The Occurrence and Spatial Dynamics of Trace Metals in the Topsoil of a Drained Meres and Mosses Habitat Area

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The Spatial Occurrence and Dynamics of Trace Metals in Topsoil of a Meres and Mosses Wetland Habitat

Abstract:

Trace metal pollution is one of the most concerning of anthropogenic impacts on meres and mosses wetland habitats due to their toxicity, persistence and potential for bioaccumulation. The topsoil of these habitats provides a transfer interface for trace metal accumulation in the environment. This study aimed to elucidate the factors driving in-situ trace metal mobility in the topsoil of these habitats once the status of the topsoil of the habitat had been determined. A screening study was undertaken which examined physiochemical properties of the topsoil and determined its trace metal content via Field Portable X-Ray Fluorescence Spectroscopy (FPXRF). This study found there to be enrichment of trace metals within the topsoil of the region being studied and relationships with physiochemical properties, such as pH and organic matter content (OM), were then determined. The enrichment factor analysis results ranged from moderate for Ga, Rb, Co, V, Zn and Sn through to significant for Pb, As and Nb. The trace metal concentrations were then placed into context by examining widely cited soil quality guidelines. The screening study showed that some of these trace metals exceeded 'target values' of these soil quality guidelines. Some soil quality guidelines require an alternative analytical methodology, however. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with reverse aqua regia digestion was therefore implemented due to the analytical methodology having superior limits of detection and adhering to the requirements of the much-cited Finnish Soil Quality Guidelines (Decree 214/2007). Following a method development study to aid in method validity, a comparison study was undertaken so to determine if the results obtained from the acid extraction process (known as pseudo-total result) could be used to indicate the 'total' trace metal concentration result obtained via FPXRF. This study found significant relationships for select trace metals between the 'pseudo-total' result and the 'total' result. A focussed mesocosm study was then conducted on two representative topsoil samples within the sampling area. This study examined the influence of pH and soil organic matter content on the trace metal mobility within the porewater of the topsoil over the course of a calendar year and found that topsoil pH had a direct influence on trace metal mobility for many of the trace metals analysed and that OM content influenced the impact that the change of pH incurs. A chemical fractionation study was conducted on these two representative samples so to ascertain potential for bioavailability and to determine potential risk to the local environment and found that Cu, Pb and Zn reached the 'Low Risk' status of the Risk Assessment Code (RAC) in the topsoil. Knowledge garnered from the studies in this thesis will aid in conservation, mitigation and remediation of these environmentally valuable habitat types.

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Acronyms and Abbreviations

Artificial Rainwater (ARW)

British Broadcasting Corporation (BBC)

Certified Reference Material (CRM)

Cluster Analysis (CA)

Collision Cell Technology (CCT)

UK Department for Environment, Fisheries and Rural Affairs (DEFRA)

Enrichment Factor (EF)

European Union (EU)

Field Portable X-Ray Fluorescence Spectroscopy (FPXRF)

Geographic Information Systems (GIS)

Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

Inductively Coupled Plasma Optical Emissions Spectroscopy (ICP-OES)

International Organization for Standardization (ISO)

Joint Research Centre (JRC-Ispra)

Kinetic Energy Discrimination (KED)

Landscape Partnership Scheme (LPS)

Loss on Ignition (LOI)

Magnetic Susceptibility (MS)

National Nature Reserve (NNR)

Nature Improvement Area (NIA)

Organic Matter Content (OM)

Potential Hydrogen (pH)

Principal Component Analysis (PCA)

Relative Standard Deviation (RSD)

Risk Assessment Code (RAC)

Sequential Pressure Digestion (SP-D)

Site of Special Scientific Interest (SSSI)

Soil Reference Material Digest (SRM)

Special Areas of Conservation (SAC)

Standard Deviation (SD)

Total Carbon (TC)

Total Inorganic Carbon (TIC)

Total Organic Carbon (TOC)

United States Environmental Protection Agency (USEPA)

X-Ray Fluorescence Spectroscopy (XRF)

Chapter 1:

Introduction

1.1 Soil as a Resource

Soil is known to undergo short-term fluctuations. These fluctuations and variations in redox potential, pH, moisture levels and organic content as well as undergoing changes in response to seasonal environmental variation and land management factors (Chao et al, 1998), mean that the soil is considered to be a 'dynamic system' (Stamenov et al, 2016). These variations in the properties of the soil affect the form of these trace metals in the soil and their consequent bioavailability and, as such, need to be considered in land management strategies (Alloway et al, 1995; Ball, 2005).

In their report 'Soil – A Key Resource for the EU' (2011), the European Commission describes soil as "the fragile, friable layer of the earth's crust that covers the continents, between the surface and the bedrock. It is formed by mineral particles, organic matter, water, air and living organisms. It is the interface between earth, air and water and hosts most of the biosphere" (European Commission, 2011). The report goes on to state that soil is a "largely non-renewable and very complex natural resource and yet it is increasingly damaged by certain human practices" (European Commission, 2011). Soil has been found to hold double the organic carbon as plants and vegetation with it being determined that EU soils contain over 70 billion tonnes of organic carbon which accounts for approximately 7 % of the total global carbon budget (Schils et al, 2008). As such, "soil is a crucial link between global environmental problems such as climate change, water management and biodiversity loss" (Rubio, 2010). "Soil degradation is the physical, chemical and biological decline in soil quality. It can be the loss of organic matter, decline in soil fertility, and structural condition, erosion, adverse changes in salinity, acidity or alkalinity, and the effects of toxic chemicals, pollutants or excessive flooding" (Mitryasova, 2022). This has resulted in degradation of soil being of major environmental, social and economic concern due to its significant impacts on ecosystem health and rural land productivity (Chapman et al, 2011). Therefore, the quality of the soil is of vital importance when considering conservation and environmental sustainability and, as a result, soil degradation has emerged as a major global agricultural problem (Rengel, 2011).

At a basic level, soil is considered to be visually and physically made up of multiple layers known as the soil profile (see Fig 1.1). These are the O-horizon, which is the layer at the soil the surface and is made up of plant residues and other organic substances in relatively undecomposed form. This is followed by the A-horizon. This horizon is made-up of organic constituents and minerals from parent material and is considered a good part of the soil profile for plants and organisms to thrive and, as such, a valuable resource (Soil Science Society of America, 2023). The B Horizon, or 'Subsoil' is rich in minerals that leached from the horizons above and accumulated in this layer. This is followed by the C-horizon which is made up of parent material and has little or no humus accumulation or structure development (Soil Science Society of America, 2023).

Topsoil is an important component of the soil profile, both as diagnostic horizons (in all global classification systems) and in terms of soil functioning (Gerasimova et al, 2010). Topsoil is the top 10 inches, excluding the surface O-Horizon (see Fig 1.1). This layer is also known as the 'A-Horizon' and has the highest levels of organic matter and microfauna and is where most of the biological activity occurs in the soil (GreenBio, 2021).

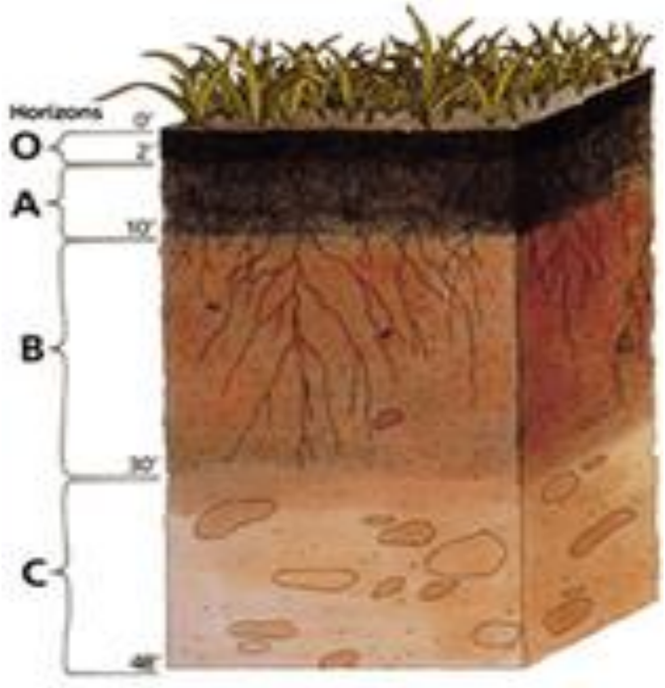

Fig 1.1: Full Soil Profile and Approximate Depth in Inches (Adapted from CK-12, 2015)

Fertile topsoil is a precious agricultural resource which, if lost or damaged, can take many decades to repair/renew. As such, many agronomists consider topsoil to be a non-renewable resource (Pimentel and Burgess, 2018). Topsoil is more sensitive to environmental conditions, changes and impacts than other parts of soil profiles. Many anthropogenic activities can impact the soil profile including agriculture, and urban development (Darmody et al, 2009). On agricultural soil and land, seasonal growing leads to impacts on the topsoil due to agricultural management as well as the seasonal variability of rainfall, crop yield and root growth. Due to these pressures, topsoil is prone to compaction and structure degradation as well as preferential pathway disconnection (Zumr et al, 2019). This sensitivity is indicated by the presence of a variety of microstructures and organic matter types found in the a-horizon as well as features related to faunal activity or to specific environmental conditions (Gerasimova et al, 2010). As such, soil is a resource that needs to be protected and closely monitored from pressures such as those posed by anthropogenic pollution and toxins within the soil profile.

1.1.1 Agricultural Soil in a Meres and Mosses Habitat

Meres and Mosses habitats are those in which a series of marsh land, bogs and fen wetlands are formed by glacial retreat following the last ice age (Cheshire Wildlife Trust 2014).   These habitats contain intact peatlands and are known for their species rich biodiversity and carbon storage (Gaudig and Tanneberger, 2023). These complexes contain wetland habitats of glacial origin and include geographically discrete, lowland meres and peatland sites (Fisher et al, 2009). These mire habitats are inherently dynamic systems (Seppa, 2002). Their development includes changes caused by local factors that may be independent of climate or other regional environmental changes (Foster & Glaser 1986).

Water and its dynamics play a vital role in the functioning of peatlands and soils within these mire complexes which makes them vulnerable to disturbance from erosion and drainage as well as the anthropogenic influences which include tourism and consequent infrastructure construction (roads and trails), pollution from agricultural and industrial practices (such as nutrient loading from sewage sludge application and trace metal toxicity) and pollution from mining (trace metals) which all have a potential to find their way into the soil system (see section 1.3 and section 1.4) and have a detrimental impact on the soil health of such a habitat(Gaudig and Tanneberger, 2023). Sewage sludge is a wastewater product from water treatment plants, and a source of organic matter and nutrients beneficial to crops (EEA, 2024). This sewage sludge is often applied to agricultural soils as a fertiliser. Regular application of sewage sludge within or near peatland and wetland habitats increases the inputs to groundwater, via surface water, of nitrogen and phosphorus to these ecosystems which can lead to nutrient enrichment that can have detrimental ecological impacts to flora and fauna through eutrophication (Verhoeven et al, 2006). Local characteristics, the type of sewage sludge applied and the characteristics of the soil it is being applied to may influence the load of pollutants and resultant risks (EEA, 2024). These types of fertiliser may also contain trace metal elements which may find their way into the soils of these peatland habitats following application to the topsoil (see section 1.2 - section1.4) for which there are strict limits when this agricultural process takes place (see section 1.7).

Anthropogenic influence has resulted in a mosaic of soil types within these mire complexes with traditional peat extraction and oxidation and erosion from agricultural practices resulting in many former peatlands rich in organic material now being extensively mineral soils with lower organic matter content (Tanneberger and Moen, 2023). Consequently, these peat rich habitats face challenges to their biodiversity and sustainability from this anthropogenic influence resulting from economic and agricultural pressures (Moss, 2014).

In Europe, healthy peatlands are being lost faster than the peat can grow with intensive agriculture, peat extraction and forestry being a driver behind this ecological destruction (Gaudig and Tanneberger, 2023) and is the continent with the largest proportional loss of actively accumulating peatlands (Tanneberger and Moen, 2023). These various interactions have the potential to lead to changes in the ecological character of these habitats and result in possible permanent loss of biodiversity and beneficial characteristics thus emphasising the requirement for a sustainable approach to agriculture in these habitats (Wood & van Halsema, 2008). In the UK, 8% of land is made up of peatland which stores approximately 5.5 billion tonnes of carbon (Gaudig and Tanneberger, 2019). One such meres and mosses habitat in England is the Meres and Mosses of the Marches (also known as ‘The Meres and Mosses’). These meres and mosses are located in Cheshire and Shropshire in the UK and are an ecologically diverse cluster of natural wetlands and peatlands (Natural England, 2007). The Meres and Mosses are facing increasing challenges to biodiversity, including afforestation, intensive agriculture and those from diffuse pollution (Duff, n.d). This is despite many of these mire complexes being under protected status from environmental designations such as RAMSAR and SSSI (see section 2.1.2 of Chapter 2). This protected status can bring challenges to agricultural management with various schemes having been set up to encourage sustainable management of agricultural land within these protected areas (see section 2.1.2 of Chapter 2). This priority on sustainability and conservation measures provides additional challenges that do not necessarily apply to agricultural areas not having these environmental protections (Galbraith & Stroud, 2022). These meres and mosses will be examined extensively throughout this thesis with an emphasis on the spatial occurrence and dynamics of trace metals in the topsoil of this wetland habitat. See section 2.1 of Chapter 2 for further details of the research area.

1.2 Trace Metal Chemistry and Terminology

Trace metals are a pollutant of concern in our natural environment due to their persistence, toxicity and their ability to bioaccumulate (Tam & Wong, 2000). Regularly referred to as 'heavy metals', these are metal elements that are known to be high in density and belong to the transition group of the periodic table (van der Perk, 2006). Most of these metals are also called trace elements and/or trace metals, in reference to their low abundance in soils (Haygarth and Jarvis, 2002; de Vries et al, 2002).

The term 'heavy metal' (or 'trace metal') "is applied to the group of metals and semi-metals (metalloids) that have been associated with contamination and potential toxicity or ecotoxicity" (van der Perk, 2006). Semi-metals are "elements that have the physical appearance and properties of a metal but behave chemically like a non-metal" (van der Perk, 2006). It has been argued that these trace metals should be defined in relation to their position in the period table because this position is related to the chemical properties of the trace metal (Sherameti and Varma, 2010; Appenroth, 2010). However, in their report 'Heavy Metals – A Meaningless Term?' the International Union of Pure and Applied Chemistry (Duffus, 2002) state that despite the term "heavy metals" as being widely used, there is no authoritative definition to be found in the relevant literature for this particular term". Some define these metal elements as "a metal with an atomic mass greater than sodium, whereas others define it as a metal with a density above 3.5-6gcm^{-3}" (Van der Perk, 2006). 'Heavy metals' and 'trace metals' can be considered a collective term (usually interchangeable) which applies to the group of metal elements and metalloids with atomic density greater than 4g/cm^3, or 5 times or more, greater than water (Nagajyoti et al, 2010; Hawkes 1997). Rather than their density it is the chemical properties of these trace metals that are thought to be the most influencing factor in their behaviour (Nagajyoti et al, 2010). Common heavy/trace metals include Lead (Pb), Zinc (Zn), Copper (Cu), Chromium (Cr), Nickel (Ni), Tin (Sn) and Arsenic (As) (van der Perk, 2006). Duffus (2002) describes a trace metal as "A metal found in low concentration, in mass fractions of ppm or less, in some specified source, e.g., soil, plant, tissue, ground water, etc.'. For reasons of clarity and conciseness, the term 'trace metals" will be used throughout this thesis.

Lone et al (2008) states that trace metals, "cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another" (Garbisu and Alkorta, 2001; Gisbert et al., 2003; Lion et al, 2016). The Dangerous Substances Directive of the European Union (76/464/EEC) defines dangerous chemicals as those which are toxic, bioaccumulate and are considered persistent (Council of the European Communities, 1976). As trace metals are elements, they cannot be broken down, therefore these toxic metal elements "will persist in the environment.

Unlike many organic pollutants, which eventually degrade to carbon dioxide and water, trace metals will tend to accumulate in the environment" (APIS, 2016). As a result, the EU sludge to land directive (86/278/EEC) sets limits for trace metals in agricultural soils that receive sewage sludge as well as guidelines now being established for these trace metals across the EU and globally (see section 1.7).

1.3 Source, Transport and Deposition of Contaminant Trace metals in Topsoil

As shown in Fig 1.2, trace metals in the environment have both natural sources and those of an anthropogenic origin (Lone et al, 2008). Such sources include mining, industrial effluent and emissions, weathering of the earth's crust, soil erosion, diffuse urban runoff, sewage effluents and agricultural practices to name but a few (Ming-Ho, 2005). Legacy trace metal toxins are also released via sediment disturbance (Ridgway, et al. 2003). The use of chemical pesticides, disposal of industrial waste and excessive use of fertilisers are all sources of agricultural trace metal pollution (Lone et al, 2008).

Topsoil, the quality of which provides habitats for fauna and micro-organisms that enhances a regions biodiversity potential, is a medium for flora to flourish. As a result, the quality of the soil is a vital parameter in the production of food, timber and fibre (Sabo et al, 2014). "The topsoil serves as the transfer interface for the accumulation of trace metals in the air, plant, and water" (Sun and Chen, 2016). Trace metals concentrations in topsoil is primarily dependent on the composition of the geological parent material (De Temmerman et al., 2003). Other factors also influence trace metal concentration in topsoil, including rainfall variability, leaching, dissolution, volatilisation, vegetation uptake, and other associated processes (Sun and Chen, 2016; Cao and Ikeda, 2000). However, normal agricultural practices can cause enrichment of these trace metals (Errecalde et al, 1991; Mantovi et al, 2003). In agricultural habitats, where animal farming and other agricultural practices can be intensive, trace metals can also reach the soil due to application of liquid slurry and soil manure (such as sewage sludge) or inorganic fertilisers (Khanlari and Jalali, 2008; Mirzae et al, 2015). These common agricultural management measures are an important source of trace metals, including Cu and Zn (Nicholson et al., 2003; Rodriguez et al, 2008). DEFRA in the UK state that "Spreading organic (e.g. composts, manure, sewage sludge) and inorganic materials (e.g. recycled gypsum from waste plasterboard) materials to land is an important part of increasing levels of organic matter in soil, reducing fertiliser requirements and diverting materials from landfill. It can have important agricultural and ecological benefits. However, on occasion, these materials can also contain low concentrations of pollutants, especially trace metals, which can accumulate in soil following repeated applications. This could pose a risk to human health and the environment" (DEFRA UK, 2009; Nicholson and Chambers, 2007).

Trace metal transport in the atmosphere is also an important factor in the distribution of trace metals in topsoil (Melaku et al, 2008). Studies have found that trace metals transferred by the atmosphere are up to three orders of magnitude greater than those transported through local geogenic processes within the parent material (Aelion et al, 2009). In a major study of trace metal inputs for England and Wales, Nicholson et al (2006) found that, "Across the whole agricultural land area, atmospheric deposition was a major source of metals ranging from 8 to 85% of total inputs, with livestock manures and biosolids also important sources as a result of the large quantities of these materials applied" (Nicholson et al, 2006). Combustion processes are the most important sources of trace metals via atmospheric transport. Power generation, smelting/steel production, and the internal combustion engine from automobiles all heavily contribute to sources of trace metal pollution in the topsoil (APIS, 2016; Duce et al, 1991; Galloway et al, 1982; Hutton & Symon, 1986; Nriagu, 1989).

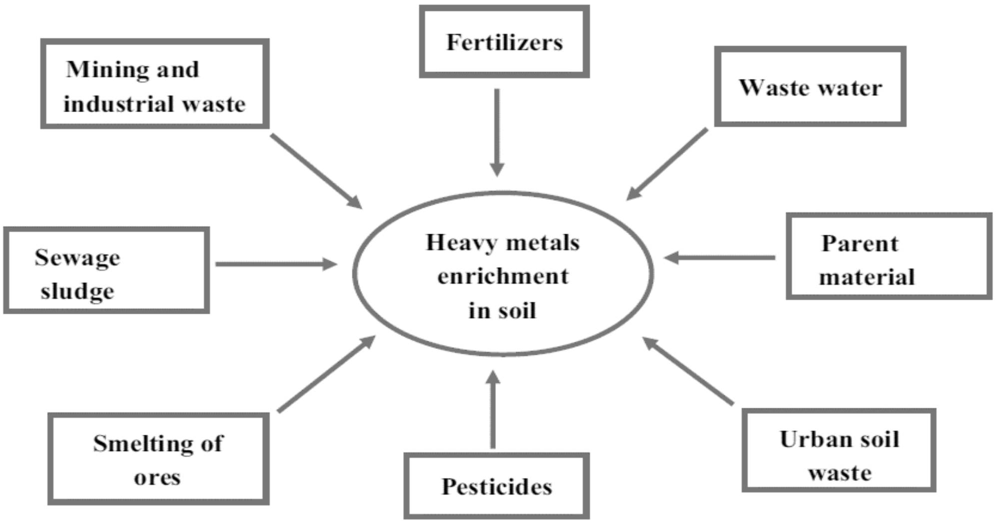

Fig 1.2: Sources of Trace Metals in Soil (Mahar et al, 2016)

Trace metal accumulation in the topsoil is, therefore, subject to many varying factors. Primarily, it is thought that the physical and chemical properties of the soil, such as levels of organic matter and pH, are directly correlated to the solubility and mobility of the trace metals within the topsoil (Sehube, 2020; Chuan et al, 1995; Sukreeyapongse et al, 2002). The properties of the trace metal element itself and the seasonal climatic conditions within a particular region also play a significant role in the behaviour of both the accumulation and the mobility/solubility of the metal element

(Davis et al, 2009; Candelone et al, 1996). As Lone et al (2008) states, “Each source of contamination has its own damaging effects to plants, animals and ultimately to human health, but those that add trace metals to soils are of serious concern due to their persistence in the environment and carcinogenicity to human beings” (Lone et al, 2008).

1.4 Trace Metals in the Topsoil of an Agricultural Environment and their Pathways

Spatial variations of trace metals in the topsoil are of increasing concern globally (Pathak et al, 2013). The topsoil acts as a transfer medium for trace metals accumulation in the air, plant, and water (Sun and Chen, 2016). Trace metals and their spatial distribution in topsoil is largely influenced by natural sources such as weathering/leaching and anthropogenic activity such as mining, production and domestic sources (Wang et al, 2012). The concentration of these trace metals in the topsoil is determined by pedogenic processes and the parent materials. However, the anthropogenic input of trace metals in various regions is becoming the primary source of trace metals in the topsoil (Davis et al, 2009). Agrochemicals and organic fertilisers, such as manure, can bring additional trace metals into the topsoil (see section 1.3) in agricultural land (Grant et al, 2013). In agricultural landscapes, accumulation of trace metals is associated with land use and crop rotation methods (Sun and Chen, 2016).

Unlike urban areas, in which it can be said that trace metals are mainly derived from anthropogenic sources such as those from industry, vehicular emissions and by-products (Guagliardi et al, 2012), the trace metal concentrations in the topsoil of agricultural areas are significantly impacted by natural conditions such as rainfall, leaching, dissolution, plant uptake, and other associated processes (de Vries and McLaughlin, 2013). Climatic conditions, such as rainfall and the impacts of weathering have been found to have an impact on the accumulation of trace metals in the topsoil (Davis et al, 2009). In addition to these anthropogenic, pedogenic and natural inputs/factors, solubility and mobility of trace metals are correlated to physical and chemical properties of the topsoil (Sukreeyapongse et al, 2002) such as pH and organic matter (see section 1.6). It is important, therefore, due to these varied sources shown in section 1.3, to determine potential receptors. It is also necessary to determine the pathways of transfer of trace metals from soil to potential receptors in order to properly manage polluted soils.

The partition of the trace metals between the solid phase and solid solution phases greatly influences the bioavailability of the trace metals (Ayoub, 2000). Robinson et al (2005) state that, “Water is the vehicle for transport of solutes, including trace metals, through soil. Although solubility is a prerequisite of mobility, various rate-limited or kinetic geochemical and hydrological processes in the rhizosphere affect the transport of trace metals" (Robinson et al, 2005). The importance of the trace metal content of soil solution as a major controlling factor in the bioavailability of trace metals is widely acknowledged (Jackson and Alloway et al, 1992; Boekhold et al, 1993; Kabata-Pendias, 1995). Sources of trace metal inputs are variable dependent upon the type of agricultural systems and where they are located (Haygarth and Jarvis, 2002). In grazing habitats, agricultural feed contributes most to the inputs of metals such as Cu and Zn, whereas animal manure is the most important contributor to the input of those trace metals on arable land (Haygarth and Jarvis, 2002; de Vries et al, 2002). Haygarth and Jarvis (2002) state that, "Major pathways are soil to solution transfer (mobilisation) followed by plant uptake (see Fig 1.3) and leaching to groundwater and surface water (see Fig 4). Soil erosion may also cause trace metal transfer to surface water" (Haygarth and Jarvis, 2002).

(Fig 1.3: Transfer of Trace Metals in Topsoil (Efremova and Isozimova, 2012)

Due to the risks and consequences of trace metal contamination in topsoil, it is important to identify the receptors and the pathways of the trace metals from soil, including topsoil, to the receptors (Groenenberg, 2011). Fig 1.4 gives an example of receptors and pathways. Some examples of pathways are the intake of metals via water uptake (such as drinking water) and food crops in the general population. In-situ organisms in the topsoil take up trace metals via soil solution intake and ingestion of the soil (Vijver et al, 2003). When studying the major trace metal pathways in agricultural ecosystems, it can be seen that soil to solution transfer is a major factor as well as plant uptake, groundwater leachate and leaching to surface water. Erosion of the topsoil may also cause trace metal transfer to surface water (Haygarth and Jarvis, 2002). The soil solid phase contains the largest pool of trace metals. Despite this it is the trace metals in the solution phase that may impose the greater risks with multiple pathways within the system to find a receptor (Fig 1.4).

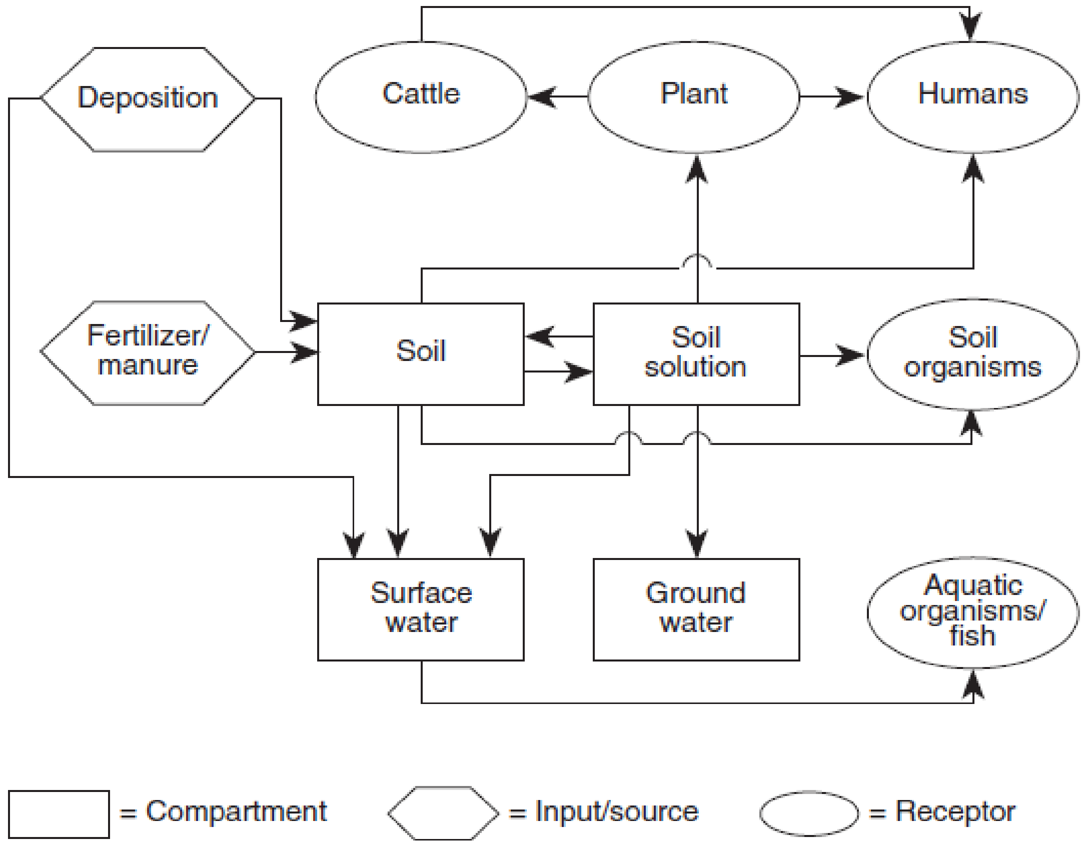

Fig 1.4: Overview of Major Pathways of Trace Metals in Agroecosystems (Haygarth and Jarvis, 2002; de Vries et al 2002).

Localised topsoil quality plays an important role in the quality of water in the local environment. Natural precipitation (rain etc..) runs into reservoirs following contact with the topsoil via surface runoff and runoff that occurs just beneath the surface via subsurface runoff. It can be said, therefore, that solid solution partitioning, which leads to the transfer of trace metals from the solid phase to the solution phase, is an important process when considering the impacts of trace metals in topsoil pollution (Groenenberg, 2011).

1.5 Geochemical Partitioning and Contaminant Trace Metal Mobility

Geochemical partitioning of trace metals has become a fundamental theme of modern geochemistry (Lastovicka, 2009). Trace metals are potentially toxic to the environment. Trace metal toxicity depends on chemical associations within the soil. As a result, the toxic impact of trace metals are determined both by their concentration and in what form they take within the topsoil (Nicholson et al, 2006). Depending on the geological and mineralogical constituents of a particular site, trace metals, including their speciation and spatial distribution, can become associated with various geochemical phases within the soil itself. Geochemical partitioning of trace metals and their behavioural patterns of these trace metals have been found to change spatially with depth (Lynch, 2015; Hurkamp et al, 2009). The determination of the chemical form of a metal in soil is, therefore, important so to evaluate the mobility and bioavailability of the trace metal. To more fully understand the dynamics of trace metals in agricultural and natural ecosystems, it is important to be able to identify the forms of trace metals in the soil (Alloway, 1992). Partitioning occurs between solid/water, air/water and air/solid interfaces, including minerals and soil particles. The Chemical species of a trace metal in the environment may be dissolved in aqueous solution. Since the total trace metal concentration provided by some analytical techniques does not provide information on trace metal mobility, the bioavailability or the source of the trace metal element, geochemical partitioning methodology is a fundamental aspect in determining bioavailability of trace metals in the topsoil and with their association with the different fractions present (Sabo et al, 2014).

For soils, the chemical fractionation study is the process of identification and of determining the amount of trace metals in phases within the soil profile, such as carbonates, oxides, organic matter and others (Silwana. et al 2017). These trace metals may be adsorbed to mineral surfaces or particulate matter in the topsoil via water or air medium and may be extracted in sequence (Jignesh et al, 2012). Sequential extraction analysis is an established method used in soil pollution studies, particularly that proposed by Tessier et al. (1979), as the procedure may be used as a first approach to assess potential phytoavailability and bioavailability within the soil (Chlopecka et al, 1996). This sequential extraction process aids in understanding the behaviour of these metal elements in the environment. It is important to determine if a particular trace metal is likely to remain in aqueous solution, precipitate in solids, adsorb to surfaces, be taken up by organisms, volatise into the air, change oxidation state, bind to another species, or break down into something else (Lastovicka, 2009). This geochemical partitioning can be determined in a laboratory setting using a chemical fractionation method for a sequential extraction study (see Fig 1.5). This analytical procedure involves the addition of increasingly and consecutively aggressive acids/reagents and thus extracting conditions to the soil sample in question (Rao et al., 2008). These reagents for the extraction of metals can range from relatively neutral deionised water to highly concentrated acids (Poulton and Canfield, 2005). The extraction reagent is chosen by which specific fractions is being studied. Sequential Extraction techniques are now established as being used for determining geochemical partitioning in the solid phase in the topsoil (Sohlenius and Öborn, 2004).

Trace metals in the a-horizon and topsoil are found in multiple fractions (Fig 1.5). These are the exchangeable, carbonate bound, Fe/Mn bound, organic matter bound and the residual fractions (Tessier et al, 1979; Sebasthiar et al, 2005). The most mobile of these fractions are the exchangeable and carbonate fractions which are highly bioavailable. Increasingly acidic conditions are required to leach out the remaining fractions including those trace metals bound to their mineral forms which are resistant to environmental flux and thus remain unavailable for uptake (Sebasthiar et al, 2005).

This sequential extraction technique shows that the risk of trace metal transfer from the topsoil to a receptor is high if it is bound to exchangeable fraction (see section 1.4). The risk decreases when the trace metal element is mainly found in either carbonate, Fe/Mn or organic fractions with very little risk being posed when the metal element is found to be bound to the residual fraction (Rao et al, 2008).

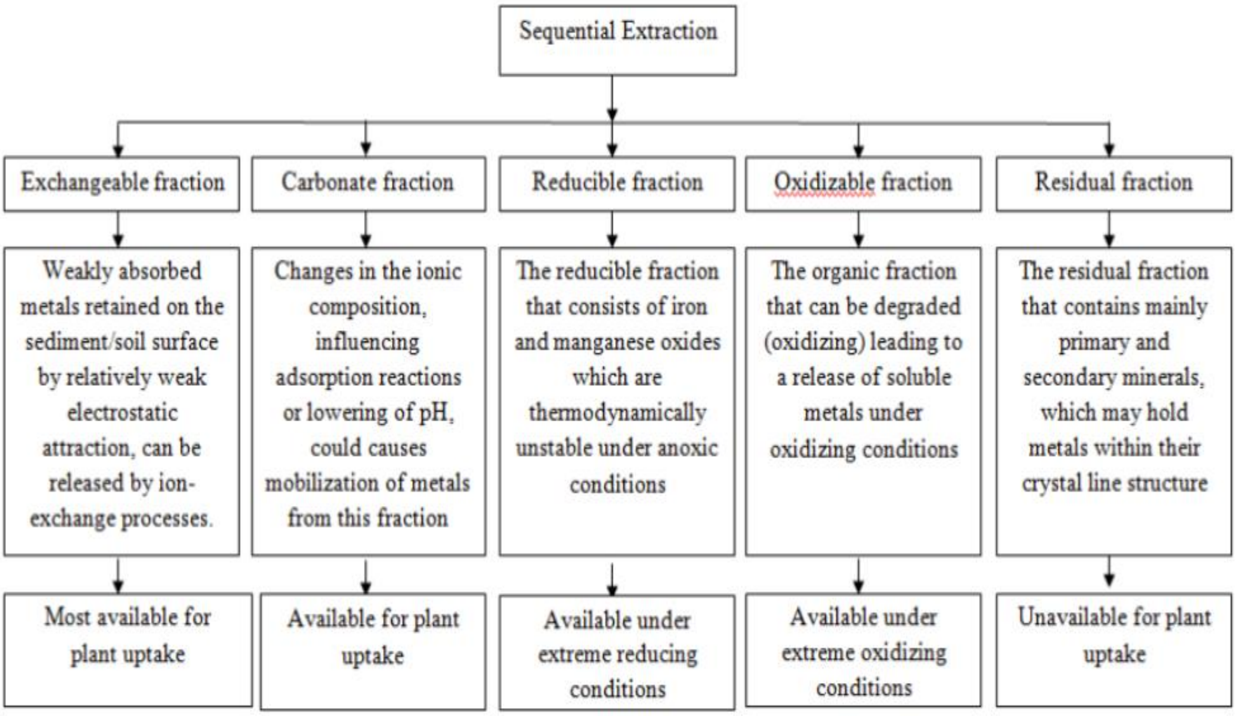

Fig 1.5: Summary of the sequential extraction method for metals, as applied to the analysis of topsoil samples (Singh and Kalamdhad, 2013)

Changes in the environmental conditions and seasonal variability could potentially alter trace metal bioavailability when the trace metal is associated with other geochemical phases (Byrne et al, 2009). Further study of how trace metals are bound within the topsoil will elucidate their current and potential mobility within agricultural topsoil. This will allow for improved remediation techniques and planning so to minimise the adverse effects caused by trace metal mobilisation in agricultural landscapes (Claff, 2011). The principles of sequential extraction and speciation will be covered in greater detail in Chapter 2 and Chapter 6 of this thesis.

1.6 The role of Organic Matter and pH in Trace Metal Mobility in Topsoil

The ability of the trace metal to bind to the soil fluctuates across different soils due to the differences in soil properties (Allen 2002) including pH and organic matter content. It can be considered that the partitioning of trace metals between the solid phase and solution phase can reduce trace metal bioavailability but that this aspect can vary due to seasonal fluctuations (Nieminen 2005, Allen 2002, Sauvé 2002). The interconnection of trace metals with soil phases and their predilection for each component of the soil (see section 1.5) is vital when determining their behaviour in soils (Kabata-Pendias, 2001).

The pH of topsoil and the texture of the topsoil itself are significant factors in the mobility of trace metals in the topsoil, with many trace metal elements (in free-ionic form) being most mobile in acidic soils (McBride, 1994). Soil pH, as a result, is of a fundamental concern in rural regions and those with agricultural land when considering the geochemical processes within agricultural landscapes. Sewage sludge is used as a fertiliser within agricultural areas, which may alter the pH of the topsoil at a local level, as well as soil liming in acidic areas which are sensitive for agricultural use.

Organic matter can potentially impact and increase metal mobility within the topsoil (see Fig 1.6) by forming humic acids via litter decay and thus lowering topsoil pH and forming complexes with trace metals (Renella et al., 2004). Increasing amounts of humic and organic acids on and within the topsoil may also impact trace metal desorption, solubility, and mobility (Krishnamurti et al., 1997; Khodadoust et al., 2005; Schwab et al., 2005).

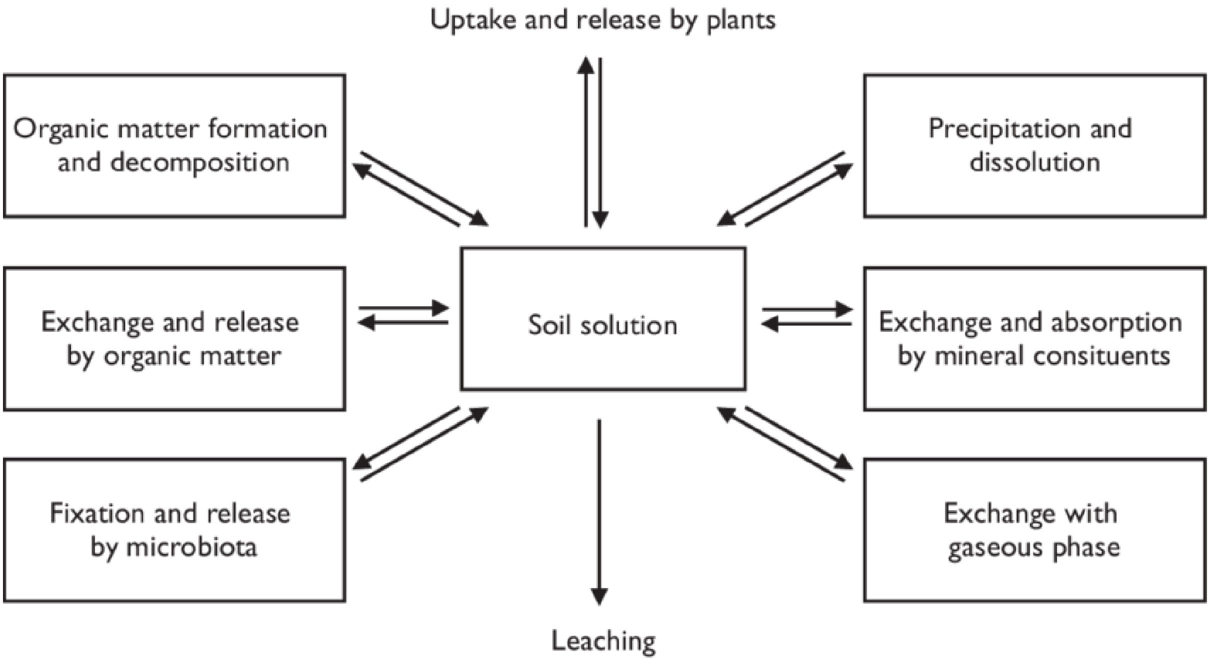

Fig 1.6: Diagram of important interactions in the soil system (modified from Kabata-Pendias 2001, Nieminen 2005)

Plants and vegetation are a primary method of environmental reclamation in respect to the impacts of land degradation via wind/water erosion and reducing downward translocation of contaminants (Kansas State University, 2002). Translocation of trace metal contaminants from roots to above the soil surface is an important process in an effective implementation of using vegetation to aid in remediation of polluted sites (Zacchini *et al*, 2009). As a result, vegetation is used frequently in the remediation of soils, especially in the abatement of the nutrient pollution of Nitrate and Phosphate via vegetative buffer strips (Saleh et al, 2017). Many agricultural regions contain vegetation buffer strips to attempt to alleviate such diffuse nutrient loadings. Vegetative Buffer strips composed of grass and/or trees can improve porewater quality by physically trapping the constituents of soil pore water, sediments and associated pollutants, and by immobilising soluble nutrients through plant uptake or microbial degradation (Randall et al, 2015). However, the impact of these plants and consequent increase in organic matter and humic acids, via litter decay, on trace metal transport throughout topsoil and the a-horizon is unclear. The impact of organic acids exuded by plants and microorganisms on trace metal transport is complex and likely is dependent upon specific trace metals, organic acids and environmental conditions (Schwab et al, 2008). Organic matter, such as leaf litter, plants and vegetation, may increase trace metal leaching through interactions with organic acids in the rhizosphere exuded by plant roots, produced by microbial activity, or as a result of decomposing soil organic matter (Schwab et al, 1989). The roots of plants have also been found to influence the mobility of trace metals in topsoil/a-horizon by reducing sorption, increasing solubility, enhancing bioavailability, and mobilising trace metals in soils (Krishnamurti et al., 1995, 1997; Liao and Xie, 2004). These interactions have potential to vary pH of the topsoil. Soil pH and soil organic matter are considered to be controlling factors in trace metal mobility within the topsoil with trace metals being most mobile in acidic soils (McBride, 1994). "The complexation of trace metals with dissolved organic matter in the environment also influences the mobility of these trace metals" (Weng et al, 2002).

Factors influencing trace metal adsorption within the soil solution include variables such as pH and the organic matter content of the soil (Alloway, 1995). These conditions are subject to change due to land-use and environmental variability. As a result, trace metal mobility may also change as a function of time (such as seasonal variation). Organic matter is a fundamental property associated with the topsoil that can adsorb trace metals such as Copper (Cu) and Cadmium (Cd), especially if the soil is acidic (Stevenson, 1982). Dissolved Organic Matter (DOM) may also bind trace metals such as copper (Benedetti et al, 1995, Temminghoff et al, 1994). Copper-DOM complexes, for example, are known to increase copper mobility as well as bioavailability (McCarthy et al, 1989). As a result, there is likely to be variation of pH across the various sites sampled within regions of agricultural soil and those studied within this thesis that is geogenic in origin but may also be influenced by anthropogenic factors. This may be a significant factor in regard to the fate of trace metals when studying these sites. Further details on the impact of pH and organic matter on trace metal dynamics in the topsoil can be found in Chapter 6 of this thesis.

1.7 Soil Quality Guidelines Overview

There are several approaches that countries in Europe chose to define risk level concentrations of trace metals in agricultural soils and topsoil (Toth et al, 2016). The terminology associated with these risks include 'target', 'intervention' and 'limit' values (Carlon et al, 2007). Most prevalent in academic study are the often cited 'Dutch Intervention Values' and 'Finnish legislation for contaminated soil' as well as the EU sludge to land directive (86/278/EEC), (Carlon et al, 2007). These are guidelines appropriate, under certain circumstances, for some trace metals in agricultural soils. The Dutch Target and Intervention Values (see Table 1) are derived from the Netherlands Soil Contamination Guidelines (Department of Soil Protection, 1994). Dutch Standards are environmental pollutant reference values, such as for trace metal concentrations in soil, are often implemented in remediation strategies for trace metal toxins in the soil. These guidelines set targets and/or thresholds for those metals most associated with anthropogenic input. They set out two sets of values which are classified as ‘target’ values and ‘intervention’ values. Target values for the topsoil quality are those which are required for sustainability and are a related to negligible risk for ecosystems or those required for the restoration of the soil’s functionality for flora and fauna in the local environment. The target value is determined to be approximately 1% of the Maximum Permissible Risk (MPR) level for a particular ecosystem, where MPR is the concentration expected to be hazardous for 5% of the species within that ecosystem (Swartjes, 1999). The Intervention values indicate the level of soil quality in which the functionality of the topsoil for local flora and fauna is potentially seriously impaired. Concentrations in exceeding these intervention values indicate potential for serious contamination (Wuana and Okieimen, 2011). This ecological Intervention Value is the concentration where “50% of the potentially present species and processes may experience negative effects” (Dutch Target and Intervention Values, 2000). Remediation and restrictive action may be required following further investigation at sites that exceed the target and intervention value concentration limits. Environmental contamination in the soil is considered to be 'serious' if >25m^3 of the soil is contaminated above the intervention value (Dutch Target and Intervention Values, 2000). It is important to note that the figures displayed in Table 1.1 are based on a standardised soil with 10% organic matter and 25% clay (fine fraction/lutite). These values will be recalculated according to the organic matter and clay content of the topsoil under investigation in this thesis as per the methodology set out in the annexes of the Dutch Target and Intervention Values (see Appendix F). The ‘Target values’ introduced here are considered background values as acknowledged in the Soil Remediation Circular 2013 of the Dutch authorities (Rijkswaterstaat Environment, 2013).

Metals	**Topsoil**	
	Target/Background value (mg/kg)	**Intervention value (mg/kg)**
Antimony (Sb)	3.0	15
Arsenic (As)	29.0	55.0
Barium (Ba)	160	625
Beryllium (Be)	1.1	30
Cadmium (Cd)	0.8	12
Chromium (Cr)	100.0	380
Cobalt (Co)	9.0	240
Copper (Cu)	36.0	190
Nickel (Ni)	35.0	210
Lead (Pb)	85.0	530
Mercury (Hg)	0.3	10.0
Molybdenum (Mo)	3.0	200
Silver (Ag)	-	15
Selenium (Se)	0.7	100
Tellurium (Te)	-	600
Thallium (Tl)	1.0	15
Tin (Sn)	-	900
Vanadium (V)	42.0	250
Zinc (Zn)	140	720

Table 1.1: Dutch Target and Intervention Values (Dutch Target and Intervention Values, 2000).

The Finnish government sets out threshold and guide values (see Table 1.2) from the Finnish legislation for contaminated soil (Decree 214/2007) based on aqua regia extractable analytical techniques which is the recommended analytical method by the Decree. Median, 90th percentile and maximum values of European topsoil, as ascertained in the FOREGS Geochemical Map of Europe (Salminen et al, 2005) are also often utilised in conjunction with these guidelines (Salminen et al, 2005; van der Voet et al, 2013), where values shown for As. Sb and Hg are total concentrations (see Table 2) with Cd, Co, Cr, Cu, Pb, Ni. Zn and V being pseudo-total concentrations (aqua regia extractable).

The Finnish standard values represent an approximate of the mean values of the differing national systems in Europe (Carlon et al, 2007; Toth et al, 2016). The Finnish guidelines have been routinely and widely used in international studies and surveys when analysing agricultural soils (UNEP, 2013). According to the Finnish Decree 214/2007 (Ministry of the Environment, Finland, 2007), soil contamination is assessed on the estimated risk to human health and to the environment of a specific site and should take into account the baseline concentrations of harmful substances in the soil. In this regard, the baseline concentration refers to the natural geological background concentration and the diffuse anthropogenic input of trace metals (Jarva et al, 2009) with the current land use and profile (see section 2.1.1 of Chapter 2 for Meres and Mosses characterisation) as well as future land use activities being taken into consideration (Ministry of Environment, Finland, 2007; Jarva at al, 2008). Based on these baseline values, three distinct categories, the threshold value and the lower and the upper guideline value, were introduced in the Government Decree (214/2007). These lower and higher guide concentration values determine the investigative and/or remedial actions required if these values are exceeded. The threshold value is deemed as a trigger value, which "if exceeded indicates the necessity for further investigations on potential contamination" (Jarva, 2016). The second set of concentration level categories is the so-called "guide values". If the guide values are exceeded, the area has a contamination level which is deemed to have an ecological or health risks. Different guide values are set depending on land use with industrial areas and those under intensive threat of pollution being placed under the higher guideline value and with all other land uses being placed in the lower guideline value category (Toth et al, 2016). The Finnish government sets out 'threshold' and 'guide' values from the Finnish legislation for contaminated soil (Decree 214/2007) based on aqua regia extractable (pseudo-total) analytical techniques which is the recommended analytical method by the Decree (Finnish Government, 2007) as laid out in Chapter 5 of this thesis.

Trace Metal Element	Threshold Value (mg/kg)	Lower Guide Value (mg/kg)	Higher Guide Value (mg/kg)	Topsoil Europe Median (mg/kg)	Topsoil Europe 90th Percentile (mg/kg)	Topsoil Europe Maximum (mg/kg)
Antimony (Sb)	2	10	50			
Arsenic (As)	5	50	100	5.0	20	562
Mercury (Hg)	0.5	2	5	0.04	0.1	1.35
Cadmium (Cd)	1	10	20	0.15	0.48	14.1
Cobalt (Co)	20	100	250	7.0	17.0	255
Chromium (Cr)	100	200	300	22	53	2340
Copper (Cu)	100	150	200	12	33	239
Lead (Pb)	60	200	750	15	38	886
Nickel (Ni)	50	100	150	14	41	2560
Zinc (Zn)	200	250	400	48	96	2270
Vanadium (V)	100	150	250			

Table 1.2. Finnish Guidelines for pseudo-total concentration for contaminated soil (Ministry of the Environment, Finland, Decree 214/2007) where values of As, Sb and Hg are total concentrations.

The EU sludge to land directive (86/278/EEC) sets out limits for trace metals in agricultural soils that receive sewage sludge (see Table 1.3a). The purpose of this Directive is to control and regulate the application of sewage sludge on agricultural soils so to prevent negative impacts on the soil flora and fauna. This is to incentivise the appropriate use of such sewage sludge in an environmentally sensitive manner. The use of sewage sludge may be prohibited if found that the concentration of the trace metals in the soil exceeds these limit values (CEC, 1986). The values set out by the EC directive 86/278/EEC are limits for trace metals that prevent further application of sewage sludge to agricultural land (DEFRA, 2018). In relation to the European Commission, The Joint Research Centre, Institute for Environment and Sustainability, Soil and Waste Unit (JRC-Ispra) has previously analysed the currently held data on trace metal concentration in European soils. The JRC-Ispra provide constant independent scientific support and advice to EU policy have previously set further proposals for limit values in regard to concentrations of trace metals in soil (see Table 3a.)

which have been implemented into the directive (Langenkamp et al, 2001). Langenkamp et al (2001) describe the JRC values as those which "establish a basis for discussion how to evaluate data on heavy metals in agricultural soils (especially against the background of the revision of the European sewage sludge directive".

Trace Metal Element	**Limit Values (mg/kg dm)**			
	Directive 86/278/EEC 6<pH<7	**JRC Proposed 5≤pH<6**	**JRC Proposed 6≤pH<7**	**JRC Proposed pH≥7**
Cadmium (Cd)	1-3	0.5	1	1.5
Chromium (Cr)	400	50	75	100
Copper (Cu)	50-140	30	50	100
Mercury (Hg)	1-1.5	0.1	0.5	1
Nickel (Ni)	30-75	30	50	70
Lead (Pb)	50-300	70	70	100
Zinc (Zn)	150-300	100	150	200

Table 1.3a: Trace Metal Limits in Soil as set out by 86/278/EEC (CEC, 1986) and Proposed by JRC-Ispra

The JRC based their guidelines for trace metal contents according to soil pH. Four pH-classes were determined: pH ≤ 5, pH > 5 - 6, pH > 6 - 7, pH > 7 (Gawlik & Bidoglio, 2006). The JRC recommends the use of aqua regia/reverse aqua regia for the digestion of soil materials and the use of analytical determination techniques of a high level of accuracy such as ICP-OES/ICP-MS. This means that the above guidelines are all using 'total' or 'pseudo-total' result (Aqua Regia soluble) dependent on the analytical procedure implemented for determining trace metal concentration. The final report by the JRC, including the proposed guideline limits therein, was implemented as a tool to aid in discussion in regard to the necessity to regulate trace metals in the revision of the European Sludge Directive 86/278/EEC. This report was then distributed among the European Soils Bureau (ESB)-Working group and the cooperating institutions for their input and feedback in early 2004 (Gawlik & Bidoglio, 2006).

In the UK, this EC directive 86/278/EEC is implemented via UK regulations Sludge (Use in Agriculture) Regulations (1989) Great Britain and Northern Ireland 1990) under the Department for Environment, Food and Rural Affairs (DEFRA). In England, Wales and Northern Ireland, under these DEFRA regulations, separate limits are dependent on the pH of the soil and also the land use of the area in question (such as arable farming or grassland) as well as placing a ‘maximum permissible average annual rate’ of trace metals over a 10 year period on land used for arable farming (DEFRA, 2018) as shown in Table 1.3b. Under these regulations, Zinc (Zn), Copper (Cu) and Nickel (Ni) are considered thusly:

Trace Metal Element	**Maximum Permissible Concentration in Soil (mg/kg dry solids) for Arable Farming**				**Maximum Permissible Concentration in Soil (mg/kg dry solids) for Grassland**				**Maximum Average Annual Rate over 10 years (kg/ha) for Arable Farming**
	pH 5<5.5	**pH 5.5<6**	**pH 6.0-7.0**	**pH >7.0**	**pH 5<5.5**	**pH 5.5<6**	**pH 6.0-7.0**	**pH >7.0**	
Zn	200	200	200	300	200	200	200	300	15
Cu	80	100	135	200	130	170	225	330	7.5
Ni	50	60	75	110	80	100	125	180	3

Table 1.3b: Trace Metal Limits for Arable Farmland and Grassland for those who produce or use sewage sludge in England, Wales and Northern Ireland (DEFRA,2018) as per UK regulations Sludge (Use in Agriculture) Regulations (1989)

DEFRA sets out guidance for select trace metal elements and ‘Potentially Toxic Elements (PTEs)’ via UK regulations Sludge (Use in Agriculture) Regulations (1989) Great Britain and Northern Ireland (1990) for pH 5 and above for both land being used for arable farming that receives sewage sludge and grassland (DEFRA, 2018) as shown in Table 1.3c

Trace Metal Element	**Maximum permissible concentration of PTE in soil (mg/kg dry solids) for pH 5.0 and above for Arable Farming**	**Maximum Average Annual Rate over 10 years (kg/ha) for Arable Farming**	**Maximum permissible concentration of PTE in soil (mg/kg dry solids) for pH 5.0 and above for Grassland**
Cadmium	3	0.15	3
Lead	300	15	300
Mercury	1	0.1	1.5
Chromium	400	15	600
Molybdenum	4	0.2	4
Selenium	3	0.15	5
Arsenic	50	0.7	50
Fluoride	500	20	500

Table 1.3c: Trace Metal and PTE limits for select elements on Arable Farmland and Grassland for those who produce or use sewage sludge in England, Wales and Northern Ireland (DEFRA,2018) as per UK regulations Sludge (Use in Agriculture) Regulations (1989)

Studies have shown, however, that environmental quality guidelines and standards based on total concentration of a trace metal may not reflect the actual effects with the pseudo-total from aqua regia digestion providing a superior estimate of the bioavailable fraction of trace metals (Santoro et al, 2017; Andersen and Kisser, 2004). The total concentration of a trace metal does not necessarily reflect the ecotoxicological effects of the trace metals in the terrestrial environment (Allen and Janssen, 2006). Identification of trace metals in phases (speciation) is a vital aspect when considering bioavailability (see section 1.5 and Chapter 6) and ecotoxicology (Silwana et al, 2017) as trace metals within different fractions of topsoil vary considerably in their chemical reactivity, mobility and bioavailability (Tokalioglu, 2004). Chemical environmental factors such as organic matter and pH may also affect the toxicity of a trace metal (Allen and Janssen, 2006). As a result, many studies, such as the 'Technical guidance for deriving environmental quality standards' (Whitehouse et al, 2011) and the **'**Technical guidance to implement bioavailability-based environmental quality standards for metals' (Bio-met, 2015) have shown that guidelines should consider other factors, such as speciation, bioavailability, pH and organic matter, when establishing soil quality guidelines. Further details and implementation of these guidelines can be found in Chapters 4 and 5 of this thesis.

1.8 Conclusion

Trace metals, as Lone et al, 2008 point out, "cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another" (Lone et al, 2008; Garbisu and Alkorta, 2001; Gisbert et al., 2003; Lion et al, 2016). This leads to their persistence in the environment. Trace metal pollution from multiple diffuse sources, such as agricultural and atmospheric, are known sources of anthropogenic trace metal pollution in agricultural soils. Topsoil has been found to be particularly sensitive to these negative impacts from both varying climate conditions and the multiple diffuse sources within an agricultural landscape.

Guidelines have been laid out both in the EU and globally to attempt to set targets and limits for trace metal toxins in agricultural soils including topsoil. Research has shown, however, that 'total' concentrations are not reflective of the ecotoxicity or potential ecotoxicity of these trace metals as they do not fully consider the bioavailability of the trace metal element under various climatic conditions or those trace metals in different fractions of the topsoil. The 'pseudo-total', which is obtained from certain acid digestion methodologies, whilst being more indicative of the bioavailability of the trace metal, may not be indicative of the total amount of the trace metal content in the soil matrices being analysed.

Research has shown that organic matter and pH play a role in trace metal mobility in topsoil. Seasonal/Monthly variations in precipitation may impact both accumulation and mobility of trace metals in the soil, impact pH and levels of organic matter dynamically. This may have an impact on the speciation, pathway and consequent bioavailability of the trace metals in the soil with these trace metal elements and soil fractions being sensitive to disturbance from both natural and anthropogenic sources. How these geochemical, geophysical and seasonal factors contribute to trace metal concentration as a result of these environmental fluctuations over time is currently not fully understood (Lynch, 2015). An understanding of the dynamics involved are important when attempting to elucidate the factors that impact trace metal mobility in the topsoil/a-horizon and how this may be implemented into best practice remediation and management techniques for threatened areas and agricultural landscapes.

1.9 Project Aim and objectives

The overarching aim of this project is to establish the magnitude and dynamics of trace metal pollution in the topsoil of an environmentally sensitive agricultural meres and mosses wetlands habitat. This will be done by determining the current levels of trace metal concentrations in the topsoil of an agricultural meres and mosses region and then to elucidate the mechanisms controlling the mobility of those trace metals from a geochemical, geophysical (such a soil pH and organic matter content) and 'annual environmental changes' perspective. The study will elucidate, by means of multivariate statistical methodology, the current situation in regard to select trace metals within the topsoil of an agricultural and environmentally sensitive region and proceed to ascertain how these trace metals function. The factors controlling trace metal mobility, with an in-depth study on how both pH of the topsoil and the levels of organic matter within the soil matrices impacts on the mobility of the trace metals, will be determined by means of a laboratory controlled mesocosm study. Validation of the analytical procedures provided within this study will be explored in depth with a method development study which will ensure that the analytical process is suitable for the research when analysing multiple trace metal elements simultaneously. A consequent comparison study will compare the outcomes of major analytical methods used within this field of study and within this research project (ICP-MS and FPXRF) and examine the impacts on adherence to soil quality guidelines.

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Within this overall aim, there are several key objectives:

1. To undertake a survey of the topsoil of an environmentally sensitive agricultural landscape via FPXRF so to determine trace metal concentrations and geophysical/geochemical factors in order to obtain a data-set that Identifies the distribution and variation of trace metals in the region sampled, how they adhere to prominently implemented soil quality guidelines and to determining the source of origin of the trace metals in-situ, be it anthropogenic or natural/geogenic by implementation of multivariate statistical analyses (see Chapter 4).

2. Validate an appropriate analytical procedure for the determination of the concentration of multiple trace metal elements simultaneously in a soil matrix via ICP-MS with reverse aqua regia digestion by means of a method development study (see Chapter 3).

3. Elucidate how these differing analytical methodologies for the determination of trace metal concentrations in a soil matrix can potentially impact the outcome of soil quality assessments (see Chapter 5).

4. Determine the link between changes in geochemical and geophysical processes with trace metal speciation, mobility and bioavailability by implementation of a mesocosm study and chemical fractionation/sequential extraction analysis (see Chapter 6).

Elaboration of each key aim and objective will be covered in Chapters 3, 4, 5 and 6 of this thesis. Each chapter of the thesis will cover these key aims with a view of determining how each aspect play a key role in elucidating the current status of an agricultural meres and mosses wetlands habitat in regard to trace metal concentrations, distribution and variation in the topsoil of this habitat and how physiochemical factors influence the spatial occurrence, dynamics and potential for bioavailability of these trace metals within the porewater of this topsoil. This determination of topsoil quality can then be placed into context against prominent soil quality guidelines for trace metals in soil and be used as an aid for conservation, mitigation and remediation in the important task of maintaining the health of these environmentally sensitive habitats.

Chapter 2

Site Background, Field Sampling and Overview of Laboratory Methods

2.1 The Meres and Mosses of the Marches

2.1.1 Background and Site Description.

In the northwest of England lies the environmentally sensitive landscape known as The Meres and Mosses of the Marches (otherwise known as The Meres and Mosses of Cheshire and Shropshire, or simply The Meres and Mosses). This is a wetland area which crosses the Shropshire and Cheshire border in the Northwest of England. The Meres and Mosses of the Marches is a unique landscape in Britain which contains the largest group of lowland lakes and wetlands in England and has “the highest density of ponds in Europe” (Boardman et al, 2012). As described by Boardman et al, 2012, “The Meres and Mosses region covers the North Shropshire, Maelor and Cheshire plain. This area extends from the Mersey Estuary in the north to the Black Country in the south, and from the Welsh Marches in the east to the Potteries” region which is in the north of the geographic county of Staffordshire, England (Boardman et al, 2012). The landscape was created at the end of the last ice age, approximately 15,000 years ago**,** at a time when glaciers were in retreat, resulting in shallow meres and peatland mosses (BBC News, 2012a). As Natural England describe in their work ‘Delivering Landscape Scale Conservation in the Meres and Mosses’, “The glaciers retreated, massive blocks of ice fell into the outwash from the glaciers. In time these melted, leaving behind water-filled basins known as ‘kettle holes’” (Natural England, n.d.) As shown in Fig 2.1).

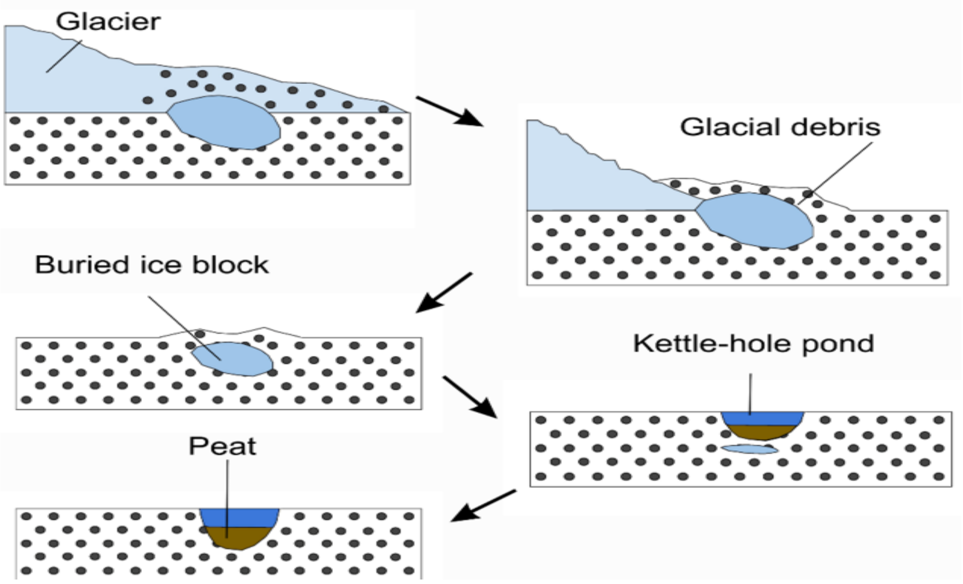


Fig 2.1: Graphic showing the formation of kettle holes (King, 2021)

The meres within the region are the deepest of these kettle holes which today exist as open waterbodies. Mosses are thought to have formed in shallow basins where the accumulation peat and the processes of succession resulted in lowland raised bog formation (Jones, 2016). Lowland raised bogs are discrete peatland entities found within agricultural landscapes (Artz et al, 2012). Lowland raised bogs in the UK have been damaged by intensive drainage and cutting activities since medieval times (Natural England, 2022). When examining the ecological characteristics of the study area (Fig 2.2) we can see that the Meres and Mosses of the Marches are a diverse series of lowland open water and peatland moss habitats. These habitats developed in depressions in the glacial drift created by the receding ice sheets which had previously covered the Cheshire/Shropshire Plain (St. Helens Borough Council, 2021). The Meres and Mosses region comprise of over sixty deep and shallow, relatively small, lowland lakes (Reynolds 1979) while the mosses cover in excess of 900 ha, making them the third largest and one of the most southerly lowland mire complexes in Britain (Natural England 2007). According to Natural England (2022) “Lowland raised peatbogs are one of the rarest habitats on earth with over 96% have been destroyed”. The mosses in the region constitute the third-largest lowland raised peatbog habitats in the UK (Natural England, 2022) which has a fundamental role in carbon storage, which is beneficial to the response against climate change, which makes it of vital importance to regenerate the Marches Mosses and monitor their ecological health (Natural England, 2022).

Fig 2.2: Glacial Landscape of the Meres and Mosses (Jones, 2015)

Because of the relative abundance of the peat bogs in the region, industry in the Meres and Mosses area has been historically based around peat cutting and extraction (Natural England, 2021). This peat extraction has had a negative impact on the landscape in areas such as Whixhall Moss, which show significant signs of scarring (see Fig 2.4).

Despite being heavily associated with agriculture and peat mining/extraction, the Meres and Mosses region also has an industrial heritage. The surrounding towns to the Meres and Mosses region include Wem, Whitchurch and Prees and contain multiple industrial estates. For many centuries, Shropshire was heavily associated with wrought iron manufacture to which the legacy of this industrial process persisted over the decades and into the 21st century (Hayman, 2004). The industrial estate that now occupies the industrial site at Wem provides a rich archaeological record of this industrial past and although some of the land has been returned to agriculture, steel production, fabrication and manufacture continues in this area (ERIH, n.d.). Because of this industrial heritage, despite the conservational importance of the region, the Meres and Mosses are facing increasing challenges to biodiversity, including afforestation, intensive agriculture and those from diffuse pollution (Duff, n.d) such as those by trace metal accumulation.

The Meres and Mosses, with their unique geological landscape within mean that they are considered one of the world's most productive environments that are essential for biological diversity due to their inter-connected network of meres, mosses and catchments (Jones, 2015).

When examining the connectivity of these habitats (see Fig 2.3), the Meres and Mosses of the Marches are considered to be "geologically 'fixed' and hydrologically isolated from one another" (Jones, 2015) with wetland and peatland sites being clustered in the landscape (Jones, 2016). Most of the lakes and meres are isolated from surface inflows/outflows and are fed by lateral flows of groundwater (Reynolds, 1997). Many of these meres and mosses are fed by groundwater in permeable sands and gravels (Environment Agency, 2023). Despite their geologically isolated features, when contextualised within the catchment features of these meres and mosses landscapes, such as surface water and groundwater that feeds these mosaic meres and mosses habitats, they can be considered an interconnected functional ecological unit (Jones, 2016). However, these wetlands have been degraded by drainage, inappropriate management and the impacts of poor water quality resulting in a modification of the water balance and fragmentation of this interconnectivity leading to pressures on biodiversity and soil health (Jones, 2015; Reynolds, 1997).

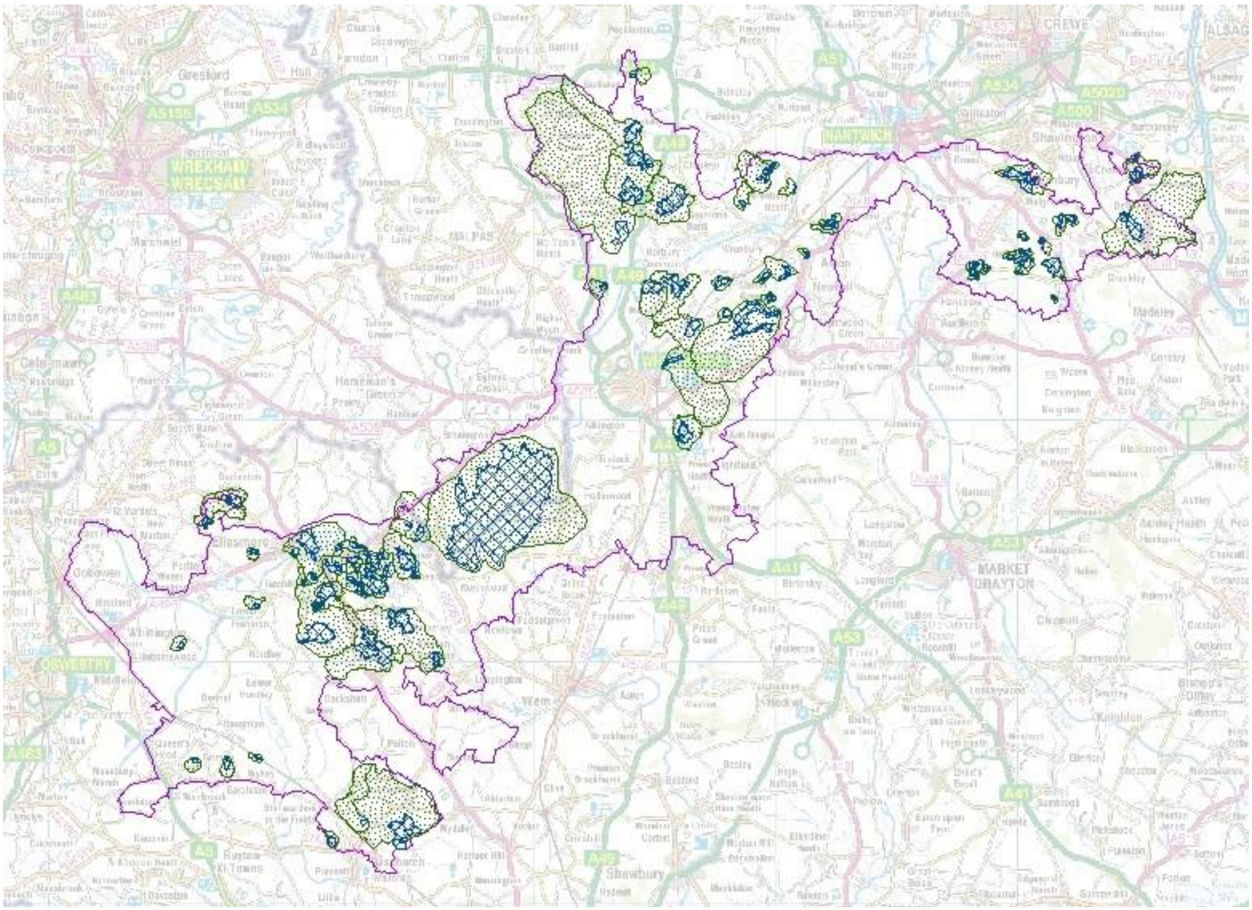


Fig 2.3: Interconnected network of Meres, Mosses and Catchments in the Meres and Mosses Region (Jones, 2015)

Because of their ecological importance, the region has multiple environmental designations with the aim to appropriately protect and conserve the sensitive region (see section 2.1.2). Due to these varying environmental designations, it is important to ascertain the current situation in regard to trace metal content of the Meres and Mosses region. Previous studies have focused on nutrient pollution in the region (Fisher et al, 2010; Kilinc & Moss, 2002; Maberly & Carvalho, 2010; Moss et al, 2005). As trace metals are becoming a toxic pollutant of increasing environmental concern (see section 1.2 of Chapter 1), a trace metal and physiochemical screening program was performed via Field Portable X-ray Fluorescence Spectrometry (FPXRF) and laboratory analytical methods to garner further information of the study area (see section 2.4.2 and Chapter 4).

2.1.2 Environmental Designations:

The Meres and Mosses of the Marches within Cheshire and Shropshire are part of the 'Natural Areas' programme as set out by Natural England. Within this 'Natural Area' are locations classified as a National Nature Reserve (NNR). "A National Nature Reserve (NNR) is the land declared under the National Parks and Access to the Countryside Act 1949 or Wildlife and Countryside Act, 1981" (Gill and Matthews, 2021). They were set up so to protect important habitats for wildlife and to conserve geological formations in Britain as well as establishing areas for scientific research (Dorset Council, 2023). In the region, Whixall Moss, Bettisfield Moss, Fenn's Moss (see Fig 2.4), Wem Moss and Brown Moss (see Fig 6) have all been established as NNRs so to protect these important habitats (Natural England, 2009).

