 ± 0.1	14.5 ± 0.4
	3	5.80 ± 0.1	14.3 ± 1.4
	5	5.70 ± 0.1	14.3 ± 1.1
PGAS	1	6.30 ± 0.1	14.7 ± 0.2
	3	6.90 ± 0.1	15.1 ± 1.1
	5	6.90 ± 0.1	15.4 ± 0.7
RDAS	1	6.80 ± 0.1	13.6 ± 1.0
	3	6.80 ± 0.1	13.8 ± 1.3
	5	6.70 ± 0.1	13.9 ± 1.3
SWAS	1	6.70 ± 0.1	15.4 ± 2.4
	3	6.60 ± 0.1	16.4 ± 1.2
	5	6.40 ± 0.1	18.3 ± 1.8

CM-chicken manure; CMAS-chicken manure amended soil, GM-growmore; GMAS-growmore amended soil; PG-phostrogen; PGAS-phostrogen amended soil; RD-rock dust; RDAS-rock dust amended soil; SW-seaweed; SWAS-seaweed amended soil; OM-organic matter; NA-not applicable; **not determined.

4.6.2 Pseudototal PTE concentration in the fertilisers

Table 4.12 shows the results of mean pseudototal concentration for PTE in the additional fertiliserss used in experiment 2. The discussion here excludes that of chicken manure since it was discussed earlier in experiment 1 (section 4.4.3).

Table 4.12 Pseudototal metal concentration (mg kg^{-1}) in the fertilisers (mean ± S.D, n = 3)

PTE	Sample				
	Chicken manure	Growmore	Phostrogen	Rockdust	Seaweed
As	0.468 ± 0.03	4.45 ± 0.3	0.209 ± 0.02	1.49 ± 0.1	4.45 ± 0.3
Cd	(0.280 ± 0.01)	2.98 ± 0.03	0.05 ± 0.06	0.07 ± 0.07	0.35 ± 0.06
Cr	(4.90 ± 0.4)	19.0 ± 0.3	0.790 ± 0.14	14.7 ± 0.3	7.18 ± 0.6
Cu	(93.0 ± 4.7)	75.2 ± 2.2	47.5 ± 2.4	11.9 ± 1.7	17.7 ± 0.8
Fe	(1360 ± 5)	10100 ± 3530	552 ± 43	47800 ± 173	4600 ± 271
Mn	(506 ± 21)	439 ± 15	234 ± 6	754 ± 2	109 ± 4
Ni	(4.80 ± 0.2)	12.9 ± 1.0	0.26 ± 0.1	10.5 ± 0.4	4.80 ± 0.2
Pb	(1.70 ± 0.1)	3.00 ± 0.5	1.16 ± 0.05	3.43 ± 0.2	1.16 ± 0.2
U	(1.20 ± 0.03)	26.9 ± 0.8	0.04 ± 0.03	1.33 ± 0.02	0.620 ± 0.05
Zn	(500 ± 17)	404 ± 8	19.6 ± 0.7	1.25 ± 1.7	104 ± 4

( ) = values already discussed in experiment 1; (n = 3)

Arsenic

The RSD values for triplicate analysis of all fertilisers were less than 8% (n = 3). The mean concentration of the element in the individual fertilisers ranged from 0.209 to 4.45 mg kg^{-1}. Growmore and seaweed gave the same mean concentration and the highest. Arsenic is a well know impurity of inorganic fertilisers and its high concentration in growmore may be attributed to the phosphate content of the fertiliser. The concentration of As in all the fertilisers fell below the maximum recommended levels of the element in commercial fertilisers as shown in Table 4.6.

Cadmium

As shown in Appendix A, the RSD of Cd measurement in all the samples were greater than 10% (n = 3) with the exception of growmore. The poor RSDs can be attributed to high uncertainty in concentration values as measurement was carried out close to the limit of detection (LOD). The concentration of Cd in the amendments ranged from 0.07 to 2.98 mg kg^{-1} with growmore containing the highest concentration. Although, Otero *et al.*[5] had observed that Cd is one of the elements that shows the lowest concentration in fertilisers, the average concentrations of Cd obtained by Modaihsh *et al.*[1] in a variety of NPK fertilisers indicated that Cd concentrations could be as high as 28 mg kg^{-1} depending on the source. The relatively high concentration of Cd in growmore may be attributed to the source of the raw materials used in its formulation which may have contained elevated content of Cd impurity compared to the other fertilisers.

Chromium

The RSD of Cr concentration in all the fertilisers were generally less than 9% (n = 3) except for phostrogen whose RSD was greater than 17%. The high RSD may be attributed to the difference in the concentration of one of the replicate sample that produced a higher signal when compared with the other two replicate samples. The concentrations of Cr in the fertiliserss ranged from 0.790 to 19.0 mg kg^{-1} with growmore having the highest concentration. Rockdust contained higher concentration (14.7 mg kg^{-1}) when compared with phostrogen and seaweed. The natural occurrence of Cr in rocks can be the reason for its high concentration in this material.

Copper

The RSD of Cu measurement in the fertilisers studied were generally less than 6% (n = 3), showing good precision during the extraction process. However, an RSD of 14.5% for Cu measurement in rockdust was observed. The pseudototal concentration of Cu in the various fertilisers ranged from 11.9 to 75.2 mg kg^{-1}. Copper is an essential nutrient for plants therefore, its high concentration in commercial fertilisers may be expected. The values of Cu obtained for growmore and phostrogen in this work were generally greater than the results reported by Otero *et al.*[5] and Milinovic *et al.*[98] in a variety of NPK fertilisers. However, a few of their results were higher than those reported in this study. The concentration of Cu in rockdust obtained in this work was higher than the

values of 8.17 and 7.30 mg kg^{-1} reported by Ramezanian *et al*[95] and Ramezanian *et al*[96] respectively for rockdust. The concentration of seaweed (17.7 mg kg) obtained in this work was similar to the concentration obtained by Al-Shwafi and Rushdi[166] in different species of seaweeds and fell below the maximum recommended concentration of Cu in organic fertilisers (Table 4.6).

Iron

The RSD of Fe concentration in all the samples were below 10% (n = 3), whereas in growmore, a higher value, greater than 30% was obtained. It was evident in that the difference in the concentration of one of the replicate samples gave a higher signal when compared with the other two replicate samples (Appendix A) possibly due to non-homogenous distribution of the element in the sample. The mean concentration of Fe in the amendments ranged from 552 to 47800 mg kg^{-1}. The highest value was obtained in rockdust. The concentration of Fe (47800 mg kg^{-1}) obtained in this study exceeded the levels reported by Ramezanian *et al.*[95] and Ramezanian *et al.*[96] The differences observed might be due to the sources from which the material was obtained. The value of 10100 mg kg^{-1} obtained for growmore exceeded the values (100 to 13000 mg kg^{-1}) reported by Otero *et al*[5] in a wide range of NPK fertilisers. The concentration of Fe specified in the seaweed product specification or declaration (1970 mg kg^{-1}) was less than the concentration obtained in this work.

Manganese

A good precision was achieved for Mn measurement in all the fertilisers as shown by the RSD values of less than 5% (n = 3). The mean concentration of Mn in the materials analysed ranged from 109 to 754 mg kg^{-1}**.** The highest concentration was obtained in Rockdust and is higher than the values of 297 and 375 mg kg^{-1} reported by Ramezanian *et al.*[95] and Ramezanian *et al.*[96] respectively. Growmore and phostrogen contained concentrations of 439 and 234 mg kg^{-1} respectively. These values were generally higher than the concentration of Mn in most of the NPK fertiliser samples analysed by Otero *et al.*[5] Seaweed contained the lowest concentration of Mn compared to the other fertilisers. This value was less than the concentration of 153 mg kg^{-1} declared in the product specification certificate but higher than the value obtained by Ryan.[167]

Nickel

The RSD of Ni measurement in all the samples showed good precision as the values obtained were less than 5% (n =3). However, an exception was observed for phostrogen whose RSD value was greater than 39%. This high value was due to the lower signal one of the replicate samples gave (Appendix A). The mean concentrations of Ni in the individual fertiliserss ranged from 0.26 to 12.9 mg kg^{-1}. The highest Ni concentration was found in growmore. The concentration of Ni in growmore and phostrogen were less than the results obtained by Senesi and Polemio[168] in a variety of NPK fertilisers. The values were also far less than the 180 mg kg^{-1} value recommended in the Canadian regulations for Ni in commercial fertilisers. The level of Ni in rockdust was second highest and the value (10.5 mg kg^{-1}) was similar to the concentrations earlier reported by Ramerazian *et al.*[95] and Ramezanian *et al.*[96] Seaweed contained 4.80 mg kg^{-1} of Ni which was relatively lower than the concentration of Ni reported by Ryan *et al.*[97] for seaweed. This value also fell below the 50 mg kg^{-1} set by EU and Czech regulations respectively.

Lead

Apart from the RSD of Pb concentration in growmore and seaweed (Appendix A), the precision was generally good with RSD values of less than 7% (n = 3). The mean concentrations of Pb in the samples ranged from 1.16 to 3.43 mg kg^{-1}. The concentration of Pb in growmore and rockdust were relatively higher when compared with the other fertilisers. These concentrations are far below the Canadian and Chinese maximum recommended levels of 500 mg kg^{-1} and 100 mg kg^{-1} in fertilisers respectively. However, the values of 3.00 mg kg^{-1} and 1.16 mg kg^{-1} for growmore and phostrogen are far less than the results reported by Senesi and Polemio[168], and Modaishsh *et al.*[1] in their separate studies of NPK fertilisers. The concentration of Pb obtained for rockdust in this study was relatively higher than the levels of (1.98 mg kg^{-1})[95] and (2.5 mg kg).[96] Morrison *et al.*[93] had earlier reported low concentrations (0.118 to 2.11 mg kg^{-1}) of Pb in a similar species of seaweed to that used in this study. The concentration of Pb obtained in this study for all the fertilisers did not exceed the maximum recommended levels as shown in Table 4.6.

Uranium

The RSD were less than 8% (n = 3) which showed good precision. However, the RSD of U concentration in phostrogen was greater than 72%. The high RSD resulted uncertainty in concentration values that was high because measurement was carried out close to LOD. The concentration of U in all the samples was generally low. However, growmore recorded a high concentration (26.9 mg kg^{-1}) compared to the concentrations of the other fertilisers. This was unexpected as the percentage of phosphate in the phostrogen amendment is 10% while that of growmore is less (7%) as shown in Table 4.1. One might expect an association between U and PO_4^{3-}, which also depends on the source and type of PO_4^{3-}. It may be assumed that the growmore contained PO_4^{3-} derived from sedimentary phosphates but phostrogen does not.

Zinc

The RSD of Zn measurement in all the materialss were less than 8% (n = 3), indicating good precision of during the analysis. The mean concentration of the element in the respective fertilisers ranged from 1.25 to 404 mg kg^{-1}. The high value of Zn (404 mg kg^{-1}) in growmore may suggest a possible addition of the element to the fertiliser during its formulation. Low concentration was observed in phostrogen and its presence might be traced to fortification as well. Rockdust contained the lowest concentration of Zn. Ramezanian *et al.*[96] reported a higher concentration in their study. This may be due to differences in the source materials. Zinc was relatively high in seaweed (104 mg kg). This value was higher than the concentrations of 85.2 mg kg^{-1} and 80.9 mg kg^{-1} reported in the product specification certificate and Ryan *et al.*[97] respectively for seaweed. Generally, the levels of Zn measured in all the fertilisers were lower than the EU and Canadian maximum recommended concentration in organic and commercial inorganic fertilisers.

4.6.3 Pseudototal PTE concentration in the amended soils

The amended soils were analysed for pseudototal PTE content after the 40-day period and results obtained are discussed below. It should be noted for all the figures that CMAS = chicken manure amended soil; GMAS = Growmore amended soil; PGAS = phostrogen amended soil; RDAS = rockdust amended soil and SWAS = seaweed amended soil.

Arsenic

Figure 4.11 shows the mean concentration of As in the amended soil samples. The RSD of As measurement were generally less than 14% (n = 3, except 3% dosage rockdust treated sample where n = 2). However, RSD values greater than 30% were recorded for the 5% chicken manure and growmore amended soil samples. This was as a result of variations in the concentrations of one replicate, signifying inhomogeneous distribution of As in the samples. The addition of all the fertiliser materials did not show any marked trend. This was expected since as shown in Table 4.13, the amounts of fertiliser added, and As concentrations therein, should not have had marked effect on As concentrations in the mixtures.

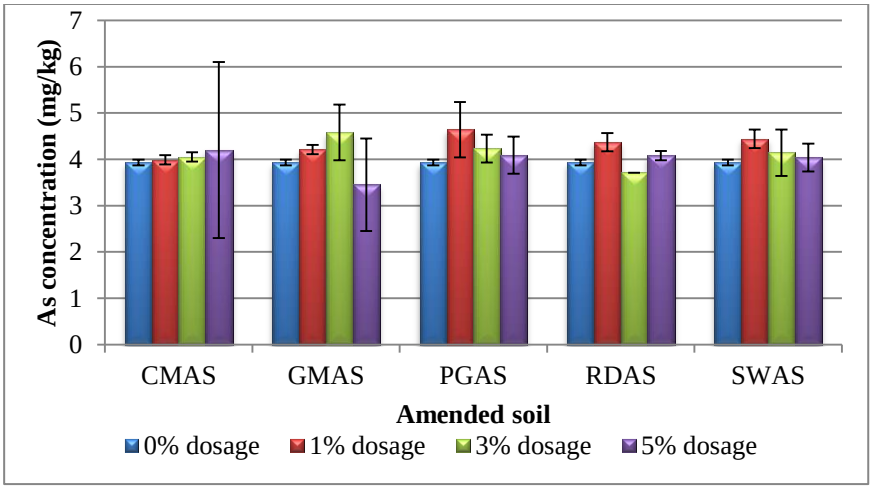


Figure 4. 11 Pseudototal concentration of As and in the amended soil samples (n = 3)

Table 4.13 Result of mass balance for As in the amended soils at all dosages

Dos.	Exptd mg kg^{-1}	Found mg kg^{-1}	Rec %	Dos.	Exptd mg kg^{-1}	Found mg/kg	Rec %	Dos.	Exptd. mg kg^{-1}	Found mg kg^{-1}	Rec. %
1%				**3%**				**5%**			
CM	3.99	3.99	100	CM	3.83	4.05	106	CM	3.76	4.20	112
GM	3.94	4.21	107	GM	3.95	4.58	116	GM	3.96	3.45	87
PG	3.89	4.64	119	PG	3.82	4.23	111	PG	3.74	3.90	104
RD	3.91	4.37	112	RD	3.86	3.71	96	RD	3.81	4.08	107
SW	3.94	4.44	113	SW	3.95	4.14	105	SW	3.96	4.04	102

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 1, 3, and 5% = dosages of each of the amendments.

Cadmium

The RSD of Cd in all the amended soil samples were greater than 55% (n =3), except for the 3% growmore amended soil sample whose RSD value was found to be 6.57%. The explanation to the poor RSD values may be attributed to measurement which was carried out close to LOD. Indeed, in most of the samples, Cd was not detected (Appendix F) and so no figure of Cd results is presented.

Chromium

Figure 4.12 shows the pseudototal concentration of Cr in the amended soil samples. The RSD of Cr in the fertiliser amended soil samples were generally less than 15% (n = 3), except in the 3% rockdust amended soil sample, and 5% chicken manure and rockdust amended soil samples – the RSD of Cr measurement in these samples were higher than 21% (n = 3) as can be seen in (Appendix F). The mean concentration of Cr on addition of the fertilisers again, did not result in any clearly defined trends. As would be expected, based on the mass balance calculations presented in Table 4.14, but any differences are generally small within experimental uncertainty, as indicated by the error bars in Figure 12.

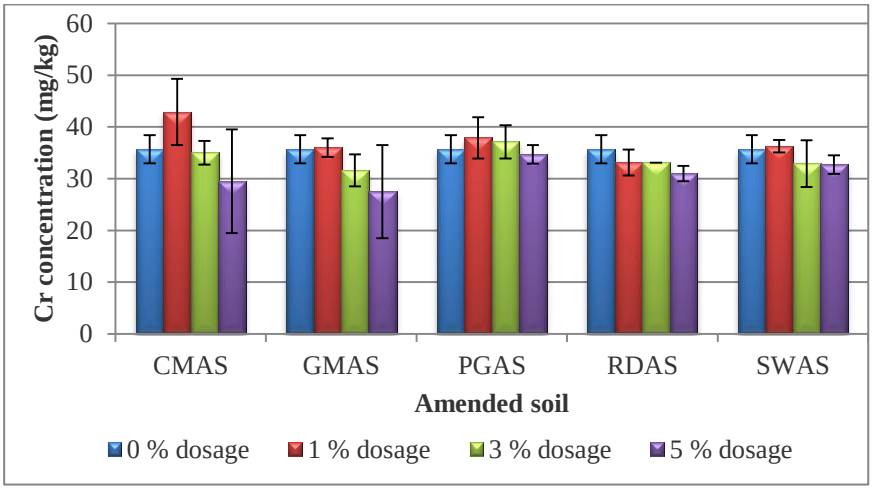


Figure 4. 12 Pseudototal concentration of Cr in the amended soil samples (n = 3)

Table 4.14 Result of mass balance for Cr in the amended soils at all dosages

Dos.	Exptd mg kg^{-1}	Found mg kg^{-1}	Rec %	Dos.	Exptd mg kg^{-1}	Found mg/kg	Rec %	Dos.	Exptd. mg kg^{-1}	Found mg kg^{-1}	Rec. %
1%				**3%**				**5%**			
CM	35.4	42.9	121	CM	34.8	35.0	101	CM	34.2	29.5	86
GM	35.5	35.9	101	GM	35.2	31.6	90	GM	35.0	27.5	79
PG	35.3	37.9	107	PG	34.7	37.1	107	PG	34.0	34.7	102
RD	35.5	33.1	93	RD	35.1	33.1	94	RD	34.4	31.0	90
SW	35.4	36.3	103	SW	34.8	32.9	95	SW	34.3	32.7	95

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 1, 3, and 5% = dosages of each of the amendments.

Copper

Figure 4.13 shows the mean concentration of Cu in the amended soil samples. The RSD of Cu measurement in the fertiliser amended soil samples were generally less than 11% (n = 3). This indicated that the result was of acceptable level of uncertainty. The addition of chicken manure, growmore and to a lesser extent, phostrogen increased the concentration of Cu in the soil when compared to the control soil sample, in line with Cu concentrations in the fertilisers (Table 4.12) into soil thereby increasing its levels in soil. Rockdust and seaweed contained the lowest amount of Cu and their addition did not result in any marked variation. Mass balance was acceptable as shown in Table 4.15, indicating no loss of analyte through leaching, nor addition through contamination.

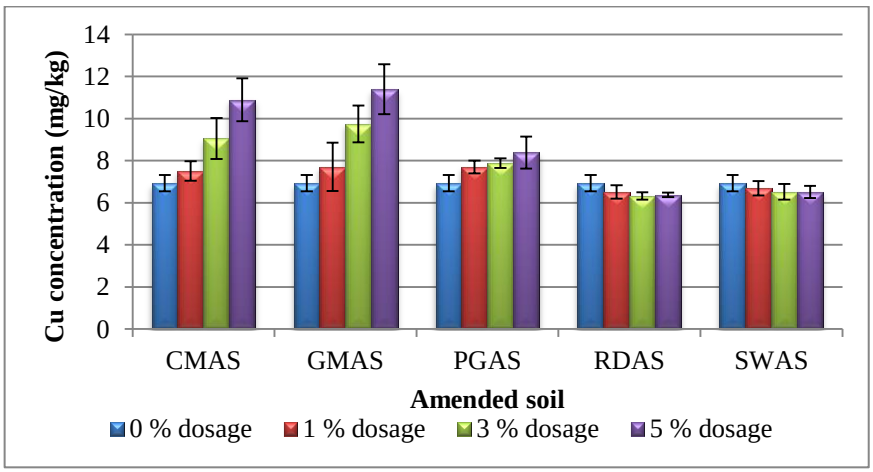


Figure 4. 13 Pseudototal metal concentration Cu in the amended soil samples (n = 3)

Table 4.15 Result of mass balance for Cu in the amended soils at all dosages

Dos.	Exptd mg kg^{-1}	Found mg kg^{-1}	Rec %	Dos.	Exptd mg kg^{-1}	Found mg/kg	Rec %	Dos.	Exptd. mg kg^{-1}	Found mg kg^{-1}	Rec. %
1%				**3%**				**5%**			
CM	7.79	7.52	97	CM	9.51	9.05	95	CM	11.2	10.9	97
GM	7.61	7.70	101	GM	9.00	9.75	108	GM	10.3	11.4	111
PG	7.33	7.70	105	PG	8.14	7.88	97	PG	8.96	8.39	94
RD	6.98	6.51	93	RD	7.08	6.32	89	RD	7.20	6.38	89
SW	7.03	6.69	95	SW	7.25	6.56	90	SW	7.47	6.52	87

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 1, 3, and 5% = dosages of each of the amendments.

Iron

Figure 4.14 shows the mean pseudototal concentration of Fe in the amended soil samples. The RSD of Fe measurement in the fertiliser amended soil samples as shown in (Appendix F) were generally less than 10% (n = 3). Although the concentration of Fe in the fertiliser amendments (Table 4.12) are relatively high, the amount of Fe in the control soil sample was much higher than in the amendments. The addition of all the materials at the various dosages resulted in a corresponding decrease in Fe levels in the soil, except for rockdust amended soil where a slight increase of Fe was perhaps observed. This was as expected as the latter fertiliser material contained the highest concentration of Fe and as confirmed by the result of the mass balance studies in Table 4.16.

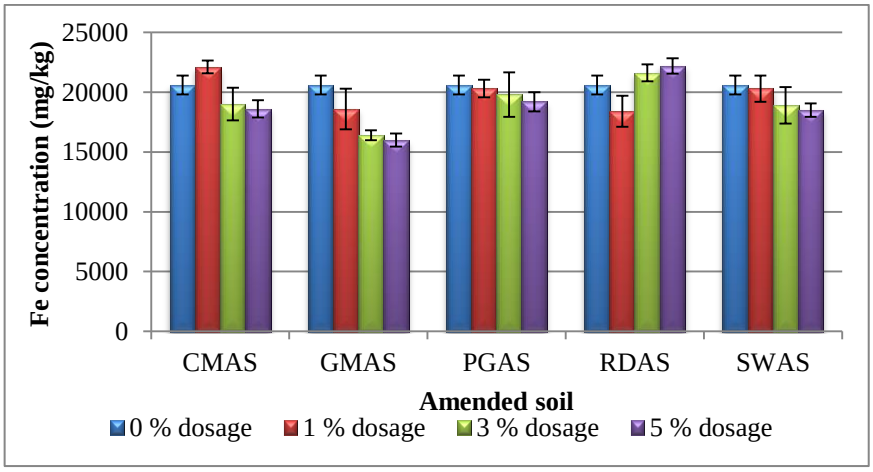


Figure 4. 14 Pseudototal metal concentration Fe in the amended soil samples (n = 3)

Table 4.16 Result of mass balance for Fe in the amended soils at all dosages

Dos	Exptd mg kg^{-1}	Found mg kg^{-1}	Rec %	Dos.	Exptd mg kg^{-1}	Found mg/kg	Rec %	Dos.	Exptd. mg kg^{-1}	Found mg kg^{-1}	Rec. %
1%				**3%**				**5%**			
CM	20400	22100	108	CM	20000	19000	95	CM	19600	18600	95
GM	20500	18600	91	GM	19900	16400	82	GM	20100	16000	80
PG	20400	20300	100	PG	20000	19800	99	PG	19600	19200	98
RD	20900	18400	88.0	RD	21400	21600	101	RD	22000	22200	101
SW	20400	20300	100	SW	20100	18900	94	SW	19800	18500	93

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 1, 3, and 5% = dosages of each of the amendments.

Manganese

Figure 4.17 shows the concentration of Mn in the amended soil samples. The RSD of Mn measurement in the amended soil samples were generally less than 11% (n = 3). The addition of all the fertiliser materials did not result in any significant changes in the the concentration of the amended soil, which is as expected considering the quantity of fertilisers added, except for seaweed. Thismaterial resulted in a marked decrease in the concentration of Mn as the material was added. This was not expected as can be seen in the mass balance calculations for Mn/SW in Table 4.17. A tentative explanation may be the fact that seaweed, being rich in organic matter, caused the soil to become anerobic, mobilising Mn and subsequently allowing it to leach out. However a similar effect was not noted for chicken manure which contains almost the same amount of organic matter.

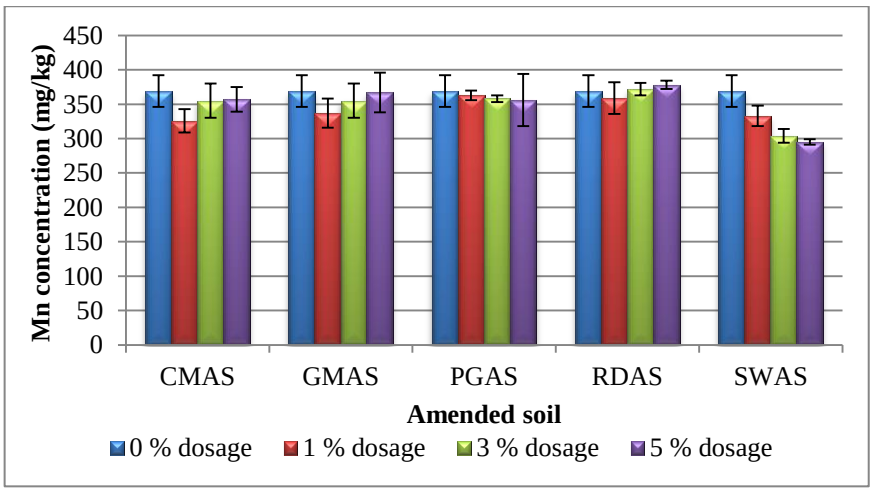


Figure 4.15 Pseudototal concentrations Mn in the amended soil samples (n = 3)

Table 4.17 Result of mass balance for Mn in the amended soils at all dosages

Dos.	Exptd mg kg^{-1}	Found mg kg^{-1}	Rec %	Dos.	Exptd mg kg^{-1}	Found mg/kg	Rec %	Dos.	Exptd. mg kg^{-1}	Found mg kg^{-1}	Rec. %
1%				**3%**				**5%**			
CM	370	326	88	CM	373	355	95	CM	376	357	95
GM	370	337	91	GM	371	355	96	GM	373	367	98
PG	368	363	99	PG	365	358	98	PG	362	356	98
RD	373	359	96	RD	381	372	98	RD	388	378	97
SW	366	333	91	SW	361	304	84	SW	356	295	83

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 1, 3, and 5% = dosages of each of the amendments.

Nickel

Figure 4.16 shows the mean pseudototal concentration of Ni in the amended soil samples. The RSD of Ni measurement in all the fertiliser amended soil samples were less than 8% (n =3). There was perhaps a slight decrease, but were no marked differences in the concentration of Ni on addition of the fertilisers. This trend was expected based on the mass balance calculations in Table 4.18.

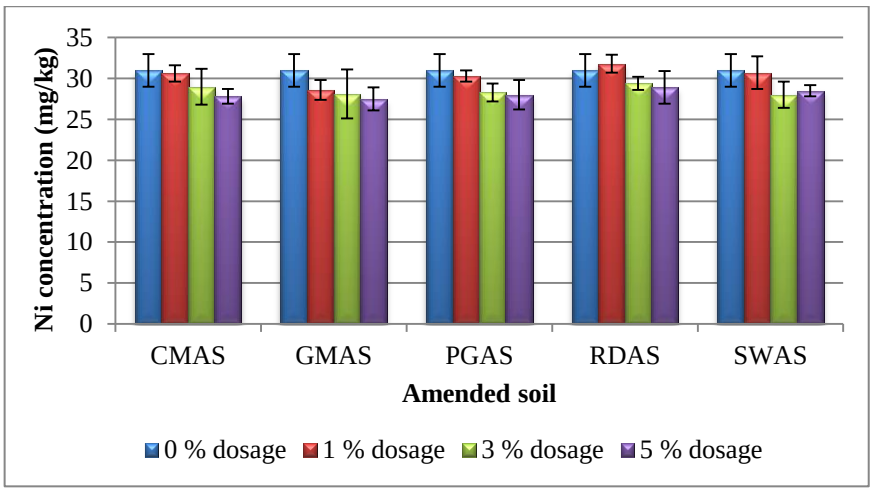


Figure 4.16 Pseudototal concentrations Ni in the amended soil samples (n = 3)

Table 4.18 Result of mass balance for Ni in the amended soils at all dosages

Dos.	Exptd mg kg^{-1}	Found mg kg^{-1}	Rec %	Dos.	Exptd mg kg^{-1}	Found mg/kg	Rec %	Dos.	Exptd. mg kg^{-1}	Found mg kg^{-1}	Rec. %
1%				**3%**				**5%**			
CM	30.7	30.6	100	CM	30.2	29.0	96	CM	29.7	27.8	94
GM	30.8	28.6	93	GM	30.5	28.1	92	GM	30.1	27.5	91
PG	30.7	30.3	99	PG	30.1	28.3	94	PG	29.5	28.0	95
RD	30.8	31.8	103	RD	30.1	29.4	98	RD	30.0	28.9	96
SW	30.7	30.7	100	SW	30.2	28.0	93	SW	29.7	28.5	96

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 1, 3, and 5% = dosages of each of the amendments.

Lead

Figure 4.17 shows the mean pseudototal concentration of Pb in the amended soil samples. The RSD of Pb measurement in the fertiliser amended soil samples were generally less than 12% (n = 3). The addition of chicken manure, growmore, rockdust and seaweed at the various dosages (1%, 3% and 5%) did not show any marked trends in the concentration of Pb. The mass balance results (Table 4.19) confirm this behaviour was expected. However, when phostrogen and seaweed were added at the same dosages, the concentration of Pb decreased very slightly in the amended soil compared to the control soil sample.

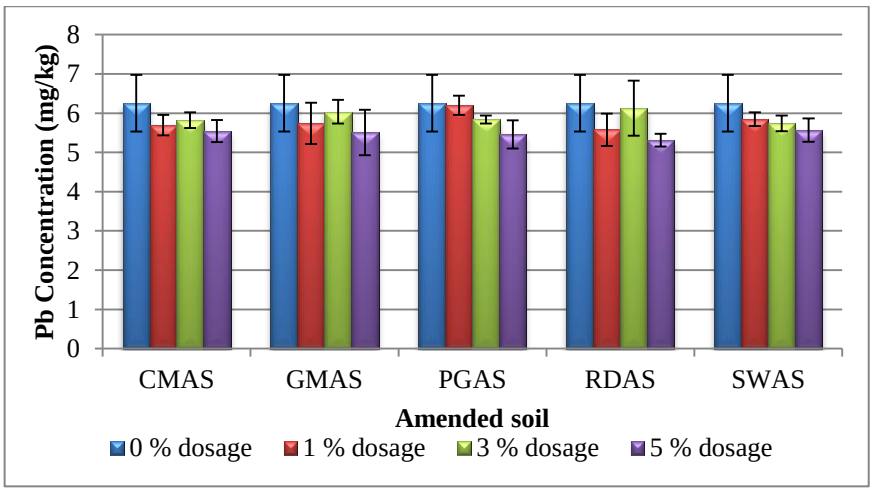


Figure 4.17 **Pseudototal concentrations Pb in the amended soil samples (n = 3)**

Table 4.19 **Result of mass balance for Pb in the amended soils at all dosages**

Dos.	Exptd mg kg^{-1}	Found mg kg^{-1}	Rec %	Dos.	Exptd mg kg^{-1}	Found mg/kg	Rec %	Dos.	Exptd. mg kg^{-1}	Found mg kg^{-1}	Rec. %
1%				**3%**				**5%**			
CM	6.20	5.70	92	CM	6.10	5.82	95	CM	6.00	5.54	92
GM	6.20	5.74	93	GM	6.20	6.04	97	GM	6.10	5.51	90
PG	6.20	6.20	100	PG	6.30	5.83	93	PG	6.00	5.46	91
RD	6.22	5.58	90	RD	6.17	6.13	99	RD	6.11	5.31	87
SW	6.20	5.85	94	SW	6.20	5.73	92	SW	6.00	5.57	93

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 1, 3, and 5% = dosages of each of the amendments.

Uranium

Figure 4.18 shows the mean pseudototal concentration of U in the amended soil samples. The RSD of U measurement in all the fertiliser amended soil samples were generally less than 13% (n = 3). There was an exception for soil samples treated with 3% seaweed, which recorded an RSD of 41%. This was due to the fact that one of the replicate samples resulted in a higher concentration value compared to others (Appendix F). The addition of growmore (Figure 4.18) resulted in a corresponding increase in the level of U in the amended soils compared to the control soil sample due to the high level of U in the material. However, no significant differences were observed with the addition of chicken manure, phostrogen, rockdust and seaweed fertilisers to the soil. This trend was expected as further confirmed by the mass balance calculations (Table 4.20).

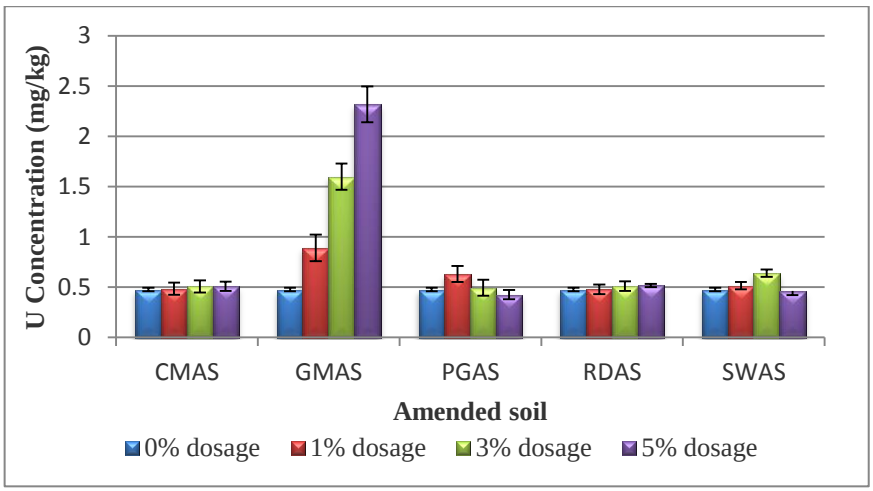


Figure 4.18 Pseudototal concentrations U in the amended soil samples (n = 3)

Table 4.20 Result of mass balance for U in the amended soils at all dosages

Dos.	Exptd mg kg^{-1}	Found mg kg^{-1}	Rec %	Dos.	Exptd mg kg^{-1}	Found mg/kg	Rec %	Dos.	Exptd. mg kg^{-1}	Found mg kg^{-1}	Rec. %
1%				**3%**				**5%**			
CM	0.483	0.484	100	CM	0.498	0.507	102	CM	0.513	0.510	99
GM	0.740	0.890	120	GM	1.30	1.60	123	GM	1.80	2.32	129
PG	0.472	0.630	133	PG	0.463	0.492	106	PG	0.454	0.425	94
RD	0.485	0.478	99	RD	0.502	0.509	101	RD	0.519	0.516	99
SW	0.478	0.514	108	SW	0.480	0.64	133	SW	0.483	0.460	95

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 1, 3, and 5% = dosages of each of the amendments.

Zinc

Figure 4.19 shows the mean pseudototal concentration of Zn in the amended soil samples. The RSD of Zn measurement in the samples were generally less than 14% (n = 3). The addition of chicken manure and growmore raised the concentration of Zn in the soil, dosages in a corresponding manner whereas the addition of phostrogen, rockdust and seaweed did not cause any significant change to the original level of Zn in the soil . The magnitude of the increase was as expected based on mass balance calculations (Table 4.21).

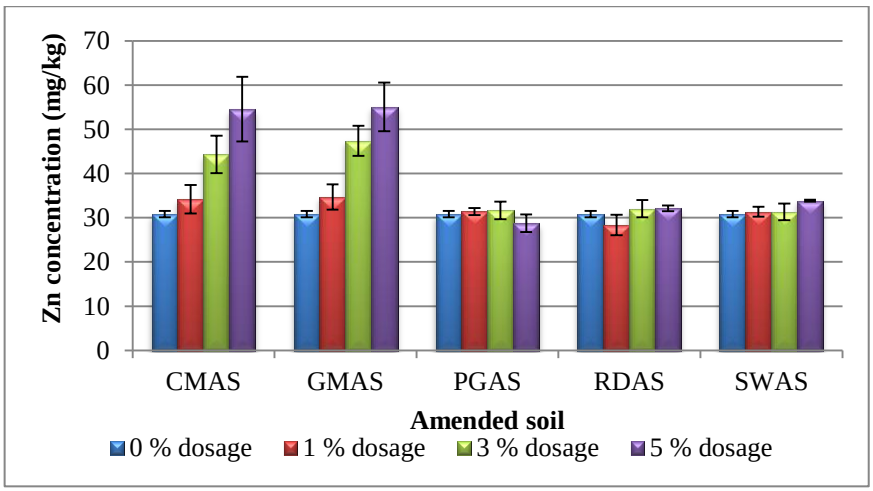


Figure 4.19 Pseudototal concentrations Zn in the amended soil samples (n = 3)

Table 4.21 Result of mass balance for Pb in the amended soils at all dosages

Dos.	Exptd mg kg^{-1}	Found mg kg^{-1}	Rec %	Dos.	Exptd mg kg^{-1}	Found mg/kg	Rec %	Dos.	Exptd. mg kg^{-1}	Found mg kg^{-1}	Rec. %
1%				**3%**				**5%**			
CM	35.5	34.2	96	CM	44.9	44.3	99	CM	54.3	54.6	101
GM	34.5	34.7	101	GM	42.0	47.4	113	GM	49.5	55.1	111
PG	30.7	31.4	102	PG	30.5	31.6	104	PG	30.2	28.8	95
RD	30.5	28.4	93	RD	30.0	32.1	107	RD	29.3	32.2	110
SW	31.5	31.4	100	SW	33.0	32.1	97	SW	34.5	33.9	98

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 1, 3, and 5% = dosages of each of the amendments.

Results reported so far have aided the development of a robust experimental protocol where there were no uncontrolled loses of analyte due to leaching, nor addition of analyte due to contamination. Pseudototal concentrations of PTE measured in amended soils were generally similar to those predicted from mass balance calculations.

4.7 The BCR sequential extraction of fertilisers and amended soils

The next, and more interesting, step in the study was to examine the impact of the fertilisers on PTE mobility. This was done by means of the BCR sequential extraction protocol.

4.7.1 Detection Limits

Table 4.22 shows the intrument and procedural detection limits of PTE measured in different metrices as described in section 3.5 after for BCR sequential extraction.

Table 4.22 Intrument and procedural detection limits of PTE measured in different metrices

	EXC.		RED.		OXID.		RES.	
	DL inst. (µg/l)	DLpro. (mg/kg)	DL inst. (µg/l)	DLpro. (mg/kg)	DL inst. (µg/l)	DLpro. (mg/kg)	DL inst. (µg/l)	DLpro. (mg/kg)
As	0.00720	0.00029	0.0670	0.0027	0.021	0.00120	0.0360	0.0036
Cd	0.00700	0.000280	0.0290	0.0120	0.015	0.00077	0.0085	0.00085
Cr	0.0520	0.00210	0.447	0.0180	0.105	0.00530	0.232	0.0230
Cu	0.106	0.00420	0.425	0.0170	0.370	0.0190	0.151	0.0150
Fe	0.139	0.00550	0.492	0.0200	0.701	0.0350	2.73	0.273
Mn	0.077	0.00310	0.177	0.0071	0.239	0.0120	0.0380	0.00380
Ni	0.109	0.00430	0.671	0.0270	0.063	0.00310	0.0230	0.00230
Pb	0.0120	0.00049	0.154	0.0062	0.131	0.00660	0.0340	0.00340
U	0.00078	0.000031	0.0037	0.00015	0.012	0.000620	0.0024	0.00024
Zn	1.16	0.0460	0.673	0.0270	0.660	0.0330	0.166	0.0170

EXC = exchangeable step; RED = reducible step; OXID. = oxidisable step; RES. = residual

4.7.2 Validation of the method (BCR sequential extraction protocol)

The quality of the data obtained by the method was assessed by the analysis of a certified reference material (CRM); BCR-701 (Lake sediment) alongside the samples. The CRM was used in this experiment because it was handy at the time of the analysis. The results and the certified/indicative values for the CRM are presented in Table 4.23. The obtained and certified/indicative values for the PTE were generally good as recoveries were (100 ± 30%) which indicated that the results obtained were of high quality. However, there were exceptions – In exchangeable step, Cr, Fe or Mn were over extracted (124%), while Pb was under extracted (83%). Manganese was over extracted (114%) again in reducible step and Pb was under extracted (78%) in oxidisable step. Chromium and Pb were again over extracted with recoveries of 121% and 152% respectively. Although Cr and Fe were overextracted in exchangeable step, less of these elements were extracted in residual step and reducible step respectively such that their sum in certified/indicative and obtained were almost the same (Table 4.23). However, the sum of Mn values in certified/indicative is considerably less than that obtained, and this confirms

overextraction. For Pb, which was under extracted from exchangeable step and oxidisable step, substantially more than expected was extracted in residual step such that the sum of certified/indicative and obtained values in all the fractions were about the same (Table 4.23).

Table 4.23 Quality control of BCR protocol using CRM-701 (Lake Sediment) showing PTE levels (mg kg^{-1}, n = 3)

		Cd	Cr	Cu	Fe*	Mn*	Ni	Pb	Zn
EXC.	Cert. value	7.34 ± 0.4	2.26 ± 0.16	49.3 ± 1.7	71.0 ± 1.0	170 ± 1	15.4 ± 0.9	3.18 ± 0.21	205 ± 6
	Obt. value	8.53 ± 0.2	2.80 ± 0.1	46.7 ± 0.8	88.0 ± 4.0	211 ± 11	14.6 ± 0.2	2.64 ± 0.05	196 ± 3
	% Rec.	116	124	95	124	124	95	83	96
RED.	Cert. value	3.77 ± 0.3	45.7 ± 2.0	124 ± 3	7700	125 ± 2	26.6 ± 1.3	126 ± 3	114 ± 5
	Obt. value	4.33 ± 0.04	44.9 ± 1.0	120 ± 4	7070 ± 60	143 ± 3	25.2 ± 0.1	129 ± 5	105 ± 0.7
	% Rec.	115	98.2	97	92	114	95	102	92
OXID.	Cert. value	0.270 ± 0.06	143 ± 7	55 ± 4	1080 ± 53	23.0 ± 1	15.3 ± 0.9	9.30 ± 2.0	46 ± 4
	Obt. value	0.295 ± 0.01	136 ± 7	59.2 ± 3.0	1150 ± 65	23.5 ± 1.3	16.3 ± 1.0	7.22 ± 0.4	49.0 ± 2.0
	% Rec.	109	95	108	106	102	107	78	107
RES.	Ind. value	11.7 ± 0.6	62.5 ± 7.4	38.5 ± 11.2	25500 ± 197	299 ± 7	41.4 ± 4.0	11.0 ± 5.2	95.0 ± 13
n = 2	Obt. value	11.3 ± 0.2	75.8	38.6	24600	306	40.9	16.8	101
	% Rec.	97	121	103	96	102	97	152	106
	∑ ind./cert. values	23.1	253	267	34400	617	98.7	149	460
	∑ obt. values	24.5	259	265	33000	683	97.0	156	451

EXC = exchangeable step; RED = reducible step; OXID. = oxidisable step; RES. = residual

*The concentrations of Fe and Mn are reported by Kubova *et al*[169] and are taken as indicative values

4.7.3 The BCR sequential extraction of fertilisers

Pseudototal analysis of the fertiliser amended soil concentration in soil confirmed literature findings that support the hypothesis that fertilisers can increase PTE levels in soil. However, it is necessary also to have knowledge of the forms of these elements in such fertiliser materials, to understand their influence on PTE mobility and availability.