The area known as the Meres and Mosses was established in 1991 with most of the boundary being owned or leased by Natural England, Natural Resources Wales and Shropshire Wildlife Trust. The remainder has been determined to be in private land ownership (Natural England, 2022).

Fig 2.4: Aerial View of Fenn's, Betisfield and Whixhall Moss Showing Signs of Scarring (Marches Mosses BogLIFE, 2022)

The Meres and Mosses consist of Sites of Special Scientific Interest (SSSI) and areas that have been Ramsar Site designated. SSSI sites are designated by Natural England within the Meres and Mosses region. Natural England describes an area that is of "particular interest to science due to the rare species of fauna or flora it contains - or even important geological or physiological features that may lie in its boundaries" as those worthy of SSSI status (Natural England, 2020; Woodland Trust, 2019). SSSI sites are considered to be those in need of environmental protection due to their high conservation value (Natural England, 2020; Woodland Trust, 2019). Ramsar sites are wetlands (and associated riparian and coastal zones) of international importance, designated under the Ramsar convention in 1971 (Goodwin, 2017). Areas designated as Ramsar sites are considered of vital importance to biodiversity and, as such, are of high priority for conservation. There are also candidate Special Areas of Conservation (SAC) under the European Habitats Directive (European Commission Council Directive 92/43/EEC) within the region.

The Meres and Mosses of the Marches have been designated as a Nature Improvement Area (NIA) and the region is one of only twelve such designated sites in England. Nature Improvement Areas (NIA) were established to create ecological networks at a local landscape scale. They are run by multiple partnerships of invested groups, including local authorities, local community groups/landowners, conservation groups and the private sector with funding supplied by Natural England and the Department for the Environment, Food and Rural Affairs (DEFRA) as per the details set out the Natural Environment White Paper (DEFRA, 2011). The Meres and Mosses nature reserve region was awarded £500,000 due to being designated as a Nature Improvement Area as part of a £7.5m government scheme (BBC News, 2012b). The area has also previously secured Heritage Lottery Fund (HLF) funding for a Landscape Partnership Scheme (see Fig 2.5). A Landscape Partnership Scheme (LPS) aims to engage multiple interested parties by engaging them with their local landscape. This LPS led to the establishment of educational facilities (including Wood Lane Learning Centre and Bickley Hall Farm Learning Centre) with the aim of engaging schools, universities and the wider community whilst educating on the role of these environmental designations (Hayek, 2018). Clarke et al, 2011 state that "Landscape Partnerships seek to conserve the landscape heritage, natural and cultural, and also to deliver 'people' benefits within and beyond the areas they cover, which are more than those that would derive from funding a series of separate projects". The overarching aim of this scheme was to bring communities and organisations together with a view of environmental engagement within a local community. The Marches Meres and Mosses Nature Reserve received £973,000 of lottery money in 2012 to aid this scheme (BBC News, 2012b). The area is now part of the 'BogLIFE project' which aims to "restore Britain's third-largest lowland raised bog within the Fenn's, Whixall & Bettisfield Mosses and Wem Moss NNRs near Whitchurch, Shropshire and Wrexham in Wales" (Natural England, 2022). This BogLIFE project is a 5-year project overseen by Shropshire Wildlife Trust, Natural England and Natural Resources Wales with the aim being to restore 665 hectares of the Marches Mosses. This is in addition to the improvements made during the lifespan of the Meres and Mosses Project, which ran prior to the Boglife Project (Shropshire Wildlife Trust, 2022).

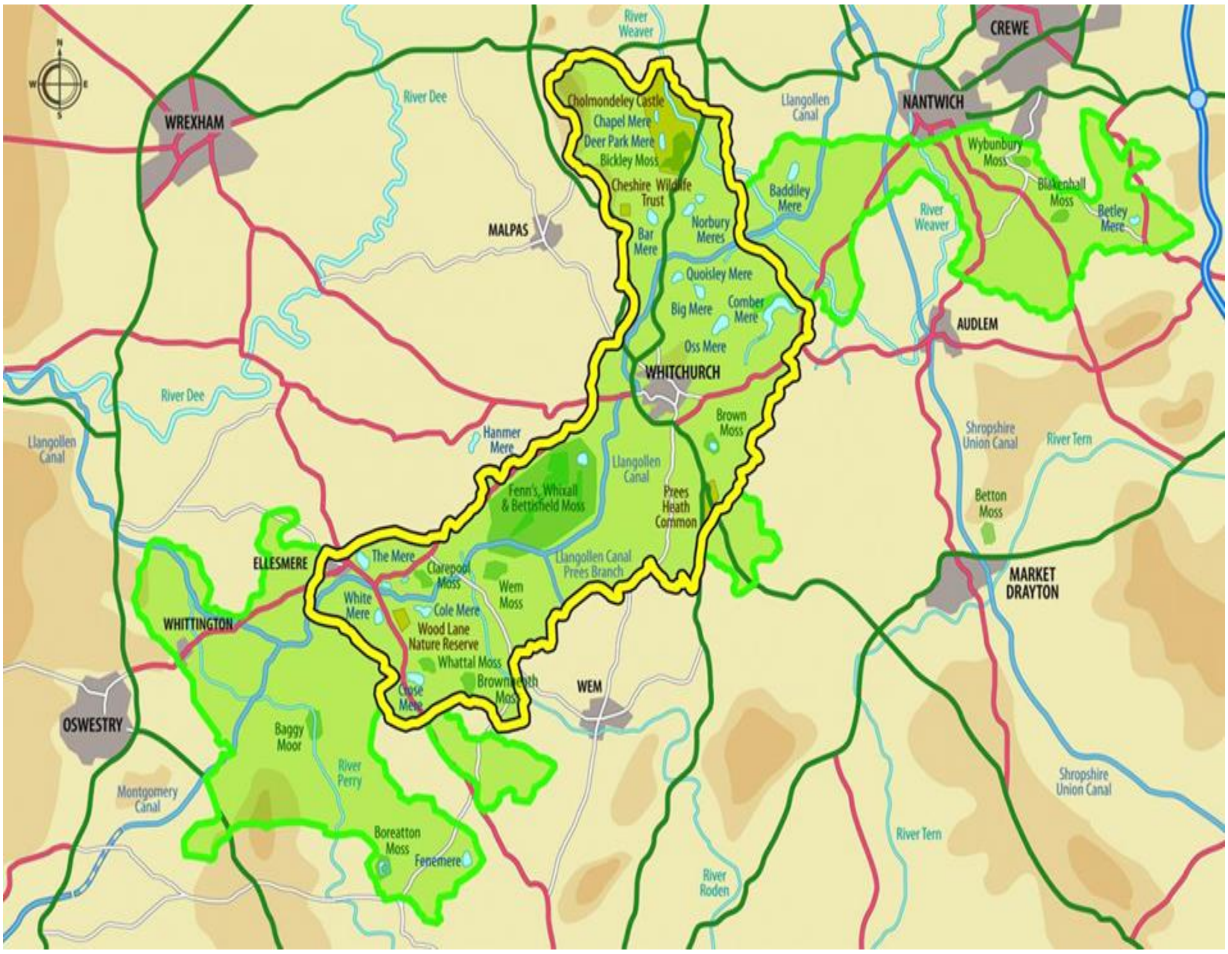


Fig 2.5: The Boundaries of the NIA (green) and LPS (yellow) schemes (Cheshire Community Action, 2014)

One such site within the NIA/LPS region is Brown Moss (Fig 2.6 and Chapter 6). Brown Moss is a Site of Special Scientific Interest and part of a Ramsar site consisting of several small and shallow pools which contain important flora and fauna, including aquatic birds, migrant and exotic species. As such the area is popular with the public as a tourist attraction and for those with interests in conservation (Chaichana et al, 2010; Whild, 2007).

Fig 2.6: Brown Moss SSSI/Ramsar Site (Charles Leventon, CC BY-SA 2.0)

The site at Brown Moss and the surrounding Meres and Mosses network are, as Chaichana et al (2010) point out, “Susceptible to other pressures, including diffuse nutrient loading from surrounding land and septic tanks, water level fluctuation and atmospheric pollution”. These factors have aided in causing dwindling flora biodiversity (Whild, 2007). As a result, it is important to conserve these areas designated as being environmentally sensitive. This site is examined more closely during the mesocosm study to ascertain bioavailability and risk of trace metals within the region (see section 2.4.4 and Chapter 6).

2.1.3 Climate Data Rainfall Characteristics in the Meres and Mosses of the Marches

The closest available climate data for the region is from the town of Shawbury (co-ordinates: 355874, 321283). Data obtained from the Shawbury Climate Station provided the average monthly amount of rainfall in the Meres and Mosses region for nearly a 30-year period (1981 – 2010). The location of this site is at the RAF Base (52.794, -2.663 or Lat/Long 52°47'38.4"N 2°39'46.8"W) which is in Shrewsbury, UK (see Fig 2.7). This climate station falls within just approximately 5 miles South from the sampling region. The climate station is 72m above sea level and is classified as an ‘observing station’ (metoffice.gov.uk).

Fig 2.7: Location of Shawbury Climate Station in context to Shropshire and the UK (Nilfanion, 2010)

The data from this climate station shows that the average annual rainfall for the region between these dates is 659.9mm (see Fig 2.8). This is lower than the average in England over the same period of 854.8mm (Met Office, 2021). The 80th and 20th percentile figures are included so to represent the wettest and dryest periods (see Fig 2.8).

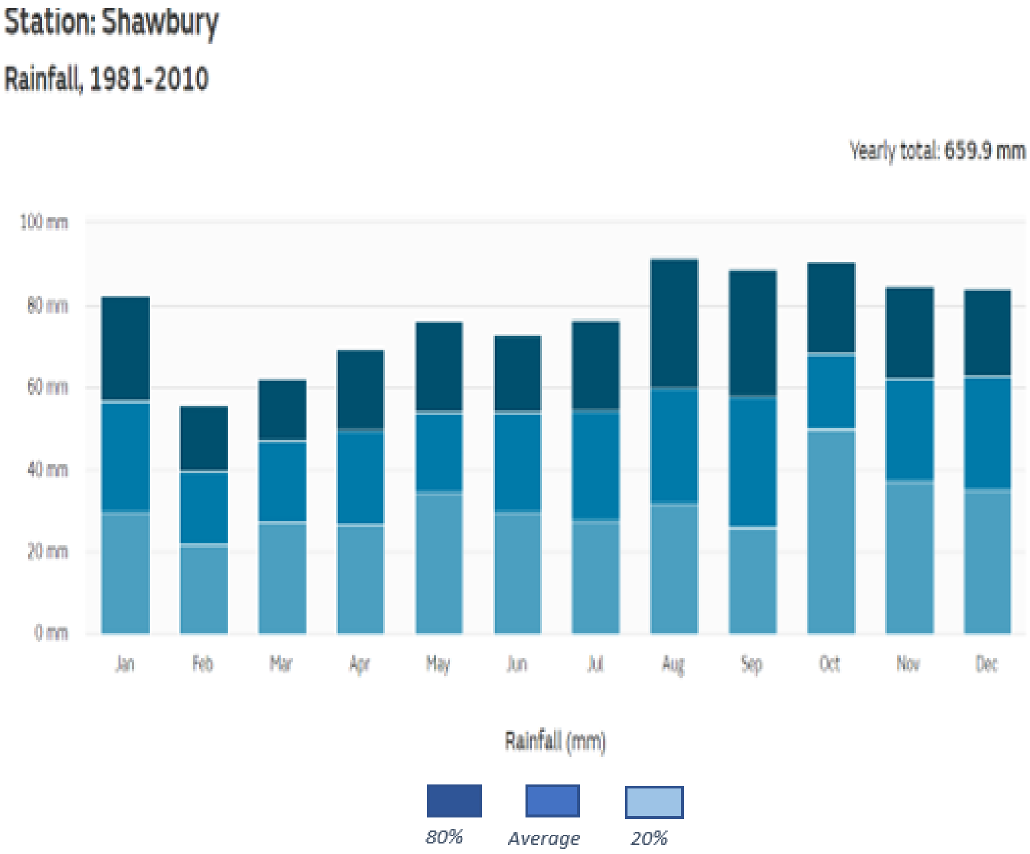


Fig 2.8: Chart of Average Monthly Rainfall for Meres and Mosses Region including *20th* and *80th* percentile values 1981-2010 (Met Office, 2021)

Also recorded were an average of 126.5 days of precipitation equal to or greater than 1mm per year (see Fig 2.9). This is also lower than the average in England of 132.8 per year (Met Office, 2021). The 80th and 20th percentile figures were included so to display the wettest and dryest days (see Fig 2.9)

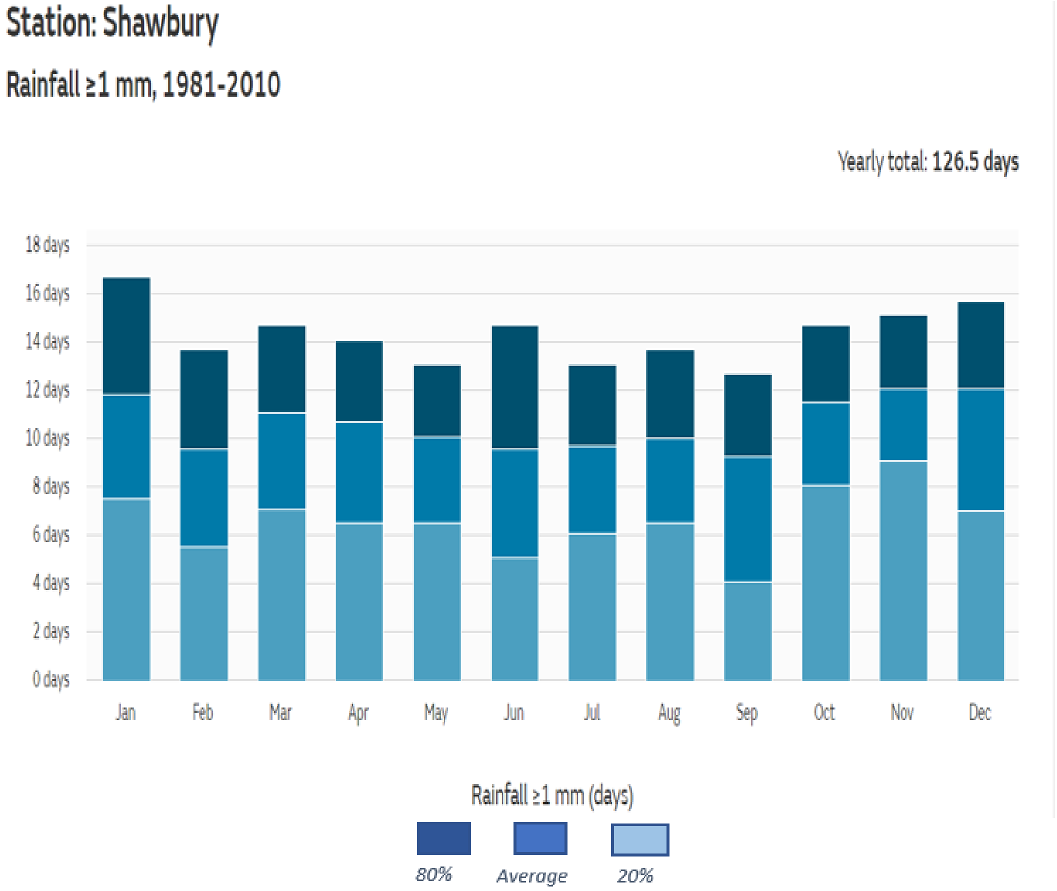


Fig 2.9: Chart of Average Days of Rainfall ≥1mm 1981-2010 including *20th* and *80th* percentile values (Met Office, 2021)

The details and data obtained from the Shawbury Climate Centre helped determine the experimental procedure in the application of Artificial Rain Water (ARW) in the mesocosm study within this thesis (see section 2.4.4). For further information on the mesocosm study, see Chapter 6 of this thesis.

2.2 Meres and Mosses Field Survey and Screening Study

2.2.1 Field Sampling Study: Sampling Rationale and Procedure

Topsoil is of fundamental importance when studying the occurrence and dynamics of trace metals in the pedosphere (see section 1.3 of Chapter 1). Simple Random Sampling (see Fig 2.10) of the topsoil (0-30cm depth) was deployed across the Meres and Mosses region so that an extensive number of samples were taken from within the boundaries that represented both the Nature Improvement Area (NIA) and Landscape Partnership Scheme (LPS) initiatives (see fig 2.5). These initiatives were long-term environmental schemes within the region (see Chapter 4). The requirement for a random sampling method for soil screening surveys has previously been recognised (Roels and Jonker, 1983; Abbitt, 2002). With a simple random system, each soil sample location is selected randomly and independently of each other within the designated sampling space. This was due to the variations in habitats and challenges in terrain and access to sites across the region. Where possible, for some of the larger sampling areas, a stratified random sampling approach was adopted within a coarse grid approach. This sampling regime involved taking a topsoil sample from a sampling area that had been divided into several smaller areas in an attempt to enhance field coverage of the overall sampling site (see Fig 2.10).

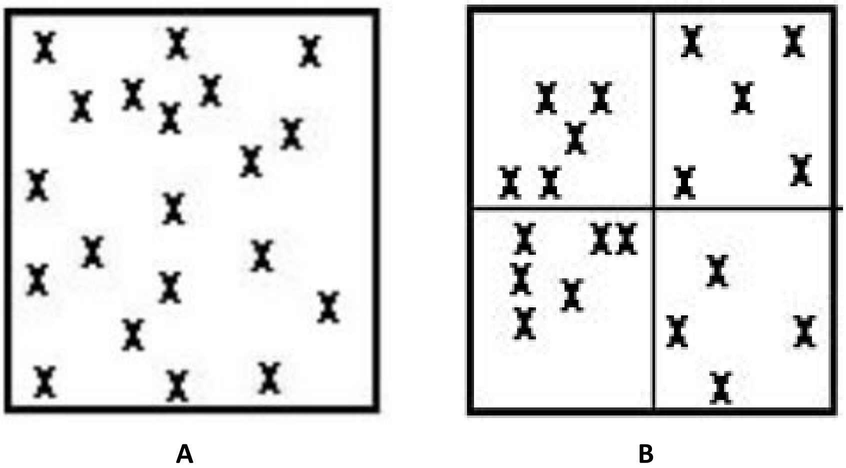


Fig 2.10: An example of Simple Random Sampling (A) and Stratified Random Sampling (B) in a Geographical Space (Mzansi Agricultural Talk, 2019)

A total of 89 samples of topsoil (A-horizon) were collected from various sites in the Meres and Mosses, using sample bags (8” x 11”) and a trowel (see Fig 2.11). These samples were taken on a random sampling regime and in a stratified coarse grid process were possible so to try and cover as much as the NIA/LPS area as possible to obtain representative coverage. Sampling sites were chosen from with a boundary designed to be representative of the NIA/LPS boundaries (see Fig 2.5 and Fig 2.11). A sample bag was filled with topsoil sample from the top 10 inches of the soil horizon from just below the O-Horizon (see Fig 1.1 of Chapter 1).

Upon collection of a sample, a coordinate sample point was recorded with a handheld GPS so to be able to accurately map the sample and attach information to an exact point (see Fig 2.11). Additional samples were taken just outside the NIA/LPS area to identify potential sources of toxic trace metals (see Fig 2.11). This was undertaken in the town of Wem, which has a history of industry (see section 2.1.1) and included the 60-acre industrial site which has steel fabricators and vehicle body builders present (Bradbury, 2019).

Upon return to the laboratory, the topsoil samples were air dried for a week and then oven dried at 50 ^{O}C for 24 hours. These samples were then initially sieved to a particle size of less than 4 mm, so that there was a standard soil sample throughout the investigation. Further sieving to the working fraction (≤2mm) was performed on all samples.

2.2.2 Mapping and Geographic Information Systems (GIS)

Following the field sampling study, the locational data for each sample site was recorded (see Fig 2.11) and, following laboratory analysis, the data obtained for geochemical factors (trace metal concentration, soil pH, organic matter content etc..) were applied to Geographical Information System (GIS) mapping software to achieve the goal of obtaining visual data for spatial occurrence for said data (see Chapter 4 of this thesis). GIS is used to interpret spatial variability and display spatial relationships within the environmental and geochemical data (Qingjie & Hejmanowska, 2013). This visualisation can be used to determine the influence of human activities on trace metal contents in a particular geographical range (Shan et al, 2013). A polygon was created to represent the NIA and LPS boundaries (see Fig 2.11). To explore the spatial distribution of the attained results (see Chapter 4) from sample to sample, the Kriging Interpolation method was used with Geographic Information Systems software, ArcGIS 10.1 and QGIS Desktop 3.16.0. This interpolation method is considered the optimal method for spatial prediction of the data-set (Goovaerts, 1999).

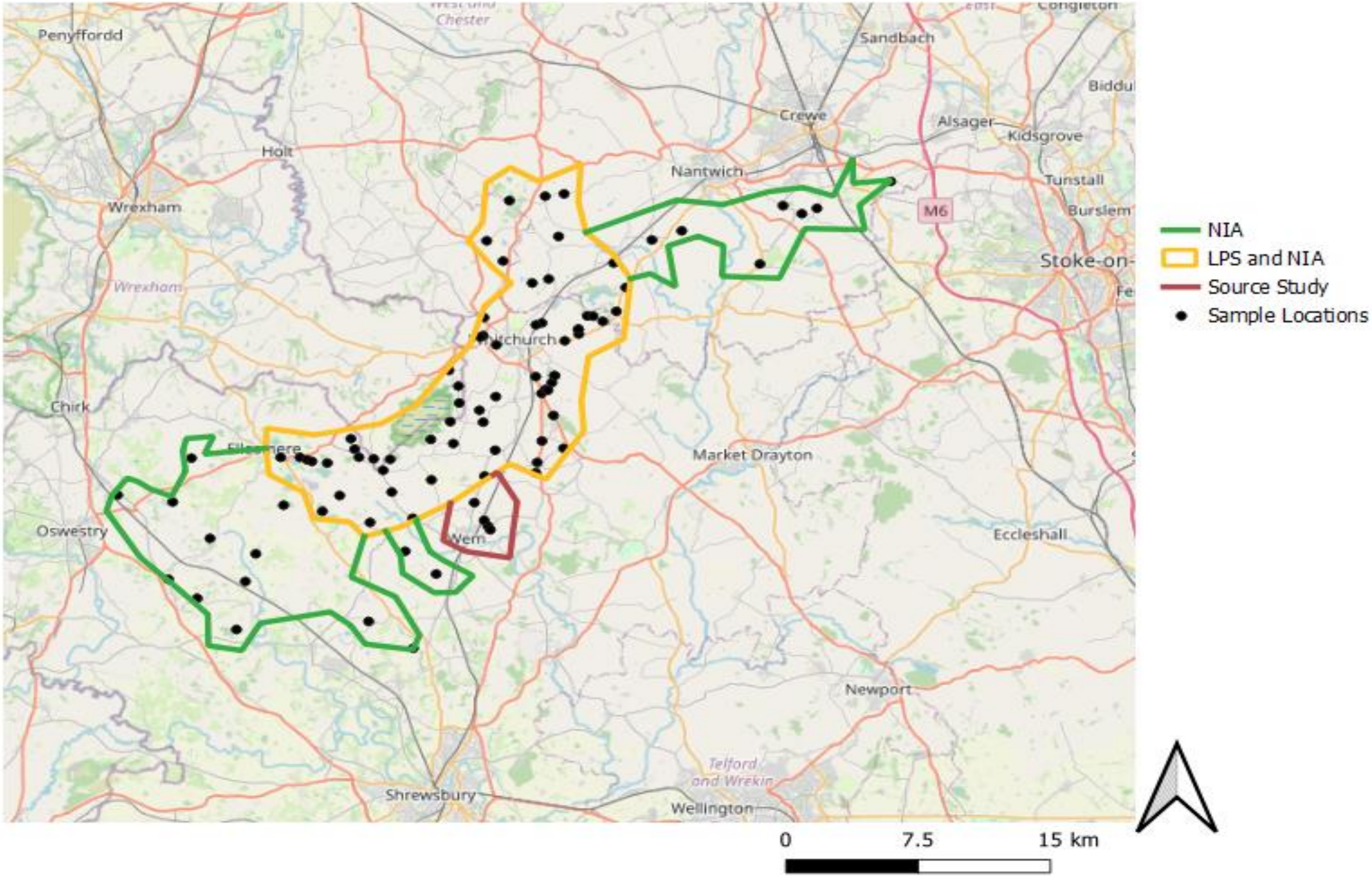


Fig 2.11: Map showing sampling locations for trace metals in the Meres and Mosses of the Marches.

2.3 Physiochemical Characterisation of Topsoil (Chapter 4)

The quality of the topsoil is an important factor in influencing its functions that are critical in promoting biodiversity and supporting agriculture (Chen et al, 2016) and is a vital aspect of determining the overall productivity of the soil (Kekane, 2015). The physiochemical properties of soil are a determining factor for the quality and health of the soil (Usharani et al, 2019). Relatively mild changes in soil chemistry and physiochemical properties of the topsoil could be important for the mobility of trace metals within the topsoil with the potential for increased leaching of certain trace metals bound in the topsoil (Linde et al, 2007). As such, the physiochemical properties of the topsoil within the Meres and Mosses of the Marches, including pH, organic matter content (OM) and total organic carbon content (TOC) were determined from samples taken within the Meres and Mosses region.

2.3.1 Granulometry

All topsoil samples in this study were dried and sieved for texture analysis. Texture analysis is the basic and most often conducted soil analysis (Rząsa et al, 2013). The objective of texture analysis is to ascertain the percentage content of each fraction of the soil, which for this study used the Wentworth classification system (see Fig 2.12); Very Coarse Sand (1-2mm), Coarse Sand (0.5-1mm), Medium Sand (0.25-0.5mm), Fine Sand (125-250µm), Very Fine Sand (62.5-125µm) and Silt (3.9-62.5µm). The Clay fraction (<2µm was also determined.

Millimeters (mm)		Micrometers (μm)	Phi (ϕ)	Wentworth size class	
	4096		-12.0		
				Boulder	Gravel
	256		-8.0		
				Cobble	
	64		-6.0		
				Pebble	
	4		-2.0		
				Granule	
	2.00		-1.0		
				Very coarse sand	Sand
	1.00		0.0		
				Coarse sand	
1/2	0.50	500	1.0		
				Medium sand	
1/4	0.25	250	2.0		
				Fine sand	
1/8	0.125	125	3.0		
				Very fine sand	
1/16	0.0625	63	4.0		
				Coarse silt	Silt
1/32	0.031	31	5.0		
				Medium silt	
1/64	0.0156	15.6	6.0		
				Fine silt	
1/128	0.0078	7.8	7.0		
				Very fine silt	
1/256	0.0039	3.9	8.0		
				Clay	Mud
	0.00006	0.06	14.0		

Fig 2.12: The Wentworth Scale of Soil Classification (Wentworth, 1922)

This granulometry analysis was implemented during the screening survey of The Meres and Mosses of the Marches with further details in Chapter 4 of this thesis.

2.3.2 Soil Reaction (pH)

Soil pH/soil reaction is the measurement of the acidity or alkalinity of soil. The soil reaction is considered to the primary controlling factor in terms of the chemical behaviour of trace metals in soil (Bullock, 992; Alloway, 1995). The pH and soil constituents, including organic matter (see section 2.3.3), play a vital role in the adsorption of trace metals in the soil (Pan, 2015; Weng et al., 2001). Heavy seasonal rainfall can cause a decrease in the surface area of the soil and its constituents which has the potential to result in an increase in trace metal solubility (Pan, 2015). Trace metal mobilisation may be further amplified during these heavy rains due to the release of this organic matter and humic acids which impact the topsoil pH and, in turn, metal mobility and binding potential of the trace metals (Koopmans and Groenenberg, 2011). Variations in pH of a topsoil can also have an impact on trace metal mobility (Pan, 2015; Weng et al, 2001) with trace metal cations being most mobile under acid conditions (Alloway, 1995). Further examination of these processes will be undertaken by way of a mesocosm study (see Chapter 6).

Soil reaction analyses were performed on each sample as part of the screening study for The Meres and Mosses of the Marches using a HANNA HI 99121 Direct to soil pH meter (see Chapter 4) as well as being part of the experimental methodology for the mesocosm study (see Chapter 6).

2.3.3 Organic Matter Content (Total Organic Carbon and Loss on Ignition)

It is important to ascertain the level of organic matter content in topsoil when studying trace metal content as organic matter can strongly interact and impact the topsoil both physically and chemically (Santschi et al. 1997). Organic matter content can contribute to the acidity of topsoil through formation of humic acids and can lead to complexation with trace metals (Zoumis et al. 2001). As such, organic matter content of the topsoil is an important factor in the environmental fate of trace metals (Robinson et al, 2005). Total Organic Carbon analysis (TOC) is an analytic procedure that is capable of measuring the organic content in topsoil (Yun 2003). The Loss on Ignition (LOI) is a common and widely used analytical procedure (Heiri et al, 2001) which provides a measurement of the organic content of the topsoil. The percent of sample mass lost following heating is reported as LOI. Analytical procedures for both these assessments on organic matter content were performed as part of the screening study of The Meres and Mosses of the Marches (see Chapter 4).

2.3.4 Magnetic Susceptibility

Magnetic susceptibility is the degree to which a substance can be magnetised (de Mello et al, 2020), and the amount of this magnetisation depends on the concentration and type of magnetic elements and minerals in the topsoil (Ghobadi et al, 2024). The measurement of magnetic susceptibility (MS) is a laboratory based analytical technique used for investigating potential anthropogenic pollution in soil matrices (Paradelo & Barral, 2014). The MS of topsoil, its relationship with topsoil properties and the concentration of trace metals, have previously been studied (Ghobadi et al, 2024) and have found MS measurements to be a useful tool for assessing trace metal pollution on soil and sediment samples (Paradelo & Barral, 2014; Reshetnikov et al, 2020; Wang, 2013). There are proven relationships between the results of MS analysis and areas with trace metal contamination when assessing geochemical data and obtained magnetic data (Paradelo & Barral, 2014).

MS analysis was performed on each sample during the screening study of The Meres and Mosses of the Marches using a Bartington MS-2 Magnetic Susceptibility Meter (see Chapter 4).

2.4 Rationale and Overview of Experimental Methods.

To fulfil the aims of the research project throughout this thesis (see section 1.9 of Chapter 1), various laboratory methods, aided by application of statistical analyses, and principles were applied so to establish and elucidate the current situation in regard to trace metal occurrence at The Meres and Mosses of the Marches. These analyses are addressed in further detail in each relevant chapter of this thesis. An overview containing the principles and reasoning for each analytical method is provided in this section.

2.4.1 Method Development for ICP-MS Analysis (chapter 3)

The requirement for, and benefits of, trace level analysis has been a significant incentive for the development of improved analytical techniques (Rowley, 2000). Prior to the use of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on obtained samples in the field (see section 2.4.3), a method development and improvement process was undertaken so to assure the validity and accuracy of the results garnered from the samples obtained from the agricultural landscape of the Meres and Mosses region. This method development/method validation process was undertaken in Chapter 3 of this thesis using a certified reference material (CRM), so to prove accuracy of the analytical techniques undertaken. Certified reference materials (CRMs) are characterised with certain known chemical or physical properties. These reference materials are often used during method development and validation so to ensure the accuracy of the method and their development (Mester and Sturgeon, 2003). With a set number of certified elements in a reference material, it is possible to determine the suitability of a method via recovery of the elements from the outcome of the analytical procedure. The elements chosen for analysis in this method development procedure were based on those highlighted for recovery in the certified reference material that were suitable for ICP-MS analysis. In this study, the CRM chosen was ERM-CC141 Loam Soil so to be as representative of the soils found in the Meres and Mosses region as feasibly possible as well as being representative of soil in agricultural landscapes (Parikh and James, 2012). Further details on the method development process can be found in Chapter 3.

2.4.2 Trace Metal Screening Study via FPXRF Analysis (Chapter 4)

X-ray fluorescence spectrometry (XRF) is a long-established analytical technique, often as a 'first order' analysis, for obtaining high-resolution elemental data (Kilbride et al, 2006). Sarkar, 2018, states that "X-ray fluorescence (XRF) analysis is based on the measurement of characteristic X-rays resulting from de-excitation of inner shell vacancy produced in a sample by means of a suitable source of incident radiation" (West et al, 2014). Because it is not destructive in its analytical design and relatively short analysis time, XRF is considered a good analytical technique when examining trace metal elements (Kobylarz et al, 2023). Whilst it does not benefit from the low level of detection capability that is attributable to analytical techniques such as ICP-MS (see section 2.4.3) it is useful for rapid screening in both a laboratory and field environment. Field Portable XRF (FPXRF) is one which can be implemented in both the laboratory and/or outdoors. The FPXRF can be used directly in contact with the surface of the soil of interest or with a clear soil sample bag in the laboratory (Kilbride et al, 2006). X-Ray Fluorescence Analysis (XRF) was carried out on the soil samples that were collected in the field (see 2.2.1 and Chapter 4). A total of 17 trace metal elements were analysed via FPXRF (Cu, Pb, Zn, Ca, K, Ti, V, Cr, Mn, Fe, Co, As, Rb, Sr, Nb, Sn, Ga) on these soil samples as part of a screening program to help aid in the determination of the spatial occurrence of trace metals in the Meres and Mosses region (see chapter 4). Further comparison with ICP-MS can be found in Chapter 5 of this thesis. Further details on the methodology implemented for FPXRF analysis can be found in Chapter 4 of this thesis.

2.4.3 Comparison Study and Trace Metal Concentration Analysis via ICP-MS (Chapter 5)

Sheppard and Caruso, 1994 describe plasma source mass spectrometry as "one technique that is currently of interest for multi - element analysis at the part per billion (ppb) and part per trillion (ppt) levels" (Sheppard and Caruso, 1994) which is advantageous when studying trace levels of elements compared to less sensitive methodologies such as XRF. The suitability of inductively coupled plasma mass spectrometry (ICP-MS) for the determination of trace metals has become widely established and is suitable when analysing from a multi-element approach (Lyon et al, 1988).

In the book, 'Handbook of the Toxicology of Metals' by Nordberg and Fowler, 2014, Smith and Nordberg state that "This method is based on measuring m/z ratios. The very low detection limits are due to the very high degree of atomization in the argon plasma at approximately 7000 K" (Nordberg and Fowler, 2014; Dean, 2005). Advantages of ICP-MS include a high sample throughput with multi-element capability (Nordberg and Fowler, 2014; Vanhaecke and Kollensperger, 2003). The use of acid digestion in this methodology, such as aqua regia or reverse aqua regia, results in only a partial result for trace metal concentration due to the inability to dissolve silicates in the soil matrices which may result in deviation from the total result obtained via XRF methodology (Declercq et al, 2019). This can lead to very concentrated strong acids, such as Hydrofluoric Acid (HF), being implemented in some acid digestion techniques which are hazardous and, as a result, time consuming and not as cost-efficient as when compared to field portable X-ray fluorescence (Schneider et al., 1999). As such, comparison of both analytical methodologies (ICP-MS and FPXRF) is explored in Chapter 5 of this thesis in which an Agilent 7900 ICP-MS, with collision cell technology, was used, in conjunction with reverse aqua regia digestion, for analysis of trace metals from the topsoil of the Meres and Mosses of the Marches. Further details of the methodological process for the ICP-MS analysis can be found in Chapter 5.

2.4.4 Mesocosm Study (Chapter 6)

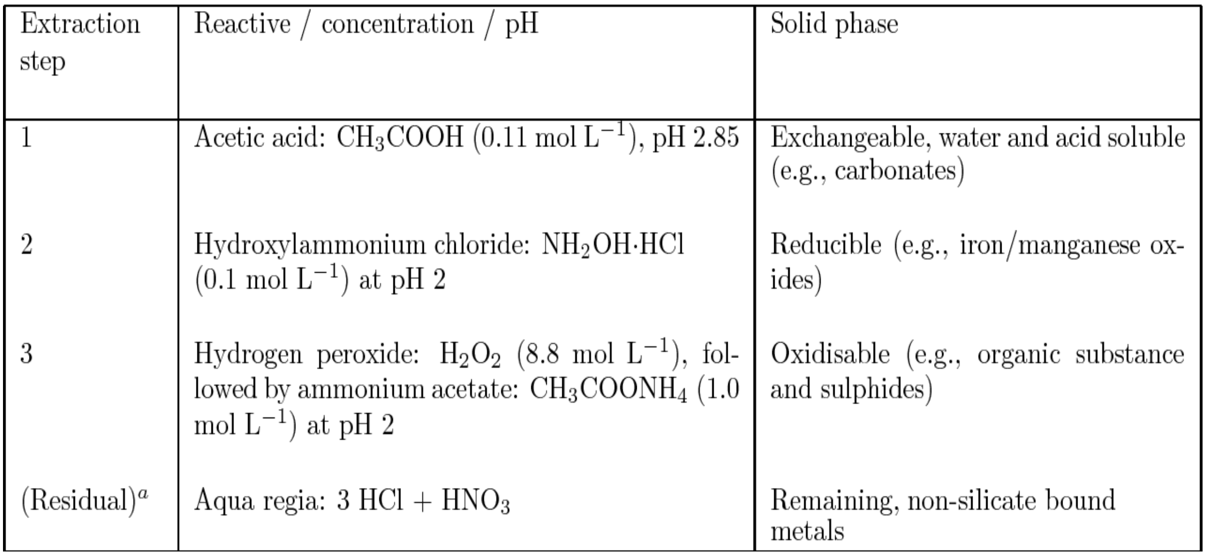
Processes in terrestrial ecosystems are often studied using mesocosm experiments that are performed in the laboratory but aim to simulate conditions in the field (Teuben & Verhoef, 1992). These mesocosm often come in the form of cylindrical columns that aim to simulate processes in soil depth with a view of elucidating the temporal and spatial variation of various aspects in the topsoil pore water of the column (Pan et al, 2021). Mesocosm experiments attempt to isolate, and then replicate, an area of an ecosystem so to examine the impacts of physiochemical conditions (Svendsen & Weeks, 1997). Physiochemical conditions, such as the pH of the topsoil, as well as organic matter content, play an important role in controlling trace metal mobility (Richards et al, 2000) with those trace metals in free ionic form within the topsoil being the most mobile when those soils are acidic (McBride, 1994).

Based on the results obtained from the screening study (Chapter 4) topsoil was collected from sites chosen within the Meres and Mosses sampling area so to be representative of those in the wider region. These samples were placed in cylindrical mesocosms which examined the impact of varying the pH levels and organic matter content when studying trace metal mobility and occurrence within the topsoil whilst applying artificial rainwater at the amount and rate determined from the local climate station (Shawbury) so to replicate field conditions. The A-Horizon (topsoil) is that most prone to disturbance from environmental weathering and anthropogenic factors (Darmody et al, 2009; Fox et al, 2010). As the A-Horizon is the singular soil horizon being studied here, disturbed soil columns were deemed suitable for this study without compromising field conditions which are naturally prone to disturbance. This implementation of disturbed soil columns has the benefit of being able to evaluate multiple columns (in duplicate) based on the potential for disturbance observed in the field (Treese et al, 2010). Further details on this study and the methodology involved can be found in Chapter 6 of this thesis.

2.4.5 Application of Sequential Extraction (Chapter 6)

When examining trace metal mobility, the principles of geochemical partitioning (see Chapter 1, section 1.5) are important so to provide an understanding of the interactions between the trace metals and the soil matrix which is required to assess their impact on the habitat being studied (Tokalıoğlu et al, 2003). Although the total concentration of trace metals in soil (such as those obtained by FPXRF) provides information on the overall level of contamination in the soil profile, it provides no insight into the bioavailability or mobility trace metals (Nur Aliya, 2017). As such, the behavioural factors of the trace metals in the topsoil, such as their mobility or bioavailability, cannot be determined based on their total trace metal concentration data obtained (Petit and Rucandio, 1999; Tokalioglu et al., 2003). Trace metals and trace elements in the a-horizon and topsoil are found in multiple fractions when taking various physiochemical forms (Silwana. et al 2017). These are the exchangeable, carbonate bound, Iron (Fe)/Manganese (Mn) bound, organic matter bound and the residual fractions (see Table 2.1). Chemical fractionation and extraction methodologies have been used so to examine the differing physiochemical forms and the fractions the trace metals are associated with so to better understand the processes that influence trace metal bioavailability and to obtain a greater understanding on the distribution of trace metals in the topsoil (Chlopecka et al, 1996).

The purpose of the Sequential Extraction method is to imitate the release of the chosen trace metals into the porewater, indicating potential for bioavailability under varying environmental conditions (Nur Aliya, 2017; Nyamangara, 1998). The procedures involve subjecting the topsoil sample to successive exposure to increasingly harsh reagents which contain different chemical properties (such as redox potential or acidity) in which each extract step represents a ‘fraction’ which contains trace metals associated with the sample and indicates their bioavailability (Tokalıoğlu et al, 2003, Kotand and Namiesnik, 2000) as shown in Table 2.1.



Extraction step	Reactive / concentration / pH	Solid phase
1	Acetic acid: CH_3COOH (0.11 mol L^{-1}), pH 2.85	Exchangeable, water and acid soluble (e.g., carbonates)
2	Hydroxylammonium chloride: $NH_2OH{\cdot}HCl$ (0.1 mol L^{-1}) at pH 2	Reducible (e.g., iron/manganese oxides)
3	Hydrogen peroxide: H_2O_2 (8.8 mol L^{-1}), followed by ammonium acetate: CH_3COONH_4 (1.0 mol L^{-1}) at pH 2	Oxidisable (e.g., organic substance and sulphides)
(Residual)^a	Aqua regia: 3 HCl + HNO_3	Remaining, non-silicate bound metals

a Digestion of the residual material is not part of the BCR protocol.

Table 2.1: The BCR/SM&T three-stage sequential extraction scheme (Tokalıoğlu et al, 2003).

The sequential extraction exercise was performed on the two representative topsoil samples chosen for the control columns in the mesocosm study aspect of this thesis (see section 2.4.4) following the results obtained in the screening survey (see Chapter 4). Further details on the methodology implemented can be found in Chapter 6 of this thesis.

2.5 Conclusion

The Meres and Mosses of the Marches are a unique landscape in the northwest of England formed by post-glacial retreat approximately 15,000 years ago. These meres and mosses habitats are considered some of the most ecologically productive environments globally and are essential for biological diversity and carbon storage. The region has faced many anthropogenic environmental pressures due to being associated with agriculture, peat mining/extraction and, latterly, industrial heritage resulting in trace metal accumulation. Due to these pressures, there is value in both studying and protecting these landscapes. Previous studies in the Meres and Mosses have focused on nutrient pollution but little is known about the presence or spatial occurrence of trace metals, a pollutant of increasing concern, in the region. The aims of this project (see Chapter 1, section 1.9) will aid in assessing the region so to establish the magnitude and dynamics of trace metal pollution in the topsoil, their potential origin (anthropogenic or geogenic) and consequent risk to ecological health.

- A screening study will establish the current physiochemical properties of the Meres and Mosses of the Marches. This screening study will help with the elucidation of varying geo-chemical aspects, including the presence and occurrence of trace metals within the topsoil of the region. This screening study will adopt established analytical methodology so to garner a greater understanding of the physiochemical properties of the sampling region.

- Random and stratified random field sampling was undertaken in the Meres and Mosses region so to maximise field coverage. The location of each sample was recorded so that implementation of GIS mapping software could be applied to each sample data point.

- A combination of analytical procedures with statistical analyses will provide a greater understanding of the factors controlling these physiochemical properties and the influencing relationships therein. Examination, development and validation of analytical procedure and comparison of analytical methodology will aid in the assessment of the most appropriate analytical tools used in this task.

- Additional laboratory analyses will then aid in not only determining the presence of these trace metals in the topsoil of the Meres and Mosses, but then help to elucidate the behaviour and mobility of these elements under varying topsoil properties (pH and OM). These additional analyses will aid in determining the bioavailability of these trace metals and resultant environmental/ecological risk in the Meres and Mosses region.

- Each data chapter in this thesis will elaborate on the rationale and analytical methodology for each laboratory method outlined here with a view of aiding in the understanding of the status of the site being studied and the processes involved in trace metal occurrence and mobility. This study will be beneficial for forming consequent mitigation measures and long-term sustainable management plans of the agricultural land and topsoil within the region.

Chapter 3:

Method Development:

Application of Method Development for Trace Metal Digestion via Reverse Aqua Regia for ICP-MS Analysis - A Simultaneous Multi-Element Approach.

Abstract

Method development, improvement and validation is a process which provides documentary evidence that an analytical procedure fulfils its pre-defined purpose. Additional information can be obtained from each analytical sample by using simultaneous multi-element methods rather than repeated single element analysis. A simultaneous multi-element analytical regime has been found to have beneficial outcomes both economically and logistically. In this study a digestion method for simultaneous determination of some of the most toxic and polluting trace metals (Co, Cr, Cu, Mn, Ni, Pb, Zn) in soil via reverse aqua regia extracts was performed using a Sequential Pressure microwave digestion technique combined with ICP-MS. A series of digestion programs were applied by varying temperature, ramp time and hold time steps. These variations implemented maximum temperatures of 180^{O}C, 200^{O}C and 220^{O}C for 20, 25 and 30 minutes when applied to approximately 0.2g of soil sample (a certified reference material) with a mixture of HCl and HNO_3 (in a 1:3 ratio) known as reverse aqua regia. Reverse aqua regia (sometimes known as inverse or LeFort Aqua Regia) has been found in various studies to give optimal recovery, with reproducible and optimal results, without the need for more hazardous or aggressive acid such as Hydrofluoric Acid (HF).

For validation purposes, a certified reference material (CRM) ERM-CC141 loam soil, was used in the experimental study in which various digestion parameters were examined using reverse aqua regia as the digestion medium (SRMs 1-18). The recovery percentages obtained from the chosen SRM samples (following the elimination process), when compared to the certified results for the CRM, situated all metals analysed in the range of 90% to 110% for ICP-MS determinations with a relative standard deviation (RSD) <10%. The results obtained in the method development process show that multiple variations of Hold time (5 or 10 minutes) and Temperature (180^{O}C or 200^{O}C) with a Ramp time of 20 minutes are suitable for use in agricultural soil analysis as well as peatland/wetland systems (due to Loam Soil CRM) when studying trace metal concentration ('pseudo-total') as in this study of a 'Meres and Mosses' wetland habitat when using reverse aqua regia as the chosen digestion medium and in the selected trace metals within this study. More specifically, with either

200^{O}C maximum temperature with Ramp time of 20 minutes and Hold time of 5 minutes or maximum temperature of 180^{O}C with a Ramp time of 20 minutes and Hold time of 10 minutes giving optimal recovery values when SP-D Microwave settings are set with Pressure at '350 psi', Power at '300 W' and Stirring setting set to 'High'. This displays the parameters required to perform a multi-element analysis for Trace Metals in soils/sediment. The analytical procedure/s determined in this method development process were then implemented in the analytical comparison study in Chapter 5 of this thesis.

3.1. Introduction

Method development is rarely used in geo-scientific studies yet is becoming increasingly important due to increasingly stringent environmental guidelines and the requirement for accurate results and reliable analytical procedures (Pye and Croft, 2004). This increasingly 'forensic geoscience' requires confidence in obtained results and use of the most appropriate techniques (Pye and Croft, 2004). Method development is used in proving that an analytical procedure is acceptable for its intended application (Green, 1996). Method development, along with method improvement and method validation, is an established process that provides the documentary evidence that an analytical method fulfils its pre-defined purpose (Tavazzi et al, 2016). In any scientific discipline, it is important to know whether the result of an experiment or analytical procedure can be accepted with confidence or rejected because it does not fulfil its purpose and/or certain parameters. It is essential, also, to know whether an analytical method is suitable for the intended use of said method. Likewise, it is more important to know if newly developed methods can be trusted and what are the criteria, in respect of its purpose, to ensure its validity. In this regard, method development is a vital aspect in many scientific disciplines as the quality of the obtained result is an important issue when analytical measurements are necessary. It is being increasingly acknowledged by those involved in the environmental sciences and its research that analytical results should be robust and open to scrutiny. It is, therefore, important to present results that are both traceable and accurate (Bulska 2009).

So to determine the potential for ecotoxicity, the accurate measurement of trace metals in soil is an important aspect so to develop appropriate environmental policy (Santoro et al, 2017; Ure et al, 1993; Quevauviller et al, 1997). Normally in the soil there are trace amounts of trace metals (hence the term 'trace metals'), but sometimes their levels are increased due to anthropogenic sources (Alloway, 1995). Trace metals are widespread pollutants of considerable environmental concern as they do not degrade as well as being cumulative, toxic and persistent (Stihi et al, 2011). The soil behaves as a deposit for the trace metals due to their ability accumulate over time (Marin et al, 2015). Trace metal content in soils is naturally determined by geochemical factors. The process that takes place for soil formation and the nature of the parent material are all important aspects of these factors (McGrath, 1998). On the other hand, input of the metals into the environment by anthropogenic activity (industry, traffic, etc.) or as a consequence of such activities (e.g. leaching from soils by acid rains) has increased over the last few decades. During the last few decades, scientific interest in the methods and techniques for determination of metal concentration in environmental samples and, among them, soil samples, has significantly increased (Shi et al, 2021). Inductively coupled plasma –mass spectrometry is a powerful technique for the determination of trace metals in various environmental samples as well as in the soil samples (Sturgeon, 2000).

Inductively coupled plasma mass spectrometry (ICP-MS) is an important analytical instrument in the determination of trace metal concentration, among other applications, due to its increased sensitivity and detection limit range when compared to alternative analytical techniques (see Fig 3.1). It is therefore highly useful when used for elemental determinations across a wide range of analyses, including environmental assessment and research applications in which only trace amounts are being analysed and/or researched (McCurdy & Woods, 2012). ICP-MS is an analytical technique for determining trace metal concentrations in a variety of matrices and is characterised by its low limits of detection (Vanhaecke and Kollensperger, 2003). A high degree of atomisation in the argon plasma due to being at approximately 7000 K results in the capability of low detection limits (Dean, 2005). This high temperature makes it more suitable than the graphite furnace, for example, as an atomisation source for atomic absorption spectrometry (Nordberg and Fowler, 2014).

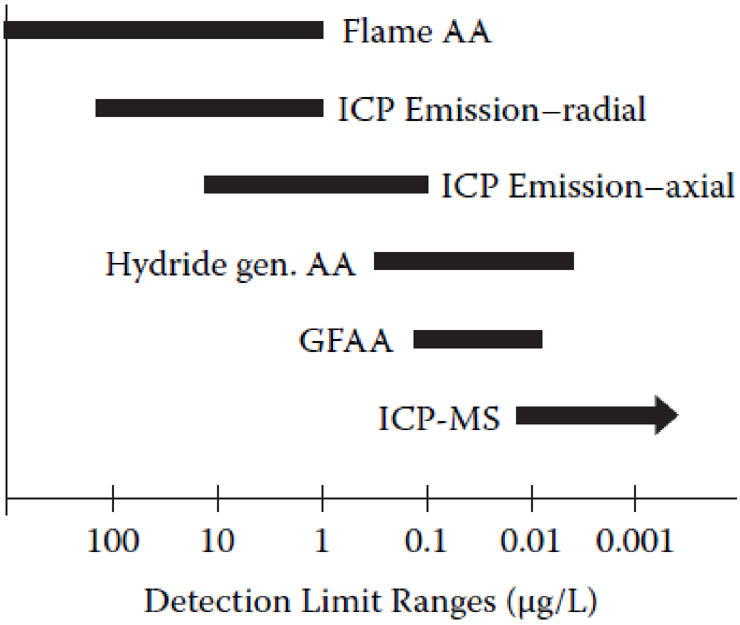


Fig 3.1: Comparison of Detection Limits (Sahin, 2020)

When using an Inductively Coupled Mass Spectrometer, there are problems known as spectral interference due to the resolution being limited to Relative Mass Differences (Nordberg and Fowler, 2014). For instance, as Table 5 shows, when measuring 52Cr, it will experience interference from the isobars such as those of $^{40}Ar^{12}C+$ and $^{36}S^{16}O+$, which can occur in samples that contain significant C, Cl, S, content (Nordberg and Fowler, 2014). Polyatomic interference, therefore, is one of the fundamental challenges in ICP-MS. This interference can be suppressed with the use of a collision/reaction cell known as Collision Cell Technology (CCT) which can be activated during a given sample run if the ICP-MS is equipped with the technology (Laemmel & Bonacina, 2009).

ICP-MS usually requires a transformation of the analyte into solution. Digestion methods are often studied and researched, this is because varying digestion parameters and techniques often yield significantly different final analytical results (Sastre et al., 2002; Melaku et al., 2005). With respect to the microwave-assisted digestion, there are diverse effects of each acid formulation. Simultaneously, the holding time and temperature will influence the results (Zhou et al, 1995). Different acid digestion methods can release a different number of elements from the soil matrix. Therefore, it is important to assess differing digestion methodologies used to determine trace metal concentrations in soils. The amount of trace metal extracted by the commonly used digestion methods might depend on the trace metal being analysed, their source and the properties of the soil itself (Chen and Ma, 2001). As Roge (2010) points out, because of their efficiency, digestion procedures using closed-vessel microwave sample preparation systems are used for various environmental investigations (Wang et al., 2006; Limbeck, 2006; Go´recka, 2006), biological investigations (Engstro¨m et al., 2004), as well as for investigating food science (Caroli et al., 1999; Bhandari and Amarasiriwardena, 2000). It has been shown, however, that venting in this type of closed vessel microwave digestion can cause losses and quantitative recoveries of volatile analytes are not possible after venting. However, if the venting process is controlled as well as the chemical reactions, losses of volatile elements can be avoided (Amaral et al, 2016).

Determination of trace metals in soils by implementation of reverse aqua regia as the digestion medium has become widely adopted due to limited application of chemical reagents with straight forward and convenient operation procedures (Lu et al, 2007). Recent studies have shown that reverse aqua regia has superior recovery of elements when compared to conventional aqua regia and produces reproducible results without the requirement for harsh and aggressive acids such as Hydrofluoric Acid (Szymczycha-Madeja et al, 2021; Lu et al, 2007). Digestion with reverse aqua regia enables the acid extractable trace metals to be determined as distinct from the total trace metal content. This acid mixture is not capable of not dissolving silicates but is aggressive enough to dissolve the trace metals which are not bound to silicate phases (Ure et al, 993). It is Important that reverse aqua regia only partially dissolves silicates which results in only a limited release of potentially interfering elements when considering ICP-MS analysis (Cheng et al, 2019). Having an increased ratio of HNO_3 in the mix (as is the case for reverse aqua regia) is preferred because of its oxidising nature. HNO_3 converts trace metals into soluble nitrate salts (Kumar, 2015). There is also less chance of loss by forming volatile compounds with reverse aqua regia than with some other methods of sample digestion (Siaka et al, 1998). The partial digestion technique provides results known as a 'pseudo-total' (Quevauviller, 1998). This pseudo-total is considered to be a good estimate of the bioavailable fraction of trace metals (Andersen and Kisser, 2003) and is often requested by some national regulations/guidelines rather than the 'total' content provided by alternative analytical procedures (Santoro et al, 2017).

The persistence and toxicity of trace metals has created an imperative need for developing swift, reliable methods for their qualitative and quantitative determination in environmental samples. This is especially the case when testing for multiple elements and/or toxic metals in a single sample. The benefits of a simultaneous multi-element approach are that more information can be obtained from each sample rather than when using repeated single element analysis as well as having been found to have beneficial outcomes both economically and logistically (Lyon et al, 1988). This multi-element approach would provide multiple advantages. (i) sample cost; a smaller volume of soil sample would be sufficient to obtain the same information that would be provided by repeated single trace metal analysis, this is important in studies in which sample is limited and/or costly/difficult to obtain. (ii) Direct multi-element analysis would simplify sample handling and thus reduce pre-analysis contamination. (iii) Investigation into various agricultural and soil related pollutants may be detected if a trace metal "profile" could be easily obtained. Therefore, producing a 'Trace Metal Profile' for a given sample or sampling area is highly advantageous for remediation/management purposes (see Chapters 4 and 5 of this thesis).

Certified reference materials can be used to aid in the validation of a method for the determination of multiple trace metals in a single sample. Certified reference materials (CRMs) are characterised with certain chemical or physical properties. These reference materials are often used during method development and validation so to ensure the accuracy of the method and their development (Mester and Sturgeon, 2003). With a set amount of certified elements in a reference material, it is possible to determine the suitability of a method via recovery of the elements from the outcome of the analytical procedure. Recovery is an essential aspect of method development and method validation (Vanhaecke et al, 1995). Hibbert (1999), in the study 'Method Validation of Modern Analytical Techniques', states that, recovery of certified reference material should be at "80% to 120% of target concentration". The "target concentration" being that listed for a particular trace metal element by the Certified Reference Material. EU Environmental Standards on certain elements are often more stringent, such as those for ambient air quality (EN 14902) which states that the recovery of Pb should be between 90 and 110% and that the recovery for Ni to be in the range of 85 and 115%. This is also the case when applying recovery rates to EU standard procedures for methods determining trace elements in soils such as CEN/TS 16171:2013 (Tirez et al, 2015). In this study, certified reference material, ERM-CC141 was used for validation. This CRM was derived from a loam type soil with a natural level of trace metal content (Birgersson-Liebich et al, 2010). The purpose of the reference material is to evaluate method performance from the obtained analytical data with information being implemented in validation studies (EVISA, 2010).

ERM-CC141 is a reference material derived from loam soil and is certified for the mass fraction of the total and aqua regia extractable content (ISO 11466) of arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), magnesium (Mg), nickel (Ni), lead (Pb) and zinc (Zn) at the mg/kg range. ERM-CC141 is primarily intended for method performance control and for validation purpose (JRC, 2010). As CCT was not implemented on all of the SRM variations (see section 3.2.2), cadmium and arsenic were excluded from this method development study as the determination of cadmium (Cd) in geological and topsoil matrices via ICP-MS is impacted by significant molybdenum and/or zirconium (Zr) based oxide interference in soil samples of this type (Xu et al, 2015) whilst arsenic (As) with its mono-isotopic ion at m/z 75 is interfered by the polyatomic ions of $^{40}Ca^{35}Cl^+$, $^{40}Ar^{35}Cl^+$, $^{39}K^{36}Ar^+$ and the doubly charged ions of $^{150}Nd^{2+}$, $^{150}Eu^{2+}$ and $^{150}Sm^{2+}$ which results in inadequate and inaccurate results for this particular trace metal when implementing this methodology without CCT (Guo et al, 2011).

The objectives of this overall thesis are to determine the trace metal dynamics in an environmentally sensitive region of agricultural land. For this task, a robust and valid analytical procedure is important. This method development will aid in this task by: (i) optimisation of a microwave digestion method for simultaneous determination of multiple trace metals in CRM soil using Inductively Coupled Plasma with Mass Spectrometry (ICP-MS) techniques and reverse aqua regia digestion. (ii) Application of this method for the agricultural topsoil analysed in this study.

This study will show that even subtle changes to temperature, ramp time, hold time and even vessel size can make significant changes to the digestion outcome and vary the suitability of a set program for analysis of metals in the soil matrices by analysing the pseudo-total result. The statistical tools applied within this study, such as standard deviation, will allow us to address all these points during the method development process (Belouafa et al, 2017).

3.2. Materials and Methods

3.2.1 Sample Preparation for Reverse Aqua Regia Extraction

For this multi-element approach, a certified reference material (CRM) was used to determine the quality of recovery when using reverse aqua-regia as the digestion matrices. Certified reference materials are an important aspect in the analytical sciences. Certified reference materials (CRMs) are those that are used for method validation purposes. These certified reference standards are widely used in forensic applications, clinical diagnostics and environmental planning and management (Gates et al, 2009). These reference materials contain stable properties with provided uncertainty parameters (Yang et al, 2016). This uncertainty parameter (see Table 3.1) is expressed in ± *x* units (such as mg/kg) of concentration (Gates et al, 2009). This 'uncertainty' is a parameter which is associated with the result of a measurement, which characterises the dispersion of the values to the trace metal being studied (De Bièvre, 2012). This uncertainty measurement was taken into consideration when establishing a valid SRM digest.

Trace Metal Element	Certified Value for 0.1g of ERM-CC141 (mg/kg)	Uncertainty in 0.1g of ERM-CC141 (±mg/kg)
Co	7.9	0.9
Cr	31	4
Cu	12.4	0.9
Mn	387	17
Ni	21.9	1.6
Pb	32.2	1.4
Zn	50	4

Table 3.1: Certified Recovery Values for ERM-CC141 and Certified Uncertainty

ERM-CC141 (European Commission Joint Research Centre) is a loam type soil CRM certified for the mass fraction of the total and aqua regia extractable content (ISO 11466) of various metals including Chromium (Cr), Magnesium (Mg), Cobalt (Co), Nickel (Ni), Lead (Pb)and Zinc (Zn) at the mg/kg level. ERM-CC141 (Fig 3.2) loam soil is intended to be a practical quality control tool in the analysis and element determinations in soils or other similar matrices (Birgersson-Liebich et al, 2010). ERM-CC141 Loam Soil was chosen so to be representative of the soils found in the Meres and Mosses region, meres and mosses habitats and as well as in agricultural landscapes (Parikh and James, 2012).

Fig 3.2: ERM-CC141 Loam Soil (JRC, 2024)

Approximately 0.2g of ERM-CC141 sample was added to 3.75ml HNO_3 and 1.25ml HCl in a 10ml pressure vessel (this was upscaled to 9ml HNO_3 and 3ml HCl for 80ml vessels later in the study) and placed into a sequential pressure microwave oven. A multi-step digestion program was performed in order to obtain suitable extracts. These extracts were then labelled sequentially following differing digestion parameters being applied to each sample. A total of 18 Soil Reference Material digests were run via SP-D Microwave and labelled as 'Soil Reference Material' digests (SRM '1' through to '18' respectively).

3.2.2 Sequential Pressure Digestion (SP-D)

The digestion method in this study used a CEM Discover SP-D Sequential Pressure Digestion unit (see Fig 3.3) which is a sequential pressurised microwave digestion system which utilises a 'closed vessel with venting' technique. This SP-D Unit has distinct advantages over other forms of digestion processes in that it can vary the digestion method per sample rather than per run on a batch sampling regime. This includes the ability to vary temperature, ramp time and hold time. As such the SP-D Unit is ideal when undertaking an exercise in method development and validation. Accurate determination of trace metals in soil samples via sequential pressure microwave digestion will aid in the enhanced development of digestion methods for trace metals in soil as well as choosing the correct parameters for obtaining accurate and reproducible results.

Digestion parameters were ascertained as per the guidelines set out for the certified reference material (ISO 11466) and for those in the use of 'Microwave Assisted Extraction' (ISO 12914). All samples were run with Pressure at '350 psi', Power at '300 W' and Stirring setting set to 'High'.

Fig 3.3: SP-D Microwave Digestion Unit (CEM Corporation, 2024)

A maximum temperature was set for between 15 and 20 minutes (depending on the desired program) with the temperature inside the vessels being increased over this set time (ramp time) to the maximum temperature, between 180°C and 220°C (depending on the desired program). The temperature was then kept steady (hold time) for between 5 to 15 minutes (depending on the desired program) at the set maximum temperature. The cooling process was applied for 30 minutes for all programs. The steps of the digestion program are given in Table 3.2, 3Table 3.3 and Table 3.4. After cooling (see Fig 3.4 and Fig 3.5), the solutions were filtered on 0.45µm porosity filter into 100ml volumetric flasks and were analysed via ICP-MS.

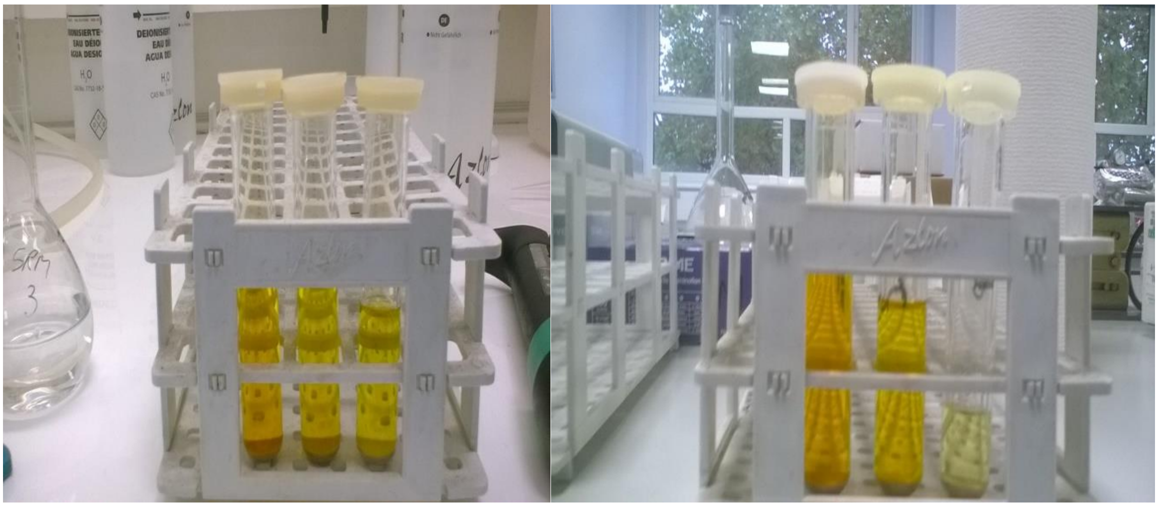


Fig 3.4: SRM 1-6 Digestions in 10ml Vessels

Sample	Ramp (Mins)	Hold (Mins)	Max Temp °C	Total Heating Time (mins)
SRM 1	20	10	180	30
SRM 2	20	10	200	30
SRM 3	20	10	220	30
SRM 4	15	5	180	20
SRM 5	15	5	200	20
SRM 6	15	5	220	20
SRM 7	20	5	180	25
SRM 8	20	5	200	25
SRM 9	20	5	220	25
SRM 10	15	10	180	25
SRM 11	15	10	200	25
SRM 12	15	10	220	25

Table 3.2: SRM 1-12 Digestion Parameter Profile

Fig 3.5: SRM 7-12 Digests in 10ml Vessels

Adjustments in digestion factors were based on progression factors and results obtained throughout the method development. This method improvement step allows further revision of the procedure with revalidation or redevelopment of the method if necessary. Collision Cell Technology (CCT) mode (Table 3.3) was performed on some SRMs (Fig 3.6) to attempt to reduce the impact of polyatomic interference.

Sample	Ramp (Mins)	Hold (Mins)	Max Temp ^{O}C	Total Heating Time (Mins)
SRM 13 CCT	20	10	180	30
SRM 14 CCT	20	5	180	25
SRM 15 CCT	20	5	200	25

Table 3.3: SRM 13-15 Digestion Parameter Profile

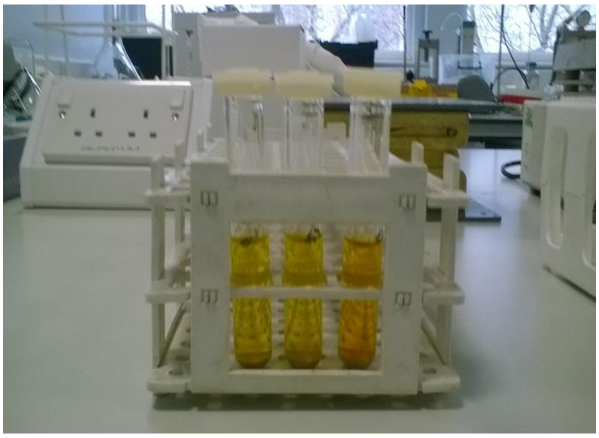


Fig 3.6: SRM 13-15 Digests in 10ml Vessels

80 ml vessels (Fig 3.7) were introduced, alongside application of CCT, on select samples (Table 3.4) to check that no solute/digest were being lost on vaporisation due to the potential of volatilisation (Amaral et al, 2016) in smaller vessels in a 'closed vessel with venting' system.

Sample	Ramp Time (mins)	Hold Time (mins)	Max Temp ^{O}C	Total Heating Time (Mins)
SRM 16 80ml CCT	20	10	180	30
SRM 17 80ml CCT	20	5	200	25
SRM 18 80ml CCT	20	10	200	30

Table 3.4: SRM 16-18 Digestion Parameter Profile

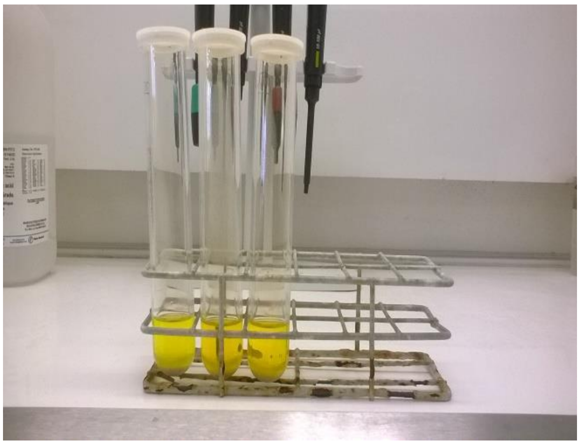


Fig 3.7: SRM 16-18 Digests in 80ml Vessels

3.2.3 The Use of ICP-MS Analysis, Reagents and Calibration.

In this study a Thermo Scientific X Series 2 instrument (Fig 3.8) which utilises a collision/reaction cell for interference removal were used for determination of Co, Cr, Cu, Mn, Ni, Pb and Zn concentrations.

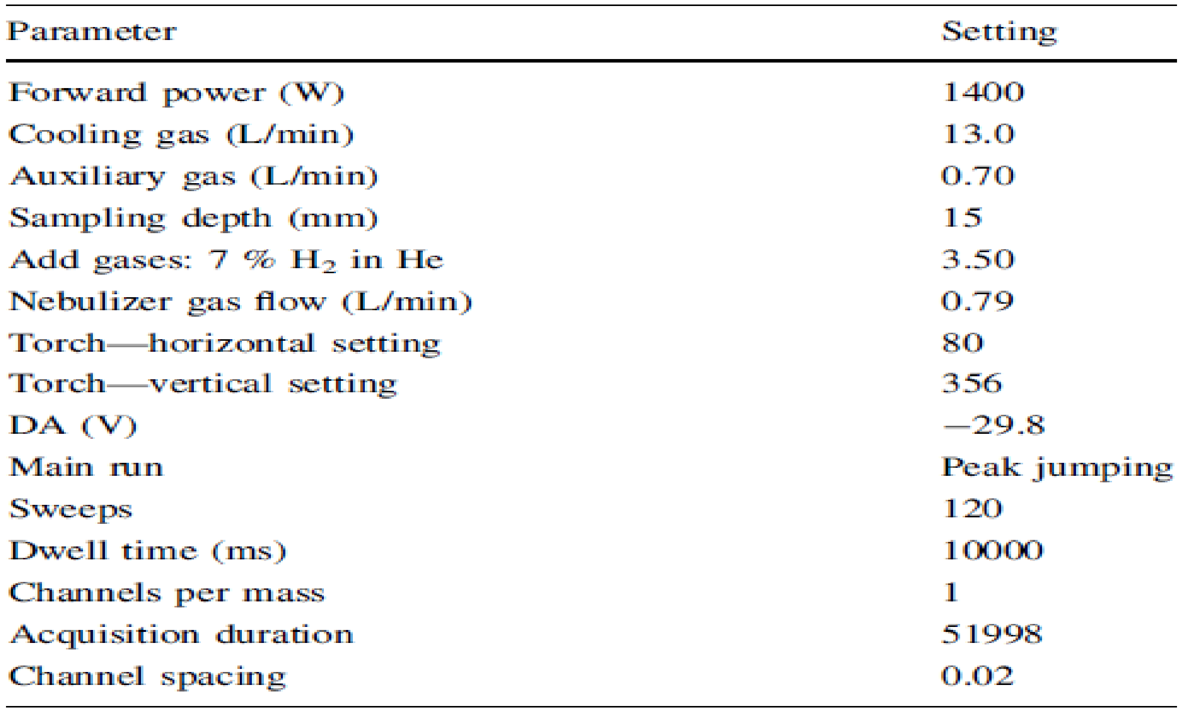
Fig 3.8: Thermo Scientific X Series 2 ICP-MS (Thermo Fisher Scientific Inc, 2010)

During the development of ICP-MS as an analytical tool, it has emerged that one of the limitations of the analytical technique is the presence of molecular ions that are known to overlap the preferred isotopes of the trace metals being analysed (McCurdy & woods, 2012). These molecular ions known as polyatomic ions (see Table 3.5) and are as a result of combinations of the elements present in the plasma or from the samples themselves in the matrix of the sample or the solvent being implemented (McCurdy & Woods, 2012). For remediation of this issue, Collision Cell Technology (CCT) was applied at the Method Improvement phase.

Trace Metal Element	Isotope	Interferences by poly-atomic ions
Lead	$^{206}\mathrm{Pb}$ $^{207}\mathrm{Pb}$ $^{208}\mathrm{Pb}$	$^{190}\mathrm{Pt}^{16}\mathrm{O}+$,$^{191}\mathrm{Ir}^{16}\mathrm{O}+$, $^{192}\mathrm{Pt}^{16}\mathrm{O}+$
Chromium	$^{52}\mathrm{Cr}$ $^{53}\mathrm{Cr}$	$^{40}\mathrm{Ar}^{12}\mathrm{C}$, $^{35}\mathrm{Cl}^{16}\mathrm{O}^{1}\mathrm{H}$ $^{37}\mathrm{Cl}^{16}\mathrm{O}$, $^{36}\mathrm{Ar}^{16}\mathrm{O}^{1}\mathrm{H}$, $^{36}\mathrm{S}^{16}\mathrm{O}+$,
Cobalt	$^{59}\mathrm{Co}$	$^{43}\mathrm{Ca}^{16}\mathrm{O}+$, ^{42}Ca16O1H+, $^{24}\mathrm{Mg}^{35}\mathrm{Cl}+$, $^{36}\mathrm{Ar}^{23}\mathrm{Na}+$, $^{40}\mathrm{Ar}^{18}\mathrm{O}^{1}\mathrm{H}+$, $^{40}\mathrm{Ar}^{19}\mathrm{F}+$
Copper	$^{63}\mathrm{Cu}$ $^{65}\mathrm{Cu}$	$^{40}\mathrm{Ar}^{23}\mathrm{Na}$, $^{31}\mathrm{P}^{16}\mathrm{O}_2$, $^{26}\mathrm{Mg}^{37}\mathrm{Cl}$, $^{49}\mathrm{Ti}^{16}\mathrm{O}$, $^{23}\mathrm{Na}^{40}\mathrm{Ca}$, $^{32}\mathrm{S}^{16}\mathrm{O}_2{}^{1}\mathrm{H}$
Nickel	$^{58}\mathrm{Ni}$ $^{60}\mathrm{Ni}$	$^{40}\mathrm{Ca}^{18}\mathrm{O}$, $^{44}\mathrm{Ca}^{14}\mathrm{N}$, $^{23}\mathrm{Na}^{35}\mathrm{Cl}$, $^{24}\mathrm{Mg}^{34}\mathrm{S}$, $^{44}\mathrm{Ca}^{16}\mathrm{O}$, $^{43}\mathrm{Ca}^{16}\mathrm{O}^{1}\mathrm{H}$, $^{23}\mathrm{Na}^{37}\mathrm{Cl}$
Manganese	$^{55}\mathrm{Mn}$	$^{40}\mathrm{Ar}^{14}\mathrm{N}^{1}\mathrm{H}+$, $^{39}\mathrm{K}^{16}\mathrm{O}+$, $^{37}\mathrm{Cl}^{18}\mathrm{O}+$, $^{40}\mathrm{Ar}^{15}\mathrm{N}+$, $^{38}\mathrm{Ar}^{17}\mathrm{O}+$, $^{36}\mathrm{Ar}^{18}\mathrm{O}^{1}\mathrm{H}+$, $^{38}\mathrm{Ar}^{16}\mathrm{O}^{1}\mathrm{H}+$, $^{37}\mathrm{Cl}^{17}\mathrm{O}^{1}\mathrm{H}+$, $^{23}\mathrm{Na}^{32}\mathrm{S}+$, $^{36}\mathrm{Ar}^{19}\mathrm{F}+$
Zinc	**$^{67}\mathrm{Zn}$**	$^{35}\mathrm{Cl}^{16}\mathrm{O}_2+$, $^{33}\mathrm{S}^{34}\mathrm{S}+$, $^{34}\mathrm{S}^{16}\mathrm{O}_2{}^{1}\mathrm{H}+$, $^{32}\mathrm{S}^{16}\mathrm{O}^{18}\mathrm{O}^{1}\mathrm{H}+$, $^{34}\mathrm{S}^{16}\mathrm{O}^{17}\mathrm{O}+$, $^{33}\mathrm{S}^{16}\mathrm{O}^{18}\mathrm{O}+$, $^{32}\mathrm{S}^{17}\mathrm{O}^{18}\mathrm{O}+$, $^{33}\mathrm{S}^{17}\mathrm{O}_2+$, $^{35}\mathrm{Cl}^{16}\mathrm{O}_2+$

Table 3.5: Table of Elements Analysed, Some of Their Stable Isotopes and Their Common Polyatomic Spectral Interferences (DIN EN ISO 17294-2, 2005)

Samples were introduced to the ICP-MS plasma by an autosampler, through a nebulizer with all samples being run in triplicate replication. Details of the ICP-MS operating conditions are shown in Table 3.6. The Internal standard Rhodium was used to compensate for analytical drift. All samples and standards were prepared with 2 % reverse aqua regia using trace analysis grade HNO_3 and HCl (3:1) (Fisher Scientific, UK) prior to introduction (the same proportion as was used for digestion step). Calibration standards included Co, Cr, Cu, Mn, Ni, Pb, and Zn, concentrations of which were in the range blank (0 ppb) up to 200 ppb (0, 10, 25, 50, 100, 150 and 200 ppb).



Parameter	Setting
Forward power (W)	1400
Cooling gas (L/min)	13.0
Auxiliary gas (L/min)	0.70
Sampling depth (mm)	15
Add gases: 7 % H_2 in He	3.50
Nebulizer gas flow (L/min)	0.79
Torch—horizontal setting	80
Torch—vertical setting	356
DA (V)	−29.8
Main run	Peak jumping
Sweeps	120
Dwell time (ms)	10000
Channels per mass	1
Acquisition duration	51998
Channel spacing	0.02

Table 3.6: Thermo-Fisher X Series 2 Operating Conditions

For the calibration curves, standards were prepared using 1000ppm single element certified reference standard materials (1000ppm in nitric acid, TraceCert, Merck) for ICP-MS. For the matrix solution, the standards were prepared and filtered using 0.45µm syringe filter. These standards were then acidified with reverse aqua regia in the same proportion as in the digestion step.

The acids used during method development, hydrochloric acid 37% and nitric acid 67%, were reagent quality and at trace metal grade whilst the ultra-purity water was produced with a Millipore Milli-Q System at 18.2 MΩ-cm.

3.2.4 The Use of Standard Deviation in a Multi-Element Approach

When studying trace metal data, the results uniform with a similar result to the mean total or they can be spread out with a wide spread of results. The wider the spread of the obtained results, the larger the result for the standard deviation. Standard Deviation is set out in Equation 3.1:

$$\mathrm{SD} = \sqrt{\frac{\sum |x - \bar{x}|^2}{n}}$$

Equation 3.1: Standard Deviation

where ∑ means "sum of", x is a value in the data set, $\bar{x}$ is the mean of the data set, and n is the number of samples analysed in the study. This statistical tool can be further applied when converting the Standard Deviation to relative standard deviation (RSD). The coefficient of variation (RSD) determines if the standard deviation (SD) of a dataset is great or small when compared to the mean. The RSD is determined as shown in Equation 3.2:

$$\mathbf{RSD = 100SD/}\ \bar{x}$$

Equation 3.2: Relative Standard Deviation

Where SD = Standard Deviation, $\bar{x}$ = Mean of the data set.

The aim of the use of the standard deviation statistical method is to find the SRM with the mean closest to the 'ideal' 100% recovery for multiple elements with the least amount of variance from that mean. An SRM sample set with a relative standard deviation of equal to or less than 10% whilst having a recovery figure close to the 'ideal' 100% is often considered acceptable in terms of method development and has been the benchmark in many studies in which proposed methods are found to be suitable for trace metal analysis in soils (Zhou et al, 2012).

3.3. Results and Discussion

Visual representation of trace metal element recovery was undertaken by producing jitter charts for each method development step (see Figs 3.9-3.12). Initially, results were studied based on their temperature profile, including max temp, ramp time and hold time (Fig 3.9, Fig 3.10 and Fig 3.11).

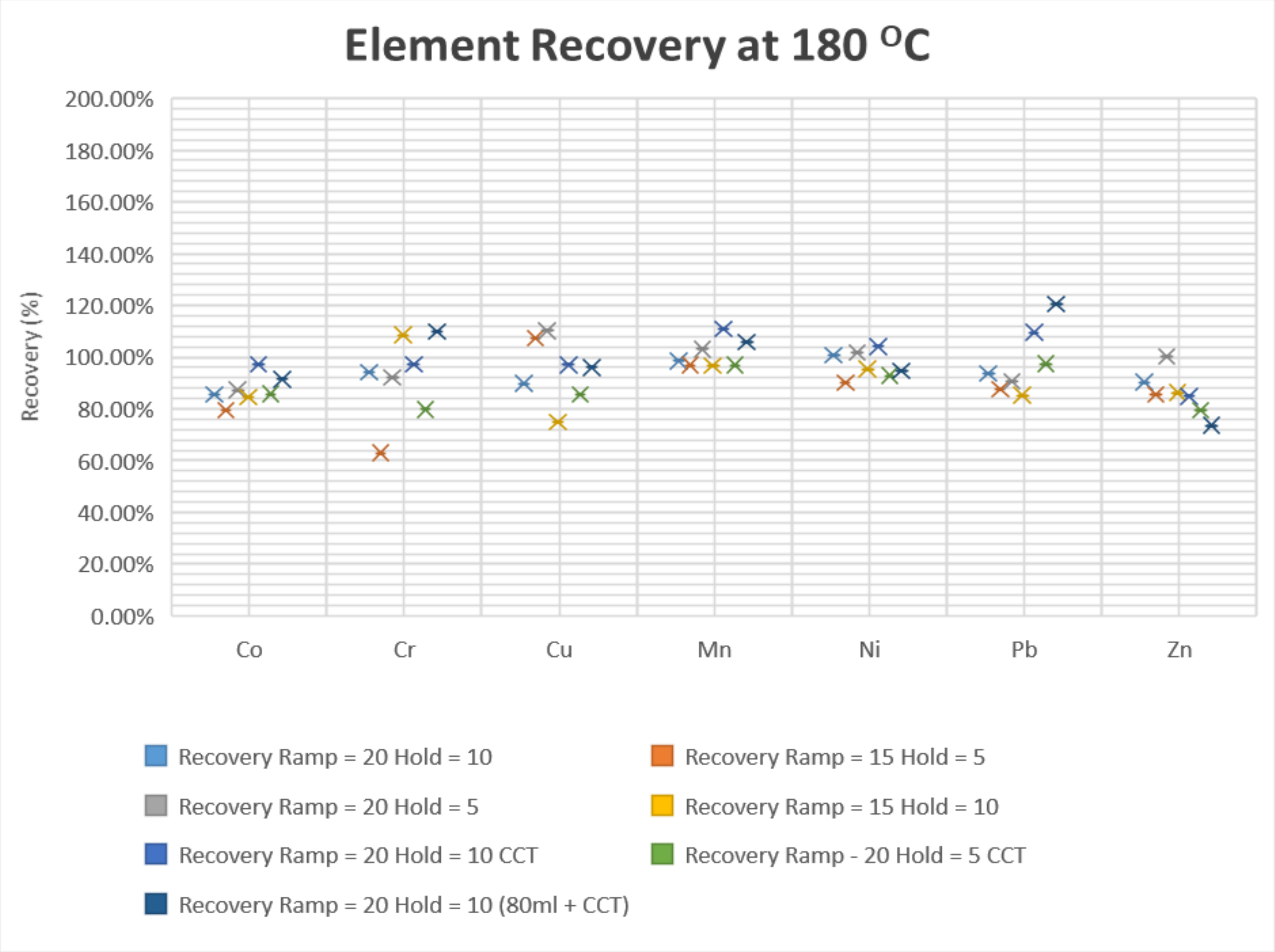


Fig 3.9: Recovery of all elements at 180^{O}C

Following collation of the individual results for each run, the minimum, mean and maximum recovery were determined for each soil reference material at temperature profile. The standard deviation (SD) of the results were then calculated (Table 3.7, Table 3.8 and Table 3.9). 'N' signifies the number of trace elements analysed.

Certified Soil Reference Material Type	**N**	**Minimum %**	**Maximum %**	**Mean %**	**Std. Deviation %**	**RSD %**
SRM 1: Recovery Ramp = 20 Hold = 10	7	85.53	100.64	93.22	5.23	5.61
SRM 4: Recovery Ramp = 15 Hold = 5	7	63.01	107.47	87.16	13.91	15.96
SRM 7: Recovery Ramp = 20 Hold = 5	7	87.28	110.26	97.91	8.16	8.33
SRM 10: Recovery Ramp = 15 Hold = 10	7	74.99	108.5	90.24	10.86	12.03
SRM 13: Recovery Ramp = 20 Hold = 10 CCT	7	84.82	110.94	100.09	8.97	8.96
SRM 14: Recovery Ramp - 20 Hold = 5 CCT	7	79.50%	97.38%	88.23%	7.56%	8.57
SRM 16: Recovery Ramp = 20 Hold = 10 (80ml + CCT)	7	73.57%	120.54%	98.90%	15.07%	15.24

Table 3.7: Recovery and Standard Deviation Results for SRMs at 180^{O}C

Following recovery analysis of these results, any SRM that had results falling below the 80% threshold or above the 120% threshold were excluded from the study. This was the case for SRM 4, SRM 10 and SRM 16 when considering those SRMs digested at 180^{O}C, SRM 5, SRM 17 and SRM 18 when considering those SRM samples digested at 200^{O}C (see Table 3.8) and SRM 3, SRM 9 and SRM 12 when considering those SRMs digested at 220^{O}C.

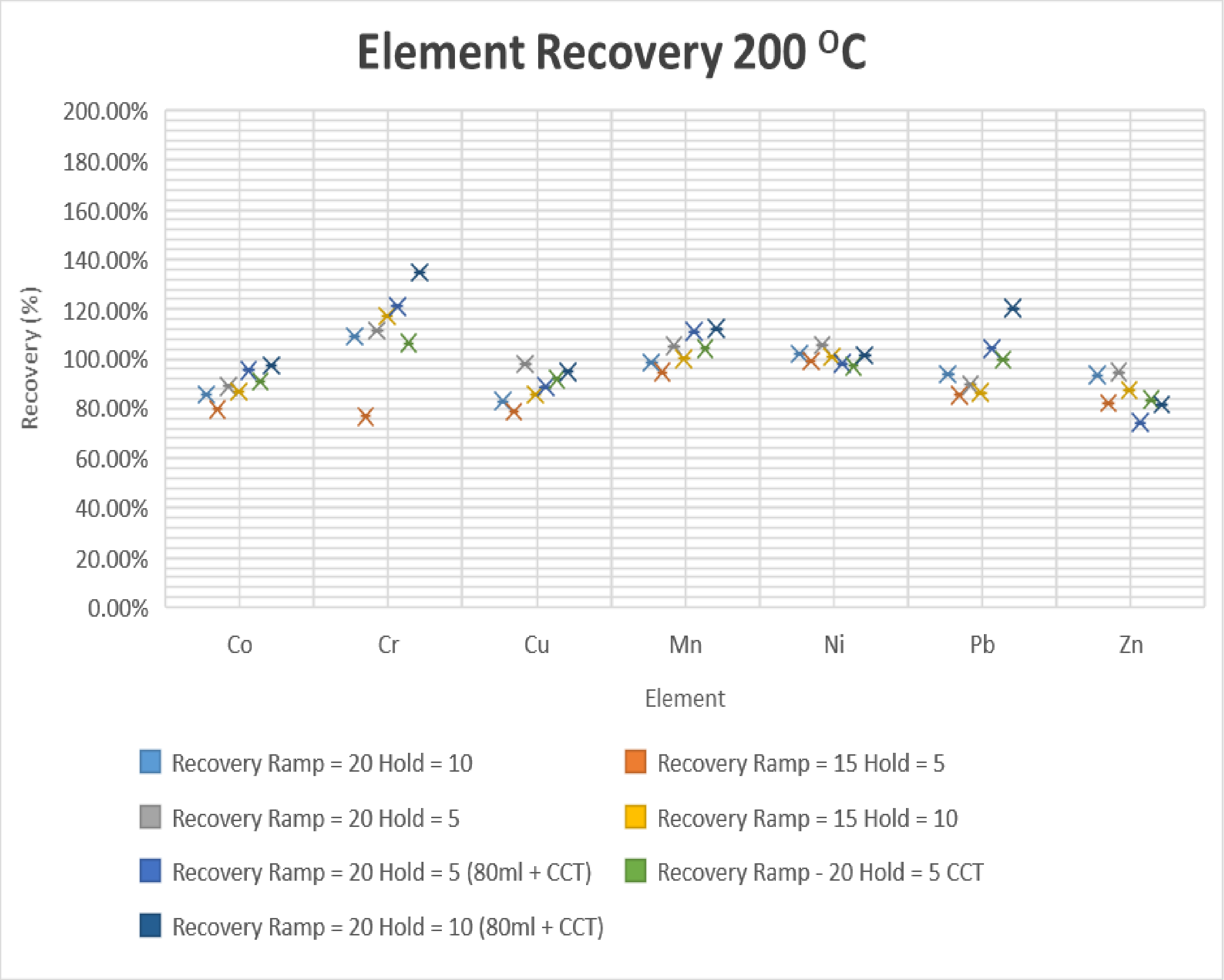


Fig 3.10: Recovery for all Elements at 200^{O}C

Certified Soil Reference Material Type	Number of Elements	Minimum %	Maximum %	Mean %	Std. Deviation %	RSD %
SRM 2: Recovery Ramp = 20 Hold = 10	7	82.98	109.01	95.08	9.09	9.56
SRM 5: Recovery Ramp = 15 Hold = 5	7	76.79	99.06	85.23	8.49	9.96
SRM 8: Recovery Ramp = 20 Hold = 5	7	88.96	111.44	99.02	8.61	8.70
SRM 11: Recovery Ramp = 15 Hold = 10	7	85.65	117.08	94.96	11.77	12.39
SRM 17: Recovery Ramp = 20 Hold = 5 (80ml + CCT)	7	74.21	121.05	98.97	15.21	15.37
SRM 15: Recovery Ramp - 20 Hold = 5 CCT	7	83.57	106.4	96.31	8.02	8.33
SRM 18: Recovery Ramp = 20 Hold = 10 (80ml + CCT)	7	81.63	134.74	106.07	17.72	16.71

Table 3.8: Recovery and Standard Deviation Results for SRMs at 200^{O}C

Following calculation of the standard deviation (SD) and conversion to relative standard deviation (RSD), any SRM sample found to have a relative standard deviation (RSD) greater than 10% were also then excluded from the study. This was the case for SRM 4, SRM 10 and SRM 16 when considering those samples digested at 180°C, SRM 11, SRM 17 and SRM 18 when considering those samples digested at 200°C and SRM 3, SRM 6, SRM 9 and SRM 12 for those SRMs digested at 220°C (see Fig 3.9, Fig 3.10 and Fig 3.11).

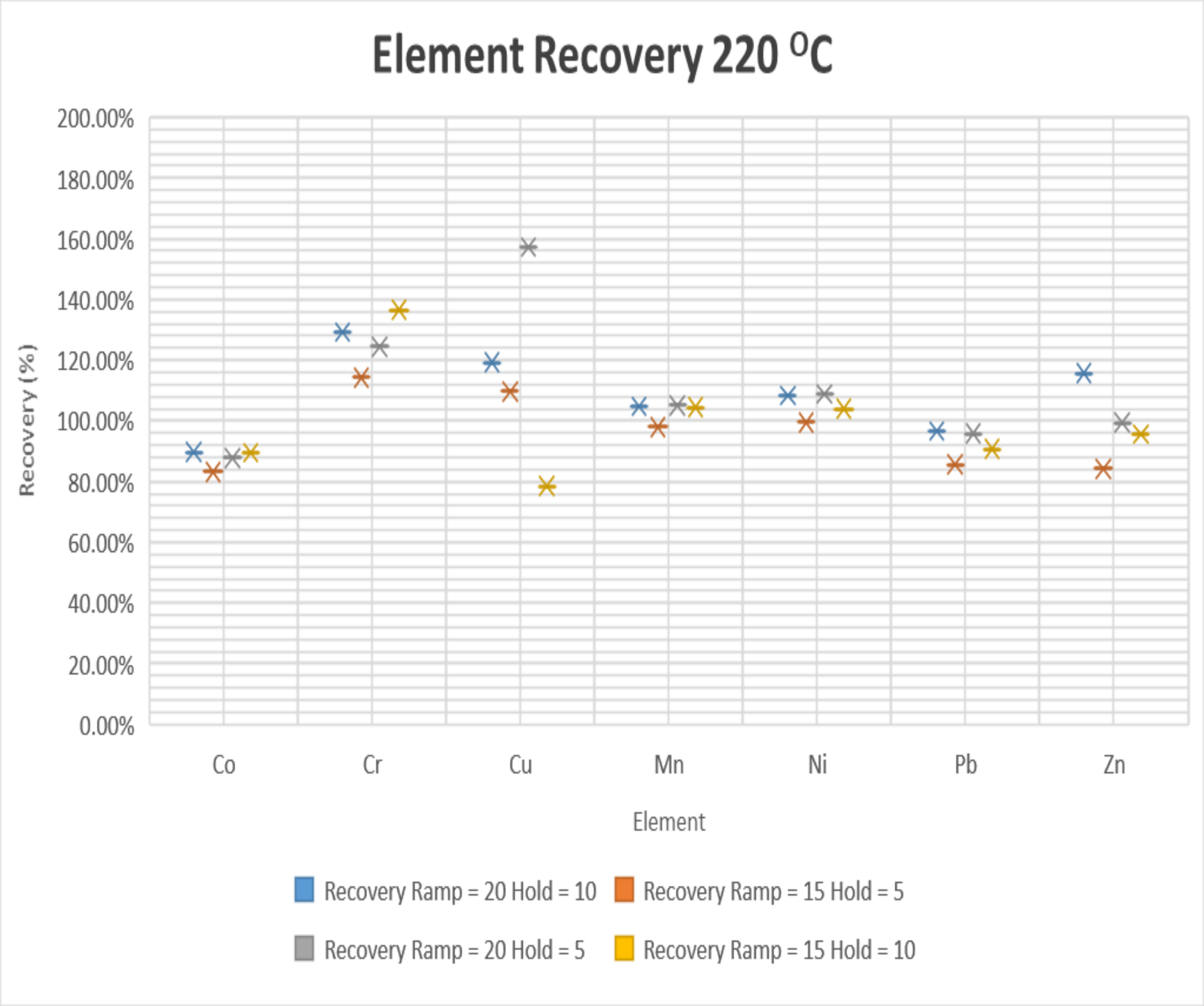


Fig 3.11: Recovery for all Elements at 220°C

Certified Soil Reference Material Type	Number of Elements	Minimum %	Maximum %	Mean %	Std. Deviation %	RSD %
SRM 3: Recovery Ramp = 20 Hold = 10	7	89.74	129.22	109.11	13.54	12.41
SRM 6: Recovery Ramp = 15 Hold = 5	7	83.26	114.25	96.36	12.54	13.01
SRM 9: Recovery Ramp = 20 Hold = 5	7	87.83	157.32	111.26	23.33	20.97
SRM 12: Recovery Ramp = 15 Hold = 10	7	78.54	136.58	99.9	18.48	18.50

Table 3.9: Recovery and Standard Deviation Results for all SRMs at 220^{O}C

When analysing the temperature profile, recovery threshold and then examining the relative standard deviation (RSD) of the remaining SRM samples it can be seen that all the SRMs that were digested at 220^{O}C were excluded from the study as, despite some individual elements giving good recovery values (as is the case for SRM 6) with all having a mean recovery of over 95% as shown in Table 3.9, there was overall too much variation when examining from a multi-element approach (Fig 3.12) which resulted in a high relative standard deviation (>10%) for all samples ran at this temperature (Table 3.9). This is most likely due to the increased temperature causing increased loss from volatilisation in the 'closed vessel with venting' system (Amaral et al, 2016) and spectral interferences from ICP-MS analysis (Nordberg et al, 2014).

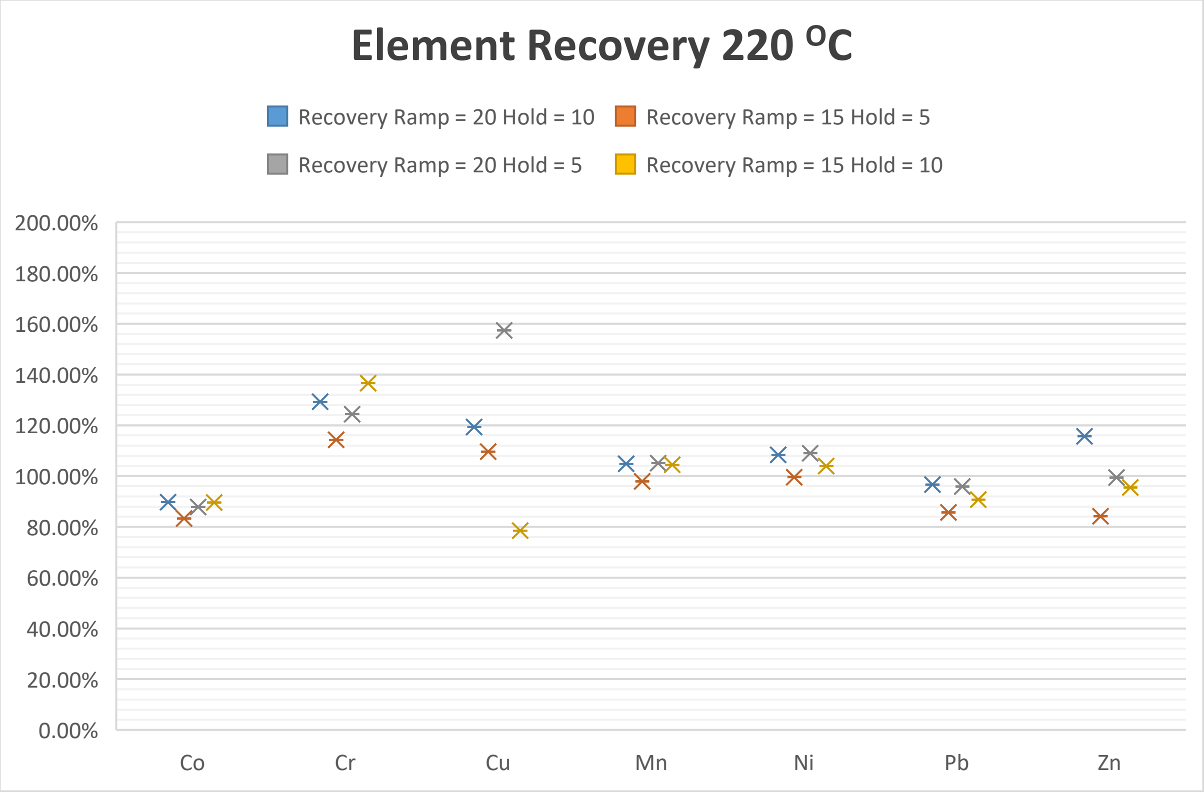


Fig 3.12: Variation of Recovery at 220ºC

3.3.1 Refinement of Method Development and Method Improvement.

From initial observation of the results, it can be seen that the samples with a higher overall digestion time (and more specifically Ramp time) give the most optimal results. This indicates that a slower, steady ramp to maximum temperature (within the set parameters) may prevent variability and also loss due to the potential for volatilisation in a 'closed vessel with venting' system. However, it is important to take mean recovery and relative standard deviation from that recovery into account when considering an appropriate SRM.

Following the analysis of the SRMs and consequent elimination from the study for those SRMs not meeting the recovery and/or RSD requirements, a Method Improvement stage was implemented. This involved application of Collision Cell Technology (CCT) to attempt to remove any polyatomic interferences. Vessel size was also increased, depending on the results obtained, to examine potential loss on volatility (Costa et al, 2009).

When examining potential suitable SRM digestion methods from the samples run at 180ºC (Table 3.7) we can see that there are 2 potential candidates (SRM 1 and SRM 7). These candidates were then further refined with CCT (SRM 13 and SRM 14). This can be seen in Fig 3.13 and Table 3.10.

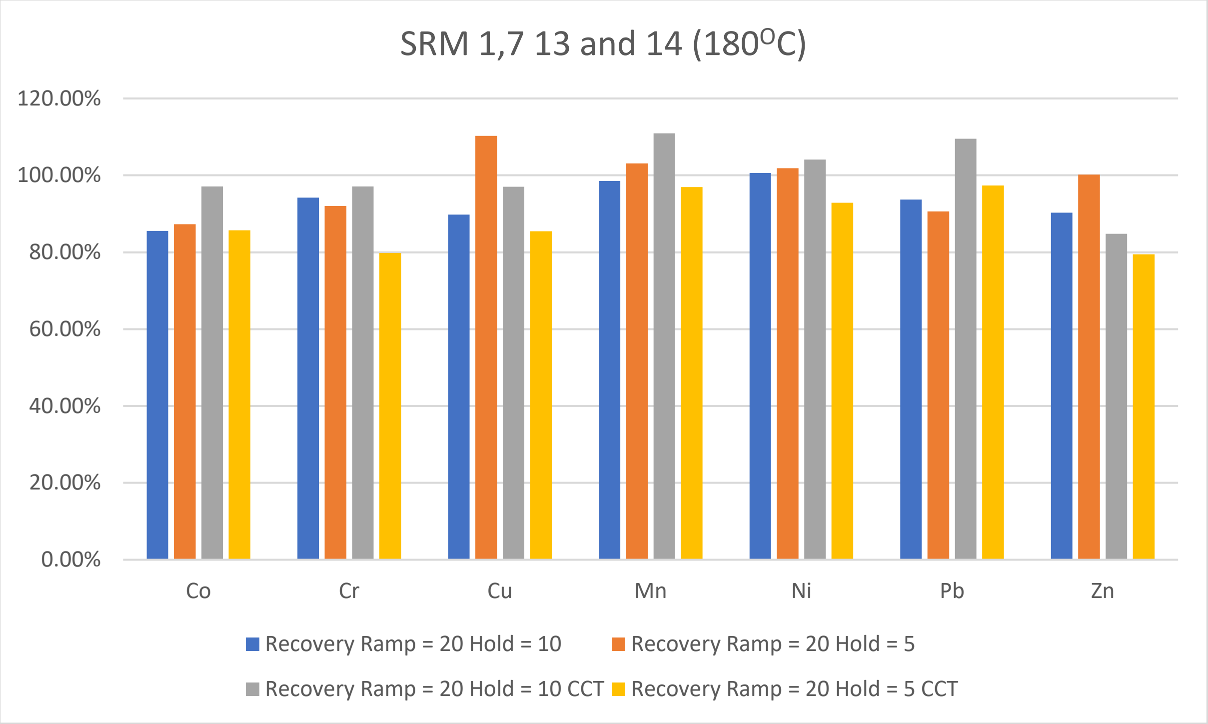


Fig 3.13: Comparison of SRMs 1, 13, 7 and 14

Certified Soil Reference Material Type	N	Mean %	Standard Deviation %	Minimum %	Maximum %	RSD %
SRM 1: Recovery Ramp = 20 Hold = 10	7	93.22	5.23	85.53	100.64	5.61
SRM 7: Recovery Ramp = 20 Hold = 5	7	97.91	8.16	87.28	110.26	8.33
SRM 13: Recovery Ramp = 20 Hold = 10 CCT	7	100.09	8.97	84.82	110.94	8.96
SRM 14: Recovery Ramp - 20 Hold = 5 CCT	7	88.23	7.56	79.5	97.38	8.57

Table 3.10: Results for SRMs 1, 7, 13 and 14

Due to the encouraging results displayed by SRM 1, further refinement of the parameters were then included to see if improved recovery could be obtained (SRM 13). This was done by applying CCT mode to reduce potential Spectral Interference (Fig 3.14).

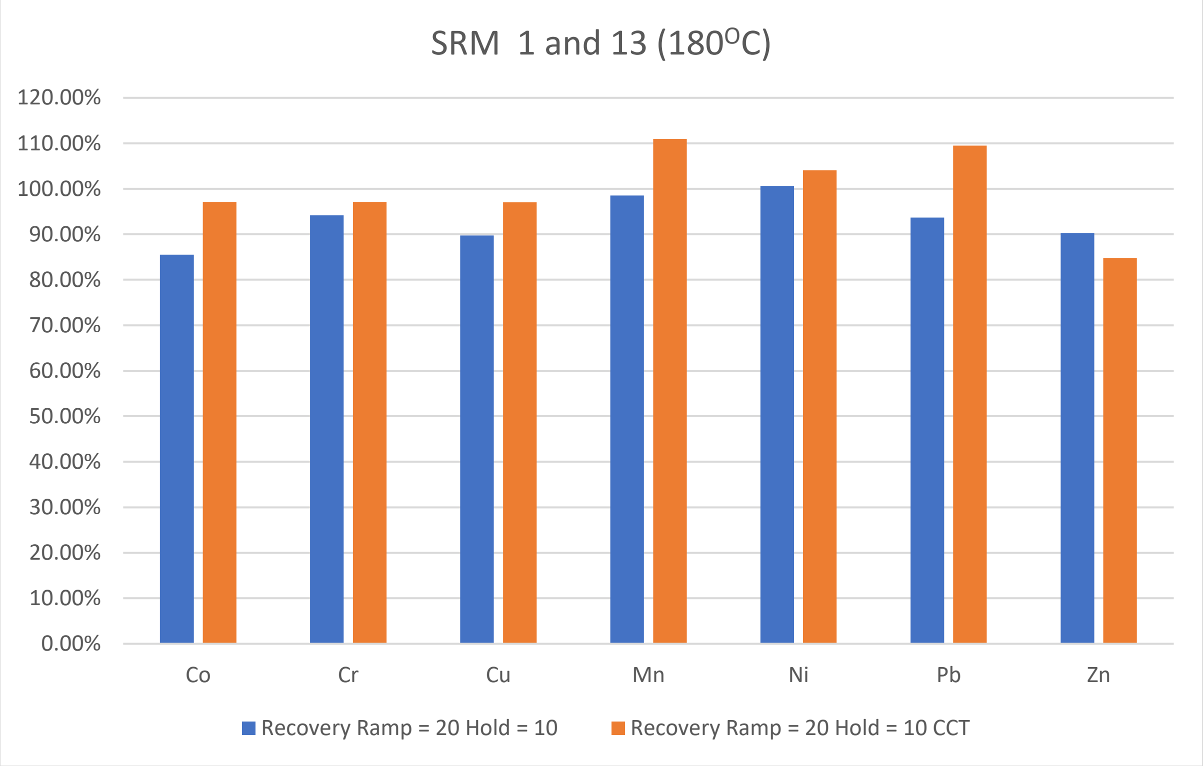


Fig 3.14: Comparison of SRM 1 and SRM 13

This further refinement by implementation of CCT resulted in the mean being closer to the 100 mark (100.09%), an overall increase in recovery for all but one of the elements (Zn) whilst still having a relative standard deviation below 10%. Both SRM 1 and SRM 13 had 6 out of 7 elements recovering at the 90-110% range, prior to uncertainty adjustment, which is desirable when looking for an appropriate SRM in proof of method validation.

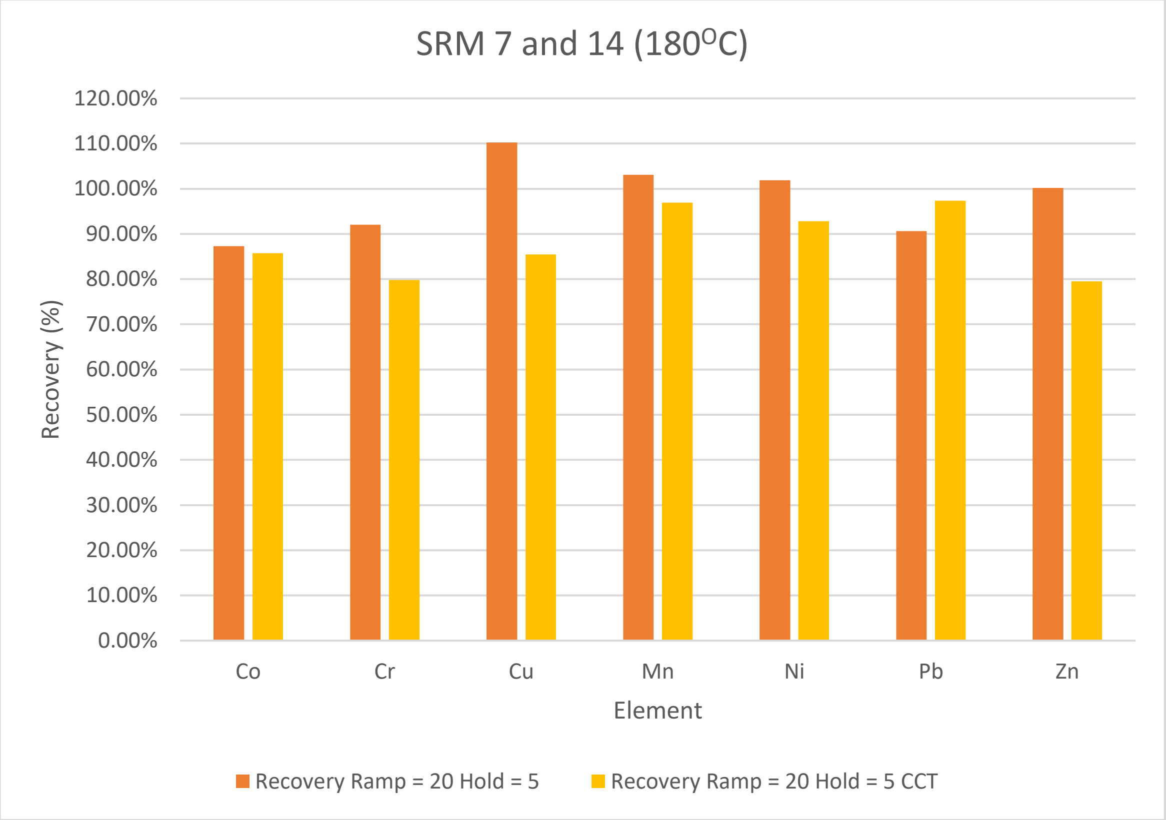


Fig 3.15: Comparison of SRM 7 and 14

SRM 7 also showed encouraging results and was further refined via the use of CCT (SRM 14) as shown in Fig 3.15. This reduced the maximum recovery, as expected, but also resulted in a lowering of the mean result and the minimum recovery hence excluding SRM 14 from the study as SRM 7 had a much superior mean recovery percentage across multiple elements. SRM 7 also had 6 of its 7 elements recovering at the 90-110% range prior to uncertainty adjustment, which is desirable when looking for an appropriate SRM in proof of method validation whereas SRM 14 only had 3 of its elements recovering at this range.

When examining potential suitable SRM digestion methods from the samples run at 200^{O}C it is observed that there are also 2 potential candidates (SRM 2 and SRM 8). These candidates were also further refined with CCT (SRM 15) mode and 80ml vessels (SRM 18) due to increased time at higher maximum temperature and thus increased chance of volatilisation. The results of which are shown in Fig 3.16 and Table 3.11.

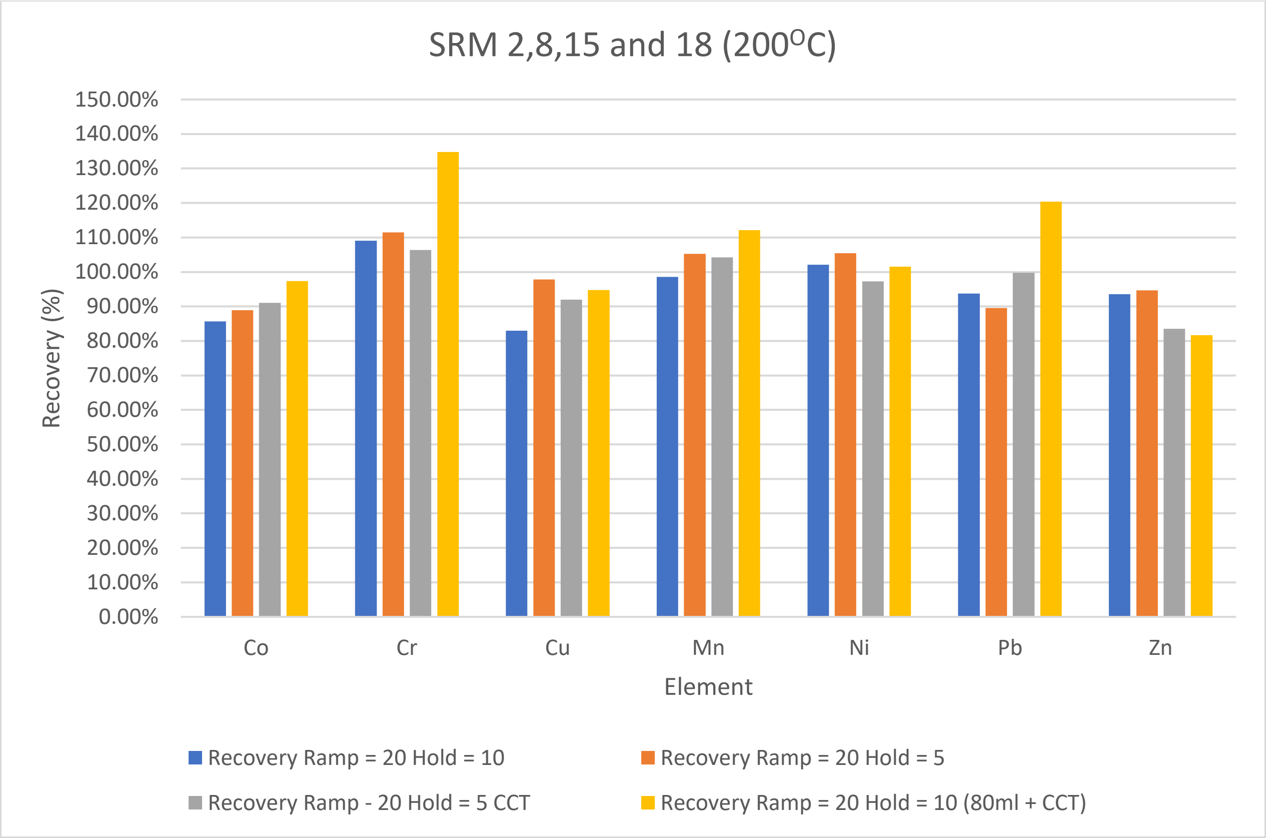


Fig 3.16: Comparison of SRMs 2, 8, 15 and 18

Certified Soil Reference Material Type	**N**	**Mean %**	**Standard Deviation %**	**Minimum %**	**Maximum %**	**RSD %**
SRM 2: Recovery Ramp = 20 Hold = 10	7	95.08	9.09	82.98	109.01	9.56
SRM 8: Recovery Ramp = 20 Hold = 5	7	99.02	8.61	88.96	111.44	8.70
SRM 15: Recovery Ramp - 20 Hold = 5 CCT	7	96.31	8.018	83.57	106.4	8.33
SRM 18: Recovery Ramp = 20 Hold = 10 (80ml + CCT)	7	106.07	17.72	81.63	134.74	16.71

Table 3.11: Recovery and Standard Deviation Results for SRMs 2, 8, 15 and 18

As Table 3.11 and Fig 3.17 show, SRM 2 showed promising results with a mean and relative standard deviation within acceptable boundaries. Further refinement with CCT (SRM 18) showed no improvement and was seen to have a maximum recovery and relative standard deviation exceeding the acceptable levels. SRM 18 was excluded as a result.

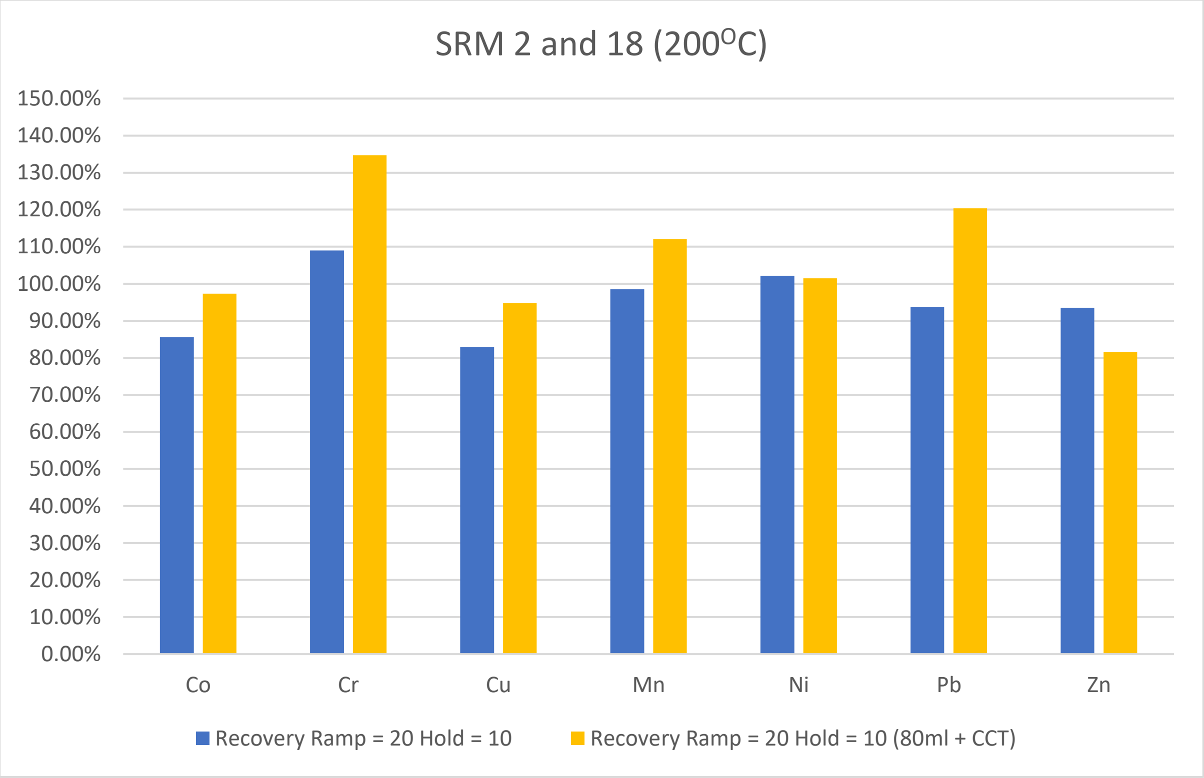


Fig 3.17: Comparison of SRM 2 and SRM 18

SRM 8 also showed promising results with a mean close to 100% (99.01%) and a relative standard deviation of less than 10%. Application of CCT mode for this SRM (SRM 15) showed very little difference in results (Fig 3.18), with a slightly reduced mean and only a minor improvement on standard deviation. This additional CCT refinement did, however, mean that 6 of the 7 elements analysed all fell within the 90-110% recovery range prior to uncertainty adjustment, making it more suitable for multi-element analysis (see Fig 3.18).

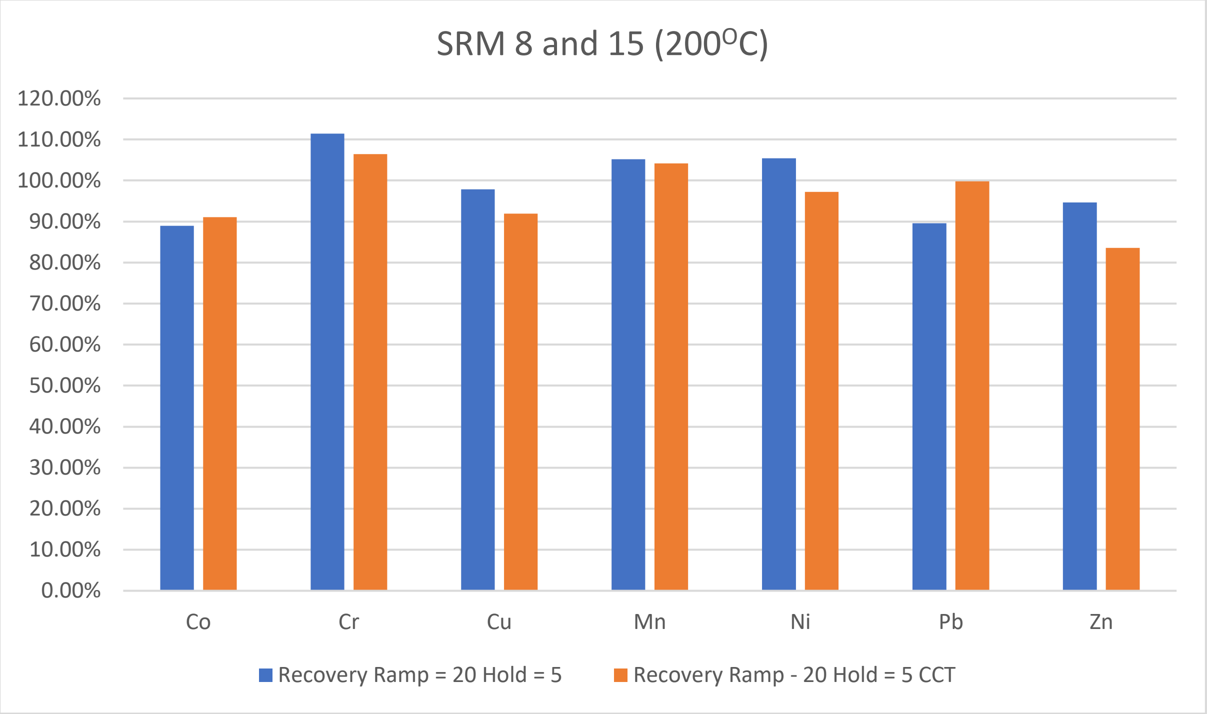


Fig 3.18: Comparison of SRM 8 and SRM 15

3.3.2 Comparison to Certified Results

The above results show that, out of the 18 analysed SRM preparations, 6 SRM samples appear to be suitable for analysing the chosen seven metals with a multi-element approach with all seven metals recovering in the 90-110% after considering certified uncertainty. These are SRMs 1, 2, 7, 8, 13 and 15 (see section 3.3.1). These SRMs were chosen for their results based on mean recovery percentage and with a relative standard deviation <10% (see Table 3.12 and Fig 3.19).

SRM Digest	Digestion Parameters	ICP-MS CCT Mode ON/OFF	Mean Recovery (%)	Relative Standard Deviation (%)
1	Temp 180^{O}C, Ramp 20 (mins), Hold 10 (mins)	OFF	93.22	5.61
2	Temp 200^{O}C, Ramp 20 (mins), Hold 10 (mins)	OFF	95.08	9.56
7	Temp 180^{O}C, Ramp 20 (mins), Hold 5 (mins)	OFF	97.91	8.33
8	Temp 200^{O}C, Ramp 20 (mins), Hold 5 (mins)	OFF	99.02	8.70
13	Temp 180^{O}C, Ramp 20 (mins), Hold 10 (mins)	ON	100.09	8.96
15	Temp 200^{O}C, Ramp 20 (mins), Hold 5 (mins)	ON	96.31	8.33

Table 3.12: The Mean Recovery and Relative Standard Deviation of Selected SRMs

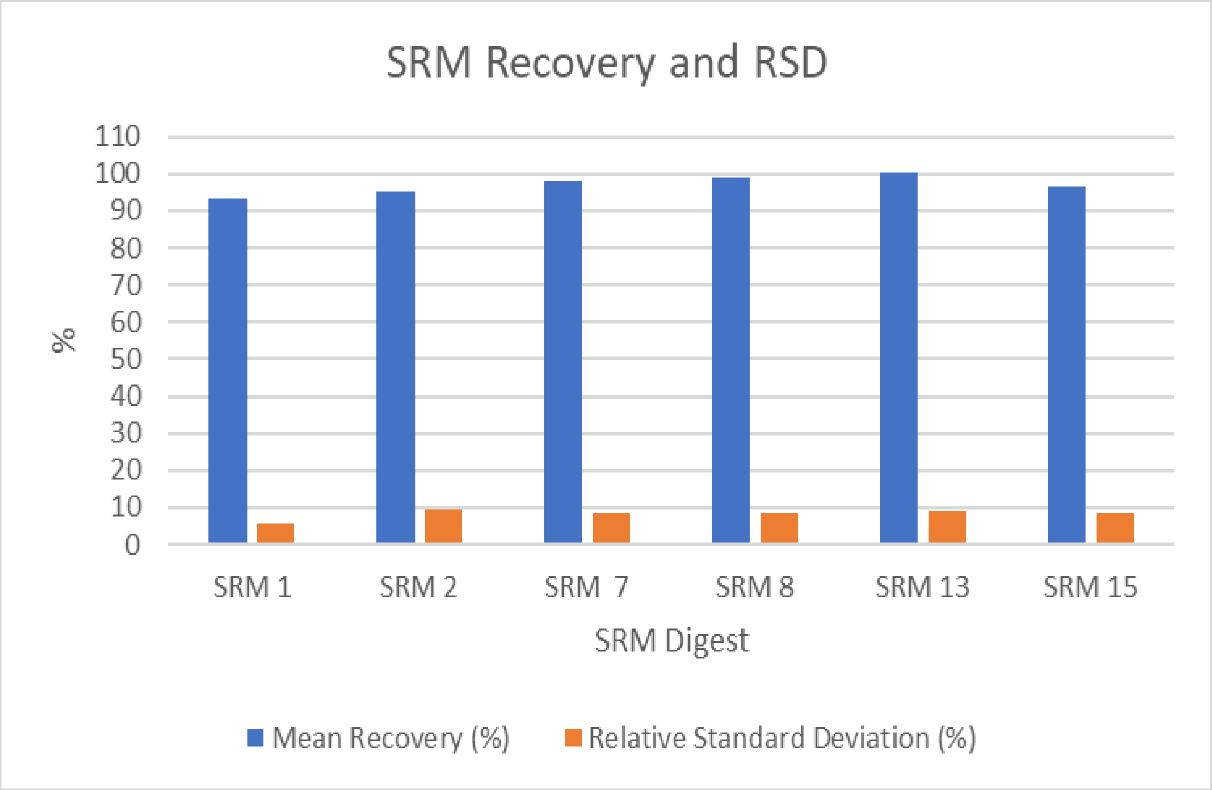


Fig 3.19: Visual Comparison of the Mean Recovery and Relative Standard Deviation of Selected SRMs

To evaluate the final selected extraction procedures, the obtained experimental values of the most promising looking SRMs (SRMs 8 and 13) were compared with certified results of the CRM ERC-CC141 (Birgersson-Liebich et al, 2010) loam soil (Table 3.13). These SRM samples were chosen as they show the best mean recovery across all 7 trace metals (close to the 'ideal' 100%) whilst having a RSD of less than 10% on the mean recovery.

The recovery percentages obtained for both SRM 8 and SRM 13 and the obtained values (mg/kg) when compared to the certified reference material (Table 3.13 and Fig 3.20) suggests that the methods involved respectively are adequate for the intended purpose of multi-element recovery of select elements when using reverse aqua regia as the digestion medium.

Metallic Element	Obtained Value for 0.2g of SRM 8 (mg/kg)	Recovery for SRM 8 %	Obtained Value for 0.2g of SRM 13 (mg/kg)	Recovery for SRM 13 %	Certified Value for 0.2g of ERM-CC141 (mg/kg)	Uncertainty in 0.2g of ERM-CC141 (mg/kg)
Co	14.06	88.99	14.40	91.12	15.8	1.8
Cr	69.11	111.47	60.22	97.13	62	8
Cu	24.27	97.86	24.06	97.02	24.8	1.8
Mn	814.59	105.24	858.58	110.93	774	34
Ni	46.20	105.48	45.58	104.06	43.8	3.2
Pb	57.72	89.62	70.52	109.50	64.4	2.8
Zn	94.65	94.65	84.81	84.81	100	8

Table 3.13: Comparison of SRM 8 and 13 to Certified Values

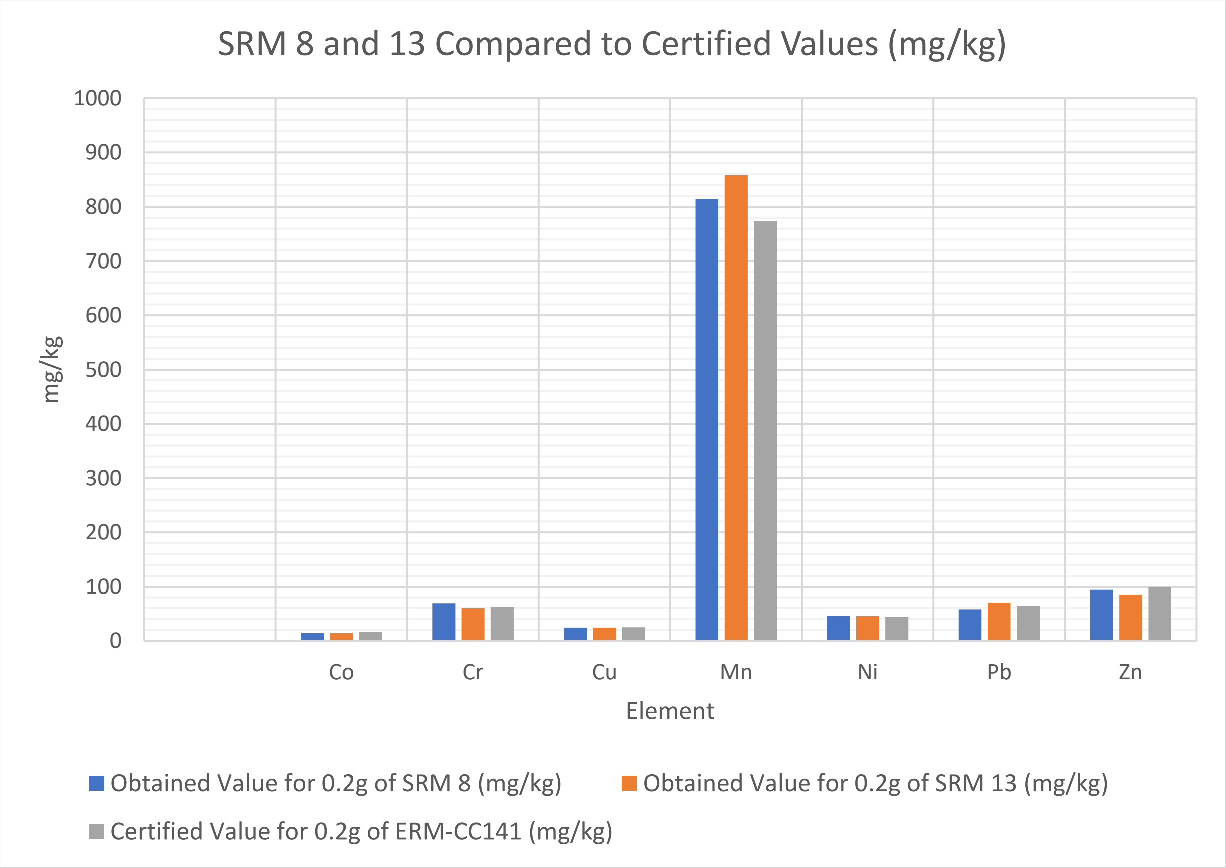


Fig 3.20: Visual Comparison of SRM 8 and 13 to Certified Values

3.4. Conclusion

Method development was undertaken for the accurate determination of seven trace metals in a soil matrix via a Sequential Pressure Microwave Digestion (closed vessel with venting) system and ICP-MS analysis using a simultaneous 'multi-element' approach when using reverse aqua regia as the digestion medium. To this end, a certified reference material (ERM-CC141) was used for validation. Recoveries close to 100 % of the certified values, within the range of the certified uncertainties, were achieved with the use of optimal digestion parameters that were determined throughout the course of the method development.

A simultaneous multi-element approach is advantageous when looking at a logistical and economic viewpoint, saving on time spent on sample preparation and analysis. This research shows, however, that when analysing multiple elements at once via ICP-MS, that even the most subtle of changes in digestion method can alter recovery rates and the variability of such (SD and RSD). Increased maximum temperature and/or decrease in temperature ramp time increases variability around the mean result (which is possibly due to acid digest losses when the acid vapor pressure exceeds the venting pressure of the vessel) and impacts the mean recovery percentage outcome, especially when examining the duration of the digestion process.

Out of a total of 18 Soil Reference Material (SRM) variations analysed (all derived from certified reference material ERM-CC141), all with subtle differences in digestion parameters, 6 of the digestion variations (SRMs) passed the set criteria with all seven elements having a recovery of between 90-110% when the certified uncertainty was taken into consideration. These SRMs had a minimum ramp time to maximum temperature of 20 minutes indicating that the longer ramp time helps with a more stable digestion process.

From the results we can see that just a subtle change in the maximum temperature (>200^{O}C) of digestion increases the standard deviation around the mean of the recovery with all SRMs analysed at >200^{O}C being excluded from the study. We can also see that a shorter ramp time (<20 minutes) seems to impact the recovery mean percentage negatively and can also impact the variation of recoveries within the digest. This is most likely due to increased volatilisation with the digests when a more rapid heating time is applied as well as an increase in maximum digestion temperature.

Various environmental standards and quality assurance stipulate that the average recovery of elements should be between 80 and 120% to be acceptable with some EU standards stating recovery for some elements should be between 90 and 110%. This is also in line with other reported ICP-MS data on these reference materials. These requirements were fulfilled for the average recovery (90-110%) obtained across all the elements analysed with a suitable RSD (<10%) for the accepted SRMs (1,2,7,8,13 and 15) with SRMs 8 and 13 achieving the most efficacious results (or SRMs 13 and 15 when using CCT mode) and, as such, these chosen method/s were suitable when using reverse aqua regia as the digestion medium. From the obtained data within this study, it is now possible to apply these analytical techniques and instrument settings when analysing agricultural soils for the pseudo-total concentration of trace metal elements (as is the purpose of the ERM-CC141 Loam Soil). This is of fundamental importance when a set of reliable data is required. As such, the findings from this method development were implemented in the analytical procedures performed in Chapter 5 of this thesis. The outcomes and findings of this method development process, and consequent comparison study (see Chapter 5), result in a robust methodology for this analytical procedure (ICP-MS analysis with reverse aqua regia digestion) that can be used in confidence when analysing topsoil of the type found in agricultural habitats and for use in those ecological assessments which require an acid digestion methodology to obtain valid data.

Chapter 4

The Occurrence and Spatial Dynamics of Trace Metals in the Agricultural Topsoil of a Meres and Mosses Wetland Habitat. A Multivariate Approach.

Abstract

Soil is a key component of the terrestrial eco-system in which rock, air and water interface and is, consequently, subject to anthropogenic input resulting in it being subject to a variety of pollutants. Trace metals are of increasing concern due to being persistent in the terrestrial environment and potential for bioavailability. The Meres and Mosses of Cheshire and Shropshire suffer from the effects of diffuse pollution in its agricultural soils. Principal Component Analysis (PCA), Cluster Analysis (CA) and Correlation Matrices were used in the task of source identification, to aid in the determination of the spatial variability of trace metals in the topsoil of the Meres and Mosses region of Cheshire and Shropshire, to determine intra-metal relationships (occurrence correlation) and to determine their relationship with soil pH and soil organic matter (OM) levels in this unique and environmentally sensitive region. To achieve this, a screening exercise of the topsoil was undertaken which included measuring the concentrations of 17 trace metals via Field Portable X-Ray Fluorescence Spectrometry (FPXRF), Magnetic Susceptibility, pH and organic matter content. These factors were assessed in a designated sampling area within the Meres and Mosses region. This sampling area was implemented so to represent the areas previously designated as the Nature Improvement Area (NIA) and Landscape Partnership Scheme (LPS) by local and national environmental governing bodies. The spatial distributions of metals in the topsoil were analysed based on the obtained data for trace metal concentrations in the topsoil using the geo-statistics and GIS mapping methods (Kriging Interpolation). The results of the trace metal concentrations were then placed into context by examining adherence to prominent soil quality guidelines. The results indicate that soil samples from the region have elevated trace metal concentrations when considering Enrichment Factor analysis (EF) and various soil quality guidelines. The Enrichment Factor results indicate that Zn, V, Cr, Co, Rb, Sn and Ga showed minimum to moderate enrichment whilst Pb, As and Nb in the topsoil of the region were moderately to significantly enriched.

For trace metals which have established target values, Pb, Zn, V, Cr and Co were seen to exceed the Dutch Standards Target/Background values by 1.02, 1.33, 14.40, 1.23 and 13.97 times the limits as

adapted to the sampling region, respectively. Significant correlations were determined to exist between many of the trace metals as well as with physiochemical factors. Trace metal concentrations were found to be mainly influenced by geogenic factors, organic matter and pH levels as well as partly by anthropogenic sources (as indicated by the PCA and spatial analysis).

4.1 Introduction

4.1.1 The Impact of Trace Metals in Soil and Topsoil

Soil is a primary component of the terrestrial ecosystem which is essential for the growth of flora and the degradation and recycling of biomass (Alloway, 1995). In this dynamic body, rock, air and water interface (Sun and Chen, 2016). As a result they are subject to a variety of pollutants due to different anthropogenic activities including impacts from agriculture, industry and transport (Facchinelli et al, 2001). Agricultural soil may be the recipient of trace metal contamination via the application of sewage sludge, fertilising chemicals and pesticides, which may contain a wide variety of trace metals as impurities (Franco-Uria et al, 2009).

The term ‘trace metals’ (sometimes known as ‘heavy metals’) is applied to the group of metals and semi-metals (metalloids) that have been associated with contamination of the environment and potential for toxicity (van der Perk, 2006). It has also been said that trace metals are those that have a high density and reside in the transition group of the periodic table (Haygarth and Jarvis, 2002). Most of these trace metals are also called heavy metals because it signifies their low abundance in soils and high density (de Vries et al. 2002). Trace metals have increasingly been the subject of much attention over recent years due to their pollutant characteristics. Tam & Wong (2000) state that trace metals “are one of the more serious pollutants in our natural environment due to their toxicity, persistence and bioaccumulation problems”. Kumar et al, 2017 state that trace metals “cannot be destroyed biologically but are only transformed from one oxidation state or organic complex to another” (Garbisu and Alkorta, 2001; Gisbert et al., 2003). Facchinelli et al (2001) state that trace metals “do not decay with time unlike many organics and radionuclides”. Trace metals can be necessary and beneficial to flora and fauna at certain levels but can also be toxic at higher concentration and when exceeding threshold limits for a given receptor. Due to pedogenesis and the natural weathering of the parent rock, trace metals are present at a background level from geogenic origin.

Trace metals can remain inert in soils for many years but can become mobile due to an environmental event or anthropogenic impact. This potential for large-scale release of trace metals into the environment is referred to as the “chemical time bomb” (Stigliani 1993). As a result, the EU sludge to land directive (86/278/EEC) sets limits for trace metals in agricultural soils that receive sewage sludge. The Dangerous Substances Directive of the European Union (76/464/EEC) defines dangerous chemicals as "those which are toxic, persistent and/or those that bioaccumulate" (Council of the European Communities, 1976). As such, trace metals are those that cannot be broken down and, as a result, will persist and accumulate in the environment (Alloway, 995). Unlike many organic pollutants, which eventually degrade to carbon dioxide and water, trace metals will tend to accumulate in the environment. As Franco-Uria et al (2009) point out, the European Commission (2006) had previously made a proposal for a Directive (COM(2006)232) which establishes a framework for the protection of soil across the EU and amending Directive 2004/35/EC. This report states that "further action is needed to ensure a high level of soil protection", sets the main aims of the strategy and sets out the measures that must be undertaken for conserving and improving the characteristics of the soil compartment, always under a sustainability criteria. Franco-Uria et al (2009) then go on say that, for this reason, the current situation of soils should be assessed and that, furthermore, it is important to identify the origin of metal content in soils in order to reduce metal inputs if necessary and/or to improve management guidelines according to the legislation.

As Kulati (2016) states, trace "metals enter the environment by natural and anthropogenic means. Such sources include natural weathering of the earth's crust, mining, soil erosion, industrial discharge, diffuse urban runoff, sewage effluents, pest or disease control agents applied to plants, air pollution fallout, and a number of others (Ming-Ho, 2005)" (Kulati, 2016). Legacy trace metal toxins are also released via sediment disturbance (Ridgway, et al. 2003). Lone et al (2008) state "that the use of insecticides/pesticides, disposal of industrial and municipal wastes in agriculture, and excessive use of fertilizers are all sources" of agricultural trace metal pollution. Trace metals are found in earth's crust and have been used in many industrial applications (Alloway, 1995). Pathways for these trace metals in the soil profile include soil to solution mobilisation from soil to solution, uptake by flora, and leaching to surface water and even the groundwater (Haygarth and Jarvis 1999). As Martin et al (2006) point out, "the natural concentration of trace metals in arable soil depends primarily on the geological parent material composition" (Martin et al, 2006; De Temmerman et al., 2003). Standard agricultural practices may cause these trace metals to become enriched (Kashem and Singh, 2001; Mantovi et al., 2003). Agricultural practices, such as application of sewage sludge and manure, as well as other commercial fertilisers can result in trace metals entering the soil system and are a significant source of trace metals, such as Cu, Zn and Cd (Nicholson et al., 2003). Lone et al (2008) go on to say that each source of contamination has its own damaging effects to the receptors of said contamination such as flora, fauna and even to humans, but that the sources that add trace metals to the soil system are of serious concern due to their persistence in the environment and potential carcinogenic properties.

Because humic acids are known for their ability to retain trace metals, soils that are peat rich and high in organic matter usually retain more trace metals than mineral soil (Borgulat et al, 2017; Lidman et al, 2014). As the study area within this piece of research is known to be abundant in peat-rich soils, elucidating the dynamics of the trace metal contents within the topsoil of the region is of valuable environmental importance. These dynamics include relationship of trace metal concentrations with geochemical factors, such as the organic matter content of the topsoil, the pH/chemical reaction of the topsoil as well as intra-trace metal relationships.

4.1.2. Study Area Background

The meres and mosses of Cheshire and Shropshire are an ecologically diverse cluster of natural wetlands and peatlands England (Natural England, 2007) and are part of the 'Natural Areas' programme as set out by Natural England. They comprise of over sixty deep and shallow, relatively small, lowland lakes (Reynolds 1979) while the mosses cover in excess of 900 ha, making them the third largest and one of the most southerly lowland mire complexes in Britain (Natural England 2007). As Boardman and Daniels (2012) state in their book 'Features of the Meres and Mosses of Shropshire, Cheshire and Staffordshire' the area "covers the North Shropshire, Maelor and Cheshire plain. This area extends from the Mersey Estuary in the north to the Black Country in the Midlands of England, and from the Welsh Marches in the east to the Potteries in the east". The Meres and Mosses region consist of sites that have been Ramsar designated as well as Sites of Special Scientific Interest (SSSI) (Woodland Trust, 2019). Ramsar sites are wetlands (and associated riparian and coastal zones) of international importance, designated under the Ramsar convention on wetlands, hence their conservation, protection and remediation from pollution and contaminants are of vital importance. The Meres and Mosses of the Marches have now been designated as a Nature Improvement Area (NIA) and is one of only twelve such designated sites in England (BBC News, 2012b). The area has previously secured Heritage Lottery Fund (HLF) funding for a Landscape Partnership Scheme (BBC News 2012a) (see Fig 4.1e).

When examining the ecological characteristics of the study area we can see that these meres and mosses form a geographically diverse series of lowland open water and peatland sites (St. Helens Borough Council, 2021). As the JNCC (2008) state, "These have developed in natural depressions in the glacial drift left by receding ice sheets which formerly covered the Cheshire/Shropshire Plain" and that the study area "sites include open water bodies (meres), the majority of which are nutrient-rich with associated fringing habitats, reed swamp, fen, carr and damp pasture. Peat accumulation has resulted in the nutrient-poor peat bogs (mosses) forming in some sites on the fringes of the meres or completely infilling basins. In a few cases the result is a floating quaking bog. The wide range of resulting habitats support nationally important flora and fauna".

The Meres and Mosses region suffers from the impacts of diffuse pollution resulting in a decline in their conservation status (James et al 2003, James et al 2005). Diffuse pollution often occurs because of agricultural practices including overgrazing and fertiliser application and whilst each individual case of this diffuse pollution may be minor, they will have a cumulative impact with the potential to result in significant loadings at a catchment level (English Nature, 2003). The main industry of the Mosses area historically has been peat cutting and extraction (Natural England, 2021). Historical peat extraction has also left a scar on the agricultural landscape in areas such as Fenn's, Whixhall and Bettisfield Mosses (Fig 4.1a-Fig 4.1c).

Fig 4.1a: Small Pond, Fenn's Moss Panoramic (Espresso Addict, 2008 CC by-SA 2.0)

Fig 4.1b: The Fenn's Moss Old Works Peat Factory (Haynes, 2007 CC by-SA 2.0; Croker, 2009 CC by-SA 2.0)

Fenn's Moss is one of the largest raised peat bogs in Britain, is part of the wider Fenn's, Whixall and Bettisfield Mosses National Nature Reserve, which fall within the geographical scope of both the NIA and the LPS (see Fig 4.1e) schemes and is an NNR which lies at the border between England and Wales, near Whixall in Shropshire.

Fig 4.1c: Lake on Whixall Moss Panoramic (Espresso Addict, 2008 CC bY-SA 2.0)

Fenn's, Whixall (see Fig 4.1c) and Bettisfield Mosses are one of the most southerly raised peat bogs in the UK. Peat cutting on an industrial scale ceased in the area in the 1990s (Natural England, 2022). The area now has SSSI status, is a designated NNR and has areas that have gained Ramsar status (Natural England, 2022). This site was sampled as part of the present study. These relics of historical industrial activity are not only an indicator of potential metal pollution but may still contribute as the structures decay over time and are weathered by the effects of run-off from precipitation (Chen et al, 2012).