Arsenic

Table 4.24 shows the results obtained for As sequential extraction, the mean pseudototal (PT) concentration and extraction recovery in the fertilisers. Figure 4.20 shows the As fractionation.

Table 4.25 Mean concentration (mg kg^{-1}) of As extracted using BCR sequential procedure in the fertilisers in each fraction.

	EXC.	% RSD	RED.	% RSD	OXID.	% RSD	RES.	% RSD	SUM	PT	%REC
CM	0.381	3.4	0.405	18.8	0.123	9.4	0.0524	6.6	0.961	0.468	205
GM	2.62	3.3	0.538	6.7	0.300	NA	0.0643	55.3	3.22	4.45	72
PG	0.512	NA	<0.0027	NA	<0.0012	NA	<0.0036	NA	0.512	0.209	245
RD	0.195	NA	0.370	6.0	0.0296	2.7	0.0290	1.0	0.430	1.49	29
SW	0.217	1.5	1.54	4.7	<0.0012	NA	<0.0036	NA	1.76	4.45	40

(*) = LOD; CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; EXC. = exchangeable frection; RED. = reducible fraction; OXID. = oxidisable fraction; RES. = residual fraction; REC. = recovery, NA = (not applicable) because concentrations values were not detected and/or duplicate values were used to obtain mean contration.

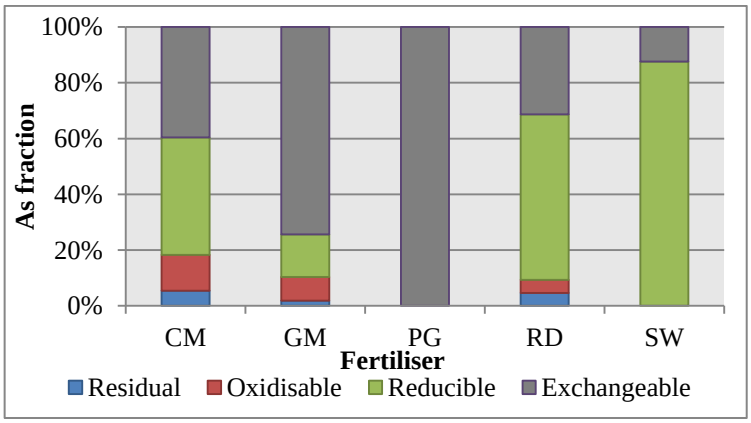


Figure 4.20 Arsenic fractionation in fertilisers by means of the BCR procedure (n = 3)

The RSD of As measurement in all the samples was generally less than 10% except for chicken manure in the reducible fraction (RSD ≈ 19%) and growmore in the residual fraction (RSD >55%). The extraction recovery of As in all the fractions was very poor (29 – 245%) as shown in Table 4.25. High level of As was found in the exchangeable and reducible fractions of the fertilisers, with almost 100% present in the exchangeable fraction of phostrogen, indicating that this element can be readily available. Smaller level of As were found in the other fractions (Figure 4.20).

Cadmium

Table 4.26 shows the results obtained for Cd sequential extraction, mean pseudototal (PT) concentration and extraction recovery in the fertilisers. Figure 4.21 shows the Cd fractionation. The RSD of Cd measurement in the samples was generally less than 10% for the first two fractions, except the reducible fraction of rockdust (RSD ≈ 14%).

Table 4.26 Mean concentration (mg kg^{-1}) of Cd extracted using BCR sequential procedure in the fertilisers in each fraction.

	EXC.	% RSD	RED.	% RSD	OXID.	% RSD	RES.	% RSD	SUM	PT	% REC
CM	0.0487	7.2	0.173	6.0	0.0460	21.0	0.0038	13.4	0.272	0.28	97
GM	2.89	1.8	0.0670	1.8	0.0188	NA	0.0031	73.9	2.98	2.98	100
PG	0.0113	9.9	0.<0.012	NA	<0.0007*	NA	<0.0008*	NA	0.011	0.05	23
RD	0.0110	NA	0.00495	14.3	0.00268	45.9	0.0052	29.5	0.023	0.07	34
SW	0.117	NA	0.193	1.6	<0.0007*	NA	<0.0008*	NA	0.310	0.35	89

(*) = LOD; CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; EXC. = exchangeable frection; RED. = reducible fraction; OXID. = oxidisable fraction; RES. = residual fraction; REC. = recovery. NA = (not applicable) because concentrations values were not detected and/or duplicate values were used to obtain mean concentration.

Poorer precision of less than 74% of Cd measurement was obtained in the oxidisable and residual fraction, probably due to closeness of measured concentrations to the LOD. The extraction recovery of Cd in all the fractions (Table 4.26) was good for chicken manure, growmore and seaweed (97, 100 and 89% respectively) while gross under extraction of Cd was observed in phostrogen and rockdust samples (23 and 34% respectively). The Cd was released in the exchangeable and reducible fractions

in most of the fertiliser samples. A relatively high concentration of Cd was present in the residual fraction of rockdust (Figure 4.21), indicating that a proportion of Cd in rockdust might not be easily available or mobilisable.

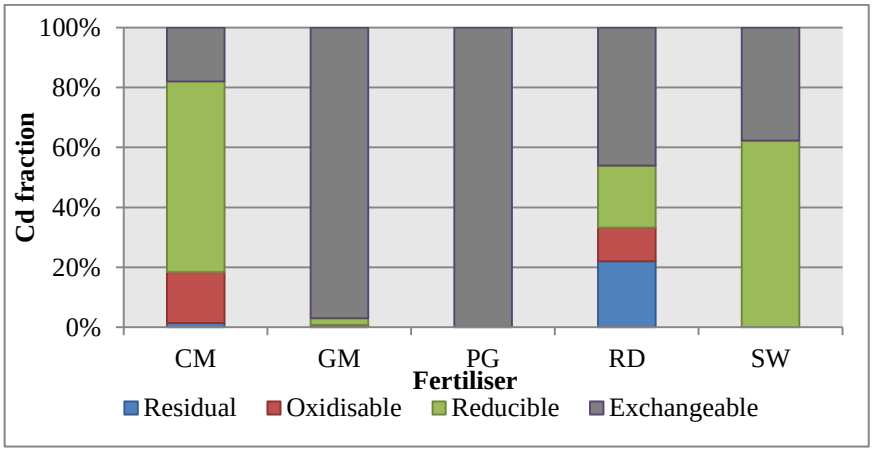


Figure 4.21 Cadmium fractionation in fertilisers by means of the BCR procedure (n = 3)

Chromium

Table 4.27 shows the results obtained for Cr sequential extraction, mean pseudototal (PT) concentration and extraction recovery in the fertilisers. Figure 4.22 shows the Cr fractionation. The RSD of Cr measurement in the fertiliser samples was generally less than 61%. However, in the exchangeable fraction, the precision was mostly less than 10%.

Table 4.27 Mean concentration (mg kg^{-1}) of Cr extracted using BCR sequential procedure in the fertilisers in each fraction.

	EXC.	% RSD	RED.	% RSD	OXID.	% RSD	RES.	% RSD	SUM	PT	%REC
CM	0.597	2.0	0.154	60.7	1.16	11.9	<0.023*	NA	1.91	4.9.0	39
GM	2.30	6.6	12.8	3.5	0.89	60.5	<0.023*	NA	16.0	19.0	84
PG	1.73	6.01	<0.018*	NA	<0.0053*	NA	<0.023*	NA	1.73	0.790	219
RD	0	NA	0.799	26.7	4.00	11.2	6.55	10.5	11.3	14.7	77
SW	1.22	NA	0.213	57.1	<0.0053*	NA	<0.023*	NA	1.43	7.18	20

(*) = LOD; CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; EXC. = exchangeable frection; RED. = reducible fraction; OXID. = oxidisable fraction; RES. = residual fraction; REC. = recovery. NA = (not applicable) because concentrations values were not detected and/or duplicate values were used to obtain mean concentration.

The recovery of Cr was generally poor – there was under extraction in all the samples (Table 4.24) except phostrogen where over extraction (219%) was observed. High levels of Cr were present in the exchangeable fractions of phostrogen and

seaweed compared to other fertilisers. Chromium was released in relatively high concentration in the oxidisable and residual fractions of chicken manure and rockdust. It is not very mobile in rockdust compared with the other amendments.

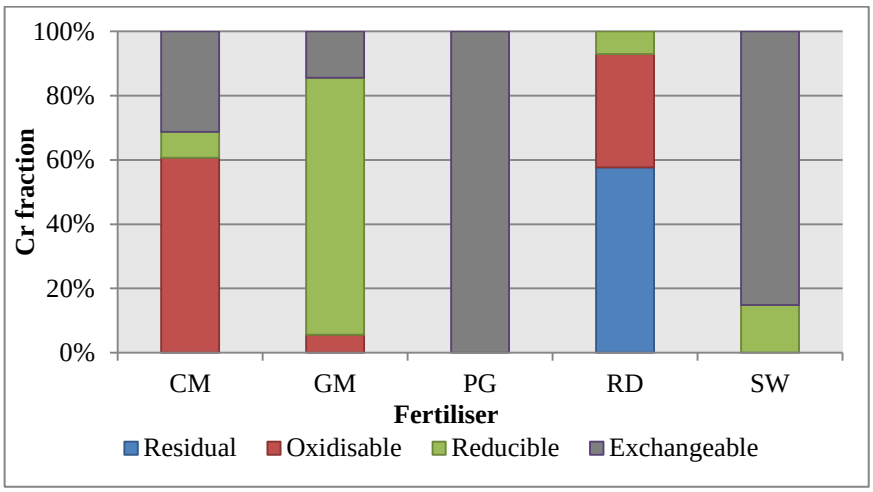


Figure 4.22 Chromium fractionation in fertilisers by means of the BCR procedure (n = 3)

Copper

Table 4.28 shows the results obtained for Cu sequential extraction, pseudototal (PT) concentration and extraction recovery in the fertilisers. Figure 4.23 shows the Cu fractionation. The RSD of Cu measurement in all the samples was generally less than 10%. The recovery of Cu in chicken manure, growmore and phostrogen were good (greater than 93%). As shown in Table 4.28, poor recoveries were obtained for rockdust (64%) and seaweed (1%) probably, due to incomplete extraction as in the case of rockdust and loss of seaweed material in the extraction of Cu in the oxidisable fraction – the reaction was quite vigorous.

Table 4.28 Mean concentration (mg kg^{-1}) of Cu extracted using BCR sequential procedure in the fertilisers in each fraction.

	EXC.	% RSD	RED.	% RSD	OXID.	% RSD	RES.	% RSD	SUM	PT	%REC
CM	17.4	5.5	1.77	12.9	61.2	5.2	5.55	8.3	85.9	92.8	93
GM	57.4	4.9	8.69	4.0	2.65	4.4	2.45	12	71.2	75.2	95
PG	51.0	0.29	<0.017*	NA	0.019*	NA	<0.015*	NA	51	47.5	107
RD	0.557	NA	1.32	5.2	0.209	2.6	5.53	3.8	7.62	11.9	64
SW	1.29	NA	0.414	5.8	<0.019*	NA	<0.015*	NA	1.7	177	1

(*) = LOD; CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; EXC. = exchangeable frection; RED. = reducible fraction; OXID. = oxidisable fraction; RES. = residual fraction; REC. = recovery. NA = (not applicable) because concentrations values were not detected and/or duplicate values were used to obtain mean concentration.

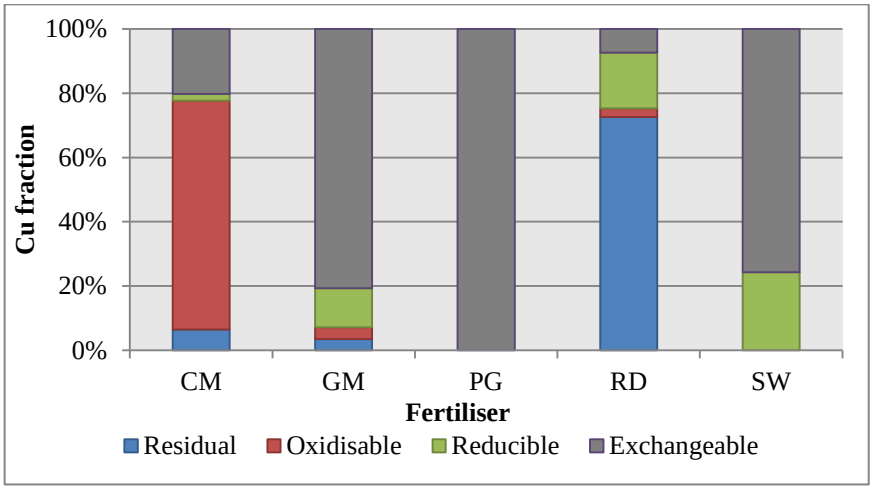


Figure 4.23 Copper fractionation in fertilisers by means of the BCR procedure (n = 3)

All of the Cu present was found in the exchangeable fraction for phostrogen and almost all of the Cu for seaweed. These two materials did not survive the conditions of the BCR extraction, with no residue remaining beyond the exchangeable step. Most of the Cu was found in the oxidisable fraction for chicken manure, and this was expected considering the known association of Cu to organic matter. Similarly, high concentration of Cu was present in the exchangeable fraction for growmore, with substantial level present in the residual fraction for rockdust. This indicates that Cu is not easily mobile in rockdust.

Iron

Table 4.29 shows the results obtained for Fe sequential extraction, the pseudototal (PT) concentration and extraction recovery in the fertilisers. Figure 4.24 shows the Fe fractionation. The RSD of Fe measurement was generally less than 61%. In the exchangeable fraction, the precision was mostly less than 10% but there were exceptions for the exchangeable and residual fractions of growmore (RSD < 42%), and the reducible fraction of chicken manure (RSD 35%). The results showed good recoveries for chicken manure, phostrogen and rockdust. Under extraction was observed for growmore (68%) and seaweed (35%). The poor recoveries obtained for growmore may be attributed to the fact that substantial amount of the material may have dissolved in deionised water used in washing residue from previous steps for subsequent extraction steps. The explanation given earlier for poor recovery of Cu in seaweed could apply to Fe too.

Table 4.29 Mean concentration (mg kg^{-1}) of Fe extracted using BCR sequential procedure in the fertilisers in each fraction.

	EXC.	% RSD	RED.	% RSD	OXID.	% RSD	RES.	% RSD	SUM	PT	%REC
CM	1150	3.6	2000	34.8	6280	3110	9.30	1.9	12500	13600	92
GM	105	13.9	3560	5.0	82.8	3120	42.4	75.7	6870	10100	68
PG	618	0.8	<0.020*	NA	<0.035*	NA	<0.273*	NA	618	552	112
RD	770	NA	8420	4.9	<0.035*	30100	1.40	7.8	39800	47800	83
SW	5660	NA	10500	5.8	<0.035*	NA	NA	NA	16200	46000	35

(*) = LOD; CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; EXC. = exchangeable frection; RED. = reducible fraction; OXID. = oxidisable fraction; RES. = residual fraction; REC. = recovery. NA = (not applicable) because concentrations values were not detected and/or duplicate values were used to obtain mean concentration.

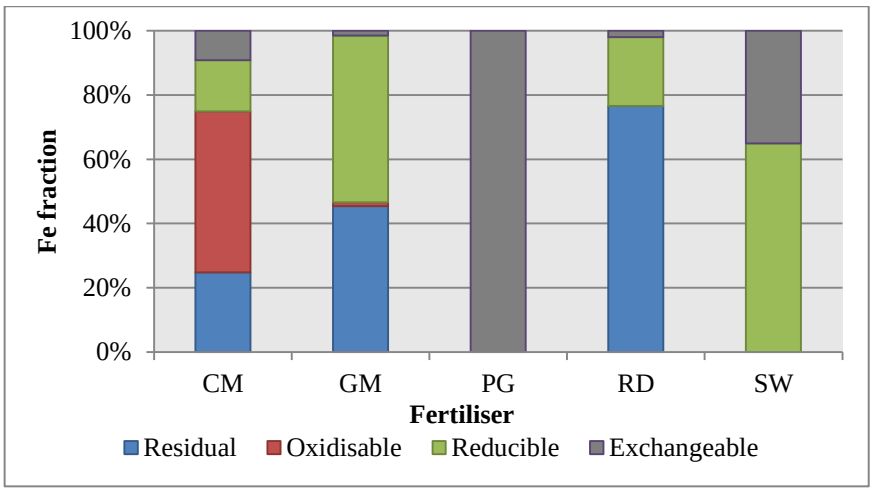


Figure 4.29 Iron fractionation in fertilisers by means of the BCR procedure (n = 3)

Iron was predominantly found in the residual fraction of rockdust, and in relatively lower concentrations in growmore and chicken manure. This indicate that Fe mobility in rockdust would be lower compared to chicken manure and growmore. Substantial level of Fe was found in the exchangeable fraction for phostrogen, indicating high mobility of Fe in the material. The reducible fraction was dominated for growmore and seaweed.

Manganese

Table 4.30 shows the results obtained for Mn sequential extraction, pseudototal (PT) concentration and extraction recovery in the fertilisers. Figure 4.25 shows the Mn fractionation. The RSD of Mn measurement was generally less than 10%. However, the results indicated that poor precision of Mn in the oxidisable and residual fractions of growmore (RSD value less than 76%), and oxidisable fraction of chicken manure (18%) – Table 4.30. Good recovery of Mn was obtained in chicken manure, growmore and rockdust (Table 4.30). However, Mn was over extracted in phostrogen (131%) probably due to contamination.

Table 4.30 Mean concentration (mg kg^{-1}) of Mn extracted using BCR sequential procedure in the fertilisers in each fraction.

	EXC.	% RSD	RED.	% RSD	OXID.	% RSD	RES.	% RSD	SUM	PT	%REC
CM	310	3.6	206	3.8	23.8	18.3	7.88	1.9	548	506	108
GM	379	8.7	46.0	6.9	1.03	28.0	14.8	75.7	441	439	100
PG	306	1.8	<0.0071*	NA	<0.012*	NA	<0.0038*	NA	306	234	131
RD	113	NA	129	5.0	41.3	2.17	347	7.80	630	754	84
SW	77.2	NA	36.1	1.6	<0.012*	NA	<0.0038*	NA	113	109	104

(*) = LOD; CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; EXC. = exchangeable frection; RED. = reducible fraction; OXID. = oxidisable fraction; RES. = residual fraction; REC. = recovery. NA = (not applicable) because concentrations values were not detected and/or duplicate values were used to obtain mean concentration.

As can be seen in Figure 4.25, most of the Mn was present in the exchangeable fraction in all the fertiliser amendments. Manganese may therefore, be readily released to the environment for uptake by plants. Large concentration of Mn was associated with the residual fraction in rockdust, and may not be readily available for uptake by plants.

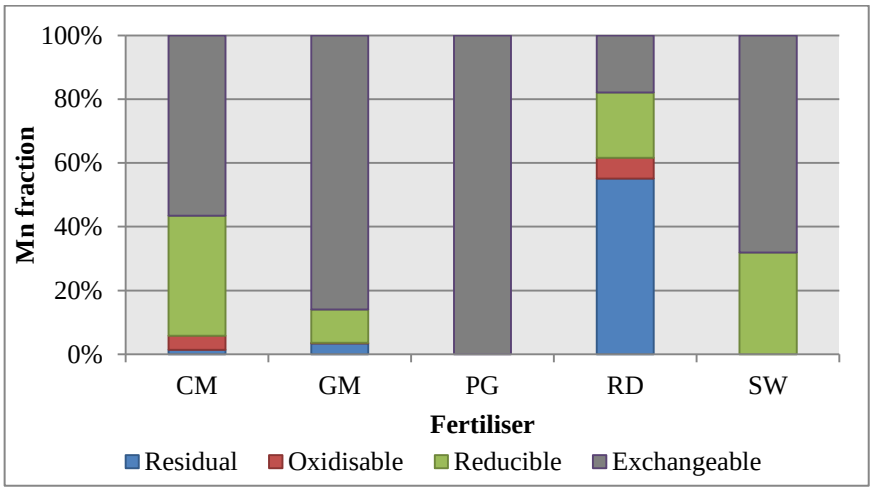


Figure 4.26 Manganese fractionation in fertilisers by means of the BCR procedure (n = 3)

Nickel

Table 4.31 shows the results obtained for Ni sequential extraction, pseudototal (PT) concentration and extraction recovery in the fertilisers. Figure 4.27 shows Ni fractionation. The precision of Ni extraction using the BCR sequential procedure was about 10%. Few exceptions were observed in the reducible fraction of chicken

manure (RSD 82%) and residual fractions of growmore (RSD 79%) probably due to measurement of concentrations close to LOD.

Table 4.31 Mean concentration (mg kg^{-1}) of Ni extracted using BCR sequential procedure in the fertilisers in each fraction.

	EXC.	% RSD	RED.	% RSD	OXID.	% RSD	RES.	% RSD	SUM	PT	%REC
CM	1.29	7	0.158	81.5	2.50	10	0.470	4.6	4.42	4.80	92
GM	9.85	2.9	0.424	5.5	0.414	4.4	0.910	79.1	11.6	12.9	90
PG	0.965	5.5	<0.671*	NA	<0.0031*	NA	<0.0023*	NA	0.193	1.30	15
RD	0.152	NA	2.35	4.7	0.554	4.0	6.43	4.8	9.50	10.5	90
SW	0.733	NA	0.861	6.1	<0.0031*	NA	<0.0023*	NA	1.59	4.80	33

(*) = LOD; CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; EXC. = exchangeable frection; RED. = reducible fraction; OXID. = oxidisable fraction; RES. = residual fraction; REC. = recovery. NA = (not applicable) because concentrations values were not detected and/or where duplicate values were used to obtain mean value.

The recovery of Ni in the fertiliser samples was generally good (90-92%). Substantially, under extraction Ni was observed in phostrogen (15%) and seaweed (33%). The poor recovery of Ni for phostrogen could be attributed to low level of the element in the material making it difficult to be fractionated easily. As explained earlier for Cu, seaweed extracton resulted in loss of sample material in the oxidisable step, due to vigorous oxidation of the organic matter by hydrogen peroxide used in this step for the material. Nickel was bound to the oxidisable fraction in chicken manure in the highest concentration compared with the other fertilisers. Significant amount of Ni was found in the exchangeable fraction in all the fertiliserss (this indicated high mobility and hence availability to plants etc) with the exception of rockdust, where it was predominantly found in the residual fraction (Figure 4.26).

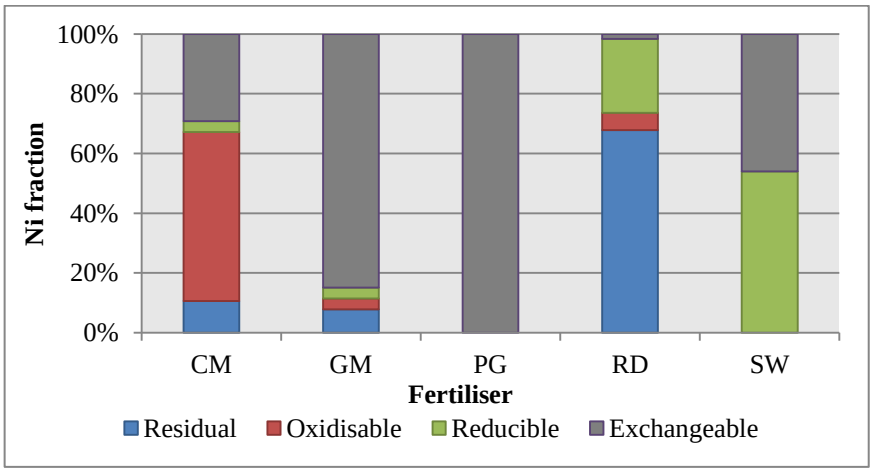


Figure 4.26 Nickel fractionation in fertilisers by means of the BCR procedure (n = 3)

Lead

Table 4.32 shows the results obtained for Pb sequential extraction, the pseudototal (PT) concentration and extraction recovery in the fertilisers. Figure 4.27 shows the Pb fractionation. The RSD of Pb measurement was generally poor (less than 93%). The poor precision could be attributed to closeness of concentration values to LOD. The recovery of Pb was generally good for chicken manure, growmore and rockdust (Table 4.32). Although, over extraction (215%) of Pb in chicken manure. Under extraction (63%), of the element in seaweed was observed. A large amount of Pb was found in the residual fraction of chicken manure and rockdust, with relatively smaller percentage in the growmore. A significant percentage of Pb was found in the growmore and seaweed. Nearly 100% of Pb (Figure 4.27) was present in the exchangeable fraction in phostrogen as it hardly survived beyond the exchangeable step.

Table 4.32 Average concentration (mg kg^{-1}) of Pb extracted using BCR sequential procedure in the fertilisers in each fraction.

	EXC.	% RSD	RED.	% RSD	OXID.	% RSD	RES.	% RSD	SUM	PT	%REC
CM	0.0652	15.2	0.045	92.3	0.930	50.4	2.55	51.5	3.59	1.67	215
GM	0.109	10	1.95	15	0.0766	22.4	0.806	89.2	2.94	3.00	98
PG	1.12	5.2	<0.027*	NA	<0.0066*	NA	<0.034*	NA	1.12	1.16	97
RD	0.0573	NA	1.04	10	0.100	10.6	2.81	13.4	4.00	3.43	117
SW	0.0330	NA	0.694	6.9	<0.0066*	NA	<0.034*	NA	0.727	1.16	63

(*) = LOD; CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; EXC. = exchangeable frection; RED. = reducible fraction; OXID. = oxidisable fraction; RES. = residual fraction; REC. = recovery. NA = (not applicable) because concentrations values were not detected and/or duplicate values were used to obtain mean concentration.

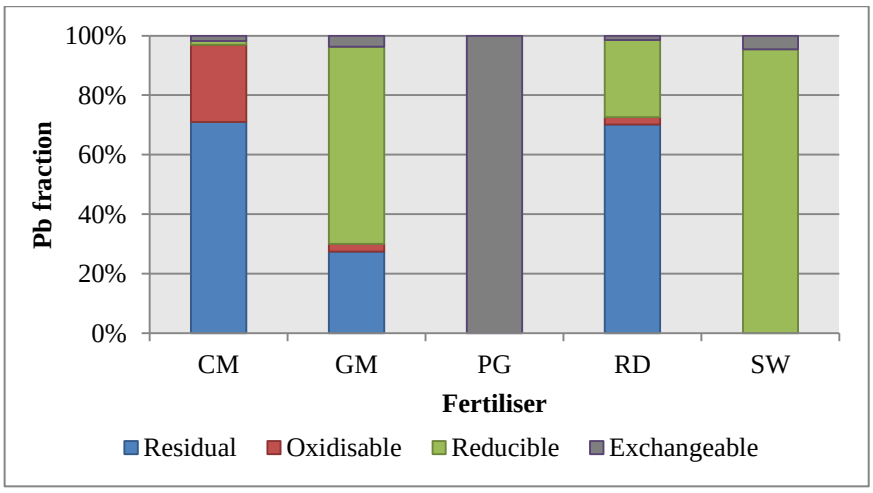


Figure 4.27 Lead fractionation in fertilisers by means of the BCR procedure (n = 3)

Uranium

Table 4.33 shows the results obtained for U sequential extraction, the pseudototal (PT) concentration and extraction recovery in the fertilisers. Figure 4.28 shows the U fractionation. The RSD of U extraction using the BCR sequential procedure was generally less than 10%. Although, poorer RSD was found in some of the fractions (residual, reducible and exchangeable) in the fertilisers (RSD < 74%), probably due to measurement which were carried out close to the detection limits. Good recovery of U was obtained in chicken manure, growmore and rockdust (Table 4.30).

Table 4.30 Average concentration (mg kg^{-1}) of U extracted using BCR sequential procedure in the fertilisers in each fraction.

	EXC.	% RSD	RED.	% RSD	OXID.	% RSD	RES.	% RSD	SUM	PT	% REC
CM	0.0706	12	1.14	2.7	0.902	5	0.231	11	1.32	1.21	109
GM	6.58	2.4	21.1	2.9	<0.00062*	NA	0.159	74.4	28.7	26.9	107
PG	0.00809	17	<0.00015*	NA	<0.00062*	NA	<0.0002*	NA	0.00809	0.04	20
RD	0.232	NA	0.370	3.3	0.0900	3.9	0.709	3.0	1.40	1.33	105
SW	0.151	NA	0.0632	6.5	<0.00062*	NA	<0.0002*	NA	0.214	0.62	35

(*) = LOD; CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; EXC. = exchangeable frection; RED. = reducible fraction; OXID. = oxidisable fraction; RES. = residual fraction; REC. = recovery. NA = (not applicable) because concentrations values were not detected and/or where duplicate values were used to obtain mean value.

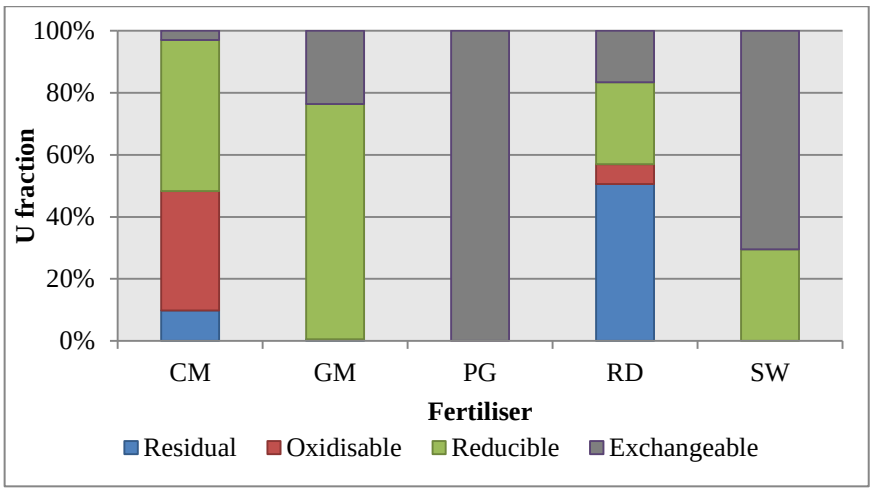


Figure 4.33 Uranium fractionation in fertilisers by means of the BCR procedure (n = 3)

Recovery of U after the analysis was generally good for chicken manure, growmore and rockdust. Uranium was under extracted when compared to the pseudototal concentration for phostrogen (20%) and seaweed (35%). Uranium was found generally in the exchangeable and reducible fractions of most of the fertilisers – growmore, which apparently contained the highest pseudototal concentration of U, released the highest concentration of the U in the reducible fraction. Large concentration of U was associated with the oxidisable fraction in chicken manure.

Zinc

Table 4.34 shows the results obtained for Zn sequential extraction, the pseudototal (PT) concentration and extraction recovery in the fertilisers. Figure 4.29 shows the Zn fractionation. The RSD of Zn extraction using the BCR sequential procedure was generally less than 10%. Poor RSD of Zn was observed in the reducible, oxidisable and residual fractions chicken manure, growmore and seaweed (RSD < 94%), which may be attributed to variations in the concentrations of one or more of the replicate samples which resulted in higher or lower signals compared to other replicate, which suggests inhomogenous distribution of Zn in the samples. Good recovery of Zn in chicken manure, growmore and phostrogen was observed.

Table 4.31 Mean concentration (mg kg^{-1}) of Zn extracted using BCR sequential procedure in the fertilisers in each fraction.

	EXC.	% RSD	RED.	% RSD	OXID.	% RSD	RES.	% RSD	SUM	PT	%REC
CM	60.0	6.1	250	28.8	133	47.7	4.17	39.2	447	500	89
GM	373	5.6	8.90	1.6	0.663	4.9	2.90	83.6	385	404	95
PG	97.5	0.6	<0.0270*	NA	<0.033*	NA	<0.170*	NA	97.5	98.0	99
RD	2.10	NA	16.1	4.3	1.71	4.1	45.6	7.4	65.5	1.25	5240
SW	57.2	NA	0.0751	93.8	<0.033*	NA	<0.170*	NA	57.3	104	55

(*) = LOD; CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; EXC. = exchangeable frection; RED. = reducible fraction; OXID. = oxidisable fraction; RES. = residual fraction; REC. = recovery. NA = (not applicable) because concentrations values were not detected and/or duplicate values were used to obtain mean concentration.

A recovery of 5240% Zn in rockdust was obtained. This was unexpected, and may be due to contamination during the extraction process. Under extraction of Zn was observed in seaweed (Table 4.34). A Large amount of Zn was found in the exchangeable fraction in growmore, phostrogen and seaweed. In chicken manure, Zn was associated with the reducible fraction. This element is very mobile in fertilisers.

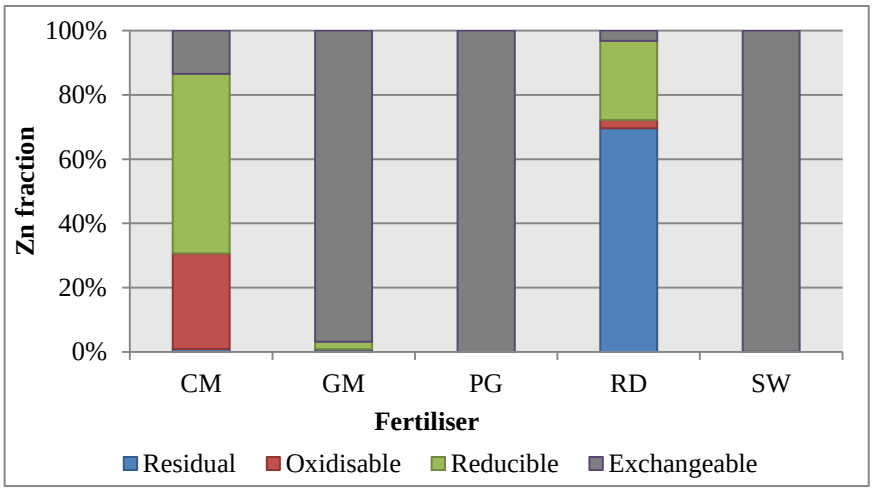


Figure 4.29 Zinc fractionation in fertilisers by means of the BCR procedure (n = 3)

The fractionation and mobility of the PTE in the amendments were assessed. The PTE were generally found in the mobile fractions in all the fertilisers except rockdust, where most of the analytes were predominantly present in the residual fraction. Growmore and phostrogen hardly survived the conditions of the BCR extraction procedure; particularly, phostrogen did dissolve immediately in the

exchangeable step and with no residue left. Whereas growmore did not survive beyond the reducible step. Due to the vigorous oxidation of seaweed in the oxidisable step, loss of material was observed, which resulted in loss of analytes hence the under extraction in most cases, contributing to the poor recoveries observed for seaweed. Iron generally proved to be refractive in rockdust..

4.7.4 The BCR sequential extraction of amended soil samples

The BCR sequential extraction was performed on the control soil (0% dosage) and the soil with 5% fertiliser dosage, and results compared. The 5% dosage was chosen because the effect of the fertilisers on the pseudototal content became most noticeable at the 5% dosage for most of the PTE. The predicted (Pr) concentrations (mg kg^{-1}) of the PTE in each step or fraction was calculated as follows:

$$\mathrm{Pr} = \frac{95}{100} \times \mathrm{conc}\left(\frac{\mathrm{mg}}{\mathrm{kg}}\right) \text{of PTE in each fraction of soil}$$

$$+ \frac{5}{100} \times \mathrm{conc}\left(\frac{\mathrm{mg}}{\mathrm{kg}}\right) \text{of PTE in each fraction of fertiliser}$$

The predicted concentration was compared with the found values, to determine whether the analyte fractions in the amended soil was simply the sum of the fractions in the soil and the fertiliser individually or whether the addition of fertiliser altered the analyte fractionation in the soil.

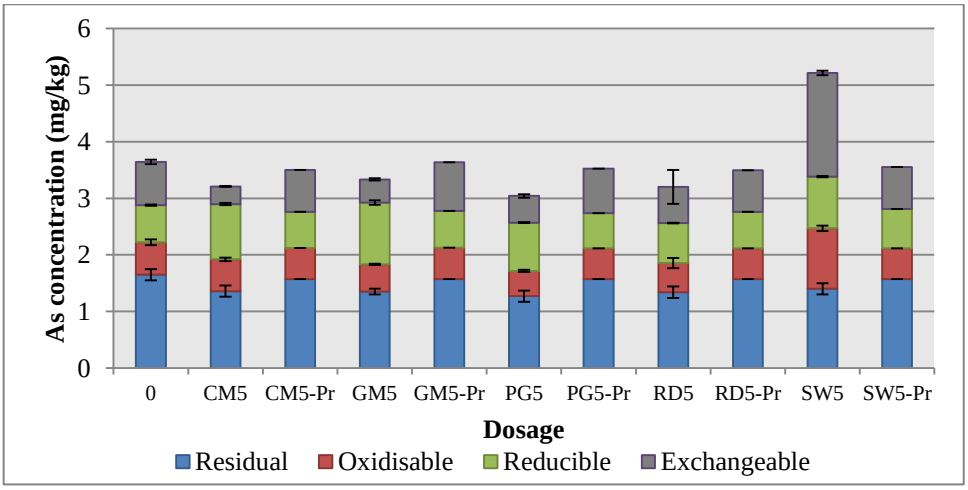
Arsenic

Figure 4.30 shows the fractionation pattern of As in the control soil and amended soil, together with the predicted patterns for the amended soil. The RSD of As measurement in control soil for all the fractions was less than 10% (n = 3). Good recovery (93%) with respect to pseudototal using *aqua regia* was obtained. Arsenic was present in all the fractions, with the highest concentration found in the residual fraction.

In the amended soil, the RSDs of As extraction was generally less than 10% except for the oxidisable fractions in rockdust and seaweed amended soil that were about 18% (Appendix G). The recovery of As with respect to pseudototal concentration in the amended soil samples was generally poor, except for growmore where excellent

recovery (97%) was obtained. With respect to the control soil, addition of the fertiliser materials at 5% dosage did not significantly affect the fractionation in the control soil due to low level of As in the fertilisers. However, an increase in the mobile fractions of the soil was noticed with addition of seaweed and a corresponding reduction in the residual fraction.

The measured fractionation patterns largely matched the predicted fractionation pattern, which indicated that As should be present in all the fraction. However, the measured concentration of As in the exchangeable and residual fractions were slightly less than in the predicted fractionation. Exceptions were observed for rockdust amended soil where both the predicted and measured fractionations were the same. Furthermore, fractionation of seaweed amended soil showed the measured exchangeable fraction was conpicuously greater than predicted. This was unexpected, suggesting possible contamination of the sample.



CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; Pr = predictated fractionation; 0 = 0% (Control); 5 = 5%

Figure 4.30 Comparison of As fractionation in the control soil and amended soils with the predicted patterns for the amended soil (n = 3)

Cadmium

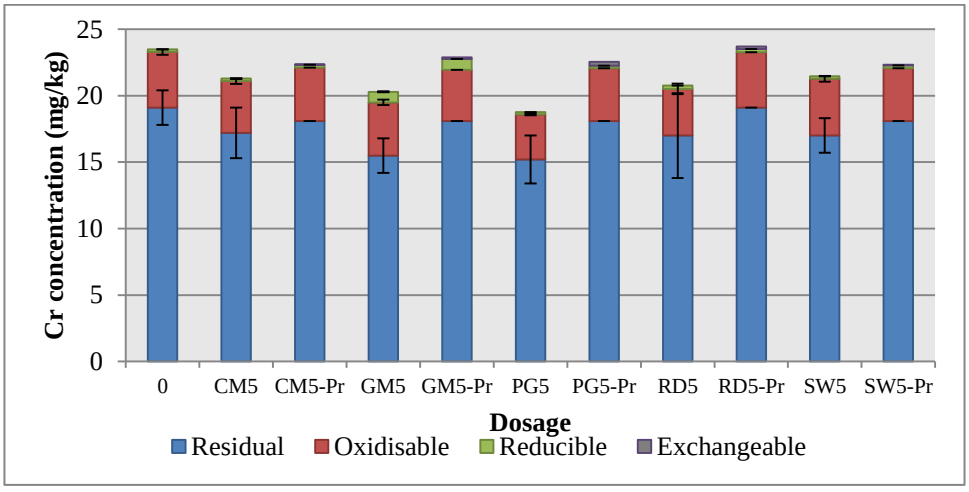
Ccadmium was not detected in the oxidisable and residual fractions of all the fertiliser amended soils (concentrations measured were less than LOD values) and is not discussed further.

Chromium

Figure 4.31 shows the fractionation pattern of Cr in the control soil and amended soil, together with the predicted patterns for the amended soil. The RSD of Cr concentration for the BCR extraction was less than 7% (n =3). Poor recovery of Cr (66%) with respect to pseudototal concentration in the control soil was obtained. The Cr present was found chiefly in the residual fraction, with small levels present in the oxidisable fraction, suggesting low overall mobility.

Good precision was obtained for the extraction of Cr in the amended soil samples as indicated by RSDs less than 10%. However poor recovery of Cr with respect to *aqua regia* extraction was found, between 54 and 74%. Chromium was found mostly in the residual fraction, and in small amounts in the oxidisable fractions in the amended soil. Addition of fertilisers did not result in any significant effect compared to the control soil. Chromium was not detected in the exchangeable fraction which could be attributed to concentration being lower than the detection limits.

The predicted fractionation confirmed the residual fraction as the dominant fraction, just like in the measured fractionation. In most cases the predicted residual fraction appeared slightly higher than the measured fraction.



CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; Pr = predictated fractionation; 0 = 0% (Control); 5 = 5%

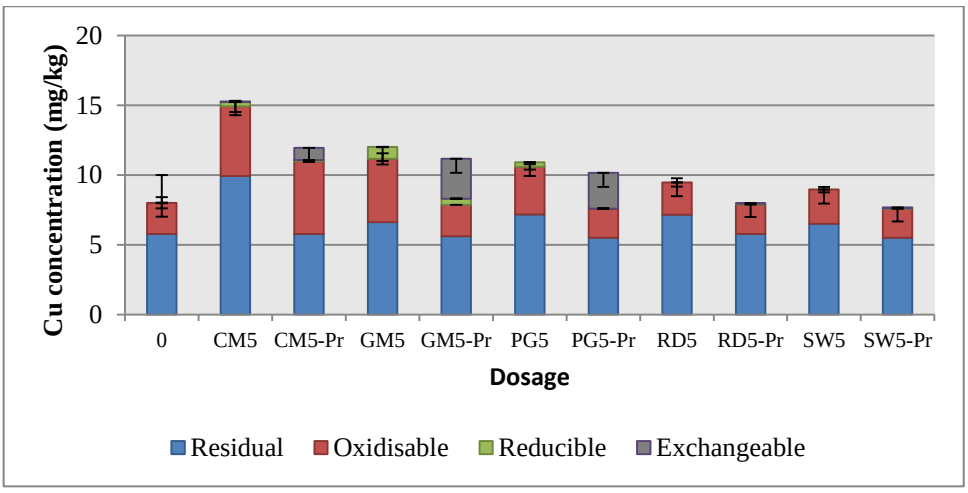
Figure 4.31 Comparison of Cr fractionation in the control soil and amended soils with the predicted patterns for the amended soil (n = 3)

Copper

Figure 4.32 shows the fractionation pattern of Cu in the control soil and amended soil, together with the predicted patterns for the amended soil. The RSD of Cu measurement in the control soil as extracted using the BCR procedure was 19% and 2% for the oxidisable and residual fractions, respectively. The recovery of Cu (115%) with respect to pseudototal concentration was good. In the control soil, more of the Cu present was found in the residual fractions, and lower level in the oxidisable fraction. This suggests low availability in soil and to plant. Copper was not detected in the exchangeable and reducible steps due to measurement close to LOD.

The RSDs of Cu in the amended soil samples was generally less than 10% (n =3) for the reducible and oxidisable fractions. However, poor precision was obtained in the exchangeable and residual fractions (RSD < 55%) probably due to concentrations being close to detection limits. Recovery of Cu with respect to pseudototal concentration in the treated samples was generally poor, except for growmore amended soil which showed 98% recovery – (Appendix G). In comparison with the control, the addition of chicken manure, where Cu was bound predominantly in the oxidisable phase, increased both the oxidisable and residual fractions in the original soil. Sahito *et al.*[170] reported a similar effect with poultry waste. Similarly, the value of Cu in the oxidisable and residual fraction increased in the growmore and phostrogen amended soil samples, respectively. This effect was less significant than in the chicken manure amended soil. Rockdust amendment, as expected resulted in slight increase in the residual fraction of rockdust amended soil.

Calculation predicted that most of the Cu should be present in the residual fraction, as was found in the measured patterns, but in slightly lower concentrations. The exchangeable fraction was larger in the predicted pattern for chicken manure, growmore and phostrogen, than in the measured fractionation pattern. This suggests that the fractions of Cu in the amended soil may not just be the sum of the fractions in the soil and the fertiliser individually.



CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; Pr = predictated fractionation; 0 = 0% (Control); 5 = 5%

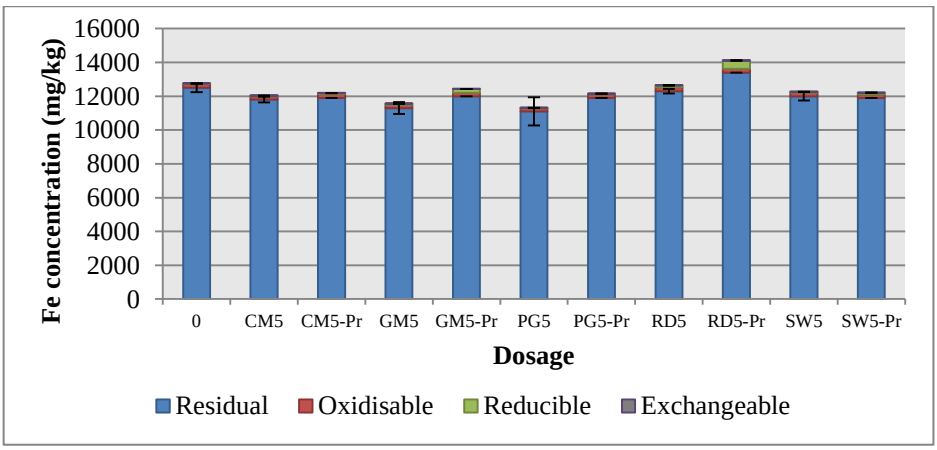
Figure 4.32 Comparison of Cu fractionation in the control soil and amended soils with the predicted patterns for the amended soil (n = 3)

Iron

Figure 4.33 shows the fractionation pattern of Fe in the control soil and amended soil, together with the predicted patterns for amended soil. The precision of Fe extracted in the control soil for all the fractions was less than 14% (n = 3). Poor recovery (62%) of Fe with respect to the pseudototal concentration was obtained in this measurement. Iron was bound chiefly in the residual fraction with markedly smaller levels in the other fractions of the soil. This behaviour was expected due to its geogenic nature.

The RSD values of Fe in the amended soil samples was generally less than 10% in most of the fractions. However, the RSD of Fe measurement in the exchangeable, oxidisable or residual fractions was observed in some cases less than 16%. Recovery of Fe with respect to *aqua regia* digestion in the various fractions for the amended soil ranged between 54 and 74%. With respect to the control soil, no marked effect was noticed, on addition of any fertiliser.

Similarly the predicted fractionation patterns for Fe did not differ from the measured patterns, with most of the Fe present in the residual fraction.



CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; Pr = predictated fractionation; 0 = 0% (Control); 5 = 5%

Figure 4.33 Comparison of Fe fractionation in the control soil and amended soils with the predicted patterns for the amended soil (n = 3)

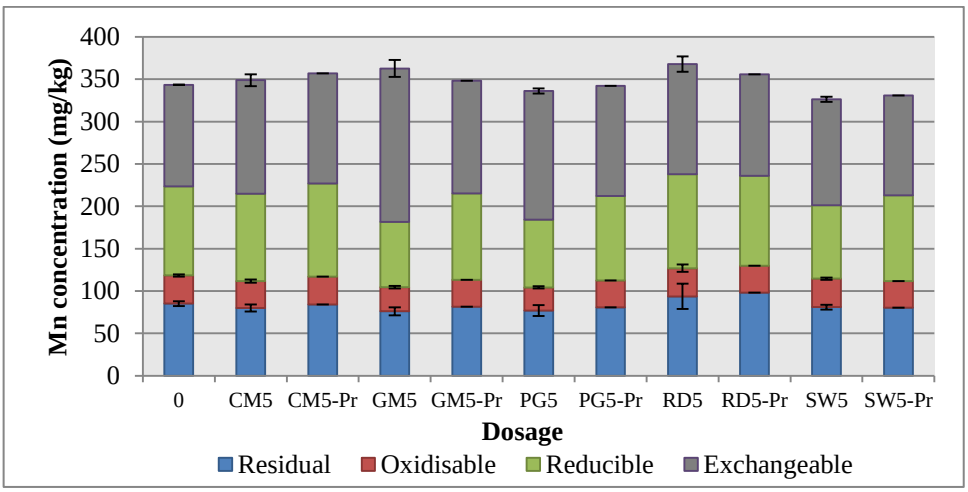
Manganese

Figure 4.34 shows the fractionation pattern of Mn in the control soil and amended soil, together with the predicted patterns for the amended soil. The RSD of Mn measurement in the control soil was good. The values were less than 4% (n = 3) for all the fractions. Good recovery of Mn (93%) with respect to pseudototal content was recorded. Manganese was present in all the four fractions in the control soil with slightly higher concentration in the exchangeable fraction suggesting higher mobility.

The RSDs of Mn for all the fractions in the amended soil samples were generally less than 10%. However, in each of the fractions, higher RSD value (14%) was occasionally found. Extraction recovery of Mn compared to the pseudotal concentration was generally good (93 to 110%) unlike in the case of Fe. With respect to the control soil, the addition of all the fertilisers did not result in any significant changes in fractionation. However, in the growmore amended soil, increased concentration (180 mg kg^{-1}) was noticed in the exchangeable fraction compared to 120 mg kg^{-1} found originally in the control soil, while a corresponding decrease in the reducible fraction was observed. A similar but less marked effect was observed for the phostrogen amended soil sample. These effects were expected as most of the

element was present in the fertilisers in the exchangeable fraction as shown in Figure 4.25.

The predicted and measured fractionation patterns for Mn were similar and showed that Mn was present in all the fractions. In some cases, the predicted values were slightly higher than the measured concentration, particularly for the exchangeable and reducible fractions.



CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; Pr = predictated fractionation; 0 = 0% (Control); 5 = 5%

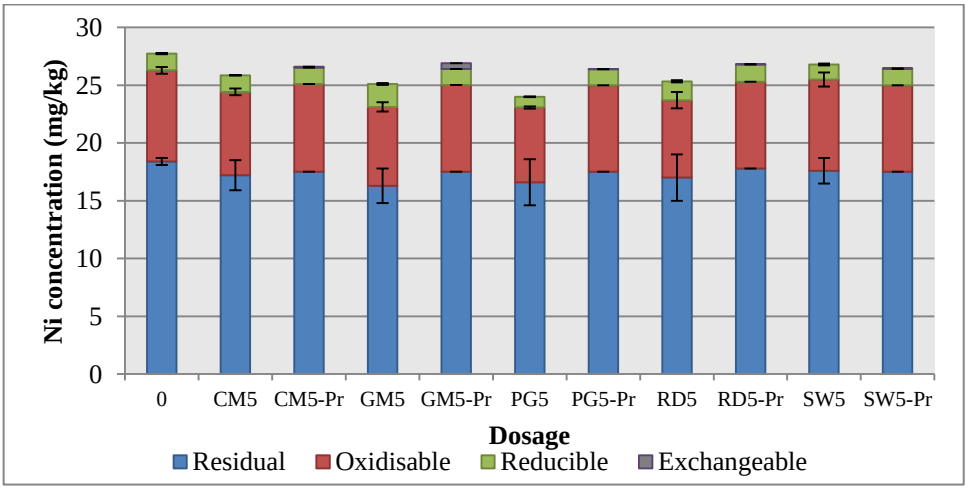
Figure 4.34 Comparison of Mn fractionation in the control soil and amended soils with the predicted patterns for the amended soil (n = 3)

Nickel

Figure 4.35 shows the fractionation pattern of Ni in the control soil and amended soil, together with the predicted patterns for the amended soil. The RSD of Ni measurement in the control soil was less than 8% (n = 3). Recovery of the analyte with respect to the pseudototal concentration was 90%. Nickel was substantially found in the residual fraction, with lesser levels in the oxidisable fraction.

The RSDs of Ni in the amended soil samples was generally less than 10% (n = 3) for all the fractions. A slightly higher (RSD > 11%) was obtained in the oxidisable and residual fractions. The extraction recovery of Ni (86 to 94%) was obtained for the amended soil. With respect to the control soil, the addition of the fertilisers generally resulted in no significant differences in Ni fractionation.

Similarly, the predicted fractionation patterns did not differ from the measured concentrations (Figure 4.35).



CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; Pr = predictated fractionation; 0 = 0% (Control); 5 = 5%

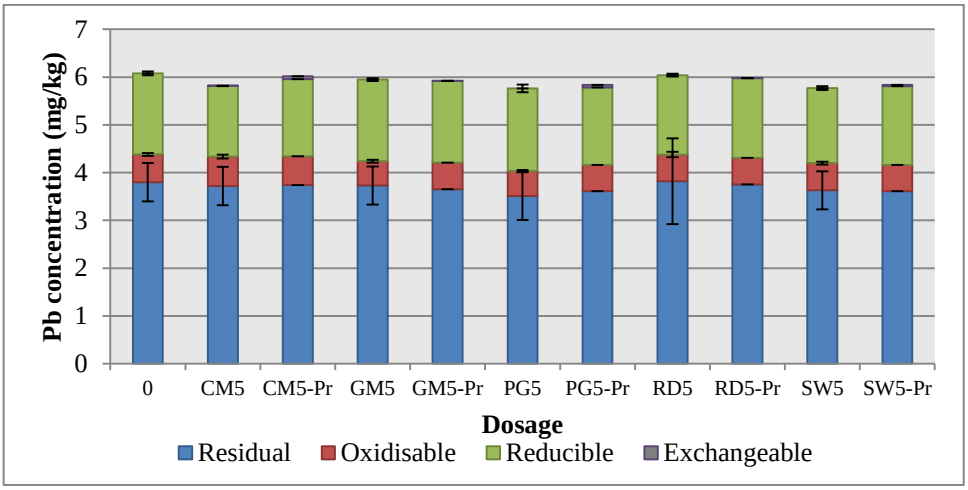
Figure 4.35 Comparison of Ni fractionation in the control soil and amended soils with the predicted patterns for the amended soil (n = 3)

Lead

Figure 4.36 shows the fractionation pattern of Pb in the control soil and amended soil, together with the predicted patterns for the amended soil. The RSDs of Pb extraction in the control soil for the fractions were less than 10% (n = 3), with excellent recovery of 97%. Lead was not detected in the exchangeable step, but present in highest amount in the residual fraction, followed by the reducible and the oxidisable fractions.

In the amended soil, RSD values of less than 10% were obtained in the reducible and oxidisable fractions, although the RSD of the oxidisable fraction in the rockdust amended soil was greater than 11%. Poor precision was found in the exchangeable fraction for chicken manure, probably due to concentration values being close to the LOD. Recovery of Pb with respect to pseudototal concentration in the various fractions for the amended soil was very good (100 to 114%). The addition of 5% fertiliser did not in any significant way alter the concentration nor fractionation of Pb in the soil (Figure 4.36).

The predicted fractionation patterns and the measured concentrations for Pb remained similar.



CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; Pr = predictated fractionation; 0 = 0% (Control); 5 = 5%

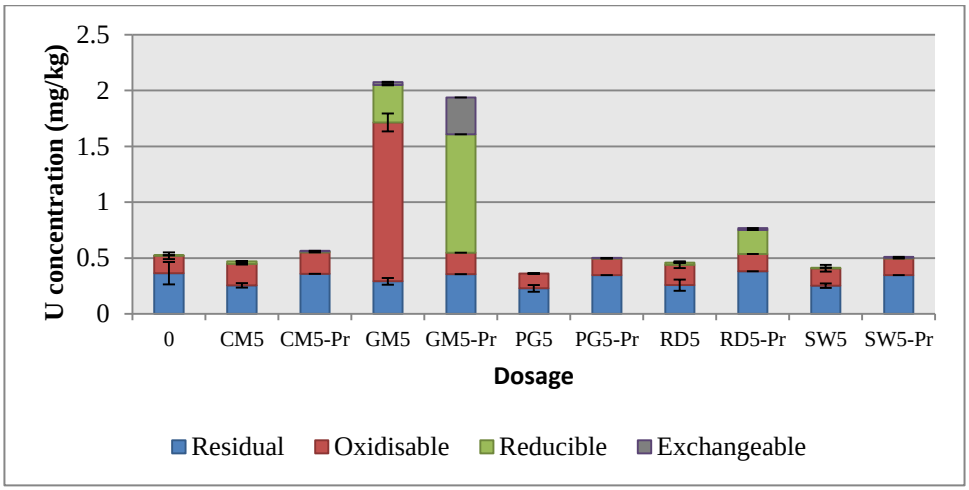
Figure 4.36 Comparison of Pb fractionation in the control soil and amended soils with the predicted patterns for the amended soil (n = 3)

Uranium

Figure 4.37 shows the fractionation pattern of U in the control soil and fertiliser amended soil, together with the predicted patterns for the amended soil. The RSD of U measurement in the control soil was less than 30% (n = 3). Recovery of U with respect to the pseudototal concentration was good (110%). The analyte was predominantly present in the residual fraction, and smaller level associated with the oxidisable fraction.

The RSD values of U in the amended soil for all the fractions were less than 21% (n = 3). Rcovery of U with respect to pseudototal analyte concentration in amended soil was in the range 85 to 110%. With respect to the control soil, addition of 5% fertiliser generally did not affect the original concentration of U in the soil. However, growmore addition caused the oxidisable fraction in soil to increase to (1.42 mg kg^{-1}), and raised the level of U in the reducible fraction. No significant effect was observed with the addition of the other fertilisers due to low concentration of the element in the materialss themselves.

The predicted and measured fractionation patterns for U were generally similar, with the exception of growmore. Here, the predicted and found exchangeable, reducible and oxidisable fractions differ with U found in less labile form than predicted. The predicted fractionation pattern for rockdust amended soil also showed U was present in the reducible fraction in contrast to the measured concentration.



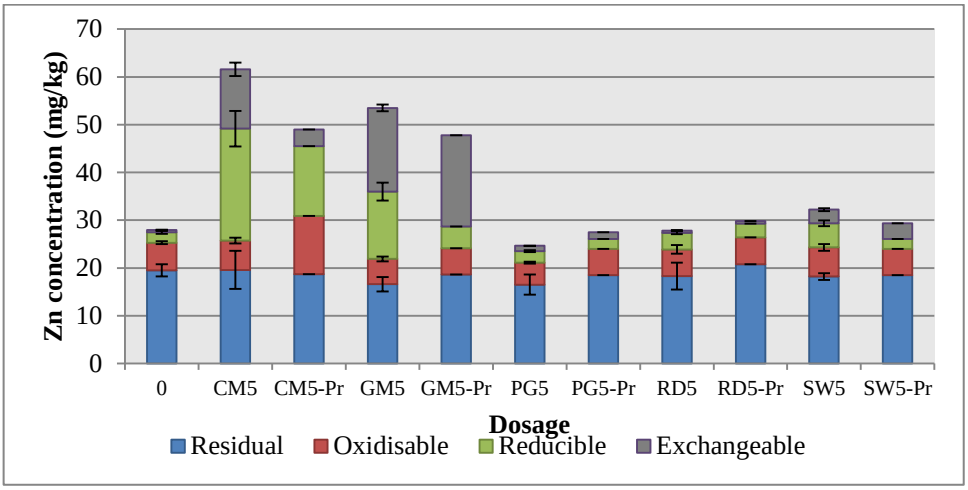
CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; Pr = predictated fractionation; 0 = 0% (Control); 5 = 5%

Figure 4.37 Comparison of U fractionation in the control soil and amended soils with the predicted patterns for the amended soil (n = 3)

Zinc

Figure 4.38 shows the fractionation pattern of Zn in the control soil and fertiliser amended soil samples, along with the predicted patterns. The RSD of Zn measurement in the control and amended soil samples was generally less than 21%. A RSD of less than 10% was found in most fractions of the control and fertiliser amended soil samples. Recovery of Zn was good for control soil, growmore, and seaweed amended soil samples. Low recovery of Zn (85 and 86%) were obtained for phostrogen and rockdust amended soil samples, respectively. The Zn in the control soil was found in the residual fraction, and lesser concentrations were found in the oxidisable and reducible fractions. Addition of chicken manure and growmore significantly incresed the the concentration of Zn in the amended soils. Further, since a high proportion of Zn in the fertiliser was in relatively available forms (Figure 4.29). Addition significantly increased Zn concentrations in exchangeable and reducible fractions. This effect was higher in the chicken manure than in the

growmore amended soil sample. The presence of other fertilisers did not result in any significant effect on Zn levels or distribution in the control soil sample, due to low level of Zn in the materials.



CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; Pr = predictated fractionation; 0 = 0% (Control); 5 = 5%

Figure 4.38 Comparison of Zn fractionation in the control soil and amended soils with the predicted patterns for the amended soil (n = 3)

4.8 Conclusions

In this work, a terrestial organic fertiliser of animal origin (chicken manure); two commercial inorganic fertilisers (growmore and phostrogen); a terrestial inorganic fertiliser of geological origin (rockdust) and a marine organic fertiliser of plant origin (seaweed), and a commercial top garden soil were studied in order to ascertain their PTE status, and assess the effect of the fertilisers on levels and potential bioavailabilities of PTE with respect to plants.

The soil was relatively unpolluted based on various soil guidelines, typical, common values and abundance of PTE in soils. Of the amendments, chicken manure contained the highest concentrations of Cu and Zn. The concentration of Cu was less than the Czech guideline value and the maximum value proposed by the EU. However, Zn in this amendment was higher than the Czech regulation, but lower than the limit proposed by the EU. Growmore contained the highest content of U – no regulation has been made for U in commercial inorganic fertilisers at the present time. Rockdust, being a geologically based amendment, contained the highest concentrations of Fe and Mn, which was expected. The amendments studied

contained relatively high concentrations of Cu, Fe, Mn and Zn except few (phostrogen and rockdust were low in Zn, while rockdust and seaweed were low in Cu). The concentrations of As, Cd, Cr, Ni, Pb and U were generally low in all the fertilisers.

When the various mixtures of soil and chicken manure were allowed to stand for 30 days in experiment 1, statistical analysis (ANOVA) revealed significant differences ($P < 0.05$) amongst the levels of Cr, Cu, Fe, Mn, Ni, U, and Zn in all the treatments. Exceptions were for Cd and Pb where changes were revealed to be non significant ($P < 0.05$). The observed trend indicated that increase in the amount of manure added to soil resulted in a corresponding increase in the levels of Cd, Cu, Mn, U and Zn compared to their levels in the control. This trend was predominant at the highest doses, confirming the expectation of these metals being accumulated when larger amounts of the material were applied, though the concentrations measured posed no threat to the environment. In contrast, the levels of Cr, Fe, Ni and Pb decreased as the manure was added due to dilution of the soil with less contaminated material.

Part of the soil from experiment 1 was taken and used in experiment 2 where the soil was next treated with all the fertiliser amendments at three dosage (1%, 3% and 5%) and compared with the control treatment. Their effect on pseudototal PTE concentration in the soil revealed that the addition of chicken manure and growmore in most cases resulted in greater influence on the concentrations of the PTE than the other fertilisers used. The levels of Cu and Zn increased prominently in the chicken manure and growmore amended soil samples. This effect was only observed in the growmore amended soil sample in the case of U. It was observed that chicken manure and growmore decreased the concentrations of Cr, Fe and Ni, and seaweed was found to decreased the concentrations of Fe and Mn in the amended soil. The level of Pb was reduced chiefly by phostrogen.

The BCR sequential extraction was performed on the fertilisers and the 5% amended soils only. The result indicated that Fe was predominantly residual in all the fertilisers. Its highest level was found in rockdust. The rest of the PTE were chiefly associated with the labile fractions.

The residual fraction of Cu in the soil increased on treatment with all the fertiliser amendments, although this effect was more in the chicken manure amended soil compared to the other four amended soils. A similar effect was seen in growmore and phostrogen amended soils but to a lesser extent. Treatment with 5% growmore introduced oxidisable forms of U to the soil. Chicken manure and growmore resulted in the redistribution of Zn to the exchangeable and reducible fractions making it highly mobile or mobilisable. Conversely, As, Cr, Fe, Mn, Ni and Pb did not show significant changes on addition of the amendments. Although the PTE concentrations in the soil after treatment with 5% amendments were not high at the dosages used in this work, the result suggested that chicken manure and growmore have the potential of altering the original distribution of some of the PTE in soil as observed for Cu, U and Zn.

5 Column leaching of potentially toxic elements in a fertiliser amended urban park soil

5.1 Introduction

Urban soils are less studied compared to agricultural soils, and are mostly used for public-access recreational areas such as parks and ornamental gardens.[171] They often contain high levels of PTE arising from different anthropogenic sources of pollution: release from industrial activity, emissions from traffic, waste disposal, as well as pesticides and fertiliser applications. These can all result in additional levels of PTE that can persist in urban soils for a very long time after their introduction.[172] They may find their way into ground water via leaching, and pollute water supplies, thereby threatening human health through the food chain.[173] The presence of PTE in organic and inorganic fertilisers together with renewed interest in use of urban soils for food production means there is need for a better understanding of the processes of potentially toxic element-soil interactions, particularly their mobility and possible retention in urban systems.[174]

One important approach to assessing mobility and leaching potential of PTE in soils is by the use of soil column experiments – which involves the application of a suitable leaching solution or fluid, either intermittently or on a continuous basis, through a column packed with the soil sample. Deionised water is the simplest and commonly used leaching agent.[175-177] The leachates are collected from time to time, and analysed for the parameters of interest. Column leaching test may be time consuming, depending on the rate at which the leachant is added. However, Anderson *et al*.[178] noted that the main advantage of column leaching experiments is that they provide information on the current PTE mobility.

The key link is that people are becoming interested in growing food in urban settings in developed countries (as well as developing countries) and application of fertiliser to urban soils is increasing and worthy of study. The aim of this study was therefore to assess the potential leaching and distribution of PTE in an urban park soil

amended with chicken manure, growmore, or a mixture of chicken manure + growmore fertilisers.

5.2 Sampling

Two stages of sampling were carried out in this aspect of the study:

A) An initial survey was performed in which surface soil samples of approximately 500 g each from a depth of 5 to 10 cm were collected, in September 2014, from five different urban parks located in Greenock, UK were taken for analysis. Table 5.1 shows a brief description of the sites sampled. These sites were selected on the basis that:

i) they may contain high level of PTE due to historical anthropogenic activities.
ii) they were not made up or reclaimed ground.
iii) they were not cemeteries.
iv) they were not likely to be already heavily amended with fertilisers.

The initial sampling was done in order to choose a site with relatively high levels of PTE for use in column leaching experiments.

Tabbe 5.1 Description and brief historical background of sampled sites

Name of site	Area covered (ha)	Type of park	Brief history
Birkmyre Park	4.75	Grassed open space	Agricultural then, park since 1897. More intensely used in the past.
Gourock Park	10.86	Ornamental and recreational	Ornamental gardens and grounds of Gourock House
Lady Octavia Park	6.89	Recreational,shrubs, and grassed open space	Park since 1912. Old railways. Old quarries and King Glens burn in the SE infilled with wastes between 1912 and 1960s.
Lyle Park	1.18	Ornamental	Greenfield then park from 1960s.
Well Park	1.75	Ornamental	Ornamental park prior to 1856.

Source: Contaminated Land Officer, Inverclyde Council, UK (5th August 2014).

B) A second sampling campaign was carried out approximately four months after the first in (January 2015) at Well Park Greenock. At this location, surface soil samples which from 18 different sampling points (Figure 5.1) were collected into small polyethene bags after cutting the turf. The surface soil was collected to a depth of 5 to 10 cm. Solid materials such as large sticks and grasses were removed from the sampled soil. The individual soil samples were then bulked together to obtain a composite sample. A total of 10 kg soil was obtained and placed in a bigger polythene bag, carefully labelled and transported to the laboratory for further processing and analysis. After that, the sample was air dried for 2 weeks, sieved through a 2 mm mesh sieve, and used without further grinding as the soil was already sufficiently fine for use.

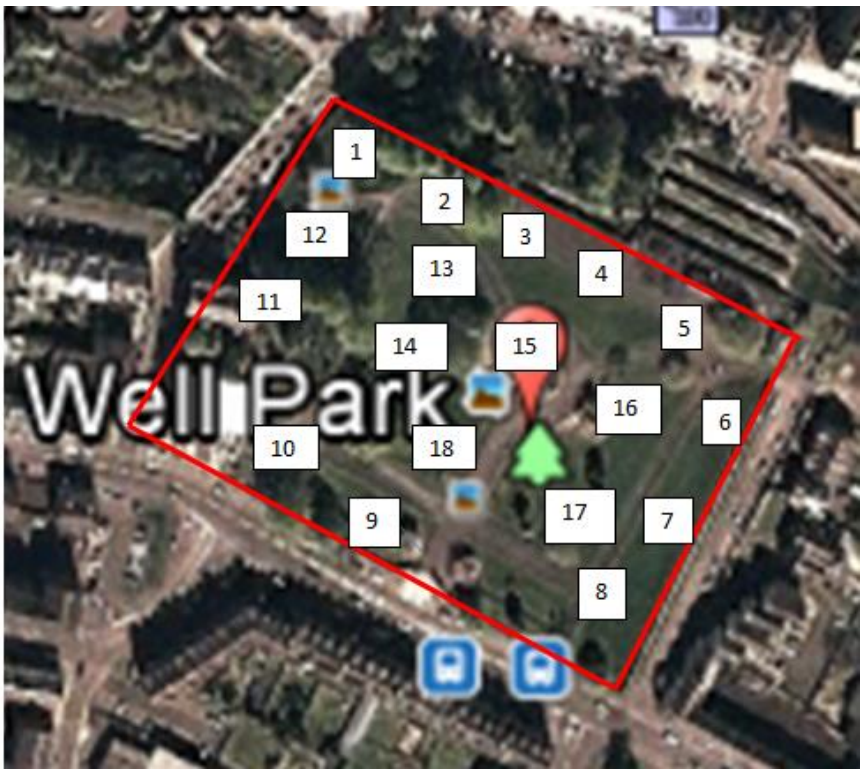


Figure 5.1 **A snap shot of Google Earth of Well Park showing the sampling points Map data: © Google / DigitalGlobe**

5.3 Pseudototal concentration

The pseudototal concentration of the soil samples was determined using *aqua regia* digestion and the digests analysed as decribed in section 3.2 in Chapter 3.

5.4 Column leaching experiment

The Well Park soil sample was used for column leaching experiments. 2% chicken manure, 5% growmore or 2% chicken manure + 5% gorwmore were mixed thoroughly with the air dried soil in order to investigate their effect on leaching of PTE in the amended soil.

The columns (Figure 5.2) used in this work were 60 cm long with internal diameter 4.0 cm. Glass beads were placed at the base of each of the leaching columns, which prevented solid material from being removed along with the leachate. Each column was filled with 550 g of the soil (control column) or amended soil. The soil was

carefully added in small aliquots and was lightly compressed after each addition. Each of the leaching experiment was performed in duplicate, except the control which was just one replicate due to limited number of columns.

Figure 5.2 Columns used and addition of amended soil into the columns

Deionised water (DW) was used as leachant, and about 200 mL were initially added manually to each of the columns in order to saturate the soil mixture over night. A volume of 50 mL was then added on a 12 hourly basis bringing the total number of volume of leachant per day to 100 mL. Addition of leachant in each case was carried out manually and addede at one go. This regime was continued for the first 3 to 5 days only and thereafter addition of 100 mLwas performed on a 24-hourly basis. The experiment lasted for 21 days, except for the chicken manure treated soil which was suspended after just 10 days due to swelling of the manure, leading to blockage of the column.

Leachate was collected in 120 mL polypropylene bottles. The volume of leachate collected was measured at the point of collection, and then it was filtered, and a portion removed for pH and electrical conductivity (EC) determination. The remaining portion was acidified with nitric acid and stored in the refrigerator pending analysis.

Measurement of PTE in the leachate was carried out on 10 mL solution using ICP-MS. The pH of leachate was measured by inserting the electrode directly into a 10 mL solution after calibration with buffer solutions as described in section 3.6 in Chapter 3. The EC in the leachate was determined using a Mettler Toledo

(SevenEasy) conductivity meter (Metller Toledo Ltd., Beaumont Leys, Leicester, UK) in 10 mL solution after calibrating the conductivity meter using the buffer solution (VWR chemicals, UK): 84, 1288 or 1413 µS/cm.

5.5 Sequential extraction of the amended soil before and after column leaching experiment

The BCR sequential extraction was applied to the amended soils before the experiment began. At the end of the leaching experiment, the residual soil was carefully removed from each of the columns and air dried for another 14 days. The air dried soil mixture was sieved, and representative samples obtained. Pseudototal digestion as described in section 3.2 and BCR sequential extraction as described in section 3.4 was performed on the residual soil to assess changes in the distribution of the PTE after leaching.

5.6 Results and Discussions

5.6.1 Pseudototal concentration of PTE in the Greenock Parks

Quality control

Table 5.2 shows the results of the pseudototal concentration of the PTE in a certified reference material, ERM® - CC141 (Loam soil) obtained during the digestion process and their indicative values. This CRM was used because the BCR®-143 (Sewage sludge amended soil) earlier used in Chapter 4 was exhausted. In general the recoveries were all good (100 ± 10%, except 88% for Cr).

Table 5.2 Pseudototal concentration of PTE (mg kg^{-1}) in ERM® - CC141 (Loam soil); n = 2

	As	Cd	Cr	Cu	Mn	Ni	Pb
Found	8.27	0.262	27.2	12.3	382	21.3	34
Target value	7.5 ± 1.4	0.250 ± 0.04	31 ± 4	12.4 ± 0.9	387 ± 17	21.9 ± 1.6	32.2 ± 1.4
Recovery (%)	110	105	88	99	99	97	106

Table 5.3 shows the pseudototal concentration of PTE found in the various urban parks initially sampled. The individual concentrations of the replicates are presented in Appendix H. As stated earlier, the purpose of measuring the concentration of the PTE at the five sites was to select from amongst them the site with a relatively high level of PTE for further sampling. The results showed that the RSD of all the PTE measurement in the samples were less than 10% (n = 3). The concentrations of, in particular As, Cu, Ni, and Pb were generally higher in the Well Park soil sample compared to the rest of the sites. Well Park was also closer to Glasgow than the other sites hence easier to access for sampling and transport of the soils back to the university. For these reasons, Well Park soil was chosen for the column leaching experiment. Table 5.4 shows the pseudototal concentration of PTE in the Well Park sampled after the initial sampling campaign. The levels of the PTE obtained in the Well Park during the initial sampling campaign did not differ markedly with the levels obtained in the second sampling campaign (Table 5.4).

Table 5.3 Pseudototal concentration (mg kg^{-1}) of PTE in some urban parks in Greenock, UK (n = 3)

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
BIR	7.80 ± 0.3	0.415 ± 0.04	23.0 ± 2.1	51.2 ± 1.9	21200 ± 925	439 ± 18	31.5 ± 1.8	229 ± 4	0.908 ± 0.05	192 ± 11
GUR	8.73 ± 0.3	0.447 ± 0.02	19.5 ± 1.8	69.7 ± 4.4	18100 ± 407	626 ± 29	25.1 ± 1.0	181 ± 12	1.02 ± 0.06	140 ± 7
LAO	7.16 ± 0.2	0.347 ± 0.01	25.1 ± 1.1	46.6 ± 1.4	19700 ± 489	356 ± 26	23.6 ± 0.8	100 ± 3	0.693 ± 0.03	167 ± 6
LYL	9.41 ± 0.7	0.178 ± 0.03	23.8 ± 1.1	45.1 ± 0.8	25300 ± 885	274 ± 5	24.6 ± 1.8	123 ± 3	0.914 ± 0.04	84.4 ± 3.9
WEL	12.8 ± 0.7	0.401 ± 0.02	22.1 ± 1.7	81.6 ± 1.8	24400 ± 1140	457 ± 29	34.8 ± 2.7	306 ± 20	1.01 ± 0.6	193 ± 9

BIR = Birkmyre Park; GUR = Gourock Park; LAO = Lady Octivia Park; LYL = Lyle Park; WEL = Well Park

Table 5.4 is the results for pseudototal concentration of PTE measured in the Well Park after the initial sampling campaign that led to selection of this site. As can be seen the PTE concentration of Well Park as shown in Table 5.4 did not differ markedly with the values obtained in the initial sampling (Table 5.3), confirming consistency in the samplings. The results obtained by Hursthouse *et al.*[179] in a number of parks and gardens in Glasgow showed higher concentrations of Cr (93.0 mg kg^{-1}), Pb (971 mg kg^{-1}) and Zn (364 mg kg^{-1}) compared to the values obtained in this work. However, the concentrations of Cu and Zn obtained in this work were less than the values reported by Kuzmanoski *et al.*[180] but they obtained similar results for Fe (30000 mg kg^{-1}) and Ni (61.5 mg kg^{-1}). Although the concentration of As, Cd, Cr and Ni were below the UK CLEA SGV, the values obtained for Pb and Zn exceeded the SGV (Table 6.8).

Table 5.4 Pseudototal concentration (mg/kg) of PTE in Well Park urban soil (n = 2, second sampling campaign for column leaching experiments)

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
WEL	14.2	0.378	32.5	103	30000	502	53.6	337	1.12	212

WEL = Well Park

5.6.2 Column leaching

The pH and the EC of the deionised water used, and the pH of the amended soils before leaching experiment commenced were measured and are presented in table 5.5. The pH of the deionised water was slightly acidic, with a relatively low electrical conductivity. The pH of the control soil and the amended soil showed they were all acidic in nature. These values were expected and particularly for the amended soils since growmore and chicken manure have been reported in Chapter 4 to be alkaline (7.70) and acid (3.90).

Table 5.5 The pH and EC of deionised water, and pH of amended soils before leaching experiment (n = 2).

	pH	EC (µS/cm)
Deionised water	6.50	0.500
Control soil	5.40	**
CMAS	5.70	**
GMAS	4.80	**
CM + GMAS	5.20	**

CMAS = 2% chicken manure amended soil; GMAS = 5% growmore amended soil; CM + GMAS 2% chicken manure + 5% growmore; ** = not determined.

5.6.2.1 The pH and electrical conductivity (EC) of leachate

The initial pH of the deionised water used 6.5. The pH and conductivity profiles for the leachate obtained for control soil, as well as the fertiliser amended soil samples in the experiment are shown in Figures 5.3 and 5.4 respectively. The pH of the leachate from the control soil ranged from 4.80 to 6.60 from the initial day of the experiment to the end of the experiment. Addition of chicken manure increased the pH of the leachates to a final value of 7.20 at the end of the 10 day period. This was expected as the pH of this fertiliser was high (7.70; Table 4.11; chapter 4). A steady increase in pH of the leachate collected in the column treated with the mixture of the materials (chicken manure + growmore) was observed for the first five days though with slight fluctuations. After that a sharp decrease was observed with a constant pH value at the end of the experiment. Leachate collected from the column amended with growmore showed a consistent decrease in pH lower than the control. This effect was observed for the first 12 day. Growmore being acidic in nature (3.90; Table 4.11; chapter 4) and as inorganic fertiliser could reduce soil pH[181]. As the leaching experiment continued, the [H^+] may have continued to decrease resulting to a corresponding increase in pH from the 13th day up to the end of the experiment.

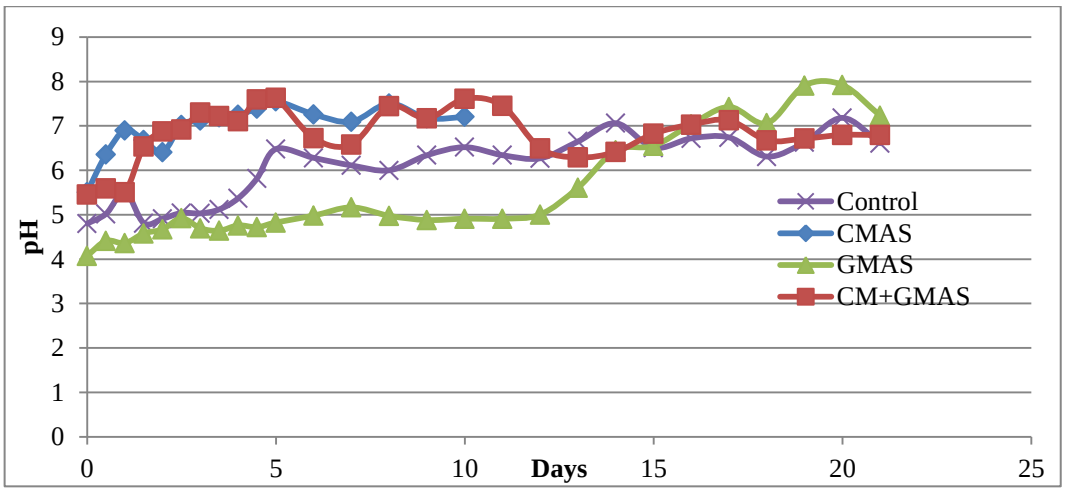


Figure 5.3 The pH profile for control soil, chicken manure, growmore and, mixture of the two fertilisers amended soil

The EC of the deionised water used was 0.500 µS/cm. The initial leachate EC of the chicken manure amended soil, growmore amended soil and chicken manure + growmore amended soil leachates were 4780, 13990 and 92000 µS/cm. These values were greater than the initial control leachate EC (469 µS/cm). Growmore being an inorganic fertiliser has high leachable salts. Addition of growmore increased sharply the level of salts in the growmore amended soil leachate for about half a day, after which substantial amounts of these species were then flushed out in the following few days to level out at about 420 µS/cm. The leachate from the chicken manure, and chicken manure + growmore showed similar trends where their initial conductivities decreased very sharply in the first few days and then throughout the period of the experiment. A similar behaviour was observed for the control soil.

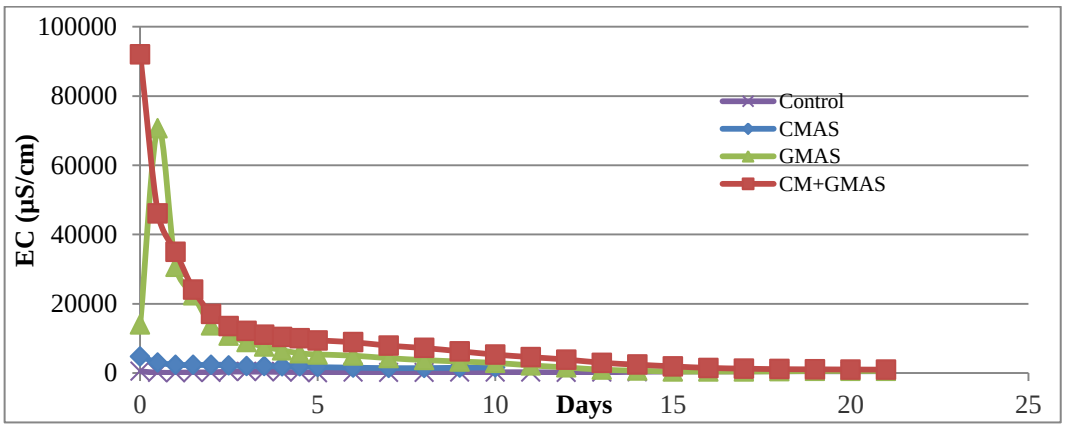


Figure 5.4 The EC profile for control soil, chicken manure, growmore and, mixture of the two fertilisers amended soil

5.6.2.2 Leaching profiles

Figures 5.5 to 5.14 show the leaching profiles for the PTE measured in the leachate of the soil treated with different dosages of the fertilisers. The amounts (mg) of PTE are presented in Appendix I. As mentioned earlier, the entire leaching process was performed for a total number of 21 days except for chicken manure amended soil where leaching had to be suspended after just 10 days due to blockage.

Arsenic

The initial concentration of As in the leachate of chicken manure + growmore amended soil was higher (8.6 µg) than in the other treatments. The amount of As leached between the first and third day of the experiment from the chicken manure amended soil column did not change until the 5th day where an increase was observed, peharps, the analyte was strongly held by the fertiliser there by delaying its release. For the growmore treatment, a sharp decrease was observed after the first day, and then increased up to the 12th day. The experience was not different for the chicken manure + growmore amended as As levels in leachate decreased until the 4th day where further increase was observed. In all cases, the amount of As released from each of the treatments was higher than the levels obtained in the control soil.

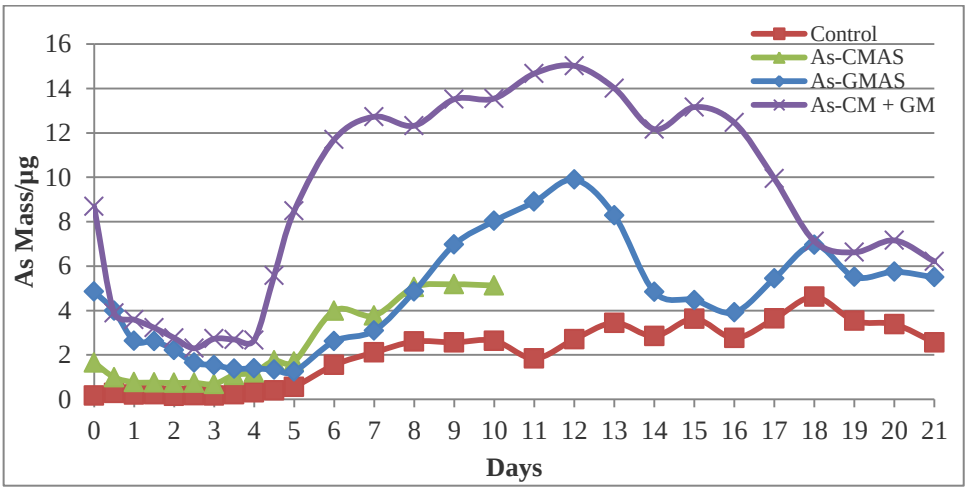


Figure 5.5 Leaching profiles for As in control soil, 2% chicken manure amended soil (CMAS) 5% growmore amended soil (GMAS) and soil amended with 2% chicken manure + 5% growmore (CM + GMAS)

Cadmium

Very low levels of Cd were released in the leachate of chicken manure amended soil with sharp decrease in the first 2 days (Appendix J) and then a steady release up to the 4.5 d. The initial amount of Cd released after addition of growmore fertiliser was highest compared to the two other treatments with a rapid decrease over 1 d period. This behaviour was expected because growmore is also acidic and very soluble, which may have contributed to the sharp release of Cd in the leachate. A similar trend was observed for the mixed amended soil. A corresponding decrease in the amount of Cd as the leaching process progressed was observed – this occurred within half a day. The leaching profiles generally showed that more of the leachate was released from the amended soils compared to the control soil.