Despite being heavily associated with agriculture and peat mining/extraction, the Meres and Mosses region also has an industrial heritage. The surrounding towns to the Meres and Mosses region include Wem, Whitchurch and Prees and contain multiple industrial estates (see Fig 4.1d).

Fig 4.1d: Wem Industrial Estate (Bulleys Bradbury, 2019)

Shropshire is historically associated with the wrought iron industry with many forges being active up until the early 1900s with demand for wire and plate manufacture (Hayman, 2004). Steel fabrication continues at these industrial sites to the present day (ERIH, n.d.). This study will explore the impact and potential contributing factor of these industrial processes to trace metal concentrations in the surrounding meres and mosses region.

As Fig 4.1e shows, the green outline is the area covered by the National Improvement Area Scheme (NIA) and within the yellow boundary is the area covered by the Heritage Lottery Fund Landscape Partnership Scheme (LPS). It is a priority region for conservation and restoration so to provide sustainable habitats so to benefit the wildlife and flora in the region (Cheshire Wildlife Trust, 2014). Natural England (2020) state that the objective of the NIA programme was to conserve and improve identified sites and link them by restoring the wetland habitats around them. By contrast, Natural England (2020) state that the "LPS scheme focused on delivering improvements for conservation, raising awareness of the landscape of the project area, engaging communities, improving access and providing opportunities for people to learn skills associated with the landscape. The conservation work concentrated on improving three Sites of Special Scientific Interest (SSSI) (Cole Mere, White Mere and Brown Moss)" (Natural England, 2020).

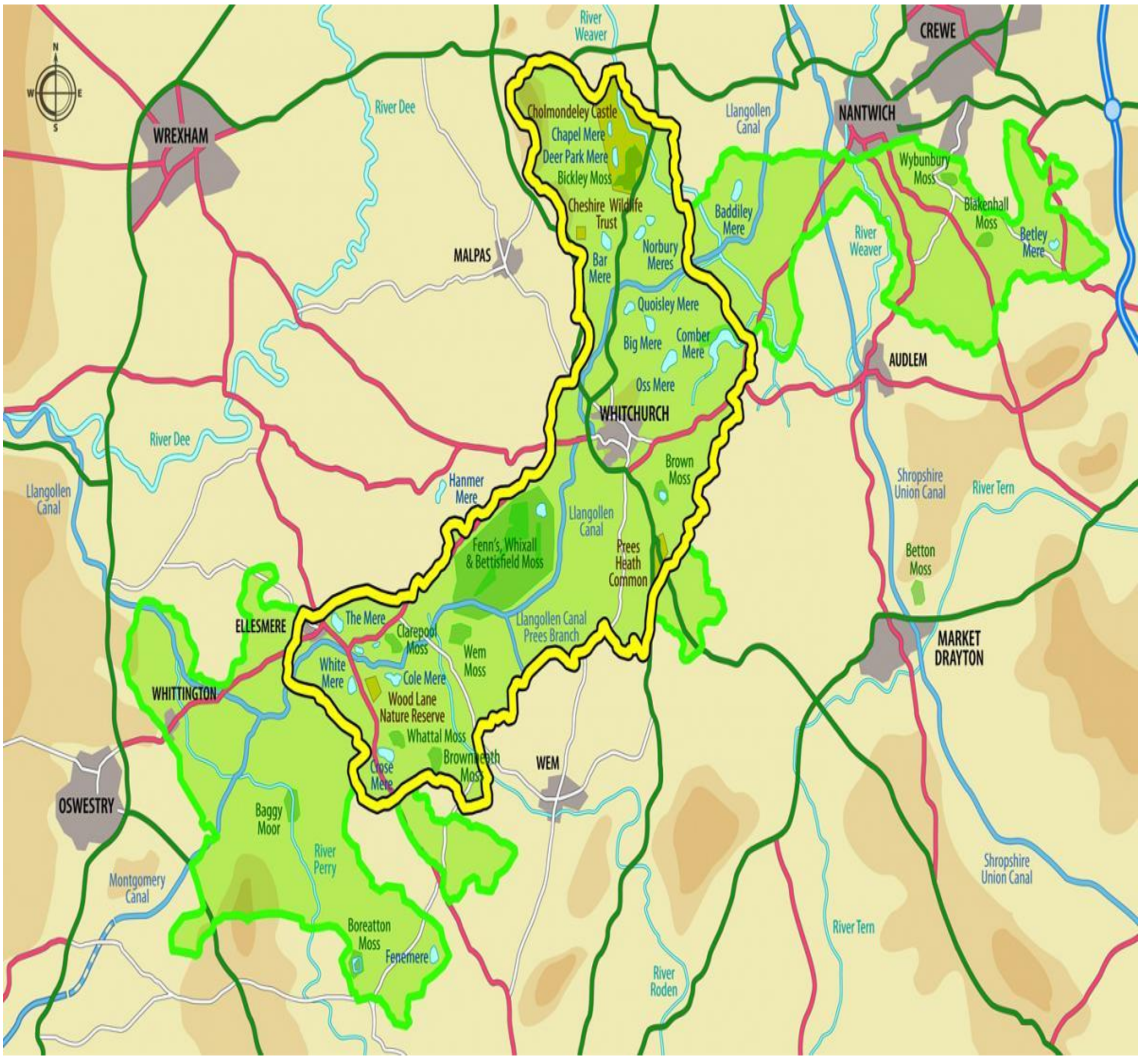


Fig 4.1e: The Boundaries of the NIA (green) and LPS (yellow) schemes (Oswestry Family and Local History Group, 2016 adapted from Cheshire Community Action, 2014)

Brown Moss (Fig 4.1f) which is located on the West side of the LPS boundary, is a biodiverse wetland area which is designated a Site of Special Scientific Interest (SSSI) and Local Nature Reserve close to Whitchurch, Shropshire (Whitchurch Rural Parish Council, 2017). It is open to visitors and contains a number of self-guided walking trails. The Shropshire's Great Outdoors Strategy Board describe Brown Moss as a "32 hectare site is one of Shropshire's most important places for plants, with over 200 species of native wildflowers, including the rare Floating Water Plantain, possibly the rarest plant in Britain, and a collection of other wetland plants found in few other sites in England. Because of its wildlife interest, Brown Moss is designated as a Local Nature Reserve, Site of Special

Scientific Interest, Special Area of Conservation (SAC) and RAMSAR site" (Shropshire's Great Outdoors, n.d.).

Fig 4.1f: Mere at Brown Moss (Darch, 2019 CC by-SA 2.0) and its Nature Reserve Boundary Extent (Shropshire's Great Outdoors, CC-2020)

To the South West of the sampling area (see Fig 4.1e)) lies Baggy Moor (see Fig 4.1g). This is one of the major features in the southerly range of the NIA boundary and was once a large area of peat bog. Currently a mixed grazing site, it was enclosed and drained in the late 18th century which led to intensive arable cultivation and used as a bombing range during the second World War (Shropshire Character Framework, 2006). Fig 4.1e displays the content with the NIA/LPS boundaries, including the various meres, mosses and other sites of sensitive environmental importance.

Fig 4.1g: Mixed Grazing on Baggy Moor (Haynes, 2007 CC by SA-2.0)

Studies in the Meres and Mosses region have tended to focus on nutrient pollution and impacts of nutrient loading (Fisher & Acreman, 2004; Fisher et al, 2010; James et al, 2005; Maberly & Carvalho, 2010; Moss et al, 2005). Previous studies regarding dynamics of trace metals in similar terrestrial wetland environments have shown that wetland soils that have been drained and converted to agriculturally managed farmland or developed for urbanisation could potentially face alterations in trace metal cycling due to the changes in the soil properties (Bai et al, 2019). Wetland soils have been found to have the increased potential to retain trace metals due to the organic matter (OM) content of the topsoil which may result in a reduction of trace metal mobility, and consequent bioavailability, in the topsoil (Wang & Xu, 2015). The reduction of this OM content in the soil through practices such as agricultural management (such as tilling and crop harvesting) has the potential to impact soil pH as well as both the occurrence and dynamics of trace metals in the soil (Reddy et al, 2010). The determination of the relationships between these physiochemical factors with trace metal occurrence and dynamics will be explored as part of the aims of this thesis (see Section 1.9 of Chapter 1 and section 4.1.3 of this chapter).

4.1.3 Study Aims

This study aims to act as a preliminary survey on trace metal contamination in soils (A-Horizon) on a regional scale in the meres and mosses region of Cheshire and Shropshire and more specifically the area that has been designated as a Nature Improvement Area (NIA) and part of the Landscape Partnership Scheme (LPS). The British Geological Survey (BGS) have undertaken analysis of deep soils (below 36cm depth) for many aspects, including trace metals, soil profile and chemistry in this region with their geochemical mapping data (Johnson et al, 2005). However, there is a monitoring gap which this study aims to fill. There is currently no data available for topsoil (A-horizon) for trace metals in this environmentally sensitive region of Cheshire and Shropshire. Topsoil is of vital importance when studying trace metal pollution and their dynamics as it is anthropogenic in nature and potentially more mobile due to erosion. This study aims to ascertain the baseline situation of trace metal pollution and potential anthropogenic impacts, including those from agricultural practices, in terms of trace metal pollution in the topsoil of the meres and mosses region and to examine their adherence to soil quality guidelines. The overarching aims of the study were:

1. To determine the concentration of select trace metals in the sampling area so to determine levels of enrichment of the trace metal concentrations found in the sampling area and to examine adherence to prominent soil quality guidelines.

2. To determine the spatial variability of the trace metals across the sampled region to aid in potential source identification.
3. To ascertain the geogenic and/or anthropogenic origin of the present in-situ trace metals by use of multivariate and statistical analyses.

The data this research will attain will build a more complete, comprehensive dataset for the NIA/LPS region as when used in conjunction with currently held data, including that from the British Geological Survey (BGS), as well as providing a vitally important dataset for trace metals and their spatial dynamics when taken as an individual piece of work and as a foundation for the consequent research in this thesis as a whole. The implementation of statistical analyses and soil quality guidelines will highlight trace metals of concern in the region whilst spatial mapping will highlight potential sources geographically.

4.2 Materials and Methods

4.2.1 Field Sampling Methods:

A total of 89 samples of topsoil (A-horizon) have been collected from various sites in the meres and mosses, using a trowel and sample bags. A sampling area was set up so as to represent the area covered both by the National Improvement Area (NIA) and Landscape Partnership Scheme (LPS).

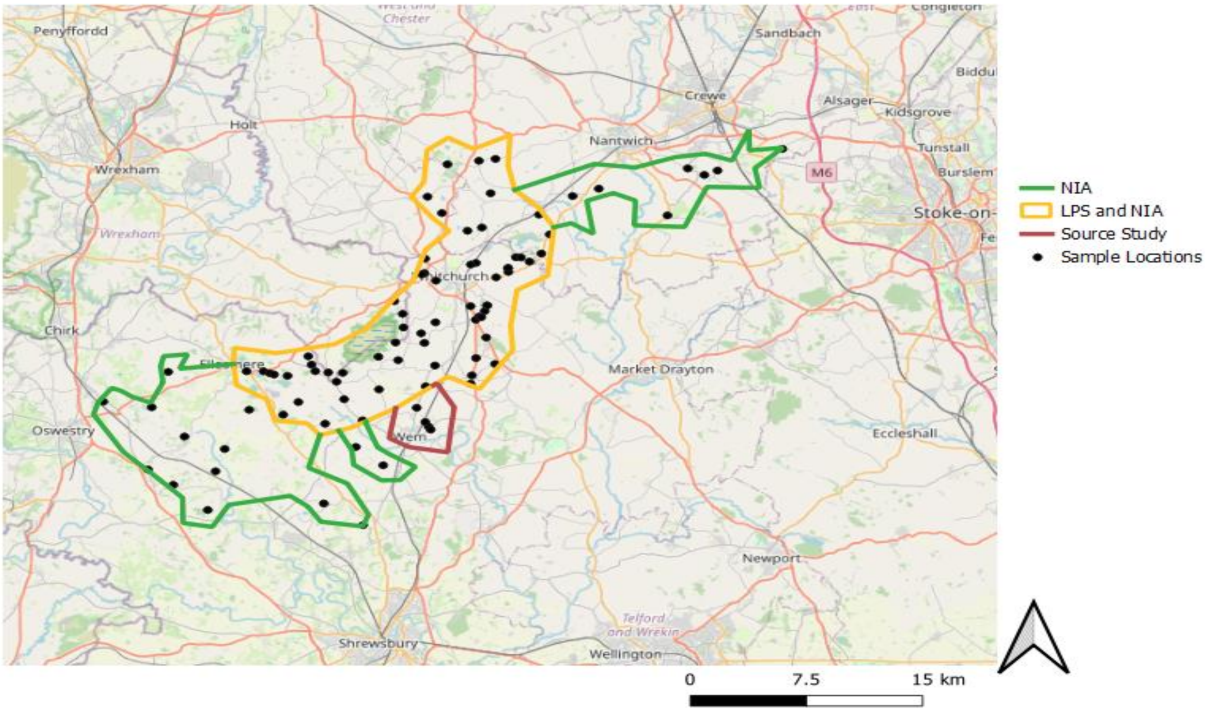


Fig 4.2: Map showing sampling locations for trace metals in meres and mosses Nature Improvement Area (NIA)/Landscape Partnership Scheme (LPS) Boundary

Samples were then taken on a random sampling regime and in a stratified coarse grid process were possible (see section 2.2.1 of Chapter 2) so as to try and cover as much as the NIA/LPS area as possible to obtain representative coverage (see Fig 4.2). Sample bags (8” x 11”) were filled with topsoil (up to 10 inches depth and below the O-Horizon) using a trowel. Upon collection of a sample, a coordinate sample point was recorded with a handheld GPS so as to be able to accurately map the sample and attach information to an exact point. Additional samples were taken just outside the NIA/LPS area to identify potential sources and examine the potential impact of industrial locations on the spatial distribution of toxic trace metals in the region, this included the extra boundary marked as ‘source study’ in Fig 4.2. This was to examine if local industry contributed to spatial concentrations of trace metal pollution in the region. The sample was then transported back to the lab in a sterile container/sample bag.

4.2.2 Laboratory Analysis and Methods:

For effective sample handling, Hoskins & Ross (2009) state that “Analysis of dried, ground, sieved soils allows for more convenient sample handling and greater reproducibility of results in comparison with analysis of field-moist soil. Sample preparation steps, although often taken for granted, have frequently been shown to significantly affect analytical results (Elk and Gelderman, 1988; Munter, 1988; Soltanpour 1976)”. Therefore, the samples were dried in an oven at Approximately 50^{o}C overnight and then sieved between 2mm down to ≤2 microns. Results for each fraction was recorded so as to ascertain the granulometric characteristics of the soil sample with FPXRF being performed on the dried soil samples at the working fraction (<2mm).

Field Portable X-Ray Fluorescence Analysis (FPXRF) was carried out on all of the samples. XRF is a non-destructive and rapid multielement analytical technique with a detection range of low ppm (Chandrasekaran & Ravisankar, 2015). This analytical method has been widely applied to the analysis and research of soil, its potential contaminants and other environmental applications (Tighe et al, 2018; Venvik and Boogard, 2020; Burlakovs, 2015). Sarker, 2018 states that “X-ray fluorescence (XRF) analysis is based on the measurement of characteristic X-rays resulting from de-excitation of inner shell vacancy produced in a sample by means of a suitable source of incident radiation” (West et al, 2014). Benefits of FPXRF analysis include the ability to directly sample from sample bags hence less preparation required for solid samples, non-destructive analysis, rapid turnaround of samples due to short analysis time, little waste produced by the technique and the inherent portability of the system (Kobylarz et al, 2023).

The portable X-ray fluorescence analysis was conducted using a Skyray Instruments Genius 9000 spectrometer (Skyray Instruments USA, 2016). The equipment uses a miniature X-ray tube with an excitation source of 50 kV/200 µA, a fast SDD detector with 128 eV energy resolution, a digital signal processor, and a micro multi-channel analysis module. The spectrometer was configured to use the "environmental soil" internal package, running for 100 s at a 45 kV voltage and 80 µA current.

Using the Skyray Genius 9000 FPXRF (see Fig 4.3), sample bags containing the whole dried sample in the working fraction (<2mm) were directly analysed. The trace metals that were chosen for analysis were those in the pre-set 'Soils' program for the Skyray Genius 9000 software built-in for environmental screening.

Fig 4.3: Skyray Genius 9000 XRF (Skyray Instruments USA, 2016)

The Skyray Genius 9000 was set to the 'Environmental Soils' program for calibration (as per factory settings) and instrumentation were calibrated before each sample run with checks and standards at regular intervals. A silver (Ag) certified reference material pellet provided with the FPXRF hardware was used as the calibration and check standard during each sample run. All results were recorded, and then statistical analysis was applied to said results. The benefit of using this FPXRF analytical technique is that a 'profile' of the total concentration of trace metals can be easily obtained with no destruction to the sample. A screening exercise, one which examines several trace metals, aids in building up a profile of this aspect of the region. Therefore, making a trace metal profile of a given sample/region highly advantageous for remediation/management purposes (Laursen at al, 2014; Lyon et al, 1988).

Additional laboratory analyses has been performed on the samples routinely. This analysis included pH, Total Organic Carbon (TOC), Loss on Ignition (LOI) and Magnetic Susceptibility (MS). The rationale for these laboratory-based analyses can be found in Chapter 2 of this thesis (section 2.3 of

Chapter 2). These properties have been measured for each sample taken to ascertain the characteristics of the soils being sampled and to be able to derive any relationships and correlations between said soil characteristics and the concentrations and mobility of metals therein. The additional laboratory analyses were performed in triplicate for each sample so to aid in statistical analyses and the median result recorded to represent the central tendency.

Alloway (1995) identifies the importance of analysing the pH of soil, especially when studying trace elements in soil. The soil pH is an important factor in controlling the chemical behaviour of trace metals and other vital processes in soil. As Alloway also points out, in general, trace metal cations are most mobile under acid conditions. The pH of soil in solution analysis was undertaken on a 1:2.5 soil suspension (10g of soil sample to 25ml H_2O) and was analysed via a HANNA HI 99121 Direct to soil pH meter.

Magnetic susceptibility (MS) measurement is a simple technique that has been widely implemented as a useful analytical methodology for assessing anthropogenic pollution, especially trace metal pollution on soil samples (Paradelo and Barral, 2014). Magnetic susceptibility has been found to have a close relationship with trace metal contamination when studying independent datasets (Paradelo & Barral, 2014). All samples were analysed for Magnetic Susceptibility using a Bartington MS-2 Magnetic Susceptibility Meter. The Meter was set to the Si /1.0 program with the sample reader set to ‘Low Frequency’ (LF). Each sample was placed in the bespoke vessel and result recorded.

Organic matter can interact strongly both physically and chemically to trace metals (Santschi et al. 1997). So, it is important to ascertain the level of organic matter content in agricultural soil when studying trace metal content. The Loss on Ignition (LOI) gives an approximate measure of the organic content of the soil. The percent of sample mass lost following heating is reported as LOI. Crucibles were dried in oven for approximately 1 hour then left to cool for 1 hour. The crucibles were weighed and the result recorded (Wc). Approximately 2g of sample was added to the crucible and this was dried overnight at 105^{O}C and then left to cool for approximately 1 hour in a desiccator. The crucibles were reweighed (Ws). Crucibles were then placed into the furnace at 550^{O}C for 4 hours and then allowed to cool in a desiccator before reweighing (Wa). From the calculation in Equation 4.1 LoI was determined for each sample and the results recorded.

$$\mathbf{LOI = (Ws\text{-}Wa) / (Ws - Wc) \times 100}$$

Equation 4.1: Determination of Organic Matter Content in Soil by Loss on Ignition

Total Organic Carbon Analysis (TOC) measures the organic content in environmental medium (Tukura et al, 2007; Yun 2003). This organic carbon can contribute to the acidity of soils through their role with organic acids (Zoumis et al. 2001). Anthropogenic inputs, as well as those from natural processes can elevate organic carbon levels in the soil (Lopez=Sanchez et al, 1996). This soil carbon is known to increase as grain size decreases due to organic matter absorbing onto mineral surfaces and having high affinity for fine grained soils (Tukura et al, 2007). As a result, increased trace metals are adsorbed onto areas and surfaces with high TOC levels (Nelson & Sommer 1982; Tukura et al, 2007).

Each sample was analysed for Total Organic Carbon (TOC) using a Shimadzu SSM-5000A combined with a TOC-VCS/CP. Combining the SSM-5000A with a TOC-VCS/CP analyser permits analysis of many solid samples including soils. Each sample was analysed for Total Carbon and Total Inorganic Carbon with the subtraction method determining the Total Organic Carbon. Prior to analysis a portion of sample was dried in the oven at $50^{O}C$ overnight along with a sufficient amount of sample vessels. Samples and vessels were then allowed to cool in a desiccator until reaching ambient temperature. Between 57mg and 100 mg of sample was weighed out into a weighing vessel and then, for Total Carbon (TC): Glass fibre wool was placed over each sample in the sample vessel and then the sample was placed into the solid sample module (TC portion) and consequently allowed to stand for 2 minutes to allow atmospheric carbon to dissipate within the system. The sample was then run on the solid sample module in accordance to the pre-loaded method. For Inorganic Carbon (IC): The Sample was placed into the solid sample module (IC portion) and was then allowed to stand for 2 minutes to allow atmospheric carbon to dissipate within the system. Approximately 1ml of Phosphoric Acid reagent was inserted into the vessel. The sample was then run on the solid sample module in accordance with the pre-loaded method. The following calculation for Total Organic Carbon was applied (see Equation 4.2):

$$\mathbf{TOC = TC - IC}$$

Equation 4.2: Determination of Total Organic Carbon

Thus, determining the Total Organic Carbon content of each sample.

For further details on the rationale for the laboratory methods used in this chapter, please see section 2.3 of Chapter 2 of this thesis.

4.2.3 Soil Quality Guidelines:

There are guidelines, under certain circumstances, for some trace metals in agricultural soils (Table 4.1a, Table 4.1b and Table 4.1c). The EU sludge to land directive (86/278/EEC) sets limits for trace metals in agricultural soils that receive sewage sludge (CEC, 1986). There are also the widely cited Dutch Target and Intervention Values, 2000 (sometimes known as the Netherlands Target and Intervention Values). These are derived from the Netherlands Soil Contamination Guidelines (VROM, Department of Soil Protection, 1994). The Dutch Standards (2000) are environmental reference values for various pollutants used in mitigation, investigation and remediation (Alijagic, 2013). These guidelines set targets and/or thresholds for those metals most associated with anthropogenic input. They are classified as 'Target' and 'Intervention' values. As the Dutch Target and Intervention Values (2000) state, "The 'Target' value is the baseline concentration value below which compounds and/or elements are known or assumed not to affect the natural properties of the soil" whereas "The 'Intervention' value is the maximum tolerable concentration above which remediation is required" (Dutch Target and Intervention Values, 2000). Whilst XRF analysis is not an approved method for soil analysis under the regime of the Dutch regulations (VROM, 2000b), these 'Dutch Targets' are a much-cited resource in studies of this nature in which XRF is the analytical method (Venvik and Boogaard, 2020; Cappuyns and Slabbinck, 2012; Dao et al, 2013). It is important to note that the figures displayed in Table 4.1a are based on a standardised soil with 10% organic matter and 25% clay (fine fraction/lutite). These values will be recalculated according to the organic matter and clay content of the topsoil under investigation as determined during this screening study (see section 4.3.1 and Table 4.1d) as per the methodology set out in the annexes of the Dutch Target and Intervention Values, (VROM, 2000b) as shown in Appendix F. In this study, the median levels of organic matter content (OM) and clay content (as ascertained in the granulometry analysis in section 4.3.2), as determined in this study, will be implemented so to represent the central tendency of these factors in the sampling region when undertaking this recalculation. The Finnish government sets out 'threshold' and 'guide' values from the Finnish legislation for contaminated soil (Decree 214/2007) based on aqua regia extractable analytical techniques which is the recommended analytical method by the Decree (Finnish Government, 2007). These Finnish guidelines will be discussed in further detail in Chapter 5 of this thesis due to the analytical procedure involved. Median, 90th percentile and maximum values of European topsoil are also often utilised in conjunction with these guidelines (van der Voet et al., 2013).

Trace Metal	Netherlands Target/Background Value Limits (mg/kg)	Netherlands Intervention Value Limits (mg/kg)	EC Directive 86/278 Limits (mg/kg)	Finnish Threshold Value (mg/kg)	Finnish Lower Guide Value (mg/kg)	Finnish Higher Guide Value (mg/kg)
Cu	36	190	100	100	150	200
Pb	85	530	300	60	200	750
Zn	140	720	250	200	250	400
V	42	250	-	100	150	250
Cr	100	380	400	100	200	300
Co	9	240	-	20	100	250
As	29	55	-	5	50	100
Sn	-	900				

- **=No available Data**

Table 4.1a: Soil Quality Guidelines

EC directive 86/278/EEC is implemented in England via the Sludge (Use in Agriculture) Regulations, 1989 (DEFRA, 2018). Under these regulations, the pH of the soil and land-use are also taken into consideration when determining the applicable limits of certain trace metal elements. These regulations also place a 'maximum permissible average annual rate' over a 10-year period for certain trace metal elements applicable to land used for arable farming receiving sewage sludge (see Table 4.1b and Table 4.1c).

Trace Metal	Maximum Permissible Concentration in Soil (mg/kg dry solids) for Arable Farming				Maximum Permissible Concentration in Soil (mg/kg dry solids) for Grassland				Maximum Average Annual Rate over 10 years (kg/ha) for Arable Farming
	pH 5<5.5	**pH 5.5<6**	**pH 6.0-7.0**	**pH >7.0**	**pH 5<5.5**	**pH 5.5<6**	**pH 6.0-7.0**	**pH >7.0**	
Zn	200	200	200	300	200	200	200	300	15
Cu	80	100	135	200	130	170	225	330	7.5
Ni	50	60	75	110	80	100	125	180	3

Table 4.1b: Trace Metal Limits for Arable Farmland and Grassland for those who produce or use sewage sludge in England, Wales and Northern Ireland (DEFRA, 2018)

The Sludge (Use in Agriculture) Regulations (1989) Great Britain and Northern Ireland (1990) sets out regulations for trace metal elements and potentially toxic elements (PTEs) for soil that is pH 5 and above on land being used for arable farming that receives sewage sludge and grassland (DEFRA, 2018) as shown in Table 4.1c.

Trace Metal	Maximum permissible concentration of PTE in soil (mg/kg dry solids) for pH 5.0 and above for Arable Farming	Maximum Average Annual Rate over 10 years (kg/ha) for Arable Farming	Maximum permissible concentration of PTE in soil (mg/kg dry solids) for pH 5.0 and above for Grassland
Cadmium	3	0.15	3
Lead	300	15	300
Mercury	1	0.1	1.5
Chromium	400	15	600
Molybdenum	4	0.2	4
Selenium	3	0.15	5
Arsenic	50	0.7	50
Fluoride	500	20	500

Table 4.1c: Trace Metal and PTE limits for select elements on Arable Farmland and Grassland for those who produce or use sewage sludge in England, Wales and Northern Ireland (DEFRA,2018)

4.2.4 Enrichment Factor Analysis (EF):

Enrichment factors (EFs) were calculated to assess the degree of anthropogenic influence. Enrichment Factors provide an estimation as to level to which trace metals may be enriched or reduced relative to a specific source (Odabasi et al., 2002). The enrichment factor analysis (EF) was performed by standardising a tested element against a reference element. A reference element is the one that is known to have low occurrence variability. The most common reference elements include Ti, Al and Fe (Quevauviller et al., 1989; Sutherland, 2000). EF analysis can be used to aid in differentiating between trace metals that arise from anthropogenic input and those from a natural origin (Meza-Figueroa et al., 2007). A value of EF close to 1 indicates natural origin, whereas values >10 are indicative of those that originate from anthropogenic sources (Han et al., 2006; Turner and Simmonds, 2006). EF ≤ 2 indicates deficient to minimal enrichment, 2 – 5 EF indicates moderate enrichment, 5 - 20 EF indicates significant enrichment, 20 – 40 EF indicates very high enrichment, and >40 EF indicates extremely high enrichment (Lu et al., 2009). EF values of 0.5-1.5 suggest that trace metal concentrations may be because of natural weathering processes, whereas EF>1.5 indicate that a non-crustal influence may be involved (Barbieri et al, 2015).

Dragovic (2008) states that, "To determine the relative degree of metal contamination, comparisons were made to background concentrations in the Earth's crust using Fe as reference element following the assumption that its content in the crust has not been disturbed by anthropogenic activity. EFs were calculated according to the equation generalized from Zoller et al. (1974)" as shown in Equation 4.3a:

$$\mathbf{EF_M = ([M]/[Fe])_{soil}/([M]/[Fe])_{crust\ or\ shale}}$$

Equation 4.3a: Determination of Enrichment of Trace Metals in Topsoil

Where [M] is the concentration of the chosen element for study, [Fe or Ti] is the concentration of Fe or Ti in the soil sample being analysed and in the reference material being used (crust or shale). The subscripts 'soil' refers to the soil sample being analysed and 'crust' or 'shale' indicates the background reference material medium being used in the study.

To be able to determine the EF result for Fe in the study, an alternative baseline element was chosen. In this case the element was Titanium (Ti) which is widely used as a baseline element in Enrichment Factor due to its low variability. EF for Fe were calculated as shown in equation 4.3b.

$$\mathbf{EF_M = ([M]/[Ti])_{soil}/([M]/[Ti])_{crust\ or\ shale}}$$

Equation 4.3b: Determination of Enrichment for Iron (Fe) in Topsoil

4.2.5 Mapping and Statistical Methods:

Geographical information system (GIS) is a tool which interprets spatial variability and can aid in identifying nonpoint source contamination (Shan et al, 2013). GIS-mapping software aids in the visualisation of spatial occurrence of environmental data and other land features which enables the investigation of anthropogenic influence on trace metals contents in the soil (Shan et al, 2013). GIS has been used extensively in soil pollution studies (Adamus and Bergman, 1995; Meinardi et al 1995). For areas that have not been directly sampled, Kriging is the primary method of spatial prediction that provides an unbiased estimator for quantities that vary in space (Goovaerts 1999; Shan et al, 2013). To explore the spatial distribution of the attained results from sample to sample, Kriging method will be used with Geographic Information Systems software, ArcGIS 10.1.

To assist in the interpretation of geochemical data, a multivariate statistical approach was adopted. The use of Principal Component Analysis (PCA) were the statistical tools used in the interpretation and elaboration of the datasets. Principal Component Analysis is a multivariate statistical technique that takes the multidimensional space of the original dataset and reduces it to a lower dimensional space known as principal components. These principal components can then be used to determine relationships within the dataset (Wold et al. 1987; Jolliffe 2002; Shan et al, 2013). Varimax with Kaiser Normalisation was used as the rotation method (Lee et al., 2006). Varimax rotation is a statistical methodology used at one factor level to ascertain the relationship among the factors (Allen, 2017). The Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy is a statistical measure that is used to assess the measure of the correlation coefficients when compared to the measure of the partial correlation coefficients (Zhao et al, 2018). Larger KMO values, those that fall between 0–1, are considered useful because correlations between pairs of variables can be explained by the other variables (Yuan et al., 2014). Bartlett's test of sphericity is used to test the hypothesis that the correlation matrix is an identity matrix with suitable significance ($P < 0.05$) (Yuan et al., 2014). In this study, the KMO (>0.5) and Bartlett's test ($P < 0.001$) show that the measured metal concentrations for the topsoil within the NIA/LPS boundaries of the Meres and Mosses region of Cheshire and Shropshire are suitable for PCA (Chen et al., 2014; Yuan et al., 2014). Trace metal to soil properties (pH, TOC, LoI and MS) and trace metal to trace metal relationships were also determined by correlation matrix (CM) so as to complete and support the results obtained by multivariate analysis.

Cluster Analysis (CA) is often used in conjunction with PCA to check results and to aid in the grouping of individual parameters and variables (Facchinelli et al., 2001). CA was developed according to Ward's method. Analysis of correlation distance was undertaken to measure the distance between clusters of similar trace metal contents. The purpose of CA is to organise observations where a groups and variables share observed properties (Lu et al., 2010). Statistical analyses were performed with SPSS 26 and OriginPro 9 for Windows. The distance measure between two clusters is calculated as shown in Equation 4.4:

$$\mathbf{D=1-C}$$

Equation 4.4: Determination of Distance Between Two Clusters

where D = Distance and C = correlation between compound clusters.

4.3 Results and Discussion

4.3.1 Descriptive Statistics for Trace Metal Contents, Soil Properties and Adherence to Soil Quality Guidelines:

The summary statistics of soil element contents are listed in Table 4.2. The arithmetic median results of the trace metal concentrations for all samples collected were determined and were implemented to describe the variation and central tendency of the data.

The soil in this region is a mixture of agricultural farmland with low organic matter content and environmentally protected countryside, woodland and vegetative strips with higher levels of organic matter. This is reflected in the varied results for Total Organic Carbon (TOC) and Loss on Ignition (LoI) in the sampling area. The median LoI and TOC values in the meres and mosses region is 6.95% and 3.23% respectively. Organic carbon in soil increases the binding capacity for metals (Boluda, 1988). Locations within the sampling area which are identified to have higher levels of organic matter and organic carbon inherent in the soil may influence the trace metal content in the soils for those locations.

The soil reaction ranged from pH 3.75 to pH 8.02 (with a median of 5.88). Trace metal mobility is strongly influenced by soil pH as is their retention ability (Alloway, 1995). Certain trace metal cations are known to be increasingly mobile as pH decreases (Martinez and Motto, 2000) and, as a result, acidic properties could facilitate the mobility of trace metals in soils (Alloway, 1995) which is due to the pH-dependence of the variable charge components of soil (Ramos-Miras et al., 2011).

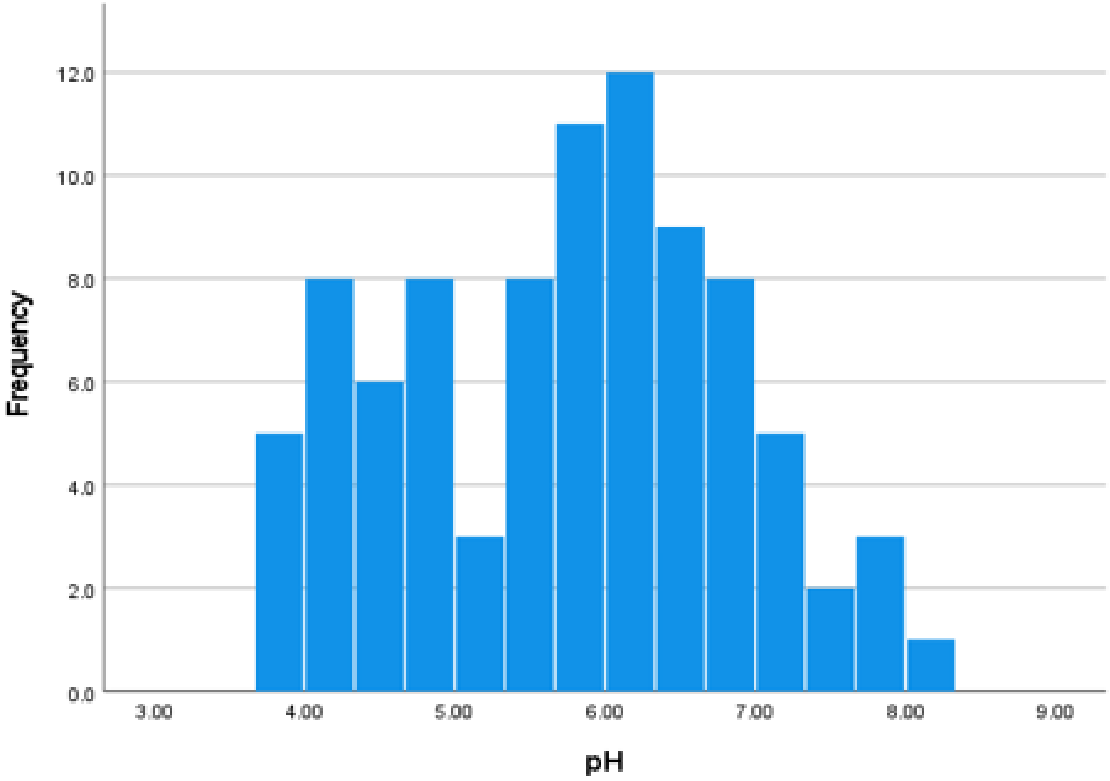


Fig 4.4: Frequency of pH Results in the Meres and Mosses Topsoil Samples

It can be seen from the pH Frequency Chart of counts for the sampling within the region that the pH of the soils sampled were mainly in the ‘neutral’ through to ‘mildly acidic’ range but with a still significant proportion of the soils being acidic (see Fig 4.4).

The Dutch Soil Quality Guidelines (VROM, 2000a) were adapted as per the methodology set out in the annexes of the Dutch Target and Intervention Values, 2000 (see Appendix F) based on the clay content/OM content as determined in the Granulometry and OM results ascertained in this chapter (see Table 4.2 and Table 4.3). This was undertaken so to make the Dutch soil quality guidelines applicable to the sampling area studied (see Fig 4.1d). Table 4.1d displays the variable nature of these Dutch guideline value limits depending on the organic matter and clay content present in the soil being studied as ascertained in this study. For the purposes and aims of this study, the median values ascertained for the 89 samples taken from across the sampling region will be referred to.

Trace Metal Element	N	Netherlands Target/Background Value Limits (mg/kg)			Netherlands Intervention Value Limits (mg/kg)		
		Min OM/Clay	Median OM/Clay	Max OM/Clay	Min OM/Clay	Median OM/Clay	Max OM/Clay
Cu	89	16.49	19.78	69.34	86.70	104.37	365.97
Pb	89	52.38	57.96	140.57	326.60	361.40	876.50
Zn	89	53.57	63.46	195.04	275.50	326.34	1003.04
V	89	12.00	15.03	30.36	71.43	89.46	180.71
Cr	89	50.00	52.02	62.24	190.00	197.68	236.51
Co	89	2.00	2.28	3.71	53.33	60.87	99.03
As	89	15.95	18.18	51.23	30.25	34.49	97.16
Sn	89	-	-	-	189.47	218.18	363.41

Table 4.1d: Amended Dutch Soil Quality Guidelines as per the methodology set out in the annexes of the Dutch Target and Intervention Values, 2000

Concentrations of various trace metals in soils of the study area, as well as geochemical and physical aspects, are given in Table 4.2. All 89 samples were analysed for seventeen elements via XRF analysis.

Descriptive statistics of the analysed samples for all seventeen elements are shown in Table 4.2. The concentrations of some trace metals associated with anthropogenic input (Pb, Zn, Co, Cr, V and Cu) as well as 11 other important trace metals in the topsoil of the meres and mosses region, are summarised in Table 4.2. The median concentration of Pb in the topsoil of the meres and mosses region (59.60 mg/kg) exceeded the median target values recommended by the Netherlands Soil Contamination Guidelines (57.96 mg/kg) when amended via the methodology set out in the annexes of the Dutch Target and Intervention Values (VROM, 2000b), so to be representative of the sampling site, with the maximum concentration of Pb found in sampling range (443.6 mg/kg) fell outside the median intervention value limit for Pb set by the amended guidelines (361.40 mg/kg). The maximum figure for lead obtained in the sample range (see Table 4.2) did exceed the values set by EC Directive 86/278 for ‘Agricultural and after sewage sludge application’ which sets the limit at 300 mg/kg (see Table 4.1a). The median Zn concentration (90.95 mg/kg) also exceeded the amended (median clay/OM) Dutch target value (63.46 mg/kg). The maximum levels of Zn found in the sample range did surpass the Dutch intervention value (as ascertained from median OM and Clay results for the topsoil) of 326.34 mg/kg and EC Directive 86/278 value of 200 mg/kg as implemented by DEFRA in England and Wales for soils with a pH 5.5<6 (see Table 4.1b) at 2330.7 mg/kg. A median value of

31.86 mg/kg of Co was found in the sampling range which exceeds the amended Dutch target/background value of 2.28 mg/kg with the maximum result obtained for Co (105.90 mg/kg) exceeding the 60.87 mg/kg 'Intervention' figure obtained when using median OM and clay data (see Table 4.1d and Appendix F) applicable to the Dutch Guidelines (2000). Cr was found to be, on average, well within both the median Dutch intervention (197.68 mg/kg) for the sampling site and EC Directive 86/278 value (400 mg/kg for Farmland and 600 mg/kg for Grassland with a pH >5) but does exceed the target/background limits (52.02 mg/kg) set by the Dutch guidelines when using the median OM/clay data for the sampling site with a median value of 63.80 mg/kg. Cu was found to be within both Dutch intervention (104.37 mg/kg) and target/background (19.78 mg/kg) with a median result of 4.90 mg/kg across the sample range. The maximum reading of 171.0 mg/kg does exceed the 'Intervention' value obtained when using median OM/clay data for the sample range (104.37 mg/kg) as well as EC Directive 86/278 value of 100 mg/kg for soils with this pH characteristic as well as the DEFRA standards for a soil with a median pH of 5.88 for both arable farmland and grassland (see Table 4.1b and Table 4.2). Many of the trace metals sampled are not covered by soil guideline values. In this case Enrichment Factor (EF) is a vital tool which can assist differentiating between anthropogenic and natural sources and can identify soils which are highly enriched in a particular element (see section 4.2.4). It is important to acknowledge that the trace metal analysis via FPXRF implemented a single element silver (Ag) standard pellet sample, as provided by the FPXRF hardware manufacturer (Skyray Instruments USA, 2016), for the calibration and validation process and, as such, the study would have benefitted from a matrix matched certified soil reference material for this purpose. This lack of quality control in this aspect should be taken into consideration as a limitation of the study.

Geochemical Property/Trace Element	Number of Samples Analysed	Minimum Result	Maximum Result	Median Result	Mean Result and Standard Deviation of the Mean (±)
pH	89	3.75	8.02	5.88	5.77 ±1.08
Magnetic Susceptibility (x10-8 m3kg-1)	89	0.00	229.53	17.13	28.68 ±34.36
TOC (%)	89	0.95	44.76	3.23	8.42 ±9.94
LOI (%)	89	2.38	84.45	6.95	16.83 ±19.02
Cu	89	0.00	171.00	4.90	23.06 ±38.20
Pb	89	31.60	443.60	59.60	114.88 ±101.29
Zn	89	23.10	2330.70	90.95	152.37 ±248.19
Ca	89	1132.50	117275.50	4945.50	9019.00 ±14477.99
K	89	594.83	18096.50	9880.20	9611.28 ±3362.36
Ti	89	269.77	4148.50	2590.30	2627.32 ±827.95
V	89	54.80	270.50	216.40	194.34 ±58.69
Cr	89	36.20	124.84	63.80	69.64 ±21.25
Mn	89	0.00	896.90	336.80	368.32 ±203.06
Fe	89	3197.32	30797.40	18768.10	18227.50 ±5816.59
Co	89	0.00	105.90	31.86	34.64 ±23.42
As	89	5.00	34.30	5.00	5.35 ±3.11
Rb	89	11.17	88.20	60.100	59.39 ±17.58
Sr	89	22.54	118.80	57.90	62.11 ±17.71
Nb	89	0.00	107.40	29.80	36.91 ±24.38
Sn	89	0.00	92.02	1.60	5.68 ±13.01
Ga	89	0.00	24.10	17.30	14.40 ±6.88

Table 4.2: Descriptive Statistics for Trace Metal Concentration and Geochemical Properties

All trace metal concentrations in Table 4.2 are in ppm or mg/kg.

4.3.2 Granulometry and Soil Type.

Soil granulometric fractions are also related with the content of trace metals in soil. Clay (particle size <2µm) is the main soil constituent relating to trace metals owing to their high affinity for clay minerals (Alloway, 1995). Contents of trace metals tend to be higher in soils with high contents of fine-grained sand, silt and clay due to the capacity of finer grains to absorb cations, which tend to retain trace metals (Chen et al., 1999; Tack et al., 2005). When using the Wentworth Scale of Soil Classification (see section 2.3.1 of Chapter 2) it was found that the soils in the study region mainly fell within the 'medium sand' to 'fine sand' range of soil texture classification, which together made up a median of 52.41% of the soil particle size in the sampling range, with 'medium sand' making up a median of 29.07% and 'fine sand' making up a median of 23.34% of the soil particle size in the topsoil samples taken in the study area (Table 4.3). The lower texture class soils, 'Very Fine Sand', 'Silt' and 'Clay', are more suitable for trace metal content analysis when examining risk to human and ecological health (Madrid et al, 2008). This is due to the finer soil particles posing an increased risk because of the possibility of ingestion and/or inhalation and the fact that many pollutants found in the topsoil, such as trace metals, are present at higher concentrations in these finer fractions than in larger size soil fractions due to the higher surface-to-mass ratio (Ajmone-Marsan et al, 2007; Qian et al, 1996). In the study area, these finer fractions make up on average a median 14.84% of the sample range (see Table 4.3). The Wentworth classification system was used (see section 2.3.1 of Chapter 2) which is as follows; Very Coarse Sand (1-2mm), Coarse Sand (0.5-1mm), Medium Sand (0.25-0.5mm), Fine Sand (125-250µm), Very Fine Sand (62.5-125µm) and Silt (3.9-62.5µm). The Clay (<2µm) fraction was also determined.

		Descriptive Statistics			
	N	**Minimum**	**Maximum**	**Median**	**Mean and Std. Deviation of Mean Result (±)**
Very Course Sand %	89	2.26%	36.41%	13.46%	14.49% ±7.68%
Coarse Sand %	89	4.78%	37.60%	17.37%	17.69% ±6.43%
Medium Sand%	89	16.48%	46.00%	29.07%	30.13% ±6.23%
Fine Sand %	89	10.85%	39.45%	23.34%	22.12% ±5.62%
Very Fine Sand%	89	0.10%	25.17%	9.25%	9.34% ±4.18%
Silt %	89	0.00%	12.50%	4.58%	4.98% ±2.98%
Clay %	89	0.00%	6.12%	1.01%	1.24% ±1.30%
Valid N (listwise)	89				

Table 4.3: Granulometric analysis of the topsoil samples taken from the Meres and Mosses region

We can see from the map of soil type (Fig 4.5) that the sampling area is mainly of seasonally wet soils with some stony soils to the Southwest of the sampling area and some sandy soils interspersed throughout the region. There is also some considerable deep loam soils in the central area of the sampling region with a patch of deep red loam soil just to the North of that.

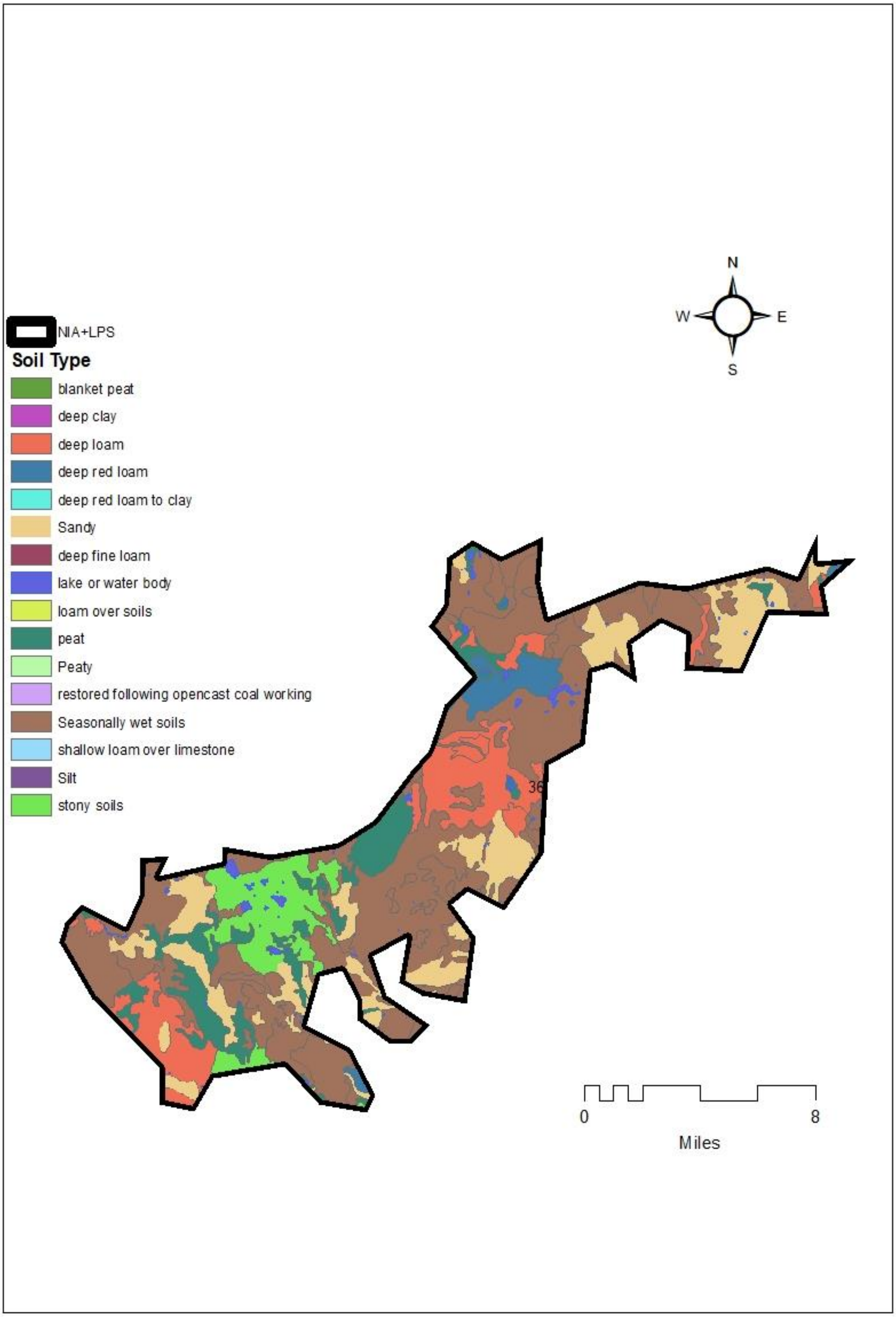


Fig 4.5: Soil Type in the Meres and Mosses Region, UKCEH (2007)

4.3.3 Enrichment Factor Analysis of Trace Metal Concentration.

The enrichment factor (EF) was performed by standardising a tested element against a reference element (see section 4.2.4). So to ascertain the degree of trace metal contamination/enrichment, the background concentrations, of a trace metal of interest, in the Earth's crust were examined by using Fe and Ti as reference elements as their content in the crust shows little variability over time (Hong et al., 2011). For this study, Fe was used as the reference element as it has high chemical stability and low spatial variability. For the same reason Ti was also used as a reference element so as to compare results and ascertain the EF for Iron (Fe) in the study. Because Fe is abundant in soil and is considered to be low anthropogenic contribution, it is commonly used for normalisation purpose. According to Sinex and Helz (1981) the EF is generally not very sensitive to the choice of baseline. The abundance of elements in the earth's crust was taken from Taylor (1964). The world average elemental concentrations reported by Turekian and Wedepohl (1961) in the earth's crust (shale) were also used as reference in this study.

If the value of EF approaches zero, crustal soils are the source of the trace metal (M) (see Equation 4.3a). If the EF value exceeds 5, the element can be considered enriched and to have a significant contribution from an anthropogenic or non–crustal source. (Sinex and Helz, 1981).

Trace Metal Element	Median Measured Concentration (PPM)	Earth's Crust (PPM)	Shale (PPM)	EF Crust (Fe)	EF Shale (Fe)	EF Crust (Ti)	EF Shale (Ti)
Cu	4.90	55	45	0.27	0.27	0.20	0.19
Pb	59.60	12.5	20	14.30	7.49	10.49	5.29
Zn	90.95	70	95	3.90	2.41	2.86	1.70
K	9880.20	20900	26600	1.42	0.93	1.04	0.66
Ca	4945.50	41500	22100	0.36	0.57	0.26	0.40
Ti	2590.30	5700	4600	1.36	1.42	1.00	1.00
V	216.40	135	130	4.81	4.19	3.53	2.96
Cr	63.80	100	90	1.91	1.78	1.40	1.26
Mn	336.80	950	850	1.06	1.00	0.78	0.70
Fe	18768.10	56300	47200	1.00	1.00	0.73	0.71
Co	31.86	25	19	3.82	4.22	2.80	2.98
As	5.00	1.8	13	8.33	0.97	6.11	0.68
Rb	60.10	90	140	2.00	1.08	1.47	0.76
Sr	57.90	375	300	0.46	0.49	0.34	0.34
Nb	29.80	20	11	4.47	6.81	3.28	4.81
Sn	1.60	2	6	2.40	0.67	1.76	0.47
Ga	17.30	15	19	3.46	2.29	2.54	1.62

Table 4.4: Enrichment Factors and Baseline Figures for trace metals in the Meres and Mosses Region.

The EF values (see Table 4.4) are <2 for Cu, K, Ca, Ti, Mn, Fe, Sr in the topsoil in the study area reflecting minimal enrichment. Some trace metals, such as Sn, Zn, Cr, Co, Rb and Ga, showed minimal (<2) to moderate (2-5) Enrichment Factor when applied to the various baseline methods. Two of the trace metal elements analysed, As and Pb, showed moderate to significant (5-20) enrichment when using the Earth's Crust figures (Taylor, 1964) as the reference material yet As showed only minor enrichment when using the Shale (Turekian and Wedepohl, 1961) figures as reference (see Fig 4.6). This can be seen clearly in the difference in the results for these elements when examining both background datasets. This may indicate that the Shale dataset (Turekian and Wedepohl, 1961) is more representative of the soils in the sample area. For the metals Nb, V, Zn and Pb, the enrichment factors ranged from moderate through to significant enrichment when the various baseline reference elements and background values were applied which shows an indication of strong anthropogenic disturbance. This indicates that there is some anthropogenic trace metal pollution in the topsoil in parts of the study area. Although the EFs for many of the elements were small, they are an indicator of trace metal accumulation (Jiao et al, 2015). Some low-level enrichments may result from variations in the composition of the soil structure in the sampling area and that of the Earth's crust and Shale baseline figures (Jiao et al, 2015).

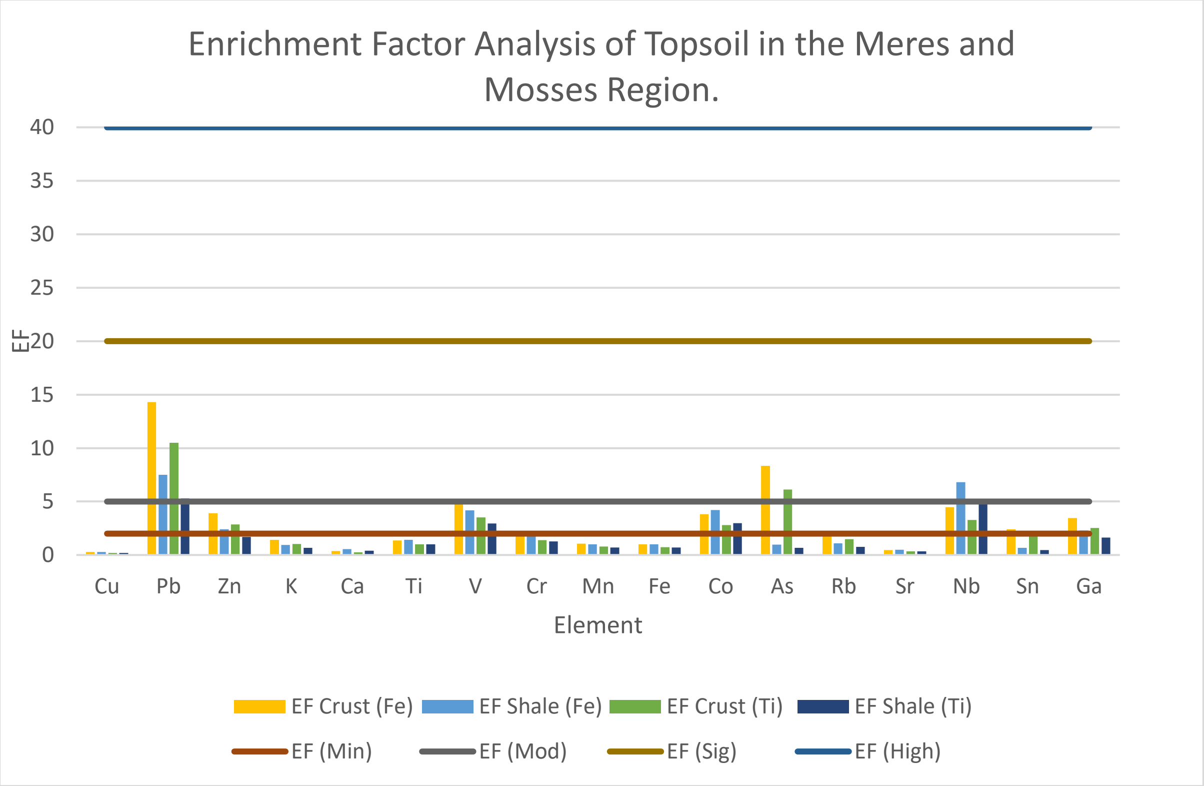


Fig 4.6: Enrichment Factor Analysis of Topsoil Samples in the Meres and Mosses Region when Compared to Enrichment Factor Levels (EF Min-EF High) Based on Median Trace Metal Concentrations.

When interpreting and evaluating the enrichment factor results of trace metals, it is important to consider these in the context of ambient background concentrations reported for unpolluted soils in England and Wales (McGrath & Zhao, 2006). Soil texture has been found to play an important role in the trace metal concentrations and distribution found within the soil (Chen et al, 2019). Meres and mosses habitats are known for their peaty texture as well as seasonally wet and loam soils (see Fig 4.5). The Meres and Mosses of the Marches, including Fenn's, Whixhall and Bettisfield Mosses are characterised by their peatland complexes with peaty and loamy soils (Lageard, 2023). The ambient background levels reported for unpolluted soils in England and Wales (McGrath & Zhao, 2006), as shown in Table 4.5, consider various soil textures including 'Peaty', 'Coarse Loamy' and 'Fine Loamy' for five of the trace metals studied in this chapter (Cu, Pb, Zn, Cr and Co).

Trace Metal Element	Median Background Concentration for Fine Loamy Soils (ppm)	Median Background Concentration for Coarse Loamy Soils (ppm)	Median Background Concentration for Peaty Soils (ppm)
Cu	18.8	14.7	15
Pb	39	36	116
Zn	89	65	52
Cr	43.5	27.4	12.2
Co	10.9	7.1	1.7

Table 4.5: Background Concentrations for Unpolluted Soils in England and Wales (McGrath and Zhao, 2006)

The median ambient background concentrations for unpolluted Loamy and Peaty soils in England and Wales (see Table 4.5), as ascertained by the Environment Agency (McGrath & Zhao, 2006), differ than those background concentrations determined globally for Earth's Crust or Shale (Taylor, 1964; Turekian and Wedepohl, 1961). This has the potential to provide different outcomes for EF analysis dependent on soil type and background concentrations determined for a particular site/region. The background value for Cu, for example, is lower in the loamy and peaty soils in England and Wales than the global figures for Earth's Crust and Shale (see Table 4.4 and Table 4.5). Likewise, ambient background concentration of Pb in Peaty soils in England and Wales is considerably higher than for the background global figures for Earth's Crust and Shale (see Table 4.4 and Table 4.5). This would potentially increase or decrease the EF respectively, dependent upon the reference element used in the calculation (see Equation 4.3a and Equation 4.3b). As both the soil type and land-use across the sampling range varied significantly, prevalence of implementation of this EF methodology in studies of this nature (Almasoud et al, 2015; Barbieri, 2016; Bourennane et al, 2010; Hernandez et al, 2003; Sutherland, 2000) along with a greater coverage of trace metal data, global average figures for background concentrations were implemented in this study for Enrichment Factor Analysis.

It is important to note that EFs only reveal the disturbance status of soil trace metals and do not necessarily indicate the pollution status and characteristics (Jiao et al, 2015). In general, it is considered that topsoil has two origins for contamination (Romic & Romic, 2003). These are either geogenic (soil parent rocks) or anthropogenic (agricultural applications, industrial waste, diffuse traffic related pollution). Many of the trace metal elements studied here were found to have an EF value >1.5 which indicates a non-crustal source for the potential origin (Barbieri et al, 2015).

4.3.4 Correlation Matrices for Trace Metals and Physiochemical Properties

Pearson correlation coefficients were used to establish the inter-relationship between trace metal levels from topsoil samples in the meres and mosses region as well as those of the geochemical characteristic of the topsoil. We can identify some definitive relationships when comparing trace metals to the geochemical and physiochemical soil characteristics which are displayed in the correlation table (Appendix A, Table A2). In general, a Pearson correlation (*r*) of <0.20 is considered ‘very weak’, 0.20-0.30 is often classified as ‘weak’, 0.30–0.40 are often classified as a ‘fair’ or ‘mild’ relationship, 0.40–0.70 as a ‘moderate’ relationship, 0.70–0.90 a ‘strong’ or ‘high’ relationship and > 0.90 as a ‘very high’ relationship (Alsaqr, 2021). According to the values of Pearson correlation co-efficient (Appendix A, Table A2) a significant relationship ($p < 0.01$) is observed between the pH of the sampled topsoil and the occurrence of various trace metal elements. Vanadium has a moderate positive correlation with pH ($r= 0.456$, $p= <0.01$) as does K ($r = 0.482$, $p<0.01$) and Mn ($r=0.508$, $p<0.01$) whereas Co shows a mild correlation with pH ($r = 0.256$, $p<0.05$). It is also observed that Lead (Pb) is moderately inversely influenced by pH with negative correlation ($r = -0.521$, $p<0.01$). This also applies for Niobium ($r=-0.618$, $p<0.01$). Soil reaction (pH) also displays a moderate inverse correlation with organic matter (TOC and LoI) which was found to be significant, as shown in Table A1 in appendix A. When examining the relationships between trace metals and organic content of the topsoil (LoI and TOC) many correlations are observed. Cu, Pb and Nb all have a moderate to very high positive correlation with organic content ($P<0.01$) whereas K, Ti, V, Mn and Rb have a moderate negative correlation with organic matter ($P<0.01$). It is observed that many trace metal elements have an affiliation with Magnetic Susceptibility Analysis (MS) including Cu, Zn, Cr, Mn, Fe, Co, Sr and Sn all having a significant positive correlation ($P<0.01$) which is inversely related in the case of Ga, whereas Ca and Ti all have weak strength correlation with MS ($P<0.05$) which is inversely so in the case of Nb. This shows that magnetic susceptibility can be a vital tool in predicting areas where some of the more environmentally important trace metals may be present.

When examining the trace metal to trace metal relationships (Appendix A, Table A3) it is observed that there are significant correlations ($p<0.01$) between many of the metal elements. It is worth examining these relationships, especially in the elements that showed a degree of enrichment (see Fig 4.6). Pb is moderately correlated to Cu and both these elements are significantly related to Nb (strongly so in the case of Pb). Pb is also moderately associated with K and Rb (negatively so). Cu is also significantly associated with Rb and Zn with mild and moderate correlations respectively. Zn and V are significantly related to Ga (strongly so in the case of V) as well as K to Sr having a mild yet significant correlation. Co is also moderately and significantly affiliated to Sr (as are Zn, Ca and Mn to Sr) as well as having significant correlations with Zn, Ti, Cr, Mn and Fe (strongly so in the case of Fe). Ti is strongly linked with Rb and moderately linked with Mn whereas Fe has significant correlations (to the 0.01 level, 2-tailed) with Zn, Mn, Sr, Co, Ti and V. V is significantly associated with Fe, Ti, Mn, and Sr and moderately so with Co (to the 0.01 level, 2-tailed). Rb also has significant correlations to K and Nb (to the 0.01 level, 2-tailed) which is strongly positive in the case of K.

Soil pH showed a positive correlation with Magnetic Susceptibility and negative correlation with Cr, Cu, and Pb and the correlation coefficient was significant ($p<0.01$) in the case of Pb. This implies that the neutral–acid soil environment will aid in enhancing the concentrations of certain trace metals (Kuo et al., 1983; Manta et al., 2002). It can also be seen that pH is significantly negatively correlated to the organic content of the topsoil and that the organic content itself is correlated with certain metals as explained above (Appendix A, Table A2). Therefore, it could be said that both pH and organic matter are fundamental controlling factors for some trace metals in the meres and mosses region.

Inter-trace metal relationships provide interesting information on the sources and pathways of the trace metals (Lu et al., 2010). In general, the significant positive correlations among the trace metals may reflect that they have geochemical affinities in soil and the constituents of that topsoil. The high positive correlations between the metals identified (or those with a moderate to strong/very strong positive correlation) may indicate that they share a similar origin, be it that they suggest the trace metals were mainly of a geogenic nature or enriched via anthropogenic impacts

4.3.5 Spatial Distribution of Trace Metal Elements in the Sampling Area.

The spatial distribution of trace metal concentrations can be used as an aid to identify their possible sources. To achieve this a shapefile (Fig 4.5, Figs 4.7-4.8f) was created to match the geographical dimensions of the sampling area (Fig 4.2). The spatial distributions of the trace metals, as well as organic matter content and magnetic susceptibility, were shown in Fig 4.8a through to Fig 4.8f. In order to explore the spatial variation of metals and geochemical properties in the topsoil, the distribution map of the varying metals concentrations and geochemical factors were interpreted using the Kriging method. Kriging is regarded as the best spatial covariance interpolation method, providing optimal interpolation (Chen et al., 2008; Chen et al, 2014). From this use of interpolation, it can be seen that the majority of the sampling area is on lightly acidic soils (as shown in Fig 4.4, Table 4.2 and Fig 4.7).

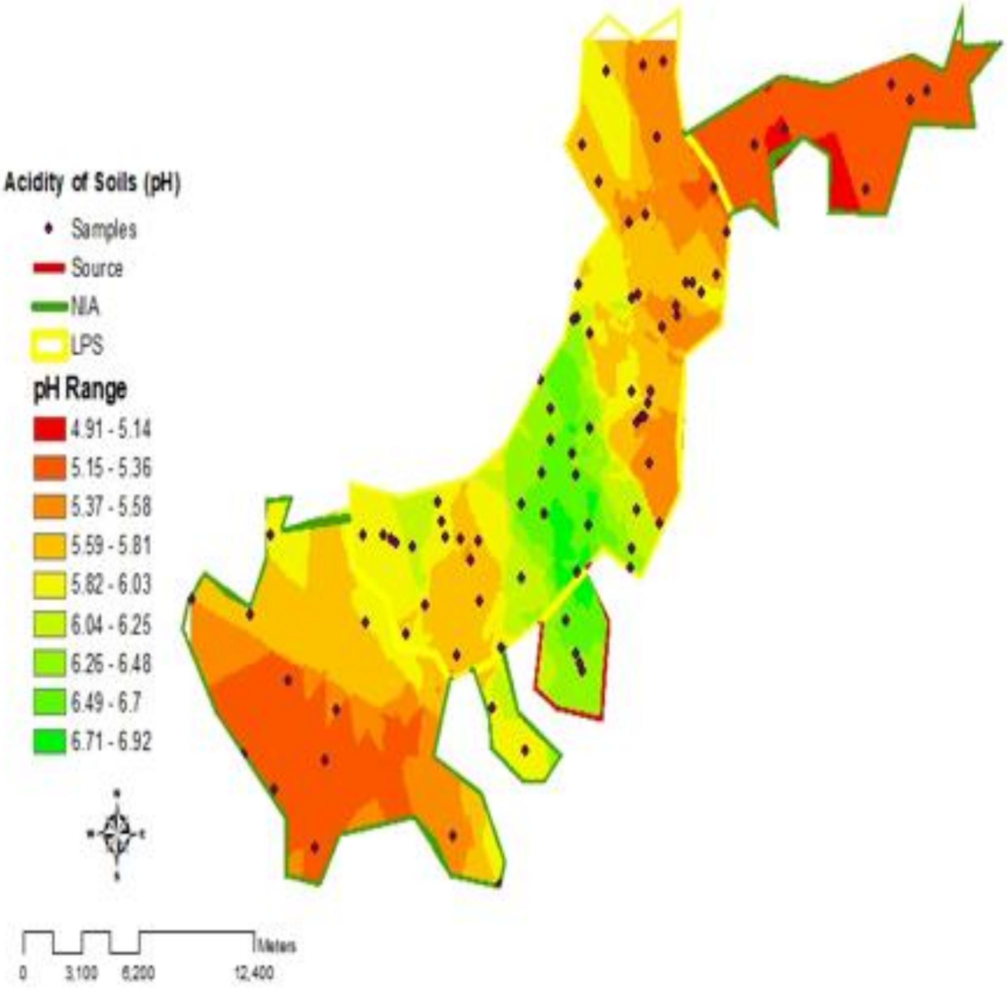


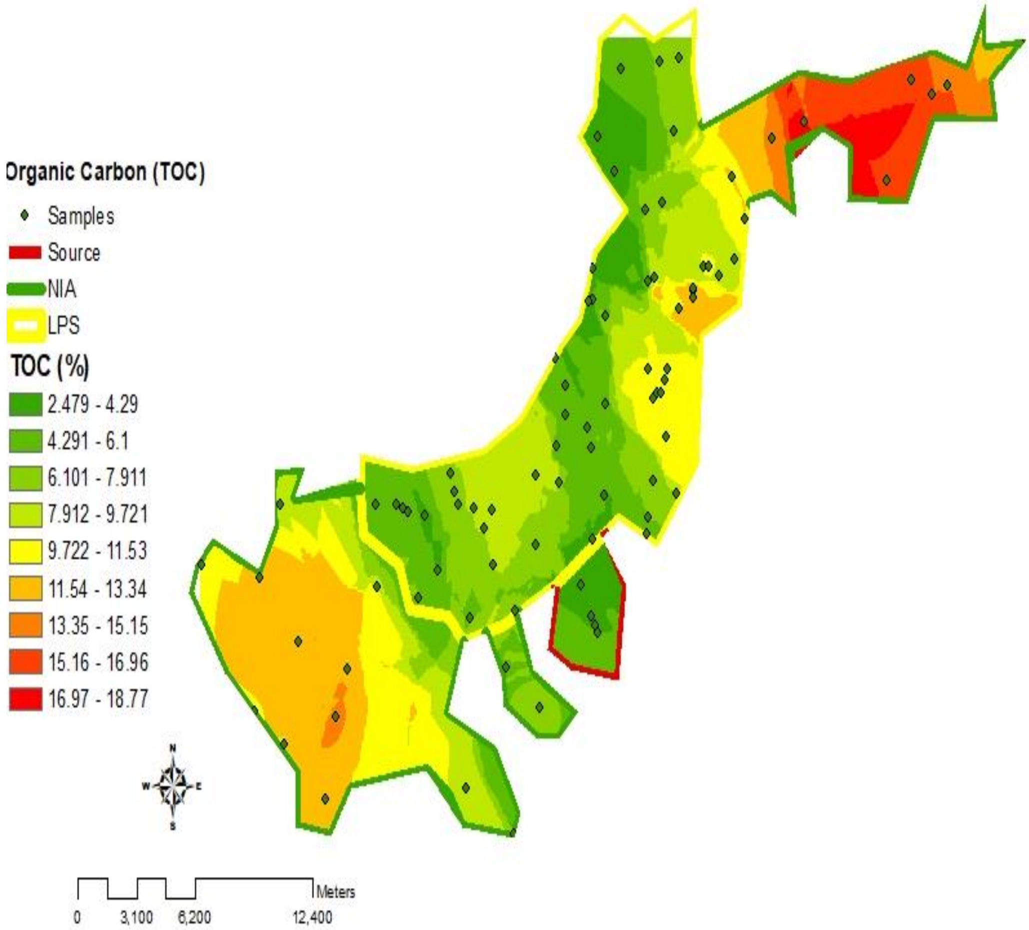
Fig 4.7: Interpolated pH

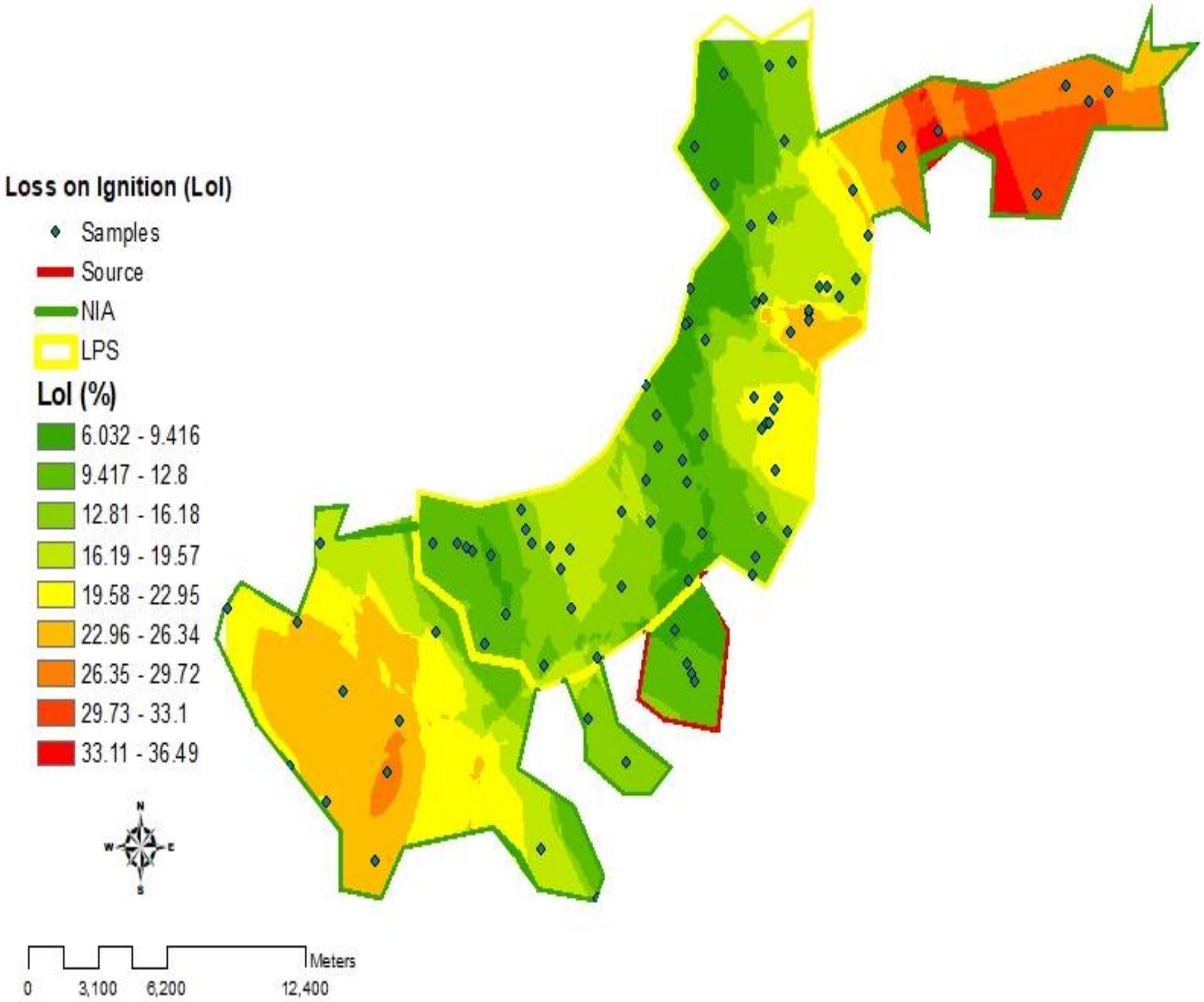
When examining the spatial distribution of the trace metals and geochemical factors we can see that some share similar distribution trends. It is observed that there is a common feature in parts of the sampling area where there are increased levels of Lead (Pb), Niobium (Nb) and Copper (Cu). We can also see this same trend apply to the organic matter in the Area (LoI and TOC). This is not surprising as all these factors were correlated to each other (see Appendix A Tables A1-A3). We can also see that they all share peaks of higher than normal and/or average concentrations in similar parts of the sampling area (see Fig 4.8a). It is interesting to note that the areas where these increased levels of trace metal occur are in the locations in close proximity with the environmentally sensitive mosses nature reserves at Brown Moss to the Eastern part of the sampling area, Fenn's, Whixhall and Bettisfield Moss to the West of the sampling area and to the North-East where Combermere is located (see Fig 4.1e). We can also see that Cu, Pb and Zn all share an increased level of concentration in the Northeast and Southwest of the sampling region.