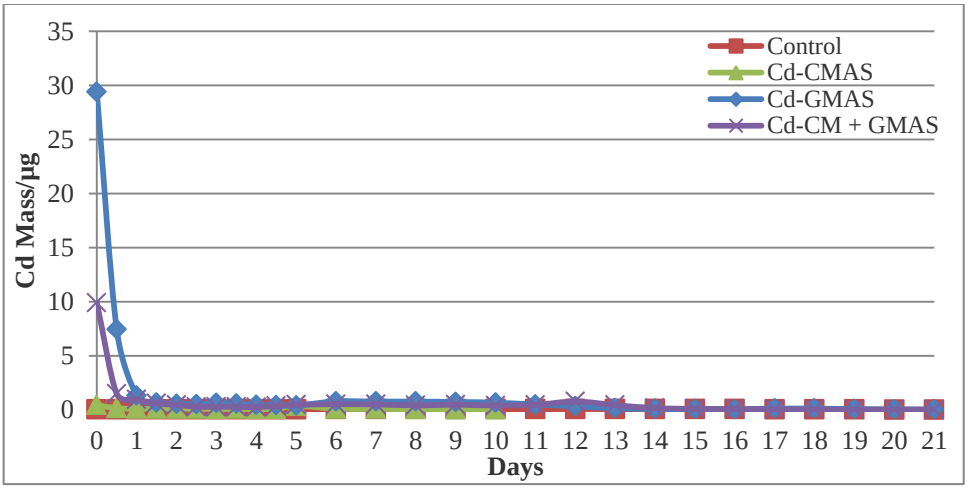


Figure 5.6 Leaching profiles for Cd in control soil, 2% chicken manure amended soil (CMAS), 5% growmore amended soil (GMAS) and soil amended with 2% chicken manure + 5% growmore (CM + GMAS)

Chromium

Relatively low levels of Cr were released in the presence of all the fertililiser amended soils at the initial stage of the leaching process. Higher amount was released from the chicken manure + growmore amended soil column. These values were higher than the amount measured in the control soil leachate. In the chicken manure amended soil column, Cr concentration in the leachate decreased steadily in the first day and later remained relatively constant until about the 4th day. A corresponding decrease in the amount of initial Cr was observed for the other two

amended soil leachates. All the profiles were higher than the control suggesting that the fertilisers had the potential of raising the levels of Cr in the soil solution.

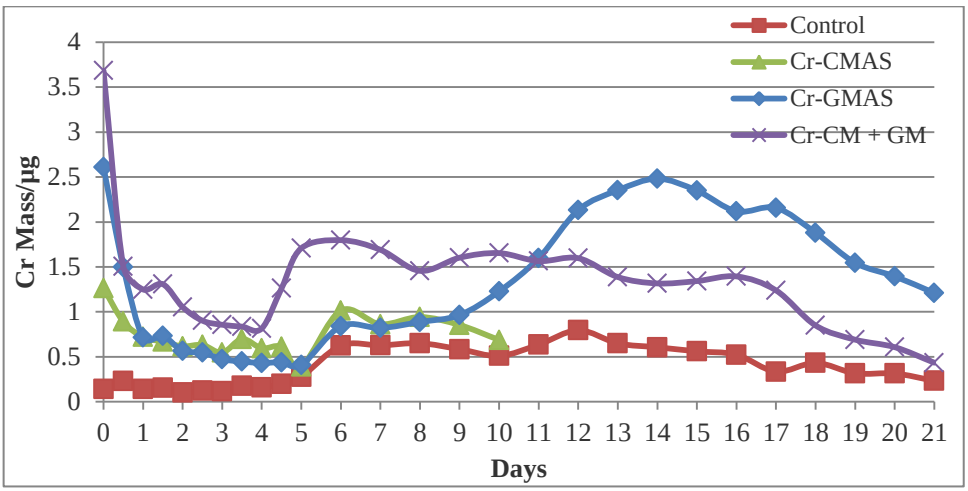


Figure 5.7 Leaching profiles for Cr in control soil, 2% chicken manure amended soil (CMAS), 5% growmore amended soil (GMAS) and soil amended with 2% chicken manure + 5% growmore (CM + GMAS)

Copper

The initial amount of Cu in the leachate obtained from chicken manure + growmore (121 µg) and this was followed by chicken manure leachate (86.4 µg) then growmore amended soils (41.1 µg). These levels were expected because the fertilisers themselves contained high concentration of Cu. The amount of Cu in all the amended soil leachate decreased during the first 3 days for chicken manure amended soil, 5 days for growmore and 4 days for the mixed fertiliser amended soil. Although higher levels were measured in the leachates from chicken manure + growmore amended soil compared to the other two, the levels of Cu in all the amended soil leachates exceeded the amount measured in the control soil leachates.

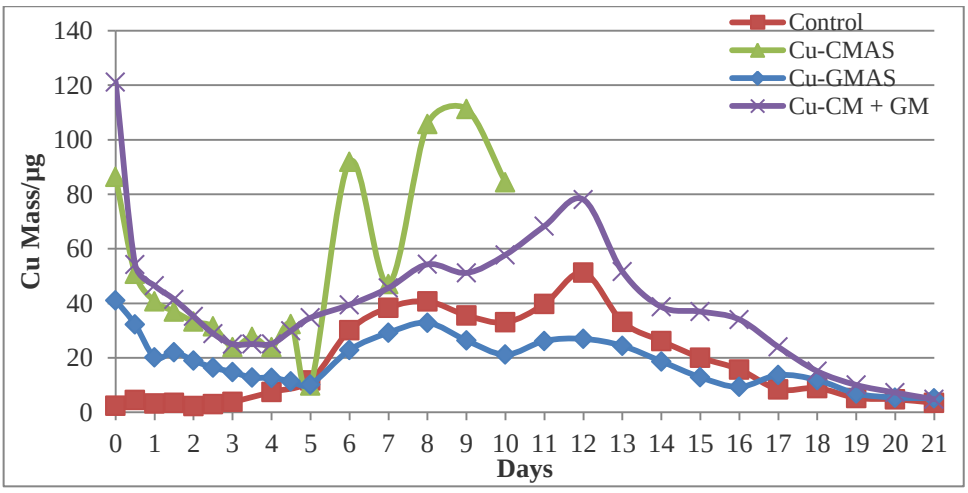


Figure 5.8 Leaching profiles for Cu in control soil, 2% chicken manure amended soil (CMAS), 5% growmore amended soil (GMAS) and soil amended with 2% chicken manure + 5% growmore (CM + GMAS)

Iron

The initial amount of Fe measured in the chicken manure amended soil leachate (198 µg) while 58.9 and 228 µg were the initial amounts obtained from growmore amd chicken manure amended soil leachates, respectively. A slight decrease in the amount of Fe was noticed in less than a day and increased steadily up to the 4.5th day, and then increased further for the entire leaching period of 10 days. The increase in the amount of Fe in the leachate corresponded to the decrease observed in the EC of chicken manure amended soil leachate (Figure 5.4). In the growmore amended soil leachate, a decrease in the amount of Fe was observed for the first 2 days despite the low pH. A rapid increase was then observed in the following 10 days and further decreased up to the 18th day. In the chicken manure + growmore amended soil leachate, a similar trend was seen where the amount of Fe in the leachate continued to increase continuously. The irregular leaching profile obtained for Fe suggested that some regions in the soil system became waterlogged which could lower the E_h (not measured in this work) resulting to reducing conditions that favour the release of Fe in such soil systems.

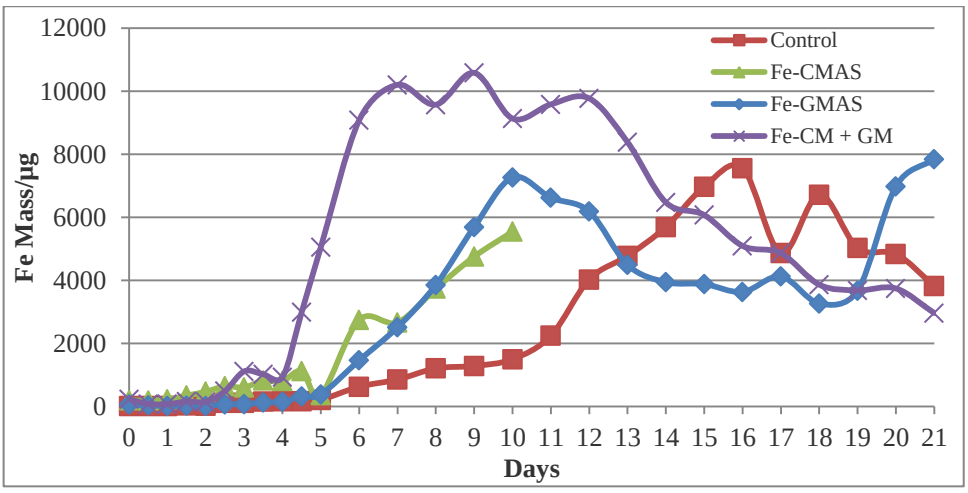


Figure 5.9 Leaching profiles for Fe in control soil, 2% chicken manure amended soil (CMAS), 5% growmore amended soil (GMAS) and soil amended with 2% chicken manure + 5% growmore (CM + GMAS)

Manganese

The initial amount of Mn in the amended soil leachate showed that Mn was highest in the growmore amended soil (6320 µg) and this was followed by leachate collected at that stage from the chicken manure + growmore (5060 µg) amended soil column. The lowest of the amended soils was from the leachate collected from the chicken manure amended soil column (657 µg). The amount measured in the control soil leachate was the lowest for the entire experiment (27.3 µg). The level of Mn released in growmore or chicken manure + growmore amended soil leachates decreased sharply for the 1st day of the leaching process. For the latter, a constant release was then observed up to the 4th day, and subsequently a rapid increase up to the 6th day. In the former, further decrease was seen towards the 2nd day, and a similar sharp increase up to the 6th day. In chicken manure amended soil, Mn was slowly and steadily released over the first 4 days, suggesting that chicken manure may have complexed it thereby preventing its releases. Another reason may be attributed to increase in pH of the leachate within this period. The amount of Mn released in the amended soil leachates was generally higher than in the control.

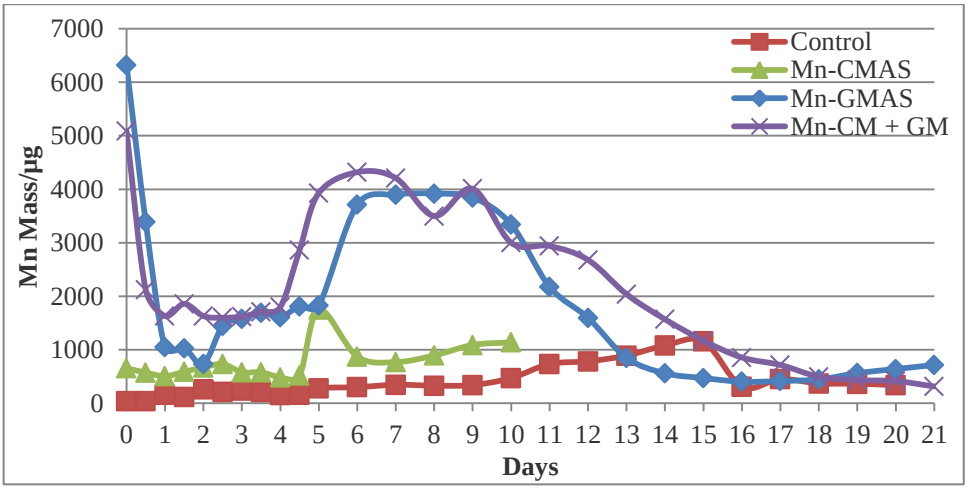


Figure 5.10 Leaching profiles for Mn in control soil, 2% chicken manure amended soil (CMAS), 5% growmore amended soil (GMAS) and soil amended with 2% chicken manure + 5% growmore (CM + GMAS)

Nickel

Low levels of Ni were released at the initial stage of the experiment. At this stage, only 12.2 µg was measured in the leachate collected from chicken manure, 67.9 µg from growmore amended soil leachate which was the highest and 31.6 µg from the chicken manure + growmore amended soil. In the chicken manure amended soil leachate, the amount of Ni decreased sharply within the half a day and assumed a steady profile up to the 5th day and then increased. A similar trend was observed for growmore amended soil leachate. Here, the initial amount of 67.9 µg decreased sharply within the first 2 days, thus indicating a high potential of the deionised water used in the experiment to solubilise Ni in the presence of growmore, which is itself highly soluble. A constant amount of Ni was then released for the following 5 days, which was followed by a sudden increase for two days and a steady amount for the remainder of the experiment. Growmore contained the highest pseudototal concentration of Ni. In the chicken manure + growmore amended soil leachate, a decrease in the initial amount of Ni was observed for the first 4 days. Similar to the individual fertiliser treatments, more Ni were released between the 5th and 10th day and later decreased towards the end of the leaching process. The control soil leachate released less Ni compared to the treated soils.

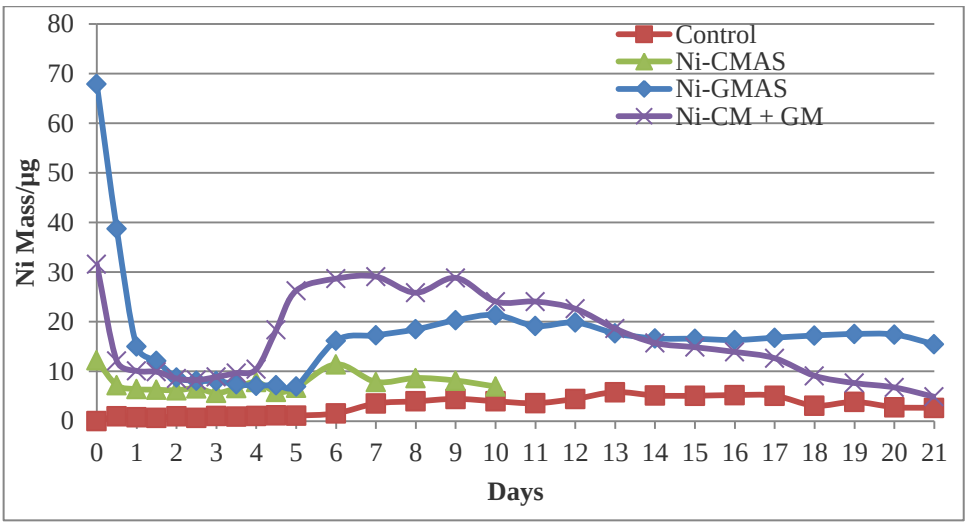


Figure 5.11 Leaching profiles for Ni in control soil, 2% chicken manure amended soil (CMAS), 5% growmore amended soil (GMAS) and soil amended with 2% chicken manure + 5% growmore (CM + GMAS)

Lead

The initial amount of Pb (163 µg) released from the column with growmore amended soil leachate was the highest, compared to the amount leached in the chicken manure or chicken manure + growmore amended soils leachate. This value decreased significantly to 27.7 µg in less than a day of the leaching process. After that a slow but steady decrease was observed throughout leaching process. From the fact that growmore contained the highest concentration of Pb and with low pH the high amount of Pb released from the initial stage was expected. The amount of Pb released from the chicken manure amended soil remained constant up to the 4th day, suggesting that the mobile species were held by the fertiliser within this period. An increase was then observed up to the 6th day before decreasing, probably due to depletion of the mobile species (Appendix J). For chicken manure + growmore amended soil leachate showed a similar trend as growmore amended soil. The amount of Pb released by the amended soil leachates exceeded the level measured in the control leachate.

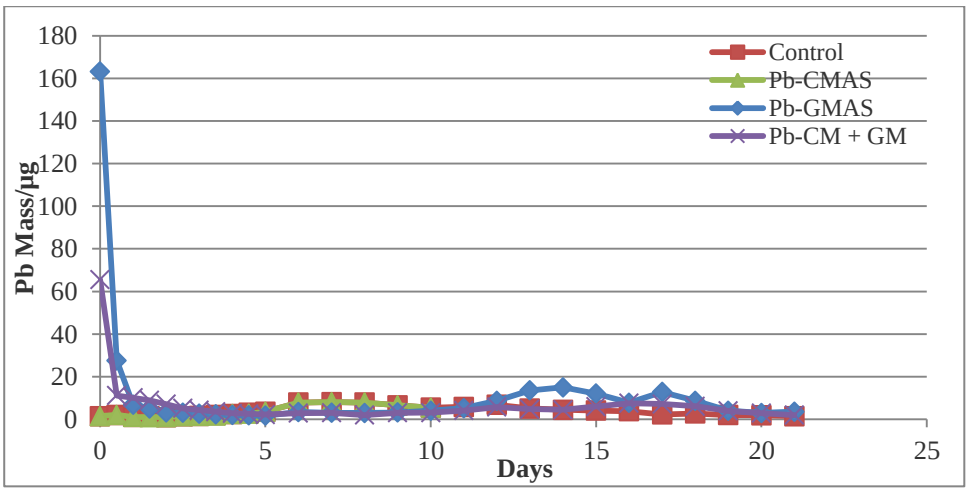


Figure 5.12 Leaching profiles for Pb in control soil, 2% chicken manure amended soil (CMAS), 5% growmore amended soil (GMAS) and soil amended with 2% chicken manure + 5% growmore (CM + GMAS)

Uranium

The initial leachates of the three treatments and the control soil contained low amount of U. The chicken manure + growmore amended soil leachate showed the highest amount (0.701 µg) compared to the other leachates including the control. A sharp decrease in this value was observed in half a day for which, a constant decrease was maintained for the most of the leaching process. In the chicken manure amended soil leachate, a decrease in the mount of U was seen until the 2nd day, after that a relatively constant release occurred up to the 5th day and then it increased slightly towards the end of the leaching process. Although the amount of U leached from the growmore was generally low, a sharp decrease was seen within the first day and no obvious difference was seen until the 5th day when a sharp and dramatic increase occurred from this day up to the 14th day. A corresponding sharp decrease was further seen during the leaching process. Growmore contained the highest pseudototal concentration of U. Further the amount of U released from the amended soil was higher than in the leachates of the control soil.

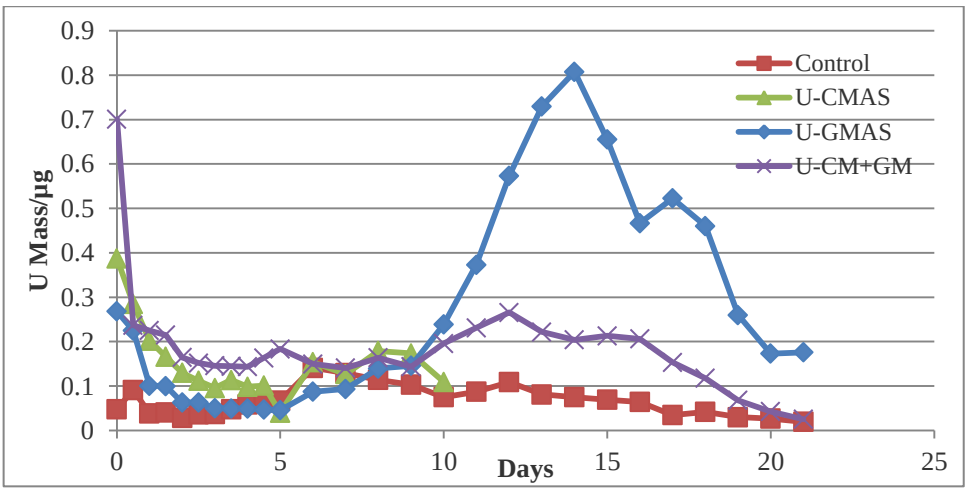


Figure 5.13 Leaching profiles for U in control soil, 2% chicken manure amended soil (CMAS), 5% growmore amended soil (GMAS) and soil amended with 2% chicken manure + 5% growmore (CM + GMAS)

Zinc

The amount of Zn in the first leachate of growmore amended soil was relatively high (1910 µg) compared to the amount released in the leachates of chicken manure + growmore amended soil (424 µg) or chicken manure amended soil (73.1 µg). There was a sharp decrease in the amount of Zn released as the experiment progressed, particularly for growmore and chicken manure amended soils. In the latter, no further significant decrease was observed as a constant and steady amount of Zn was realeased throughout the entire leaching process. Growmore amended soil released the highest amount of Zn. This may be attributed again to the low pH and high solubility of the amendment – these may have favoured Zn mobilisation and release from the soil system. The release of Zn from chicken manure amended soil was suppressed, as a result lower amount of Zn was observed for the 10 day period of leaching compared to the other two treatments. The amount of Zn measured in the amended soil leachates was higher than in the control leachate.

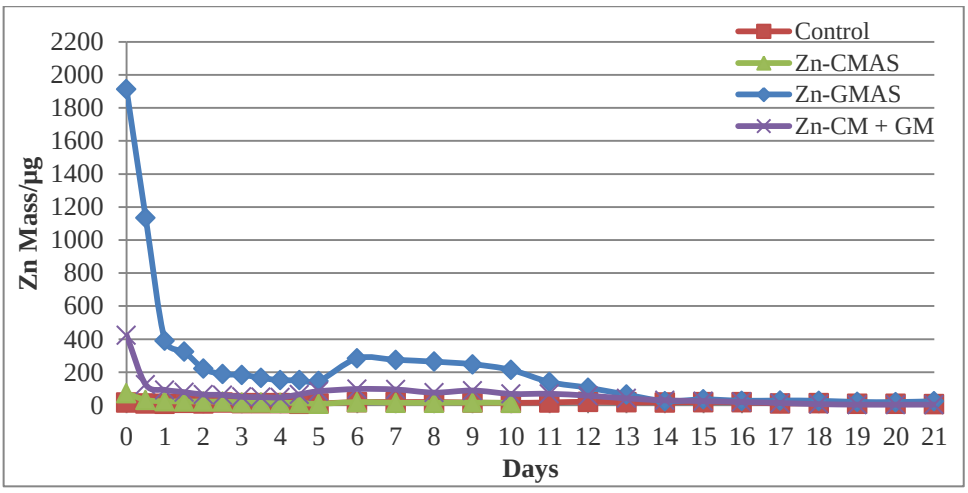


Figure 5.14 Leaching profiles for Zn in control soil, 2% chicken manure amended soil (CMAS), 5% growmore amended soil (GMAS) and soil amended with 2% chicken manure + 5% growmore (CM + GMAS)

5.6.2.3 Inter-element relationship

The various leaching profiles for the PTE were further assessed with the view to identifying any marked similarities. It was observed that Cd, Pb, U and Zn showed similar behaviour which was characterised by high initial release and and flattering out. This trend was wholly expected since relatively high concentrations of these PTE were already present in the samples, and is usually of anthropogenic sources.

The next category of PTE that showed similar behaviour was As, Cr, Ni, Fe and Mn. In this category, release of the elements was delayed and had started coming out about the 5th day, indicating how strongly they were held in the soil matrix. Further their geogenic nature is another reason for delay compared to some of the other PTE. It is well-known that Fe minerals bind As in soil hence the similarity.

Iron and manganese also showed the highest concentration in the leachates compared to the other PTE studied in this work. The high level of these elements in the leachates may be attributed to waterlogged conditions at some regions in the soil columns, which could favour Fe/Mn mobilization due to the dissolution of Mn (hydr)oxides or Fe (hydr)oxides.[182]

5.6.2.4 Total levels of PTE (mg/kg) removed from soil during leaching experiment

Table 5.4 shows the accumulated mass of PTE leached from the columns during the leaching experiment. This was calculated by the adding up all the individual amounts (µg) for the entire period of the leaching process and dividing by the total weight of soil mixture (in this case, 550 g). The individual masses of the PTE (µg) are shown in (Appendix J).

The results as presented in Table 5.4, revealed that low concentration of PTE were released from the chicken manure amended soil column compared to the total levels leached from the control soil. Iron and Mn showed the highest concentrations compared to the rest of the other PTE in this treatment. This was expected because high concentrations of these elements were found in the amendment and soil. Further the total concentrations of all the PTE released from the chicken manure amended soil were lower than in the leachates of chicken manure + growmore amended soil, probably due to the higher pH recorded for the chicken manure amended soil leachate during the leaching process.

The total levels of PTE obtained from the growmore amended soil and chicken manure + growmore leachates showed a similar trend as observed in chicken manure amended soil, with Fe showing the highest value. Apart from As, Cu, Fe and Mn, growmore released higher levels of PTE when alone but not when in the presence of chicken manure. This may be attributed the high pseudototal contents of these elements in growmore and presumably they get bound to the chicken manure thereby preventing their release. In each case the levels obtained from the control remained consistently lower, confirming that these materials can mobilise PTE in soil.

Table 5.6 Total mass of analytes (mg kg^{-1}) removed from soil during leaching experiment

Treatments	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
CS-10d	0.0261	0.00126	0.00872	0.421	11.7	5.58	0.0547	0.102	0.00201	0.387
CMAS	0.0640	0.00360	0.0218	1.52	45.1	21.5	0.220	0.3440	0.00450	0.618
GMAS	0.218	0.0865	0.0630	0.92	139	90.9	0.8580	0.602	0.0127	12.3
CS-21d	0.0898	0.00219	0.0186	0.817	115	18.5	0.1380	0.177	0.00318	0.676
CM+GMAS	0.416	0.0359	0.0651	1.87	221	98.2	0.803	0.351	0.00912	3.13

CS-10d = leachate collected from the control soil column (applicable to chicken manure amended soil only); CMAS = leachate collected from the 2% chicken manure amended soil column for the 10 days; GMAS = leachate collected from the 5% growmore amended soil column; CM+GMAS = leachate collected from the 2% chicken manure + 5% growmore amended soil column and CS-21d = leachate collected for the entire 21 day period of the experiment (applicable to GMAS and CM + GMAS only).

5.6.2.5 Sequential extraction

The BCR sequential extraction was performed on the soil sample before the column leaching experiment began and on the leached soil after the the experiment. This was done in order to assess redistribution of the PTE (if any) in the leached fertiliser amended soil. Appendix K shows the distribution of the PTE in the soils before and after leaching.

Quality control

Table 5.7 contains PTE concentration measured in a certified reference material, BCR®-601-CRM (Lake Sediment) and their respective indicative values. This CRM was used during the extraction process because it was the only one available at the time of this experiment. The recovery with respect to the indicative values was and within 100 ± 20 %.

Table 5.7 Results of the pseudototal concentration of a certified reference material, BCR®-601-CRM; n = 2 (Lake Sediment)

		Cr	Cu	Ni	Pb	Zn
EXC.	Ind. value	0.35 ± 0.08	10.5 ± 0.8	7.82 ± 0.8	2.28 ± 0.4	260 ± 13
	Obt. value	0.333	9.68	6.68	1.8	225
	% Rec.	95	92	85	79	87
RED.	Ind. value	10.6 ± 0.9	72.8 ± 4.9	10.6 ± 1.2	205 ± 11	266 ± 17
	Obt. value	8.68	61.3	9.84	197	226
	% Rec.	82	84	93	96	85
OXID.	Ind. value	14.4 ± 2.6	78.6 ± 8.9	6.04 ± 1.3	19.7 ± 5.8	106 ± 11
	Obt. value	13.9	64.3	6.4	13.9	99.1
	% Rec.	97	82	106	71	93.4
RES.	Ind. value	78.2 ± 6.5	60.4 ± 4.9	50.5 ± 6.1	38.0 ± 8.7	161 ± 14
	Obt. value	80.4	59.5	51.8	44.2	167
	% Rec.	103	99	103	116	103

EXC. = exchangeable fraction; RED. = reducible fraction; OXID. = oxidisable fraction; RES. = residual fraction; Ind. = indicative value; Obt. = obtained value.

As mentioned in section 5.4.3, the soils were subjected to sequential extraction before and after the experiment. The distribution of the PTE is shown in Figures 5.15 to 5.22. In the Figures, CSB = control soil before leaching, CSA = control soil after leaching, CMASB = chicken manure amended soil before leaching; CMASA = chicken manure amended soil after leaching; GMASB = growmore amended soil before leaching, GMASA = growmore amended soil after leaching; CM + GMASB = chicken manure + growmore amended soil before leaching and CM + GMASA = chicken manure + growmore amended soil before leaching.

Arsenic

Arsenic in the control soil before the leaching experiment was present in all the fractions with the residual fraction dominating. At the end of the experiment, the exchangeable and the reducible fractions of the control soil increased, and correspondingly, the oxidisable and residual fractions were observed to increase in small proportion. The expectation was to possibly see a decrease in the exchangeable fraction due to leaching but the reduction in the residual fraction in particular was unexpected.

In the chicken manure amended soil, there was significant variation in the concentration of As in the exchangeable fraction after the leaching. This indicated that As was mobilized from the reducible fraction by the deionised water. Table 5.6 indeed showed that some of the As were actually removed, though in small amount. Although there was significant reduction in the concentration of As in the residual fraction for CM + GMAS at the end of leaching, this effect was unexpected because As is well- known to associate with Fe minerals, may not be easily removed by a mild extractant such as deionised water used in this experiment. The recovery of As relative to pseudototal concentrations was between (84 and 114%).

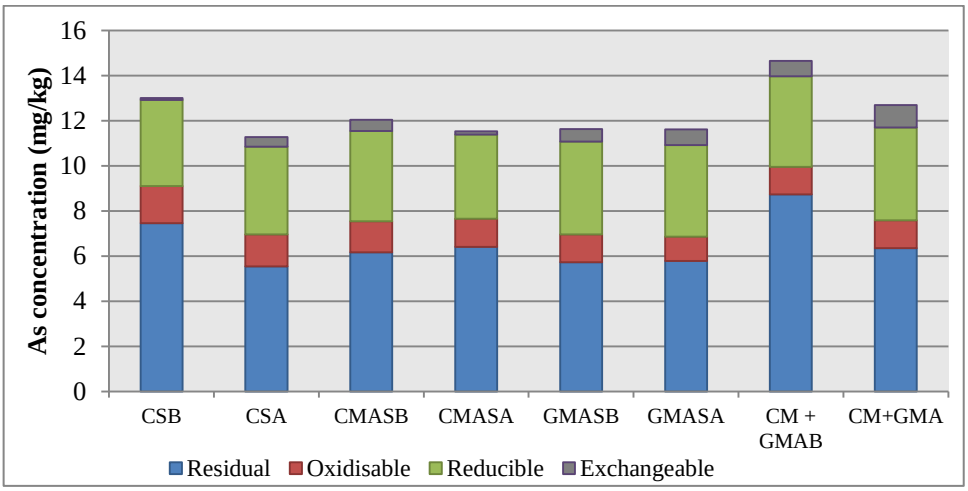


Figure 5.15 Arsenic distribution in the control soil and the amended soils before and after column leaching according to the BCR sequential extraction procedure (n = 2, except control; n =1)

Cadmium

The distribution of Cd in the control soil before and after the experiment showed that most of the Cd was in the reducible and exchangeable fractions. The residual fraction was below the detection limit. Only small amount of Cd was leached from the control and CMAS columns (Table 5.6) and hence no significant change was observed before and after the leaching process for these soils. There was variation in the distribution of Cd in the remaining soil samples – cadmium concentration decreased in the exchangeable fractions of GMAS and CM + GMAS after the leaching process, indicating that some of the exchangeable bound Cd in both soil were removed from this phase during the leaching process (Table 5.6). Table 5.6 show relatively higher level being leached out. The recovery of Cd with respect to the pseudototal concentration was generally good for all the samples.

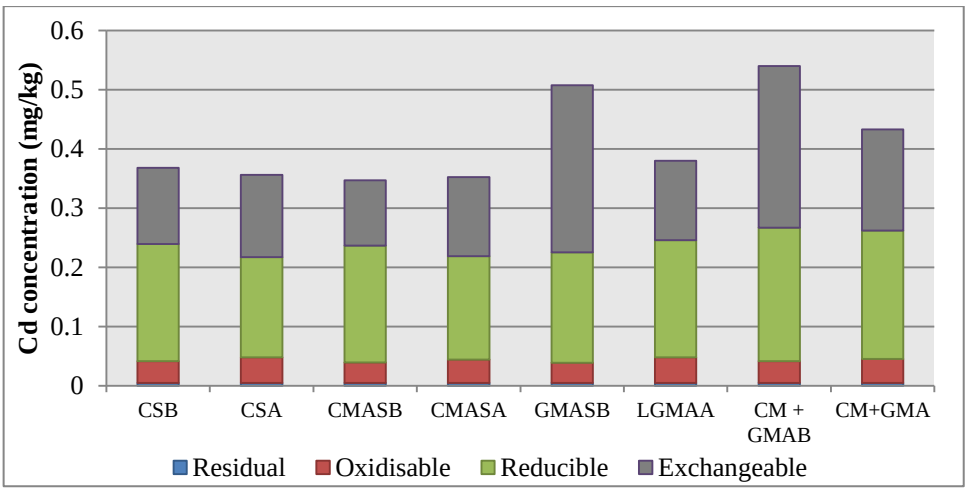


Figure 5.16 Cadmium distribution in the control soil and the amended soils before and after column leaching according to the BCR sequential extraction procedure (n = 2, except control; n =1)

Chromium

Chromium was predominantly distributed in the residual fraction in all the control and amended soils before and after the experiment. There appeared to be no significant difference in Cr concentration in the control soil after the experiment due to small amount leached from the column (Table 5.6).

Chicken manure amended soil did not show any significant difference just like in the control soil. A significant decrease in the exchangeable fraction in GMAS was observed, with no variation in the other phases. This indicated that the deionised water has the potential to leach Cr from this phase (Table 5.6). It was unexpected to see a dramatic decrease in the residual fraction in the CM + GMASB. Deionised water is a mild extractant which may not be expected to have such an effect on the residual fraction – this effect is likely to be unreal, and may be due to loss of material during the extraction process. The recovery of Cr in relation to the pseudototal concentration was generally poor (65 to 81%) perhaps due to losses during extraction process.

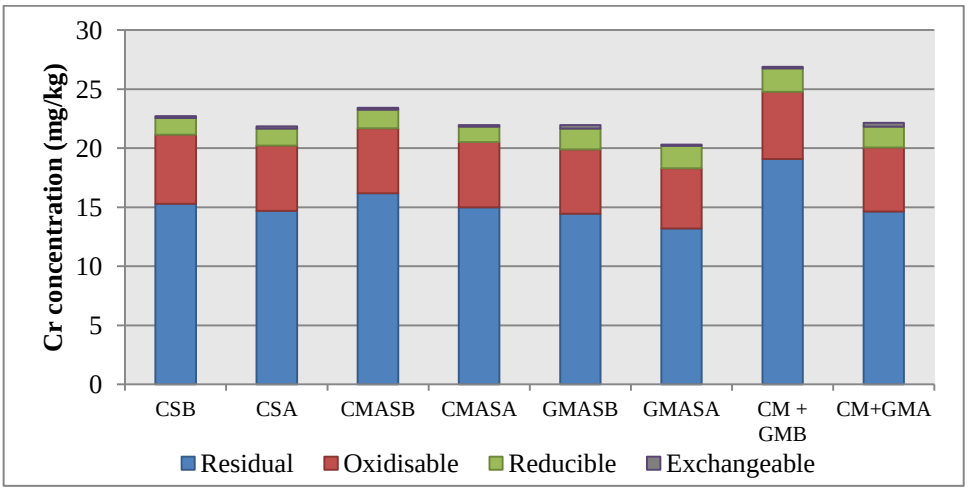


Figure 5.17 Chromium distribution in the control soil and the amended soils before and after column leaching according to the BCR sequential extraction procedure (n = 2, except control; n =1)

Copper

Copper was present in all the fractions in the control and amended soils, and in relatively equal concentration. There appeared to be mobilisation of Cu from both the reducible and oxidisable fractions to the exchangeable fraction for the control soil, and probabaly from the reducible fraction to the exchangeable fraction for the chicken manure amended soil at the end of leaching. The expected result was to see a decrease in the exchangeable fractions in all the soils since some of the Cu was removed from the soils by deionised water during the leaching process as shown in Table 5.6. It is however suspected that the increase in the exchangeable Cu may be connected to possible dissolution of Fe and Mn oxides (which are important carriers of PTE) in the columns at some point, and subsequently resulting to release of Cu to the exchangeable fraction. Further the mobilization of Cu from the oxidisable fraction to the exchangeable fraction for the control soil may be due to dissolution of soluble organic ligands formed with the element during leaching process. The recovery of Cu in the soil samples on the basis of pseudototal concentrations was generally not good (76 to 92%).

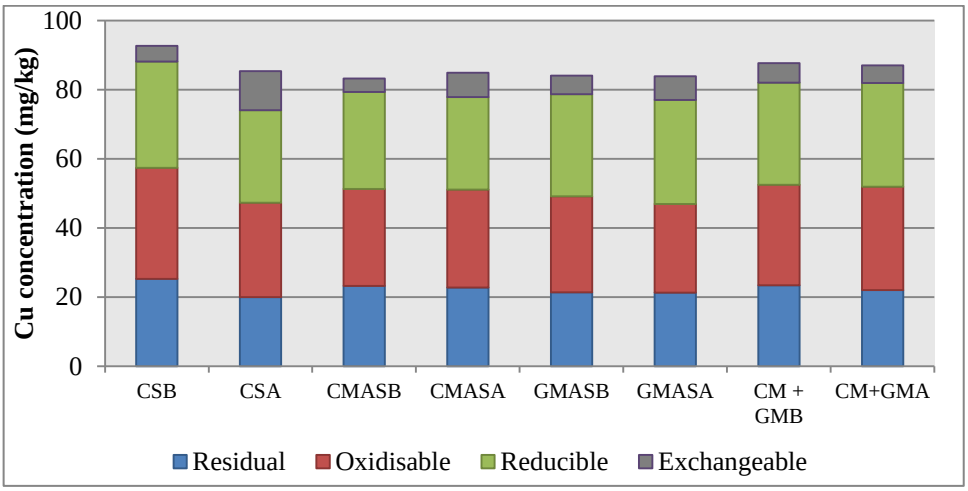


Figure 5.16 copper distributions in the control soil and the amended soils before and after column leaching according to the BCR sequential extraction procedure (n = 2, except control; n =1)

Iron

Iron was predominantly present in the residual fraction which is expected as it is a well-known major constituent of soil minerals. The concentration of Fe in the exchangeable and reducible fraction of the control soil increased at the end of the experiment (Appendix K). This trend was unexpected, since relatively high concentration Fe (µg kg^{-1}) was leached from the soil (Table 5.6). A similar behaviour was observed in the CMAS (Appendix K). In GMAS, a significant decrease in the concentration Fe was seen in the exchangeable fraction after the leaching experiment.– high concentration of Fe was leached from this fraction as shown in Table 5.6. A sudden decrease in the residual fraction was observed. The effect appeared to be unreal and could be attributed chiefly to loss of material during the BCR extraction. The recovery of Fe after the BCR extraction in the samples on the basis of pseudototal concentration was generally good except in the GMASB where it was 55%, confirming that loss had occurred during extraction..

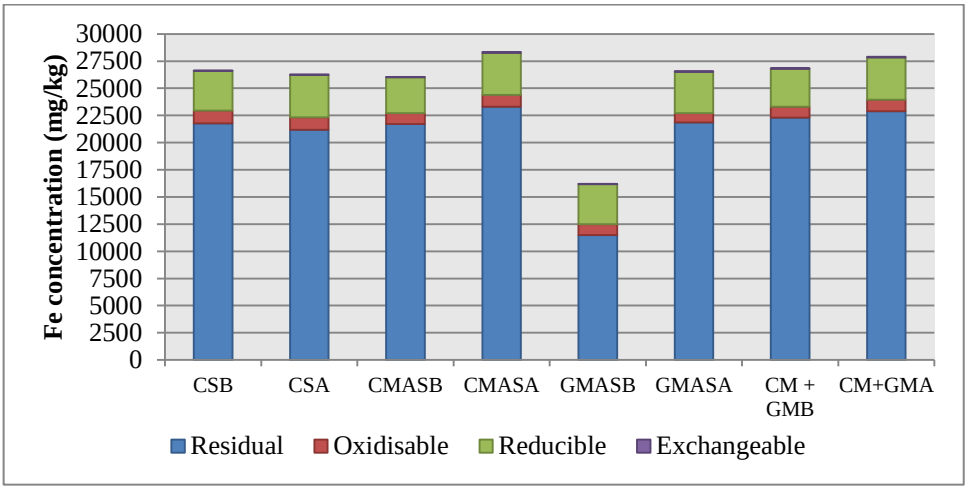


Figure 5.17 Iron distribution in the control soil and the amended soils before and after column leaching according to the BCR sequential extraction procedure (n = 2, except control; n =1)

Manganese

The distribution pattern of Mn in the soil and amended soil before leaching showed that most of the manganese was in the residual and reducible fractions. There was slight evidence that the deionised water mobilised Mn directly from the reducible fraction in all the fertiliser amended soils, except GMAS as the overall pseudototal concentration remained approximately the same. There was however, a strong evidence of redistribution of Mn from the reducible fraction to exchangeable forms in the GMAS during the leaching process, and this may be attributed to development of reducing conditions at some regions in the soil column which favoured dissolution of Mn (hydr) oxides leading to increased mobility of the element in the soil system. The recovery of Mn on the basis of pseudototal concentration in the soil samples was good.

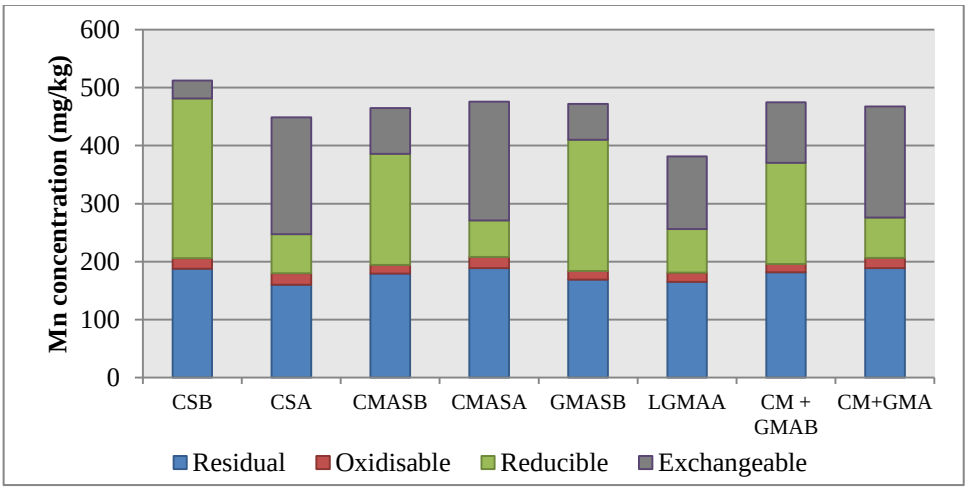


Figure 5.18 Manganese distribution in the control soil and the amended soils before and after column leaching according to the BCR sequential extraction procedure (n = 2, except control; n =1)

Nickel

Nickel was distributed chiefly in the residual fraction before and after the leaching process. This confirms how difficult this element can be mobilised especially by deionised water as seen in Figure 5.11. The exchangeable and reducible, as well as the oxidisable fractions for all the soil samples were not affected significantly after the experiment. The recovery of Ni in the soil samples on the basis of pseudototal concentration was relatively poor (73 to 96%).

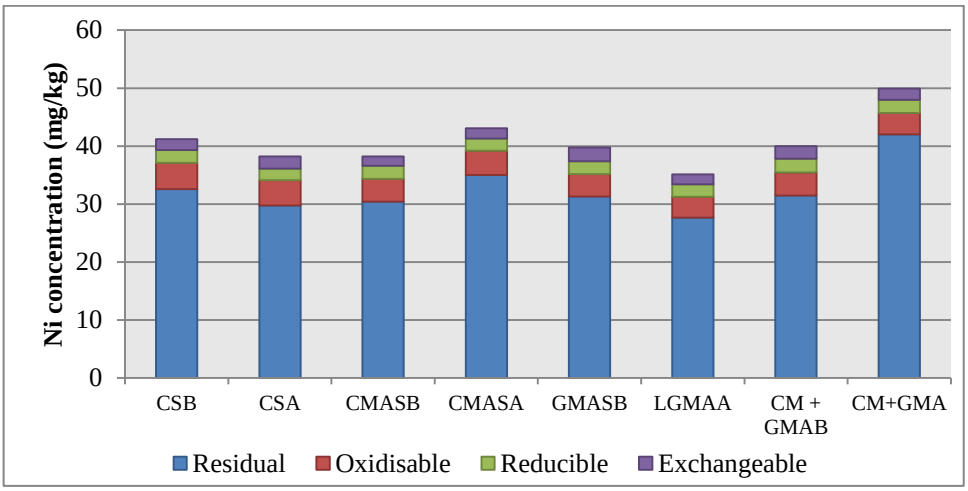


Figure 5.19 Nickel distribution in the control soil and the amended soils before and after column leaching according to the BCR sequential extraction procedure (n = 2, except control; n =1)

Lead

Larger concentration of Pb was found in the residual fraction of all the soil samples before and after the experiment. Apart from the control soil, where the there was an increase in the concentration of the soil after the leaching experiment, the exchangeable fraction showed a consistent decrease at the end of the experiment, confirming that the deionised water was able to remove Pb from the exchangeable phase. Higher level of Pb was removed from the exchangeable fraction of GMAS compared to the other amended soils. Contrary, the reducible fraction did not show any major shift in all samples before and after the experiment. Similarly, the oxidisable fraction was unaffected. The residual Pb present in the CM + GMAS before leaching appeared to have decreased but no other changes were observed for these samples. The recovery of Pb in relation to pseudototal concentration in the samples was generally good (95 to 113%)

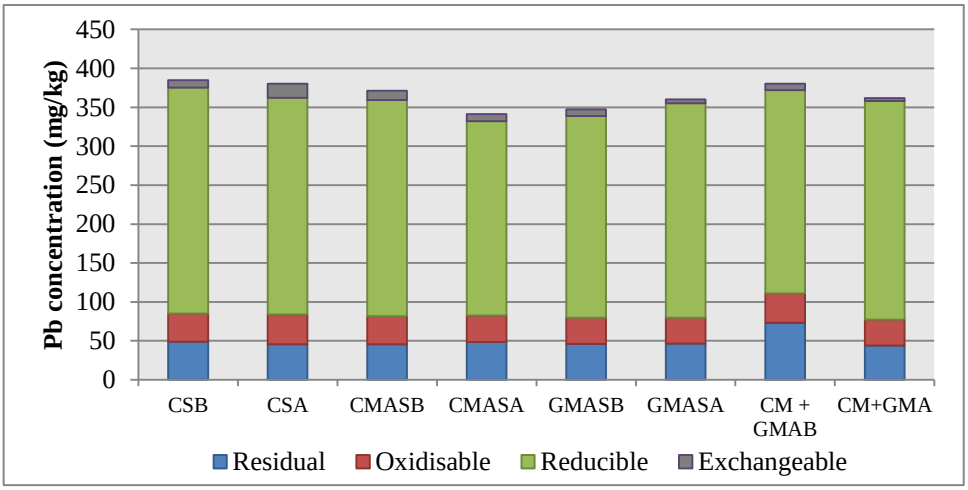


Figure 5.20 Lead distribution in the control soil and the amended soils before and after column leaching according to the BCR sequential extraction procedure (n = 2, except control; n =1)

Uranium

Uranium was present in larger concentration in the oxidisable and residual fraction before and after the experiment compared to the other fractions. It was observed that much U was released from the growmore amended soil column during the leaching experiment. It was therefore expected that a bigger change in the exchangeable fraction for this treatment at the end of the leaching would be observed but contrary the chicken manure + growmore amended soil that released less concentration of U seemed to show marked decrease in the exchangeable fraction between the start and end of leaching. The recovery of U in the soils was generally not good (80 to 135%)

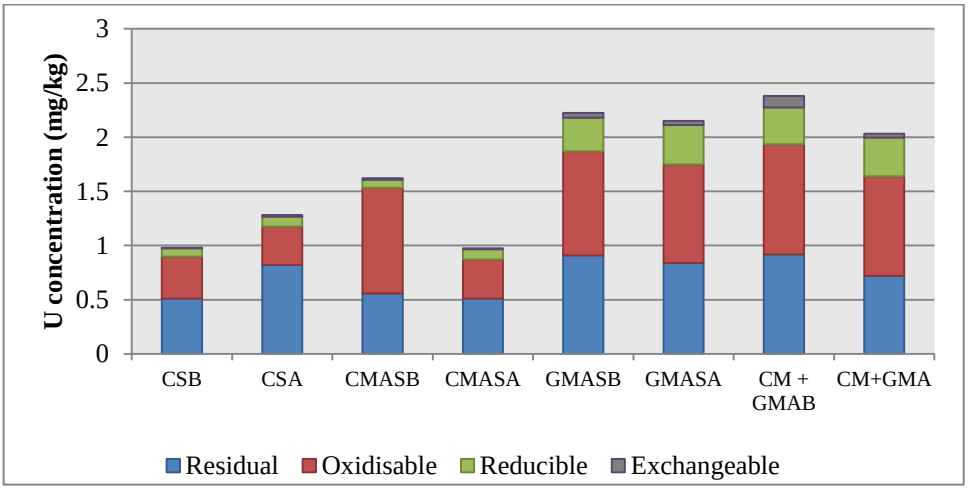


Figure 5.21 Uranium distribution in the control soil and the amended soils before and after column leaching according to the BCR sequential extraction procedure (n = 2, except control; n =1)

Zinc

Zinc was predominantly distributed in the entire soil sample before and after the experiment in the residual fraction. There appeared that no significant variation occurred in the various fractions before and after leaching, except in GMAS where Zn appeared to have been successfully leached from the exchangeable fraction as expected (Figure 5.14). Growmore has shown to have supported the removal of Zn probably because of its low pH and its high solubility. The concentration of Zn leached in the presence of growmore was significantly higher than the other PTE. The recovery on the basis of pseudototal concentration of Zn was generally good (81 to 90%)

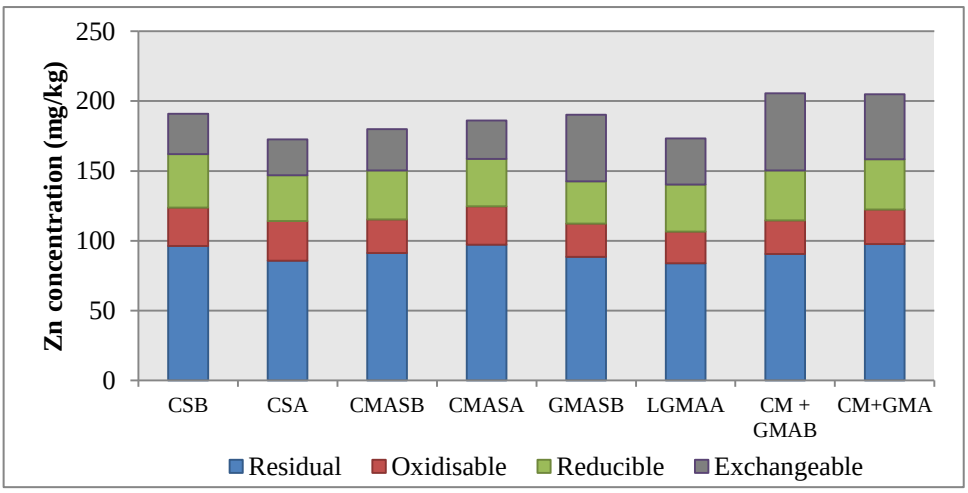


Figure 5.22 Zinc distribution in the control soil and the amended soils before and after column leaching according to the BCR sequential extraction procedure

5.7 Conclusions

The result of the physicochemical parameters of the leachates from the amended soils showed that chicken manure addition to the soil generally increased the pH of the leachate due to its high pH compared to the other fertiliserss. Growmore which was acidic in nature correspondingly decreased the pH of the leachates from the amended soil for most of the period. The effect exhibited by the mixture of the two amendments appeared to be similar to that of chicken manure. In general, the pH of all the leachates including that of control converged towards the pH of the leachant – deionised water used (6.5). High initial salts level were measured in the leachates of growmore and chicken manure + growmore amended soils and reduced significantly towards the end of the leaching. Chicken manure and control leachates gave lower EC values.

There was evidence of increased levels of PTE in leachates of the amended soils, as well as higher release when compared with the leachates obtained from the control soil. This confirmed the potentials of deionised water and fertilisers to leach PTE from soils and alter the mobilities of PTE in soil. Addition of 5% growmore to the soil resulted in higher release of most of the PTE studied: Cd, Cr, Ni, Pb, U, and Zn. This fertiliser contained relatively higher pseudotal content of these elements, and more importantly, was highly acidic and soluble which confirms its relatively higher

effect compared to the other fertilisers. 2% chicken manure showed much tendency of reducing the release of the PTE, although leachates from this treatment were collected for just 10 days for reasons provided earlier. There was a comparative higher release of As, Cu, Mn and Fe when the soil was mixed with 2% chicken manure + 5% growmore fertilisers and this was expected considering the fact that higher levels of these elements were found in this mixture of the materials. Further, Fe and Mn release is favoured by reducing condition e.g via waterlogging and could also lead to release of other elements such as As and Cu.

Relationships amonst elements leached from the treated soils revealed some interesting trends where As, Cr, Fe, Mn and Ni were not released until about the 5th day after their initial release that relatively higher amounts were seen coming out of the columns, which was attributed to their association with soil minerals. Iron as can be seen in Figure 5.9 was held most tightly compared to Cd, Pb, U and Zn which were flushed out much easily and faster, as they are particularly of anthropogenic sources. Generally the levels of PTE released in the leachates of the amended soils exceeded the amounts measured in the control soil leachate, further confirming these materials can increase mobilities of PTE in urban soil systems.

The result of the BCR sequential extraction performed on the amended soils before and after leaching showed evidence of transformation of PTE in the leached soil. Very prominently the levels of Cd, Cr, Fe, Pb and Zn were seen to decrease in the exchangeable fractions of growmore amended soil at the end of leaching. Growmore was earlier shown to release most of the PTE during the leaching process. Similar reduction of Cd concentration was observed in chicken manure + growmore amended soil. This indicated that the deionised water did leach some of the exchangeable metals in the soil (Table 5.6). Other significant outcome of this study was strong evidence for mobilisation of Mn from the reducible fraction of the same growmore amended soil to the exchangeable form, which was attributed significantly to possible development of reducing conditions in the column at some point which favours dissolution of Mn (hydr) oxides and release of Mn to the mobile form. Copper was mobilised from both the reducible and oxidisable fraction to the exchangeable but in the chicken manure amended soil after leaching.

6 The accumulation and uptake of PTE by vegetable plants grown in fertiliser amended soil.

6.1 Introduction

As discussed in detail in chapter 1, the application of fertilisers to soil can lead to accumulation of PTE and may result in the risk of their uptake by vegetable plants. This has remained a source of great concern to food quality, animal and human health. The increasing cognizance of the benefit of vegetables in the human diet warrants a continuous assessment of uptake of PTE by vegetable crops.[183]

Potentially toxic element accumulation and uptake by different vegetable plants grown on fertiliser amended soils have been reported. Zhou *et al*.[117] studied the effect of livestock and poultry manures on Cu and Zn uptake by radish and pakchoi using a pot experiment. They reported that Cu and Zn levels in both plant tissues increased when the manures were added. Similarly Muchuweti *et al*.[184] reported excessive levels of Cd, Cu, Pb and Zn in crops – maize, beans, peppers and sugarcane – which were grown in biosolid amended soils. In their recent study on the effect of biosolid on PTE uptake of five different vegetable plants, Sridhar *et al*.[185] reported that the concentrations of all the PTE increased both in the soil and plant parts as the level of the material added increased. In their efforts to understanding the effect of fertilisers on accumulation and uptake of PTE and other essential elements by bean and maize plants, Ina *et al*.[186] applied two organic based fertilisers (biochar and processed poultry manure) to a calcareous soil. They reported that both the processed poultry manure and biochar increased the levels of Cu, Fe, Mn, and Zn in the bean plants. Loland and Singh[187] applied different fertilisers including farmyard manure (FYM) to contaminated orchard soils in order to investigate extractability and uptake of Cu by beans and maize crops in the amended soils. They reported that banana compost significantly reduced Cu levels in both plants, while FYM decreased the level of Cu in the bean plants only, as a result of their different effects on soil pH. Sato *et al.*[188] assessed bioavailability of Cd to spinach in the presence of three different animal-based composts – derived from cattle, poultry and swine. They reported a significant reduction (34 to 38%) in the level of Cd in the vegetable compared to the control plant

sample. These findings have further confirmed the "double-edged" effect (as explained in section 1.3.2, in Chapter 1) that fertilisers can exhibit on PTE availability in soils and uptake by plants – under different conditions they may inhibit or enhance PTE uptake.

Bauddh and Singh[189] carried out a study on the effects of inorganic and organic fertilisers on uptake and growth of castor bean and Indian mustard in a pot experiment. They reported that the addition of inorganic fertilisers (urea and diammonium phosphate) enhanced the metal uptake, whilst the organic material (vermicompost) reduced the bioaccumulation of Cd by the Indian mustard when compared with the control sample. Singh and Agrawal[190] also applied different fertilisers which include (FYM), NPK and FYM + N to assess the uptake of Cd, Cu, Cr, Mn, Ni, Pb and Zn by radish plants (*Raphanus sativus* L.). They reported that the FYM and FYM + N reduced the uptake of all the PTE by the plant. However a corresponding increase in the concentrations of the elements in the plant grown in the NPK amended soil was reported. Previous works on the influence of fertilisers on uptake of PTE in soil by vegetable plants have mostly focused on the use of polluted agricultural and contaminated soils. Information on the effect of these materials on PTE uptake by plants grown in soils or soils not likely to be highly contaminated, nor already heavily amended with fertilisers, are lacking.

6.2 Experimental

The experimental work in this chapter is divided into two parts:

i) a pot experiment to investigate the effect of chicken manure, growmore and a mixture of both fertilisers on accumulation and uptake of PTE by runner beans (*Phaseolus coccineus*) planted during Summer 2015.

ii) a pot experiment to study the effect of the same fertilisers on accumulation and uptake of PTE by radish (*Raphanus sativa*) planted during Autumn 2015.

6.2.1 Pot experiment 1

Part of the soil collected for use in column studies (Chapter 5) in the second batch of sampling from Well Park, Greenock, UK was kept and used for this experiment.

Runner beans (*Phaseolus coccineus*) was used in this study based on the recommendation by a professional hulticulturist who happened to be a staff at the University of Strathclyde on the account that it is a common leguminous crop that can grow conveniently indoors, especially in Summer.

6.2.2 Plant, growth conditions and amendments

The air-dried soil samples were amended in the laboratory with 2% chicken manure, 5% growmore or a mixture (2% chicken manure + 5% growmore) of the two amendments. The fertilisers were thoroughly mixed with the soil samples in four replicates in plastic containers. After that distilled water was added up to field moisture (27% *w/w*). Each of the treatments was then transferred into four individual plastic flower pots (9.5 cm × 7.0 cm). Control sample was also prepared in four replicates with no added fertilisers. The pots containing each of the mixture were placed in the laboratory to equilibrate for 24 hours before planting.

One seed of the plant was placed in each pot containing the soil mixture and a saucer was placed under to avoid leaching of analyte. Any time this did occur, the saucer was thoroughly washed and the solution poured back into the pot. The plants were grown for 12 weeks indoors, in a laboratory, at ambient room temperature and lighting conditions (approximately 15-h day and 9-h night period, with day temperature of 22 °C and night temperature of 18 °C). The crops were watered once every day with a minimum of 15 mL of distilled water to keep the soil continuously moist but avoid as much as possible, leaching of solution from the bottom of the pot. All the runner bean seedlings sown in the 2% chicken manure amended soils germinated well and grew steadily to maturity. However, all the seeds sown in the 5% growmore amended soil as well as two of the control seedlings, did not germinate. As a result, the duplicate plants in the control soils (Figure 6.1A) and the four replicate plants in 2% chicken manure amended soil (Figure 6.1B) only are considered in this experiment.

A B

Figure 6.1 (A) Runner bean sample grown in control soil (B) Runner beans in 2% chicken manure amended soil after 12 weeks growth.

The plants were harvested 12 weeks after sowing, washed with tap water and rinsed thoroughly with deionised water. The soil which adhered to the roots was washed away carefully in order not to damage the root system. The leaves, stems and roots were together weighed in order to obtain fresh weight (FW), and then oven dried at 70 °C[191] for 72 hrs[192, 193] and weighed again to obtain dry weight (DW) at that temperature. Soil samples were collected from each pot after the harvest, air dried, digested in *aqua regia* and extracted with 0.05 EDTA solution at pH 7 as described in section 3.2 and 3.3 respectively.

6.2.3 Extraction of PTE from the plant samples

The oven dried plant samples were ground into powdered form using a ceramic pestle and mortar. Approximately 0.5 g of sample in duplicate (in case of the control samples) and four replicates (in case of the samples in chicken manure amended soil) were weighed into high pressure digestion vessel and 10 mL concentrated HNO_3 "for trace element analysis" (supplied by Aldrich, Gillingham, UK) was added. The mixture was allowed to stand in a fume cupboard overnight. The digestion vessels containing the sample mixed with HNO_3 were then placed in the MARS Xpress™ microwave assisted digestion system and digested using a power of 400 W, ramp time

of 20 mins, holding time, 20 mins and temperature of 160 °C. At the end of the digestion, the vessels were allowed to cool, and the contents filtered into 100 mL volumetric standard flasks using Fisher Brand FB 59023 filter papers. The solution was made up to the mark with deionised water resulting in 10% HNO_3 solution. One milliliter of the solution was further diluted in a 10 mL volumetric flask to obtain a 1% HNO_3 solution which was suitable for introduction in the ICP-MS. Certified reference material (Strawberry Leaves, LGC7162) as well as procedural blanks were digested alongside the samples in a similar manner. Digests were transferred into 10 mL transport tubes and stored in a refrigerator prior to ICP-MS analysis.

6.3 Results and discussions

6.3.1 Biomass of beans plant affected by chicken manure application

Table 6.1 shows the biomass of beans plant in the 2% chicken manure amended (CMAS). The biomass of the plant in CMAS was higher than the mass obtained for the control plant sample – about 2.3 times higher (dry weight). This behaviour was expected as the chicken manure may have increased the organic matter and nutrient contents of the soil which are major soils component influencing healthy plants growth. Wong *et al.*[194] have reported the effect of manure compost on the growth of plant. They found that the manure treated soil sample resulted in higher dry weight of plant compared to the control, indicating better supply of nutrients in the manure amended soil. The moisture content of the plant in control and amended soil were relatively the same (Table 6.1).

Table 6.1 Biomass of beans planted in control and CMAS

	FW	DW	MC
Bean plant in control soil	22.0 g	2.86 g	87%
Bean plant in 2% CMAS	66.0 g	6.6 g	90%

CMAS = chicken manure amended soil; FW = fresh weight; DW = dry weight; MC = moisture content

6.3.2 Detection limits

The detection limits of the instrument (DLinst.) and the procedure (DLpro.) for the PTE measured in the plant material and EDTA extracts from soil samples as found using equations 3.8 and 3.10 respectively in section 3.9.1 are presented in Table 6.1.

Generally these values were lower than the concentrations obtained for the analytes in the samples and as expected.

Table 6.2 Instrument detection limits (µgL^{-1}) and procedural detection limits (µg kg^{-1} d.w) of PTE measured in the plant samples (digested in HNO_3) and EDTA extracts of soil.

	Plant HNO_3 extractable		EDTA extractable	
	DLinst.	DLpro.	DLinst.	DLpro.
As	0.00225	2.25	0.0179	17.9
Cd	0.000288	0.288	0.00335	3.35
Cr	0.00128	1.28	0.0210	21.0
Cu	0.0312	31.2	0.0364	36.4
Fe	0.700	700	0.294	29.4
Mn	0.000667	0.667	0.0674	67.4
Ni	0.00587	5.87	0.0363	36.3
Pb	0.000567	0.567	0.0156	15.6
U	0.0000384	0.0384	0.0189	18.9
Zn	0.0603	60.3	0.173	173

6.3.3 Pseudototal concentrations (mg kg^{-1}, dw) of PTE in the plant, control and amended soil, and EDTA extractable PTE in the amended soil after bean plant harvest.

This section discusses the PTE content of the plant and soil, EDTA extractable PTE in the soil when no amendments were added and in the amended soil samples after the plants were harvested. In addition, bean transfer factor (TF) for each of the elements from soil to plant was calculated and reported. This was calculated as follows:

$$TF = \frac{C_{plant}}{C_{soil}}$$

Where $C_{plant}\ and\ C_{soil}$ are the concentrations of PTE (mg kg^{-1}) in the plant and soil (Table 6.4) respectively on the basis of dry weight. Transfer factor is used to predict the capability, plants to take up PTE from soil. It is one important factor which is utilised in environmental studies to evaluate the movement of these elements through the food chain.

Quality control

Table 6.3 shows the results of the analysis of a certified plant reference material LGC7162 (Strawberry Leaves). The number of replicates used for certification of the reference material was not reported and the concentration of Cu was given as an indicative value. Good recovery was obtained for all the elements except Fe, Ni and Zn, which were relatively low.

Table 6.3 PTE content of a certified plant reference material LGC7162 (Strawberry Leaves)

	Obtained value (n = 2)	Certified value	% Recovery
As	0.293	0.280 ± 0.07	105
Cd	0.156	0.170 ± 0.04	92
Cr	2.09	2.15 ± 0.4	97
Cu	10.1	10.0	101
Fe	628	818 ± 48	77
Mn	166	171 ± 10	97
Ni	1.60	2.60 ± 0.7	62
Pb	1.80	1.80 ± 0.4	100
U	0.0800	NG	NA
Zn	15.6	24.0 ± 5	65

NG = not given, NA = not applicable

6.3.3.1 Pseudototal PTE concentration (mg kg^{-1}) in the bean plant

Table 6.4 shows the mean pseudototal concentrations of PTE in the bean plant grown in soil with no added chicken manure and in the amended soil. The control plant samples were analysed in duplicate therefore no RSD value was determined for the control plant samples. The RSD values referred to in this section are for the bean plants grown in the chicken manure. The individual concentrations are shown in Appendix L.

Table 6.4 Pseudototal concentrations (mg kg^{-1}) of PTE in soil and beans plant, and permissible level of somePTE (mg kg^{-1}) in vegetable plants

	Soil	Control bean plant (n =2)	Bean plant in CMAS (n = 4)	Permissible limits of PTE in vegetable plants		
				EU[195]	WHO/FAO[196]	IS[197]
As	14.2	2.00	1.69 ± 0.05	-	-	-
Cd	0.378	0.683	0.418 ± 0.01	0.200	0.200	1.50
Cr	32.5	2.98	2.57 ± 0.02	-	-	20.0
Cu	103	40.6	36.5 ± 1.0	-	40.0	30.0
Fe	30000	3670	1580 ± 10	-	-	-
Mn	502	223	186 ± 11	-	-	-
Ni	53.6	37.4	6.60 ± 0.2	-	-	1.50
Pb	337	109	52.7 ± 1.3	0.300	5.00	2.50
U	1.12	0.231	0.151 ± 0.02	-	-	-
Zn	212	197	115 ± 3	-	60	50.0

IS = Indian standard

The RSD measurements of PTE in the bean plant grown in 2% chicken manure amended soil were generally less than 7% (n =4), except for U whose value was greater than 11% (n = 4). The concentrations of As Cd and Mn in the plant grown in the amended soil were less than the concentrations obtained in the control plants respectively. The Arsenic values obtained in all the bean plant were generally within the typical range (0.02 to 7.00 mg kg^{-1})[198] found in terrestrial plants. The level of Cd found in the plant samples exceeded the limits given by the EU and FAO/WH (0.2 mg kg^{-1} each.) but was lower than the 1.50 mg kg^{-1} level set by the Indian authority. Zheljazkov and Warman[115] observed a reduction Mn concentration in peppermint plant grown in a municipal solid waste treated soil compared to the control soil. The transfer factors for As and Mn in the control (0.14 and 0.44) and amended soil grown bean plant (0.12 and 0.37) did not differ significantly, but Cd had its values reduced from 1.8 to 1.1 in the amended soil grown bean plant.

The concentrations obtained for Cr and Cu in the control bean plant were slightly greater than the levels measured in the plant grown in the chicken manure amended soil. Chromium concentration in the plants was far below the permissible limit of 20.0 mg kg^{-1} for Cr in plants published by the Indian authority. Kamari *et al.*[199] observed a corresponding decrease in the concentration of Cu in shoots of water spinach plant grown in chicken manure amended soil at three different dosages (0%, 1% or 3%) compared to the control plant samples. The concentration of copper in the bean plants was close to the maximum allowable limit for plants set by FAO/WHO but higher than the Indian standard (Table 6.4)

The results further showed that the concentrations of Fe, Ni, Pb, U and Zn measured in the control bean plant were much higher than in the beans plant grown in the chicken manure amended soil. Iron showed the highest concentration compared to the remaining PTE. Antonious *et al.*[183] have reported higher concentration of Ni in leaves of cabbage plants grown in chicken manure amended soil compared to levels in the plants grown in a control soil sample. Similarly, Kamari[199] reported that application of chicken manure resulted in decrease in the uptake of Pb and Zn in both shoots and roots of water spinach grown in the amended soil compared to the plant grown in the unamended soil.

The transfer factors of these elements decreased from the control bean plant to the bean plant grown in the amended soils as follows: Fe (0.12 to 0.05); Ni (0.70 to 0.1); Pb (0.32 to 0.16); U (0.21 to 0.14) and Zn (0.93 to 0.50). The concentrations of Ni, Pb and Zn exceeded the permissible limits set by all the relevant bodies as presented in Table 6.4.

6.3.3.2 Pseudototal PTE concentration (mg kg^{-1}) in control soil and chicken manure amended soil after the harvest.

Table 6.5 shows the results of PTE concentration in the control soil before the experiment, as well as the levels measured in the control soil and chicken manure amended soil after the experiment. The individual values are presented in Appendix M. In all the samples, the concentration of Fe was the highest, and this was expected. Manganese, Pb and Zn were the next elements found in high concentrations while Cu was present in a relatively higher concentration compared to the remainder of the other PTE measured (Table 6.5). The levels of PTE obtained in the control soil and chicken manure amended soil before and after harvest did not differ markedly due to the small amount of chicken manure added (Table 6.5). Much higher level of treatment could result in plant damage and retard healthy growth of the crop.

Table 6.5 Pseudototal concentration (mg kg^{-1}) PTE in the control soil before planting, and 2% chicken manure amended soil after harvest.

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Control soil before planting (n = 2)	14.2	0.378	32.5	103	30000	502	53.6	337	1.12	212
Control soil after harvest (n = 2)	14.6	0.446	32.5	108	29040	513	53.4	350	1.28	216
CMAS after harvest (n = 3)	14.3 ± 0.2	0.419 ± 0.03	35.1 ± 1.8	103 ± 4	29400 ± 1780	513 ± 22	53.4 ± 2.7	354 ± 9	1.28 ± 0.05	228 ± 5

6.3.3.3 Plant – available PTE

The EDTA extraction was performed on the chicken manure, the control soil before and after the experiment, and in the amended soil after the experiment, in order to determine the bean plant-available PTE and compare this with the results reported in section 6.3.2.1. Unfortunately, no data was obtained for amended soil before the experiment and the EDTA extractable PTE in the 2% chicken manure amended soil before planting was calculated theoretically as follows:

$$EDTA\ extractable\ PTE_{CMAS-B} = \frac{2}{100} \times EDTA\ extractable\ PTE\ in\ fertiliser + \frac{98}{100} \times EDTA\ extractable\ in\ the\ original\ soil$$

Where CMAS-B = chicken manure amended soil before planting.

Table 6.6 shows the EDTA extractable PTE in chicken manure, the soil, and the chicken manure amended soil samples, before and after the experiment. Appendix N contains the individual concentrations.

The EDTA extractable PTE measured in the chicken manure showed that Zn was the analyte with the highest (373 mg kg^{-1}) plant available content in the material. Iron and Mn were the second highest, with Cu showing a relatively higher content compared to the remaining PTE (Table 6.6). The plant available PTE measured in the chicken manure was less than the values obtained in the soil except for Mn, U and Zn whose plant available concentrations were higher than in the soil (Table 6.6). This may be attributed to the fact that the pseudototal content of Mn, U, and Zn in chicken manure were higher than in the soil whereas the pseudototal concentration of the remaining PTE were higher in soil than in the amendment. Comparison between the plants available PTE measured in the control soil and the calculated chicken manure amended soil before planting did not generally differ, probably as a result of the small amount of chicken manure applied to the soil (Tables 4.12 and 6.5).

Table 6.6 The EDTA extractable PTE (mg kg^{-1}) in chicken manure, and soil and chicken manure amended soil before and after planting

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Chicken manure (n = 3)	0.0296 ± 0.007	0.191 ± 0.002	0.554 ± 0.03	46.6 ± 1.4	291 ± 12	333 ± 12	2.33 ± 0.1	0.121 ± 0.01	0.308 ± 0.02	373 ± 4
Control soil before planting (n = 3)	0.592 ± 0.007	0.346 ± 0.007	0.797 ± 0.04	66.8 ± 0.7	1100 ± 16	102 ± 2	36.4 ± 0.7	286 ± 6	0.0259 ± 0.002	49.1 ± 1.5
Control soil after harvest (n =2)	0.294	0.132	0.436	29.3	652	42.6	31.1	137	0.0121	20.7
*CMAS before planting (n = 2)	0.581	0.343	0.792	66.4	1080	107	35.7	280	0.0315	55.6
CMAS after harvest (n = 4)	0.332 ± 0.03	0.191 ± 0.03	0.328 ± 0.03	33.7 ± 1.1	535 ± 39	74.1 ± 12.2	35.5 ± 1.1	149 ± 4	0.0123 ± 0.004	25.2 ± 1.8

*Not measured but calculated as shown in section 6.3.3.

6.3.3.4 Amount of plant available PTE loss in soil

Table 6.7 shows the amount of EDTA-extractable PTE lost by the control and amended soils at the end of the experiment. This was calculated by subtracting the EDTA extractable PTE in the control soil and in the chicken manure amended soil after the experiment, from the EDTA extractable PTE in the original soil and the chicken manure amended soil preceding the experiment respectively.

The EDTA-extractable As and Pb lost in the control soil was greater than the amount found in the chicken manure amended soil. Arsenic level in both soils was lower than the values obtained for Pb.

Similarly, the results revealed that the amount of Cr, Fe and U found in the chicken manure amended soil at the end of the experiment were higher compared to the control values. Iron gave the highest value for both soils, which was followed by Cr (Table 6.7). Uranium showed the lowest amount compared to the remainder of the PTE in both soils.

Further comparison of the EDTA-extractable PTE between the control soil and the amended soil showed that Cu and Zn were the least affected as the values obtained in the control and chicken manure amended soil did not generally show a wide difference compared to the other PTE discussed earlier.

Cadmium, Mn and Ni levels lost from the control soil were much much higher than the difference obtained in the chicken manure amended soil. In this category, and comparing with the rest of the PTE studied, Ni produced the largest difference, which was followed by Mn.

As can be seen, some of the EDTA-extractable PTE in both soils showed that there were losses from the soils; however it was unclear as to the possible route through which these losses had actually occurred. The idea was to compare the values obtained here (mg kg^{-1}) for each element with the concentration (mg kg^{-1}) of each PTE measured in the bean plant grown in the control and chicken manure amended soil to see whether they correlated or not. To achieve this, the amount of PTE taken up by the beans plant (Table 6.4) was further calculated on mass (mg) basis, as well as the amount of EDTA-extractable PTE lost from the soil (Table 6.7), since the weight of soil used (200 g), and plants weight (control plant = 2.86 g and plant grown on CMAS = 6.6 g) were different. Table 6.8 shows the result of the amount of PTE taken up by

plant and the amount of EDTA-extractable PTE presumably lost from the soil after harvest. Comparison of these result revealed that, the amount of EDTA-extractable PTE released from the soil was much more than the amount of PTE taken up by the plants, except for Ni where the amount lost from soil (1.08 mg) was similar to the amount taken up (1.10 mg) in the control plant.

Table 6.7 Difference in amount of EDTA extractable PTE (mg kg^{-1}) in soil between start and end of experiment

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Control soil*	0.298	0.214	0.361	37.4	448	59.4	5.40	149	0.0138	28.4
CMAS*	0.249	0.152	0.464	32.7	549	32.9	0.219	131	0.0192	30.4

CMAS = chicken manure amended soil; * Calculated as described in section 6.3.3.3

Table 6.8 The amount of EDTA extractable PTE (mg) lost from soil and amount taken up (mg) by plant

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Amount lost from control soil	0.0596	0.0428	0.0722	7.48	89.6	11.8	1.08	29.8	0.00276	5.68
Amount taken up by plant control soil	0.00572	0.00195	0.00852	0.116	10.5	0.634	1.12	0.312	0.000661	0.563
Amount lost from CMAS	0.0498	0.0304	0.0928	6.54	110	6.58	0.0438	26.2	0.0038	6.08
Amount taken up by plant in CMAS	0.0112	0.00276	0.0170	0.241	10.4	1.23	0.0436	0.348	0.000997	0.759

CMAS = chicken manure amended soil

6.4 Conclusions

In this work, the accumulation and uptake of PTE in a bean plant grown in chicken manure, growmore or a mixture of the two fertilisers at different dosages [(2%, 0.2% or (2 + 0.2)%] were assessed.

The bean plant germinated and grew to maturity in the control and chicken manure amended soil samples only. Comparison between the control bean plant and bean plant grown in the amended soil showed that the biomass produced by the latter was higher than the former. The moisture content of both plant samples did not differ significantly.

The HNO_3 extraction of PTE from the plant samples revealed that the control bean plant contained higher levels of PTE than in the bean plant grown in the chicken manure amended soil. This indicated that the chicken manure may have reduced the availability of the PTE to the plants when mixed with the soil.

The pseudototal content of Cd, Pb and Zn in the bean plant samples exceeded most of the permissible levels set by some authorities. Arsenic and Cr were found to be within the typical levels in plants and FAO/WHO standard respectively. The transfer factor for As, Cr, Cu and in the control bean plant and in the amended soil did not vary markedly, suggesting that their availability to plant was similar in both soils. Cadmium, Fe, Mn, Ni, U and Zn, in the soils differ greatly compared to the other elements, reflecting different availability in both soils.

Comparing the amount of plant available PTE "lost" in the control and chicken manure amended soil showed that As and Pb differences in the control soil were greater than in the amended soil. Similarly, higher levels of Cr, Fe, and U were lost from the amended soils compared to the control soil. Only slight differences were observed with Cu and Zn as they were the least affected. Cadmium, Mn and Ni showed a trend where much much higher amounts of the elements were lost from the control soil than in the chicken manure amended soil. Comparison between the fraction of EDTA-extractable PTE "lost'' from the soil (mg) and the amount of PTE taken up (mg) by the plants showed that PTE levels in the plant significantly exceeded the amount released from the control and amended soil. Although low dosage of chicken manure was used, chicken manure as an organic fertiliser has shown that it could reduce PTE uptake in a bean plant.

6.5 Experiment 2

As explained earlier in experiment 1, the beans plant did not germinate, in the 5% growmore and the (2% chicken manure + 5% growmore) treatments. It therefore became necessary to modify experiment 1 based on this outcome. To achieve this objective, radish (*Raphanus sativus*) variety was used in experiment 2. In addition, the 5% dosage for growmore was reduced to 0.2%. Radish was selected because it is one of the common vegetable plants that could grow conveniently indoors, especially in autumn. It is widely consumed by many citizens in the UK and has been used previously in plant uptake studies.[191, 200]

Additional soil was collected from Well Park, Greenock, UK since the quantity left after performing experiment 1 was insufficient for use in experiment 2.

6.5.1 Soil sampling

Soil samples were collected from Well Park, Greenock in a similar manner as described in chapter 5. However, in this batch of sampling, a total of 5 kg of soil was collected and brought to the laboratory. The field moisture content was determined immediately before it was air-dried, ground and passed through a 2-mm sieve and stored in polyethene sample bags pending the pot experiment.

6.5.2 Plant growth experiment

The air-dried soil samples were amended in the laboratory with 2% chicken manure, 0.2% growmore or the mixture (2% chicken manure + 0.2% growmore). The fertiliser amendments were thoroughly mixed with the soil samples in four separate plastic containers. Distilled water was then added up to field moisture (30% *w/w*). Each of the treatments was then transferred into four individual plastic flower pots (9.5 cm × 7.0 cm). Control sample was also prepared in four replicates without any addition of the amendments. The pots containing each of the mixture were placed in the laboratory to equilibrate for 24 hours before planting.

Three seeds of the radish plant were placed in each pot containing the soil mixture with a saucer underneath to avoid leaching of analyte – when this occured, the saucer was washed and solution poured back into the pot. After germination, the seedlings

were thinned to one plant per pot. The plants were allowed to grow in the laboratory for 8 weeks under approximately 10-h day and 14-h night period, with day temperature of 20 ± 2 °C and night temperature of 16 ± 2 °C. The crops were watered once a day with a minimum of 20 mL of distilled water to keep the soil continuously moist. Figures 6.2A to D shows the radish plant after the 8 weeks.

Figure 6.2 A

Figure 6.2 B

Figure 6.2 C

Figure 6.2 D

Figure 6.2 A **Radish grown in control soil**

Figure 6.2 B **Radish grown in 2% chicken manure (CM) amended soil**

Figure 6.2 C **Radish grown in 0.2% growmore (GM) amended soil**

Figure 6.2 D **Radish grown in the mixed (CM + GM) amended soil**

The plants were harvested 8 weeks after sowing, washed with tap water and rinsed thoroughly with deionised water. The leaves and the various globes were weighed together in order to obtain fresh weight (FW), and then oven dried at 70 °C[191] for 72 hrs[192, 193] and weighed again to obtain dry weight (DW) at that temperature. Soil samples were collected from each pot, air dried and extracted with 0.05 EDTA solution at pH 7 to obtain plant-available PTE concentration and with *aqua regia* for pseudototal PTE determination as described in sections 3.2 and 3.3 respectively.

6.5.3 Extraction of PTE from the plant samples

The oven dried plant samples were ground into powdered form using a ceramic pestle and mortar. The individual replicates from the same treatment were then mixed together and approximately 0.4 g of sample in four replicates were weighed into high pressure digestion vessel and 10 mL concentrated HNO_3 for "trace element analysis"(supplied by Aldrich, Gillingham, UK), digested and analysed as described in section 6.2.3.

6.6 Results and discussions

6.6.1 The pH and organic matter content

Table 6.7 shows the pH values and organic matter content of the soil, and fertiliser amended soils after radish was harvested. The pH of the soil was acidic. The organic matter content of the soil was within the typical range (5 to 15%)[21] of organic matter content of most soils suitable for planting. The organic matter content of the soil as shown in Table 6.7 did not significantly change after harvest probably due to low percentage of the amendments added.

The pH of the soil increased slightly (Table 6.7) after application of chicken manure, whose pH was higher (7.70) compared to growmore (Table 4.11; Chapter 4) and higher than that of the soil. Slight decrease in soil pH was observed in growmore amended soil and this was expected due to the low pH value of growmore (3.90) as shown in Table 4.11 in chapter 4. Addition of chicken manure + growmore did not affect the pH of the soil since the increase is due to chicken manure and decrease due to growmore cancelled out.

Table 6.7 The pH and organic matter content of soil and amended soil after harvest

Before radish planting	Dosage (%)	pH	OM (%)
Soil	0	5.65	15.5
After harvest of radish			
Control	0	5.60	15.1
CMAS	2	5.90	15.0
GMAS	0.2	5.21	14.5
CM + GMAS	2 + 0.2	5.61	14.8

CMAS = 2% chicken manure amended soil; GMAS = 0.2% growmore amended soil; CM + GMAS = 2% chicken manure + 0.2% growmore; OM = organic matter

6.6.2 Pseudototal concentration of PTE in the soil

The pseudototal concentration of PTE in the soil used in this experiment is presented in Table 6.8. The RSD measurements of all the PTE in the soil were generally less than 11% except Cd whose RSD value was higher (15.3%) than the other elements. Iron presented the highest level in the soil. High concentrations of As, Cu, Mn, Ni, Pb and Zn were also obtained with the concentration of Cd being lowest. The pseudototal concentration measured in this batch of the soil did not differ significantly with the results obtained in chapter 5 (Tables 5.3 and 5.4) except for As, Cd, Pb and Zn where relatively higher values were obtained.

Table 6.8 Pseudototal concentration (mg kg^{-1}; n = 4) of Well Park soil, Greenock, UK (Second batch of sampling)

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Soil	24.6 ± 1.2	0.691 ± 0.1	27.6 ± 1.4	157 ± 3.2	32400 ± 585	529 ± 23	60.3 ± 1.6	520 ± 52	1.42 ± 0.1	304 ± 1.8
†SGVs	32	10	200	-	-	-	130	150	-	138

† UK CLEA guideline values

6.6.3 Biomass of radish plant affected by the treatment

Table 6.9 shows the biomass of radish plant in the control soil, 2% chicken manure amended (CMAS), 0.2% growmore amended soil (GMAS), and in the mixture of the two fertilisers (CM + GMAS). The total biomass obtained for radish plant grown in

the chicken manure amended soil presented the highest biomass both as fresh weight and dry weight compared to radish grown in the control and other fertilisers. This was also seen in experiment 1 with bean plants – the chicken manure may have increased the organic matter and nutrient content of the soil which is a major soil component for healthy plant growth. Wong *et al.*[194] have reported the effect of manure compost on the growth of plants. They found that the manure treated soil sample resulted in higher dry weight of plant compared to the control, indicating supply better supply of nutrients in the manure amended soil. Similarly, Singh *et al.*[190] applied FYM and other fertilisers, and reported that the FYM amended resulted in the highest yield for a radish (*Raphanus sativa* L) due to organic matter content. The addition of growmore and the mixture of the fertilisers resulted in slight decrease in the fresh and dry weights of the plant samples grown in them compared to the control plant. The moisture contents of all the plant samples were relatively the same (Table 6.9). All the harvested plants were fresh, healthy or free from any decay or mechanical disorder.