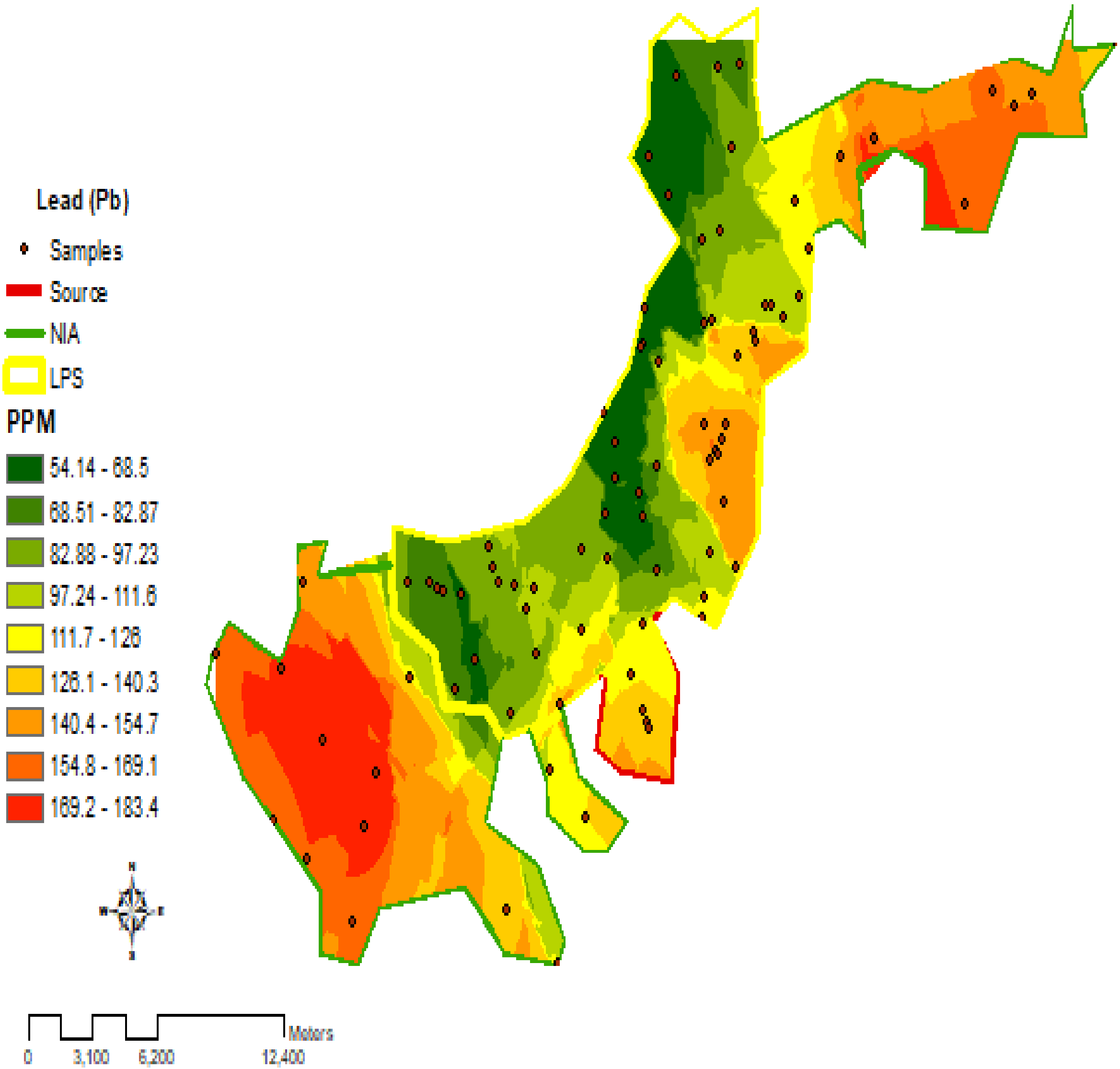
From the interpolated map for lead (Fig 4.8a) we can see that the majority of the study area is above the 22.6ppm (median) and 32.6 (mean) European average level for Pb in topsoil (Salminen et al, 2005) with the minimum concentration of Pb in the study being 31.6ppm, a maximum being 443.6ppm and an overall median across the sampling range of 59.60ppm. This median figure, 59.60ppm, also exceeds the Netherlands target/background value for Pb in soils of 57.96ppm when using median OM/clay results as applied to the sampling area (see Table 4.1d, Table 4.2 and Appendix F). The Pb in the study area also shows significant enrichment (see section 4.3.3). This indicates that there is an anthropogenic source of Pb in the study area. The area southwest on the map showing increased levels of Pb may be explained by the military application during World War 2 with Baggy Moor being used as a bombing and gunnery range at that time (ARG, 2016).

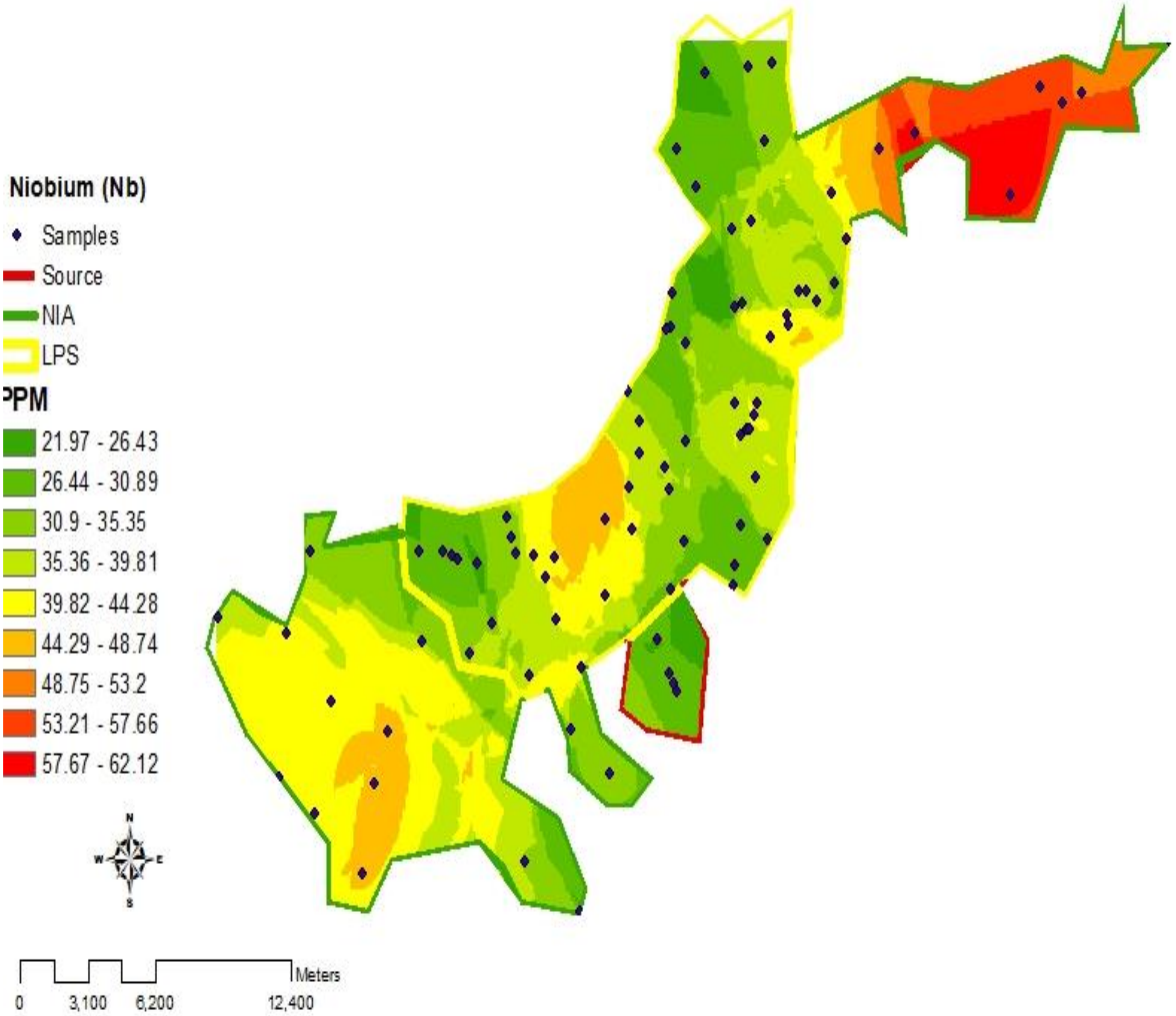
Copper values were found to be low in the study area. The copper levels in soil samples ranged from 0ppm detectable copper to 171.0ppm with a median value of 4.90 ppm. It can be seen from the interpolated map for Cu (see Fig 4.8a) that the vast majority of the study area is well within the Netherlands target/background values for Cu in agricultural soils of 19.78ppm (see Table 4.1d) when using median OM/clay for the sampling range. We can see that this target/background value is surpassed at certain points on the interpolated map for Cu (see Fig 4.8a). These elevated concentrations correspond to samples taken in the vicinity of the meres and mosses nature reserves and urban/industrial source areas (as indicated in Fig 4.1e and Fig 4.2). The EF for Cu shows minimal enrichment which indicates little anthropogenic influence of the presence of this trace metal element overall in the studied sampling area. Cu is known to accumulate in the surface layers, as a result of bioaccumulation and influence from anthropogenic sources (Kebata-Pendias, 2004). The relative levels of copper follow a similar pattern to those attained for Nb and Pb as well as levels of organic matter (LoI, TOC) indicating a relationship between these factors. It is also worth noting that the mean value of Cu in this study of 23.1ppm exceeds the European average for copper in topsoil, as ascertained by the Geochemical Atlas for Europe (Salminen, et al. 2005) 12ppm (via ICP-MS with Aqua-Regia extraction) but the central tendency, as determined by the median, indicates a result of 4.9ppm which indicates the presence of outlier values (as shown in Table 4.2).

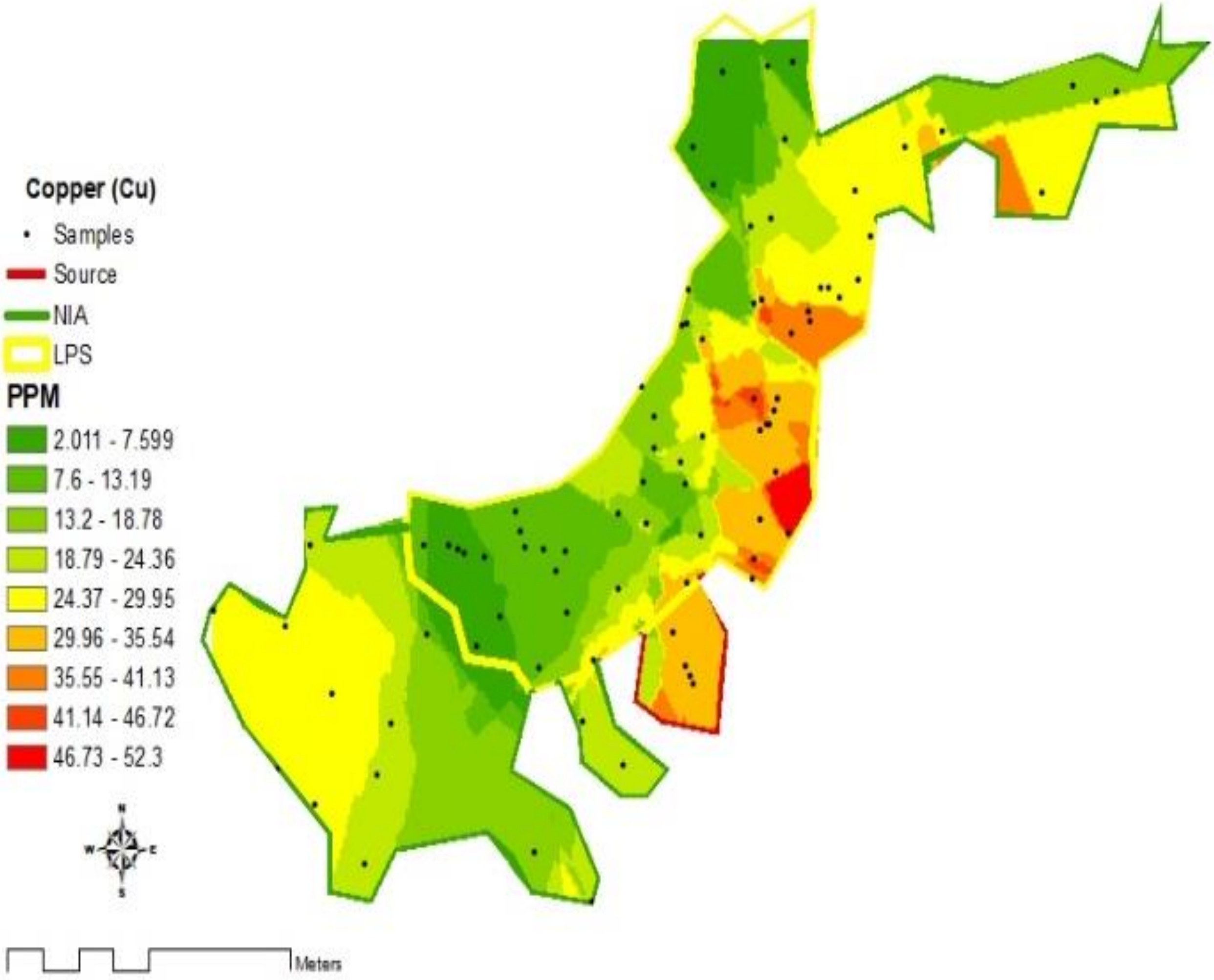
The interpolated map for Zn (Fig 4.8a) shows that there are increased levels of the element closest to samples taken near the urban area. Results show that concentrations range from a minimum found concentration of 23.1ppm to a maximum of 2330.7ppm. The median across the study range is 90.95ppm which exceeds the Netherlands target/background value of Zn in soils of 63.46ppm when applying the median OM content and clay composition ascertained for the sampling range (see Table 4.1d, Table 4.2 and Appendix F). Zn also shows moderate to significant EF. This indicates there is an anthropogenic source of Zinc in the region.


Organic Carbon (TOC)
Samples
Source
NIA
LPS
TOC (%)
2.479 - 4.29
4.291 - 6.1
6.101 - 7.911
7.912 - 9.721
9.722 - 11.53
11.54 - 13.34
13.35 - 15.15
15.16 - 16.96
16.97 - 18.77
N
W
E
S
0
3,100
6,200
12,400
Meters


Loss on Ignition (LoI)
Samples
Source
NIA
LPS
LoI (%)
6.032 - 9.416
9.417 - 12.8
12.81 - 16.18
16.19 - 19.57
19.58 - 22.95
22.96 - 26.34
26.35 - 29.72
29.73 - 33.1
33.11 - 36.49
N
W
E
S
0
3,100
6,200
12,400
Meters


Lead (Pb)
Samples
Source
NIA
LPS
PPM
54.14 - 68.5
68.51 - 82.87
82.88 - 97.23
97.24 - 111.6
111.7 - 126
126.1 - 140.3
140.4 - 154.7
154.8 - 169.1
169.2 - 183.4
N
W
E
S
0
3,100
6,200
12,400
Meters


Niobium (Nb)
Samples
Source
NIA
LPS
PPM
21.97 - 26.43
26.44 - 30.89
30.9 - 35.35
35.36 - 39.81
39.82 - 44.28
44.29 - 48.74
48.75 - 53.2
53.21 - 57.66
57.67 - 62.12
N
W
E
S
Meters
0
3,100
6,200
12,400


Copper (Cu)
Samples
Source
NIA
LPS
PPM
2.011 - 7.599
7.6 - 13.19
13.2 - 18.78
18.79 - 24.36
24.37 - 29.95
29.96 - 35.54
35.55 - 41.13
41.14 - 46.72
46.73 - 52.3
N
W
E
S
Meters

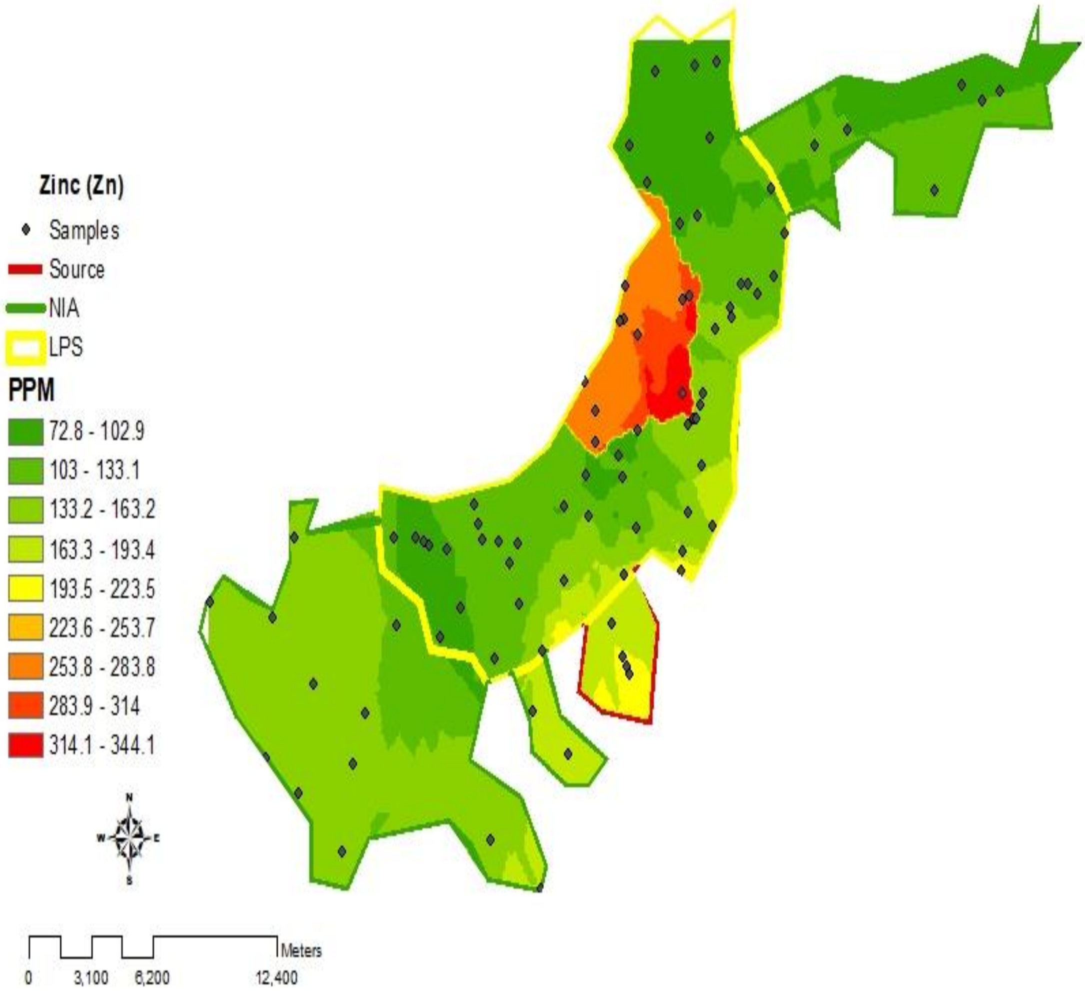
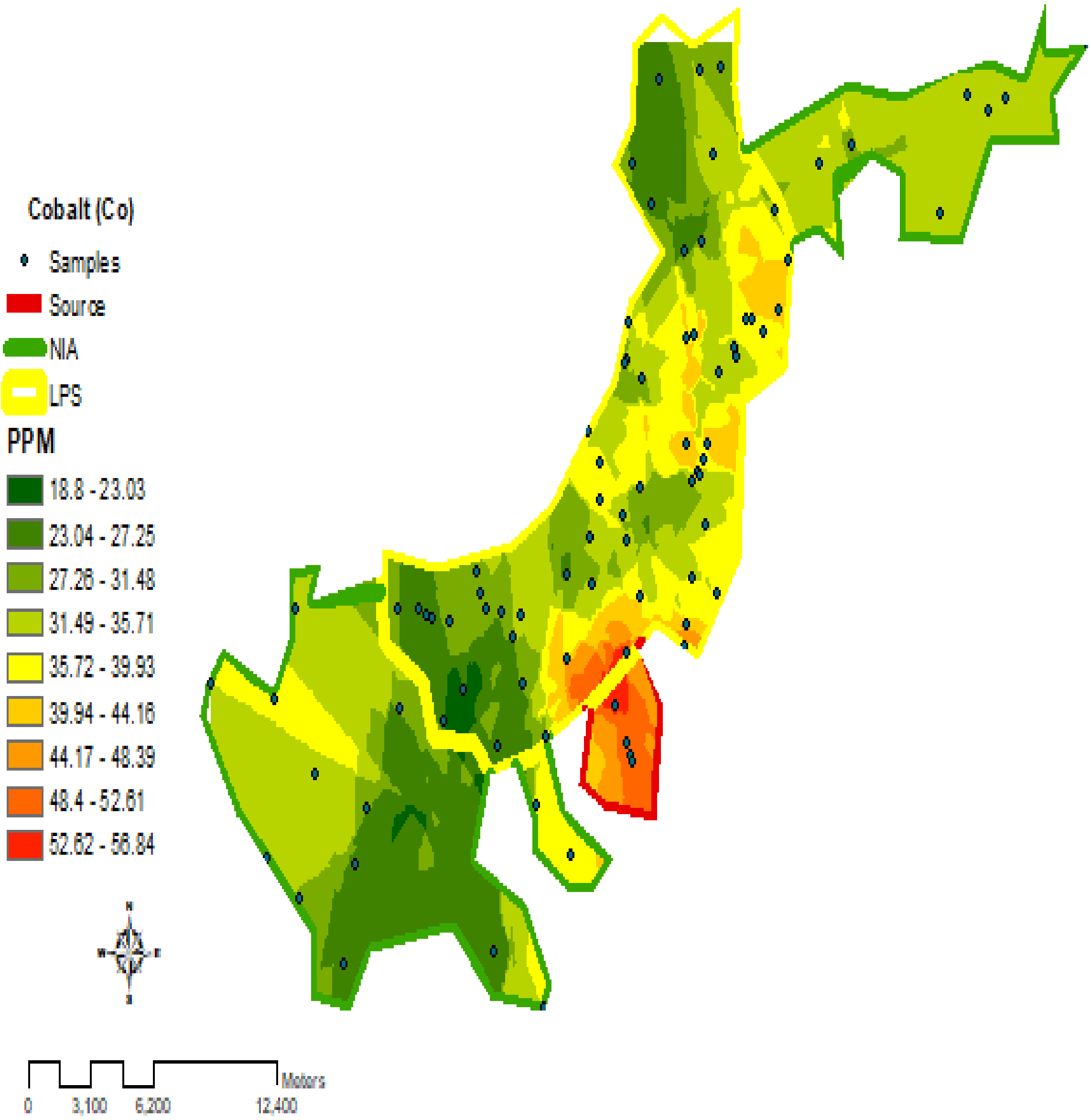


Fig 4.8a: Interpolated Pb, Nb, Cu, Zn and Organic Matter (LOI and TOC)

Schieb and Flight (2012) state that "Niobium (Nb) is of growing interest because of its application and use as an alloying element in high-grade structural steel, as well as in electronic components. However, there is very little published information on Nb concentration levels in the environment and its potential health effects". According the Scheib and Flight (2012), in their study 'Distribution and mobility of Niobium in European soils', which examined results garnered for Nb via XRF, the median baseline concentration of Nb in the topsoil of Europe is 12 ppm (XRFS). In this study it was found results for Nb ranged from a minimum of 0ppm detectable to a maximum of 107.4ppm and a median of 29.80ppm. This exceeds the average from the study performed by Scheib and Flight (2012). There was also seen to be some moderate and even significant enrichment of Niobium (see section 4.3.3). This indicates an anthropogenic input of Nb in the study area. The spatial distribution of Nb is displayed in Fig 4.8a.

In the case of Co and Cr we can see higher intensities of metal concentration across the area that is covered by the town of Wem and its industrial estate which were covered in the 'source' portion of the sampling regime (see Fig 4.1e and Fig 4.8b). This is almost certainly down to anthropogenic input with the urban layout of the area (and industry) and the amount of roads leading to the town. Increased levels for Co can also be seen for nature reserve areas such as Combermere and Brown Moss Nature Reserve to the centre and Northeast of the sampling region. This may be due to Cobalt occurring naturally in soils due to the breakdown of organic matter, and the weathering of the local minerals into soil particles as well as increased traffic flow in areas that can be considered tourist attractions (Maja et al 2019).



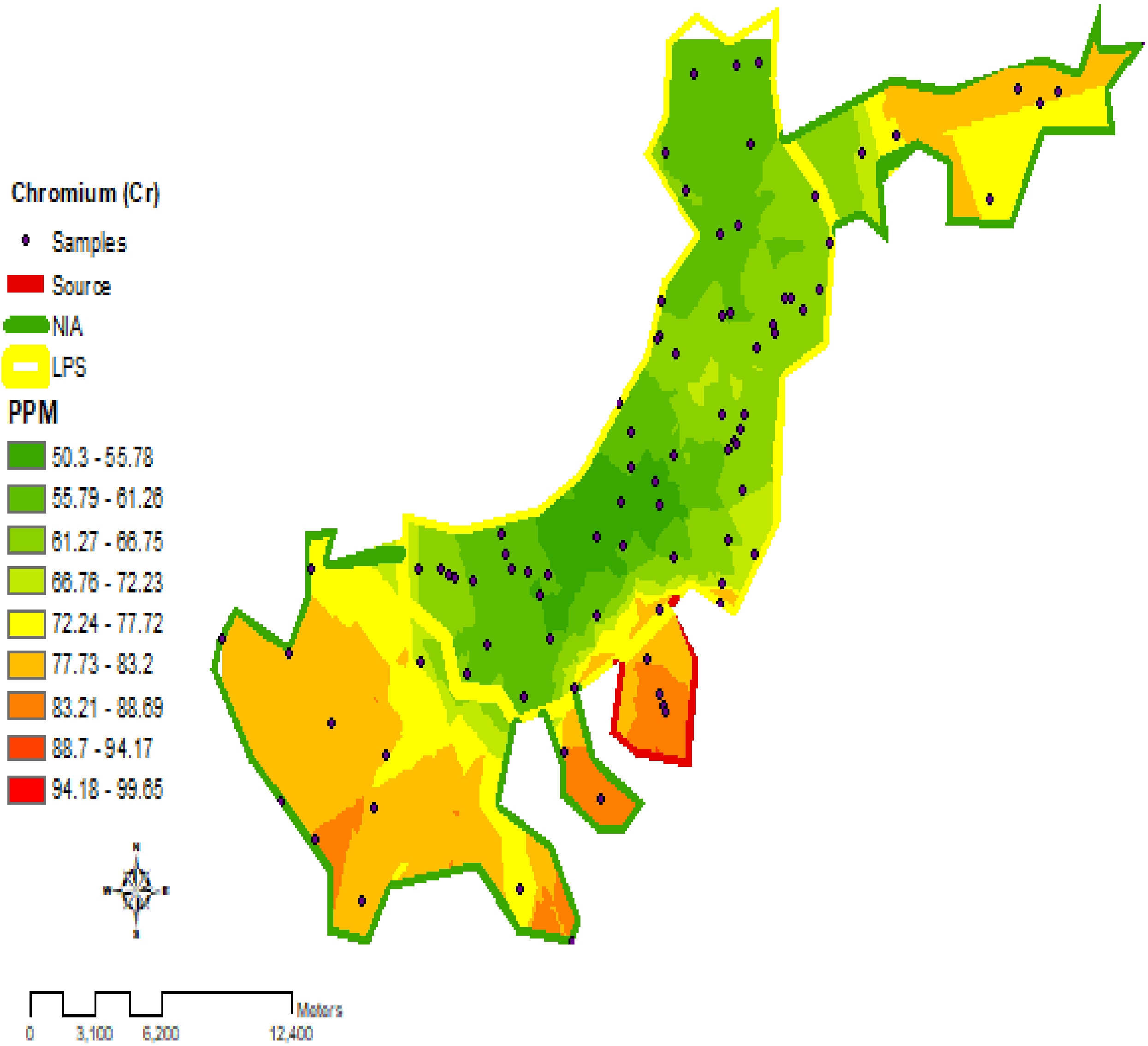


Fig 4.8b: Interpolated Co and Cr

From the above spatial maps, we can see that both Chromium and Cobalt show similar spatial distribution (see Fig 4.8b). Table A3 in Appendix A depicts the correlation coefficient matrix, listing the Pearson's correlation coefficient. A very significant correlation ($p<0.01$) was found between Cr and Co ($r = 0.330$). Chromium, in the areas that show the highest concentration within the sampling area, exceeds the Netherlands target/background values for trace metals in soils which is 52.02ppm (when using the median OM and clay composition of the sampling area, as outlined in Table 4.1d) when examining the spatial distribution following Kriging Interpolation (despite having samples above this concentration in the sample range). Chromium is a trace metal characterised by low mobility at near-neutral pH values (Kayalvizhi et al, 2015). Chromium is also a component of steel alloys and is used in metallurgy for coating steel for protection from corrosion (Minolfi et al, 2018). Cr is toxic for biological systems (Kabata-Pendias, 2001). Chromium levels in the study area ranged from 36.2 to 124.85 ppm, with a median of 63.80 ppm (Table 4.2). The EF obtained for Cr fall under the class of minimal to moderate enrichment. Therefore, the source of Cr appears to be geogenic in the main with the increased levels (see Fig 4.8b) being in the urban and industrial areas (partly identified in the red outline on the map) where there will likely be anthropogenic input of chromium and its compounds. The ambient background concentrations reported for Cr in unpolluted soils in England and Wales was found to be a median of 39.3mg/kg (McGrath & Zhao, 2006). This figure is seen to be consistently exceeded in the sampling area (see Fig 4.8b) with the ascertained background values for Cr in 'peaty' and 'loamy' soils also being consistently exceeded when studying the interpolated data (see Fig 4.8b and Table 4.5).

Cobalt shows higher values than those conveyed by the Netherlands target/background values (2.28ppm) across the sampling range when considering the median clay composition and OM levels determined for the region (see Table 4.1d, Table 4.2 and Appendix F). Co levels in the study area ranged from 0ppm detectable Co to a maximum of 105.9ppm. The median figure Cobalt is 31.86ppm and it can be seen from the Kriging interpolated map for Co that the area is consistently above the target/background value (2.28ppm) across the entire sampling area. The EF obtained for Co falls under the class of moderate enrichment. Therefore, the source of Co appears to be both geogenic and anthropogenic with the increased levels (see Fig 4.8b) being in the urban and industrial areas where there will likely be anthropogenic input of Cobalt and its compounds (as displayed by the increased spatial concentration of Co in the 'source' aspect of the map with the red outline). Anthropic sources of Co in the environment include coal combustion, fertilisers, Pb, Fe and Ag mining and processing (Reimann and de Caritat, 1998). Co becomes mobile in the soil surface following weathering and under acidic conditions (Taylor, 1968). Cobalt is also known to bind to humic and fulvic acids (Qian et al. 1998).

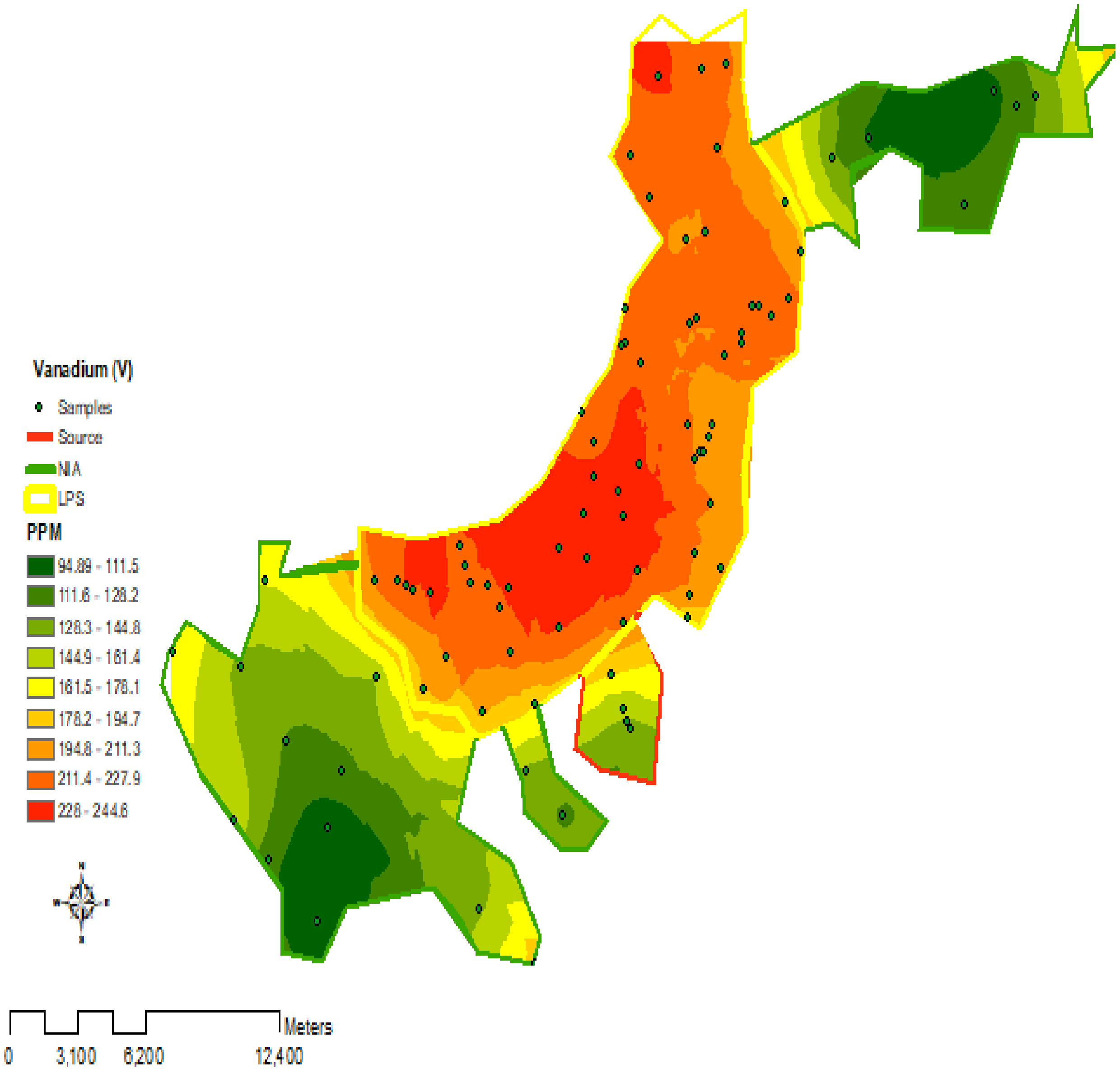


Fig 4.8c: Interpolated V

Vanadium, and its various metallic compounds are used for a wide variety of purposes. 85% of mined metal is utilised in the steel industry, and is heavily used in industry for many applications including metallurgy and electronics (Gowd et al, 2009). It is also added to some pesticides. The amount of vanadium in the soil depends upon the pedogenic process of the parent material (Hasnine et al, 2017). V in soil is also linked with the presence of Fe oxides in the soil (Kabata-Pendias, 2001). The UK Soil and Herbage Pollutant Survey (UKSHS, 2007) reported that the median concentration of V in UK soil was found to be 39.2mg/kg. The results obtained for V in topsoil in the study area consistently exceed this median concentration and show a minimum concentration of 54.8ppm, a maximum of 270.5 and a median of 216.40ppm. This median figure exceeds both the Netherlands target/background limits and the Netherlands intervention limits (15.03ppm and 89.46ppm respectively) when applying the median OM levels and median clay content ascertained for the region (see Table 4.1d, Table 4.2 and Appendix F). Fig 4.8c displays the spatial distribution (interpolated) throughout the sampling region of V. When studying the results for Enrichment Factor (EF) we can also see that the area shows moderate levels of enrichment. This indicates a source of anthropogenic and/or geogenic (weathering etc..) input of Vanadium in the topsoil of the study area.

Possible anthropogenic sources of V in the meres and mosses region include coal burning (fly ash), fly ash amended soils, sewage sludge amended soils, cement processing, and steel production which is prevalent in the area.

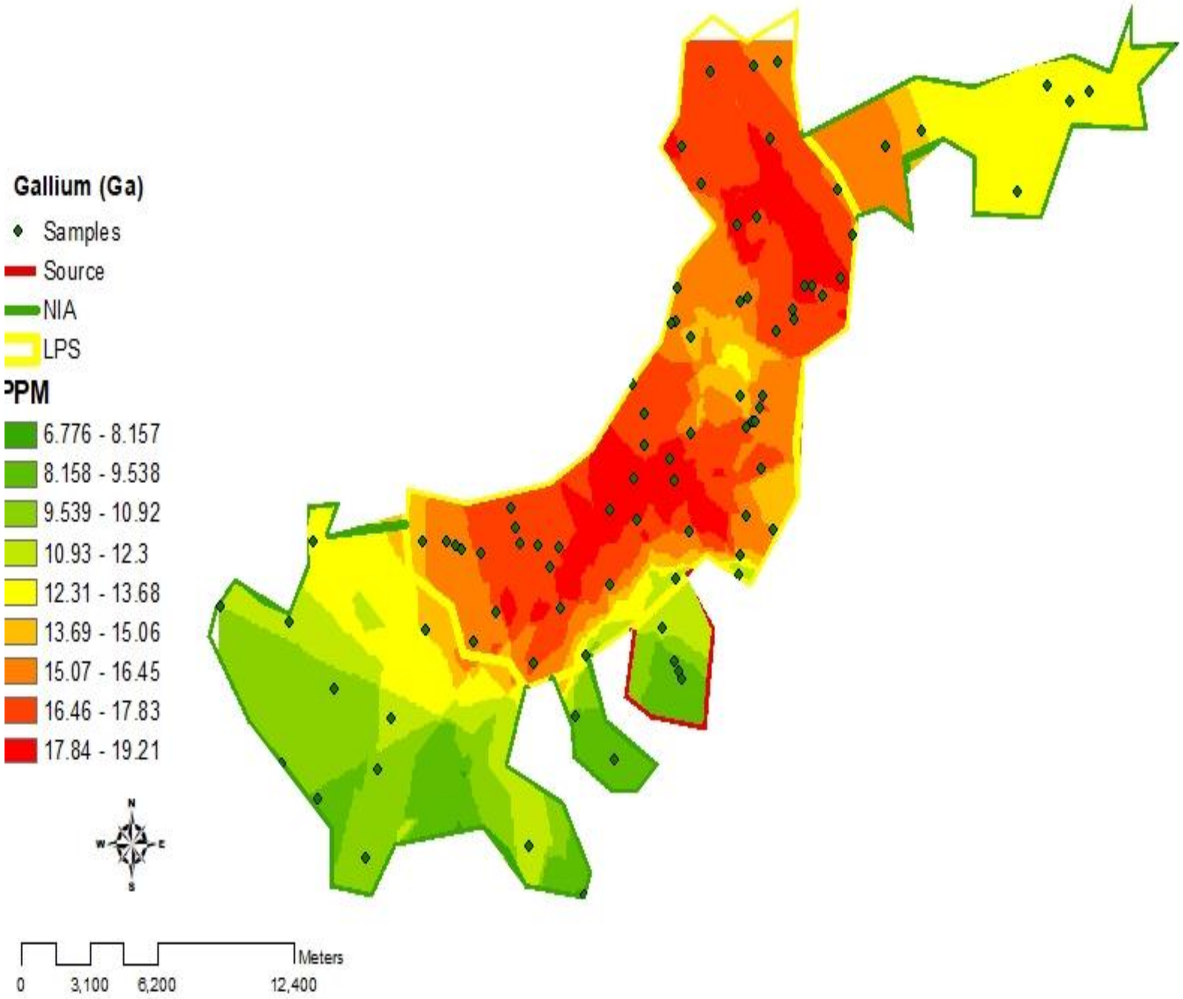
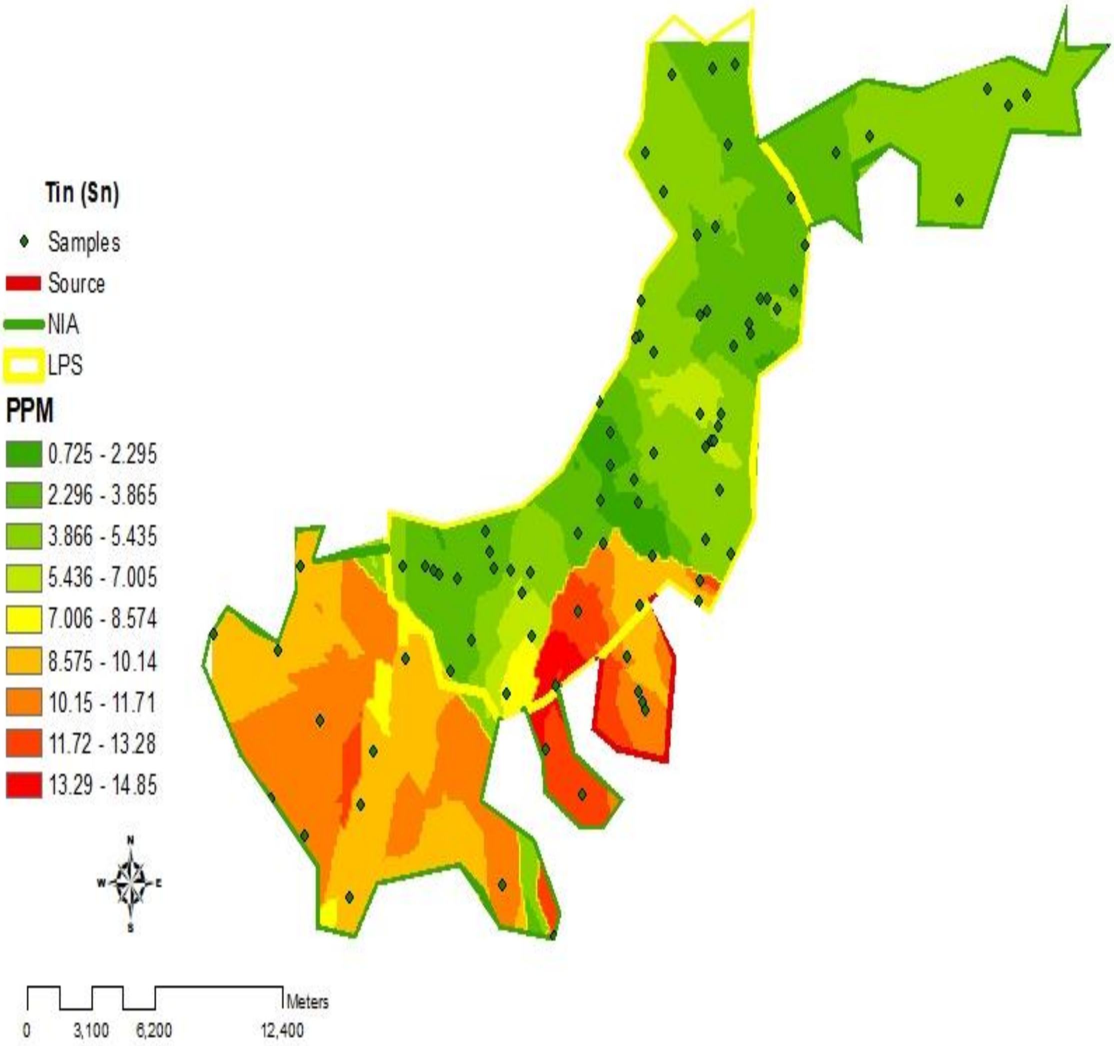


Fig 4.8d: Interpolated Ga

Fig 4.8d displays the spatial distribution (interpolated) throughout the sampling region of Gallium (Ga). In the soil environment, Ga content ranges from 1 to 70 mg kg-1 with an average of 28 mg kg-1 (Kabata-Pendias 2001). According to the FOREGS Geochemical Atlas of Europe (Salminen et al, 2005), the average amount of Ga in topsoil is 13.5ppm. Ga in this study showed moderate enrichment when using crust as the reference substrate baseline and minimal enrichment when using shale as the substrate baseline. In general, the levels of Ga are in line with what is expected for background levels (median Ga = 17.30ppm) with the enrichment being most likely due to the industry in the local area or geogenic enrichment. Anthropogenic sources of Ga include Zn and Cu smelting, chemical manufacture, waste incineration and the combustion of coal (Reimann and de Caritat 1998, Schroll 1999).



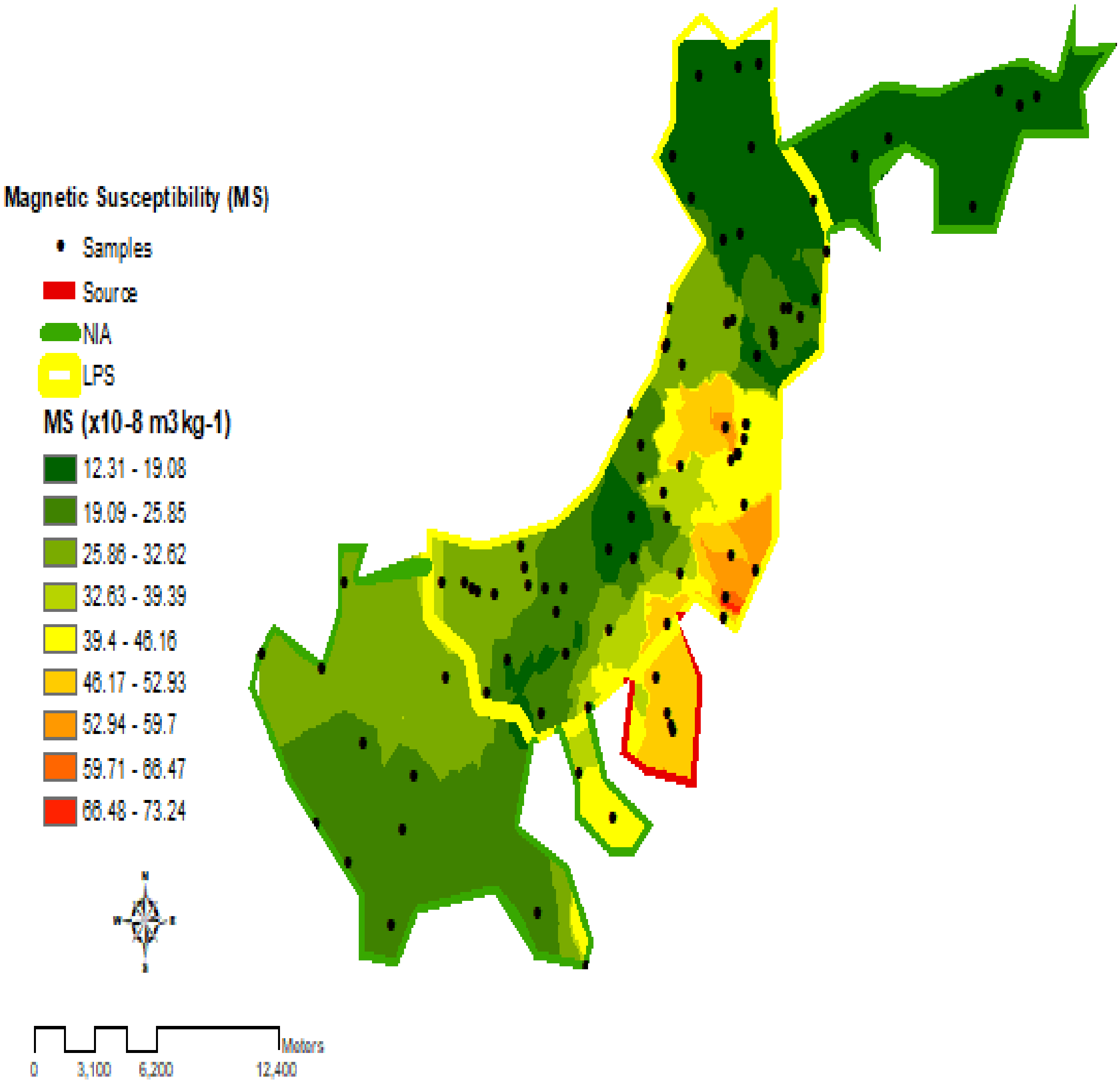
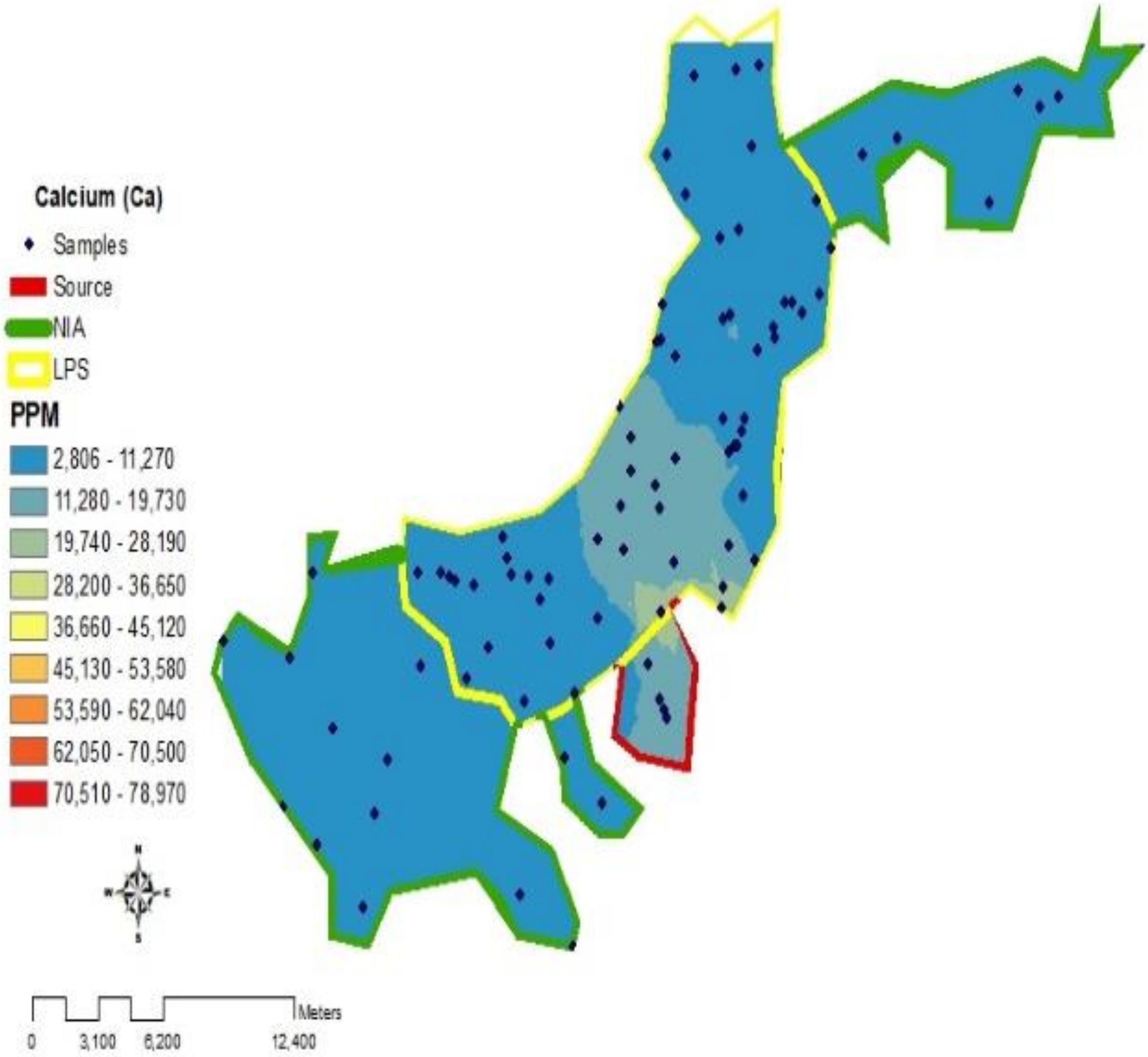
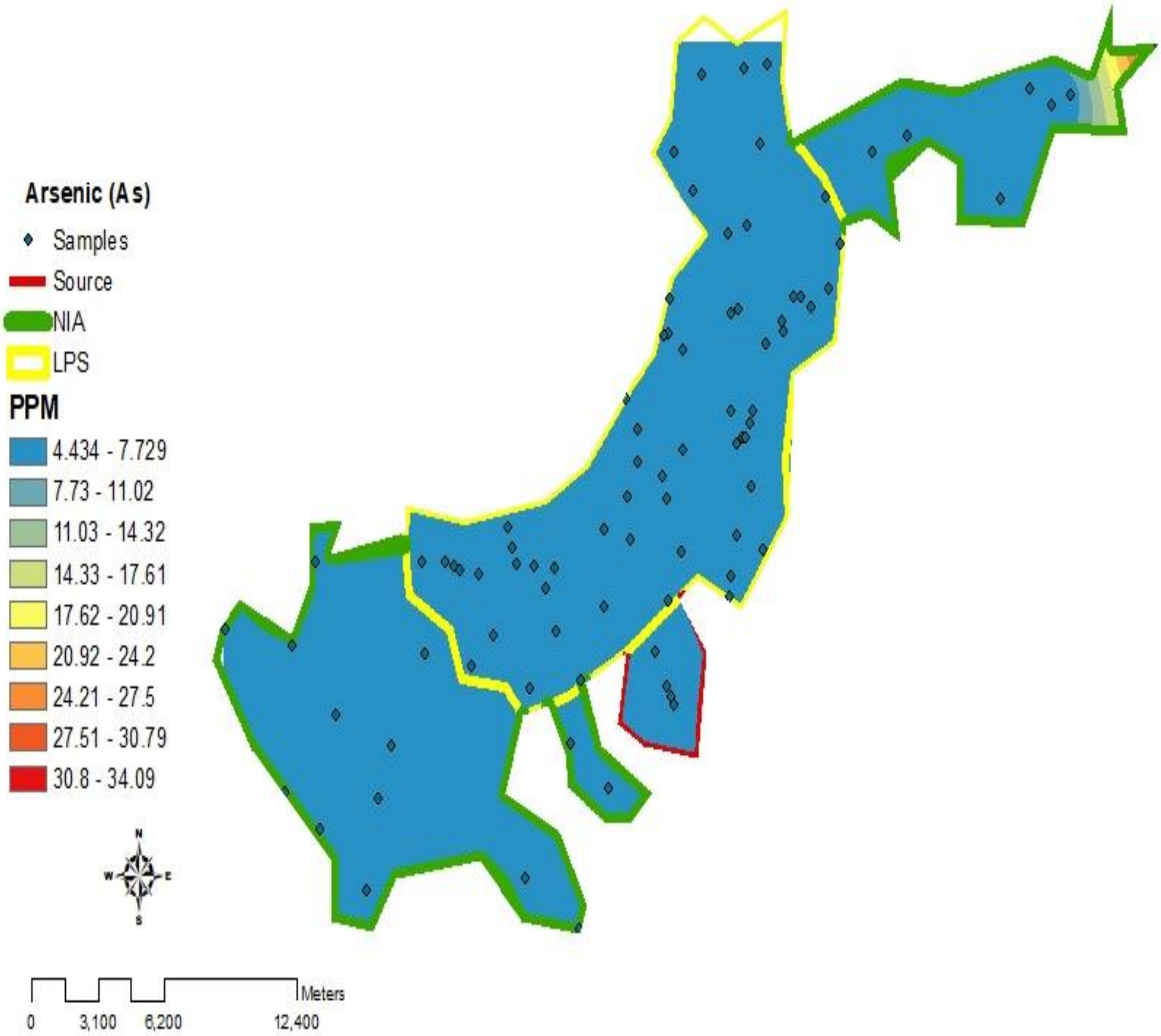


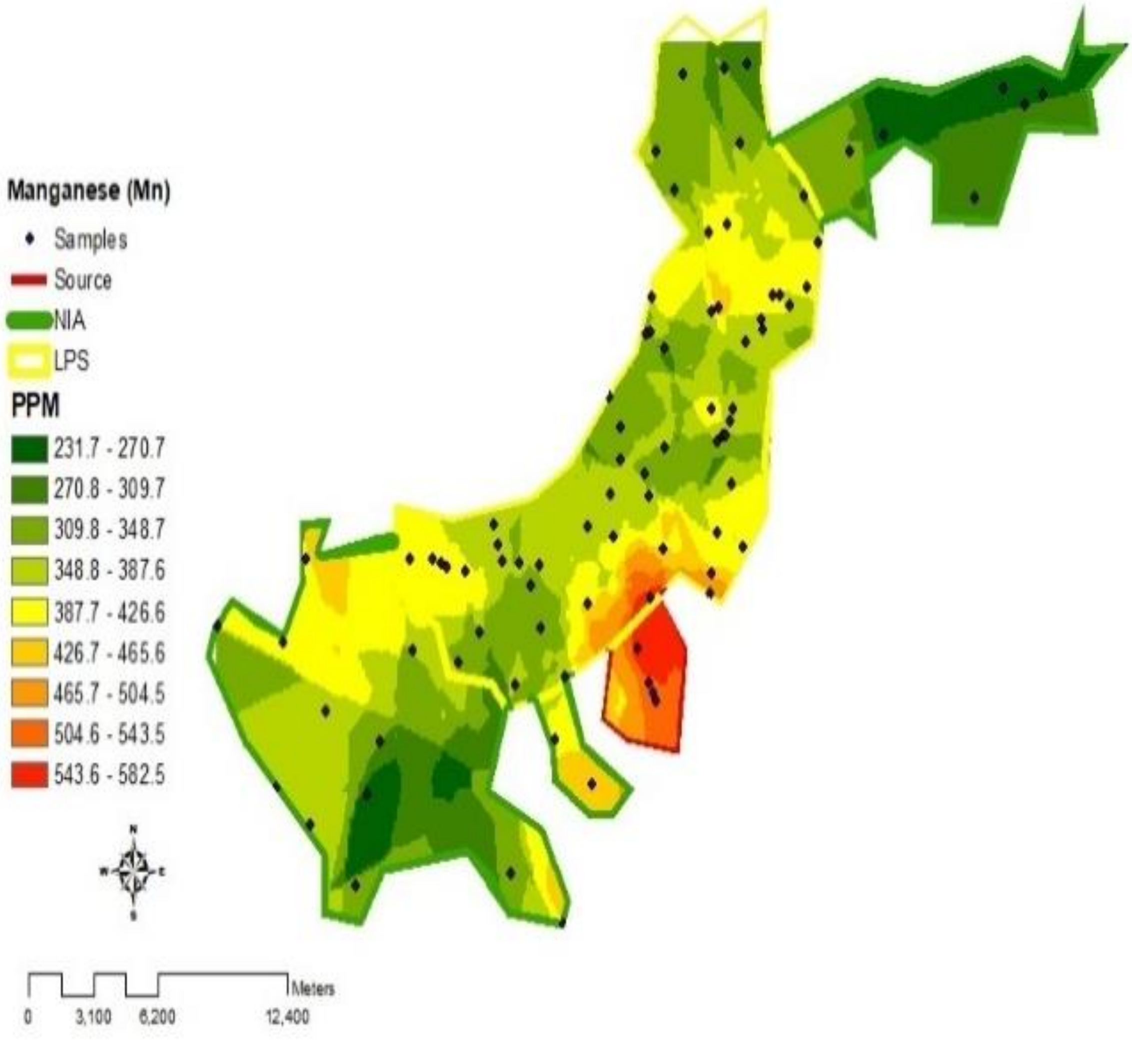
Fig 4.8e: Interpolated Sn and MS

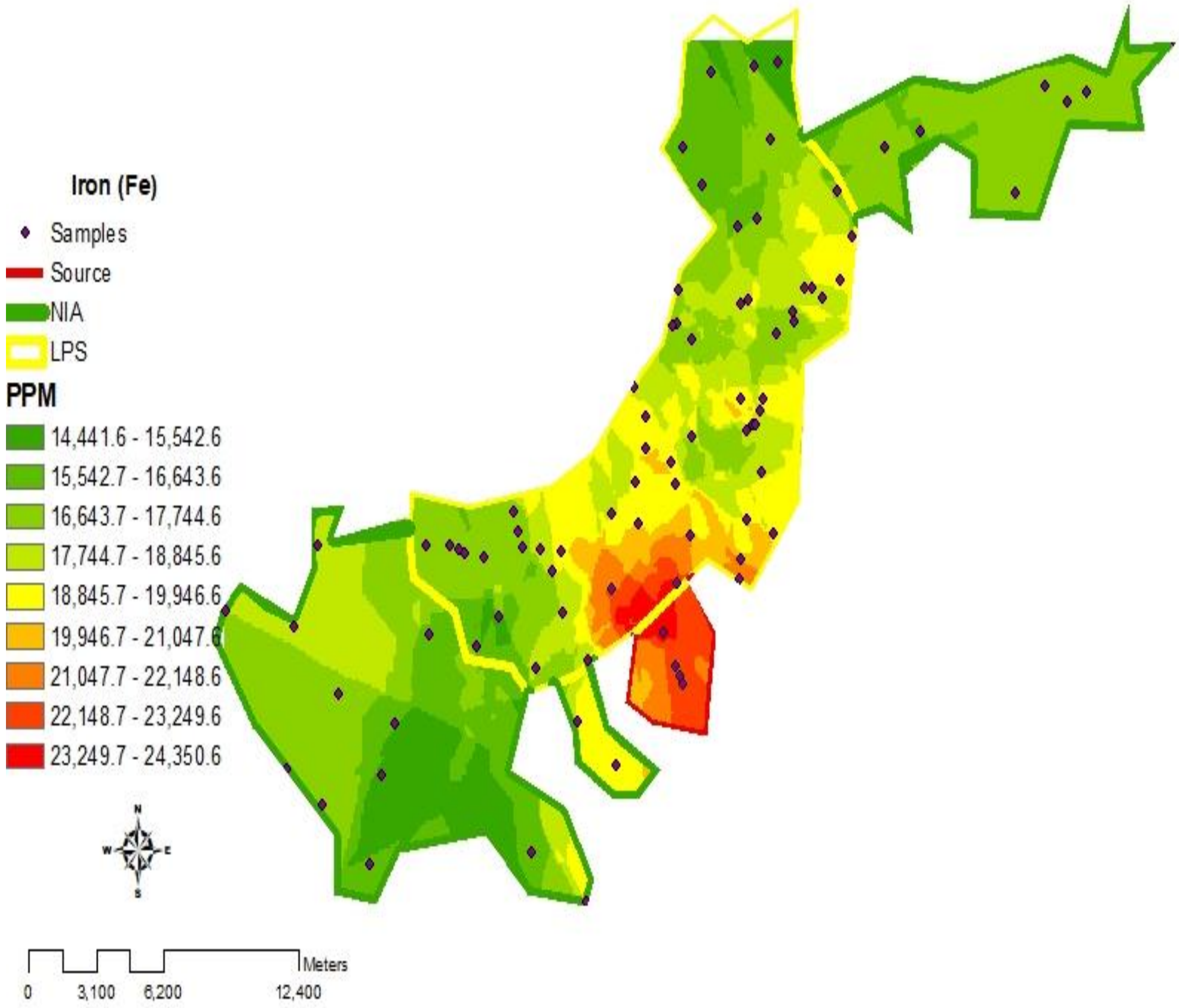
Fig 4.8e displays the spatial distribution (interpolated) for Tin (Sn) and Magnetic Susceptibility (MS) within the sampling area. Tin (Sn) shows some interesting charactistics as, at a median of 1.60ppm in the sampling area (Table 4.2), it is marginally lower than the European median for topsoil as derived by the FOREGS Geochemical Atlas for Europe (Salminen, et al. 2005) of 3ppm. Salminen et al (2005) report that “The occurrence of Sn in soil has not received much study”. Kabata-Pendias (2001) found that Sn ranged from 1 to 11 ppm with an average of 1.10 ppm. The median figure for Sn concentration in topsoil as ascertained by FOREGS Geochemical Atlas of Europe found that, whilst the European median for Tin in topsoil is 3 ppm (Salminen, et al. 2005), that there are low Sn values in subsoil (<2ppm) in Northern Britain (Salminen et al, 2005). Sn shows significant relationships ($p<0.01$) in the correlation matrix with Chromium (Cr) and Cobalt (Co) (see Appendix A, Table A3) and also shows a positive relationship with Magnetic Susceptibility ($p<0.01$). Sn has a number of anthropogenic sources including from coal, alloys, pesticides and sewage sludge (Reimann and de Caritat 1998; Salminen et al, 2005). The levels of Sn found in the sampling area show moderate enrichment. Kabata-Pendias (2001) found “that although Sn in soils is largely derived from the bedrocks, its increased level due to pollution has been noticed”. This can be seen in the sampling area were, despite overall levels being relatively low, there are higher levels recorded in and around the more urban and industrial areas such as Wem, and Prees (which is displayed in the ‘source’ portion of the map fig 4.8e and Fig 4.2) indicating that industrialised locations have an impact on the spatial distribution of these trace metals.

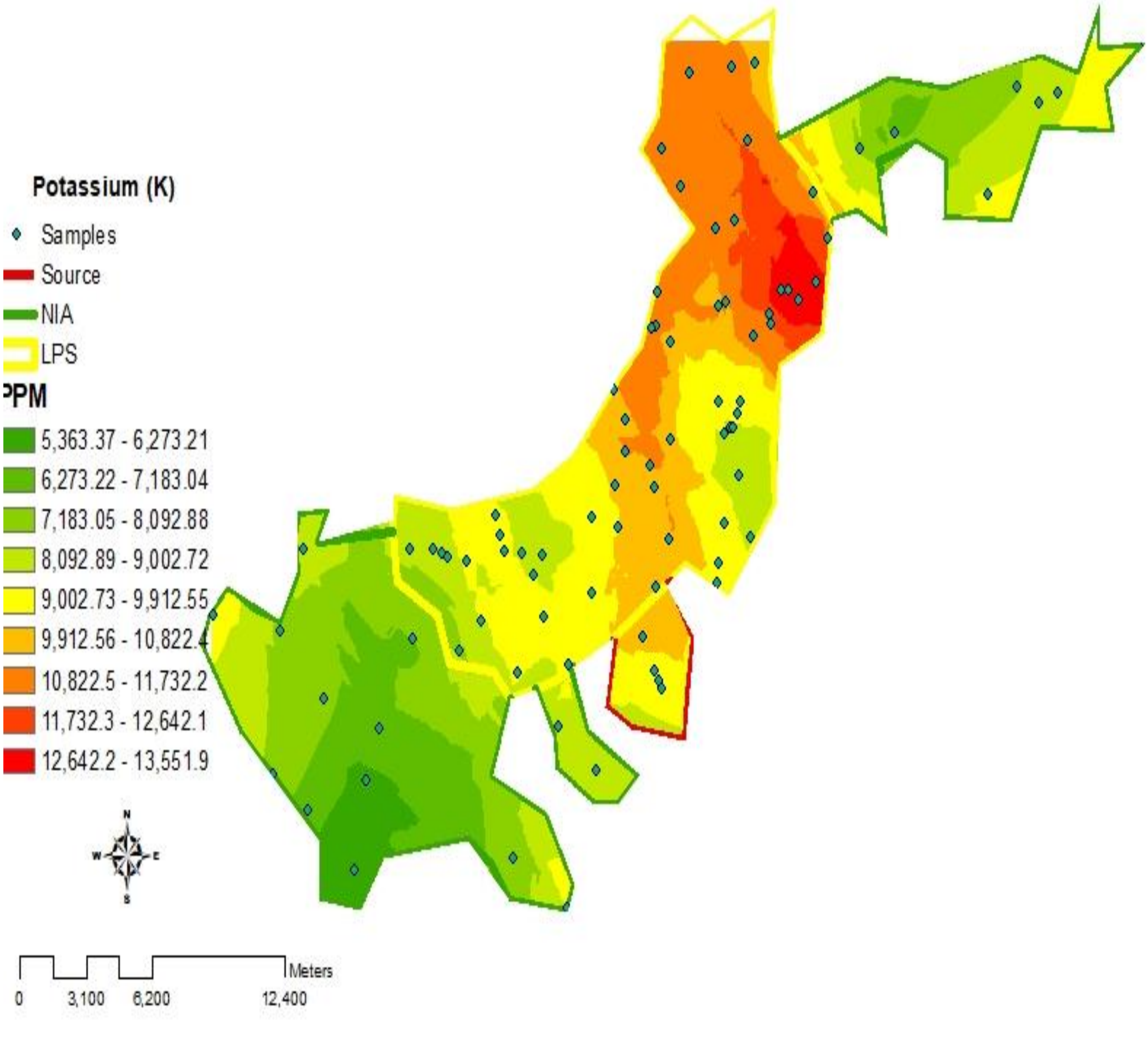
The following elements (Fig 4.8f), due to their lack of enrichment and the correlational relationships therein, indicate that they are mainly of geogenic origin. This is useful when establishing the geochemical make-up of the soils in the study area.