Table 6.9 Biomass of radish planted in the control and amended soil

	FW	DW	MC
Radish plant in control soil	7.63 ± 2.4	0.502 ± 0.03	93
Radish plant in 2% CMAS	12.6 ± 3.3	0.684 ± 0.2	94
Radish plant in 0.2% GMAS	7.03 ± 1.7	0.530 ± 0.1	93
Radish plant in CM-GMAS	7.61 ± 2.5	0.450 ± 0.1	94

CMAS = chicken manure amended soil, GMAS = growmore amended soil; FW = fresh weight; DW = dry weight; MC = moisture content

6.6.4 The PTE concentration (mg kg^{-1}, dw) in the radish plant

Quality control

Table 6.10 shows the results of the PTE content of a certified plant reference material LGC7162 (Strawberry Leaves) obtained during the analysis. The number of replicates samples used for the reference material was not reported and the concentration of Cu was given as an indicative value. Generally good recovery was obtained for all the elements, except for Cr, Fe, and Pb which were relatively low when compared with the other elements.

Table 6.10 PTE content of a certified plant reference material LGC7162 (Strawberry Leaves)

	Obtained value (n = 3)	Certified value	%Recovery
As	0.298 ± 0.008	0.280 ± 0.07	106
Cd	0.157 ± 0.005	0.170 ± 0.04	92
Cr	1.51 ± 0.009	2.15 ± 0.4	70
Cu	10.4 ± 0.09	10.0	104
Fe	567 ± 11	818 ± 48	69
Mn	168 ± 3	171 ± 10	98
Ni	2.40 ± 0.1	2.60 ± 0.7	92
Pb	1.58 ± 0.06	1.80 ± 0.4	88
U	0.0163 ± 0.002	NG	NA
Zn	24.7 ± 0.7	24.0 ± 5	103

NG = not given, NA = not applicable

Arsenic

The RSD of As measurement in the radish plant grown in all the treated and control soil samples were less than 8% (n = 4; Appendix O), indicating good precision during the analysis. Low concentration of As was obtained in all the plant samples. Addition of the various fertilisers resulted in a decrease in the level of As in the radish plants compared to the concentration obtained in the control radish plant (Figure 6.3). Statistical analysis (ANOVA; Appendix P) revealed that the differences were significant among the treatments for all the PTE in the radish plant samples. This effect was highest in the plant grown in the mixture of chicken manure and growmore amended soil (Figure 6.3). The level of As obtained in all the plant samples were within the typical range (0.02 to 7.00 mg kg^{-1})[198] found in terrestrial plants.

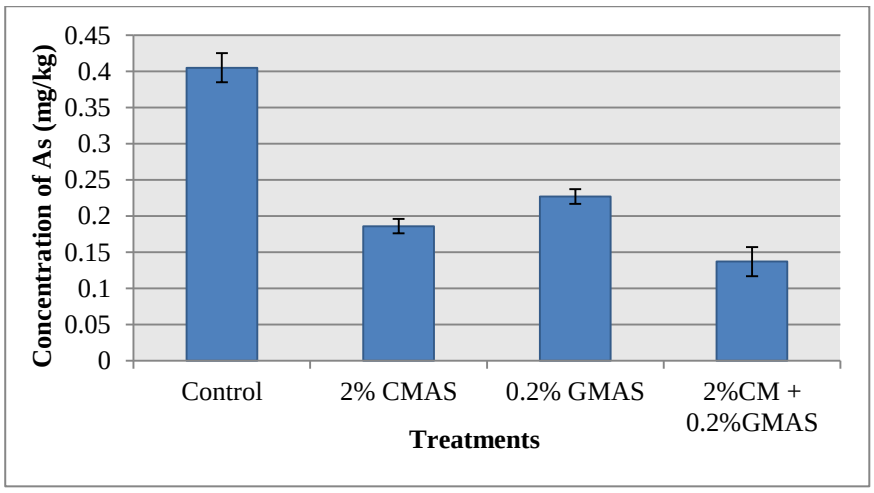


Figure 6.3 Concentration of As in radish grown in the control and fertiliser amended soils (n = 4)

Cadmium

The RSDs of Cd determination in the radish plant grown in all the fertiliser amended and control soils did not exceed 12% (n = 4). The concentration of Cd in radish plant grown in 0.2% growmore amended soil and control soil were similar within the experimental error, but higher than the concentrations obtained in radish plant grown in the chicken manure amended soil and the mixture of the two fertiliserss – two percent chicken manure and the mixture of the two materialss reduced the level of Cd in the plant samples. Chicken manure showed the highest effect, which may be attributed to addition of functional groups to the soil, capable of binding Cd through cation exchange or specific adsorption.[201] Most of the Cd in chicken manure were also found in the reducible fraction (Figure 4.21; Chapter 4), which is relatively less mobile. Growmore fractionation showed that most of the Cd present was in the exchangeable form which is highly mobile, and may result to more Cd being taken up by the plant. Statistically, the changes observed were significant ($P < 0.05$) for . The concentration of Cd measured in the various plant samples exceeded the EU and WHO/FAO permissible value (0.02 mg kg^{-1}) but lower than the Indian standard.

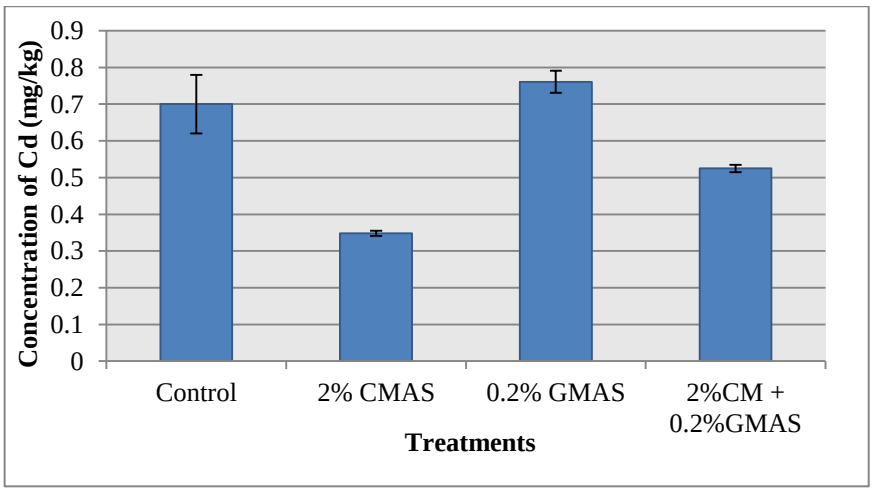


Figure 6.4 Concentration of Cd in radish grown in the control and fertiliser amended soils (n = 4)

Chromium

The RSD of Cr measurement in the radish plant grown in the control and the amended soil were all less than 9% (n = 4), except for the plant sample grown in the control soil which gave an RSD value of greater than 16% (n = 4). This was as a result of the higher signal obtained in one of the four replicates (Appendix O). The concentration of Cr in the radish plant after application of the fertilisers resulted in slight decrease in the 2% chicken manure amended soil grown plant, compared to the control plant, but much more in the 0.2% growmore and in the chicken manure + growmore amended soil grown radish plant. This trend was unexpected, as the concentration of Cr in growmore was about four times higher than in the chicken manure (Table 4.12). Similarly, the fractionation of growmore indicated that more Cr was found in the exchangeable fraction of that material compared to chicken manure. A lower level of Cr in the chicken manure amended soil grown radish plant might therefore have been expected. The differences were significant ($P < 0.05$). The concentration of Cr in the plants was far below the 20.0 mg kg^{-1} limit reported in the Indian standard.

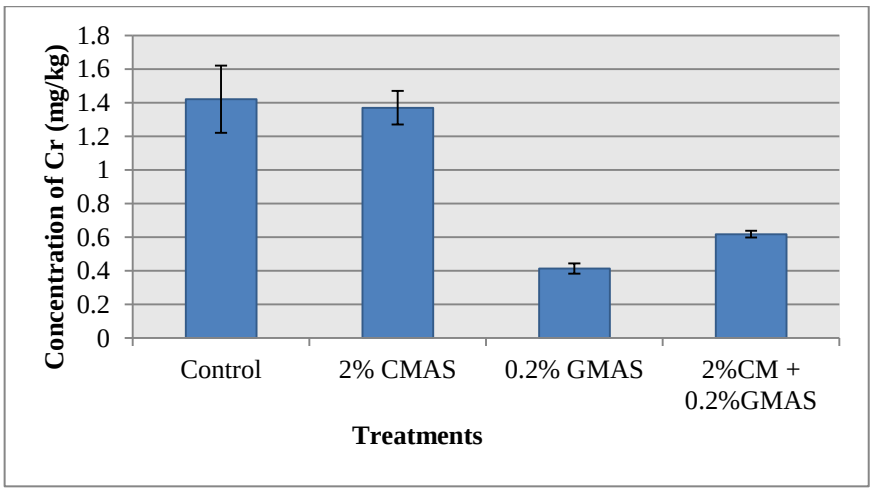


Figure 6.5 Concentration of Cr in radish grown in the control and fertiliser amended soils (n = 4)

Copper

The RSDs of Cu determination in all the radish plant grown in the control, 2% chicken manure, 0.2% growmore and the mixture of two fertilisers amended soils were less than or equal to 13% (n = 4). The concentration of Cu in the radish grown in the control soil was similar to the concentration obtained in the sample grown in growmore amended soil, within the experimental error. These values were greater than the concentration of Cu measured in the plant samples grown in the chicken manure and in chicken manure + growmore amended soil, suggesting that Cu may have been complexed by organic matter, making it difficult for its release to the plant.. Differences were also significant ($P < 0.05$) as revealed by the statistical analysis. Fractionation of growmore (Figure 4.22 in chapter 4) revealed that, more of the Cu was present in the exchangeable phase, which may have resulted in higher levels of Cu in the plant grown in the soil amended with this fertiliser. In all the plant samples, the concentration of Cu found was below the limits of 40.0 mg kg^{-1} and 30 mg kg^{-1} specified by WHO/FAO and Indian authority respectively for plants

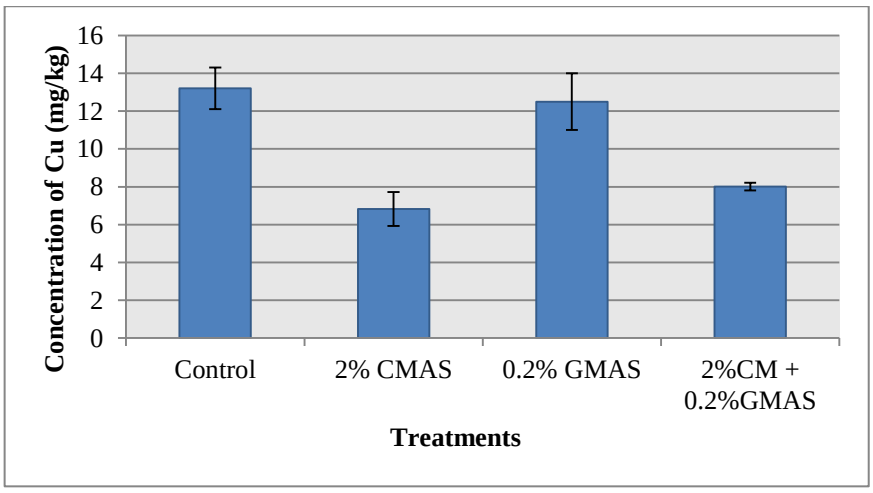


Figure 6.6 Concentration of Cu in radish grown in the control and fertiliser amended soils (n = 4)

Iron

The RSD of Fe measurement in all the radish plant grown in the various fertiliser amended soils, as well as in the control plant were less than 10% (n = 4). The application of fertiliserss to soil, and subsequent planting of radish resulted in a corresponding decrease in the level of Fe in the plant grown on chicken manure amended and chicken manure + growmore amended soils compared to the level found in the control radish plant to a similar extent. The level of Fe in the radish plant grown in growmore amended soil was similar to the concentration measured in control plant within the experimental error (Figure 6.7). These changes were significant ($p < 0.05$) as revealed by the statistical analysis.

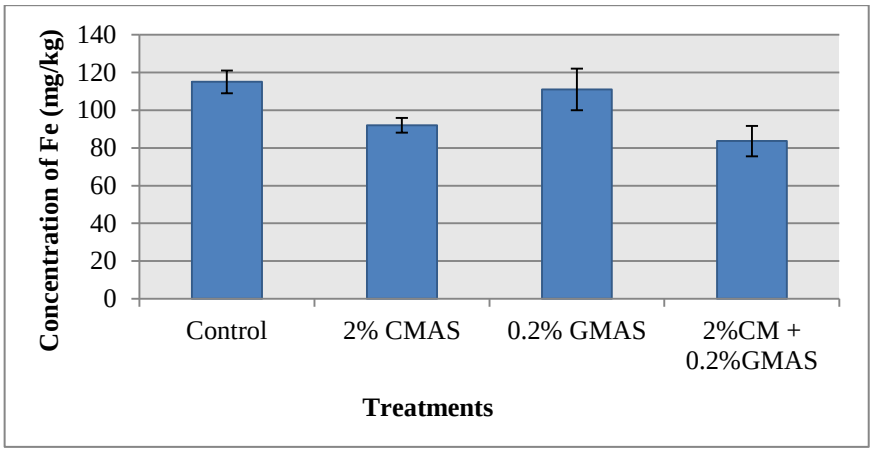


Figure 6.7 Concentration of Fe in radish grown in the control and fertiliser amended soils (n = 4)

Manganese

The RSD values of Mn determination in the radish plant grown in the fertiliser amended soils were less than 5% (n = 4). Generally the addition of the fertiliser materials resulted in a corresponding increase in the concentration of Mn taken up by the radish plant compared to the control plant sample. The difference between the concentration of Mn in the control plant, and radish grown in the chicken manure and chicken manure + growmore amended soil were small and relatively similar to one another. Growmore greatly enhanced the uptake of Mn in the radish plant compared to the other fertilisers that only slightly increased the level of Mn in the plant (Figure 6.8). The strong effect of growmore may be attributed to the fact that Mn was found mostly in the exchangeable phase in this fertiliser (Figure 4.26; chapter 4), known to be highly mobile thereby making Mn readily available for uptake by the plant. Statistical analysis revealed that the differences were significant ($P < 0.05$).

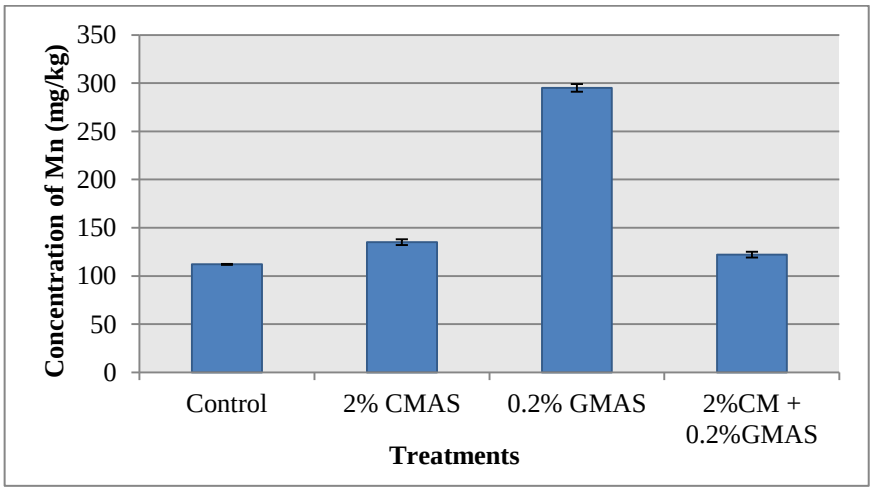


Figure 6.8 Concentration of Mn in radish grown in the control and fertiliser amended soils (n = 4)

Nickel

Good precision was obtained for Ni measurement in the radish plant as typified by low RSDs of less than 6% (n = 4). Statistical analysis revealed that significant differences occurred ($P < 0.05$) among the treatments for the radish plant grown in the amended soils. The addition of the fertilisers showed a small general decrease in the level of Ni taken up by the radish plant in all treatment compared to the control plant. However, chicken manure gave rise to the highest decrease in the concentration of Ni in the radish plant –. the fractionation of chicken manure showed that Ni was predominantly present in the oxidisable fraction (Figure 4.26; Chapter 4)

which is less mobile. Further, nickel concentration was generally low in chicken manure and it is possible that the Ni which was already in the soil gets bound to the chicken manure limiting its availability to the radish plant. The concentration of Ni found in all the radish plant exceeded the permissible limit of 1.5 set by the Indian authority.

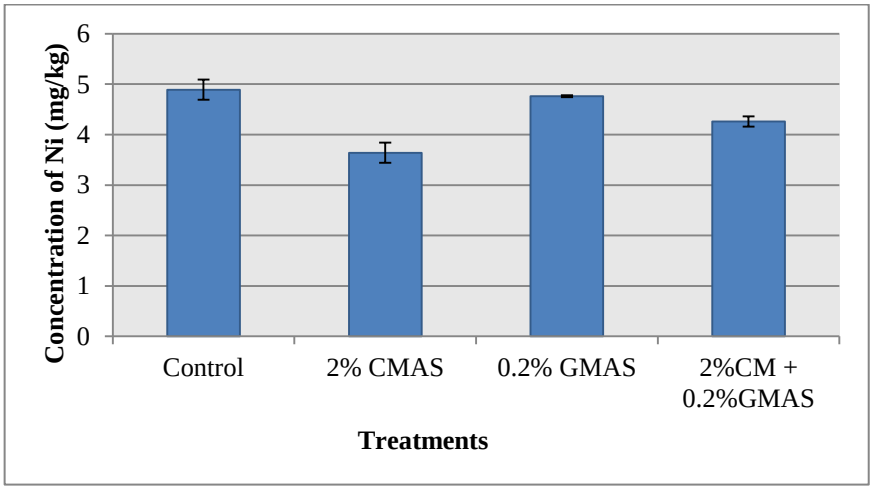


Figure 6.9 Concentration of Ni in radish grown in the control and fertiliser amended soils (n = 4)

Lead

The RSD of Pb measurement in the radish plant grown in the amended soils were equal or less than 8% (n = 4). There was a reduction in the level of Pb taken up by the radish plant grown in chicken manure, growmore and chicken manure + growmore amended soil when compared with the control radish plant. Statistical analysis also revealed significant differences existed among the treatments for Pb for all the radish plant grown in the amended soils compared with the control plant. The radish plant grown in chicken manure and chicken manure + growmore amended soil contained the lowest concentration of Pb – most of the Pb present in chicken manure was found in the oxidisable and residual fractions and these are relatively less mobile compared to the Pb fractions found in growmore (Figure 4.27; Chapter 4) making it more difficult for Pb to be transferred to the plant. The concentration of Pb obtained in all the plant samples was far above the EU permissible levels in vegetable plants. Radish grown in the control and growmore amended soils showed higher Pb values

than the WHO/FAO and Indian standard. The radish grown in chicken manure and the mixture of fertilisers amended soil had Pb contents lower than WHO/FAO values.

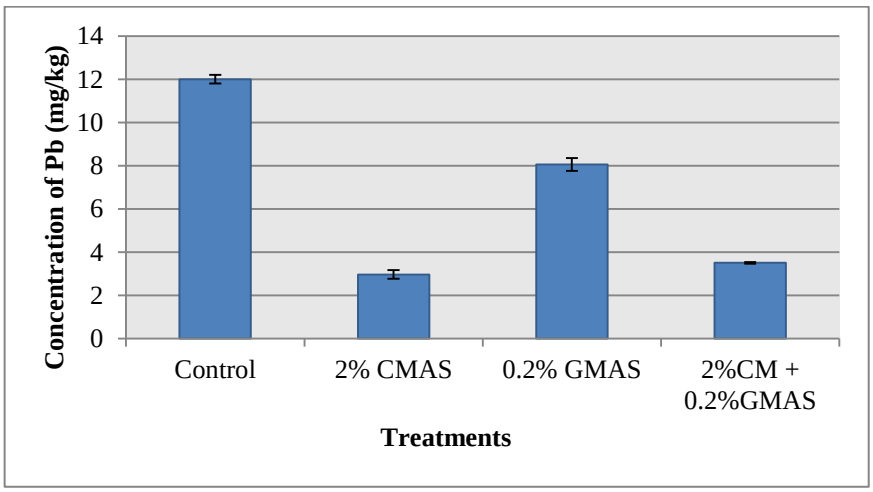


Figure 6.10 Concentration of Pb in radish grown in the control and fertiliser amended soils (n = 4)

Uranium

There was no measurable uptake of U by the radish plant and this analyte is not discussed further as the concentrations were below the LOD (0.00198 mg kg^{-1}). This result has allayed earlier fears that the high concentration of U, particularly in growmore, could be available for uptake by plants.

Zinc

The RSD of Zn measurement in the radish plants were good as shown by values of less than or equal to 8% (n = 4). Chicken manure reduced the concentration of Zn in the radish plan grown in the amended soil. Although chicken manure contained high level of Zn, some of the Zn were bound to the oxidisable fraction while relatively higher amount was in the reducible phase (Figure 4.29) which are relatively less mobile, only a small fraction was in the exchangeable, making Zn less available to the plant. However the addition of growmore and a mixture of chicken manure and growmore enhanced to some extent the uptake of Zn by the radish plant grown in the amended soil. Growmore fractionation indicated that Zn was mostly in the

exchangeable phase and this may have explained the increased level in the radish plant. The differences were significant ($P < 0.05$) as revealed by the statistical analysis. Levels of Zn in all the radish plant exceeded the limits of 60.0 mg kg^{-1} and 50.0 mg kg^{-1} set by WHO/FAO and Indian authority respectively.

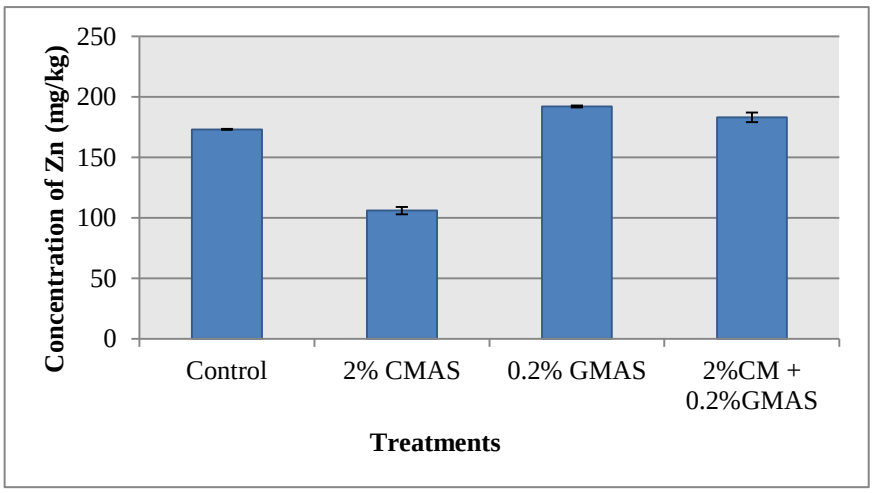


Figure 6.11 Concentration of Zn in radish grown in the control and fertiliser amended soils

6.6.5 Transfer factor from soil to radish plant

The transfer factor (TF) was determined for PTE transfer from soil to the radish plants. Table 6.11 shows the transfer factors for the PTE studied. As explained in experiment 1 in section 6.3.3, transfer factor is the measure of the potential of PTE from soil to plant. It is used to predict the ability of plants to take up PTE from soil. It is one important factor which is utilised in environmental studies to evaluate the movement of these elements through the food chain. The trend of the TF generally showed that addition of 0.2% growmore resulted in the higher TF values for As, Cd, Cr, Mn, and Zn, with Cd showing the overall highest TF value of 1.1. These values were comparable with the result obtained for the control soil. Two percent CMAS showed slightly lower TFs of PTE compared to the control soil and the 0.2% GMAS for most of the PTE. However, 2% CMAS + 0.2% GMAS showed generally the least TFs for PTE. It is well known that higher TF value indicates higher availability of PTE and possible accumulation, and toxicity to plant, and vice versa. Considering the overall effect of the fertilisers on the TFs, the result suggests that chicken manure which is organic in nature and/or a mixture of the two fertilisers are relatively better materials to be added to soil inorder to avoid excessive uptake of potentially toxic

elements such as As and Cd compared to growmore (inorganic) fertiliser even at lower levels of its (growmore) application.

Table 6.11 Transfer factor from soil to radish plant

Soil	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Control	0.016	1.0	0.051	0.084	0.0035	0.21	0.081	0.023	0.57
2%CMAS	0.00076	0.5	0.05	0.084	0.0028	0.26	0.060	0.0057	0.35
0.2%GMAS	0.092	1.1	0.45	0.080	0.0034	0.56	0.080	0.015	0.63
2%CM + 0.2%GM	0.0056	0.76	0.022	0.051	0.0026	0.23	0.071	0.0068	0.60

CMAS = chicken manure amended soil; GMAS = growmore amended soil; CM + GM = mixture of chicken manure and growmore amended soil

6.6.6 Pseudototal concentration of PTE (mg kg^{-1}) in control soil and fertiliser amended soils after the harvest

As stated in section 6.4.2, the amended soil was analysed after harvest to assess the level of PTE after possible uptake by the radish plant. Table 6.12 shows the results of the analysis and individual concentrations are shown in Appendix Q.

The RSD of all the PTE measurement in the various soil treatments were generally less than 12% (n = 4), except Pb in CM + GMAS where the RSD was found to be 21% (n = 4). This was expected, because one of the replicate measurements resulted to a higher signal compared to the others (appendix Q).

Iron as usual had the highest concentration in all the amended soil. Manganese, Pb and Zn were the next elements present in high concentrations when compared with the other PTE as shown in Table 6.12.

The PTE concentrations in the soil (Table 6.8) before planting the radish and the control soil after the experiment (6.12) were generally similar except for Cd, Cr and Ni where differences were seen. This was unexpected, as the concentrations of PTE in the latter should have decreased or remained unchanged relative to the former.

Comparing the levels of PTE in the amended soils and the control soil after harvesting the plants, the concentration of Cd significantly increased probably due to contamination but no significant changes were generally seen as was observed in

experiment 1 (Table 6.5) . This can again be attributed to low amount of these materials added to soil.

Table 6.12 Pseudototal concentration (mg kg^{-1}) of PTE in the control soil and fertiliser amended soils after harvest

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Control	23.2 ± 0.2	0.455 ± 0.04	41.9 ± 4.8	154 ± 16	30950 ± 843	555 ± 22	71.0 ± 3.0	528 ± 34	1.34 ± 0.1	303 ± 6
CMAS	22.3 ± 0.7	1.19 ± 0.05	35.2 ± 3.2	127 ± 8	26800 ± 1020	454 ± 20	58.7 ± 4.0	427 ± 34	1.50 ± 0.1	261 ± 22
GMAS	23.7 ± 1.2	0.436 ± 0.05	37.5 ± 2.1	142 ± 6	33300 ± 2050	543 ± 16	68.5 ± 2.0	488 ± 23	1.32 ± 0.01	303 ± 28
CM + GMAS	24.7 ± 2.4	0.394 ± 0.03	37.2 ± 2.0	141 ± 7	32200 ± 1470	542 ± 43	68.5 ± 3.0	521 ± 110	1.28 ± 0.04	304 ± 12

CMAS = 2% chicken manure amended soil; GMAS = 0.2% amended soil; CM + GMAS = 2% chicken manure + 0.2 growmore amended soil

6.6.7 The EDTA extractable PTE

The EDTA extraction was carried out on the control soils, the chicken manure and growmore fertilisers before the experiment, and on the amended soils after harvesting the radish plant, so that the amount of radish plant-available PTE could be estimated and compared with the result obtained earlier in section 6.6.2. The replicate values are shown in Appendix R. Unfortunately again, the data for all the amended soil preceding the experiment was not obtained. Therefore, the EDTA extractable PTE in the various fertiliser amended soils before planting was calculated according to the relationship shown in section 6.3.3.3 in experiment 1.

Table 6.13 shows the results obtained for EDTA extractable PTE in the soils, chicken manure, growmore, chicken manure + growmore (obtained by the summation of the amounts of the two fertilisers), and the amended soil samples before and after the experiment.

The EDTA-extractable PTE for the original control soil and control after the experiment did not show any major change, except for Cd and Mn whose amount in the latter were much lower than in the former. The chicken manure amendment was lower in EDTA-extractable As and Pb, and higher in Mn and Zinc, than the soil, the addition of 2% of the amendment was seen not to change markedly the concentration of the plant-available PTE. The levels obtained at harvest were therefore similar to, or slightly less than the calculated values at the beginning of the experiment.

Growmore showed the highest plant-available Fe and Mn, and Cu level was relatively higher in this fertiliser compared to the remainder of the PTE. As seen in the chicken manure amended soil, addition of 0.2% growmore did not change the EDTA-extractable such that levels at harvest were similar to, or slightly less than those (calculated) at the start.

It was observed for chicken manure + growmore that this fertiliser was lowest in plant-available Pb, and highest in Fe, Mn and Zn, and addition of 2% chicken manure + 0.2% growmore, resulted in no marked change (as seen in the other treatments) in the concentration of all the EDTA-extractable PTE (Table 6.13). The amounts obtained at the end of the experiment were therefore similar or close to those calculated at the start of the experiment except for Mn. Few other exceptions

where observed for plant-available Cu, Ni and Pb as their levels at the start of the experiment were less than the amounts measured at harvest.

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Table 6.13 The EDTA extractable PTE (mg kg^{-1}) in original soil (no plant), control soil, the amendments, and chicken manure amended soil, growmore amended soil and the mixture of the fertilisers amended soil after harvest of the radish

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Original soil before planting (n = 4)	0.518 ± 0.03	0.183 ± 0.02	0.350 ± 0.01	36.5 ± 1.7	591 ± 31	68.5 ± 6.4	2.14 ± 0.06	170 ± 8	32.5 ± 2.5
Control soil after harvest (n = 4,)	0.494 ± 0.04	0.151 ± 0.02	0.294 ± 0.04	34.9 ± 3.3	526 ± 63	30.5 ± 0.8	2.00 ± 0.2	167 ± 8	29.0 ± 2.4
Chicken manure	0.0296 ± 0.007	0.191 ± 0.002	0.554 ± 0.03	46.6 ± 1.4	291 ± 12	333 ± 12	2.33 ± 0.1	0.121 ± 0.01	373 ± 4
*CMAS before planting (n =4)	0.508	0.183	0.351	36.7	590	73.8	2.14	167	39.3
CMAS after harvest (n = 4)	0.498 ± 0.05	0.154 ± 0.02	0.329 ± 0.03	32.6 ± 2.9	556 ± 25	43.6 ± 17.7	2.00 ± 0.1	162 ± 8	29.3 ± 2.0
Growmore	2.59 ± 0.07	2.73 ± 0.09	2.19 ± 0.08	46.7 ± 2.1	282 ± 44	236 ± 14	7.57 ± 0.3	0.821 ± 0.1	323 ± 16
*GMAS before planting (n = 4)	0.560	0.234	0.384	36.7	590	71.9	2.25	167	38.3
GMAS after harvest (n =4)	0.503 ± 0.03	0.169 ± 0.03	0.301 ± 0.02	33.2 ± 2.9	492 ± 26	50.8 ± 12.7	1.99 ± 0.1	164 ± 11	32.7 ± 3.9
Chicken manure + growmore	2.62	2.92	2.74	93.3	573	569	9.90	0.942	696
*CMAS + GMAS before planting (n =4)	0.560	0.238	0.398	38.0	591	78.6	2.19	167	46.0
CMAS + GMAS after planting (n =4)	0.537 ± 0.03	0.194 ± 0.01	0.375 ± 0.06	40.8 ± 3.1	567 ± 56	27.9 ± 0.4	2.27 ± 0.09	174 ± 6	33.5 ± 6.0

CMAS = 2% chicken manure amended soil; GMAS = 0.2% amended soil; CM + GMAS = 2% chicken manure + 0.2 growmore amended soil

*Not measured but calculated as shown in section 6.3.3.3.

6.6.8 Fraction of EDTA extractable PTE loss in soil

As described in experiment 1, this fraction was calculated, by subtracting the EDTA extractable PTE in the control soil and in each of the three treatments after the experiment, from the EDTA extractable PTE in the original soil and each of the amended soil before the experiment respectively.

The amount of plant-available Cu, Ni, Pb and Zn lost in the chicken manure amended soil was higher than the amount from the control soil. The remaining PTE showed higher values in the control than in the chicken manure amended soil. Fe and Zn were the PTE with the highest differences compared to the remaining PTE. Similarly, the levels of available PTE lost in growmore amended soil exceeded the amount lost in the control soil, except for Mn (Table 6.14). With the exception of Cd, Mn and Zn, the control values were seen to be consistently higher than the levels estimated for chicken manure + growmore amended soil.

As mentioned in 6.3.3.4 in experiment 1, the losses estimated from the various treated soils were compared in (mg) with the HNO_3 extractable levels in plant (mg) since in this experiment too, the weight (300 g) of soil used were different from the weights of radish plants obtained in the various treatments as shown in Table 6.9.

Table 6.15 shows the comparison of the lost PTE in the various soils and the amount measured in the radish plant. The result showed that the amount of all the plant-available PTE lost in the control were much higher than the amount actually taken up by the control radish plant. This trend was also observed in all the treated soils as their EDTA-extractable PTE exceeded greatly the amount taken up by the radish plant grown in each of them, suggesting that EDTA extracted poorly the available PTE in the control soil, as well as the fertiliser amended soils.

Table 6.14 Difference in amount of EDTA-extractable PTE (mg kg^{-1}) in soil between start and end of experiment

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
control soil	0.0340	0.0330	0.0530	1.80	70.0	38.0	0.140	3.00	3.50
CMAS	0.0102	0.0292	0.0221	4.10	34.0	30.2	0.144	5.00	39.3
GMAS	0.0570	0.0650	0.0830	3.50	98.0	21.1	0.260	3.00	5.60
CM+GMAS	0.0230	0.0440	0.0230	- 2.80	24	50.7	- 0.0800	- 7.00	12.5

CMAS = 2% chicken manure amended soil; GMAS = 0.2% amended soil; CM + GMAS = 2% chicken manure + 0.2 growmore amended soil

Table 6.15 The amount of EDTA extractable PTE (mg) lost from soil and amount taken up (mg) by plant

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Amount lost from control soil	0.0102	0.00990	0.0159	0.540	21.0	11.4	0.0420	0.900	1.05
Amount taken up by radish in control	2.03×10^{-4}	3.54×10^{-4}	7.12×10^{-4}	6.63×10^{-3}	0.0577	0.0562	2.45×10^{-4}	6.02×10^{-3}	0.0869
Amount lost from CMAS	0.00306	0.00876	0.00663	1.23	10.2	9.06	0.0432	1.50	11.8
Amount taken up by radish in CMAS	1.27×10^{-4}	2.38×10^{-4}	9.37×10^{-4}	4.66×10^{-3}	0.0629	0.0923	2.49×10^{-3}	2.03×10^{-3}	0.0725
Amount lost from GMAS	0.0171	0.0195	0.0249	1.05	29.4	6.33	0.0780	0.900	1.68
Amount taken up by radish in GMAS	1.20×10^{-4}	4.03×10^{-4}	2.19×10^{-4}	6.63×10^{-3}	0.0588	0.156	2.52×10^{-3}	4.27×10^{-3}	0.102
Amount lost from CM + GMAS	0.00690	0.0132	0.00690	**	7.20	15.2	**	**	3.75
Amount taken up by radish in CM+GMAS	6.16×10^{-4}	2.36×10^{-4}	2.78×10^{-4}	3.60×10^{-3}	0.0376	0.549	1.92×10^{-3}	1.58	0.0824

CMAS = 2% chicken manure amended soil; GMAS = 0.2% amended soil; CM + GMAS = 2% chicken manure + 0.2 growmore amended soil

****** = not calculated because the difference < 0.00 (see Table 6.14)

6.7 Conclusions

The accumulation and uptake of a suite of PTE by a radish plant in an urban soil mixed with different commonly used fertilisers at various dosages (2% chicken manure, 0.2% growmore, and the mixture 2% chicken manure + 0.2% growmore) were assessed. Unlike in experiment 1 where no germination or growth was observed in some treatments in the case of bean plant, radish germinated and grew steadily to maturity and was free from any decay or mechanical disorder.

Radish grown in the chicken manure amended soil produced the highest biomass both as fresh weight and dry weight compared to the amount produced by the control radish plant. It generally appeared, there were no significant differences between the biomass produced by the other amended soil grown plant with respect to the control.

Generally there was decrease in the concentration of PTE in the radish plant on addition of fertilisers. This effect was predominant with the plant samples grown in the chicken manure and chicken manure + growmore amended soils – the effect of these two amendments on PTE appeared to be the same except, for Cd, Cr and Zn where differences existed compared to each other. Radish plant grown in 0.2% growmore amended soil consistently showed higher accumulation of PTE than in the other treatments, probably due to increased mobility of most of the PTE in the material – it was observed that growmore significantly ($P < 0.05$) increased the accumulation and uptake of Mn in radish compared to the control plant – a similar but lesser effect was observed with Zn. The pseudototal concentrations of Mn and Zn were the highest in this amendment (Table 4.12) which may be another reason for this behaviour.

The transfer factors were in agreement with the effect of these materials on PTE as lower values were generally obtained for soils treated with chicken manure and chicken manure + growmore, compared to the control, while higher TF were found for growmore amended soil. Generally, the PTE concentration in the control and amended soil before planting and after harvest appeared not to be significantly different from levels shown in Table 6.8 before planting due to the low amount of fertiliser added to the soil. A similar trend was observed when EDTA extraction was performed on the control soil and the amended soil before and after harvest.

The amount of plant-available Cu, Ni, Pb and Zn lost in the chicken manure amended soil was higher than the amount found in the control soil. The remaining PTE showed higher values in the control than in the chicken manure amended soil. Fe and Zn were the PTE with the highest differences compared to the remaining PTE. Similarly, the levels of available PTE lost in growmore amended soil exceeded the amount lost in the control soil, except for Mn (Table 6.14). With the exception of Cd, Mn and Zn, the control values were seen to be consistently higher than the levels estimated for chicken manure + growmore amended soil.

Comparing the EDTA-extractable PTE lost from the soil (mg) with the actual amount taken up (mg) by the radish plant, it was found that the amount of PTE taken up by the plant significantly exceeded the estimated loss from the various amended soils, as well as the control soil, suggesting that EDTA may have poorly estimated the plant available PTE in the amended soils used in this work. Further, EDTA therefore gave conservative estimate of availability for toxic metals.

7 Conclusions and further work

This thesis had provided new insight into the effects of fertilisers on the availability of potentially toxic elements (PTE) in soils, with particular emphasis on urban soils, which are rarely studied in this context but are becoming increasingly important as their use for food production increases.

In the early part of this study, a terrestial organic fertiliser of animal origin (chicken manure); two commercial inorganic fertilisers (growmore and phostrogen); a terrestial inorganic fertiliser of geological origin (rockdust) and a marine organic fertiliser of plant origin (seaweed), were added to a commercial top soil to assess the effect of the fertilisers on levels and potential bioavailabilities of PTE. The pseudototal concentrations of PTE in the soil and fertilisers were generally low with respect to environmental guideline values, where available. The concentrations of Cu and Zn were highest in the chicken manure; rockdust (being a geologically-based fertiliser) contained the highest concentrations of Fe and Mn; whilst levels of As, Cd and U were highest in growmore.

A preliminary experiment was conducted in which chicken manure was added to soil at various dosages and the mixtures allowed to stand for 4 weeks in the laboratory with addition of 5 mL water per day. Fertiliser gave rise to significant differences ($P < 0.05$) in PTE concentrations, except for Cd and Pb. Levels of Cd, Cu, Mn, U and Zn increased as the amount of manure added increased, which is expected because these element were present at higher concentrations in the fertiliser than in the soil, whereas the concentrations of Cr, Fe, Ni, and Pb decreased due to dilution, again as expected since these elements had lower concentrations in the fertiliser than the soil. The PTE concentrations measured at the end of the experiment and those calculated based on levels in the mixture components were similar, indicating no significant loses due to leaching had occurred. The experimental design was therefore considered robust.

A second experiment was conducted that involved treatment of the same commercially-available topsoil with chicken manure and additional fertiliser materials, individually, at three dosages, 0% (control), 1%, 3% and 5%). The mixtures were allowed to stand for 40 days in the laboratory with addition of 10 mL water per day. It

was found that chicken manure and growmore generally affected the concentrations of PTE more than the other fertilisers used, giving rise to increases in pseudototal concentration of Cu and Zn, but decreases in Cr, Fe and Ni, in the amended soil. The concentration of U increased with addition of growmore only. Seaweed dereased the levels of Fe and Mn in the amended soil, while the level of Pb was reduced by phostrogen.

The fractionation of PTE in the fertilisers, the control soil, and the 5% amended soils was determined using the BCR sequential extraction to determine whether changes in availability occured during the experiment. In the fertilisers themselves, Fe was chiefly associated with the residual fraction, the rest of the PTE were bound mostly in more the labile fractions that would be expected to show relatively high mobility. The fractionation patterns in the 5% amended soils were similar to those in the control soil for As, Cr, Fe, Mn, Ni and Pb, but differences were observed for Cu, U and Zn. Addition of, in particular, chicken manure increased levels of Cu in oxidisable and residual fractions; addition of growmore increased levels of U in the reducible fraction, and addition of either chicken manure or growmore increased levels of Zn in exchangeable and reducible fractions. Most significantly, the results suggested that chicken manure and growmore have the potential to alter the original distribution of some of the PTE in soil i.e. the fractionation patterns measured did not correspond to those predicted based on the fractionation patterns of the PTE in the mixture components.

In the next part of the work, column leaching experiments were performed in which soil from an urban park in West Central Scotland was amended with 2% chicken manure, 5% growmore, or a combination thereof, and leached for 21 days with deionised water. Leachates were analysed for pH, electrical conductivity and PTE content, and the BCR sequential extraction performed on the soil mixtures before and at the end of the experiment.

Chicken manure addition initially increased the pH of the leachates, whilst growmore decreased the pH – reflecting the high and low pH values of the fertilisers (7.8 and 3.9, respectively) – but the pH of all the leachates (including that from the control soil column) later converged towards the pH of the leachant used (deionised water at pH

6.5). High initial conductivity values were measured in the leachates of growmore and chicken manure + growmore amended soil columns but values reduced significantly towards the end of the leaching process. Chicken manure and control soil leachates gave lower EC values.

There were increased levels of PTE in leachates of the amended soils compared with the leachates obtained from the control soil, and larger total amounts of PTE were released.

Addition of 5% growmore resulted in higher release of most of the PTE studied (Cd, Cr, Ni, Pb, U, and Zn) compared with both the control soil and the 2% chicken manure amended soil). This is as expected since growmore contained higher pseudototal contents of these elements and is more acidic than chicken manure. The PTE concentrations in the leachate from the mixed fertiliser (2% chicken manure + 5 % growmore) were lower, based on the results for the individual fertiliserss (although comparison is limited because the column containing chicken manure amended soil became blocked after 10 days and leaching had to be suspended). Relationships amongst elements leached from the treated soils revealed some interesting trends. For example, As, Cr, Fe, Mn and Ni concentrations increased in leachates after about the 5^{th} day of leaching. The dissolution of Fe and Mn (hydr) oxide minerals is favoured by reducing condition e.g the onset of waterlogging, and this may have occurred, leading to release of associated elements. Cadmium, Pb and Zn were flushed out much easily and faster, as might be expected given that they are probably from anthropogenic sources. Overall, the experiment confirmed that fertiliser s can introduce labile PTE to soils that are easily mobilised, for example by percolating rainwater.