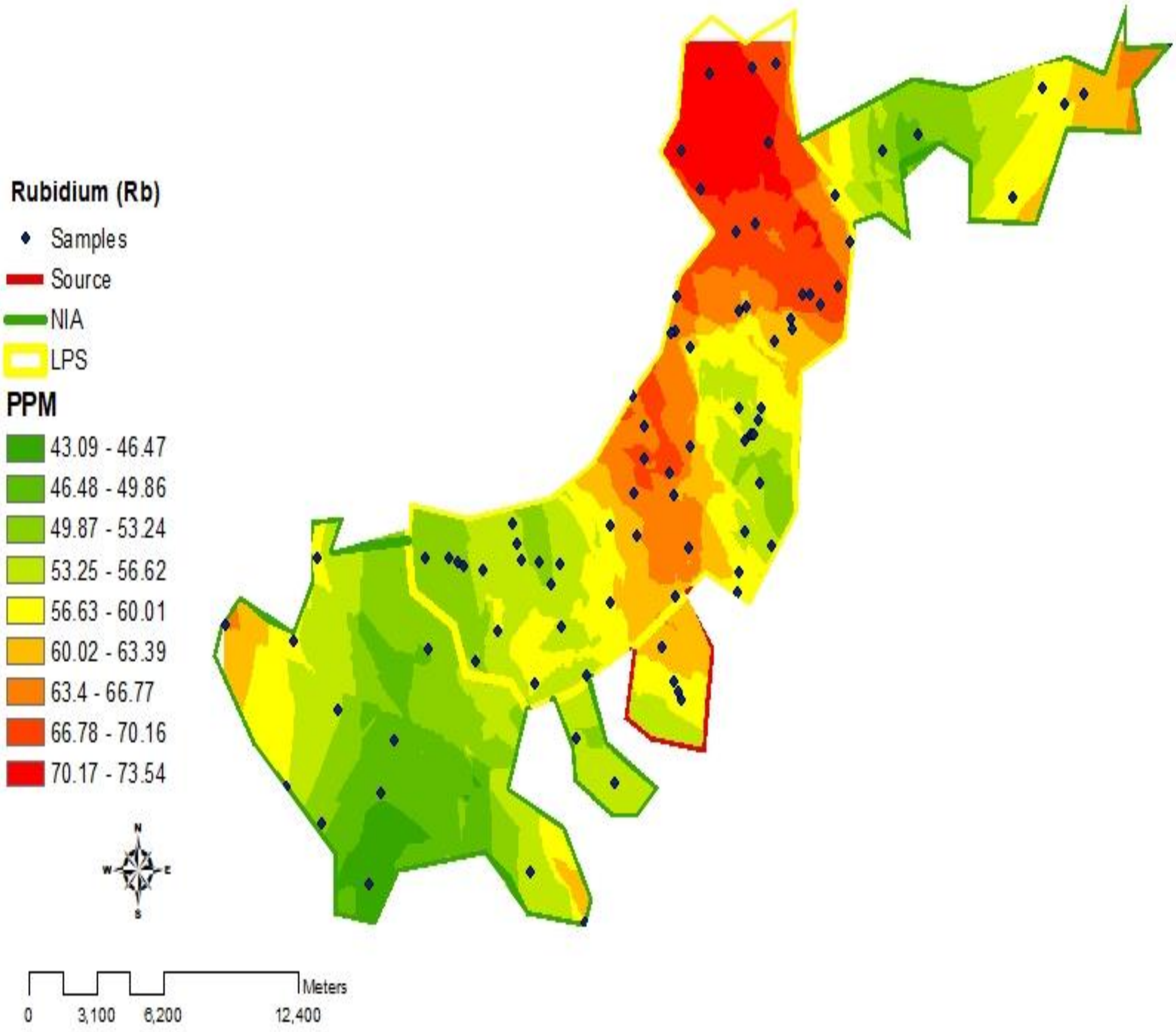

Calcium (Ca)
Samples
Source
NIA
LPS
PPM
2,806 - 11,270
11,280 - 19,730
19,740 - 28,190
28,200 - 36,650
36,660 - 45,120
45,130 - 53,580
53,590 - 62,040
62,050 - 70,500
70,510 - 78,970
N
W
E
S
0
3,100
6,200
12,400
Meters

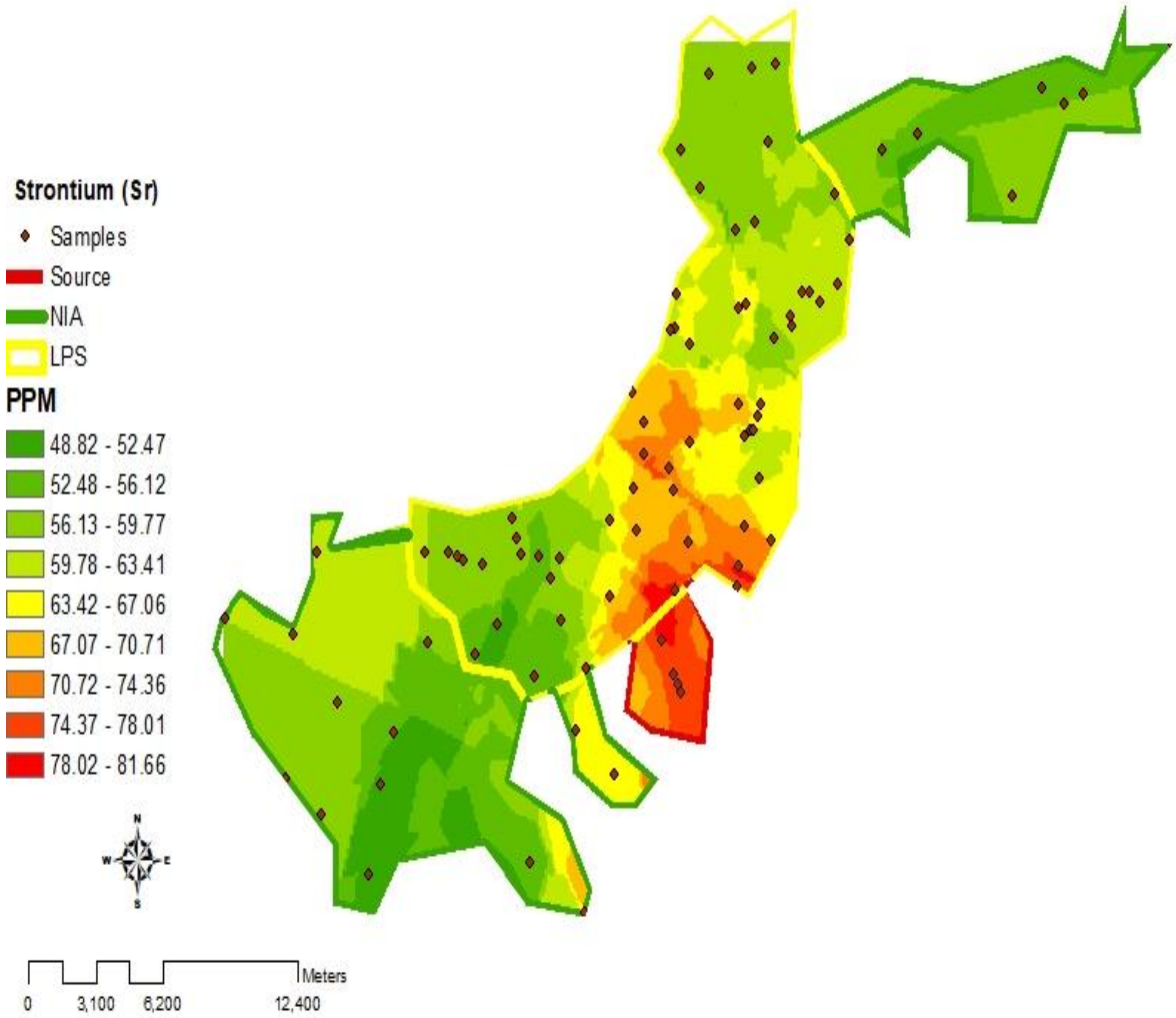

Arsenic (As)
Samples
Source
NIA
LPS
PPM
4.434 - 7.729
7.73 - 11.02
11.03 - 14.32
14.33 - 17.61
17.62 - 20.91
20.92 - 24.2
24.21 - 27.5
27.51 - 30.79
30.8 - 34.09
N
W
E
S
0
3,100
6,200
12,400
Meters


Manganese (Mn)
Samples
Source
NIA
LPS
PPM
231.7 - 270.7
270.8 - 309.7
309.8 - 348.7
348.8 - 387.6
387.7 - 426.6
426.7 - 465.6
465.7 - 504.5
504.6 - 543.5
543.6 - 582.5
N
W
E
S
0
3,100
6,200
12,400
Meters


Iron (Fe)
Samples
Source
NIA
LPS
PPM
14,441.6 - 15,542.6
15,542.7 - 16,643.6
16,643.7 - 17,744.6
17,744.7 - 18,845.6
18,845.7 - 19,946.6
19,946.7 - 21,047.6
21,047.7 - 22,148.6
22,148.7 - 23,249.6
23,249.7 - 24,350.6
N
W
E
S
Meters
0
3,100
6,200
12,400


Potassium (K)
Samples
Source
NIA
LPS
PPM
5,363.37 - 6,273.21
6,273.22 - 7,183.04
7,183.05 - 8,092.88
8,092.89 - 9,002.72
9,002.73 - 9,912.55
9,912.56 - 10,822.4
10,822.5 - 11,732.2
11,732.3 - 12,642.1
12,642.2 - 13,551.9
N
W
E
S
Meters
0
3,100
6,200
12,400


Rubidium (Rb)
Samples
Source
NIA
LPS
PPM
43.09 - 46.47
46.48 - 49.86
49.87 - 53.24
53.25 - 56.62
56.63 - 60.01
60.02 - 63.39
63.4 - 66.77
66.78 - 70.16
70.17 - 73.54
N
W
E
S
Meters
0
3,100
6,200
12,400


Strontium (Sr)
Samples
Source
NIA
LPS
PPM
48.82 - 52.47
52.48 - 56.12
56.13 - 59.77
59.78 - 63.41
63.42 - 67.06
67.07 - 70.71
70.72 - 74.36
74.37 - 78.01
78.02 - 81.66
N
W
E
S
Meters
0
3,100
6,200
12,400

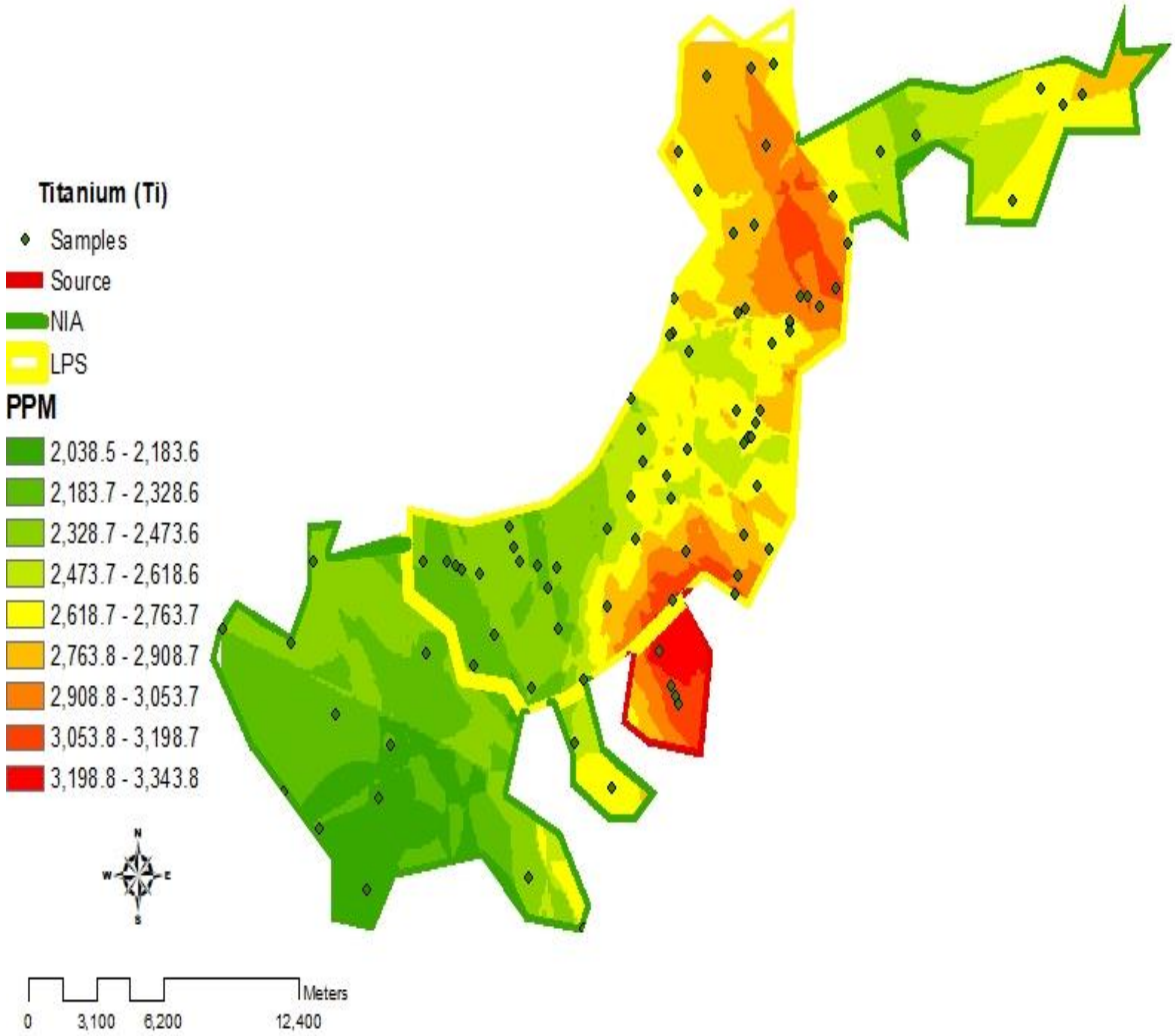


Fig 4.8f: Interpolation of geogenic associated trace metals

The above elements (see Fig 4.8f) all display what could be described as geogenic concentrations in the topsoil of the sampling region. There is little enrichment of these trace metal elements and the relationships between many of these elements, looking at both the Correlation Matrix (Appendix A, Table A3) and Principal Component Analysis (see section 4.3.6), indicate that they are inter-related and likely controlled by the parent material. Arsenic shows no correlation with any other elements and with a median measured concentration of 5.0ppm (Table 4.2) is in the region of the median concentration for European As in soils of 6.0ppm (Salminen, et al. 2005) indicating no significant anthropogenic input across the sampling range. Calcium also shows background levels of enrichment. Some areas show slightly elevated levels in concentration which can be seen on the interpolated map for Ca (Fig 4.8f) and is most likely due to samples being taken from areas that have recently undergone the agricultural process of topsoil so amend soil acidity.

We can see that some metal elements also show similar spatial distribution to pH (Fig 4.7). This tends to be an inverse relationship in which metal concentrations are higher in areas where pH is lower. This applies to metals such as Pb, Nb, Cu and Cr (Fig 4.7, Fig 4.8a, Fig 4.8b). This similarity in spatial distribution indicates a significant intra-elemental correlation (such as with Pb and Nb) between these metals and possible common source.

The spatial data for trace metal concentration and physiochemical properties (see Figs 4.7-4.8f), in conjunction with the Kriging interpolation, showed that some trace metal elements shared similar spatial patterns (Pb, Cu, Nb) and that some of these trace metals are those that have been found to display some enrichment (Pb, Zn, Nb). The organic matter content analysis for the topsoil (LoI and TOC) also display similar spatial patterns, with higher levels found near the meres and mosses protected areas (as with Pb, Cu and Nb). This is backed up when examining the results found in the Correlation Matrix and examining the relationships between the metals and the geochemical and physiochemical properties of the topsoil in the sampling area (see Appendix A, Table A2). This data shows strong relationships between Pb, Cu and Nb with the organic content analysis (LoI and TOC) as well as reasonably strong interaction with pH for some metals including Pb and Nb. This indicates that, perhaps, not only do these metals share a common source but that the level of organic matter and acidity of the soil may be a controlling factor.

4.3.6 Principal Component Analysis and Cluster Analysis

Multivariate analysis, such as Principal Component Analysis (PCA) and correlation matrices (CM), has proven to be an important analytical technique for source identification of trace metals (Han et al., 2006; Mico et al., 2006). PCA analysis was applied in this study so to assist in the source identification of, and establish relationships between, trace metals (Appendix B, Table B2). For the purposes of this study, a loading of above 0.5 was deemed important for the 4 components extracted as the square of each loading represents the proportion of variance (Abdi & Williams, 2010) with 0.5 representing 25% of the variance (Appendix B, Table B3). The results for the Kaiser-

Meyer-Olkin (KMO) Test for Sampling Adequacy (0.789) and the Bartlett's Test of Sphericity ($p < 0.001$) show that the dataset within this study is suitable for PCA analysis (see Appendix B, Table B1).

A Scree chart visually assesses components or factors explaining most of the variability in the data (Appendix B, Fig B1). The Scree chart shows that there are 4 components in which the Eigenvalue is greater than 1. Along with the Total Variance Explained (Appendix B, Table B2) we can see that these 4 factors/components make up 73.2% of the variability and with the first 2 components making up of over half (55.3%) of the total variance across all identified components. Therefore, it is vital to ascertain the nature of these two components.

Principal Component Analysis was undertaken to ascertain the factors influencing trace metal concentrations so to determine the origin of the trace metals. For the meres and mosses region, based on eigenvalues (eigenvalue > 1), four principal components (PCs) explained 73.2% of the total variance. The first principal component, PC1, explaining 33.3% of the total variance, was strongly and positively related to Rb, Ti, K, pH, V. Mn, Fe, Co and Sr. This component is also strongly negatively related to LoI, TOC, Nb, and Pb (Appendix 2, Table 3). The second principal component, PC2, explaining 21.5% of the total variance, showed highly positive factor loadings on Pb, Fe, Cu, Ms, Co, Sr, Zn and Cr. The third principal component (PC3), which explains 11.0% of the total variance, shows highly positive factor loadings for V and Ga and moderately positive loading for LoI, TOC, Nb, K and Cu. This component also shows moderately highly negative loadings for Cr. The fourth principal component (PC4), which explains 6.9% of the total variance, is made up of only 6 elements/factors, three of these have a moderate positive loading, Rb, Ti and Cr. There is one highly negative loading which is Ca and two moderate negative loading, Ph and Sr.

When examining the first 2 principal components (see Appendix B, Table B2 and Appendix B, Table B3), which account for 55.3% of the overall variation) we can see that in the first PC, PC1, we have a very strong negative relationship with those elements representing organic matter (LoI and TOC) with trace metals Pb, Nb and moderately so with Cu as well as a strong positive relation to pH. This may indicate that for these metals, pH and OM are influencing factors for their occurrence due to the potential impacts on the mobility of the trace metal elements that pH and OM can have (see Chapter 6). This is also emphasised by the fact that OM factors are the main correlations in this component (-0.848 and -0.839 for LoI and TOC respectively) which is not found in any other principal component. This, with the correlated relationships between these metals and similarities in spatial variation (see Appendix A, Table A3) indicates that Pb, Nb and Cu have a common source. This component indicates that the presence of these select trace metals are heavily influenced by organic matter and pH. Kabata-Pendias (2001) found that, despite the fact that the behaviour of V in soil has received little attention, Fe oxides hold a reasonable fraction of soil V. Norrish (1975) also found V to be linked with Mn. These relationships can be observed from the obtained data via the CM, which shows V, Mn and Fe to have a strong positive relationship (see Appendix A, Table A3) and in PC1 (Appendix B, Table B3) in which these 3 elements are observed. These elements are also significantly linked to OM factors and pH (Appendix A, Table A2). In the second PC, strong relationships between MS and the elements Pb Fe, Cu Co, Sr, Zn and Cr are observed. Magnetic Susceptibility, whilst not necessarily being an indicator of trace metal contamination, is a useful tool for indicating the anthropogenic input into the soil (Reshetnikov et al 2020). The elements found in this component are usually associated with anthropogenic factors, with elements such as Zn and Co showing some enrichment (see Fig 4.6). This may indicate that there may also be a contributing anthropogenic source/s for these elements. The assessment of anthropogenic impact in the environment can be evaluated according to trace metal contents of soils such as Pb, Cu and Zn. These trace metals are more likely to establish a metallic bond with ferrous material leading to an increase of soil magnetic susceptibility (El Baghdadi et al 2011). It is interesting to note that some trace metal elements are found with both a strong correlation in PC2 and a strong correlation in PC1 which indicates there may be both geogenic and separate anthropogenic source of elements in PC1 and mainly an enhanced anthropogenic input in PC2. The graphical representation of the three components is shown in Fig 4.9, where the relationships between these trace metals and physiochemical soil characteristics are observed. The results show that PCA leads to a reduction of the initial dataset to three components which explain 66.23% of the data variation. The first principal component (PC1) explains 33.73% of the total variance and loads heavily on the organic matter factors (LoI and TOC) as well as the metals Cu, Pb and Nb with also a strongly positive relationship with both pH and Rb. The second principal component (PC2), dominated by MS, Pb, Fe, Cu Co, Sr, Zn and Cr accounts for 21.53% of the total variance. The third principal component (PC3) is correlated very strongly with Ga, V and Cr. This PC accounts for 11.0% of the total variance.

We can see in Fig 4.9, for example, V and Pb separated by a large distance in the 3-D PCA loading plot, which indicates that the two trace metals, though both showing signs of enrichment, are inversely correlated and may have different sources or have separate controlling factors (such as acidity of the soil) when considering their presence and mobility with pH being adjacent to V suggesting a strongly positive relationship between the two.

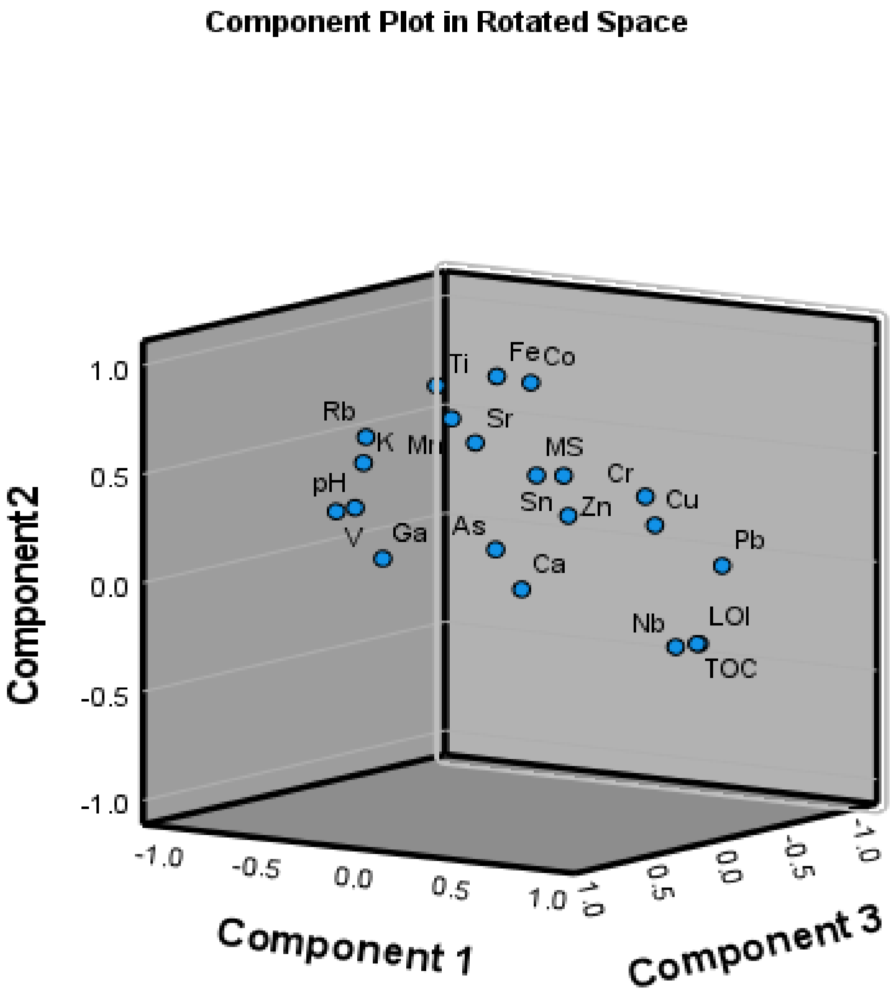


Fig 4.9: Principal components analysis loading plots (PC 1, 2 and 3) for 17 Trace Metal Elements and Physiochemical Factors of Meres and Mosses Topsoil Samples

Several studies that have shown that the association of trace metals and soil characteristics with the PCs can indicate if it is anthropogenic influence or geogenic and pedogenic characteristics (Han et al., 2006; Martin et al., 2006; Mico et al., 2006). Mn and Fe are considered geogenic in origin (Mico et al., 2006), whereas Cu and Pb are often thought as having an anthropogenic source (Han et al., 2006; Martin et al., 2006).

When trying to explain the content of the first two Principal Components, which make up over 55% of the total variance, we can say that PC1 could be defined as containing a geogenic component due to the strong correlation with Mn and Fe which appear to be controlled by parent rocks (Mico et al., 2006) but also may display a relationship in the pedogenic processes associated with their development (Gowd et al, 2009). However, due to the presence of Pb and Cu and also having OM and pH as contributing, or even controlling, factors, we can see other factors being significant apart from simply the parent material. This PC indicates that the metals are heavily influenced by both OM and soil pH as a result. The presence of Nb and V also indicate an anthropogenic input via steel production as both are by-products in that process, a process that is prevalent in the sampling area.

PC2 seems to be an anthropogenic component with factors such as MS and elements such as Cu and Pb heavily present. The controlling factors in this particular PC (Cu and MS) indicate an anthropogenic input contributing to the metals in the sampling area. PC2 also included Co, Cr and Zn, which all show some degree of enrichment (see Fig 4.6). This could also indicate that PC2 could be an anthropogenic component as the variabilities for these elements seem to be controlled by anthropogenic input. This was confirmed by the geostatistical analyses in the present study (Fig. 4.8a and Fig 4.8b), in which the spatial distribution of soil Zn, Co and Cr showed higher concentrations in urban and industrial areas as did Cu. This suggests anthropogenic input of Co, Zn and Cr in the study area and the enrichment therein may be due to a common source/s. Agro-chemicals and industrial/urban processes are major factors in the amounts and distribution of Co and Cr (Lu et al., 2012; Micó et al., 2006).

Cu and Pb feature in both PC1 and PC2. As explained above, high Cu values can come from Cu-based agrochemicals related to specific agronomic practices, whereas vehicle and industrial fumes and wastewater used to irrigate some agricultural areas can also be the source for the high Pb values found in some soils. In fact, some plots that contain high Pb contents are located near roads that have a high traffic density due to being tourist sites (meres and mosses nature reserves) and are, or have been, potentially affected by pollutants associated with traffic density.

Implementing PCA, Facchinelli et al. (2001) also drew conclusions about the trace metals in their study in which Cu and Zn were found to be associated with agricultural practices and that Pb was derived from vehicle emissions, and all three metals were related with human activities. On the other hand, Cr and Co were associated with and controlled by parent rocks in the main (Facchinelli et al, 2011). In this study, it seems reasonable to conclude that Cu and Pb are part of an anthropogenic component (PC1 and 2), whereas the elements in PC2 appear to be associated with parent rocks, albeit with some form of anthropogenic input. Some of these elements do show enrichment in this PC which is why MS can be seen to be one of the controlling factors of the PC.

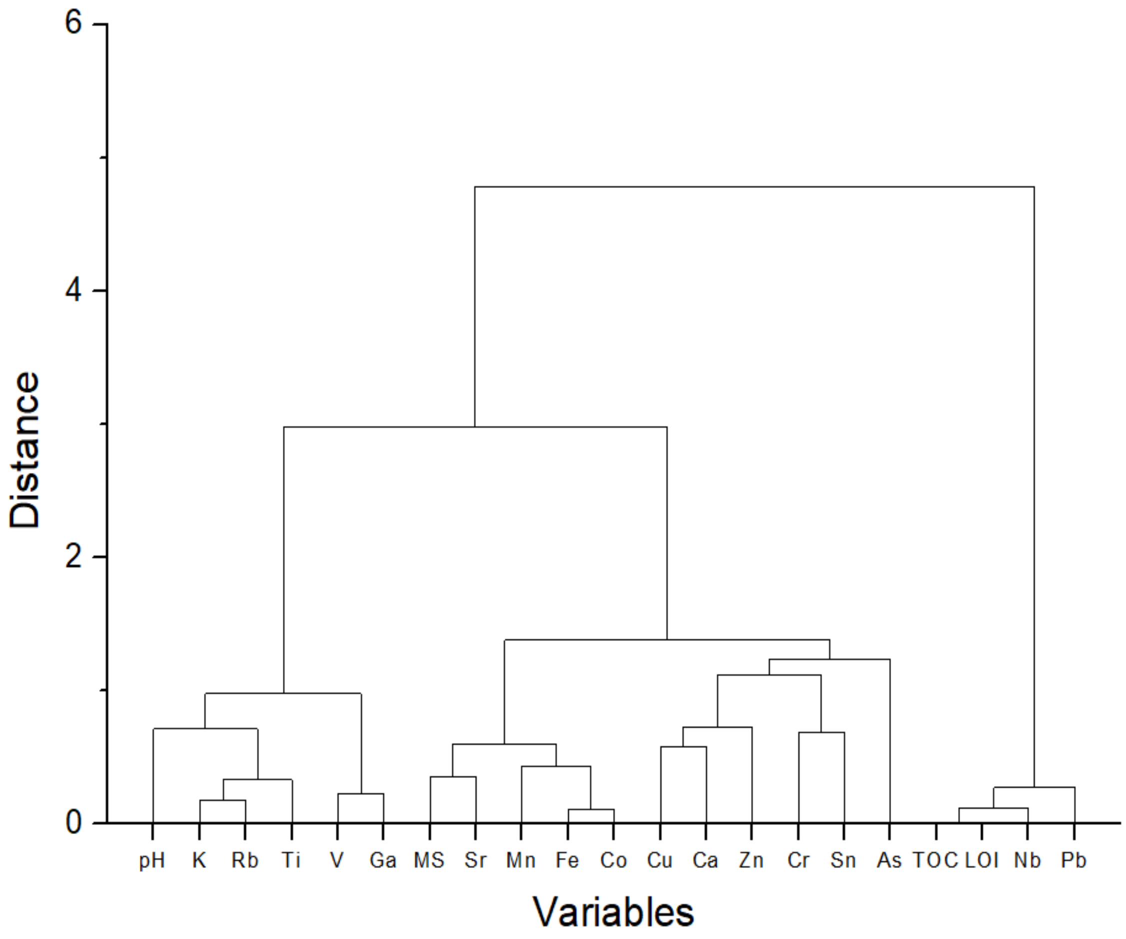


Fig 4.10: Cluster Analysis Dendrogram with Ward's Method of Hierarchal Cluster Analysis and Correlation Distance for 17 Trace Metal Elements, Organic matter, pH and MS in the Meres and Mosses Topsoil Samples.

Results for the Cluster Analysis are shown in Fig 4.10 as a dendrogram. Huang at al (2018) state that "The dendrogram is a visual representation of the compound correlation data". The trace metal elements, organic matter, pH and MS are displayed on the X-Axis of the Dendrogram (see Fig 4.10) and are known as leaf nodes. The Y-axis is the distance and represents the distance between compounds or clusters of compounds.

CA and Correlation distances for similarities in the variables were calculated (Tokalıoğlu and Kartal, 2006; Lu et al., 2010). The hierarchical clustering by applying Ward's method was then performed on the variables of the dataset. Fig 4.10 displays four clusters: (1) pH–K–Rb–Ti-V-Ga; (2) Ms–Sr-Mn-Fe-Co; (3) Cu-Ca-Zn-Cr-Sn-As; (4) TOC-LoI=Nb-Pb. Which is in close agreement with the PCA results.

4.3.7 Trace Metal Source Identification

Principal Component Analysis, Spatial Variability (GIS Mapping), Cluster Analysis and Correlation Matrices were used in aiding in source identification of some of the important metals identified in this study. it can be said that It is likely that agricultural activities (sewage irrigation, chemical pesticide usage, etc.) in the agricultural areas of the region and industrial activities in and around the urban and industrial areas have caused metal accumulation in the top soil. This is evident when examining the spatial variability of some of the most prominently studied trace metals such as Pb, Cu, Zn, Co, Cr and Sn. These elements show somewhat similar areas of high concentration when examined spatially and show inter-metal relationships when performing statistical analysis. Three main factors can be identified according to correlation coefficient analysis, PCA, CA and the spatial distribution characteristics of metals in the topsoil of the meres and mosses region. The following metals are heavily present (>+/-.500) in PC1: Rb, Ti, K, V. Mn, Fe, Co, Sr, Nb and Pb. These metal elements appear to be heavily influenced by organic content and soil pH, as indicated by their high presence in the PC, and have mixed sources, most likely from natural and geogenic aspects as well as anthropogenic inputs from traffic, fossil fuel combustion and weathering/disturbance of topsoil materials. V is a good example of the source characteristics of these metals. V mainly originates from natural sources and is spread out in distribution in the earth's crust at an average concentration of approximately 100ppm (Byerrum, 1991) but can also be present as a by-product of industrial activities, is heavily used in construction and the process of petroleum crude oil refinement (van Zinderen Bakker and Jaworski, 1980). When compared to other trace metal elements, vanadium is found to be mobile in soils that are circumneutral and has decreasing mobility in acid soils (Van Zinderen Bakker and Jaworski 1980). Using Co as another example, we can see that Co has a high presence in both PC1 and PC2 (Appendix B, Table B3) indicating mixed sources. Cobalt occurs naturally in soils via the natural weathering of minerals and the breakdown and degradation of organic matter (Kim et al, 2006). When examining the spatial variability (Fig 4.8b) we can see, however, that high levels of concentration are found in the industrial 'source' areas. This indicates an anthropogenic input such as industrial processes and construction. The presence of Co in both PC1 and PC2 indicates an anthropogenic influence on the concentrations of this metal element. This is similar for Mn which is known to be heavily influenced by organic matter and pH (Shuman and Anderson, 1978).

The spread of Cobalt (Co) on the Frequency Distribution chart (see Fig 4.11) shows that the levels of concentration are not simply due to outlier results and that many of the samples exceed the Netherlands target value of 9ppm (see Table 4.1).

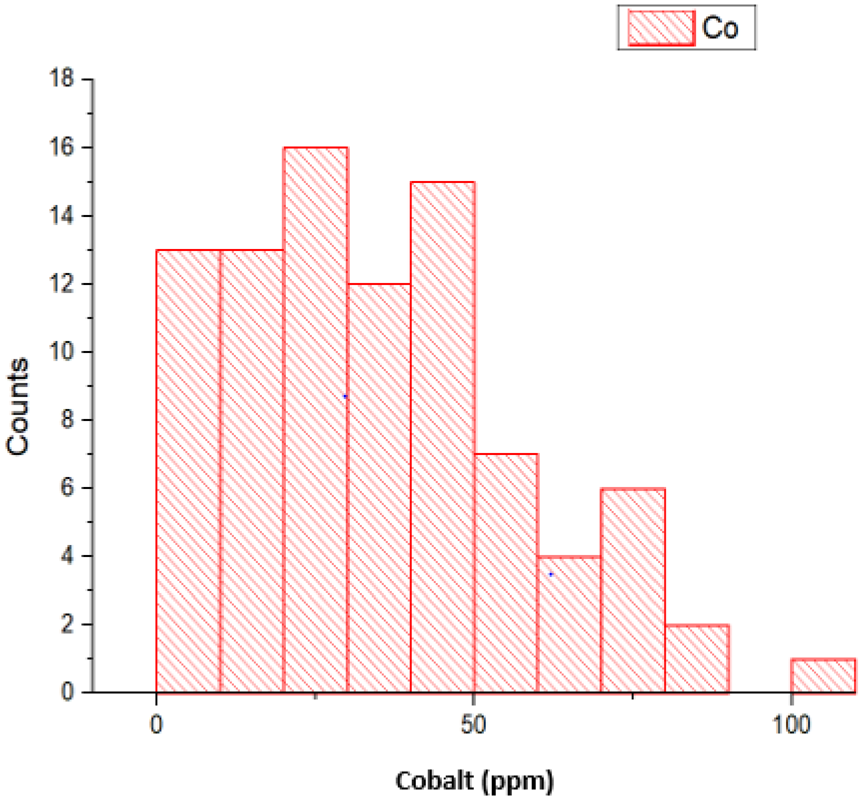


Fig 4.11: Count of Sites/Samples for Co Results (ppm) in Meres and Mosses Topsoil Samples Across All 89 Sample Sites.

When examining the inter-element relationships of these elements in the correlation matrix we can see that Pb, Nb Zn, Cu, Ca and Cr have significantly positive correlation in the correlation coefficient analysis. Pb, Nb and Cu belong to Factor 1 in PCA with Pb and Nb being classified together in CA. This indicates common influencing factors, most likely associated with organic content and the role of pH in the mobility of these metals (the major drivers of PC1). This will be explored in further detail in Chapter 6 of this thesis. Many of the metals in PC1 show a high correlation with pH and organic matter and similar spatial patterns (see Fig 4.7, Fig 4.8a). Cu is also found in PC2 along with Ca, Cr and Zn in the PCA and are classified together in the CA. This indicates a common anthropogenic source and, indeed, some of these elements show signs of enrichment and exceed target values for concentration (see Fig 4.6 and Table 4.1). Steel fabrication production is also present in the region and would explain increased levels of Nb, Cr, Mn, Sn and V. These metals show varying levels of inter-element correlation in the correlation matrix. Cu, Pb and Zn are metals associated with traffic and transport (Chen et al, 2014). Corrosion of motor vehicles and their parts and the degradation of vehicle tyres are the main anthropogenic sources of Zn in urban environments (Han et al., 2006; Lu et al., 2010). Zinc alloy is regularly used in the manufacture and construction of motor vehicles. Zinc compounds are also used as dispersant enhancers for lubricating oils (De Miguel l., 1997). Tyre thread can also contain up to 4.3% Zn, which is added to tyres during the vulcanising process (Chen et al., 2012). Under EU law, leaded petrol was banned in the UK in the year 1999. Despite this ban, due its persistence, Pb sis still found in significant quantities in the soil profile (Yang et al., 2011). Pb may also enter the soil as a result of Pb in paints and coatings present in industrial processes due to the effects of run-off from rain on the buildings and urban facilities (Chen et al., 2012). Military processes, such as those undertaken on Baggy Moor during World War 2 (ARG, 2016), may also have resulted in long-lasting Pb contamination of the soil. Copper alloy is often used in manufacture of various heavy goods due to its corrosion resistance and strength (Chen et al., 2012). Degradation of these mechanical parts as time passes, especially in vehicles, results in Cu being released to the environment via a number of pathways (Li et al., 2004). These elements showed varying levels of enrichment (from minimal for Cu to high for Pb). They also show similar spatial patterns with increased levels in industrial areas and those associated with the meres and mosses. They feature prominently in PC2 and they are significantly correlated to each other in the CM and also show significant relationship with pH and/or organic matter in the case of Cu and Pb.

The concentration of Cr in the topsoil of the region shows moderate enrichment when using Fe as the reference element showing that Cr concentration in the topsoil was mainly caused by human activities. Elevated concentrations of Cr were reported in the topsoil collected around industrial areas. Cr is extensively used in both steel production and in automobile parts and is highly correlated with Cu, Pb and Co. When examining the concentration (EF) and spatial distribution, it can be concluded that Cr in the topsoil of the region mainly originates from industrial processes and traffic emissions/fuel combustion.

V is also regularly used in the manufacture of steel. It shows a similar spatial pattern to other anthropogenic associated metals but inversely so. It shows a significant positive correlation with pH, significant negative correlation with organic matter and It shows a moderate level of enrichment. It is heavily present in PC 1 in which it displays similar relationships to pH and organic matter. This strong relationship with pH is also shown in the PCA loading plots. Studies have shown that soil properties control the potential for bioavailability of V (Agnieszka & Barbara 2012; Reijonen et al, 2016). Soil organic matter content has been found to reduce V(+V) to V(+IV) and lowered the bioavailability of V via sorption (Agnieszka & Barbara, 2012), whereas a high pH, favoured the V(+V) increased the bioavailability of V (Reijonen et al, 2016). Due to the significant industrial heritage that is contained within the Meres and Mosses of the Marches, as well as use of agricultural machinery and traffic from tourism (see section 4.1.2), there is both a history of anthropogenic input as well as contemporary pressures that are anthropogenic in origin. This would explain the presence (enriched) of V at the study site as well as the enriched and or elevated levels for some of the other trace elements in this study.

4.4 Conclusion

The present study set out to ascertain the trace metal contents and their sources in the agricultural topsoil of the Meres and Mosses of Cheshire and Shropshire by means of a screening study implementing FPXRF analysis for the determination of trace metal concentration, with a view of examining adherence to prominent soil quality guidelines, with additional analyses for physiochemical properties. From examining the results of the screening study, and consequent multivariate statistical analyses performed in this study, it is observed that the Meres and Mosses of Cheshire and Shropshire do have certain levels of trace metal enrichment and that this enrichment is found both from anthropogenic and geogenic sources. The descriptive results clearly show that some elements (such as Pb, Co, V, Cr and Zn) exceed various targets/limits as set from varying agencies, including those of the Dutch Target/Background and Intervention Values (2000) when adapted for the median clay and organic matter content of the soil analysed as ascertained for the region in this study. The Enrichment Factor Analysis (EF) indicates that some trace metals are enriched in the sampling area which indicates there may well be an anthropogenic input of certain trace metals. This EF also found these same elements (Pb, Co, V, Cr and Zn) to display various levels of enrichment, ranging from minimum to moderate enrichment for Zn, V, Cr, Co, Rb, Sn and Ga to moderate to significant enrichment for Pb, As and Nb. However, these figures were determined using global averages for the background and reference concentrations and may vary when using localised data.

The correlation matrix shows significant relationships between various trace metals and geochemical/physiochemical factors such as pH and OM. The multivariate analyses all indicate that these are inter-related correlations between the trace metals and also geochemical/physiochemical factors (pH and OM) and has separated them into Principal Components. Spatial mapping indicates that the anthropogenic sources seem to be mainly based around the industrial area and the Meres and Mosses nature reserves themselves. This could be due to increased traffic throughput and anthropogenic activity as well as the natural tendency for meres to act as sinks due to the surrounding peat rich soils with high levels of OM. The ability of trace metals to accumulate in peatland being well established (Lidman et al, 2014). Results show that organic matter content (OM) and pH of the topsoil play a role in these areas with very similar spatial patterns and highly positive correlations to these factors and metals such as Pb (a highly cited anthropogenic pollutant) and Nb. The presence of Nb can be explained by the fact that it is often a by-product of the steel fabrication manufacturing process. A process that is prevalent in the region. Nb shows some enrichment and significant relationships with Pb, Cu as well as organic matter. Further research would help elucidate the reasons as to why Nb is both present in this quantity (it is considered a rare earth element) and shows these aforementioned relationships. It is also worth considering that Pb, Zn and Cu are also known to be present in agricultural applications, such as sewage sludge, and this may account for their highly interlinked positive correlations and similar spatial patterns. Many of the enriched trace metal elements in the study are also associated with steel fabrication production and industrial processes that are local and identified in the sampling regime. This is worth further investigation when considering the source of these trace metals. Overall, this study has aided in the elucidation of the occurrence and relationships of and between trace metals and physiochemical characteristics in agricultural soils within a meres and mosses habitat. These relationships can help begin to understand the dynamics of these trace metal elements in these habitat types with a view of assessing ecological risk and aid in establishing and implementation of appropriate mitigation measures. The findings of this study concur with previous studies in similar habitat types that found that wetland soils that have previously been drained and converted to agriculturally managed farmland or developed for urbanisation could potentially face alterations in trace metal cycling due to the changes in the soil properties with the potential to impact soil pH, the occurrence and dynamics of trace metals in the soil and the relationships with physiochemical properties therein (Bai et al, 2019; Reddy et al, 2010; Wang & Xu, 2015).

To further elucidate the relationships considered and revealed within this chapter, Chapter 5 will examine alternative analytical methodology when determining trace metal content in topsoil and how they impact adherence to differing soil quality guidelines and their potential for bioavailability. It is important to acknowledge that the data obtained via FPXRF within this Chapter did not implement a matrix matched multi-element certified reference material as is implemented on the ICP-MS methodology in this thesis (see Chapters 3 and 5) and would benefit from a robust validation methodology. Chapter 6 will examine the annual trace metal mobility in the topsoil of the region under varying pH and organic matter content influences by means of a mesocosm study and consequent analysis to determine potential for bioavailability and ecological risk.

Chapter 5:

A Comparison Study Between Portable X-ray Fluorescence Spectrometry and Inductively Coupled Plasma-Mass Spectrometry to Assess Environmental Quality in the Topsoil of a Meres and Mosses Habitat.

Abstract

Two methodologies to determine the concentration of trace metals in the topsoil of a meres and mosses habitat were assessed so to determine statistical correlations and differences between the two. Field Portable X-Ray Fluorescence (FPXRF) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) with acid digestion are both established analytical techniques in determining trace metal concentration in a soil matrix but have different methodologies with varying benefits and drawbacks when implemented depending on the situation and analytical requirement. FPXRF is rapid, non-destructive and capable of screening areas both in the field and in the laboratory which produces a total concentration result but suffers from lack of sensitivity with limits of detection in the ppm range. ICP-MS with reverse aqua regia has the benefit of being a highly sensitive technique with low limits of detection that are in the ppb range with the pseudo-total result also providing an indication of bioavailability of the in-situ trace metals being analysed. This methodology is more time consuming than FPXRF with significantly longer time for sample preparation, which is also destructive to the sample meaning that re-use of soil sample is not viable. Following analysis of eight trace metals (Cu, Pb, Zn, Co, V, Sn, As, Cr) via FPXRF and ICP-MS with reverse aqua regia digestion, Pearson Correlation and Coefficient of Determination was undertaken on the raw dataset to ascertain if the ICP-MS methodology was indicative of the total result obtained by FPXRF. A linear regression model and a Wilcoxon Signed Ranks Test on the obtained data was then undertaken so to determine relationships and differences between the two datasets. The statistical analyses found significant relationships between the two analytical procedures for Pb, Zn, Cu, Co and Cr when using Pearson Correlation with Cu, Zn and Pb showing weak to moderate coefficient of determination with all three having an R^2 of 0.3 or greater following Linear Regression thus explaining over 30% of the variance From the results of the Wilcoxon Signed Ranks Test, a significant difference ($p<.001$) between the analytical methodologies for V, Cr, Co, Zn, As, Sn and Pb was observed indicating a disparity between the obtained results for total concentration and pseudo-total for these trace metal elements. Cu was the exception to this, however this may be explained by previously reported studies showing FPXRF to underestimate Cu concentrations. The obtained results from the ICP-MS

methodology were collated with the previously obtained FPXRF data and compared to the relevant and most widely cited soil quality guidelines to assess the environmental quality of the Meres and Mosses of Cheshire and Shropshire. The study found that Pb, Zn and Co exceeded 'Target' values of the Dutch Target and Intervention Values when implementing a total result via FPXRF with Pb exceeding the Finnish 'Threshold Value' when implementing a pseudo-total when using the ICP-MS methodology, thus highlighting the difference in outcome when implementing differing methodologies for determination of trace metal concentration.

5.1 Introduction

5.1.1 Background

When examining analytical procedures suitable for determination of the concentration of trace metal elements in soils, both XRF and ICP-MS are often used to determine the concentration levels of these trace metals for a particular soil sample (Moor et al, 2001; Kilbride et al, 2006) with each analytical procedure providing both advantages and disadvantages depending on the analytical requirements and the known constituents of the sample site. Environmental standards, limits and targets are frequently determined based on acid digest (aqua regia or reverse aqua regia) trace metal extractable metal concentrations, often known as pseudo-total, instead of a total trace metal concentration that would be determined by laboratory or field-based X-ray fluorescence (XRF) (Kilbride et al, 2006). Acid digestible methods using aqua regia/reverse aqua regia do not perform a complete digestion of the soil sample because of components in the soil matrix that are difficult to digest, such as those with poorly soluble components in acid (Voica et al, 2012), and, as such, cannot be detected within the analytical result (Voica et al, 2012) whereas when using an analytical method which adopts an XRF detection for soil samples, the analytical results obtained are representative of the total matrix of the soil sample and thus represent a total analysis (Potts et al., 1995). The disadvantage of this acid digestion procedure is that sample preparation times along with sample run times on the ICP-MS instrumentation can be time consuming and expensive when compared to X-ray fluorescence (XRF) or field portable X-ray fluorescence (FPXRF) (Schneider et al., 1999). Financial aspects may play a role (instrumentation costs are expensive for ICP-MS but relatively inexpensive for FPXRF) as well as facilitation issues which is especially the case for acid digestion which requires increased health and safety measures as well as laboratory space for digestion techniques and for the ICP-MS itself. Multi-element analysis is swift and inexpensive with FPXRF (Anderson et al., 1998; Kuharic and Cole, 1995) and, as a result, suitable for a trace metal screening exercise for a given area (see Chapter 4). Acid digestion with ICP-MS technology does have certain challenges which may require to be overcome due to spectral interference and sample preparation variations/inconsistencies (see section 5.2.3 and Chapter 3 of this thesis). Total trace metal concentrations and those obtained by aqua regia extraction (pseudo-total) have been studied and implemented in the Geochemical Atlas of Europe (Salminen et al. 2005; Tarvainen et al, 2009) and other geochemical mapping studies (Reimann et al, 2003; Tarvainen et al, 2009). This Chapter explores the relationship between these analytical procedures so as to determine the viability of each in respect to analysing topsoil samples for trace metal concentrations in a meres and mosses wetland habitat. More specifically, this study will examine the results obtained from the trace metal screening exercise via FPXRF in Chapter 4 of this thesis and, using analytical procedures determined in the 'Method Development' Chapter of this thesis (see Chapter 3), determine pseudo-total trace metal concentrations for the topsoil of the 'Nature Improvement Area' and the land covered by the 'Landscape Partnership Scheme' in the Meres and Mosses of the Marches in Cheshire and Shropshire in the United Kingdom (see Chapter 2 and Chapter 4).

5.1.2 Trace Metal Concentration Determination via Field Portable X-Ray Florescence Spectroscopy (FPXRF)

When examining trace metal concentration in topsoil, X-ray fluorescence spectrometry (XRF) is a long-established analytical technique for obtaining high-resolution trace metal data (Kilbride et al, 2006). The Field Portable XRF is a versatile analytical tool that can be implemented in outdoor settings or controlled laboratory conditions. The FPXRF can be used directly in contact with the soil surface of interest when in the field or with a clear sampling bag returned to the laboratory for multiple analyses (Kilbride et al, 2006) with research showing that FPXRF spectrometry has provided acceptable results for multi-element analysis (Al Maliki et al, 2017).

Due to short analysis time and non-destructive methodology, FPXRF is a good analytical technique when examining trace metals in environmentally sensitive regions. Because of these factors, FPXRF is useful for screening exercises that can be undertaken in both a laboratory and field environment. FPXRF is not as sensitive as other laboratory methods, however (see section 5.2.3). Advantages of FPXRF analysis include low preparation time with no waste due to direct, non-destructive analysis which is cost-effective and environmentally friendly and rapid turnaround of samples due to short analysis time and the portable nature of the instrumentation (Kobylarz et al, 2023). Field Portable X-ray fluorescence (FPXRF) instruments also provide an inexpensive screening analytical procedure for measuring trace metal contamination in soils giving it a potential financial benefit (Kilbride et al, 2008). Some acid digestion methods, such as those with nitric acid or aqua regia (see section 5.1.3), do not constitute a complete digestion because of the 'stubbornness' of the least acid-soluble components (Voica et al, 2012). Metal silicates, for example, are not readily digested and, therefore, impacts the result whereas results obtained by FPXRF are derived from all the materials within the matrix of a sample (Kilbride et al, 2006; Potts et al, 1995). This result from XRF methodology represents a total result when considering trace metal analysis (Potts et al., 1995). To obtain a total trace metal concentration result by alternative methodologies from XRF can lead to very concentrated strong acids, such as HF, being used in some acid digestion techniques so to obtain a total trace metal concentration result which are of increased risk to the analyst due to the handling of harsh reagents and, as a result, time consuming and expensive when compared to the total trace metal concentration result obtained via field portable X-ray fluorescence (Schneider et al., 1999).

The United States Environmental Protection Agency have produced a test method (SW-846 Test Method 6200) for FPXRF for the determination of elemental concentrations in soil and sediment (USEPA, 2007). This test method provides guidelines for detection limits when using an FPXRF (although varying instrument models may differ). Burlakovs, et al (2015) also studied the lower detection limits of liquid samples (soil digests) for ICP-MS compared to solid samples (homogenised soil samples) for FPXRF for 'key metallic elements' (see Table 5.1). This comparison of differing detection limits for differing matrices is important as ICP-MS, for example, requires the release of trace metal elements of interest from the solid sample matrix ($^{e.g.}$ soil) via acid digestion resulting in a transfer to a liquid analytical solution (Burlakovs et al, 2015). Examination of these detection limits may aid in the determination of suitability of an analytical methodology for a particular trace metal element being studied in a given sample type, study area composition and in application to 'real-world' situations (Sturgeon, 2000).

Trace Metal Element	Lower Limit of Detection in Soil and Sediments for FPXRF (USEPA, 2007) (mg/kg)	Lower Limits of Detection in Homogenised Soil for FPXRF (Burlakovs et al, 2015) (mg/kg)	Lower Limits of Detection in Soil Acid Digest for ICP-MS (Burlakovs et al, 2015) * (µg/L)
Copper	0.05	0.006	0.0002
Lead	0.02	0.003	0.00004
Zinc	0.05	0.004	0.0003
Chromium	0.15	0.008	0.0002
Cobalt	0.06	-	-
Arsenic	0.04	-	-
Tin	0.06	-	-

- = no data available in source material

Note: * Detection limits for ICP-MS is given for liquid samples, but for FPXRF is for solid samples.

Table 5.1: Lower limits of detection for analytes of interest in environmental applications for FPXRF and ICP-MS (USEPA, 2007; Burlakovs et al, 2015)

Whilst technological advancements have improved these detection limits since the production of this test method, as shown by Burlakovs et al (2015) and displayed in Table 5.1, it does demonstrate the higher limits of detection values when compared to alternative methodologies for determining trace metal concentrations in a soil matrix and a soil solution matrix such as the low limits of ICP-MS instrumentation (see section 5.2.3). Indeed, examination of these limits of detection may indicate that, for certain trace metal elements, FPXRF methodology is at a disadvantage, especially in areas in which soils are considered unpolluted or have a lack of anthropogenic influence. This analytical method is widely adopted in investigative studies of this type, however, due to its portability, rapid analysis providing a ‘total’ result and cost-effective with a non-destructive procedure (Schneider et al, 2016). Previous studies have examined the applicability of FPXRF in an investigative setting for trace metal content in agricultural soils and have found that, with improvements in technology and appropriate preparation of samples ex-situ (as is the case in this study), a high degree of linearity and similar outcomes for spatial distribution analysis is obtained (when compared to ICP-MS) indicating that FPXRF is suitable for screening of agricultural soils (Hu et al, 2014; Piorek, 2021).

A consideration with FPXRF analysis is that many environmental trace metal concentration targets and limits are based around acid digestion. For example, the Finnish government sets out threshold and guide values (see section 5.1.5) from the Finnish legislation for contaminated soil (Decree 214/2007) based on aqua regia extractable analytical techniques which is the recommended analytical method by the Decree (Tarvainen et al, 2009). This means that regulatory requirements in soil quality assessment often require laboratory analysis with increased controls than would be found in the field. As such, it can be said that the FPXRF is a powerful analytical tool which can be useful in screening sites for trace metal contaminants with both advantages and limitations depending on the required application.

5.1.3 Trace Metal Concentration Determination via Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Application of Inductively coupled plasma mass spectrometry (ICP-MS) for the determination of trace metals concentrations in soil is now an established analytical technique and is suitable when analysing from a multi-element approach (Lyon et al, 1988). The major benefit of ICP-MS is that it can detect trace metals at concentrations in the parts per billion range (ppb) unlike many other forms of spectroscopic analysis such as X-Ray Fluorescence Spectroscopy (XRF) and Graphite Furnace Atomic Absorption (GFAA). A high degree of atomisation in the argon plasma results in being able to achieve very low detection limits for trace metals in environmental medium (Dean, 2005). The ICP-MS method is based on measuring mass-to-charge (m/z) ratios. Advantages of ICP-MS include high sample throughput whilst having a multi-element capability (Vanhaecke and Kollensperger, 2003).

Depending on the acid digestion technique during the sample preparation phase, what is known as a pseudo-total concentration is achieved due to the limitations of the acid/s being used. Aqua regia/Reverse aqua regia, a mixture of HCl and HNO_3, does not digest fully the compounds in a soil sample due to the mineral composition. The term 'pseudo-total' reflects this partial result for trace metal concentration in topsoil. The pseudo-total is a good estimate of the bioavailable fraction of trace metals (Andersen and Kisser, 2004; Al-Hwaiti et al, 2010). Bioavailable, in this context refers to the relationship between the amounts of trace metals in the soil system and how much is absorbed by plants and potentially available to enter the food chain (Xu et al, 2022). This bioavailability of these trace metals can be further elucidated via sequential extraction methodologies which, in turn, can aid in determining the 'risk' to a local environment (see Chapter 6). A further limitation in the ICP-MS analytical procedure is caused by spectroscopic interferences which are caused by atomic or molecular ions that have the equivalent mass-to-charge ratio as the trace metals being analysed (May and Wiedmeyer, 1998). When these molecular ions are present, there is overlap for the preferred isotopes of several analytes. These molecular ions can be present due to the presence of certain elements which may be found in the sample solution matrix (May and Wiedmeyer, 1998). To aid in the suppression and remediation of this issue, Collision Cell Technology (CCT) was applied for all samples and at relevant parts of the Method Development/Improvement phase (see Chapter 3). CCT mode uses a non-reactive gas to eliminate interference. CCT does this via a process known as kinetic energy discrimination (KED) which diminishes polyatomic interferences based on size and cross-sectional area (Wilschefski & Baxter, 2019). The table below (Table 5.2) shows Polyatomic interferences according to DIN EN ISO 17294-2 (2005).

Trace Metal Element	**Isotope**	**Interferences by poly-atomic ions**
Lead	206Pb, 207Pb, 208Pb	190Pt16O+,191Ir16O+, 192Pt16O+
Chromium	52Cr, 53Cr	40Ar12C, 35Cl16O1H 37Cl16O, 36Ar16O1H
Cobalt	59Co	43Ca16O+, 42Ca16O1H+, 24Mg35Cl+, 36Ar23Na+, 40Ar18O1H+, 40Ar19F+
Copper	63Cu, 65Cu	40Ar23Na, 31P16O2, 26Mg37Cl 49Ti16O, 23Na40Ca, 32S16O21H
Nickel	58Ni, 60Ni	40Ca18O, 44Ca14N, 23Na35Cl, 24Mg34S 44Ca16O, 43Ca16O1H, 23Na37Cl
Zinc	67Zn	35Cl16O2+, 33S34S+, 34S16O21H+, 32S16O18O1H+, 33S34S+, 34S16O17O+, 33S16O18O+, 32S17O18O+, 33S17O2+, 35Cl16O2+
Vanadium	51V	34S16O1H+, 35Cl16O+, 38Ar13C+, 36Ar15N+, 36Ar14N1H+,
Tin	118Sn 120Sn	102Ru16O+, 102Pd16O+, 104Ru16O+, 104Pd16O+
Arsenic	75As	40Ar35Cl+, 59Co16O+, 36Ar38Ar1H+, 38Ar37Cl+, 36Ar39K, 43Ca16O2, 23Na12C40Ar, 12C31P16O2+

Table 5.2: Table of elements analysed, some of their stable isotopes and their common polyatomic spectral interferences.

An Agilent 7900 ICP-MS, with collision cell technology, was used for analysis of trace metals from the topsoil of the Meres and Mosses region of Cheshire and Shropshire during the analytical stage of this thesis when implementing this acid digestion methodology (see section 5.2.3).

5.1.4 Reverse aqua regia Digestion

When determining the trace metal concentration in soil, reverse aqua regia digestion has been found to be beneficial due to reductions in costly reagent handling of harsh acids with easy-to-follow and safer operation procedures (Lu et al, 2007). Reverse aqua regia has been shown to have superior recovery of trace metals when compared to conventional aqua regia and produces reproducible outcomes (Szymczycha-Madeja et al, 2021; Lu et al, 2007). Digestion with reverse aqua regia does not dissolve silicates but is harsh enough to extract the trace metals which are not bound to silicate phases within the soil thus providing a pseudo-total trace metal concentration (Quevauviller, 1998), distinct from a total concentration result obtained from FPXRF and other methodologies and also provides an indication of the bioavailability of the trace metals being analysed (Santoro et al, 2017; Andersen and Kisser, 2004). Having an increased ratio of HNO_3 in the mix (as is the case for reverse aqua regia) is preferred because of its oxidising properties. Adding HNO_3 converts metal ions into soluble nitrate salts (Kumar, 2015). Implementation of reverse aqua regia has also shown there to be less chance of digest loss due to the formation of volatile compounds when compared to alternative methods of sample digestion (Siaka et al, 1998). Method development was undertaken in Chapter 3 of this thesis so to determine the optimal results for multiple element recovery using microwave assisted digestion with reverse aqua regia when using ICP-MS instrumentation. The results of this method development step were implemented in the analytical procedure in this chapter (see section 5.2.3) so to determine the pseudo-total trace metal concentration in the topsoil of the Meres and Mosses region of Cheshire and Shropshire to help ascertain if this methodology was suitable for habitats of this nature compared to the long-established but less sensitive FPXRF methodology and to determine if the pseudo-total result obtained from the reverse aqua regia acid digestion methodology with ICP-MS was representative of the total result obtained from FPXRF analysis on the same topsoil samples.

5.1.5 Soil Quality Trace Metal Guidelines, Targets and Limits

There are guidelines for trace metals in soils which apply to many parts of the world that have been used as a tool in the use of evaluating a sustainable eco-system response (Karlen et al, 2001). As outlined in Chapter 1 of this thesis (see section 1.7 of Chapter 1) there are different guideline values for trace metal concentration in soil according to land-use as differing types of land contain differing exposure risks. Finnish mires are comparable in structure to the meres and mosses of Cheshire and Shropshire and other similar habitats (Seppa, 2002). The Finnish government sets out threshold and guide values from the Finnish legislation for contaminated soil (Decree 214/2007). These values are based on aqua regia extractable analytical techniques which is the recommended analytical method by the Decree. As such, these values are particularly applicable when using ICP-MS as the analytical instrument for trace metal determination due to the application of aqua regia/reverse aqua regia acid digest within the methodology. Median, 90th percentile and maximum values of European topsoil are also often utilised in conjunction with these guidelines (Finnish Government, 2007). The Finnish guideline values describe the maximum acceptable risk to the environment in a typical Finnish landscape but are widely cited in global studies in regards to soil quality (Reyes, 2020;Reinikainen, 2007; Jarva et al., 2010). The lowest of these guideline values, known as the 'Threshold Value' has been determined on that in which risks to humans, wildlife and the environment can be considered negligible and they are used as trigger values for soil contamination assessment (Taivainen et al, 2009). If data is available, then regional background values may also be used as the 'Threshold Value' (Reinikainen, 2007). Socio-economic values were also considered when defining the threshold values due to potential implications for relocation and re-use of excavated soil that was considered 'slightly contaminated' which are also considered in risk-based assessments when assessing potentially contaminated land in the UK (Reinikainen, 2007). The 'Lower Guideline Value' and 'Upper Guideline Value' are based on "significant risks to human health or soil-eco-system" (Reinikainen, 2007). The 'Threshold Value' and 'Guideline' values are displayed in Table 5.3a.

Trace Metal Element	Finnish Threshold Value (ppm)	Finnish Lower Guide Value (ppm)	Finnish Higher Guide Value (ppm)
Cu	100	150	200
Pb	60	200	750
Zn	200	250	400
V	100	150	250
Cr	100	200	300
Co	20	100	250
As	5	50	100
Sn	-	-	-

- Denotes no available data

Table 5.3a: Finnish Soil Quaqlity Guidelines (Finnish Government, 2007)

The Dutch Target and Intervention Values, 2000 and the EU 'sludge to land directive' (86/278/EEC) are set out in Chapter 1 of this thesis (see section 1.7 of Chapter 1) and Chapter 4 of this thesis (see section 4.2.3 in Chapter 4). The Dutch values were amended as per the methodology set out in the annexes of the Dutch Target and Intervention Values, 2000 (see Appendix F) during the screening study (see Chapter 4) so to be representative of the sampling region using the median OM and clay content, as ascertained from the screening study, so to represent the central tendency of these factors (see Table 4.2 in Chapter 4). These amended limits are displayed in Table 5.3b.

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Trace Metal Element	N	Netherlands Target/Background Limits (ppm)	Netherlands Intervention Value Limits (ppm)
Cu	89	19.78	104.37
Pb	89	57.96	361.40
Zn	89	63.46	326.34
V	89	15.03	89.46
Cr	89	62.24	197.68
Co	89	2.28	60.87
As	89	18.18	34.49
Sn	89	-	218.18

- Denotes no available data.

N = Number of Samples

- Table 5.3b: Dutch Soil Quality Guidelines Representing the Sample Site with Median OM and Median Clay Content of all samples.

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5.1.6 Study Aims:

This study will aim to compare the analytical methods of ICP-MS with reverse aqua regia digestion and FPXRF in terms of their statistical difference and correlation when measuring trace metal concentrations in the topsoil of a wetland meres and mosses region. This involves the comparing the total concentration results from the FPXRF method to the pseudo-total obtained from reverse aqua regia digestion employed by the ICP-MS analysis. This statistical comparison will aid in establishing whether the pseudo-total result is a good indicator of the total result for trace metal concentration. The results from the ICP-MS portion of this study are assessed against environmental standards for trace metals in soil when compared to the outcomes of the same assessment for FPXRF during the screening study (see Chapter 4 of this thesis). This is with the aim of exploring how the differing analytical techniques can impact the results of a topsoil quality assessment for trace metals. The aims of this study can be summarised thusly:

1. Determine the relationship between the total concentration obtained by FPXRF and the pseudo-total obtained by ICP-MS with reverse aqua regia digestion of the topsoil sample by means of statistical analyses.

2. To determine if the pseudo-total is representative of the total result for trace metal concentration.
3. To examine the adherence of the pseudo-total results to appropriate soil quality guidelines for trace metal concentrations in soil.
4. To examine the potential impact these differing methodologies may have on compliance with soil quality guidelines.

5.2. Materials and Methods:

5.2.1 Sample Collection.

A total of 89 samples of topsoil (A-horizon) were taken from various sites in the Meres and Mosses region of Cheshire and Shropshire, using a trowel and sample bags. Samples were taken on a randomised sampling regime and on a stratified coarse grid process to try and cover as much as the NIA/LPS area as possible to obtain representative coverage (see Chapter 2 and Chapter 4 of this thesis). Upon collection of a sample, a coordinate sample point was recorded with a handheld GPS so as to be able to accurately map the sample and attach information to an exact point (see Appendix C, Fig C1). Samples were then returned to the laboratory and dried in an oven at approximately 50°C overnight and then sieved between 2mm down to 0.063mm. Prior to FPXRF and ICP-MS analysis, physiochemical analysis was undertaken including granulometric profile, pH, magnetic susceptibility and organic matter content (see Chapter 2 and Chapter 4 of this thesis).

5.2.2 Trace Metal Analysis via FPXRF and Choice of Trace Metals

Trace metal screening using FPXRF was carried out on all 89 of the topsoil samples that were collected in the field (see Appendix C, Fig C1 and Chapters 2 and 4) via FPXRF in controlled laboratory conditions. This FPXRF analysis was undertaken on the whole of the <2mm fraction of the topsoil samples that had been homogenised. The instrument used was a 'Skyray Genius 9000 FPXRF Heavy Metals in Soil Handheld Analyser'. A total of 17 trace metal elements were analysed via FPXRF (Cu, Pb, Zn, Ca, K, Ti, V, Cr, Mn, Fe, Co, As, Rb, Sr, Nb, Sn, Ga) on these soil samples as part of the screening exercise to help aid in the determination of the spatial occurrence of trace metals in the Meres and Mosses region (see chapter 4). These trace metals were chosen as they were those pre-set in the 'Soils' program for the Skyray Genius 9000 software built-in for environmental screening.

Sample bags of topsoil collected from the meres and mosses region were placed on the laboratory testing stand. The Skyray Genius 9000 was set to the ‘environmental soils’ program (factory settings) for the appropriate calibration method. A silver (Ag) standard pellet certified reference material provided with the FPXRF hardware was used in the calibration of the instrument and for periodic checking of instrument accuracy. Once the trace metal concentration data had been obtained, statistical analyses were employed so to aid in the elucidation of the spatial occurrence of the trace metals (see Chapter 4).

This screening exercise highlighted some trace metals to be ‘enriched’ following Enrichment Factor Analysis and prevalent in soil quality guidelines/limits (see Chapter 4). As a result of these findings, the following trace elements were chosen so to compare with ICP-MS analysis:

As, Pb, V, Cu, Zn, Co, Cr and Sn

These trace metals were chosen based on their determined enrichment from the screening study (see Chapter 4) and their viability to be undertaken in a multi-element ICP-MS study with use of a certified soil reference material for validation of the method on certain trace metal elements (see Chapter 3 and section 5.2.3). Ni, which was not analysed in the screening part of the study due to limitations with the FPXRF software and widely reported poor accuracy when reporting Ni via FPXRF methodology (Kilbride et al, 2008), was added to this study to be analysed by ICP-MS due to its established properties as a toxic trace metal in soil (Flem et al, 2022). Whilst not part of the comparison study, Ni will be assessed against the relevant environmental targets/limits implemented in this study and thesis as with the other trace metals being studied and will also be included in the spatial mobility mesocosm study (see Chapter 6).

5.2.3 Trace Metal Analysis via ICP-MS and Method Development

Topsoil samples from the Meres and Mosses region (see Chapter 4 and Appendix C, Fig C1) were analysed via ICP-MS with reverse aqua regia as the digestion medium so to obtain a pseudo-total result for trace metal concentration. These samples were ground to pass a 2mm sieve and then homogenised before digestion and analysis. The ICP-MS methodology was used to determine the concentration of trace metals in soil samples that had been digested using reverse aqua regia via microwave digestion using specific parameters (see Chapter 3 and table 5.4) so to determine the pseudo-total concentration of trace metal pollutants As, Pb, V, Cu, Ni, Zn, Co, Cr and Sn. These parameters were found to be suitable in both standard operating mode and CCT mode (see Chapter 3 of this thesis) meaning that arsenic could be included in the analysis, considering the spectral interference issues known with arsenic. Interferences based on mass, caused by atomic or polyatomic ions and having the same mass/charge ratio of the trace metal being studied, can be a limitation in the analysis of trace metals by ICP-MS (D'Ilio et al, 2011). Arsenic is a mono-isotopic element (m/z = 75) that suffers from the overlapping of polyatomic species $^{40}Ar^{35}Cl^{+}$(see Table 5.2). CCT mode on the ICP-MS instrumentation aids in eliminating this interference if available (see Chapter 2 and Chapter 3 of this thesis) but reduced recovery may still be observed. As such, CCT mode was activated on the ICP-MS instrumentation when determining the concentrations of As and Co due to the known argon-based spectral interferences impacting the recovery of As and Co (see Table 5.2 and Chapter 3). This CCT technology is implemented so to mitigate the influence of polyatomic interference (Tanner et al, 2002). These setting are in alignment with SRM 8 and SRM 15 in the method development study (see Chapter 3).

Digestion Method	Ramp Time (mins)	Hold Time (mins)	Max Temperature (°C)	Power (W)
Reverse aqua regia	20	5	200	1300

Table 5.4: Microwave Parameters for Digestion of Topsoil Samples with Reverse Aqua Regia.

The choice of trace metals were based on their considered toxicity in the environment, the relevant trace metals certified in the soil reference material (see Chapter 3 of this thesis) and the findings from Chapter 4 from this thesis in which soil samples from the Meres and Mosses region were analysed for a wide range of trace metal elements via FPXRF (see Chapter 4). In this study an Agilent 7900 ICP-MS instrument, which utilises a collision/reaction cell for interference removal, was used for determination of the trace metals during the analysis of topsoil in this chapter.

Trace Metal Standard solutions were prepared in the range 0 to 1000 ppb using certified SpexR ICP-MS multi-element solutions. A certified reference material (CRM) was also implemented to ensure the validity of results. This CRM was 'ERM-CC141 Loam Soil' (see Chapter 3). This was applied to every analytical run to ensure the method achieved suitable recovery values. Recovery of certified reference material should be at concentrations of 80% to 120% of target concentration (Hibbert, 1999). This 'target concentration' is that which is listed in the datasheet of the certified reference material. To remove any suspended solids and reduce risk of damage to instrumentation, soil sample digests were filtered through a 0.45 µm PTFE syringe filter as per established standard operating procedures (EPA 3050b, 2006). The calibration standards and CRM were prepared and filtered also using 0.45µm syringe filter. The soil digest samples and CRM (0.2g) were prepared as per the methodology set out in section 3.2.1 of Chapter 3 then made up to volume in a 50ml volumetric flask and acidified with reverse aqua regia in the same proportion as in the digestion step so to stabilise the elements in the soil digest matrix. The acids used, Hydrochloric acid 37% and

Nitric acid 67%, were reagent quality and at trace metal grade whilst the ultra-purity water was produced with a Millipore Milli-Q System at 18.2 MΩ-cm.

The ICP-MS instrument was tuned and calibrated at the beginning of every analytical run using internal tuning procedure and standards prepared from certified SpexR ICP-MS multi-element solutions in the range 0 to 1000ppb, as per the prepared standards, with a minimum accepted calibration of 0.9990 R^2. In addition, mixed element standards at 500 ppb and 200ppb were inserted during the analytical run at regular intervals so to correct any observed drift in instrument sensitivity. In order to reduce the potential of contamination, the samples were analysed without any addition of internal standard (Smyth and Johnson, 2013; Young et al, 2016).