The result of the BCR sequential extraction showed evidence of redistribution of PTE in the soil during leaching. Very prominently, the levels of Cd, Cr, Fe, Pb and Zn decreased in the exchangeable fractions of growmore amended soil, which correlates with the higher concentrations of most of these PTE in column leachates, discussed above. A similar reduction of Cd concentration in the exchangeable fraction was observed in chicken manure + growmore amended soil. This indicated that metals recovered in the deionised water leachate had originated in the exchangeable fraction,

as would be expected. Another interesting observation was transformation of Mn from the reducible fraction of growmore amended soil to exchangeable forms. This was attributed to the possible development of reducing conditions in the column, which favoured dissolution of Mn (hydr) oxides and conversion of of Mn to more mobile forms. Copper was redistributed from both the reducible and oxidisable fractions to the exchangeable fraction in the chicken manure amended soil after leaching.

The final stage of the work involved plant uptake experiments. First, the accumulation and uptake of PTE in bean plants grown in 2% chicken manure, 5% growmore or 2% chicken manure + 5% growmore amended soils were studied. Unfortunately the bean seeds did not germinate in either the 5% growmore amended soil or the soil amended with both fertilisers. The bean plants germinated and grew to maturity in the control and chicken manure amended soil samples only.

The biomass of the plants grown in chicken manure amended soil exceeded that of plants grown in control soil, but the pseudototal concentrations of PTE in the plants grown in chicken manure amended soil were consistently lower than the concentrations found in the control bean plants. This suggests that the chicken manure may have reduced the availability of the PTE to the plants when mixed with the soil. The pseudototal content of Cd, Cu, Pb and Zn in the bean plants at harvest exceeded permissible levels set by some authorities.

The amounts of EDTA-extractable PTE were similar in the soil and the chicken manure, except for Zn where concentrations were almost an order of magnitude higher in the fertiliser. However, because the amount of fertiliser added was small (2 %) levels of EDTA-extractable PTE in amended and control soils were similar at the beginning of the plant growth period. By harvest, the amounts of EDTA-extractable PTE in the soils had generally decreased to about 50% of initial levels. A decrease would be expected if readily phytoavailable species were being taken up by the plants. However, it is important to note that the decrease in EDTA-extractable PTE during the growth period overestimated the amounts actually measured in the plants, indicating that EDTA gave a conservative estimate of availability for toxic species.

A further plant uptake experiment was conducted with the same soil but using a lower dosage of growmore and radish plants, which grow well indoors in Autumn. Germination and growth were successful in soil amended with 2% chicken manure, 0.2% growmore, or 2% chicken manure + 0.2% growmore. The biomass of the radish plants grown in all the amended soils appeared to be similar, except for the plant grown in chicken manure amended soil, which was greater. As with the beans, there was a reduction in the PTE content of plants grown in the chicken manure amended soil, compared with the plants grown in control soil (except for Mn). Radish plant grown in 0.2% growmore amended soil consistently showed higher accumulation of PTE than plants grown in the other amended soils, with levels similar to control plants. In particular it was observed that growmore significantly ($P < 0.05$) increased the accumulation and uptake of Mn in radish compared to the control plant – a similar but lesser effect was observed with Zn. The pseudototal concentrations of Mn and Zn were the highest in this amendment (Table 4.12) which may be a reason for this behaviour. Plants grown in the mixed amendment soils were similar in PTE content to those in chicken manure amended soil, providing further evidence that chicken manure can bind PTE in soil and reduce their availability to plants.

Higher soil-to-plant transfer factors were obtained for soils amended with growmore and lower values were obtained for the remainder of the soils. All of the soil mixtures contained similar levels of EDTA-extractable PTE at the beginning of the experiment, and levels had reduced by the time of harvest, although by different amounts for different analytes. The reduction in the amount of plant-available (EDTA-extractable) Cu, Pb and (especially) Zn over the course of the experiment was greater in the chicken manure amended soil than in the control soil. Similarly, the levels of available PTE "lost" from the growmore amended soil exceeded the amount lost from the control soil, except for Mn and Pb. When the total amounts of plant available PTE "lost" from the amended soil (in mg) were compared with the amounts taken up by the plant (in mg), the same outcome was observed as with the bean experiment – the amounts of PTE found in the plants were lower than the amounts of PTE released from the amended soils, confirming the conservative nature of EDTA in estimating availability of toxic species.

In this work, simple laboratory-based approaches were developed and applied to assess the effects of fertilisers commonly used in the UK on levels, distribution and mobility of PTE in urban soil. If further, similar, experiments were undertaken, some simple procedural modifications would be desirable e.g. to undertake the pot experiments in a greenhouse and to use a peristaltic pump for continuous automated delivery of the leachant to the soil columns. Unfortunately neither was available during the current study.

There is debate in literature over the preferred extractant to use in determining phytoavailability. Therefore, other single extraction procedures such as the use of $CaCl_2$, DTPA etc could be compared with EDTA so as to establish whether they display a similar degree of conservativeness with respect to estimating actual plant uptake. Other leachates could also be considered for use in the column leaching studies, for example, artificial rainwater.

It would be useful to compare results obtained under laboratory conditions with field trials. For example, information could be sought from allotment and urban garden owners/users on the actual dosages of manures or fertilisers they apply, in order to assess the levels and the effect of such materials on their soil and crops, and possible implications for human health. This could be linked to human bioaccessibility studies where approaches such as physiologically-based extractions were applied to urban soil samples in parallel to plant-availability studies, to investigate multiple routes for human exposure to PTE in soil.

More generally, there is a need to extend this type of study to additional soil and plant species, in order to gain a global understanding of the effects of fertiliserss on PTE bioavailability and plant uptake. Soils with different physicochemical characteristics and levels of PTE should be studied, including soils from different climatic regions. For example, Lagos, Nigeria is one of the fastest growing cities in the world and the combination of rapid industrialisation and population growth is putting huge pressure on the soil resource. A wider range of food plants need to be investigated, including those typically grown in urban garden for human consumption, not only in the UK but – once again – in different parts of the world.

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Appendices

Appendix A Mean, SD, RSD concentration (mg/kg, dw) of PTE in amendments and soil samples

	Rep.	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Soil	1	3.91	0.0821	45.3	11.3	21000	368	33.0	6.77	0.667	39.9
	2	4.00	0.0851	41.5	10.0	19200	343	30.5	6.30	0.617	34.3
	3	3.88	0.150	43.8	10.6	19400	334	30.1	6.22	0.605	37.1
	Mean	3.93	0.106	43.5	10.6	19900	348	31.2	6.43	0.630	37.1
	SD	0.06	0.04	1.9	0.7	969	18	1.6	0.3	0.03	2.8
	RSD	1.5	36	4.3	6.3	4.9	5.0	5.0	4.6	5.3	7.6
CM	1	0.432	0.268	4.74	97.6	1360	519	4.74	1.64	1.23	517
	2	0.502	0.290	5.37	92.4	1370	518	4.97	1.77	1.23	499
	3	0.470	0.281	4.60	88.3	1360	482	4.60	1.59	1.18	483
	Mean	0.468	0.280	4.90	92.8	1360	506	4.77	1.67	1.21	500
	SD	0.03	0.01	0.4	4.7	4.7	21.0	0.2	0.1	0.03	17
	RSD	7.4	3.9	8.4	5.1	0.3	4.1	3.8	5.7	2.3	3.4
GM	1	4.75	2.95	18.8	77.5	10100	438	13.1	3.13	27.4	413
	2	4.30	2.99	19.1	73.1	13600	455	13.7	3.45	27.2	399
	3	4.31	3.00	18.5	75.1	6510	425	11.9	2.41	25.9	400
	Mean	4.45	2.98	18.8	75.2	10100	439	12.9	3.00	26.9	404
	SD	0.3	0.03	0.30	2.2	3560	15.4	0.9	0.5	0.8	7.8
	RSD	5.8	0.9	1.6	2.9	35.0	3.5	7.3	17.8	3.0	1.9
PG	1	0.221	0.020	0.695	50.1	552	233	0.329	1.22	0.081	19.5
	2	0.215	0.017	0.722	45.3	594	240	0.304	1.16	0.025	19.0
	3	0.190	0.120	0.952	47.3	509	228	0.140	1.11	0.026	20.4
	Mean	0.209	0.05	0.790	47.5	552	234	0.258	1.16	0.044	19.6
	SD	0.02	0.06	0.1	2.4	42.6	5.9	0.1	0.1	0.03	0.7
	RSD	7.8	112	17.9	5.0	7.7	2.5	39.8	4.6	72.7	3.7

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed

Appendix A. Mean, SD, RSD concentration (mg/kg, dw) of PTE in amendments and soil samples continued …….

RD	1	1.37	0.0773	14.5	13.8	47798	756	10.7	3.63	1.35	72.8
	2	1.48	0.876	15.1	11.4	47900	756	10.7	3.44	1.31	75.0
	3	1.62	0.0539	14.6	10.4	47600	752	10.0	3.21	1.33	72.8
	Mean	1.49	0.07	14.7	11.9	47800	754	10.5	3.43	1.33	73.5
	SD	0.1	0.07	0.3	1.7	173	2.3	0.4	0.21	0.02	1.25
	RSD	8.3	100	2.3	14.5	0.4	0.3	3.7	6.11	1.46	1.70
SW	1	4.62	0.319	7.51	17.2	4750	108	4.98	1.04	0.601	105
	2	4.06	0.425	7.55	18.5	4760	114	4.68	1.38	0.666	109
	3	4.67	0.321	6.47	17.4	4300	106	4.66	1.067	0.579	99.7
	Mean	4.45	0.355	7.18	17.7	4600	109	4.774	1.16	0.615	104
	SD	0.3	0.1	0.6	0.7	271	4	0.12	0.19	0.045	4.4
	RSD	7.6	17.0	8.5	3.8	6	3.7	3.8	16.6	7.4	4.2

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed

Appendix B The F-test analysis for OS and W0

Cd	W0	OS		Cr	OS	W0		Cu	W0	OS
Mean	0.170	0.106		Mean	42.7	48.5		Mean	11.8	10.6
Variance	0.00676	0.00149		Variance	2.69	0.984		Variance	19.9	0.452
Observations	3	3		Observations	2	2		Observations	3	3
df	2	2		df	1	1		df	2	2
F	4.54			F	2.74			F	44.2	
P(F<=f) one-tail	0.180			P(F<=f) one-tail	0.346			P(F<=f) one-tail	0.0221	
F Critical one-tail	19			F Critical one-tail	161			F Critical one-tail	19	
Fe				Mn	OS	W0		Ni		
Mean	19878	21495		Variance	42.1	5.82		Mean	31.2	35.5
Variance	939784	291819		Observations	2	2		Variance	2.43	0.134
Observations	3	3		df	1	1		Observations	3	3
df	2	2		F	7.24			df	2	2
F	3.22			P(F<=f) one-tail	0.227			F	18.1	
P(F<=f) one-tail	0.237			F Critical one-tail	161			P(F<=f) one-tail	0.0523	
F Critical one-tail	19							F Critical one-tail	19	
Pb	W0	OS		U	W0	OS		Zn	OS	W0
Mean	11.4	6.43		Mean	0.739	0.630		Mean	37.1	40.3
Variance	51.3	0.0863		Variance	0.00437	0.00110		Variance	7.93	4.50
Observations	3	3		Observations	3	3		Observations	3	3
df	2	2		df	2	2		df	2	2
F	594			F	3.99			F	1.76	
P(F<=f) one-tail	0.00168			P(F<=f) one-tail	0.201			P(F<=f) one-tail	0.362	
F Critical one-tail	19			F Critical one-tail	19			F Critical one-tail	19	

Appendix B1 The t-test analysis for OS and W0

Cd	W0	OS
Mean	0.170	0.106
Variance	0.00676	0.00149
Observations	3	3
Pooled Variance	0.00413	
Hypoth. Mean Diff.	0	
df	4	
t Stat	1.22	
P(T<=t) one-tail	0.144	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.288	
t Critical two-tail	2.78	

Cr	OS	W0
Mean	43.5	48.9
Variance	3.57	1.04
Observations	3	3
Pooled Variance	2.30	
Hypoth. Mean Diff.	0	
df	4	
t Stat	4.31	
P(T<=t) one-tail	0.00627	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.0125	
t Critical two-tail	2.78	

Mn	OS	W0
Mean	348	399
Variance	307	5.82
Observations	3	2
Pooled Variance	207	
Hypoth. Mean Diff.	0	
df	3	
t Stat	3.88	
P(T<=t) one-tail	0.0152	
t Critical one-tail	2.35	
P(T<=t) two-tail	0.0304	
t Critical two-tail	3.18	

Cu	W0	OS
Mean	11.8	10.6
Variance	19.9	0.452
Observations	3	3
Hypoth. Mean Diff.	0	
df	2	
t Stat	0.459	
P(T<=t) one-tail	0.346	
t Critical one-tail	2.92	
P(T<=t) two-tail	0.692	
t Critical two-tail	4.30	

Fe	OS	W0
Mean	19878	21495
Variance	939784	291819
Observations	3	3
Pooled Variance	615801	
Hypoth. Mean Diff.	0	
df	4	
t Stat	2.52	
P(T<=t) one-tail	0.0326	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.0652	
t Critical two-tail	2.78	

Ni	OS	W0
Mean	31.2	35.5
Variance	2.43	0.134
Observations	3	3
Pooled Variance	1.28	
Hypoth. Mean Diff.	0	
df	4	
t Stat	4.68	
P(T<=t) one-tail	0.00473	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.00946	
t Critical two-tail	2.78	

Appendix B1 The t-test analysis for OS and W0 continued……

Pb	W0	OS
Mean	11.4	6.43
Variance	51.3	0.0863
Observations	3	3
Hypoth. Mean Diff.	0	
df	2	
t Stat	1.21	
P(T<=t) one-tail	0.175	
t Critical one-tail	2.92	
P(T<=t) two-tail	0.350	
t Critical two-tail	4.30	

U	W0	OS
Mean	0.739	0.630
Variance	0.00437	0.00110
Observations	3	3
Pooled Variance	0.00273	
Hypoth. Mean Diff.	0	
df	4	
t Stat	2.57	
P(T<=t) one-tail	0.0311	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.0623	
t Critical two-tail	2.78	

Zn	OS	W0
Mean	37.1	40.3
Variance	7.93	4.50
Observations	3	3
Pooled Variance	6.22	
Hypoth. Mean Diff.	0	
df	4	
t Stat	1.56	
P(T<=t) one-tail	0.0973	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.195	
t Critical two-tail	2.78	

Appendix C Mean, SD, RSD concentration (mg kg^{-1}) of PTE in chicken manure amended soil samples

Dosage	Rep.	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
W0	1	0.131	49.7	9.29	21900	398	35.8	7.34	0.698	40.2
	2	0.265	47.7	17.0	20900	397	35.1	19.7	0.816	42.4
	3	0.115	49.2	9.22	21700	401	35.7	7.27	0.705	38.2
	Mean	0.170	48.9	11.8	21500	399	35.5	7.31	0.739	40.3
	SD	0.0822	1.0	4.5	540	1.8	0.366	0.047	0.066	2.1
	% RSD	48.4	2.1	37.7	2.5	0.5	1.0	0.6	8.9	5.3
W1	1	0.117	49.5	15.9	21400	417	35.5	7.451	0.688	66.9
	2	0.115	48.0	13.4	21200	409	34.9	7.123	0.687	63.0
	3	0.0878	50.0	13.5	21800	417	36.1	7.231	0.671	63.6
	Mean	0.106	49.2	14.3	21500	414	35.5	7.268	0.682	64.5
	SD	0.0162	1.1	1.4	294	4.4	0.6	0.167	0.009	2.1
	% RSD	15.2	2.2	9.8	1.4	1.1	1.7	2.302	1.389	3.3
W2	1	0.189	45.5	17.6	20600	415	34.0	6.789	0.674	87.1
	2	0.0783	44.6	21.2	20000	380	33.7	6.922	0.679	90.9
	3	0.256	45.8	19.3	19900	394	34.0	6.934	0.690	89.0
	Mean	0.174	45.3	19.4	20100	396	33.9	6.881	0.681	89.0
	SD	0.0895	0.6	1.8	432	17	0.2	0.081	0.008	1.9
	% RSD	51.4	1.3	9.4	2.1	4.4	0.5	1.2	1.1	2.1

Where W0, W1, W2, W3 and W4 are treatment mixtures corresponding to 0, 2.5, 5 .0, 12.5 or 25 g of chicken manure + 50 g of soil.

Appendix C Mean, SD, RSD concentration (mg kg^{-1}) of PTE in chicken manure amended soil samples continued……

W3	1	0.127	41.7	26.3	18800	409	31.5	6.521	0.755	136
	2	0.346	43.9	29.4	18500	419	30.9	6.785	0.963	147
	3	0.186	40.4	32.4	18000	485	30.8	6.747	0.873	167
	Mean	0.220	42.0	29.4	18400	438	31.1	6.684	0.864	150
	SD	0.113	1.8	3.0	392	42	0.4	0.143	0.104	12.6
	% RSD	51.6	4.2	10.4	2.1	9.5	1.3	2.1	12.1	8.4
W4	1	0.238	34.9	49.1	15300	519	26.1	5.95	1.09	268
	2	0.222	32.0	68.8	13000	470	22.8	5.96	0.968	260
	3	0.354	36.6	48.0	15200	507	26.5	5.97	1.00	254
	Mean	0.271	34.5	55.3	14500	499	25.1	5.96	1.02	260
	SD	0.012	2.3	11.7	1310	25	2.0	0.01	0.06	7.2
	% RSD	4.3	6.7	21.2	9.0	5.1	8.0	0.2	5.9	2.8

Where W0, W1, W2, W3 and W4 are treatment mixtures corresponding to 0, 2.5, 5 .0, 12.5 or 25 g of chicken manure + 50 g of soil**.**

Appendix D Analysis of variance (ANOVA) for the chicken manure amended soil results

Anova: Single Factor			Cadmium			
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Wo	3	0.510157	0.170052	0.006762		
W1	3	0.319337	0.106446	0.000261		
W2	3	0.522477	0.174159	0.008012		
W3	3	0.658746	0.219582	0.012845		
W4	3	0.814212	0.271404	0.005173		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.045354	4	0.011338	1.715207	0.222689	3.47805
Within Groups	0.066105	10	0.006611			
Total	0.111459	14				

Anova: Single Factor			Chromium			
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Wo	3	146.6369	48.8789796	1.041381		
W1	3	147.4905	49.1635018	1.14651		
W2	3	135.8947	45.2982409	0.3375		
W3	3	126.0948	42.0315928	3.077818		
W4	3	103.4588	34.4862709	5.285569		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	439.5999	4	109.899969	50.46479	1.35E-06	3.47805
Within Groups	21.77756	10	2.17775555			
Total	461.3774	14				

Anova: Single Factor			Copper			
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Wo	3	35.50693	11.83564	19.94683		
W1	3	42.77021	14.25674	1.955052		
W2	3	58.05631	19.3521	3.283956		
W3	3	88.17658	29.39219	9.276516		
W4	3	165.9646	55.32153	137.3399		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3761.945	4	940.4864	27.3712	2.29E-05	3.47805
Within Groups	343.6044	10	34.36044			
Total	4105.55	14				

Anova: Single Factor			iron			
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
wo	3	64484.07	21494.69	291819		
w1	3	64393.38	21464.46	86610.19		
w2	3	60429.78	20143.26	186450.3		
w3	3	55285.07	18428.36	153906		
w4	3	43570.88	14523.63	1712428		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.01E+08	4	25309827	52.05185	1.17E-06	3.47805
Within Groups	4862426	10	486242.6			
Total	1.06E+08	14				

Anova: Single Factor			manganese			
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Wo	3	1195.628	398.542577	3.37924		
W1	3	1242.88	414.293333	18.95996		
W2	3	1189.371	396.45706	297.5277		
W3	3	1312.865	437.621575	1740.746		
W4	3	1495.917	498.638858	644.5029		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	21380.47	4	5345.11829	9.879646	0.001676	3.47805
Within Groups	5410.232	10	541.023233			
Total	26790.71	14				

Anova: Single Factor			nickel			
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Wo	3	106.5889	35.52962	0.134245		
W1	3	106.3854	35.46181	0.368325		
W2	3	101.7412	33.91372	0.028869		
W3	3	93.21318	31.07106	0.156104		
W4	3	75.37717	25.12572	4.066974		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	227.9413	4	56.98533	59.92757	5.97E-07	3.47805
Within Groups	9.509034	10	0.950903			
Total	237.4503	14				

Anova: Single Factor			lead			
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Wo	3	34.32236	11.44079	51.26283		
W1	3	21.80477	7.268256	0.028001		
W2	3	20.64407	6.881356	0.006495		
W3	3	20.05243	6.684143	0.020369		
W4	3	52.36458	17.45486	396.5175		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	257.4583	4	64.36458	0.718619	0.598328	3.47805
Within Groups	895.6704	10	89.56704			
Total	1153.129	14				

Anova: Single Factor			uranium			
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Wo	3	2.217966	0.739322	0.00437		
W1	3	2.047031	0.68234377	8.98E-05		
W2	3	2.043469	0.68115631	6.13E-05		
W3	3	2.591793	0.86393101	0.010871		
W4	3	3.057693	1.01923116	0.003581		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.251276	4	0.06281912	16.55498	0.000208	3.47805
Within Groups	0.037946	10	0.00379457			
Total	0.289222	14				

Anova: Single Factor			zinc			
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
Wo	3	120.7765	40.25884	4.500114		
W1	3	193.6287	64.5429	4.418715		
W2	3	491.0547	163.6849	16735.5		
W3	3	450.4661	150.1554	237.575		
W4	3	781.2243	260.4081	52.34543		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	92149.52	4	23037.38	6.762041	0.006663	3.47805
Within Groups	34068.68	10	3406.868			
Total	126218.2	14				

Appendix E1 Result of pH and organic matter content (%) of some soil and some of the fertiliser amendment (mean ± SD, n = 3)

pH						
	1	2	3	Mean	SD	RSD
Top soil	6.58	6.54	6.61	6.60	0.04	0.53
Chicken manure	7.69	7.67	7.68	7.70	0.01	0.13
Growmore	3.91	3.92	3.90	3.90	0.01	0.26
Phostrogen	4.06	4.09	4.10	4.10	0.02	0.51
Rockdust	9.95	9.93	9.91	9.90	0.02	0.20
Seaweed	5.19	5.22	5.21	5.20	0.02	0.29
Organic matter						
Top soil	11.8	12.4	13.3	12.1	0.43	3.57
Chicken manure	58.0	61.0		59.5	2.14	3.59
Rockdust	0.800	0.760	0.710	0.78	0.03	3.63
Seaweed	62.0	62.2	63.2	62.1	0.12	0.19

Appendix E2 Result of pH and Organic matter (OM) content (%) of fertiliser amended soil samples (Mean ± SD, n= 3)

pH							
Dose (%)		1	2	3	Mean	STD	RSD
0	CSO	6.65	6.64	6.72	6.67	0.04	0.65
1	CMAS	6.90	6.7	6.69	6.76	0.1	1.75
	GMAS	5.99	6.02	6.04	6.02	0.03	0.42
	PGAS	6.29	6.25	6.27	6.27	0.02	0.0032
	RDAS	6.76	6.76	6.74	6.75	0.01	0.17
	SWAS	6.67	6.78	6.63	6.69	0.08	1.2
3	CMAS	6.81	6.85	6.84	6.83	0.02	0.30
	GMAS	5.78	5.81	5.81	5.80	0.02	0.30
	PGAS	6.9	6.88	6.92	6.9	0.02	0.29
	RDAS	6.72	6.76	6.77	6.75	0.03	0.39
	SWAS	6.61	6.54	6.52	6.56	0.05	0.72
5	CMAS	6.75	6.94	6.98	6.89	0.1	1.78
	GMAS	5.65	5.67	5.68	5.67	0.02	0.27
	PGAS	6.88	6.95	6.91	6.91	0.04	0.51
	RDAS	6.66	6.79	6.75	6.73	0.07	0.99
	SWAS	6.43	6.37	6.39	6.40	0.03	0.48

CSO = control soil; CMAS = chicken manure amdnded soil; GMAS = growmore amended soil; PGAS = phostrogen amended soil; RDAS = rockdust amended soil; SWAS = seaweed amended soil

Appendix E2 Result of pH and Organic matter (OM) content (%) of fertiliser amended soil samples (Mean ± SD, n= 3) continued...

Organic matter content							
Dosage (%)	Sample	1	2	3	Mean	STD	RSD
0	CSO	16.2	12.8	15.8	14.9	1.9	12.4
1	CMAS	16.8	14.4	15	15.4	1.2	8.1
	GMAS	15	14.3	14.2	14.5	0.4	3.0
	PGAS	14.9	14.6	14.6	14.7	0.2	0.012
	RDAS	14.6	13.5	12.7	13.6	1.0	7.0
	SWAS	14.4	18.1	13.7	15.4	2.4	15.4
3	CMAS	16.8	14	16.5	15.8	1.5	9.8
	GMAS	13	14	15.8	14.3	1.4	9.95
	PGAS	14.7	16.4	14.3	15.1	1.1	7.4
	RDAS	12.8	13.3	15.2	13.8	1.3	9.20
	SWAS	15	17.1	17	16.4	1.2	7.24
5	CMAS	15.4	18.5	16.8	16.9	1.6	9.2
	GMAS	13.6	13.7	15.6	14.3	1.1	7.88
	PGAS	16.2	14.9	15.2	15.4	0.7	4.4
	RDAS	13.0	13.3	15.3	13.9	1.3	9.02
	SWAS	17.5	17.1	20.4	18.3	1.8	9.82

CSO = control soil; CMAS = chicken manure amdnded soil; GMAS = growmore amended soil; PGAS = phostrogen amended soil; RDAS = rockdust amended soil;SWAS = seaweed amended soil

Appendix F **Mean, SD and RSD of PTE concentration (mg kg^{-1}) in control soil (CSO) and soil treated with 1, 3 or 5% fertiliser amendment**

3% dosage		As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
CMAS	1	3.93	ND	32.4	9.88	20600	399	31.5	6.06	0.526	47.9
	2	3.90	ND	35.8	7.97	18500	326	27.5	5.67	0.458	39.7
	3	4.31	ND	36.7	9.3	18000	341	27.8	5.74	0.536	45.4
	Mean	4.05	NA	35	9.05	19000	355	29	5.82	0.507	44.3
	SD	0.2	NA	2.3	1.0	1400	39	2.2	0.2	0.04	4.2
	% RSD	5.8	NA	6.5	10.8	7.2	10.9	7.6	3.6	8.4	9.5
GMAS	1	4.00	0.139	34.8	9.45	16800	341	28.8	6.05	1.55	44.9
	2	4.48	0.125	31.2	9.07	16200	341	28.2	5.71	1.49	46
	3	5.27	0.141	28.7	10.7	16000	384	27.4	6.37	1.75	51.3
	Mean	4.58	0.135	31.6	9.75	16400	355	28.1	6.04	1.6	47.4
	SD	0.6	0.009	3.1	0.9	418	25.1	0.7	0.3	0.1	3.4
	% RSD	14.0	6.6	9.7	8.9	2.6	7.1	2.5	5.5	8.3	7.2
PGAS	1	4.51	NA	40.6	7.96	18800	355	28.3	5.69	0.486	32.9
	2	3.98	ND	34.3	8.06	18600	364	27.2	5.92	0.483	31.1
	3	4.21	ND	36.4	7.63	21900	354	29.3	5.89	0.508	30.9
	Mean	4.2	NA	37.1	7.88	19800	358	28.3	5.84	0.492	31.6
	SD	0.265	NA	3.2	0.2	1900	5	1.1	0.123	0.01	1.1
	% RSD	6.3	NA	8.7	2.9	9.4	1.5	3.7	2.1	2.8	3.6

CSO = control soil; CMAS = chicken manure amdnded soil; GMAS = growmore amended soil; PGAS = phostrogen amended soil; RDAS = rockdust amended soil; SWAS = seaweed amended soil; NA = not applicable; ND = not detected

Appendix F Mean, SD and RSD of PTE concentration (mg kg^{-1}) in control soil (CSO) and soil treated with 1, 3 or 5% fertiliser amendment continued.....

		As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
RDAS	1	LS	ND	32.9	6.49	22000	382	30.3	6.97	0.509	32.2
	2	4.41	ND	40.2	6.35	22000	371	28.9	5.63	0.52	32.6
	3	3.01	ND	26	6.13	20800	363	28.9	5.8	0.498	31.4
	Mean	3.71	NA	33.1	6.32	21600	372	29.4	6.13	0.509	32.1
	SD	NA	NA	7.1	0.2	709	9	0.8	0.7	0.01	0.6
	% RSD	NA	NA	21.5	2.9	3	2.5	2.8	11.9	2.2	2.0
SWAS	1	4.68	ND	31.6	6.63	17000	305	26.4	5.69	0.526	30.3
	2	4.00	ND	38	6.16	19500	311	28.2	5.56	0.454	31.8
	3	3.74	ND	29.2	6.89	20000	296	29.5	5.96	0.939	34
	Mean	4.14	NA	32.9	6.56	18900	304	28	5.74	0.64	32.1
	SD	0.488	NA	4.5	0.4	1500	7.7	1.6	0.2	0.3	1.9
	% RSD	11.8	NA	13.8	5.7	8.1	2.5	5.6	3.6	40.9	5.9

LS = Lost sample; CSO = control soil; CMAS = chicken manure amended soil; GMAS = growmore amended soil; PGAS = phostrogen amended soil; RDAS = rockdust amended soil; SWAS = seaweed amended soil; NA = not applicable; ND = not detected

Appendix F Mean, SD and RSD of PTE concentration (mg kg^{-1}) in control soil (CSO) and soil treated with 1, 3 or 5% fertiliser amendment continued.....

5% dosage		As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
CMAS	1	2.05	ND	21.1	10.6	18400	350	27.7	5.73	0.51	51
	2	5.37	ND	28.5	10.1	17900	343	26.9	5.22	0.46	49.7
	3	5.17	ND	38.9	12	19400	378	28.6	5.68	0.56	63
	Mean	4.20	NA	29.5	10.9	18600	357	27.8	5.54	0.51	54.6
	SD	1.86	NA	8.94	1	729	18	0.9	0.3	0.05	7.3
	% RSD	44.3	NA	30.3	9.4	3.9	5.1	3.1	5.1	9.1	13.4
GMAS	1	2.30	0.132	17.2	10.4	16300	334	26.2	5.07	2.14	49
	2	3.78	0.569	30.7	12.7	15300	389	29	6.17	2.49	59.6
	3	4.26	0.191	34.6	11.1	16100	377	27.4	5.28	2.32	56.7
	Mean	3.45	0.297	27.5	11.4	16000	367	27.5	5.51	2.32	55.1
	SD	1.0	0.2	9.1	1.2	544	29	1.4	0.6	0.2	5.5
	% RSD	29.7	79.8	33.2	10.4	3.4	7.9	5.1	10.5	7.7	10
PGAS	1	3.81	ND	34.5	9.22	19400	332	29	5.63	0.461	29.6
	2	4.55	ND	36.6	8.22	20000	400	29.1	5.7	0.44	30.2
	3	3.90	ND	33.1	7.73	18400	336	25.9	5.05	0.373	26.5
	Mean	4.09	NA	34.7	8.39	19200	356	28	5.46	0.425	28.8
	SD	0.405	NA	1.8	0.8	801	38	1.8	0.4	0.05	2
	% RSD	9.9	NA	5.1	9.1	4.2	10.7	6.5	6.6	10.8	6.9
RDAS	1	4.23	ND	32.7	6.33	22600	379	31.2	5.47	0.51	32.6
	2	3.99	ND	30.1	6.5	21400	383	27.5	5.32	0.534	31.4
	3	4.04	ND	30.3	6.3	22500	372	28	5.14	0.505	32.4

CSO = control soil; CMAS = chicken manure amdnded soil; GMAS = growmore amended soil; PGAS = phostrogen amended soil; RDAS = rockdust amended soil; SWAS = seaweed amended soil; NA = not applicable; ND = not detected

Appendix F Mean, SD and RSD of PTE concentration (mg kg^{-1}) in control soil (CSO) soil treated with 1, 3 or 5% fertiliser amendment continued.....

		As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
	Mean	4.08	NA	31	6.38	22200	378	28.9	5.31	0.516	32.2
	SD	0.1	NA	1.5	0.1	642	6	2	0.2	0.02	0.7
	% RSD	3.10	NA	4.7	1.7	2.9	1.5	7.0	3.1	3.1	2.0
SWAS	1	4.38	ND	34.6	6.87	18500	297	28.7	5.84	0.428	34.1
	2	3.78	ND	32.2	6.54	17700	285	29.2	5.19	0.436	33.9
	3	3.97	ND	31.2	6.16	19100	304	27.5	5.68	0.517	33.6
	Mean	4.04	NA	32.7	6.52	18500	295	28.5	5.57	0.46	33.9
	SD	0.3	NA	1.8	0.3	556	9.7	0.7	0.3	0.04	0.2
	% RSD	7.5	NA	5.4	4.4	3.0	3.3	2.4	6.1	8.7	0.7

CSO = control soil; CMAS = chicken manure amdnded soil; GMAS = growmore amended soil; PGAS = phostrogen amended soil; RDAS = rockdust amended soil; SWAS = seaweed amended soil; NA = not applicable; ND = not detected

Appendix G Mean concentration (mg kg^{-1}) of As and Cd in sequential extracts of control (0%) and 5% fertiliser amended soil samples

As	RESIDUAL FRACTION		OXIDIDISABLE FRACTION		REDUCIBLE FRACTION		EXCHANGEABLE FRACTION			PSEUDOTOTAL		
Dosage (%)	Mean	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	SUM	MEAN	%RSD	% RE
0	1.65	7	0.572	9.2	0.657	1.5	0.764	5.5	3.64	3.93	0.06	93
CM5	1.36	7.7	0.562	11.8	0.976	2.5	0.311	4.1	3.21	4.20	44.3	76
GM5	1.35	4	0.484	4.5	1.09	4.1	0.41	4.2	3.33	3.45	29.7	97
PG5	1.27	8.5	0.446	3	0.854	1.5	0.472	5.4	3.04	4.09	9.9	74
RD5	1.34	9.4	0.516	3.5	0.707	0.9	0.64	5.2	3.2	4.08	3.1	78
SW5	1.4	2.9	1.07	17.3	0.914	10.2	1.83	7	5.21	4.04	7.5	129

Cd	RESIDUAL FRACTION		OXIDIDISABLE FRACTION		REDUCIBLE FRACTION		EXCHANGEABLE FRACTION			PSEUDOTOTAL		
Dosage (%)	Mean	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	SUM	MEAN	%RSD	% REC.
0	ND	NA	ND	NA	0.0270	7.2	ND	NA	0.027	0.0102	67.0	265
CM5	ND	NA	ND	NA	0.0410	4	0.000805	75.2	0.0418	ND	NA	NA
GM5	ND	NA	ND	NA	0.1310	15.4	0.107	4.1	0.238	0.297	NA	80
PG5	ND	NA	ND	NA	0.0188	8.8	0.00437	14.1	0.0232	ND	NA	NA
RD5	ND	NA	ND	NA	0.0269	15.1	0.00281	67.1	0.0297	ND	NA	NA
SW5	ND	NA	ND	NA	0.0340	17	0.00519	34.1	0.0392	ND	NA	NA

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 0 = 0%, 5 = 5%; NA = not applicable; ND = not detected

Appendix G Mean concentration (mg kg^{-1}) of Cr and Cu in sequential extracts of control (0%) and 5% fertiliser amended soil samples continued.....

Cr	RESIDUAL FRACTION		OXIDIDISABLE FRACTION		REDUCIBLE FRACTION		EXCHANGEABLE FRACTION			PSEUDOTOTAL		
Dosage (%)	Mean	%RSD	Mean	%RSD	MEAN	%RSD	MEAN	%RSD	SUM	MEAN	%RSD	% REC.
0	19.1	6.7	4.19	5	0.202	NA	0	NA	23.5	35.7	7.6	66
CM5	17.2	11	3.9	8.2	0.189	7.4	0	NA	21.3	29.5	30.3	72
GM5	15.5	8.1	4	5.3	0.799	3.4	0	NA	20.3	27.5	33.2	74
PG5	15.2	11.7	3.41	4.7	0.162	1.3	0	NA	18.8	34.7	5.1	54
RD5	17	19.2	3.51	2	0.258	5.3	0	NA	20.8	31	4.7	67
SW5	17	6.8	4.27	12.6	0.208	5.5	0	NA	21.5	32.7	5.4	66

Cu	RESIDUAL FRACTION		OXIDIDISABLE FRACTION		REDUCIBLE FRACTION		EXCHANGEABLE FRACTION			PSEUDOTOTAL		
Dosage (%)	Mean	%RSD	Mean	%RSD	MEAN	%RSD	MEAN	%RSD	SUM	MEAN	%RSD	% REC.
0	5.79	19.2	2.22	1.6	0	NA	0	NA	8	6.93	5.5	115
CM5	9.93	43.3	4.99	9.5	0.310	7.0	0.0517	56.4	15.3	10.9	9.4	140
GM5	6.64	17.9	4.52	9	0.854	3.3	0	NA	11.2	11.4	10.4	98
PG5	7.18	50.9	3.41	9.2	0.334	3.0	0	NA	11.0	8.39	9.1	131
RD5	7.16	35.3	2.32	5.9	0	NA	0	NA	9.5	6.38	1.7	149
SW5	6.51	36.2	2.45	13	0	NA	0	NA	9.00	6.52	4.4	138

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 0 = 0%, 5 = 5%; NA = not applicable; NA = not applicable; ND = not detected

Appendix G Mean concentration (mg kg^{-1}) of Fe and Mn in sequential extracts of control (0%) and 5% fertiliser amended soil samples continued.....

Fe	RESIDUAL FRACTION		OXIDIDISABLE FRACTION		REDUCIBLE FRACTION		EXCHANGEABLE FRACTION			PSEUDOTOTAL		
Dosage (%)	Mean	%RSD	Mean	%RSD	MEAN	%RSD	MEAN	%RSD	SUM	Mean	%RSD	% REC.
0	12500	2.1	211	4.1	44.1	1.9	0.884	13.1	12800	20600	3.8	62
CM5	11800	1.4	192	11.1	30.7	3.3	1.35	12.8	12000	18600	3.9	65
GM5	11300	3.1	181	6.9	68.7	6.1	1.74	7.3	11600	16000	3.4	73
PG5	11100	7.5	166	5.5	39.7	3.7	6.6	5.2	11300	19200	4.2	59
RD5	12300	11.1	226	3.6	116	6.6	1.13	2.8	12600	22200	2.9	57
SW5	12000	5.1	210	14.9	44	5	4.09	15.1	12300	18500	3.0	66

Mn	RESIDUAL FRACTION		OXIDIDISABLE FRACTION		REDUCIBLE FRACTION		EXCHANGEABLE FRACTION			PSEUDOTOTAL		
Dosage (%)	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	SUM	MEAN	%RSD	% REC.
0	85.1	3.3	33.3	3.9	105	2.4	120	2.6	343	369	6.2	93
CM5	79.9	5.1	31.9	5.7	103	2.4	134	5.3	349	357	5.1	98
GM5	76.1	6.2	28.6	5.2	76.9	9.3	181	5.6	363	367	7.9	99
PG5	77.1	8.4	27.2	3.1	79.8	16.9	152	3.7	336	356	10.7	94
RD5	93.7	16	33.2	13	111	5.9	130	6.9	381	378	1.5	101
SW5	81	6.1	33.6	7	86.5	7.4	125	14.3	326	295	3.3	111

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 0 = 0%, 5 = 5%

Appendix G Mean concentration (mg kg^{-1}) of Ni and Pb in sequential extracts of control (0%) and 5% fertiliser amended soil samples continued.....

Ni	RESIDUAL FRACTION		OXIDIDISABLE FRACTION		REDUCIBLE FRACTION		EXCHANGEABLE FRACTION			PSEUDOTOTAL		
Dosage (%)	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	SUM	MEAN	%RSD	% REC.
0	18.4	1.8	7.88	3.9	1.46	3.1	0	NA	27.7	31	3.0	89
CM5	17.2	7.5	7.22	3.9	1.44	0.7	0	NA	25.9	27.8	3.1	93
GM5	16.3	9	6.81	5.6	1.99	4.7	0	NA	25	27.5	5.1	91
PG5	16.6	12.1	6.47	1.9	0.926	3.5	0	NA	24	28	6.5	86
RD5	17	11.5	6.7	11.1	1.63	5.9	0	NA	25.3	28.9	7.0	88
SW5	17.6	6.3	7.89	7	1.29	5.9	0	NA	26.8	28.5	2.4	94

Pb	RESIDUAL FRACTION		OXIDIDISABLE FRACTION		REDUCIBLE FRACTION		EXCHANGEABLE FRACTION			PSEUDOTOTAL		
Dosage (%)	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	SUM	MEAN	%RSD	% REC.
0	3.8	9.2	0.58	5.1	1.7	2.2	0	NA	6.08	6.25	11.6	97
CM5	3.72	11.2	0.615	6	1.48	2.2	0.00273	86.7	5.82	5.54	5.1	105
GM5	3.73	11.2	0.51	6.5	1.71	1.6	0	NA	5.95	5.51	10.5	108
PG5	3.51	13.8	0.523	3.8	1.73	4.8	0	NA	5.78	5.46	6.6	106
RD5	3.82	23	0.559	11.4	1.66	1.9	0	NA	6.02	5.31	3.1	113
SW5	3.63	9.7	0.57	7.7	1.57	2.5	0	NA	5.77	5.57	6.1	104

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 0 = 0%, 5 = 5%; NA = not applicable; ND = not detected

Appendix G Mean concentration (mg kg^{-1}) of U and Zn in sequential extracts of control (0%) and 5% fertiliser amended soil samples continued.....