5.2.4 Linear regression

Linear regression is a useful tool to evaluate data sets (Bernick et al, 1995). A linear regression model was implemented so to assess the relationship between trace metal concentrations determined by reverse aqua regia extractable analysis in ICP-MS and the portable X-ray Fluorescence Spectrometer FPXRF instrument. The analysis produces a linear model (see Equation 5.1), which reduces the square of the differences between the regression model and the dependent variable (Kilbride et al, 2008). So to adhere to the linear regression model, reduce skewness and standardise variance (EPA 2007; Havukainen et al, 2019), all raw data was log transformed (SPSS Statistics Software v26.0).

$$Y = mx + c + \mathcal{E}$$

Equation 5.1: Linear Regression

Where Y is the transformed FPXRF concentration, x is the aqua regia extractable metal concentration, c is the y-intercept of the regression line, m is the slope and Ɛ is the residual.

R-squared (R^2), or Coefficient of determination (see 5.2.5), indicates the percentage of the variance in the dependent variable that is explained by the independent variable and is a 'goodness of fit' measure when applied to linear regression (McKelvey & Zavoina, 1975).

5.2.5 Pearson Correlation, Coefficient of Determination and Significance

Statistical Analyses software (SPSS Statistics 26.0) was used to determine the bivariate correlation between the elements studied for total trace metal concentration (FPXRF) and for those elements analysed for pseudo-total trace metal concentration analysis via reverse aqua regia digestion (ICP-MS). Undertaking a bivariate Pearson Correlation analysis via SPSS software, produces a correlation coefficient denoted by the letter 'r' which "measures the strength and direction of linear relationships between pairs of variables" (Tsokanta, 2015) which, in this study, were the trace metal concentration results obtained via the respective analytical methods. The SPSS software also determines the statistical significance of these correlation results. This was undertaken by performing a two-tailed test, sometimes known as the 'probability value' or 'ρ-value'. Coefficient of Determination (R^2) was also determined and visually displayed via Microsoft Excel 365. This R^2 is a "statistical measure in the regression model that determines the proportion of variance in the dependent variable that can be explained by the independent variable" (Mustafa, 2022) thus determining if any relationship exists or can be predicted when examining the data from one variable (in this study this is the trace metal concentration data).

5.2.6 Test for Normal Distribution and Wilcoxon Signed Ranks Test

When comparing two sets of data from the same samples, a non-parametric test is required if the data is found not to be normally distributed (see Section 5.3.3 and Table 5.7). A Shapiro-Wilk test for Normality was undertaken on the obtained data for ICP-MS and FPXRF trace metal concentrations in the topsoil of the Meres and Mosses of the Marches. The results obtained for many of the trace metals indicated that the data was not normally distributed (see Table 5.7). Under these conditions, a Wilcoxon Signed Ranks Test is undertaken so to examine differences. The Wilcoxon Signed Ranks Test is the non-parametric test equivalent to the Paired Samples t-Test and is considered a more powerful statistical tool than the *t*-test for non-normal data (Kitani & Murakami, 2020). The Wilcoxon Signed Ranks Test is used to determine whether two matched datasets are statistically different (Dao, 2022). The Wilcoxon Signed Ranks Test does not assume normality in the data and, as such, it can be used when this assumption of normality has been violated (such as in the dataset presented here) and the use of the Paired Samples t-test is, consequently, inappropriate (Bernick et al, 1995). The Wilcoxon Signed Ranks Test has previously been used in studies to compare the differences in two sets of scores that come from the same participants in soil studies implementing FPXRF and ICP analyses (Caporale et al, 2018; Sikora et al, 2021). When undertaking a Wilcoxon Signed Ranks Test, a p-value is generated in the output. If the p-value is less than the significance level, the null hypothesis is rejected and it is determined that there are significant differences between the results obtained for ICP-MS when compared to the results from FPXRF. If the p-value is greater than the significance level, it indicates that there is no significant difference between the two data sets. This Wilcoxon Signed Ranks Test was performed on all of the trace metals in the study that underwent both ICP-MS analysis via reverse aqua regia digestion and FPXRF analysis (see screening study in Chapter 4). This statistical analysis was undertaken on SPSS Statistics 26.0 software.

5.3 Results and Discussion:

5.3.1 Certified Reference Material Recovery

The implementation of a certified reference material (CRM) was undertaken during the ICP-MS analysis so to verify the recovery of select trace metals during the acid digestion process. A total of 20 certified reference material digests were run in conjunction with the topsoil sample digests during the ICP-MS analytical process. This was undertaken, using the same digestion parameters as for the topsoil samples (as determined in Chapter 3 of this thesis), to show the validity of the method when analysing several different trace metals simultaneously via ICP-MS and using reverse aqua regia with microwave as the digestion method. The recovery results all fell within the 80% lower threshold and 120% upper threshold for recovery (see Chapter 3 of this thesis) and produced an overall recovery for all elements analysed at 103.72% (see Fig 5.1).

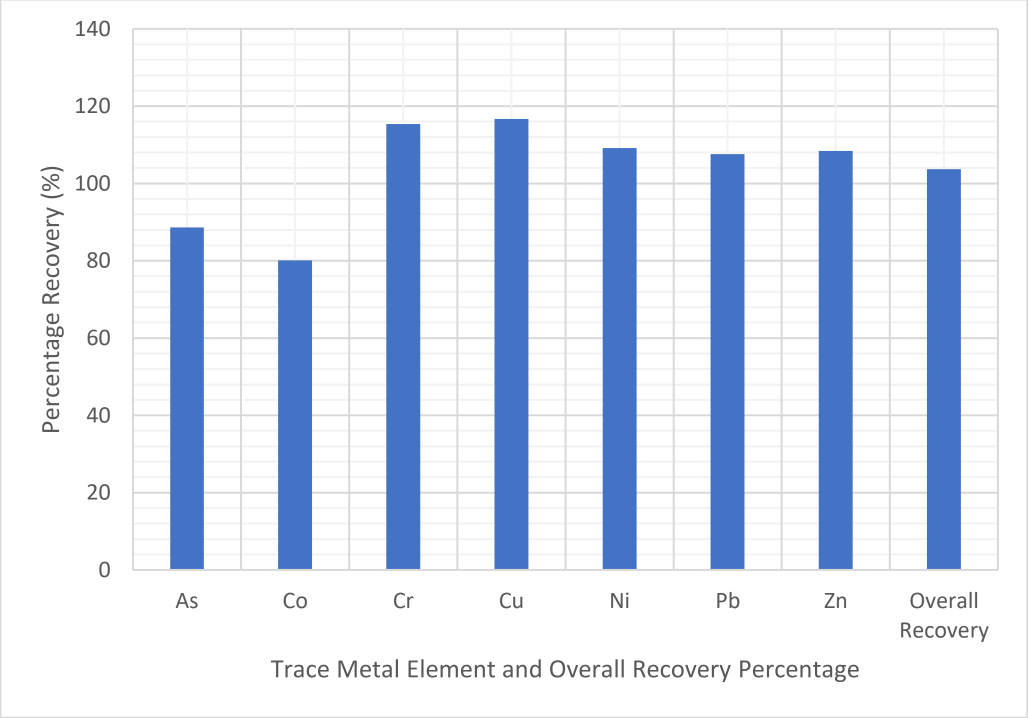


Fig 5.1: Recovery Data for ERM-CC141 and Overall Recovery Percentage

Both As and Co are known to suffer from low recovery due to argon based polyatomic interference (Nenni et al, 2012;McCurdy and Woods, 2012). This was mitigated by implementing Kinetic Energy Discrimination (KED) with Collision Cell Technology (CCT) which minimises or eliminates many argon-based polyatomic interferences (Tanner et al, 2002). This explains lower recovery figures for Co and As at 80.13% and 88.62% respectively. These lower recoveries may have a small impact on the correlation results for Co and As in this comparison study due to resulting in lower results in the ICP-MS trace metal concentration data-set for these two trace metals.

It is also worth considering, when examining the certified reference material (ERM-CC141), the data provided by the JRC (2024) for total content concentrations obtained for these trace metal elements and how they vary to the aqua regia extractable content certified (see Appendix G, Table G1). The data provided with the certified reference material indicates that the aqua regia extractable content for all of the trace metal elements being studied here are greater than 70% of the certified results obtained for the total content with the exception of Cr which displays only 36.05% of the total content when using aqua regia extractable methodology. This data may help explain the statistical differences observed between the ICP-MS methodology implemented in this study and the total trace metal concentrations determined via FPXRF analysis (see Section 5.3.3).

5.3.2 Statistical Comparison of ICP-MS and FPXRF Datasets

All 89 samples were analysed for select trace metal concentration. The descriptive statistics were determined via SPSS 26.0 (see Table 5.5 and Table 4.2 in Chapter 4). These descriptive statistics clearly display the disparity in the obtained results from the two analytical methodologies for trace metal concentration.

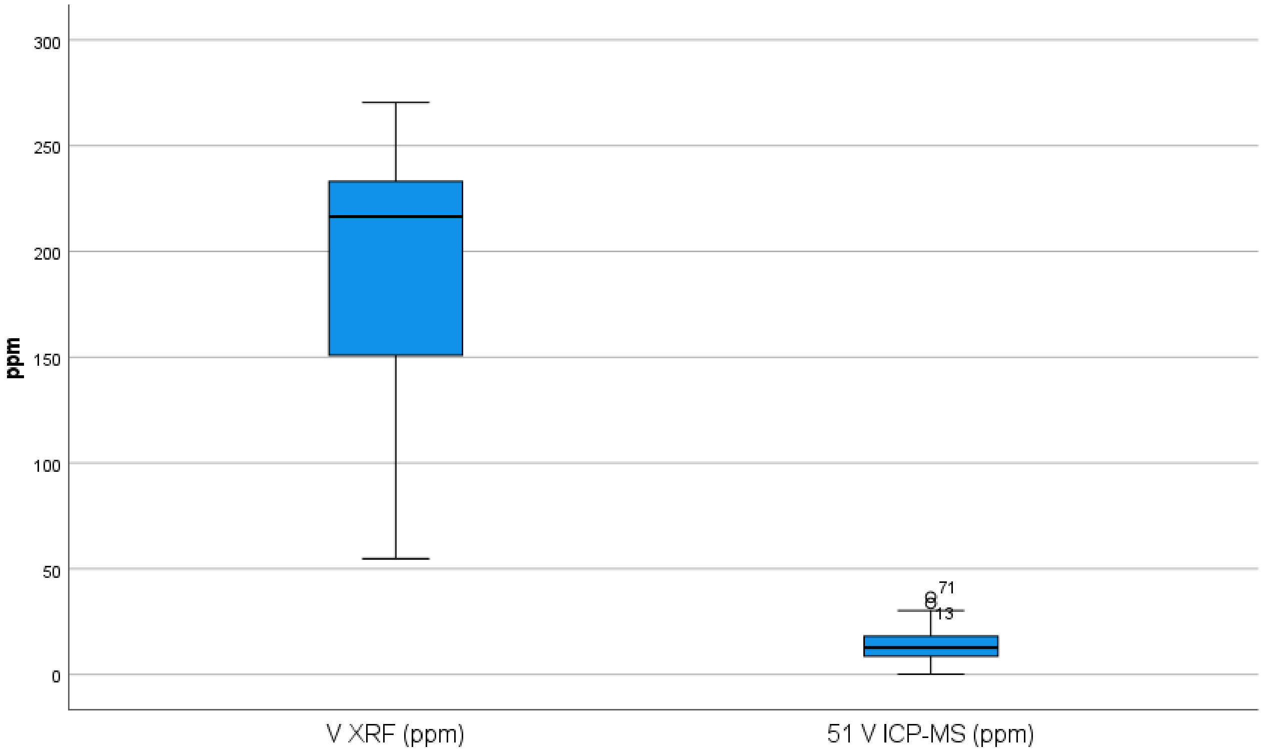
Trace Metal Element	Minimum Result ICP-MS (ppm)	Minimum Result XRF (ppm)	Median Result ICP-MS (ppm)	Median Result XRF (ppm)	Maximum Result ICP-MS (ppm)	Maximum Result XRF (ppm)	Mean Result ICP-MS (ppm)	Mean Result FPXRF (ppm)	Std. Deviation ICP-MS	Std. Deviation XRF
Cu	5.49	0.00*	14.7	4.9	77.83	171.00	20.50	23.06	14.82	38.20
Pb	2.51	31.60	35.9	59.6	267.51	443.60	60.09	114.88	56.28	101.29
Zn	6.73	23.10	48.6	90.95	892.71	2330.70	78.28	152.37	102.09	248.19
V	0.203	54.80	12.8	216.4	36.64	270.50	14.01	194.34	7.38	58.69
Cr	2.07	36.20	16.3	63.8	97.76	124.84	18.03	69.64	11.22	21.25
Co	0.050	0.00*	2.8	31.86	7.19	105.90	2.97	34.64	1.52	23.42
As	0.277	5.00	3.5	5	9.37	34.30	3.58	5.35	2.00	3.11
Sn	0.00	0.00*	0.6	1.6	6.00	92.02	1.07	5.68	1.19	13.01
Ni	1.96	-	11.5	-	23.40	-	11.61	-	4.45	-

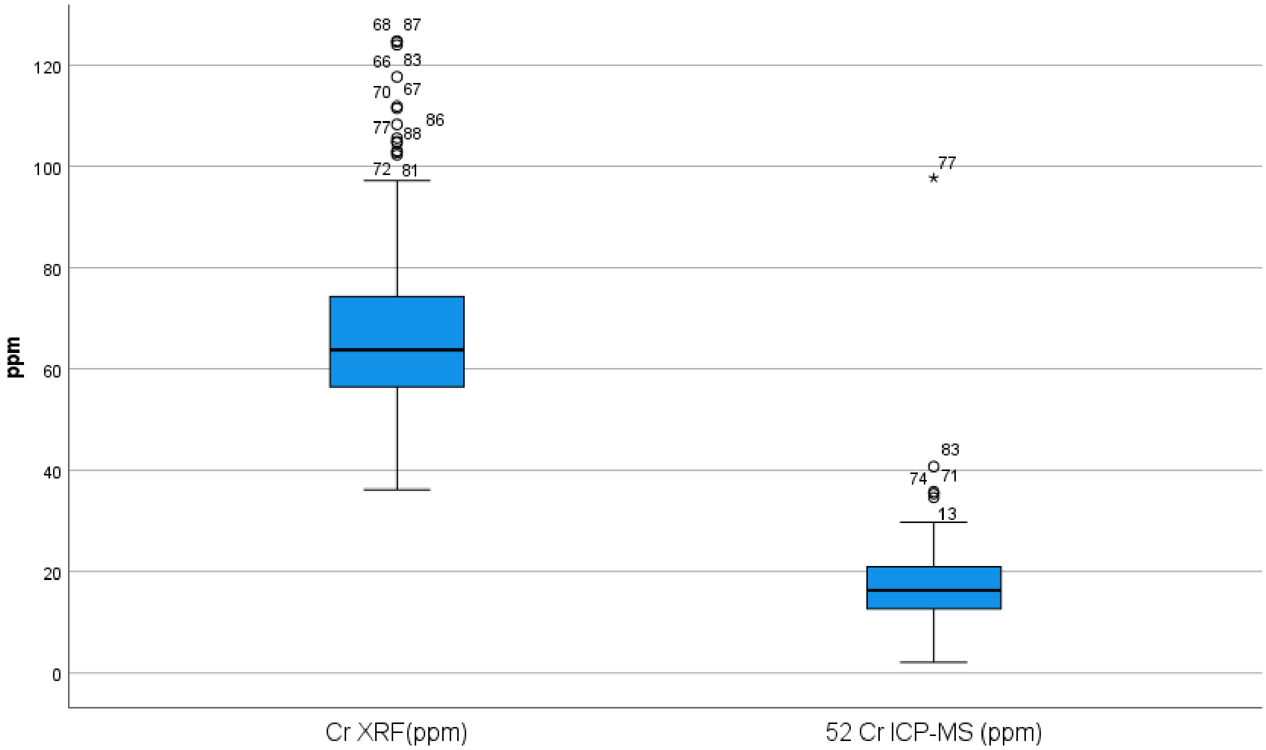
- For FPXRF methodology, a reading of 0.00 = below detectable limit.

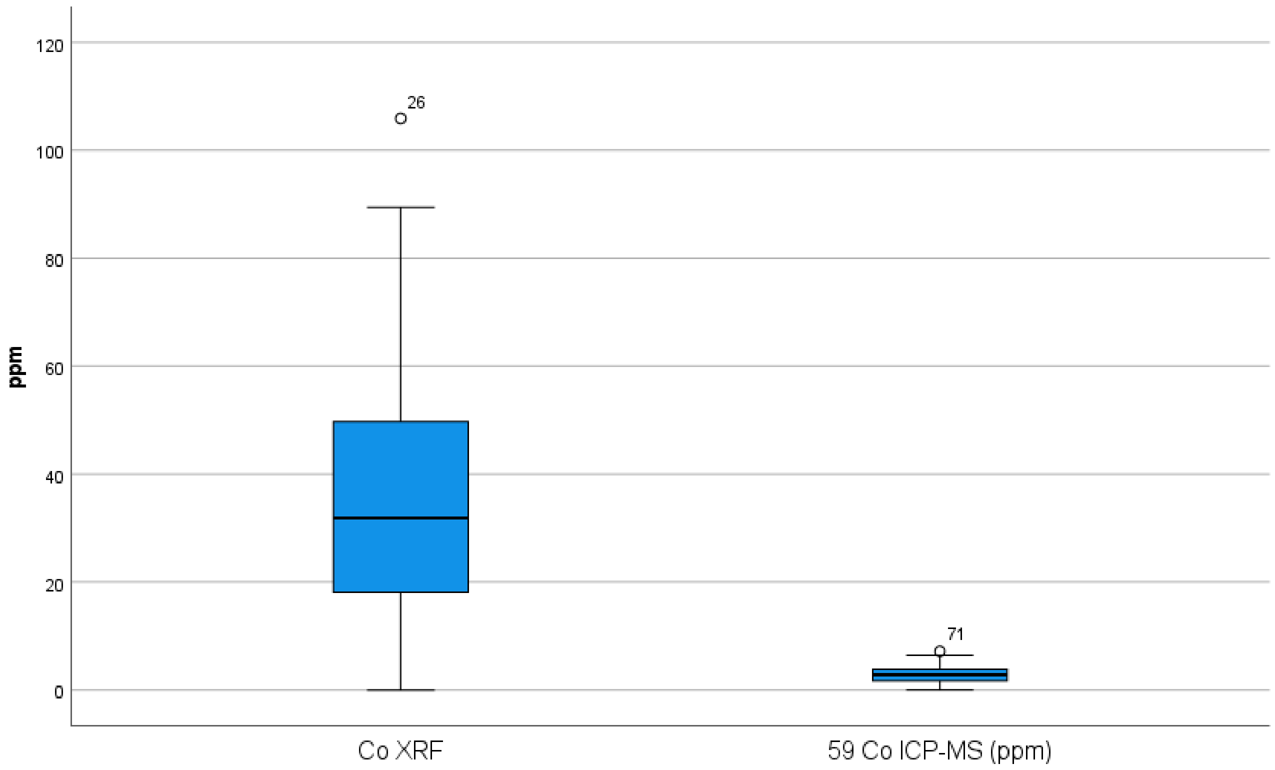
Table 5.5: Descriptive Statistics for Trace Metal Concentrations in FPXRF and ICP-MS

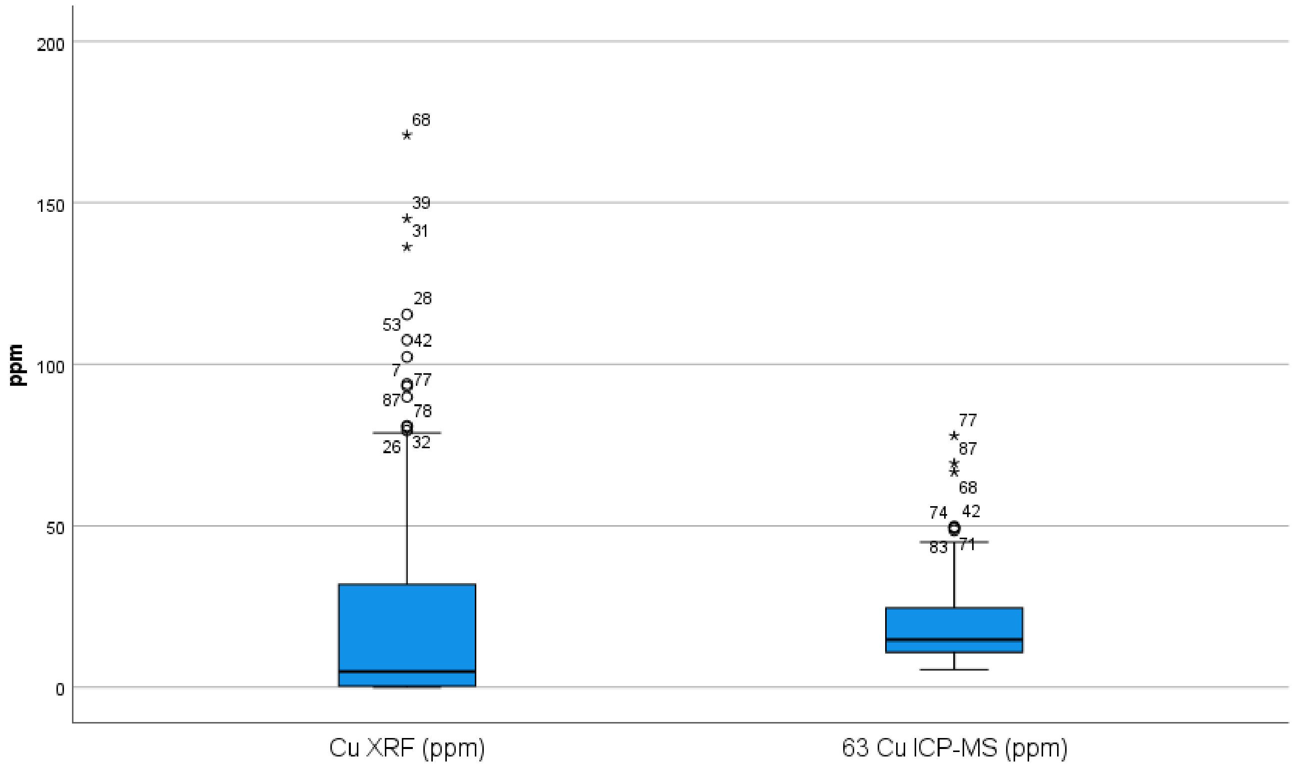
Boxplots (sometimes known as box and whisker plots or box plots) were implemented so to visually display the distributions of the obtained trace metal concentration from both the analytical procedures (FPXRF and ICP-MS) as shown in Fig 5.2. The boxplot is an effective illustrative method of summarising sets of data and can be used in identifying outliers (Schwertman et al, 2004). This illustrative method is less susceptible to outlier influence due to its use of quartiles and interquartile ranges to define the box and whisker plot (Mazerei et al, 2025). The box plot illustrative method has become a standard approach in many fields of data analysis due to this ability to simply identify and display outlier data (Walker et al, 2018). The outer limits of the box itself (see Fig 5.2) represent the range of the central 50% of the dataset encompassing the range between the first (25th percentile) and third (75th percentile) quartile, known as the 'interquartile range' (IQR). The intersecting line within the box itself represents the median (50th percentile) value of the dataset. The lines extending either side of the box represents the range of the remaining data excluding outliers and are considered to be 1.5 times the IQR at their limit (Spitzer et al, 2014). Dots that are placed beyond the range of these lines represent outliers and are those data points from the analysed samples that deviate significantly from the underlying distribution of a dataset (Aggarwal, 2017). These outliers are defined as those that are more than 1.5 times the IQR below the first quartile or more than 1.5 times the IQR above the third quartile (Sun & Genton, 2012).

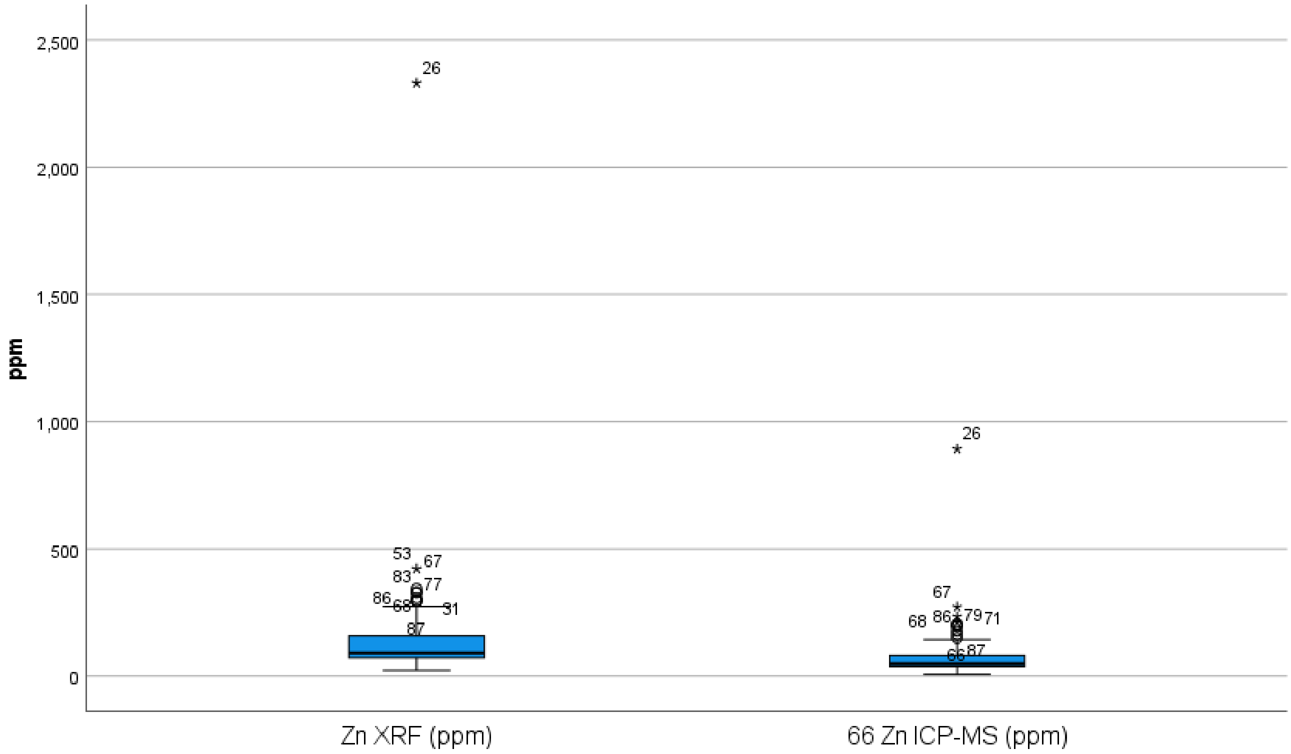
When examining the boxplots taken from the descriptive statistics (see Fig 5.2), it is observed that FPXRF methodology (denoted as XRF here) is nearly always a higher value than the ICP-MS with reverse aqua regia methodology (denoted as ICP-MS here) as is to be expected with the difference between the total result for FPXRF and the pseudo-total result obtained from ICP-MS. The exception for this is when examining Cu which shows a marginally higher result for ICP-MS. The spread of the data is nearly always seen to be greater for FPXRF than for ICP-MS. This will be due to the limited concentration range of the pseudo-total when compared to the more expansive total result of the FPXRF despite the increased sensitivity of the ICP-MS methodology. The exception here is for As which consistently showed a result of 5ppm in all but one of the 89 samples analysed by FPXRF.


300
250
200
150
100
50
0
ppm
71
13
V XRF (ppm)
51 V ICP-MS (ppm)


120
100
80
60
40
20
0
ppm
68
87
66
83
70
67
77
88
86
72
81
77
83
74
71
13
Cr XRF(ppm)
52 Cr ICP-MS (ppm)


ppm
120
100
80
60
40
20
0
26
71
Co XRF
59 Co ICP-MS (ppm)


ppm
200
150
100
50
0
68
39
31
28
53
42
7
77
87
78
26
32
77
87
68
74
42
83
71
Cu XRF (ppm)
63 Cu ICP-MS (ppm)


ppm
2,500
2,000
1,500
1,000
500
0
26
53
67
83
77
86
68
31
87
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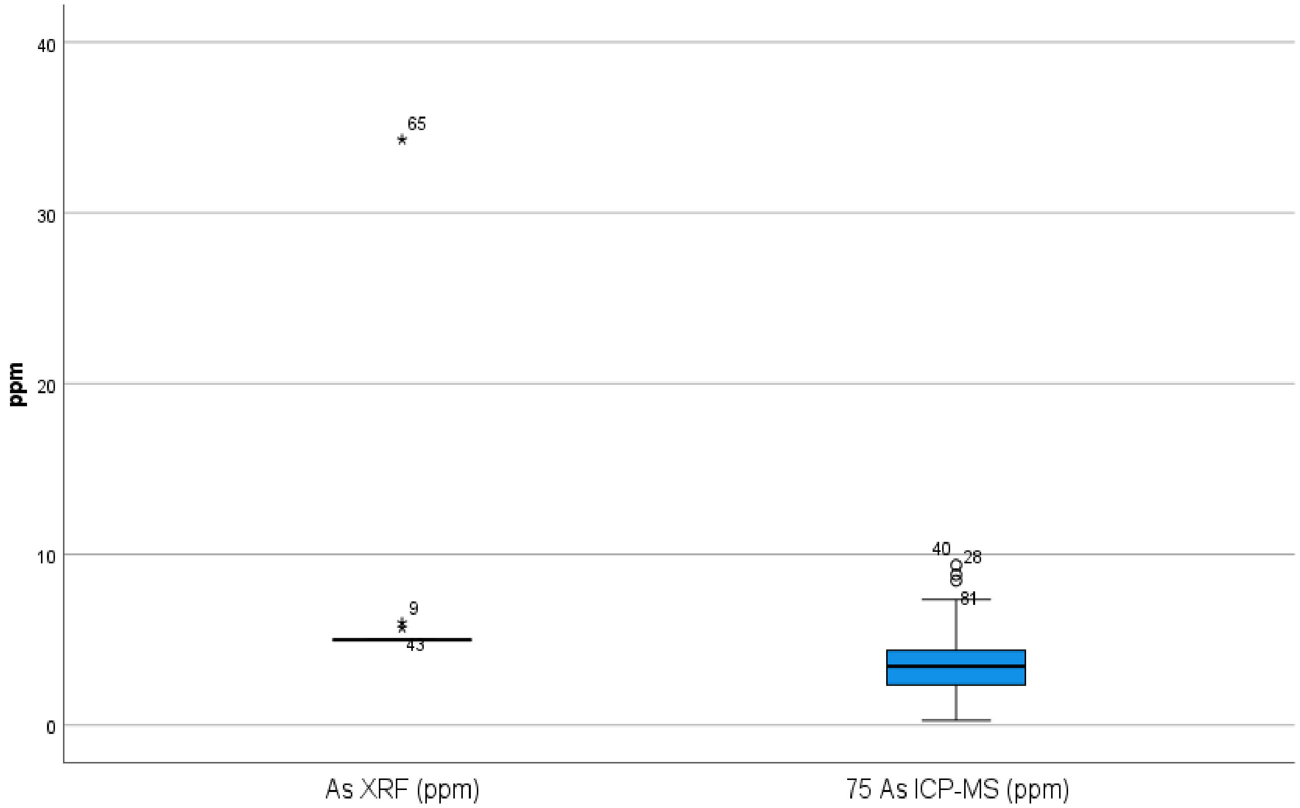
67
68
86
79
71
66
87
Zn XRF (ppm)
66 Zn ICP-MS (ppm)


ppm
40
30
20
10
0
65
9
43
40
28
81
As XRF (ppm)
75 As ICP-MS (ppm)

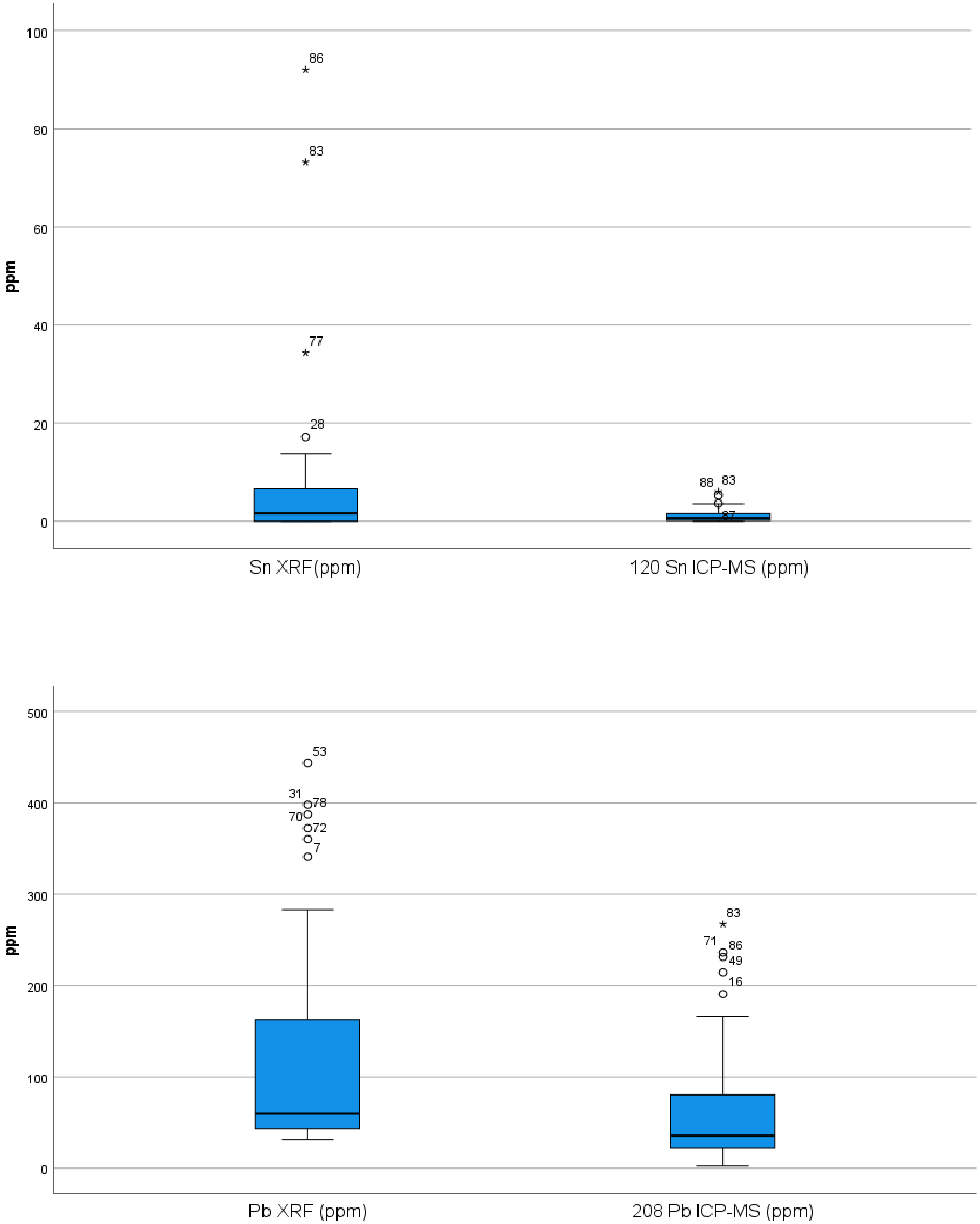


Fig 5.2: Boxplots Displaying Trace Metal Concentrations in ppm.

It is observed in Table 5.5 and Fig 5.2 that there are multiple outliers in both sets of data. These outliers are included in the raw data and implemented in the statistical analysis due to being a comparison of the same samples on two differing methodologies. The number adjacent to the outlier signifier in Fig 5.2 is the identifier code for the sample being analysed (samples 1-89), thus easily identifying the location and physiochemical characteristics of these samples based on the results and outcomes of the screening study (see Chapter 4). A chart was produced so to help visually examine these outliers, which are noticeable when examining the median and maximum results (see Fig 5.3). These outliers are extremal values that have the potential to cause the skewness of the dataset to be far from zero (Heymann et al, 2012). Due to these outliers having the potential to increase skewness in the dataset when undertaking this comparison study, the data was log-transformed to aid in reducing the skewness (West, 2022) as sown in Fig 5.4.

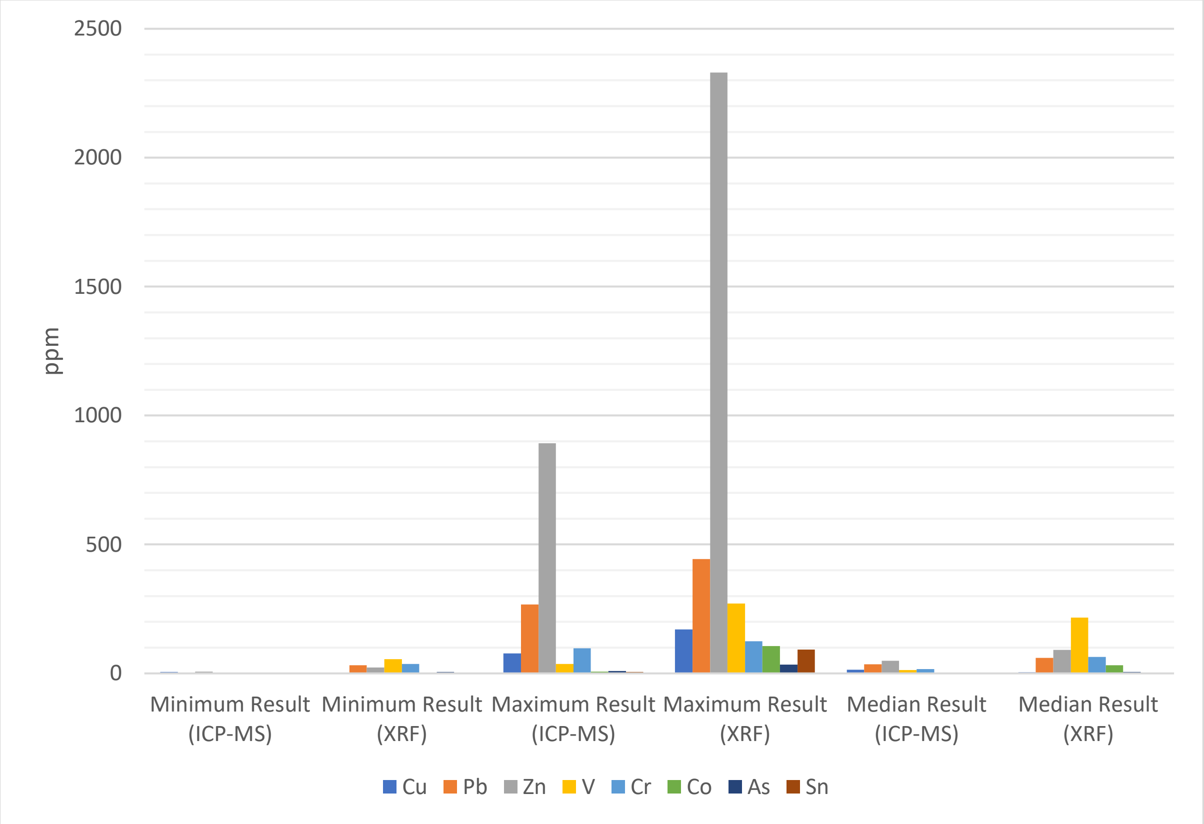


Fig 5.3: Descriptive Statistics for Average Trace Metal Concentrations Throughout the Sample Range for 8 trace metals.

The descriptive statistics show a similar pattern for those trace metals analysed for the pseudo-total concentration to those same samples analysed for total concentration. We can see that the FPXRF has beneficial use in determining the total result as it has values for minimum, median and maximum results that are significantly higher than the ICP-MS acid digestion methodology. This indicates the presence of a particular trace metal that may be difficult to disassociate from the mineral state and thus not readily detectable from the aqua regia digestion despite being present in the topsoil matrix. As such, this ICP-MS methodology represents the acid extractable, pseudo-total, content and can be seen as distinct from the total result (Tarvainen et al, 2009). An example of this is for Vanadium (V) in which the median result is higher for FPXRF at 216.4ppm than for median result for the same trace metal via the ICP-MS with reverse aqua regia digestion methodology which resulted in 12.8ppm (see Table 5.5). The results for Vanadium also show the advantage of the ICP-MS methodology however, as the minimum recorded result for this method is 0.203ppm. This result would be too low for the FPXRF to detect. The ICP-MS result is useful as it indicates how much of the trace metal may become mobile or, potentially, bioavailable under acidic conditions (Santoro et al, 2017; Andersen and Kisser, 2004). Cu has a similar mean concentration for both methodologies (see Table 5.5) with the boxplots showing a higher median value in the ICP-MS methodology with an increased spread in the FPXRF methodology (see Fig 5.2). This highlights how implementing the mean to represent the central tendency can be influenced by outlier results. This ICP-MS result for Cu appears to indicate that most of the Cu in the soil matrices is acid extractable and potentially bioavailable. This comparison outcome for Cu should be treated with caution, however, as this similarity in result is, potentially in part, most likely due to the reported issue of FPXRF underestimating Cu concentrations (Kilbride et al, 2008; Potts et al, 1995) combined with detection limitations for the FPXRF methodology as ICP-MS recorded 15 samples with concentrations for Cu of <10ppm and 22 samples were recorded as 0ppm (or below detectable limits) for FPXRF. The validation results for Cu via ICP-MS with reverse aqua regia methodology returned a recovery within the acceptable 80%-120% range (see Fig 5.1). This indicates that the ICP-MS methodology is performing as intended here.

For this study, it is important to note that a certified reference material, ERM-CC141 (see section 5.3.1) was implemented for the validation of the ICP-MS analytical process. This certified reference material was implemented due to having the characteristics of a loam soil and, thus, being representative of the soil type in the study area. The FPXRF analysis used a single element silver standard reference material for both calibration and validation and, as such, would benefit from the additional and robust validation measures that a multi-element and matrix-matched reference material can provide so to ascertain suitability for implementation in this type of investigation in which only trace amounts of a particular trace metal element may be present in the topsoil.

Pearson Correlation was undertaken so to ascertain the relation between the results obtained for each element analysed by FPXRF and the results obtained for pseudo-total by reverse aqua regia digestion for ICP-MS analysis (see Table 5.6).

Trace Metal Element	Pearson Correlation (r)	Significance, ρ-value (2-tailed)
Arsenic (As)	-0.007	.948
Lead (Pb)	0.518	0.000**
Vanadium (V)	-0.064	0.548
Zinc (Zn)	0.907	0.000**
Copper (Cu)	0.521	0.000**
Cobalt (Co)	0.527	0.000**
Chromium (Cr)	0.359	0.001**
Tin (Sn)	0.230	0.030*

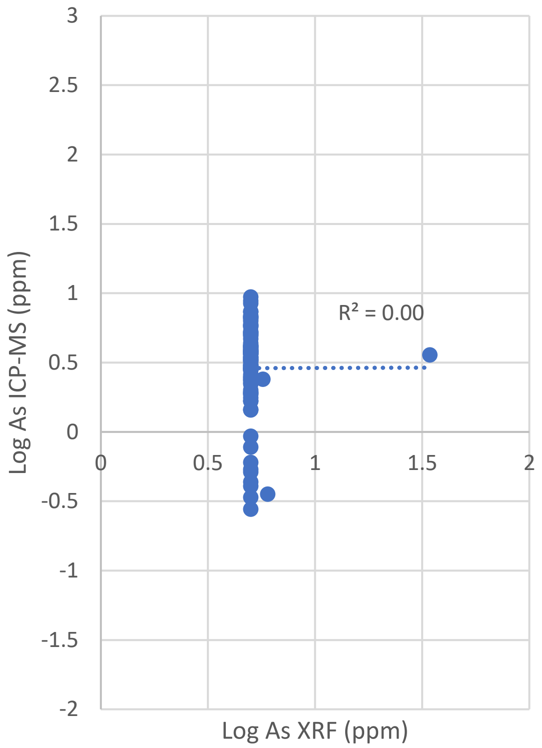
* Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

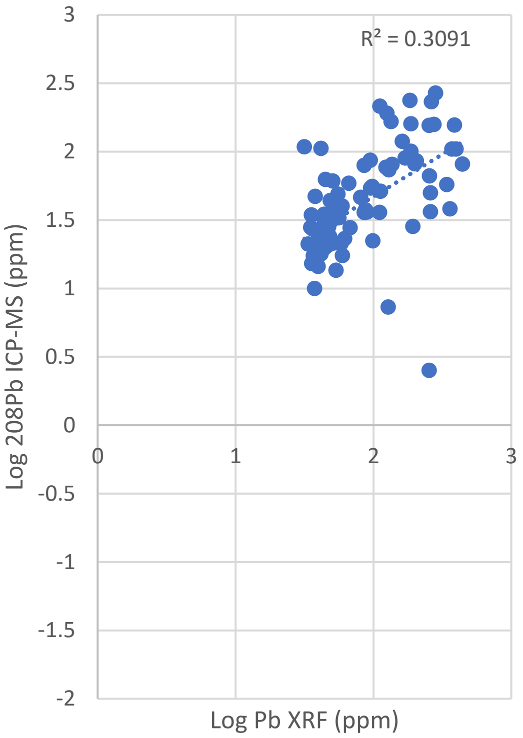
Table 5.6: Pearson Correlation for Select Trace Metals for Two Analytical Methodologies

The obtained data for trace metal concentrations show significant correlations between the FPXRF total concentration result and the result obtained using a reverse aqua regia soil digestion method followed by ICP-MS analysis (pseudo-total). This indicates that, for certain trace metal elements (Pb, Zn, Cu, Co, Cr and Sn) the total result obtained by the FPXRF method can be a good indicator of the pseudo-total trace-metal concentrations obtained under methodological conditions which benefitted from the robust validation of a multi-element, matrix-matched CRM (see section 5.3.1). This is especially the case with those trace metal elements that show a strong Pearson Correlation result (those that have $r > 0.7$) such as Zn or, to a lesser extent, those with a moderate strength of relationship ($0.4 < r < 0.7$) such as Pb, Cu and Co. These correlation coefficients have a significance/probability (ρ-value), which shows the probability that the relationship between the two variables is equal to zero and, thus, no relationship (Mindrila & Balentyne, 2017). Those elements showing moderate to strong correlations will have low ρ-values as this displays that the probability that there is no relationship between them is low. Correlations are statistically significant if the ρ-value is lower than 0.05. The results for the Pearson Correlation show that Pb, Zn, Cu, Co, Cr and Sn all have statistically significant correlations between the two analytical techniques. As and V, however, showed no significant correlation in the results obtained for each analytical method. The Reason for the low correlation in these two trace metal elements can be explained by a number of factors. One factor is due to the limitations of the methodology itself with reverse aqua regia digestion not being aggressive enough to fully disassociate the trace metal from its mineral state within the soil matrix. ICP-MS also has limitations with certain elements due to spectral interference, one of which is As and is often cited as such due to its monoisotopic properties (Colon et al, 2009). Collision Cell Technology (CCT) can mitigate this to some extent but still may impact correlation to the total result via FPXRF not suffering such methodological limitation. The validation of the ICP-MS methodology via use of a certified reference material (ERM-CC141) displays that, for those trace metals in the reference material (As, Co, Cr, Cu, Ni, Pb and Zn), acceptable recoveries of 80%-120% obtained from the analytical process indicate that the methodology is functioning as intended (see Fig 5.1) resulting in confidence of the results obtained and that the FPXRF methodology may benefit from the same level of robust validation via a matrix matched CRM.

Some further reasons for low correlations in samples of this nature is due to the low concentrations of some trace metals in some of the sample sites which may result in them being less suitable for FPXRF analysis due to sensitivity limitations. Sn, for example show only very low concentrations on average across the dataset with many results returning as zero which indicates below detectable limits with 37 of the 89 samples returning this figure for FPXRF whilst only 1 of the 89 samples returned a zero reading for Sn when implementing ICP-MS analysis. It is likely that correlations for the concentration of these trace metal elements in their respective analytical procedures would improve when outliers are omitted. Linear Regression and co-efficient of determination statistical analyses were performed on the obtained data. Indeed, despite both analytical procedures offering their own advantages and disadvantages, previous studies have found that correlation strength between the results of each analytical technique diminishes when studying agricultural soils, in which lower levels of trace metals are more likely to occur, than when compared to the majority of studies that tend to examine areas that are likely to have elevated levels of trace metals (Hu et al, 2014). This diminishing and/or variable correlation between the two analytical techniques need to be considered when assessing applicability for the investigation being undertaken.

As the concentration measurements for many of the trace metals compared here span more than one order of magnitude, the data was log-transformed so to standardise variance which is proportional to the magnitude of the measurement (EPA, 2007) and so to ensure that a correlation was obtained that was equally influenced by both high and low values (Barago et al, 2022). The trace metal concentration results were also log transformed so to remove skewness in the dataset (West, 2022). The coefficient of determination (R^2) was then deduced from the log transformed dataset (see Fig 5.4).


R² = 0.00
Log As ICP-MS (ppm)
Log As XRF (ppm)
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
0
0.5
1
1.5
2


R² = 0.3091
Log 208Pb ICP-MS (ppm)
Log Pb XRF (ppm)
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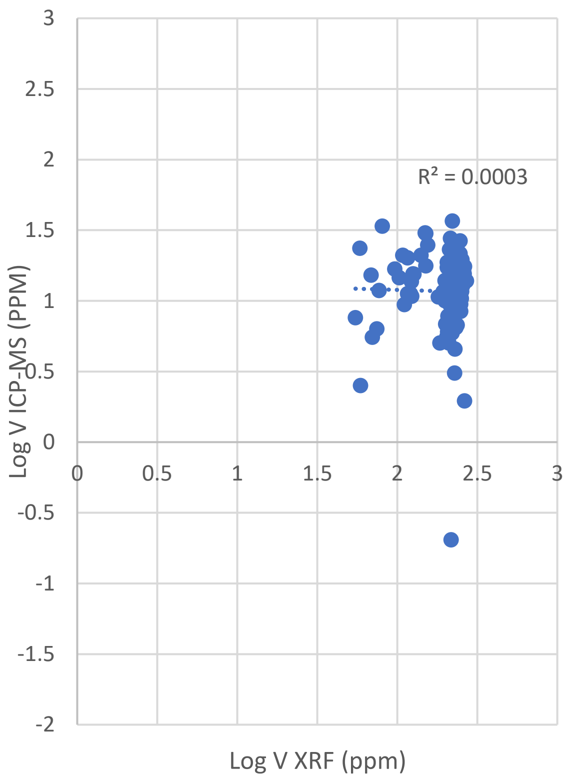
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
0
1
2
3


R² = 0.0003
Log V ICP-MS (PPM)
Log V XRF (ppm)
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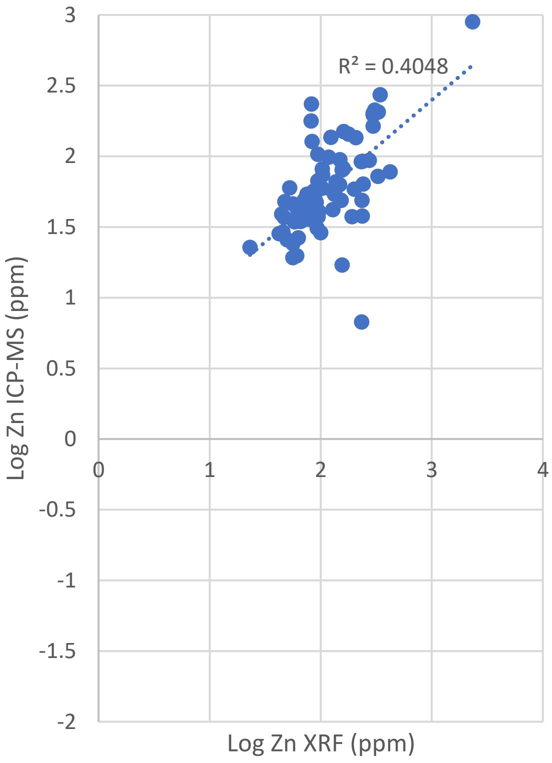
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
0
0.5
1
1.5
2
2.5
3


R² = 0.4048
Log Zn ICP-MS (ppm)
Log Zn XRF (ppm)
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2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
0
1
2
3
4

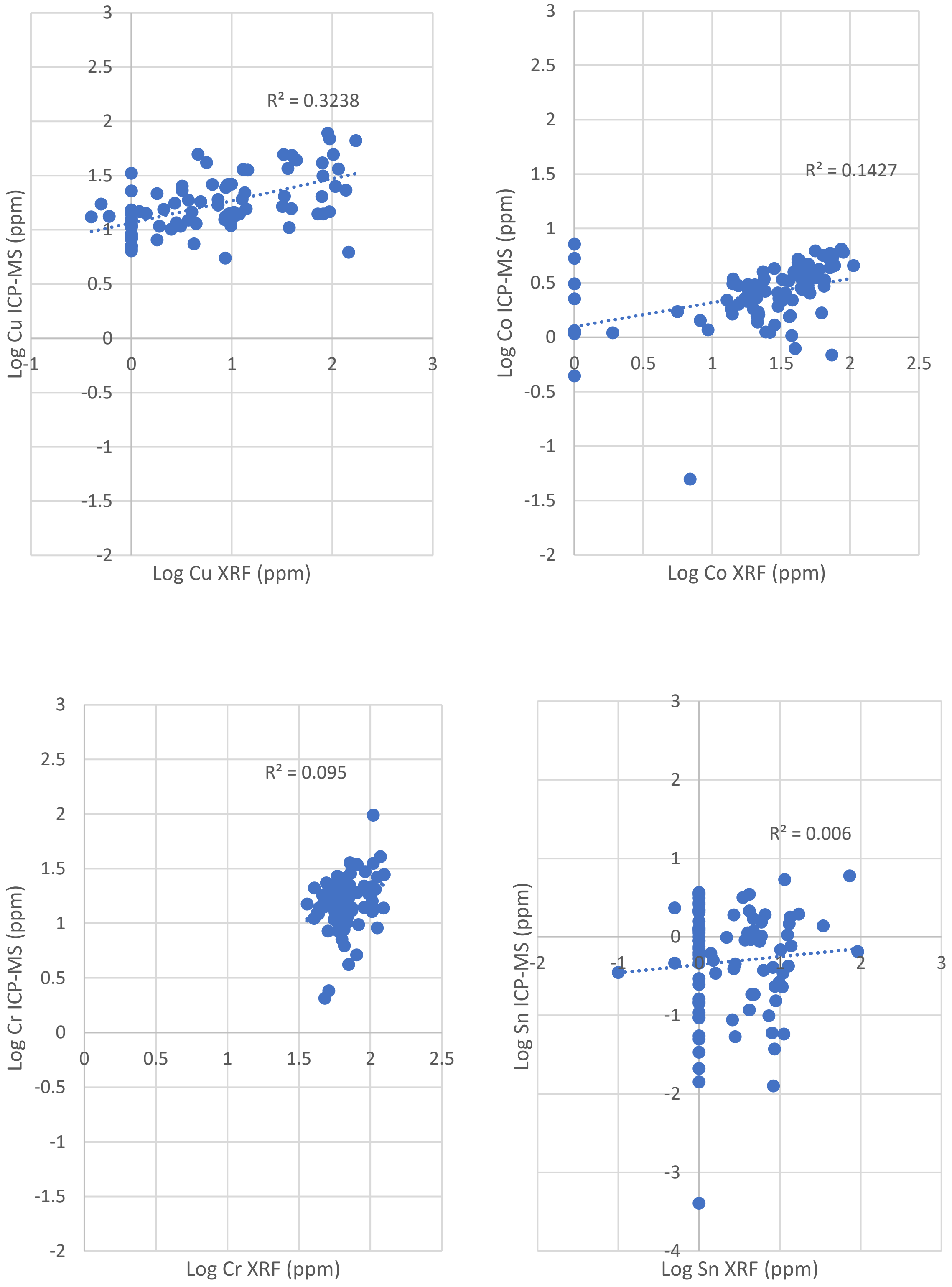


Fig. 5.4. Linear Regression of FPXRF data and reverse aqua regia extractable metals via ICP-MS analysis for As, Pb, V, Zn, Cu, Co,
Cr, and Sn.

As previously explained in section 5.2.5, the R^2 value explains to what extent the variance of one variable explains the variance of the second variable. Linear regression and consequent coefficient determination (R^2) were used to ascertain if the results obtained from the pseudo-total analysis via ICP-MS could be used to explain the variance of the results obtained for the FPXRF trace metal analysis. R^2 can be expressed as the percentage variance explained by the study model. We can see from the results that two of the elements (Cu and Pb) displayed what is considered a weak positiveR^2 correlation by showing an R^2 figure of >0.2-0,4, or over 20% of the variance being explained. Zn displayed a moderate positive coefficient of determination having an R^2 of 0.4 or over, thus explaining 40% of the variance. The remaining trace metal elements (As, V, Co, Cr and Sn) showed very weak positive correlation. It is likely that these correlations will improve once the observed outliers are omitted (see Fig 5.2 and Fig 5.3) when studying a regression model (Cappuyns and Slabbinck, 2012). These results, along with those obtained for Pearson Correlation (see Table 5.6) show that the data obtained for the pseudo-total trace metal concentration do provide a weak, moderate to very strong indication of the total trace metal concentration obtained via FPXRF for 6 of the 8 trace metals analysed.

5.3.3 Test for Normal Distribution and Wilcoxon Signed Ranks Test

To ascertain a suitable test for difference between the two analytical techniques, data was assessed for normal distribution (see Table 5.7). If the p-value of the Shapiro-Wilk Test is greater than 0.05, the data is normal. If it is below 0.05, the data significantly deviate from a normal distribution (Khatun, 2021).

Tests of Normality			
	Shapiro-Wilk		
	Statistic	df	p-value
V XRF (ppm)	.855	89	<.001
51 V ICP-MS (ppm)	.964	89	.015
Cr XRF(ppm)	.899	89	<.001
Cr ICP-MS (ppm)	.679	89	<.001
Co XRF	.963	89	.013
Co ICP-MS (ppm)	.972	89	.049
Cu XRF (ppm)	.652	89	<.001
Cu ICP-MS (ppm)	.790	89	<.001
Zn XRF (ppm)	.314	89	<.001
Zn ICP-MS (ppm)	.447	89	<.001
As XRF (ppm)	.089	89	<.001
As ICP-MS (ppm)	.961	89	.009
Sn XRF(ppm)	.423	89	<.001
Sn ICP-MS (ppm)	.804	89	<.001
Pb XRF (ppm)	.769	89	<.001
Pb ICP-MS (ppm)	.770	89	<.001

Table 5.7: Test for Normalisation (Shapiro-Wilk) on Data Obtained from FPXRF and ICP-MS

In the Shapiro-Wilk test, a p-value of <0.001, requires the rejection of the hypothesis that the data is from a normal distribution (EPA, 2016). As the result of the Shapiro-Wilk test indicated that the raw data obtained from both analytical techniques were not normally distributed for the majority of the trace metals studied (see Table 5.7), a Wilcoxon Signed Ranks Test (see Table 5.8) was undertaken on the data so to ascertain the difference between the two analytical techniques.

	Z-Score	**p-Value** **Asymp. Sig (2-tailed)**
V ICP-MS (ppm) – V FPXRF (ppm)	-8.193[a]	<.001
Cr ICP-MS (ppm) – Cr FPXRF (ppm)	-8.193[a]	<.001
Co ICP-MS (ppm) – Co FPXRF (ppm)	-8.001[a]	<.001
Cu ICP-MS (ppm) – Cu FPXRF (ppm)	-2.539[b]	.011
Zn ICP-MS (ppm) – Zn FPXRF (ppm)	-7.305[a]	<.001
As ICP-MS (ppm) – As FPXRF (ppm)	-5.599[a]	<.001
Sn ICP-MS (ppm) – Sn FPXRF (ppm)	-3.953[a]	<.001
Pb ICP-MS (ppm) – Pb FPXRF (ppm)	-6.475[a]	<.001

[a] Based on Positive Ranks

[b] Based on Negative Ranks

Table 5.8: Wilcoxon Signed RanksTest on ICP-MS and FPXRF Data

The Z-value in Table 9 is the average ranks from the two analytical techniques. This value will be lower if there is no difference between the groups. V, Co and Cr show the three most pronounced scores indicating the greatest difference between the two datasets. In part, the disparity between the outcomes of the two methodologies for Cr content in soil is explained when examining the certified results of the soil reference material (see Appendix G, Table G1) which shows that the aqua regia extractable content is only 36.05% of the total content under the methodology implemented by the JRC (2024). The lowest Z-score in the dataset was found for Cu based on negative ranks. This is confirmed in the descriptive statistics in Table 5.5 which displays that Cu is the only trace metal element analysed in which the median result for all samples for ICP-MS (14.7ppm) exceeds the median result obtained via FPXRF (4.9ppm). The trace metals showing the least difference (Cu, Sn and As) indicate that the pseudo-total result obtained via ICP-MS is closer to the total result obtained via FPXRF which indicates that a larger proportion of these three trace metals are acid extractable and, thus, potentially bioavailable in the topsoil analysed. This potential for bioavailability will be examined further in Chapter 6 of this thesis.

When testing for difference, the lower the Asymp. Sig (2-tailed) figure, the greater the statistical significance of observed difference (Krzywinski and Altman, 2013). As the Asymp Sig (2-tailed), which is the p-value for this test, is less than the significance level (0.01), the null hypothesis is rejected and it is determined that there are significant differences between the data sets. This applies to all trace metals studied here except for Copper (Cu). This and the low Z-score for Cu may be due to limitations of the FPXRF methodology as FPXRF has been shown to underestimate Cu concentrations (see section 5.3.2) and result in less of a difference being determined when compared to the pseudo-total obtained via the ICP-MS analytical procedure undertaken here. FPXRF has previously been evaluated for measuring trace metals in soils (Kilbride et al, 2008; Potts et al, 1995) and reported strong linear trends for many trace metals analysed via the FPXRF instrumentation when comparing against aqua regia digestion and Inductively Coupled Plasma analysis (Kilbride et al, 2008). However, poor linearity for Cu has previously been reported (Potts et al, 1995) which was determined to be "due to the Cu concentration in the materials being analysed at or below the detection limit of the FPXRF" (Potts et al, 1995). The descriptive statistics (see Table 5.5) show that the samples used in the current study displayed a wide range of Cu concentrations (0-171ppm) and therefore indicates that the results in this study are both definitive and quantitative despite the reported methodological limitations.

5.3.4 Soil Quality Guidelines, Targets and Limits

The results of the ICP-MS analysis for the pseudo-total of trace metals were assessed against the leading environmental targets and limits that are currently implemented in varying legislation and cited in relevant studies (see Table 5.9). The Finnish legislation for contaminated soil (Decree 214/2007) is of particular interest as they are based on acid extractable analytical techniques which are the recommended analytical methods by the Decree and in alignment with the methodology implemented in this study (Finnish Government, 2007). The Netherlands Target/Background and Intervention values were adapted to the sampling region and determined from the median clay and median organic matter content as determined in the screening study (see Chapter 4) as per the methodology set out in the annexes of the Dutch Target and Intervention Values, 2000 (see Appendix F).

Trace Metal	**Median Result FPXRF (ppm)**	**Maximum Result FPXRF (ppm)**	**Median Result ICP-MS (ppm)**	**Maximum Result ICP-MS (ppm)**	**Netherlands Target/Background Value Limits (ppm)***	**Netherlands Intervention Value Limits (ppm)***	**EC Directive 86/278 limits (ppm)**	**Finnish Threshold Value (ppm)**	**Finnish Lower Guide Value (ppm)**	**Finnish Higher Guide Value (ppm)**
Cu	4.9	171.00	14.7	77.83	19.78	104.37	100	100	150	200
Pb	59.6	443.60	35.9	267.51	57.96	361.40	300	60	200	750
Zn	90.95	2330.70	48.6	892.71	63.46	326.34	250	200	250	400
V	216.4	270.50	12.8	36.64	15.03	89.46	-	100	150	250
Cr	63.8	124.84	16.3	97.76	52.02	197.68	400	100	200	300
Co	31.86	105.90	2.8	7.19	2.28	60.87	-	20	100	250
As	5	34.30	3.5	9.37	18.18	34.49	-	5	50	100
Ni	-	-	11.5	23.40	11.1	66.06	30-75	50	100	150
Sn	1.6	92.02	0.6	6.00	-	218.18	-	-	-	-

- Denotes no available data., * = when using median Clay/OM

Table 5.9: Trace Metal Concentration Data compared to Soil Quality Guidelines for Trace Metals in Soil.

The Finnish Government produced the now widely cited “Government Decree on the Assessment of Soil Contamination and Remediation” (Finnish Government, 2007). When examining the results of the ICP-MS analysis for all sample sites in the region of the Meres and Mosses of the Marches compared to the limits set by the Finnish guidelines, it is observed that all trace metals analysed are below the Finnish Threshold Value, the value at which there is deemed to be negligible risk to soil eco-systems, when examining the median results obtained for each trace metal element. It is also observed that the maximum result for Pb in the sampling area exceeds the Finnish Lower Guideline Value which is the guideline value at which it is considered there is significant risk to a soil eco-system. Nearly a third of all samples taken from the Meres and Mosses region (32.6%) exceeded the Finnish ‘Threshold Value’ for Pb. Whilst Zn has a median value that is below the Threshold Value, it is observed that the maximum value of Zn exceeds the Higher Guide value of 400ppm with 6.7% of the samples taken in the region exceeding the Threshold Value for Zn.

During the screening study, the median results for Pb, Zn, V, Cr and Co were seen to exceed the Netherlands/Dutch Target/Background Value limits by 1.02, 1.33, 14.40, 1.23 and 13.97 times respectively with V also exceeding the Intervention Value limits (see Table 5.9 and Chapter 4) when using FPXRF as the analytical procedure for trace metal concentration. When examining the median results obtained for trace metal concentrations from the ICP-MS with reverse aqua regia digestion methodology and comparing to the Finnish guidelines which implement a similar methodology, it is determined that all the trace metals fall below the Threshold Value. It can be observed, however, that the median result obtained for Co and Ni via ICP-MS does exceed the ascertained Netherlands Target/Background Value for the region. When examining the median and maximum results obtained when using the ICP-MS methodology (see Table 5.9), it is observed that all trace metal elements fall below the Dutch Intervention levels when examining the median results. One trace metal element, Zn, is seen to exceed the Dutch Intervention value when examining the maximum results with 892.7ppm being detected when compared to the Dutch Intervention value of 326.34ppm when using the median clay and organic matter content of the sampling region (see Table 5.9 and Appendix F). It is important to note that whilst FPXRF methods have been widely used in conjunction with the Dutch soil quality standards (VROM, 2000b) for studies of this kind (Venvik and Boogaard, 2020; Cappuyns and Slabbinck, 2012; Dao et al, 2013), the FPXRF analytical procedure is not approved for use by the Dutch Soil Quality Standards (VROM, 2000b) whereas the ICP-MS analytical methodology is approved when implementing these Dutch regulations. When examining the obtained data against the EC Directive 86/278 which applies to land receiving sewage sludge (Council Directive EC 86/278) the median results for both analytical methodologies fall within the limit values for all the relevant trace metals listed. It is important to note that not all sample sites in this study would be applicable for the EC 86/278 guidelines as not all sample sites received sewage sludge application. This study highlights how application of different methodology for determination of trace metal concentration, along with appropriate implementation of soil quality guidelines, can potentially impact the outcome of a soil health survey/assessment.

5.4 Conclusion

The aim of this study was to determine if there was a relationship that could be established between two analytical techniques for determining trace metal concentration in the topsoil of a wetland meres and mosses habitat (FPXRF and ICP-MS via reverse aqua regia extraction) so to determine if the pseudo-total result obtained from the ICP-MS with acid extraction methodology is representative of the total result obtained from the FPXRF analysis implemented in the screening study (see Chapter 4) and vice versa. The findings from the ICP-MS analysis, which is an approved method for the Dutch Soil Quality Standards (VROM, 2000b), can then be placed into context against widely cited environmental targets and limits similar to that undertaken for the FPXRF screening study in Chapter 4 of this thesis but are appropriate when using acid digestion with ICP-MS methodology such as the Finnish Soil Quality Guidelines (Decree 214/2007) and Dutch Soil Quality Standards (VROM, 2000b). This study displayed that differing analytical techniques widely used in the determination of trace metal concentration in a soil matrix can result in significantly different results (as proven by statistical analyses) which have the potential for differing outcomes for adherence to varying soil quality guidelines.

Whilst both FPXRF and ICP-MS are both commonly used for analysis of trace metal concentrations in soil, the two methods have disadvantages and advantages for a given analytical situation. The ICP-MS analytical procedure requires considerable sample preparation, which the FPXRF does not. This sample preparation for ICP-MS also destroys the sample meaning that it requires extra sampling resources than the FPXRF. This ICP-MS procedure, whilst not giving us a full total result due to the limitations of the acid mix being able to break down trace metals from their mineral state, it does provide a good indication of the bioavailability of these trace metals in the natural environment when used in conjunction with leaching studies and sequential extraction techniques (as explored in Chapter 6 of this thesis). The main advantage of the ICP-MS, however, is found in its ability to accurately analyse very low concentrations of trace metals. The limits of detection for ICP-MS are a magnitude lower than in FPXRF and can detect trace metals in the ppb range as compared to the ppm range in FPXRF. This sensitivity can elucidate a greater understanding of the spatial mobility of these trace metals.

The results obtained from this comparison study help elucidate the differences in the analytical methods deployed and ascertain the relationships between the methodologies in terms of how the two datasets correlate and the statistically determined differences between the two. Nine trace metals were chosen for analysis in this study. These trace metals were Cu, Pb, Zn, Co, Cr, V, As, Sn and Ni with the first eight of these being chosen from the outcomes of the screening study (see Chapter 4) and Ni also being latterly chosen for assessment due to its potential impact from anthropogenic sources. This study found that, despite the differences in methodological procedure, the pseudo-total obtained by reverse aqua regia digestion and consequent analysis on ICP-MS does have variable correlation with the total concentration results obtained from FPXRF for the trace metals with all trace metals analysed other than V and As showing significant relationships at either the 0.05 level (2-tailed) or 0.01 level (2-tailed) when implementing statistical analyses on both data-sets (Pearson correlation and co-efficient of determination). The Wilcoxon Signed Ranks Test was performed on the dataset was used to aid in determination of the difference between the two analytical methodologies and determined a significant difference between the ICP-MS and FPXRF methodologies in all trace metals analysed except for Cu. This statistical methodology can aid in estimating the total result from the pseudo-total when differences are observed from the two analytical procedures.

The methodology for the ICP-MS study, using reverse aqua regia, was validated using a certified reference material. This reference material was used so to reflect the type of soil found in the Meres and Mosses region (ERM-CC141 Loam Soil) as determined in the screening study (see Chapter 4). All the relevant trace metals in the certified reference material were recovered at 80-120% of target certified concentration as is standard for method validation recovery of certified reference material (Hibbert, 1999). All instrumentation (FPXRF and ICP-MS) were calibrated for each sample run with checks and standards at regular intervals being implemented using certified trace metal standards. This implementation of a soil reference material in the ICP-MS methodology provides a more robust validation method than implemented in the FPXRF screening study (see Chapter 4) which used a single-element reference material (Ag) for calibration, checks and validation.

The results of both analytical methodologies were assessed against the most cited environmental guidelines. These guidelines are based around potential risk to both humans and the relevant eco-systems. Many factors, including land-use and socio-economic values. The Finnish Government decree on the assessment of soil contamination and remediation needs (Decree

214/2007) recommends analytical methods implementing acid/aqua regia extraction via microwave assisted acid digestion when investigating potential cases of soil contamination (Ministry of the Environment, 2007). When the results of the ICP-MS analysis for nine trace metals were assessed against these guidelines, it was found that the median results for all trace metals across the sampling region fell below the Finnish Threshold Value. The maximum results obtained for Pb and Zn within the sampling region did exceed the Finnish Lower Guide Value with the maximum result for Zn also exceeding the Higher Guide Value. This means that these trace metals exceed the value at which the risk to humans and soil eco-systems can be considered 'negligible' and, under these guidelines, would trigger assessment for soil contamination at those particular sampling sites. Likewise, the Dutch 'Intervention and Target/background' Values are designed to indicate when the functional properties of the soil system are impaired in relation to flora and fauna (see Chapter 4). These guidelines have been widely adopted in academic studies examining potential soil contamination for both the implementation of FPXRF and ICP-MS analytical methodologies despite the FPXRF not being an approved analytical procedure under the Dutch regulations for use with these soil quality guidelines (VROM, 2000b). The results show that the majority of the data obtained from ICP-MS analysis fall below all of the outlined values set in the Dutch standards as amended for the sampling region when using median OM and Clay content (see Appendix F). The exceptions to this are for Co and Ni in which the obtained median pseudo-total result marginally exceeds the Target/Background Values as ascertained for the sampling sites in this study. It was observed that the median results for Pb, Zn, V, Cr and Co exceeded the Dutch 'Target/Background Value' when implementing FPXRF on the topsoil of the Meres and Mosses region with V also exceeding the Dutch 'Intervention Values' which, under the conditions of these guidelines, would warrant further investigation. All median trace metal concentrations for both ICP-MS and FPXRF fell within the EC 86/278 limits relevant to the median pH of the topsoil in the study area (5.88). Due to these increasingly stringent environmental guidelines/limits and with the inherent low concentrations found in meres and mosses type habitats, ICP-MS analysis, with its low limits of detection, should be the preferred analytical procedure for these habitats when studying mobility and occurrence of these trace metals in detail. The advantage of the pseudo-total result is that, not only can it be said to be more accurate due to the low limits of detection, but it can also be used as an indicator of risk due to the reverse aqua regia digestion methodology giving an indication of the amount of trace metal that can be considered as bioavailable. However, there are advantages to the FPXRF methodology. Primarily, FPXRF is rapid and non-destructive. This is beneficial when examining sensitive and protected environments, such as meres and mosses habitats, despite their lack of anthropogenic input. It also means that the same sample can be analysed multiple times for varying properties both in the field and in the laboratory. An increasingly elaborated assessment of these types of habitats can be garnered when both analytical procedures are implemented.