U	RESIDUAL FRACTION		OXIDIDISABLE FRACTION		REDUCIBLE FRACTION		EXCHANGEABLE FRACTION			PSEUDOTOTAL		
Dosage (%)	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	SUM	MEAN	%RSD	% REC.
0	0.365	29.4	0.157	19.7	0.00318	19.3	0	NA	0.525	0.476	3.5	110
CM5	0.256	6.5	0.193	3	0.022	19.5	0	NA	0.471	0.51	9.1	92
GM5	0.293	8.9	1.42	5.3	0.337	12	0.0237	15.2	2.07	2.32	7.7	89
PG5	0.229	14.2	0.133	2	0	NA	0	NA	0.362	0.425	10.8	85
RD5	0.258	17.6	0.182	15	0.0207	24.1	0	NA	0.461	0.516	3.1	89
SW5	0.252	6.8	0.157	17.5	0.00436	9	0	NA	0.413	0.46	8.7	90

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 0 = 0%, 5 = 5%; NA = not applicable; ND = not detected

Zn	RESIDUAL FRACTION		OXIDIDISABLE FRACTION		REDUCIBLE FRACTION		EXCHANGEABLE FRACTION			PSEUDOTOTAL		
Dosage (%)	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	MEAN	%RSD	SUM	MEAN	%RSD	% REC.
0	19.5	6.9	5.79	5.8	2.16	11.8	0.488	9.2	27.9	30.8	2.4	91
CM5	19.6	20.6	6.16	9.1	23.4	15.7	12.4	11.2	61.6	54.6	13.4	113
GM5	16.6	9.1	5.29	8.6	14.1	13.1	17.5	4.2	53.5	55.1	10.0	97
PG5	16.5	12.9	4.58	4.1	2.43	9.7	1.12	4	24.6	28.8	6.9	85
RD5	18.3	15.3	5.58	15.9	3.47	8.9	0.489	11.5	27.8	32.2	2.0	86
SW5	18.2	3.7	6.12	11.9	5.05	11.9	2.83	10.8	32.2	33.9	0.7	95

CM = chicken manure; GM = growmore; PG = phostrogen; RD = rockdust; SW = seaweed; 0 = 0%, 5 = 5%

Appendix H **Mean concentration (mg kg^{-1}) of PTE in the various sites sampled for column leaching experiments**

Site	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Birkmyre Park 1	7.90	0.469	21.9	52.6	21100	458.6	30.8	232	0.913	199
Birkmyre Park 2	8.07	0.402	25.9	52.4	22400	443.4	34.0	232	0.966	202
Birkmyre Park 3	7.43	0.374	21.1	48.6	20100	415.4	29.8	224	0.845	177
Mean	7.80	0.415	23.0	51.2	21200	439.2	31.5	229	0.908	192
SD	0.3	0.04	2.1	1.9	942	17.9	1.8	4.0	0.05	11.2
RSD	3.4	9.6	9.1	3.6	4.4	4.1	5.6	1.7	5.4	5.8
Gourock Park 1	9.19	0.479	20.4	75.8	18500	667	25.5	198	1.08	149
Gourock Park 2	8.39	0.436	17.1	65.4	18300	604	23.7	170	0.943	133
Gourock Park 3	8.62	0.427	21.0	68.0	17600	606	25.9	176	1.03	138
Mean	8.73	0.447	19.5	69.7	18100	626	25.1	181	1.02	140
SD	0.3	0.02	1.8	4.4	386	29.2	1.0	12	0.06	6.5
RSD	3.8	5.1	9.0	6.3	2.1	4.7	3.8	6.6	5.6	4.7
Lady Octavia Park 1	7.46	0.364	26.3	48.4	20400	393	24.7	101	0.724	176
Lady Octavia Park 2	6.93	0.339	25.3	44.9	19300	336	23.3	104	0.699	164
Lady Octavia Park 3	7.09	0.338	23.6	46.4	19400	340	22.9	95.8	0.656	162
Mean	7.16	0.347	25.1	46.6	19700	356	23.6	100	0.693	167
SD	0.2	0.012	1.1	1.4	497	26.1	0.8	3.5	0.03	6.1
RSD	3.1	3.5	4.4	3.0	2.5	7.3	3.2	3.5	4.0	3.6
Lyle Park 1	8.8	0.157	22.3	44.3	24300	268	22.5	120	0.853	79.5
Lyle Park 2	10.4	0.219	24.3	46.2	26500	280	24.6	127	0.956	89.1
Lyle Park 3	9.04	0.158	25.0	44.8	25300	275	26.7	123	0.932	84.5
Mean	9.41	0.178	23.8	45.1	25400	274	24.6	123	0.914	84.4
SD	0.7	0.03	1.1	0.78	900	5.1	1.7	3.1	0.0441	3.9
RSD	7.4	16.4	4.8	1.72	3.5	1.8	6.9	2.5	4.8	4.7
Well Park 1	13.7	0.422	23.7	83.8	26000	470	38.2	332	1.10	199
Well Park 2	12.8	0.399	23.0	79.4	24000	484	34.6	303	0.987	199
Well Park 3	11.9	0.382	19.7	81.5	23300	416	31.5	284	0.961	181
Mean	12.8	0.401	22.1	81.6	24400	457	34.8	306	1.01	193
SD	0.7	0.02	1.7	1.8	1140	29	2.7	20	0.06	8.5
RSD	5.7	4.1	7.7	2.2	4.7	6.4	7.8	6.5	5.8	4.4

Appendix I Mean pH/EC values of leachates from control, chicken manure amended soil, growmore amended soil

and soil amended with chicken manure and growmore

Days	Contr pH	pH-CMAS	pH-GMAS	pH-CM + GMAS	Contr EC	EC-CMAS	EC-GMAS	EC-CM + GMAS
0	4.8	5.51	4.08	5.46	469	4781	14000	92000
0.5	5.02	6.36	4.41	5.60	86.3	3000	70700	46100
1	5.51	6.90	4.36	5.51	78.3	2360	30600	35000
1.5	4.81	6.68	4.58	6.54	168	2300	22400	24100
2	4.91	6.41	4.67	6.88	170	2300	13700	17000
2.5	5.04	7.02	4.92	6.92	396	2180	10800	13500
3	5.03	7.12	4.70	7.30	379	2000	8890	12100
3.5	5.12	7.20	4.64	7.22	364	2000	7620	11000
4	5.37	7.25	4.76	7.11	286	1870	6550	10400
4.5	5.82	7.39	4.72	7.60	235	1850	5690	10000
5	6.48	7.56	4.83	7.63	70.5	1690	5350	9410
6	6.28	7.26	4.98	6.73	174	1530	5000	8890
7	6.11	7.10	5.17	6.58	170	1400	4270	7870
8	6.00	7.51	4.98	7.45	165	1400	3720	7200
9	6.34	7.18	4.88	7.17	181	1510	3300	6260
10	6.52	7.21	4.91	7.61	200	1600	2900	5300
11	6.34		4.91	7.45	214		2180	4570
12	6.27		5.01	6.50	121		1590	3870
13	6.65		5.61	6.29	181		943	2940
14	7.06		6.45	6.42	340		672	2400
15	6.51		6.55	6.83	423		407	1900
16	6.72		7.04	7.03	447		405	1460
17	6.74		7.43	7.13	465		418	1250
18	6.31		7.06	6.68	502		469	1100
19	6.63		7.91	6.72	495		566	1050
20	7.18		7.92	6.80	488		567	997
21	6.61		7.23	6.80	514		567	967

CMAS = chicken manure amended soil leachate, GMAS = growmore amended soil leachate
CM + GMAS = chicken manure + growmore amended soil leachate; Contr = control soil leachate

Appendix J Amount (µg) of PTE measured in leachate of 2% chicken manure amended soil

Days	As	Control As	Cd	Control Cd	Cr	Control Cr	Cu	Control Cu	Fe	Control Fe
0	1.66	0.164	0.437	0.0485	1.26	0.143	86.4	2.46	198	11.1
0.5	0.995	0.288	0.158	0.0281	0.892	0.229	50.9	4.59	184	15.0
1	0.772	0.217	0.0894	0.0254	0.720	0.142	40.7	3.22	225	9.85
2	0.739	0.156	0.104	0.0306	0.617	0.104	33.3	2.33	463	24.3
2.5	0.745	0.177	0.100	0.0452	0.632	0.125	31.5	3.01	639	115
3	0.689	0.163	0.0778	0.0383	0.546	0.117	23.9	3.84	600	108
3.5	1.097	0.226	0.0809	0.0389	0.698	0.179	27.6	4.41	819	152
4	1.21	0.319	0.0691	0.0371	0.595	0.159	23.9	7.47	803	165
4.5	1.75	0.402	0.0691	0.0294	0.611	0.199	32.4	8.17	1120	168
5	1.69	0.565	0.372	0.0322	0.391	0.277	9.8	11.7	360	207
6	3.99	1.55	0.106	0.0630	1.01	0.625	91.9	30.1	2740	623
7	3.77	2.12	0.0843	0.0659	0.862	0.628	47.0	38.4	2660	849
8	5.05	2.60	0.0543	0.0658	0.943	0.651	106	40.7	3740	1200
9	5.18	2.57	0.0437	0.0624	0.854	0.585	111	35.6	4750	1280
10	5.13	2.64	0.0362	0.0551	0.687	0.510	84.5	33.0	5540	1500
	34.5	14.2	1.88	0.666	11.3	4.67	801	229	24800	6430
	0.062	0.025	0.0034	0.00121	0.0206	0.0085	1.46	0.416	45.1	11.7

Appendix J Amount (µg) of PTE measured in leachate of 2% chicken manure amended soil continued.....

Days	Mn	Control Mn	Ni	Control Ni	Pb	Control Pb	U	Control U	Zn	Control Zn
0	657	27.3	12.2	0.954	44.4	1.48	0.388	0.0483	73.1	15.9
0.5	573	39.4	7.25	0.758	20.1	2.06	0.285	0.0913	31.4	9.89
1	508	42.3	6.43	0.645	14.1	1.10	0.202	0.0383	23.7	8.48
2	670	119	6.20	0.670	11.4	1.06	0.130	0.0285	21.7	10.6
2.5	734	261	6.61	1.03	11.4	1.54	0.112	0.0366	21.3	15.4
3	581	217	5.65	0.913	8.90	1.58	0.096	0.0373	17.1	12.7
3.5	581	239	6.63	1.01	8.49	1.83	0.114	0.0434	15.9	13.1
4	486	216	7.87	1.19	7.87	2.49	0.099	0.0590	14.3	12.3
4.5	521	154	5.89	1.12	7.63	2.59	0.101	0.0582	13.2	9.07
5	1750	159	6.70	1.55	1.67	3.59	0.040	0.0673	8.28	10.1
6	874	280	11.4	3.55	11.3	7.86	0.154	0.141	18.7	18.6
7	770	302	7.90	3.97	8.25	8.08	0.127	0.129	14.7	18.7
8	894	346	8.67	4.47	8.60	7.89	0.179	0.114	14.5	19.0
9	1090	330	8.16	3.99	6.68	6.61	0.174	0.103	15.4	15.9
10	1140	342	6.96	3.60	5.53	5.44	0.109	0.075	13.9	13.9
	11800	3070	115	29.4	176	55.2	2.31	1.07	317	204

Appendix J Amount (µg) of PTE measured in leachate of 5% growmore amended soil continued.....

Days	As	Control As	Cd	Control Cd	Cr	Control Cr	Cu	Control Cu	Fe	Control Fe
0	4.86	0.164	29.4	0.0485	2.61	0.143	41.1	2.46	58.9	11.1
0.5	3.99	0.288	7.43	0.0281	1.50	0.229	32.2	4.59	31.9	15.0
1	2.63	0.217	1.32	0.0254	0.715	0.142	20.2	3.22	15.3	9.8
1.5	2.61	0.231	0.661	0.0409	0.732	0.156	22.0	3.42	16.4	31.3
2	2.22	0.156	0.549	0.0306	0.566	0.104	18.9	2.33	14.7	24.3
2.5	1.65	0.177	0.518	0.0452	0.547	0.125	16.4	3.01	65.0	115
3	1.54	0.163	0.632	0.0383	0.473	0.117	14.7	3.84	68.4	108
3.5	1.38	0.226	0.568	0.0389	0.447	0.179	12.7	4.41	124	152
4	1.39	0.319	0.443	0.0371	0.431	0.159	12.7	7.47	146	165
4.5	1.33	0.402	0.418	0.0294	0.439	0.199	11.3	8.17	316	168
5	1.25	0.565	0.395	0.0322	0.408	0.277	10.2	11.6	375	207
6	2.62	1.55	0.781	0.0630	0.841	0.625	22.8	30.1	1460	623
7	3.10	2.12	0.763	0.0659	0.821	0.628	29.2	38.4	2510	849
8	4.86	2.60	0.766	0.0658	0.888	0.651	32.8	40.7	3850	1200
9	6.97	2.57	0.726	0.0624	0.965	0.585	26.4	35.6	5690	1280
10	8.03	2.64	0.668	0.0551	1.23	0.510	21.2	33.0	7260	1500
11	8.90	1.84	0.487	0.0603	1.60	0.635	26.2	39.7	6620	2240
12	9.89	2.71	0.334	0.0769	2.13	0.795	26.9	51.3	6180	4030
13	8.29	3.45	0.145	0.0656	2.35	0.650	24.4	33.2	4490	4770
14	4.84	2.85	0.0887	0.0601	2.48	0.606	18.7	26.2	3940	5690
15	4.47	3.63	0.0547	0.0556	2.35	0.561	12.8	20.1	3880	6970
16	3.92	2.77	0.0472	0.0503	2.12	0.524	9.30	15.7	3630	7560
17	5.44	3.64	0.115	0.0356	2.16	0.335	13.7	8.39	4120	4860
18	6.96	4.62	0.111	0.0369	1.88	0.432	11.8	8.88	3260	6710
19	5.51	3.54	0.0703	0.0246	1.55	0.317	6.86	5.28	3670	5020
20	5.75	3.38	0.0369	0.0177	1.40	0.314	5.39	4.76	6980	4840
21	5.50	2.56	0.0410	0.0145	1.21	0.233	5.11	3.48	7840	3820

Appendix J Amount (µg) of PTE measured in leachate of 5% growmore amended soil continued.....

Days	Mn	Control Mn	Ni	Control Ni	Pb	Control Pb	U	Control U	Zn	Control Zn
0	6320	27.3	67.9	0.954	163	1.48	0.268	0.0483	1910	15.9
0.5	3390	39.4	38.8	0.758	27.7	2.06	0.225	0.0913	1130	9.89
1	1050	42.3	15.0	0.645	6.87	1.10	0.101	0.0383	391	8.48
1.5	1030	159	12.1	0.960	5.03	1.41	0.100	0.0410	324	14.8
2	736	119	8.74	0.670	3.33	1.06	0.0629	0.0285	221	10.6
2.5	1440	261	8.10	1.03	3.09	1.54	0.0633	0.0366	188	15.4
3	1580	217	8.04	0.913	2.70	1.58	0.0502	0.0373	184	12.7
3.5	1690	239	7.43	1.01	2.31	1.83	0.0494	0.0434	166	13.1
4	1610	216	7.12	1.19	2.14	2.49	0.0494	0.0590	151	12.3
4.5	1810	154	7.25	1.12	1.95	2.59	0.0474	0.0582	151	9.07
5	1830	159	7.00	1.55	1.75	3.59	0.0455	0.0673	146	10.1
6	3710	280	16.2	3.55	3.47	7.86	0.0875	0.141	283	18.6
7	3900	302	17.3	3.97	3.07	8.08	0.0936	0.129	275	18.7
8	3920	346	18.5	4.47	2.98	7.89	0.140	0.114	263	19.0
9	3850	330	20.3	3.99	3.22	6.61	0.146	0.103	248	15.9
10	3340	342	21.4	3.60	4.02	5.44	0.239	0.0752	213	13.9
11	2180	472	19.1	4.48	5.26	5.85	0.373	0.0875	140	16.5
12	1600	735	19.8	5.83	8.71	6.80	0.574	0.109	106	20.8
13	849	783	17.7	5.15	13.6	5.07	0.730	0.0812	63.8	17.4
14	561	892	16.7	5.09	15.0	4.52	0.808	0.0751	24.7	17.0
15	472	1080	16.6	5.25	12.1	4.14	0.655	0.0695	34.8	18.4
16	403	1160	16.3	5.11	7.81	3.81	0.467	0.0643	26.7	17.4
17	416	309	16.8	3.10	12.8	2.30	0.523	0.0347	28.4	9.90
18	446	453	17.2	3.86	8.70	2.79	0.460	0.0418	26.6	12.5
19	564	372	17.5	2.80	4.04	2.00	0.260	0.0301	19.7	8.94
20	640	367	17.4	2.66	3.01	1.92	0.173	0.0268	18.9	8.25
21	717	338	15.5	2.02	3.57	1.48	0.176	0.0196	23.6	6.19

Appendix J Amount (µg) of PTE measured in leachate of 5% growmore + 2% Chicken manure amended soil continued.....

Days	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
0	8.68	9.91	3.68	121	228	5090	31.6	65.5	0.701	424
0.5	3.88	1.49	1.51	54.2	76	2120	12.1	11.2	0.237	124
1	3.59	0.955	1.25	46.5	68	1630	10.1	10.1	0.225	92.4
1.5	3.23	0.604	1.31	41.5	153	1860	9.92	8.90	0.215	78.9
2	2.77	0.460	1.05	35.2	146	1630	8.57	7.15	0.165	64.4
2.5	2.30	0.287	0.902	28.9	484	1600	8.26	5.17	0.152	61.7
3	2.73	0.282	0.857	25.0	1110	1620	8.88	4.34	0.146	50.6
4	2.67	0.256	0.815	25.1	925	1800	10.5	2.83	0.144	46.8
5	8.49	0.466	1.71	34.7	5050	3930	26.3	2.24	0.184	84.4
6	11.7	0.524	1.80	39.5	9080	4320	28.7	2.92	0.150	98.0
7	12.7	0.495	1.69	45.6	10200	4210	29.1	3.05	0.141	95.0
8	12.3	0.410	1.46	54.3	9570	3500	25.8	2.04	0.164	76.0
9	13.5	0.472	1.60	51.1	10580	4010	28.9	3.11	0.143	88.8
10	13.5	0.397	1.65	57.8	9130	3001	24.1	3.14	0.196	68.4
11	14.7	0.461	1.56	68.3	9580	2940	24.1	4.11	0.231	69.0
12	15.0	0.776	1.60	78.1	9770	2680	22.6	5.80	0.266	57.5
13	14.0	0.438	1.39	51.7	8380	2040	18.6	4.81	0.222	40.8
14	12.2	0.163	1.32	38.7	6470	1570	15.7	4.51	0.204	29.4
15	13.2	0.0921	1.34	37.0	6070	1170	14.9	6.03	0.213	24.6
16	12.5	0.0817	1.39	34.1	5090	856	13.9	7.58	0.206	19.4
17	9.94	0.0448	1.24	24.0	4860	717	12.7	7.19	0.153	12.9
18	7.12	0.0420	0.849	15.1	3860	497	9.12	6.12	0.118	6.00
19	6.62	0.0185	0.691	10.0	3680	434	7.66	4.00	0.0678	3.23
20	7.16	0.00780	0.607	7.22	3740	415	6.77	2.93	0.0426	2.93
21	6.21	0.000522	0.435	4.79	2960	315	4.89	2.00	0.0253	3.18

Note the control values are not included here because they are the same for 5% growmore amended soil

Appendix K Distribution of PTE (mg kg^{-1}) in amended soil before and after leaching

As		Exchangeable	Reducible	Oxidisable	Residual	SUM	PT	% REC.
	CSB	0.078	3.83	1.64	7.46	13.0	14.2	92
	CSA	0.434	3.88	1.42	5.54	11.3	13.5	84
	CMASB	0.501	4.00	1.37	6.17	12.0	13.7	88
	CMASA	0.138	3.74	1.25	6.410	11.5	13.3	87
	GMASB	0.560	4.11	1.23	5.73	11.6	12.0	97
	GMASA	0.697	4.06	1.07	5.79	11.6	13.6	86
	CM + GMAB	0.688	4.01	1.22	8.74	14.7	12.9	114
	CM+GMA	0.986	4.12	1.23	6.35	12.7	14.1	90

Cd		Exchangeable	Reducible	Oxidisable	Residual	SUM	PT	% REC.
	CSB	0.129	0.198	0.037	<0.00476	0.364	0.378	96
	CSA	0.139	0.169	0.044	<0.00476	0.352	0.335	105
	CMASB	0.110	0.197	0.035	<0.00476	0.347	0.388	89
	CMASA	0.133	0.175	0.039	<0.00476	0.352	0.388	91
	GMASB	0.283	0.186	0.034	<0.00476	0.508	0.482	105
	GMASA	0.134	0.198	0.043	<0.00476	0.380	0.427	89
	CM + GMAB	0.273	0.225	0.037	<0.00476	0.540	0.528	102
	CM+GMA	0.171	0.216	0.041	<0.00476	0.433	0.483	90

dl

Cr		Exchangeable	Reducible	Oxidisable	Residual	SUM	PT	% REC.
	CSB	0.158	1.40	5.86	15.3	22.7	32.5	70
	CSA	0.183	1.45	5.51	14.7	21.8	31.3	70
	CMASB	0.166	1.56	5.50	16.2	23.4	35.7	66
	CMASA	0.132	1.32	5.53	15.0	22.0	31.9	69
	GMASB	0.284	1.76	5.47	14.4	22.0	29.4	75
	GMASA	0.112	1.91	5.10	13.2	20.3	30.9	66
	CM + GMB	0.118	1.98	5.69	19.1	26.9	33.0	81
	CM+GMA	0.323	1.76	5.41	14.6	22.1	34.2	65

Cu		Exchangeable	Reducible	Oxidisable	Residual	SUM	PT	% REC.
	CSB	4.53	30.7	32.1	25.3	92.7	103	90
	CSA	11.3	26.8	27.3	20.0	85.4	92.9	92
	CMASB	3.85	28.1	28.1	23.2	83.2	110	76
	CMASA	7.02	26.8	28.3	22.8	84.9	106	80
	GMASB	5.36	29.6	27.77	21.4	84.1	96.4	87
	GMASA	6.86	30.0	25.7	21.3	83.9	105	80
	CM + GMB	5.61	29.5	29.06	23.5	87.7	102	86
	CM+GMA	5.08	30.0	30.0	22.0	87.0	109	80

Appendix K Distribution of PTE (mg kg^{-1}) in amended soil before and after leaching continued.....

Fe		Exchangeable	Reducible	Oxidisable	Residual	SUM	PT	% REC.
	CSB	7.25	3650	1200	21800	26600	30000	89
	CSA	81.6	3890	1100	21190	26300	29500	89
	CMASB	9.55	3300	1010	21700	26000	28600	91
	CMASA	83.8	3880	1100	23300	28400	28700	99
	GMASB	13.8	3670	996	11500	16200	29200	55
	GMASA	43.8	3800	865	21900	26600	29100	91
	CM + GMB	20.5	3500	995	22300	26800	27100	99
	CM+GMA	43.7	3900	1057	22900	27900	29200	96

Mn		Exchangeable	Reducible	Oxidisable	Residual	SUM	PT	% REC.
	CSB	31.2	275	18.2	188	513	502	102
	CSA	201	67.4	20.0	160	449	500	90
	CMASB	78.7	192	15.1	179	465	540	86
	CMASA	205	63.2	19.0	189	476	490	97
	GMASB	61.8	226	15.0	169	472	485	97
	GMASA	126	74.9	16.1	165	382	452	84
	CM + GMAB	104	174	14.19	182	475	499	95
	CM+GMA	192	69.4	17.5	189	468	476	98

		Exchangeable	Reducible	Oxidisable	Residual	SUM	PT	% REC.
Ni	CSB	1.89	2.23	4.49	32.6	41.2	53.6	77
	CSA	2.07	1.99	4.35	29.8	38.2	51.1	75
	CMASB	1.56	2.29	3.93	30.4	38.2	50.8	75
	CMASA	1.78	2.10	4.22	35.0	43.1	52.2	83
	GMASB	2.36	2.21	3.89	31.3	39.8	47.9	83
	GMASA	1.75	2.11	3.58	27.7	35.1	48.2	73
	CM + GMAB	2.14	2.39	3.95	31.5	40.0	49.0	82
	CM+GMA	1.91	2.29	3.71	42.0	49.9	51.8	96

		Exchangeable	Reducible	Oxidisable	Residual	SUM	PT	% REC.
Pb	CSB	9.53	290	36.4	48.8	385	355	108
	CSA	18.3	278	38.1	45.7	380	337	113
	CMASB	12.1	277	36.2	45.6	371	338	110
	CMASA	9.03	250	34.0	48.7	341	360	95
	GMASB	8.84	259	33.5	46.1	347	315	110
	GMASA	5.13	275	33.0	46.6	360	359	100
	CM + GMAB	8.21	261	37.7	73.2	380	351	108
	CM+GMA	3.93	281	33.2	44.0	362	345	105

CSB = control soil before leaching, CSA = control soil after leaching, CMASB = chicken manure amended soil before leaching; CMASA = chicken manure amended soil after leaching; GMASB = growmore amended soil before leaching, GMASA = growmore amended soil after leaching; CM + GMASB = chicken manure + growmore amended soil before leaching and CM + GMASA = chicken manure + growmore amended soil before leaccching.

Appendix K Distribution of PTE (mg kg^{-1}) in amended soil before and after leaching continued.....

U		Exchangeable	Reducible	Oxidisable	Residual	SUM	PT	% REC.
	CSB	0.00621	0.0791	0.386	0.510	0.981	1.12	88
	CSA	0.0150	0.0902	0.355	0.820	1.28	1.15	111
	CMASB	0.0148	0.0740	0.975	0.558	1.62	1.20	135
	CMASA	0.0109	0.0921	0.362	0.510	0.975	1.14	85
	GMASB	0.0443	0.310	0.962	0.908	2.22	2.60	86
	GMASA	0.0365	0.364	0.908	0.840	2.15	2.70	80
	CM + GMAB	0.107	0.339	1.02	0.917	2.38	2.60	92
	CM+GMA	0.0352	0.355	0.920	0.720	2.03	2.55	80

Zn		Exchangeable	Reducible	Oxidisable	Residual	SUM	PT	% REC.
	CSB	28.9	38.2	27.6	96.3	191	212	90
	CSA	25.6	32.8	28.4	85.8	173	200	86
	CMASB	29.6	35.1	24.0	91.2	180	215	84
	CMASA	27.7	34.0	27.4	97.2	186	221	84
	GMASB	47.6	30.3	23.7	88.5	190	216	88
	GMASA	33.1	33.7	22.7	83.9	173	215	81
	CM + GMAB	55.2	35.7	24.1	90.6	206	232	89
	CM+GMA	46.6	35.9	24.8	97.6	205	236	87

CSB = control soil before leaching, CSA = control soil after leaching, CMASB = chicken manure amended soil before leaching; CMASA = chicken manure amended soil after leaching; GMASB = growmore amended soil before leaching, GMASA = growmore amended soil after leaching; CM + GMASB = chicken manure + growmore amended soil before leaching and CM + GMASA = chicken manure + growmore amended soil before leaching.

Appendix L **Pseudototal concentrations (mg kg^{-1}, dw) of PTE in bean plant grown in chicken maure (n =4, except control; n =2)**

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
RB-control1	2.001	0.653	3.01	41.5	3690	228	34.5	110	0.234	193
RB-control2	1.99	0.713	2.95	39.6	3650	219	40.2	109	0.228	200
Mean	2.00	0.683	2.98	40.6	3670	223	37.4	109	0.231	197
RB-2%CMAS1	1.67	0.408	2.60	37.9	1580	182	6.4	54.3	0.161	118
RB-2%CMAS2	1.63	0.432	2.55	35.5	1580	202	6.6	51.4	0.170	111
RB-2%CMAS3	1.74	0.407	2.56	36.6	1600	184	6.7	53.2	0.139	115
RB-2%CMAS4	1.70	0.424	2.56	36.0	1570	175	6.8	51.9	0.135	114
Mean	1.69	0.418	2.57	36.5	1580	186	6.6	52.7	0.151	115
SD	0.04	0.01	0.02	1.0	9.9	11	0.2	1.3	0.02	3
RSD	2.7	2.9	0.8	2.8	0.6	6.14	2.7	2.5	11.3	2.4

RB-control = runner bean grown in control soil; RB-CMAS = runner bean grown in chicken manure amended soil

Appendix M **Pseudototal concentration (mg kg^{-1}) of control soil and chicken manure amended soil after harvest of bean plant (n =4, except control; n =2)**

Replicates	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Control1	14.7	0.416	34.6	110	29546	523	54.8	354	1.28	219
Control2	14.5	0.476	30.5	107	28530	503	52.1	346	1.27	212
Mean	14.6	0.446	32.5	108	29038	513	53.4	350	1.28	216
CMAS1	14.0	0.403	35.1	98.4	28745	531	51.5	360	1.28	226
CMAS2	14.5	0.384	36.9	101	31158	495	54.6	348	1.23	223
CMAS3	14.4	0.445	35.8	105	30550	532	56.6	363	1.35	232
CMAS4	14.2	0.444	32.7	107	27245	493	50.9	345	1.28	233
Mean	14.3	0.419	35.1	103	29425	513	53.4	354	1.28	228
SD	0.2	0.03	1.8	4	1780	22	2.7	9	0.05	5
RSD	1.6	7.2	5.1	4	6	4.2	5.0	2	3.7	2

CMAS = chicken manure amended soil

Appendix N **The EDTA extractable PTE (mg kg^{-1}) of soil and chicken manure amended soil after harvest of bean plant (n = 4; except control; n = 2)**

Replicates	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Before planting										
Control soil 1	0.312	0.177	0.561	35.1	652	46.5	36.8	146	0.0205	24.3
Control soil 2	0.313	0.164	0.466	34.7	614	43.0	36.4	137	0.0182	21.8
Control soil 3	0.309	0.179	0.544	35.1	626	45.5	36.9	149	0.0153	24.8
Control soil 4	0.298	0.167	0.495	33.6	634	42.9	35.4	139	0.0156	22.0
Mean	0.592	0.346	0.797	66.8	1111	102	36.4	286	0.0259	49.1
SD	0.007	0.007	0.04	0.7	16	1.8	0.7	6	0.002	1.5
RSD	1.2	2.1	5.5	1.0	1.4	1.8	1.9	2.0	9.4	3.1
After harvest										
Control1	0.282	0.121	0.428	28.1	633	40.4	29.9	130	0.0119	18.1
Control 2	0.306	0.144	0.445	30.5	671	44.7	32.3	144	0.0122	23.2
Mean	0.294	0.132	0.436	29.3	652	42.6	31.1	137	0.0121	20.7
CMAS 1	0.346	0.171	0.337	33.9	568	85.6	35.7	148	0.0103	23.0
CMAS 2	0.362	0.236	0.339	34.7	558	78.9	36.5	153	0.0179	27.3
CMAS 3	0.284	0.174	0.281	32.2	480	57.1	34.0	143	0.00854	25.9
CMAS 4	0.336	0.183	0.357	34.0	535	74.7	35.8	151	0.0125	24.7
Mean	0.332	0.191	0.328	33.7	535	74.1	35.5	149	0.0123	25.2
SD	0.03	0.03	0.03	1.1	39	12.2	1.0	4	0.004	1.8
RSD	10.2	15.9	10.1	3.2	7.0	16.4	3.0	2.8	33.0	7.1

CMAS = chicken manure amended soil

Appendix O **Pseudototal concentrations (mg kg^{-1}, dw) of PTE in radish plant grown in the amended soils (n =4)**

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Control 1	0.428	0.661	1.28	14.4	117	112	5.07	11.9	ND	174
Control 2	0.399	0.646	1.30	12.3	108	113	4.82	12.1	ND	174
Control 3	0.387	0.794	1.67	12.9	120	112	4.79	11.8	ND	173
Control 4	0.389	0.660	1.29	13.0	118	112	4.78	11.7	ND	172
Mean	0.405	0.700	1.42	13.2	115	112	4.89	12.0	ND	173
SD	0.02	0.08	0.2	1.1	6	0.4	0.2	0.2	NA	0.4
RSD	5.1	11.6	15.5	8.2	6	0.4	3.1	1.3	NA	0.3
CMAS 1	0.200	0.355	1.50	5.82	96.3	138	3.39	3.14	ND	110
CMAS 2	0.172	0.342	1.29	7.42	88.6	133	3.79	2.70	ND	104
CMAS 3	0.185	0.346	1.32	7.23	90.8	133	3.73	3.08	ND	105
CMAS 4	0.184	0.343	1.33	7.25	91.0	134	3.75	3.09	ND	106
Mean	0.186	0.348	1.37	6.82	91.9	135	3.64	2.97	ND	106
SD	0.01	0.007	0.1	0.9	3.9	3	0.2	0.2	NA	3
RSD	7.6	2.0	8.1	12.8	4.3	2.1	5.8	8.0	NA	3.1
GMAS 1	0.237	0.771	0.443	11.7	109	300	4.75	8.01	ND	192
GMAS 2	0.226	0.779	0.385	11.4	122	292	4.76	7.81	ND	193
GMAS 3	0.218	0.731	0.414	14.2	101	294	4.79	8.36	ND	192
GMAS 4	0.222	0.776	0.444	11.5	103	294	4.77	7.77	ND	193
Mean	0.227	0.761	0.414	12.5	111	295	4.76	8.06	ND	192
SD	0.01	0.03	0.03	1.5	11	4.1	0.02	0.3	NA	0.7
RSD	4.3	3.361	7.080	12.4	9.6	1.40	0.487	3.48	NA	0.4
CM + GMAS 1	0.142	0.531	0.619	7.77	92.5	125	4.22	3.48	ND	185
CM + GMAS 2	0.151	0.536	0.642	8.06	81.2	120	4.17	3.55	ND	186
CM + GMAS 3	0.119	0.508	0.593	8.21	77.0	120	4.40	3.52	ND	178
CM + GMAS 4	0.150	0.537	0.620	8.19	82.1	121	4.19	3.51	ND	177
Mean	0.137	0.525	0.618	8.01	83.6	122	4.26	3.51	ND	183
SD	0.02	0.01	0.02	0.2	8.1	3	0.1	0.04	NA	4
RSD	11.8	2.8	4.0	2.8	9.6	2.4	2.8	1.02	NA	2.3

CMAS = 2% chicken manure amended soil; GMAS = 0.2% amended soil; CM + GMAS = 2% chicken manure + 0.2 growmore amended soil, ND = not detected; NA not applicable

Appendix P **ANOVA results for the various treatments for radish grown in control soil and various fertiliser treatments**

			As							Cd			
SUMMARY							SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*			*Groups*	*Count*	*Sum*	*Average*	*Variance*		
Control	4	1.60	0.401	0.000348			Control	4	2.76	0.690	0.00481		
CMAS	4	0.741	0.185	0.000132			CMAS	4	1.39	0.347	0.0000363		
GMAS	4	0.902	0.226	0.0000677			GMAS	4	3.06	0.764	0.000495		
CM + GMAS	4	0.562	0.140	0.000216			CM + GMAS	4	2.11	0.528	0.000180		
Source of Var.	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Source of Var.*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.156	3	0.0520	272	2.7E-11	3.49	Between Groups	0.413	3	0.138	100	9.40E-09	3.49
Within Groups	0.00229	12	0.000191				Within Groups	0.0166	12	0.00138			
Total	0.158	15					Total	0.430	15				
			Cr							Cu			
SUMMARY							SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*			*Groups*	*Count*	*Sum*	*Average*	*Variance*		
Control	4	5.54	1.38	0.0360			Control	4	52.6	13.2	0.800		
CMAS	4	5.44	1.36	0.00870			CMAS	4	27.7	6.93	0.557		
GMAS	4	1.69	0.421	0.000798			GMAS	4	48.9	12.2	1.81		
CM + GMAS	4	2.47	0.618	0.000403			CM + GMAS	4	32.2	8.06	0.0422		
Source of Var.	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Source of Var.*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.98	3	0.995	86.6	2.12E-08	3.49	Between Groups	112	3	37.3	46.6	0.0000006	3.49
Within Groups	0.138	12	0.0115				Within Groups	9.62	12	0.802			
Total	3.12	15					Total	122	15				

CMAS = chicken manure amended soil grown radish; GMAS = growmore amended soil grown radish; CM + GMAS = cchicken manure + growmore anended soil grown radish

Appendix P **ANOVA results for the various treatments for radish grown in control soil and various fertiliser treatments continued....,**

			Fe							Mn			
SUMMARY							SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*			*Groups*	*Count*	*Sum*	*Average*	*Variance*		
Rad-Control	4	463	116	29.5			Control	4	449	112	0.139		
CMAS	4	367	91.7	10.6			CMAS	4	539	135	5.65		
GMAS	4	435	109	90.0			GMAS	4	1179	295	11.6		
CM + GMAS	4	333	83.2	43.8			CM + GMAS	4	486	121	5.86		
Source of Var.	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Source of Var.*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2698	3	899	20.7	4.93E-05	3.49	Between Groups	89850	3	29950	5149	6.41E-19	3.49
Within Groups	522	12	43.5				Within Groups	69.8	12	5.82			
Total	3220	15					Total	89920	15				
			Ni							Pb			
SUMMARY							SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*			*Groups*	*Count*	*Sum*	*Average*	*Variance*		
Control	4	19.5	4.86	0.0189			Control	4	47.6	11.9	0.0325		
CMAS	4	14.7	3.67	0.0333			CMAS	4	12.0	3.00	0.0409		
GMAS	4	19.1	4.77	0.000368			GMAS	4	32.0	7.99	0.0737		
CM + GMAS	4	17.0	4.24	0.0107			CM + GMAS	4	14.1	3.51	0.000863		
Source of Var.	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Source of Var.*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.64	3	1.21	76.9	4.20E-08	3.49	Between Groups	210	3	69.9	1891	2.59E-16	3.49
Within Groups	0.190	12	0.0158				Within Groups	0.444	12	0.0370			
Total	3.83	15					Total	210	15				

CMAS = chicken manure amended soil grown radish; GMAS = growmore amended soil grown radish; CM + GMAS = echicken manure + growmore anended soil grown radish

Appendix P ANOVA results for the various treatments for radish grown in control and various fertiliser treatments continued....,

			Zinc			
SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
CTR	4	692	173	0.613		
CMAS	4	425	106	7.24		
GMAS	4	770	192	0.440		
CM + GM	4	727	182	22.0		
Source of Var.	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	18178	3	6059	799	4.46E-14	3.49
Within Groups	91.0	12	7.58			
Total	18269	15				

CMAS = chicken manure amended soil grown radish; GMAS = growmore amended soil grown radish;

CM + GMAS = chicken manure + growmore anended soil grown radish

Appendix Q **Pseudototal concentration (mg kg^{-1}) of control soil and fertiliser amended soil after harvest of radish plant (n =4)**

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Control 1	23.5	0.497	43.4	145	30400	550	72.0	519	1.36	306
Control 2	23.2	0.445	38.6	147	31900	560	69.6	503	1.32	301
Control 3	22.9	0.474	48.1	179	30100	581	74.5	578	1.42	308
Control 4	23.3	0.402	37.7	147	31400	529	67.7	510	1.28	295
Mean	23.2	0.455	41.9	154	30950	555	71.0	528	1.34	303
SD	0.2	0.04	4.8	16	843	22	3	34	0.1	6
RSD	1.1	9.0	11.4	10	3	4	4	7	4.36	2
CMAS 1	22.5	0.389	42.3	141	30500	536	69.7	486	1.28	321
CMAS 2	23.9	0.468	43.5	157	32200	572	74.0	536	1.43	336
CMAS 3	24.1	0.370	36.8	143	31200	529	66.2	465	1.28	293
CMAS 4	23.3	0.463	38.2	156	32800	558	74.1	528	1.38	344
Mean	19.2	1.19	35.2	127	26800	454	71.0	427	1.50	256
SD	0.7	0.05	3.2	8	1020	20	4	34	0.1	22
RSD	3.7	4.3	9.2	7	4	4	5	8	4.9	9
GMAS 1	23.8	0.379	39.2	139	31500	552	67.3	500	1.31	287
GMAS 2	25.3	0.424	39.2	137	35500	561	72.2	515	1.34	301
GMAS 3	22.8	0.502	35.0	149	31600	526	67.2	469	1.33	343
GMAS 4	23.0	0.438	36.5	141	34600	532	67.3	470	1.32	282
Mean	23.7	0.436	37.5	142	33300	543	68.5	488	1.32	303
SD	1.2	0.05	2.1	6	2050	16	2	23	0.01	28
RSD	4.9	11.7	5.6	4	6	3	4	5	1.0	9
CM + GMAS 1	22.7	0.375	38.5	137	31400	517	72.7	456	1.29	308
CM + GMAS 2	24.2	0.394	37.1	140	33600	601	64.9	492	1.25	313
CM + GMAS 3	23.7	0.440	34.5	151	33200	546	69.2	684	1.33	308
CM + GMAS 4	28.2	0.368	38.7	135	30500	503	67.2	452	1.24	287
Mean	24.7	0.394	37.2	141	32200	542	68.5	521	1.28	304
SD	2.4	0.03	2.0	7	1470	43	3	110	0.04	12
RSD	9.8	8.3	5.3	5	5	8	5	21	3.4	4

CMAS = 2% chicken manure amended soil; GMAS = 0.2% amended soil; CM + GMAS = 2% chicken manure + 0.2 growmore amended soil

Appendix R **The EDTA extractable PTE (mg kg^{-1}) of control soil before and fertiliser amended soil after harvest of bean plant (n = 4)**

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
Soil 1	0.474	0.162	0.358	35.1	606	66.0	2.10	175	ND	30.9
Soil 2	0.534	0.205	0.339	38.4	554	76.3	2.23	174	ND	35.5
Soil 3	0.547	0.184	0.353	37.3	612	70.4	2.14	171	ND	33.5
Soil 4	0.519	0.182	0.340	35.1	614	61.3	2.09	158	ND	30.1
Mean	0.518	0.183	0.347	36.5	596	68.5	2.14	170	ND	32.5
SD	0.03	0.02	0.01	1.7	28.2	6.4	0.06	8	NA	2.5
RSD	6.1	9.6	2.7	4.5	4.7	9.3	2.9	4.6	NA	7.6
Control soil 1	0.539	0.171	0.355	38.6	619	30.8	2.18	176	ND	32.2
Control soil 2	0.457	0.137	0.274	32.1	498	30.9	1.89	156	ND	26.4
Control soil 3	0.486	0.146	0.273	33.9	507	29.2	2.02	166	ND	28.5
Control soil 4	0.456	0.147	0.274	34.1	482	31.0	1.90	168	ND	29.0
Mean	0.484	0.150	0.294	34.7	526	30.5	2.00	167	ND	29.0
SD	0.04	0.01	0.04	2.76	63	0.83	0.14	8.13	NA	2.38
RSD	8.0	9.8	13.9	7.9	12	2.7	6.8	4.9	NA	8.2
CMAS 1	0.568	0.183	0.281	36.7	526	69.7	2.14	168	ND	32.2
CMAS 2	0.489	0.146	0.352	31.9	583	33.9	1.92	166	ND	28.4
CMAS 3	0.473	0.144	0.342	32.0	567	39.0	1.97	166	ND	29.2
CMAS 4	0.460	0.143	0.343	29.9	548	31.7	1.85	150	ND	27.5
Mean	0.498	0.154	0.329	32.6	556	43.6	2.0	162	ND	29.3
SD	0.05	0.02	0.03	2.9	25	17.7	0.1	8.2	NA	2.0
RSD	9.7	12.5	10.0	8.8	4	40.5	6.3	5.0	NA	7.0

CMAS = 2% chicken manure amended soil; GMAS = 0.2% amended soil; CM + GMAS = 2% chicken manure + 0.2 growmore amended soil; ND = not detected; NA; not applicable.

Appendix R **The EDTA extractable PTE (mg kg^{-1}) of soil and amended soil after harvest of radish plant (n = 4) continued.....**

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
GMAS 1	0.453	0.131	0.333	29.0	531	31.8	1.81	148	ND	27.0
GMAS 2	0.527	0.177	0.287	33.9	483	56.0	2.00	167	ND	33.5
GMAS 3	0.526	0.188	0.303	35.6	480	57.2	2.08	171	ND	35.0
GMAS 4	0.506	0.179	0.279	34.4	475	58.4	2.06	169	ND	35.3
Mean	0.503	0.169	0.301	33.2	492	50.8	1.99	164	ND	32.7
SD	0.03	0.03	0.02	2.9	26	12.7	0.1	11	NA	3.9
RSD	6.8	15.1	7.9	8.7	5	25.0	6.1	6.6	NA	11.9
CM + GMAS 1	0.521	0.214	0.285	36.4	485	28.3	2.36	180	ND	42.1
CM + GMAS 2	0.580	0.187	0.416	42.2	601	28.1	2.25	174	ND	32.4
CM + GMAS 3	0.520	0.181	0.391	40.8	579	27.8	2.15	165	ND	28.5
CM + GMAS 4	0.526	0.193	0.409	43.6	604	27.5	2.33	176	ND	31.1
Mean	0.537	0.194	0.375	40.8	567	27.9	2.27	174	ND	33.5
SD	0.03	0.01	0.06	3.1	56	0.4	0.09	6	NA	6.0
RSD	5.4	7.3	16.2	7.6	10	1.3	4.1	3	NA	17.8

CMAS = 2% chicken manure amended soil; GMAS = 0.2% amended soil; CM + GMAS = 2% chicken manure + 0.2 growmore amended soil ND = not detected; NA; not applicable.