This study has emphasised that, whilst correlations and relationships have been found between the results obtained from the two analytical methodologies at varying strengths, care must be taken to apply the correct guidelines to each analytical technique as each methodology can lead to different outcomes depending on the trace metal being studied. This is especially important in studies in which adherence to soil quality guidelines are the remit of the investigation/study and only approved methodologies for trace metal content are applicable to a set of soil quality guidelines that are being implemented (such as ICP-MS for the Dutch Soil Quality Standards and in Decree 214/2007 Finnish guidelines). Chapter 6 of this thesis will demonstrate that differing soil properties can also have an influence on the bioavailability and occurrence of these trace metals and, consequently, the ecological risk posed.

Chapter 6:

The Influence of Soil Properties on the Leaching of In-Situ Trace Metals in the Topsoil of a Meres and Mosses Habitat.

Abstract

A mesocosm column assay was devised to elucidate the influence of soil properties on the leaching of in-situ trace metals in topsoil over the course of a calendar year (simulated to 12 weeks with one year's equivalent leaching events applied) and their potential for bioavailability in a meres and mosses habitat. Two representative topsoil samples were chosen from the Brown Moss region of the Meres and Mosses of Cheshire and Shropshire following physio-chemical analysis (see Chapter 4 of this thesis). These topsoil samples varied in organic matter content (OM) with one sample of low organic matter content (<15%) and one sample of high organic matter content (>25%). Mesocosms were set up so to represent the topsoil as found in the field (control columns) and with additional pH variations to study the potential impact on the trace metal mobility within the porewater due to anthropogenic influence. The topsoil with >25% OM showed signs of resilience to pH adjustment in comparison to the topsoil with <15% OM with the >25% topsoil type showing less variability of trace metal concentrations in general, with pH variation, than in the topsoil with <15% OM. Examining the trace metal concentration with soil depth found that the >25% OM topsoil showed an increase in trace metal concentration with soil depth in all but two of the column pH variations (Ni in topsoil with >25% OM under circumneutral conditions and Ni in topsoil with >25% OM under basic conditions) for which no change was detected in these two variations which remained at 0ppb (or undetected). This indicates that there is mobility of trace metals through the porewater for the majority of the trace metals analysed for the >25% OM topsoil type. The topsoil sample with <15% OM showed greater influence by pH with soil depth with all trace metals studied showing declining concentrations in porewater when increasing pH with Zn, Pb, Cr, Co and Ni not being detected (on average) under the mildly basic conditions. When decreasing the pH for this circumneutral soil type, results showed that the concentration of Zn, Pb, Co, Sn and Ni increase with depth, with all trace metals studied having increased concentrations under these conditions indicating an increase in magnitude and mobility of these trace metals. A sequential extraction procedure was undertaken on the two topsoil samples so to examine the behaviour of these topsoil under increasingly acidic conditions and to aid in the determination of bioavailability and consequent risk to ecological health. It was determined that the majority of the trace metal concentrations are found in the residual fraction for both of these topsoil types and, as such, undergo little anthropogenic influence in terms

of trace metal contribution. Only Cu, Pb and Zn in the <15% OM topsoil reached the 'low-risk' threshold for the RAC with the remainder being classified as 'zero-risk' for both topsoil types. The garnered results from the mesocosm study aid in the understanding of the spatial mobility and bioavailability of select trace metals which will aid in devising appropriate management, mitigation and remediation methodologies.

6.1. Introduction

Trace metal contamination in agricultural environments and habitats are of considerable ecological concern due to their toxicity, persistence and potential for accumulation (Wong et al, 2002). As a result, these trace metals can adversely influence the plants and soils of the agricultural eco-system and, potentially, human health (Alengebawy, et al, 2021). The properties of soil are known to fluctuate in the short-term.   These variable properties include variations in moisture status, pH and organic content. Soil properties, such as pH, are also prone to variation due to land management changes and environmental factors which can impact the bioavailability of trace metals (Alloway et al, 1995).

The soil profile (sometimes known as 'layers') is defined as "the vertical section of the soil from the ground surface downwards through to where the soil meets the underlying rock" (Sposito, 1999). Each layer is distinctive and show differing characteristics and texture. The topsoil is the top 10 inches, of the soi profile (not including the surface O-Horizon. Known as the A-Horizon (see Fig 6.1), this layer is considered and important component of the soil profile and overall soil functioning due to having the highest concentration of organic matter as well as plentiful microfauna (Gerasimova et al, 2010).

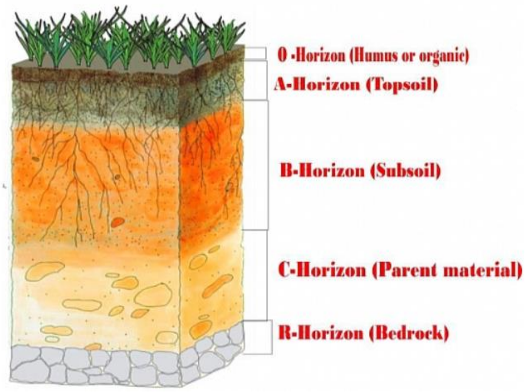


Fig 6.1: Soil Profile (Brown, 2023)

Topsoil is also of vital importance when studying trace metal pollution and their dynamics as it is anthropogenic in nature and potentially more mobile due to erosion and disturbance (Sun and Chen, 2016). Vegetation is often used for reclamation purposes in respect to erosion from air and water and has also been used for mitigation purposes in terms of the downward translocation of pollutants in the soil (Kansas State University, 2002). Plants and vegetation may reduce the leaching of trace metals through decreased infiltration of soil solution, adsorption of trace metals to root surfaces the uptake of trace metals by plants, a process sometimes known as phytoremediation (Yan et al, 2020). Plants may, however, increase trace metal leaching through reactions with soil organic acids which are a result of decomposition of soil organic matter (Schwab et al, 1990) which, as well as potentially altering topsoil pH, may result in increased trace metal mobility (Alloway, 1995).

The pH and OM contents in soil play a vital role in the adsorption of trace metals in these agricultural ecosystems (Weng et al., 2001). Fluctuations in seasonal rain can lead to increase in the solubility of these trace metals (Pan, 2015). Changes in the reactive surface area of the topsoil constituents during seasonal rains can alter the solubility of trace metals (Pan, 2015). The mobilisation and consequent bioavailability of trace metals are potentially further influenced by the release of organic matter following those months that have heavier rainfall during the annual seasons. This is due to certain trace metals, including Cu and Pb, being known to bind strongly to dissolved organic matter (DOM) following heavier rain events (Amery et al., 2007; Koopmans and Groenenberg, 2011).

Studies have previously shown that peat rich soil and those high in organic matter content usually retain more trace metals than mineral soil (Borgulat et al, 2017;Lidman et al, 2014). It has also been shown that an “increase in the pH of acidic soils can increase the adsorption of trace metals to the reactive surfaces of soil organic matter while a decrease in the pH of alkaline soils can lead to desorption of trace metals from these surfaces” (Weng et al., 2001). The interaction between pH and soil organic matter has been researched with studies showing that dissolved organic matter and soil organic matter seems to have impacts on mobilisation factors dependent on pH due to physiochemical properties (Vance and David, 1989) with most trace metals (in free-ionic form) being most mobile in acidic soils (McBride, 1994). Previous studies have shown that pH is a factor in the transport and sorption of trace metals in soils (Chotpantarat et al 2011; Mesquita and Vieira, 2002; Scoullos and Pavlidou, 2003).

In the environmental and geo-sciences, bioavailability is the term used to describe the mechanisms in which various substances in that environment may enter into living organisms and, potentially, the food chain (Semple et al, 2004). These mechanisms are diverse, complex and take many forms (Anderson and Hillwalker, 2008). Bioavailability, in the context of this thesis/study, refers to availability of trace metals in soil systems and is implemented when determining the risk posed by these trace metals and their infiltration/mobility within the topsoil (Hinsinger,2001) and how pH and OM may influence this bioavailability.

The Meres and Mosses of Cheshire and Shropshire have experienced a loss of biodiversity across the area due to increased environmental pressures resulting in reduction in habitat quality (Whild, 2007). Soil pH is of a fundamental concern in the mere and mosses region. Sewage sludge is used as a fertiliser within the region (Milburn, 2018) and widely across the UK (Kirchmann et al, 2017), as well as the application of soil liming in managed areas in which the soil is known to be acidic (Buckle et al, 2024). The area being studied has been recognised as one in which further calcium-based liming would be of benefit for ecological health by the British Geological Survey (BGS, 2023) with the data being available at British Geological Survey agronomy map on the UK Soil Observatory website (BGS, 2023), in accordance with the methodology set out in Bide et al, 2021. Vegetative buffer strips are present that have been established to alleviate diffuse run-off from nutrient loads (Fisher & Acreman, 2004; Fisher et al, 2010). These factors and soil amendments have the potential to alter pH. As a result, there is a variation of pH across the various sites sampled (during the screening study in Chapter 4 of this thesis) within the meres and mosses region that is not naturogenic/geogenic in origin and may be heavily influenced by anthropogenic factors. This may be a significant factor in regard to the fate of trace metals at these sites. It is understood that trace metal mobility is impacted by pH levels in the soil (Richards et al, 2000). The results from the screening study (see Chapter 4) indicate that increasing pH will lower the mobility of these trace metals (and vice versa) and that certain OM levels can alter both the levels of mobility and the fate of metals in the soil due to the lowering of pH on decay, the binding properties of organic matter and the formation of complexes of these trace metals with organic acids (Kalbitz et al, 2000).

6.1.1 Study Aims

The aim of this study is to determine the link between changes in geochemical and geophysical processes with trace metal speciation and bioavailability in topsoil. This will be undertaken by means of a mesocosm study and implementation of chemical fractionation analysis. Specifically, this study will examine this relationship between soil organic matter and the mobility of the in-situ trace metals within the topsoil of a meres and mosses wetland habitat region as well as examining the influence and impacts of varying pH in acid and circumneutral soils by implementation of a column assay experiment. This will be followed by a sequential extraction study which will determine the bioavailability of the trace metals in the topsoil and determine potential risk to the local environment by implementing the risk assessment code (RAC). Risk Assessment Code has been widely used in the environmental sciences for trace metal toxicity assessment (Zhang et al, 2017; Perin et al. 1985; Jain and Ran 2004; Huang et al. 2011).

6.2. Materials and Methods

6.2.1 Study Area and Sampling

Chapter 4 of this thesis revealed that two important drivers for spatial mobility in trace metals in the Meres and Mosses region to be pH and OM. As such, two sampling locations were chosen to be representative of these drivers. A topsoil sample from a region with high OM levels (>25%) in the topsoil was chosen, as is commensurate in constituents with an ‘organo-mineral’ soil that are prominent in the region, as well as a sampling area known to have low levels of OM (<15%) which are commensurate with ‘mineral soils’ that are prominent in the region.

One such site that fulfils these criteria within the region is Brown Moss (see Fig 6.2 and Fig 6.3). Brown Moss (Lat/Long: 52.951081, -2.6533511) is a 77-acre English wildlife and nature reserve site located to the south-east of Whitchurch, in northern Shropshire, UK, as shown in Fig. 6.3 and Fig 6.4. Since 1953, Brown Moss has been designated as a Site of Special Scientific Interest under the stewardship of Shropshire County Council and part of the Midlands Meres and Mosses (Meres and Mosses of the Marches) Ramsar site. The area consists of shallow pools which contain a multitude of flora and fauna, including aquatic birds, migrant and exotic species. The area is popular with those with interests in conservation due to being a local nature reerve (Chaichana et al, 2010). Latterly, the area fell within the boundaries of both the ‘Nature Improvement Area’ (NIA) and ‘Landscape Partnership’ (LPS) schemes with a community focus on conservation (see Chapter 2 and Chapter 4 of this Thesis). For further details on the environmental protection designations of this region, see section 2.1.2. of Chapter 2.

Fig 6.2: Brown Moss Aerial View (Whitchurch Rural Parish Council, 2017)

The site at Brown Moss and the surrounding Meres and Mosses network are, known to be susceptible to anthropogenic pressures, including diffuse nutrient loading from surrounding land and septic tanks, water level fluctuation, pressures from agricultural land management and atmospheric pollution (Chaichana et al, 2010). As a result of these environmental and ecological pressures, Brown Moss (and the wider Meres and Mosses region) has dwindling flora biodiversity (Whild, 2007) which emphasises the importance to conserve and study these areas designated as being environmentally sensitive.

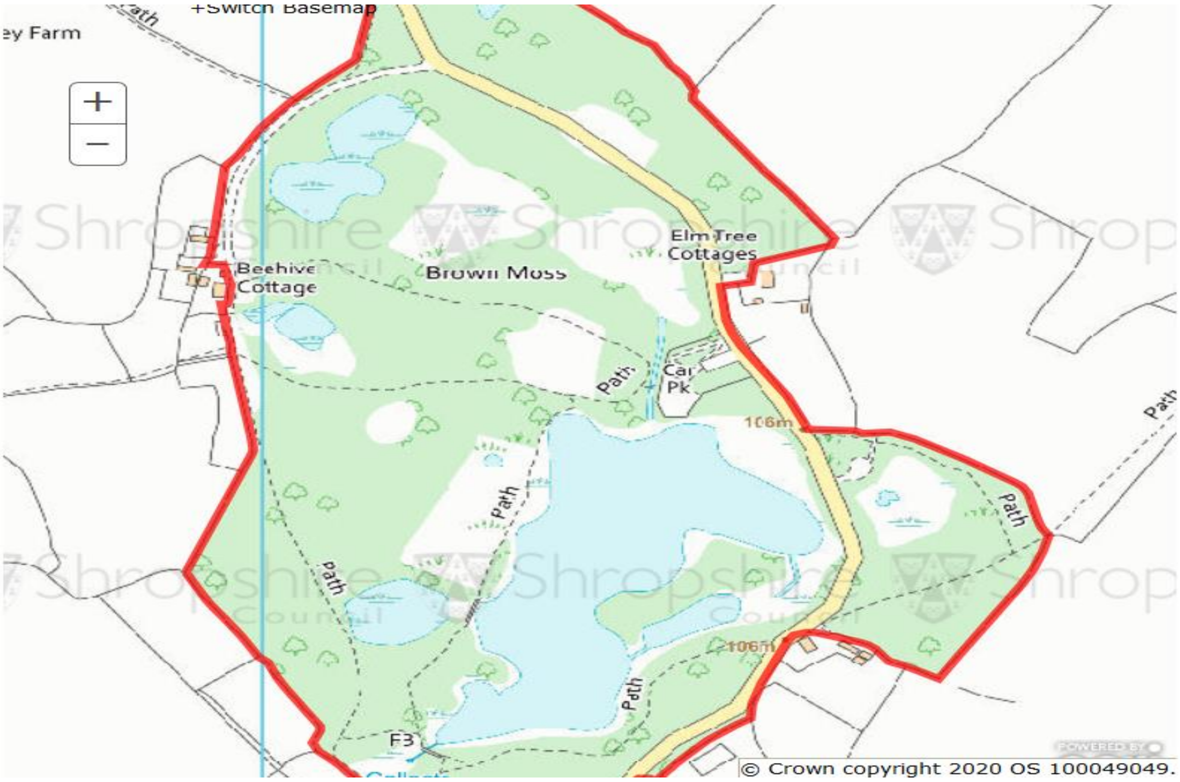


Fig 6.3: Brown Moss RAMSAR Site Boundary Extent (Shropshire's Great Outdoors, CC-2020)

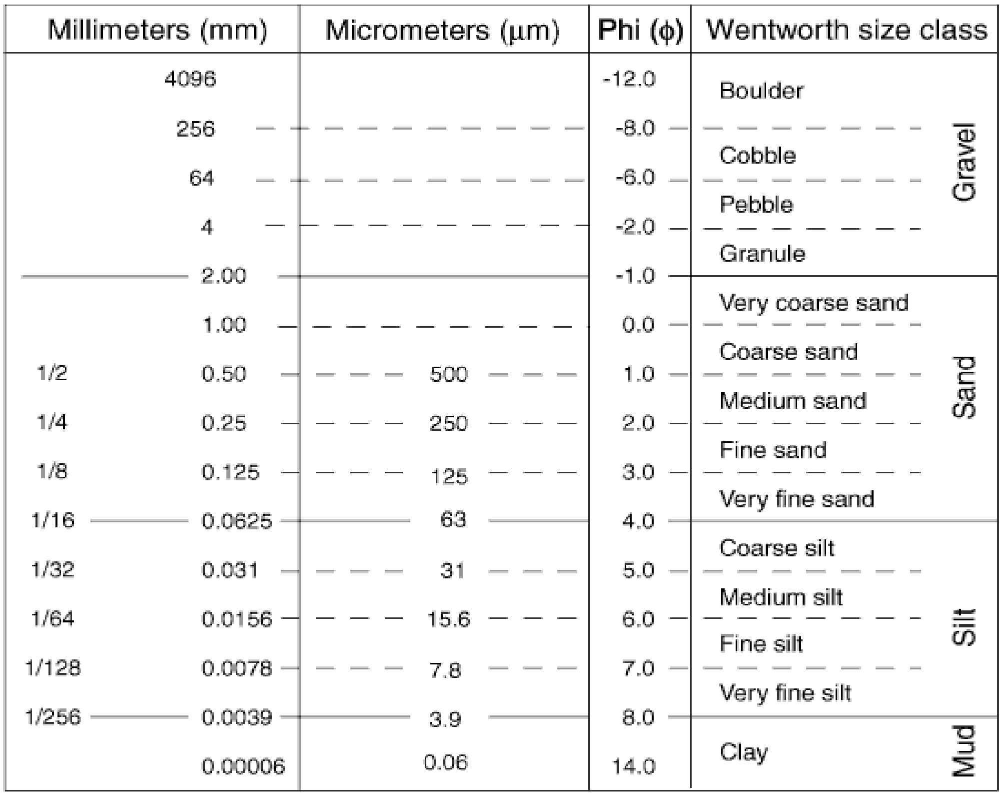
During the FPXRF and physiochemical field screening study for trace metal total concentrations and topsoil characterisation (see Chapter 4), sites of interest were identified, following the obtained results, in which certain factors had relationships (pH, Organic Matter, Trace Metal concentration). The strength of these relationships were determined through Principal Component Analysis (PCA) and, from the physiochemical analysis, two sites were chosen as 'representative' sites for types of soil and their characteristics so that a focussed study could be undertaken on the spatial mobility of trace metals under varying physio-chemical conditions of the topsoil. Sites for the two control samples were chosen based on their organic matter content and pH differences (Padoan et al, 2020). The sites chosen (see Fig 6.4) were those that were of sandy loam type soil that is prevalent (see Chapter 4), circumneutral pH (pH=6-7.5) and low organic matter content (<15%). Sites with these factors are abundant across the region and are representative of managed agricultural areas, often tilled with little tree cover, prone to weathering and open to anthropogenic influence (location Easting 356042 Northing 338278).

Fig 6.4: Map Displaying Sampling Area for Control Columns

Also examined are those sites with a deep, fine loam type soil with an acidic pH (<6) and high levels of organic matter (>25%) as determined in the screening study (chapter 4 of this thesis). These are sites prevalent in the landscape that are associated with the meres and mosses in the region, often with heavy tree and vegetation cover and undergo little anthropogenic impacts, due in part to their protected status (location Easting 355414 Northing 339330). In all, 60% of the overall samples collected in the screening study (see Chapter 4 of this thesis), from a random sampling methodology (see Chapter 2 of this thesis), fulfilled the criteria proposed for the control columns in this mesocosm study (one column with circumneutral pH with OM <15% topsoil and one column with pH<6, with OM >25% topsoil). These two topsoil types were analysed for trace metal concentrations via ICP-MS as part of the analytical comparison study (see Chapter 5 of this thesis) and the pseudo-total results for these two samples can be found in Table 6.7 in section 6.3.4 of this chapter.

6.2.2 Soil Characterisation

The topsoil samples chosen for the mesocosm study underwent granulometric analysis (see Fig 6.5), pH analysis and tests to determine organic content (further details can be found in Chapter 2 and Chapter 4 of this thesis). Soil samples from these sites were bagged up, returned to the laboratory where they were oven dried at 50°C overnight and then sieved to the working fraction (≤2mm). Separately, sub-samples were then sieved between 2mm down to <2µm using a set of sieves and a sieve shaker. Results for each fraction were recorded so to ascertain the granulometric characteristics of the soil sample.



Millimeters (mm)		Micrometers (µm)	Phi (ϕ)	Wentworth size class	
	4096		-12.0	Boulder	Gravel
	256		-8.0	Cobble	
	64		-6.0	Pebble	
	4		-2.0	Granule	
	2.00		-1.0	Very coarse sand	Sand
	1.00		0.0	Coarse sand	
1/2	0.50	500	1.0	Medium sand	
1/4	0.25	250	2.0	Fine sand	
1/8	0.125	125	3.0	Very fine sand	
1/16	0.0625	63	4.0	Coarse silt	Silt
1/32	0.031	31	5.0	Medium silt	
1/64	0.0156	15.6	6.0	Fine silt	
1/128	0.0078	7.8	7.0	Very fine silt	
1/256	0.0039	3.9	8.0	Clay	Mud
	0.00006	0.06	14.0		

Fig 6.5: The Wentworth Scale of Soil Classification (Wentworth, 1922)

Following sieving to the working fraction, the oven dried soil samples were then placed into columns and were identified as control samples due to no further pre-treatment or pH regulation of the samples (see Fig 6.6).

The pH of soil in solution was analysed via a HANNA HI 99121 Direct to soil pH meter (see Chapter 2 and Chapter 4) so to be able to directly measure the pH within the column. The pH of the pore water from the sampling taps was also recorded during each week of sampling.

A Shimadzu SSM 5000A TOC analyser with Solid Sample Module (TOC-Vcs/cp) was used in the determination of Total Organic Carbon in the topsoil samples (see Chapter 2 and Chapter 4 of this thesis).

Samples and sample vessels were oven dried at 50 ^{O}C for 24 hours followed by cooling in a desiccator. Approximately 100mg of soil sample was weighed out. The following procedure was then adopted:

Total Carbon (TC):

Glass fibre wool was placed over each sample in the sample vessel; The sample was placed into the solid sample module (TC portion) and consequently allowed to stand for 2 minutes to allow atmospheric carbon to dissipate within the system. The sample was then run on the solid sample module so to ensure full conversion to CO_2 by undergoing combustion at 900^{o}C in an oxygen stream.

Inorganic Carbon (IC):

When using the Shimadzu SSM 5000A. the Sample was placed into the solid sample module (IC portion) and was then allowed to stand for 2 minutes to allow atmospheric carbon to dissipate within the system. 1ml of Phosphoric Acid reagent was inserted into the vessel producing CO_2 that was purged at 200^{o}C (Shimadzu Scientific Instruments, 2021a). The sample was then run on the solid sample module in accordance with the pre-loaded method. The following calculation was applied (see Equation 6.1) where TC is the result obtained for 'Total Carbon' and IC is the result obtained for Inorganic Carbon (see section 4.2.2 of Chapter 4 for more details):

$$\mathbf{TOC = TC - IC}$$

Equation 6.1: Determination of Total Organic Carbon from Total Carbon and Inorganic Carbon Levels

The results obtained where expressed as 'percentage Total Carbon (TC)' and 'percentage Total Inorganic Carbon (TIC)' that allowed the 'percentage Total Organic Carbon (TOC)' to be calculated from the above calculation (see Equation 6.1) with the results of both the samples being incorporated into the screening study (see Chapter 4 of this thesis)

The Loss on Ignition (LOI) is widely used analytical procedure to determine the overall organic content in a particular soil sample (Heiri et al, 2001). The calculated amount of mass lost following heating is reported as percentage LOI. The following procedure was performed:

Crucibles were oven dried at 50°C for approximately 1 hour then left to cool for 1 hour. The crucibles were then removed and weighed with the result recorded (Wc). Approximately 2g of soil sample was then added to the crucible and this was dried overnight at 105°C and then left to cool for approximately 1 hour in a desiccator. The crucibles were reweighed (Ws). Crucibles were then placed into the furnace at approximately 550°C for 4 hours and then allowed to cool in a desiccator before reweighing (Wa). From the following calculation (see Equation 6.2):

$$\mathbf{LOI = (Ws\text{-}Wa) / (Ws - Wc) \times 100}$$

Equation 6.2: Determination of Organic Matter Content via Loss on Ignition

Loss on Ignition of each sample was thus determined via the calculation above and results recorded. Further details on the above two analytical procedures can be found in Chapter 2 and Chapter 4 of this thesis with the relative magnitude of the results of the LOI for each of the two soil types being referenced throughout this chapter (OM>25%=High, OM<15%=Low)

6.2.3 Mesocosm Column Topsoil Porewater Leachate Study

As outlined in Chapter 2 (section 2.4.4), mesocosm experiments are those that are undertaken in controlled laboratory conditions with a view of simulating the conditions in the field. For these experiments, columns are used as proxies for natural habitats (Bleuven et al, 2020). These soil column studies aim to mimic the composition of the natural habitat being studied whilst affording control over physicochemical factors which aids in elucidating the mechanisms within the topsoil (Fraser & Keddy, 1997). Disturbed, homegenised soil columns were implemented in this study. As the A-horizon of the soil (topsoil) is constantly under flux from environmental and anthropogenic pressures (Darmody et al, 2009; Fox et al, 2010), disturbed soil columns could be implemented without compromising field topsoil conditions, which are prone to disturbance. This implementation of disturbed columns has the benefit of being able to evaluate multiple columns (in duplicate) based on the potential for disturbance observed in the field (Treese et al, 2010).

Following oven drying at 50°C overnight and sieving to ≤2mm, topsoil samples from the identified locations were added to a total of 12 mesocosms (6 variations with 1 duplicate each for replication purposes). The mesocosms were constructed as per Lynch, 2015 and were made out of uPVC (unplasticised polyvinyl chloride) drainpipe and were approximately 30cm in height with a base attached and sealed. Holes were drilled into the mesocosms to represent 10-12cm below soil surface and at the bottom (22-24cm) of the mesocosm to represent the base of the A-Horizon to be able to determine metal mobility through the A-Horizon. Sampling port taps were attached to polytetrafluoroethylene (PTFE) tubes and fitted into the designated sampling holes. To prevent leakage from tube bases, sealant was applied. These mesocosms were designed to vary the pH levels for a given soil depending on their levels of organic matter and to receive the appropriate amount of artificial rainwater (ARW) based on the precipitation data for the region in question determined from the data obtained from the local climate centre to the sampling region (Shawbury). Each column had a duplicate for analytical replicability and were set up as follows (see Fig 6.6):

Fig 6.6: Photo (above) and Schematic (Below) showing layout and content of Soil Columns in duplicate in which Low pH = <6, ≈Neutral pH = 6-7.5, High pH = >7.5, Low OM = <15%, High OM = >25%.

Each mesocosm was filled with soil 24 cm deep using a plastic trowel. All sample points were numbered. Sample points were located at the top (0-2cm deep), middle (10-12cm deep) and bottom (22-24cm deep) of the soil depth (Lynch, 2015; Lynch et al, 2018). Soil solution was sampled directly from the top of the soil (0-2cm deep) using a filter syringe (MacroRhizon soil sampler) and from sampling ports for the middle sampling point (10-12cm deep) and the bottom sampling port (22-24cm deep) using a filter syringe with the sampling ports. Control columns were determined via the physio-chemical properties (see Chapter 4) of topsoil under 'no addition/amendment' (Janus et al, 2018) conditions ($^{i.e.}$ High OM = Low pH). pH variation was undertaken by the addition of HNO_3 (Chotpantarat et al, 2011) or $CaCO_3$ (Janus et al, 2018) to adjust pH levels to the level desired for a particular column/mesocosm so as to represent the hypothetical varying environmental pressures that the topsoil in the region is under. Calcium Carbonate ($CaCO_3$) was also used to represent the agricultural process of 'liming' in the study (Alkaline/High pH, Low OM) which remains the principal input to neutralise soil acidity in the UK to enhance crop production (Holland et al, 2019). So to maintain the pH level at the correct parameters, a pour-through technique was undertaken with HNO_3/$CaCO_3$ solution until the pH reading required was obtained for all sampling points/ports on each column (Chotpantarat et al, 2011; Janus et al, 2018; Lynch, 2015). The range of both the pH and the organic matter levels were based upon the results obtained from the screening exercise of the topsoil in the region in Chapter 4. This screening showed a minimum pH of 3.75 within the region and a maximum pH of 8.02 for the topsoil when examining the 89 sampling locations (see Chapter 4).

In order to simulate the natural seasonal precipitation in the Meres and Mosses region, artificial rainwater (ARW) was implemented on the mesocosm study. Based on the Plynlimon rainwater chemistry study (Neal et al, 2001), ARW with a pH 4.9-5.2, was formulated and added to the mesocosms so to represent monthly average rainfall and to simulate the natural annual environment in the Meres and Mosses region in a simplified fashion (see Table 6.1). This was done to show how monthly variation can also impact the metal mobility in the Topsoil/A-Horizon with results recorded corresponding to each calendar month (see Appendix D, Fig D1). The ARW was applied over the course of the week/month using a 500ml watering canister with sprinkler attachment so to represent the monthly figures obtained from the Shawbury Climate station, spread across the week to represent a calendar month and in proportion so to represent days of rainfall ≥1mm (see Table 6.2). Prior to commencement of the experiment, all mesocosms were kept at field capacity for three weeks (Lynch et al, 2018). As per Lynch (2015) "Twice a week 500ml ARW was added via the top of the mesocosm until water percolated through the bottom tap". This was performed so to let the soil equilibrate (Lynch, 2015; Lynch et al, 2017). Araya (2005) states that, "Field capacity refers to the water content of soil that has been allowed to drain freely from saturation with negligible loss due to evaporation" (Townend et al., 2001; Araya, 2005).

Salt	(mg/L)
K_2SO_4	0.2
$CaSO_4.2H_2O$	0.74
$MgSO_4.7H_2O$	0.56
NaCl	4.47
$MgCl_2.6H_2O$	1.36
NH_4NO_3	0.726
$(NH_4)2SO_4$	0.18

Table 6.1: Composition of ARW based on Plynlimon Rainwater Chemistry (Neal et al, 2001; Lynch, 2015)

ARW was then added, over the course of the week/month, to each column commensurate with the rainfall data obtained from the Shawbury Weather Centre (see section 2.1.3 of Chapter 2 and Table 6.2) and converted to the area of each mesocosm (78.54cm^2) as shown in Table 6.2. This ARW was allowed to settle prior to each sampling session. The experiment lasted 12 weeks with each week representing 1 calendar month in the field. To determine the ARW required in ml for each column, the catchment area, in this case the area of the column, is multiplied by the depth of rain that falls in the Meres and Mosses region (data provided by Shawbury Weather Centre, see Table 6.2) to give the total volume of water produced (see Equation 6.3). Factors such as evaporation, wetting, and soaking into the ground are not considered here. The following equation was implemented:

Catchment Area (m^2) x Average Monthly Rainfall (m) = Total Rainwater Falling on the Catchment in an Average Month (m^3)

Equation 6.3: Determination of ARW Volume Required per Column (adapted from Lancaster and Marshall, 2013)

When applied to the mesocosm study, the catchment area is column area in m^2 x weekly rainfall figure (m) = Total weekly with each week representing a calendar month (^{ie} where January = week 1, February week 2 etc..) and data taken from the average monthly figures for rainfall in Shawbury between 1981-2010 (see section 2.1.3 of Chapter 2 of this thesis). Days of rainfall ≥1mm were taken into consideration when applying the ARW throughout each week (see Table 6.2).

Data	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Shawbury Average Rainfall Data 1981-2010 (mm)	56.3	39	46.5	49.1	53.5	53.4	53.9	59.4	57.2	67.8	61.5	62.3
Days of Rainfall ≥1mm	11.73	9.53	11.03	10.6	10.00	9.53	9.57	9.93	9.20	11.40	12.00	11.98
ARW added to Column over the course of a week (ml)	442	306	365	386	420	419	423	467	449	533	483	489

Table 6.2: Addition of ARW to Columns per Week

At the end of each week, the leachate from each mesocosm sampling point was collected into a 50ml centrifuge tube. This leachate sample was then filtered through a 0.45µm syringe filter, acidified (approximately 2%) with trace metal grade HNO_3, and stored/refrigerated in a 15ml centrifuge tube for further analysis. The leachate was analysed for trace metals (Pb, Zn, Cu, Co, Cr, Ni, As, Sn and V) via ICP-MS analysis as per the methodology for the ICP-MS instrument as set out in Chapter 3 and Chapter 5 of this thesis. In total, 36 leaching samples were taken for each column over the course of the study.

6.2.4 Chemical Fractionation of Trace Metals and Risk Assessment Code

In the topsoil, trace metals are found in multiple fractions when taking various physiochemical forms (Silwana. et al 2017). These fractions are known as the exchangeable, carbonate bound, Iron (Fe)/Manganese (Mn) bound, organic matter bound and the residual fractions (see Chapter 1, Fig 1.5). As outlined in Chapter 1 (section 1.5), The most mobile of these fractions are the exchangeable and carbonate fractions which are highly bioavailable (Singh and Kalamdhad, 2013). Increasingly acidic conditions are required to leach out the remaining fractions including those trace metals bound to their mineral forms which are resistant to environmental flux and thus remain unavailable for uptake (Sebasthiar et al, 2005).

For the purposes of this study, a sequential extraction procedure was performed in accordance to the methods set out by the Standards, Measurements and Testing programme of the European Union (SM& T) so to garner additional information on the potential effects of environmental or anthropogenic influence on the lability/mobility of the trace metals in the topsoil by introducing increasingly harsh conditions in a step-wise process (de Andrade Passos et al, 2010; Padoan, 2020) to the control topsoil samples. This was for the determination of 9 trace metals, based on those trace metals known to be commonly from anthropogenic sources such as agricultural practices (Panagos et al, 2018, Mirzaei et al, 2015, Gasparatos et al, 2018) such as Cu and Ni as well as those trace metals found to be enriched in samples analysed during the field study in chapter 4 of this thesis (Pb, V, As, Zn, Co, Cr, Sn) in the topsoil samples collected from the Meres and Mosses region of Cheshire and Shropshire at the sites that were identified as sites that are representative of those in the wider region (see section 6.2.1). These samples were designated control samples in the mesocosm study and were characterised by having high organic matter levels with low pH and with low organic matter with approximately neutral pH levels (see section 6.2.1). Trace metals associated with the more readily soluble fractions, such as exchangeable and carbonate fractions, are more likely to be of anthropogenic origin whereas trace metals in the residual fraction are generally of natural origin (de Andrade Passos et al, 2010).

The procedure was undertaken in the following steps:

Step 1 (exchangeable/carbonate fraction):

40ml of 0.11M acetic acid were added to 1.0g of dry soil in a 250ml PTFE vessel. The vessel was then shaken for 16 hours (overnight) at ambient temperature by means of a rotary shaker at the speed of 80rpm. Centrifugation apparatus were then deployed and the extract was separated from the solid residue at 3500 rpm for 20 minutes. The remaining supernatant was decanted into a sampling tube. This sample was then analysed. The remaining residue was rinsed by adding 10ml of deionised water, shaking for 15 minutes and further centrifuging for 20 minutes. The supernatant of this rinse was then discarded, whilst retaining the solid residue which underwent ‘Step 2’.

Step 2 (reducible fraction):

40ml of 0.1M hydroxylammonium chloride (adjusted to pH=2 with nitric acid) were added to the residue in the centrifuge tube and then transferred to a 250ml PTFE vessel. The extraction procedure was then performed as described in step 1. After rinsing, the solid residue underwent ‘Step 3’.

Step 3 (oxidisable fraction):

10 ml of 8.8M hydrogen peroxide were added dropwise to the residue which had been placed into a 250ml beaker. The beaker was covered with a watch glass and left at ambient room temperature for

1 hour whilst shaking periodically. This procedure continued for a further 1 hour at 85°C and the until the volume of the residue was reduced to a few ml by heating of the uncovered beaker on a hot plate. A further 10ml of 8.8M hydrogen peroxide was then added to the residue. The beaker was covered, heated to 85°C for 1 hour. The cover was then taken off and the volume reduced almost to dryness. After cooling, 25ml of 1M ammonium acetate (adjusted to pH 2 with nitric acid) were added to the residue. The contents were transferred back to the 250ml PTFE shaker vessel and the extraction procedure was the carried out as per steps 1 and 2.

A residual step was also performed in accordance with ISO11466 using aqua regia as the digestion medium. The remaining material from the 3-step sequential extraction was reduced to dryness prior to sub-sampling for digestion to determine contents of the Residual fraction. After a period of cooling at room temperature for approximately 1 hour. Approximately 0.3g of the remaining sample/residue were placed into a microwave vessel followed by addition of 9ml of Aqua Regia (3 parts HCl to 1 part HNO_3). Aqua regia was the chosen digestion medium for this aspect so to be consistent with previous studies implementing a residual fraction when adapting the BCR protocol (Abollino et al, 2011; Tokalioğlu et al, 2003). Aqua regia and reverse aqua regia have been found to give similar and comparable recovery results when measured against values of standard reference materials (Lu et al, 2007). Microwave settings were as follows in Table 3:

Digestion Method	**Ramp Time (mins)**	**Hold Time (mins)**	**Max Temperature (°C)**	**Power (W)**
Aqua Regia (12ml)	20	5	200	1300

Table 6.3: Microwave Digestion Settings

The residual step was determined through the method determined in chapter 3 following aqua regia digestion using ICP-MS analysis (see Chapters 2, 3 and 5) and in accordance with ISO11466. Upon completion of the 4 sequential extraction steps, the RAC could be determined (see Equation 6.4).

When determining the Risk Assessment Code (see Equation 6.4), the following equation is implemented:

$$RAC(\%) = \frac{C_m}{C_t} \times 100$$

Equation 6.4: Risk Assessment Code

Where Cm is the concentration of the trace metal found in the exchangeable/carbonate fraction and the Ct being the 'total' concentration of the trace metal in the sample obtained from the sum of the fractions for a particular trace metal in the sequential extraction procedure (Soares et al, 2015; Ates et al, 2020).

The RAC has the following classifications for environmental risk:

Zero (RAC < 1), low (1 < RAC < 10), medium (10 < RAC < 30), high (30 < RAC < 50) or very high (RAC > 50) risk (Bo et al, 2015; Perin et al. 1985).

A mass balance calculation will be performed on each element when comparing the outcome of the total of the sum of each fraction for the trace metals of interest when comparing to the result obtained from the pseudo-total (ICP-MS with reverse aqua regia digestion. This mass balance recovery was calculated by summing up the concentration of each of the trace metals in the different fractions and then comparing with the pseudo-total concentrations obtained for the trace metals (Oyeyiola et al, 2011) as shown in Equation 6.5 and Table 6.7).

$$= \frac{[\sum \text{sequential extraction procedure}]}{\text{single digestion with strong acids}} \times 100$$

Equation 6.5: Mass Balance Equation for Trace Metals

Correlations will be determined between the pseudo-total results obtained for each sample and the sum of the fractionation steps so to assess if one analytical procedure can predict the outcome of the other (coefficient of determination) and to determine the validity of the methodology (Soares et al, 2015). This will be done by determining the Pearson correlation coefficient between the two sets of data so to determine the linear correlation. Statistical assessment within this chapter was undertaken using SPSS Statistics 26.0 software with charts and visual representation from use of Microsoft Excel 365 software.

6.3. Results and Discussion

6.3.1 Topsoil Properties

Granulometric analysis was undertaken on the two control column topsoil samples so to establish their constituent makeup (see Fig 6.7).

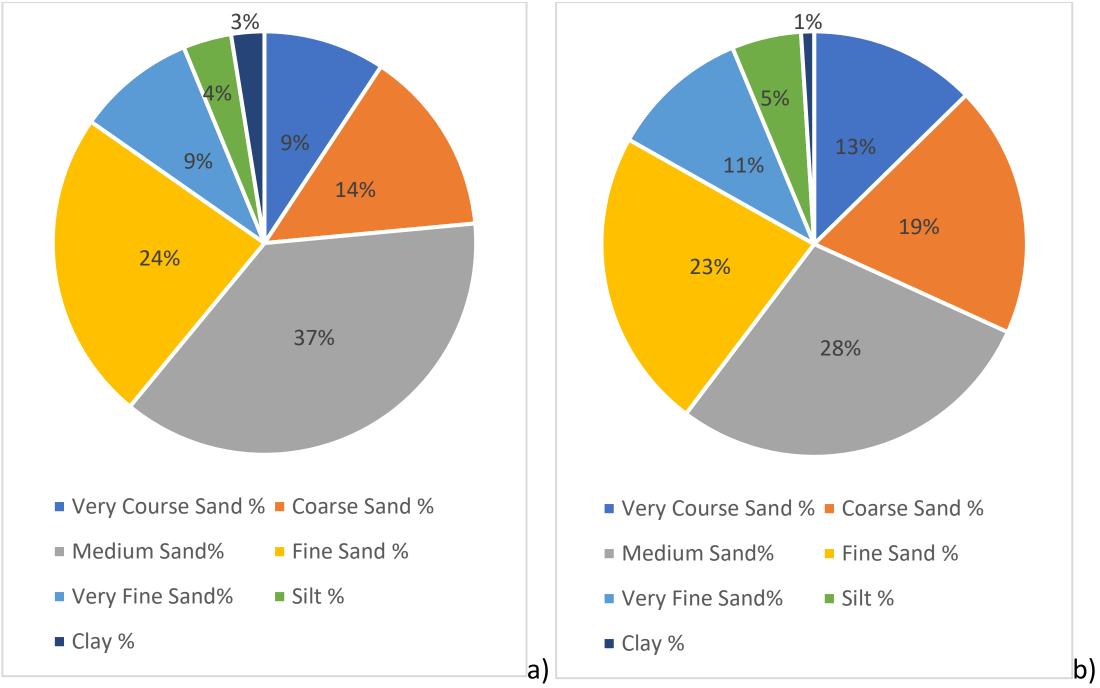


Fig 6.7: Constituent Makeup of Topsoil Control Column Sample with OM>25% (a) and OM<15% (b).

Granulometric analysis of the chosen topsoil samples show that both soils majorly consist in the ‘medium sand’ category (250 microns). The two topsoil samples appear similar in constitution, however it is observed that control column with low OM (<15%) has larger proportions of ‘Coarse Sand’ and ‘Very Coarse Sand’ compared to control column with high OM (>25%) which has a larger proportion of ‘Medium Sand’ based on the Wentworth size classification system (see Fig 6.5). Both topsoil samples had a similar proportion of silt, very fine sand and fine sand in regard to the Wentworth Classification System. The composition of the high OM (>25%) control column is consistent in the pH variation columns with high OM whereas the composition of the low OM (<15%) control column is consistent with the pH variation columns for low OM.

Control Column	LOI (%)	TOC (%)	pH
OM<15% with Circumneutral pH	8.79	4.56	6.65
OM>25% with pH<6	42.1	24.18	4.10

Table 6.4: Median Organic Content and Median pH of Control Column Samples

From the median results of the descriptive statistics (as per the methodology described in section 4.2.2 of Chapter 4), it can be seen that the pH of the control columns are as expected, with the topsoil with OM content >25% also having acidic properties, most likely due to decaying OM and consequent release of humic acids and the <15%OM soil having circumneutral pH (see Table 6.4). The pH of each column was maintained and recorded every week (see appendix E Table E1) except for the pH of the control columns which were simply recorded due to having no additions other than ARW (see section 6.3.2).

6.3.2 Mesocosm Column Topsoil Porewater Leachate Study

The median results of the trace metal concentration in the porewater that were recorded weekly from each column variation, and their duplicate, were analysed, creating an annual depth profile for trace metal concentration in the pore water, so to observe the trends in mobility of trace metals under varying pH and OM conditions representative of a calendar year's sampling corresponding with depth. When the results of the columns are compared, we can see how pH and OM impact the potential for spatial mobility and consequent bioavailability of the trace metals within the A-Horizon. Following the recording of weekly data for each column and its duplicate for trace metal concentration in the pore water (see Appendix D, Fig D1), median concentrations with depth were determined using the results obtained for the three sampling points, top point (0-2cm depth), middle port (10-12 cm depth) bottom port (22-24cm depth) respectively. The parameters for this study were that the organic matter content had a high OM = >25% LOI and a low OM = <15% LOI with soil pH ranging from low/acidic (<6), circumneutral (6-7.5) and basic/high (>7.5). The organic matter content remained unchanged for each pH variation column to that of their control (see Fig 6.6). The control samples were found to have a median LOI of 8.79% for the low OM (<15%) soil type and median 42.1% for the high OM (>25%) soil type (see Table 6.4) with the pH range being 3.6-4.4 for the high OM (>25%) control and 6.4-7.2 for the low OM (<15%) control. The pH range of all columns studied was found to be the following: High pH columns (>7.5) = 7.7-8.4, circumneutral pH columns (6-7.5) = 6.4 – 7.3 and low pH columns (<6) = 3.3-4.3 (see appendix E, Table E1 for weekly pH results).

Graphs were drawn up to provide a visual representation of the central tendency of the occurrence of trace metals at depth over the course of the 12-week study so to represent 12 months in the field. Observations could then be made on the impact of pH variation on these two soil types in studying trace metal concentration in topsoil porewater with the depth of the topsoil. This was done by determining the median result of trace metal concentration (over the 12-week study) of the obtained median result (for the duplicate columns) for each weekly sample from each sampling port (see Appendix D) so to ensure outlier results did not influence the data.

The control columns were those first studied. These were the columns with low pH (<6) with High OM (>25%) and Circumneutral pH (6-7.5) with Low OM (<15%). The observations from these control columns were then compared with the columns undergoing pH adjustment on the same soil type with reference to both the temporal fluctuations observed in the weekly analysis (see Appendix D, Fig D1) and the obtained pseudo-total results via ICP-MS analysis for the control samples as per the methodology set out in Chapter 5 of this thesis (see Chapter 5 and Table 6.7).

6.3.2.1 Observations for Soil Columns with >25% Organic Matter (OM). Soil columns with >25% OM content were set-up so to have a column with no pH adjustment (pH<6) as the control with two further columns having their pH adjusted, one with circumneutral pH (6-7.5) and one with basic pH (>7.5). These columns were run in duplicate over the course of the 12-week study (see Fig 6.6) and median results recorded to represent the central tendency of the obtained results for the concentration of each trace metal element in the porewater. Average annual concentration with topsoil depth were then determined from these temporal porewater concentrations (see Figs 6.8-6.16 and Appendix D, Fig D1).

As Figs 6.8-6.16 shows, median trace metal concentration increases with depth for all of the trace metals studied in the zero additions control column with a pH<6 and OM>25%. This increase of concentration with depth indicates that these trace metals are mobile under these physiochemical conditions.

Zn concentrations are found to increase with depth with all pH variations studied (see Fig 6.8). The concentration of Zn in the porewater is most abundant under acidic (pH<6) conditions (the control of this soil type) and decreases with increasing alkalinity. When examining the temporal variations of these trace metal elements (see Appendix D, Fig D1), Zn is observed to be detected in the porewater from all 3 sampling areas of the column when compared to the topsoil of this type that has had its soil pH adjusted via $CaCO_3$ to alleviate the soil acidity. This observation is reinforced when examining the median concentration results for Zn of the mesocosm studies with OM>25% and pH adjustment with the control column (pH<6) showing Zn present in the porewater from the top through to the bottom of the column. This indicates that pH plays a role in Zn mobility in the porewater of a topsoil of this type.

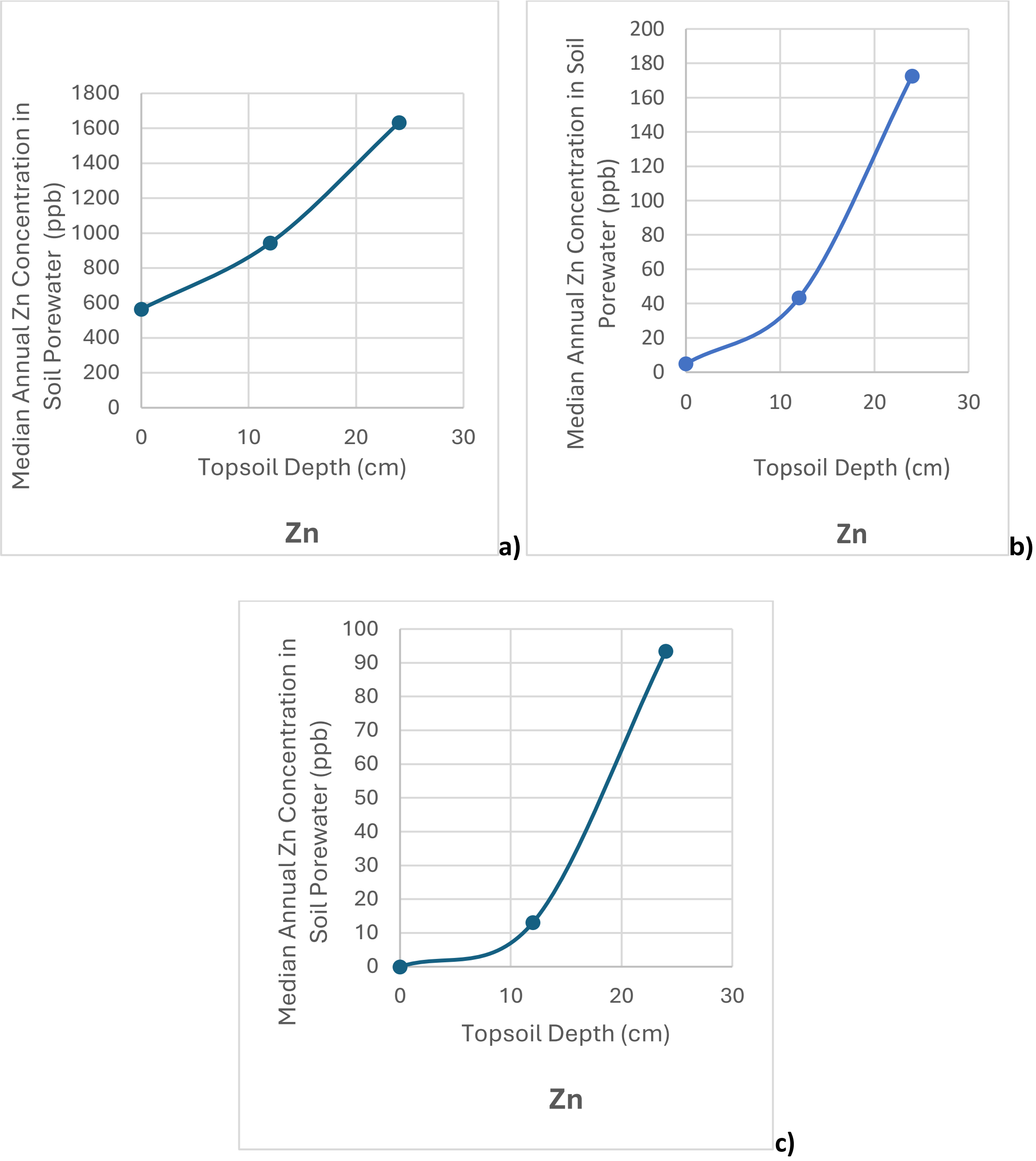


Fig 6.8: Median Annual Concentration of Zn with Depth in Topsoil Porewater for Columns with OM>25% and pH<6 (control) (a), pH=6-7.5 (b) and pH >7.5 (c)

Pb concentrations are found to increase with depth with all pH variations studied (see Fig 6.9). The concentration of Pb in the porewater is most abundant under basic (pH>7.5) conditions with the acidic control column (pH<6) showing the least amount of Pb in the porewater of this topsoil type. When studying the temporal fluctuations, Pb displays an initial concentration that dissipates over the course of the study (see Appendix D, Fig D1) for all pH variations in this topsoil type. This indicates that Pb is released from the topsoil readily and mobile within the porewater. The control sample with zero additions (pH<6) shows a rapid decline (over the first 3 weeks of analysis) in Pb concentration in the pores from the bottom sampling areas of the column when compared to the soil that has had its soil pH adjusted via $CaCO_3$ to alleviate the soil acidity (see Appendix D, Fig D1), which show an increased level of Pb detected for this soil type as the soil becomes less acidic (see Fig 6.9). This indicates that Pb concentrations found in the porewater of the topsoil with >25% OM is heavily influenced by topsoil pH.

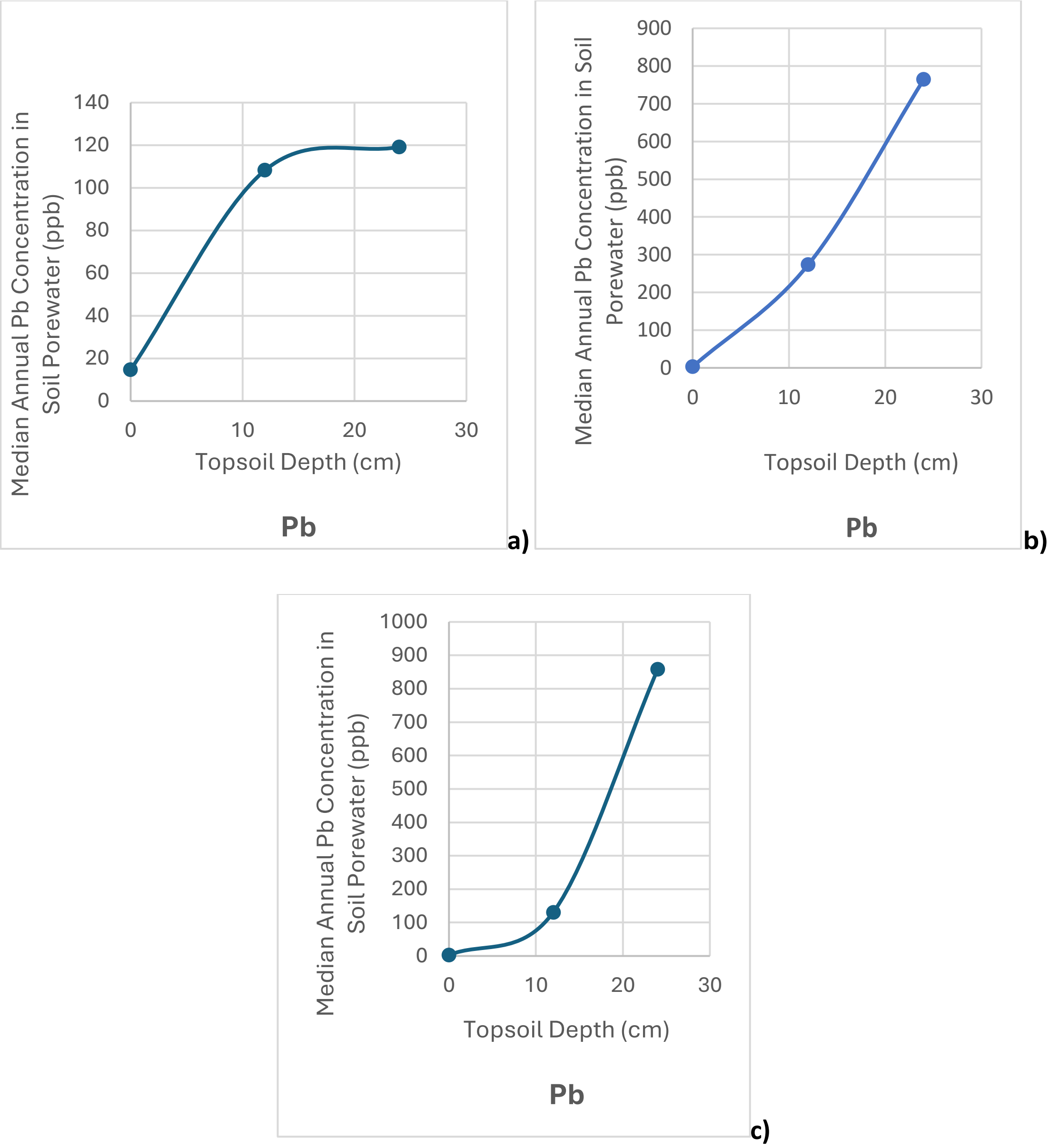


Fig 6.9: Median Annual Concentration of Pb with Depth in Topsoil Porewater for Columns with OM>25% and pH<6 (control) (a), pH=6-7.5 (b) and pH >7.5 (c)

All column varieties of pH in a topsoil with OM>25% show an increase in average V concentration with depth (see Fig 6.10), although this increase is less pronounced in the acidic, control topsoil. The circumneutral and basic pH columns have similar concentrations of V with depth which are both at a slightly increased level from the control for OM>25%. The results show how V concentrations vary depending on the pH of a topsoil with OM>25%. All columns display only low amounts of V concentration on average (<15ppb) when analysing the pore water. From examining the pseudo-total results for this sample (see Table 6.7), we can see that V is present at 2.51ppm. This indicates that V is not readily released into the pore water under the potential conditions applied by the column study (OM>25%). There is a slight increase of V concentration in the porewater as pH is raised with both the circumneutral Column and the pH>7.5 column showing increased V concentration in the porewater throughout the 12-week study when compared to the the control, 'no additions' column (see Fig 6.10). The soils that were adjusted to a circumneutral (pH=6-7.5) or basic conditions (pH>7.5) show similar behaviour to each other with V being found up to approximately 20ppb range for the latter 7 weeks of analysis from the bottom sampling port with the middle and top ports only showing trace amounts of V (<10ppb) for the duration of the study when examining temporal variation (see Appendix D, Fig D1).

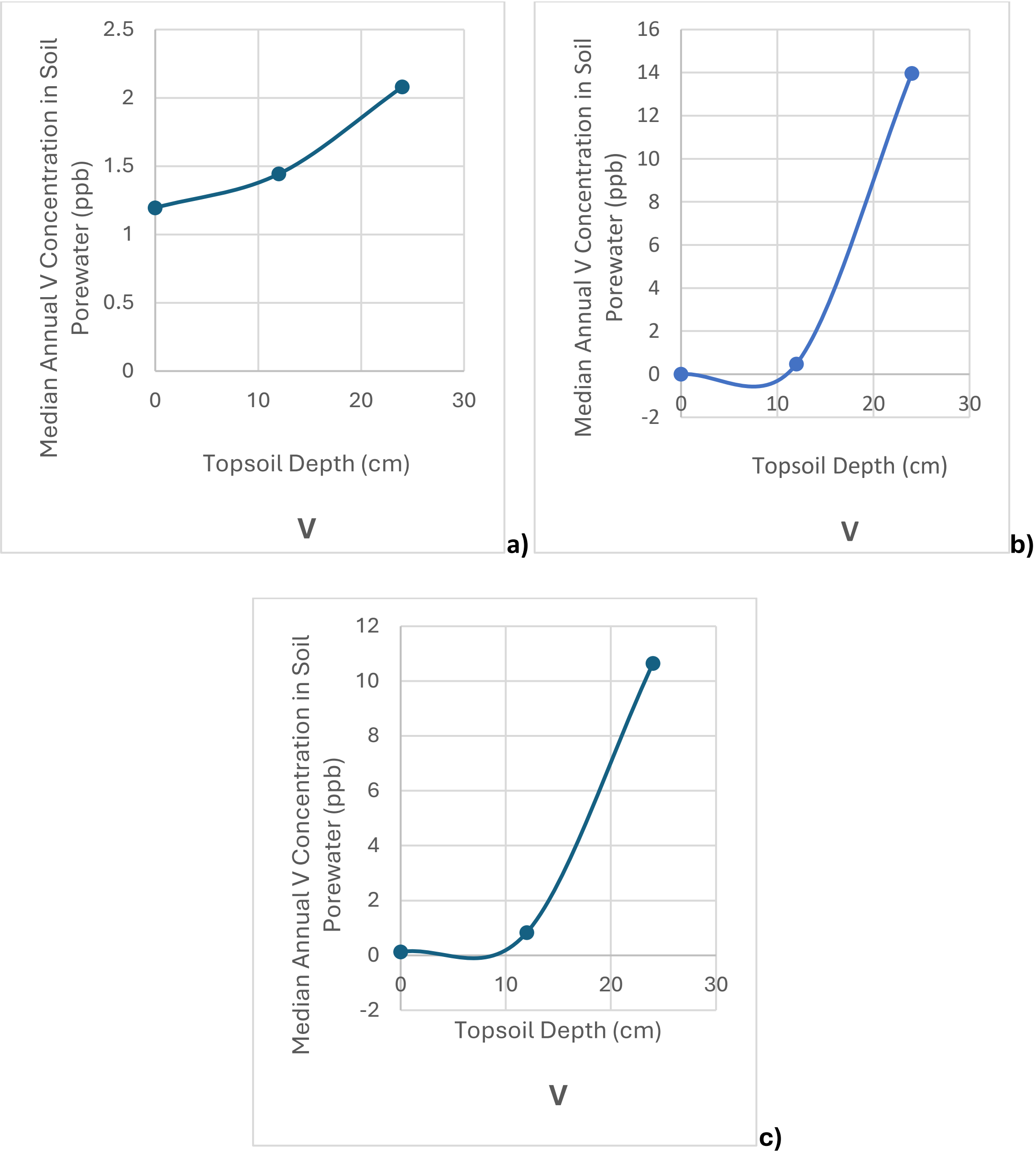


Fig 6.10: Median Annual Concentration of V with Depth in Topsoil Porewater for Columns with OM>25% and pH<6 (control) (a), pH=6-7.5 (b) and pH >7.5 (c)

When studying the concentrations of Cu in the topsoil porewater with the depth of the topsoil (see Fig 6.11), it is observed that the concentration of Cu increases with depth for all pH variations of this topsoil type and that these concentrations are higher in the circumneutral (pH=6-7.5) and basic (pH>7.5) pH variations than for the control (pH<6). Cu concentrations vary depending on the pH of a topsoil with OM>25%. It is seen that the control column for the topsoil with OM>25% (pH<6) displays a pattern that would indicate a short (during the third week of analysis) but significant release of Cu into the porewater (see Appendix D, Fig D1). ICP-MS analysis with reverse aqua regia digestion showed a pseudo-total of 10.49ppm (see Table 6.7) for this soil type. This analysis indicates that the conditions in the column enable Cu to be released into the porewater. When examining the temporal variation, we can see that, apart from this release of Cu in the third week, only very low amounts of Cu are detected (see Appendix D, Fig D1). There does appear to be a low but steady Cu concentration in the porewater as pH is raised with both the column with circumneutral pH and the pH>7.5 column showing increased levels of Cu in the porewater when compared to that of the control column (see Fig 6.11). In these 2 columns, which have undergone pH variation, it can be seen that, when examining temporal fluctuations (see Appendix D, Fig D1), the concentrations of Cu are consistently higher in the bottom sampling port indicating that Cu is moving down the column through the porewater under the conditions in the column over time.

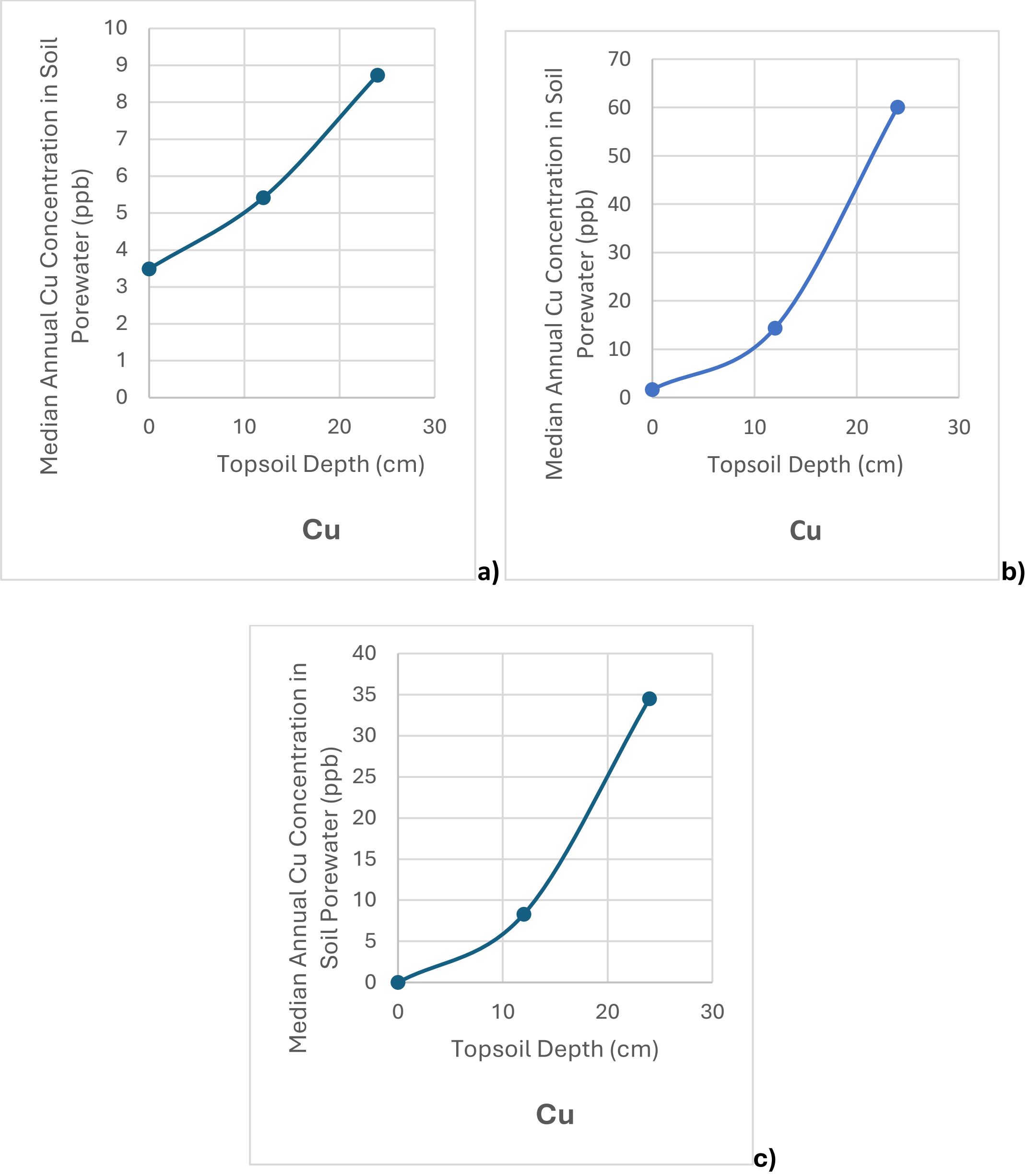


Fig 6.11: Median Annual Concentration of Cu with Depth in Topsoil Porewater for Columns with OM>25% and pH<6 (control) (a), pH=6-7.5 (b) and pH >7.5 (c)

Cr concentrations are seen to vary depending on pH of a topsoil with OM>25% (see Fig 6.12). We can see that the control column for the topsoil with OM>25% (pH<6) displays only a low level of Cr concentration in the porewater over the 12-week study (<12ppb) when examining temporal variation (see Appendix D, Fig D1). The pseudo-total of Cr in this soil type was found to be 5.1ppm (see Table 6.7). This indicates that Cr is not readily released under the conditions of the column. Increasing the pH seems to make little difference with both the circumneutral column and column with pH>7.5 showing only trace levels of Cr concentration over the 12-week study (<20pb) as shown when examining the temporal fluctuations (see Appendix D, Fig D1). When examining the median concentration with soil depth for Cr in topsoil with high OM (see Fig 6.12), it is observed that there is an increase with depth in Cr concentration in all the pH variations for the study. This increase is more pronounced in the circumneutral and basic columns. This indicates increased mobility vertically down the profile of the topsoil under these conditions.

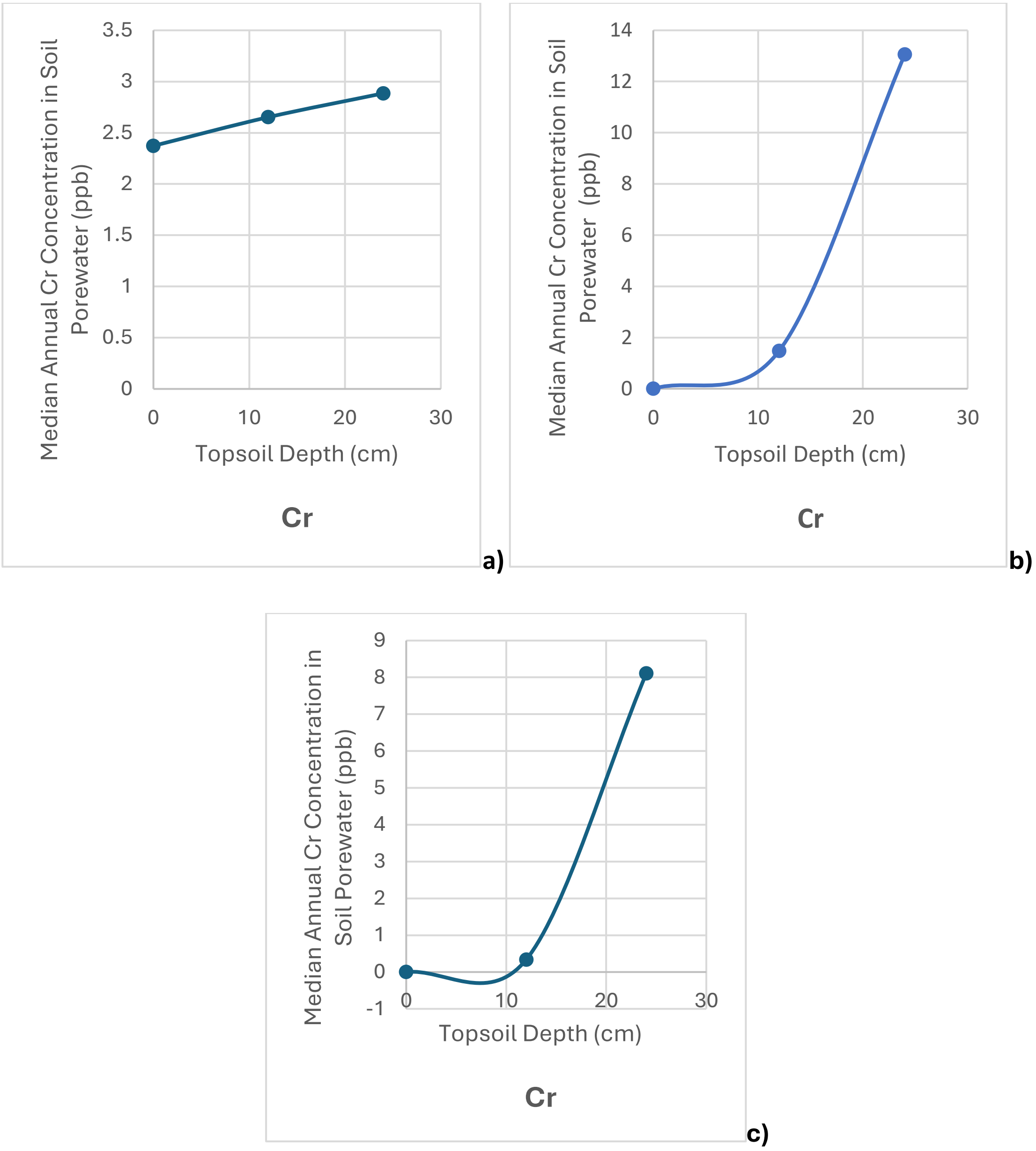


Fig 6.12: Median Annual Concentration of Cr with Depth in Topsoil Porewater for Columns with OM>25% and pH<6 (control) (a), pH=6-7.5 (b) and pH >7.5 (c)

Co concentrations vary depending on the pH of a topsoil with OM>25% (see Fig 6.13). We can see that the control column for the topsoil with OM>25% (pH<6) displays only a low level of Co concentration (<30ppb) in the porewater over the 12-week study (See Fig 6.13 and Appendix D, Fig D1). The pseudo-total concentration for Co in this topsoil sample was found to be 0.44ppm (see Table 6.7). This indicates that Co is not readily released under the conditions of the column. Increasing the pH seems to make little difference with both the circumneutral pH column and pH>7.5 column showing only trace levels of Co concentration (<5ppb) over the 12-week study (see Appendix

D, Fig D1) and <1ppb on average with depth (see Fig 6.13). When examining the average concentration with soil depth for Co in topsoil with OM>25%, it is observed that there is an increase in Co concentration in all the pH variations for the study (see Fig 6.13). This increase is more pronounced in the acidic column (control) than in those with pH adjustment (circumneutral and basic columns) in which only very low levels of Co are observed. This indicates increased mobility vertically down the profile of the topsoil under these conditions.

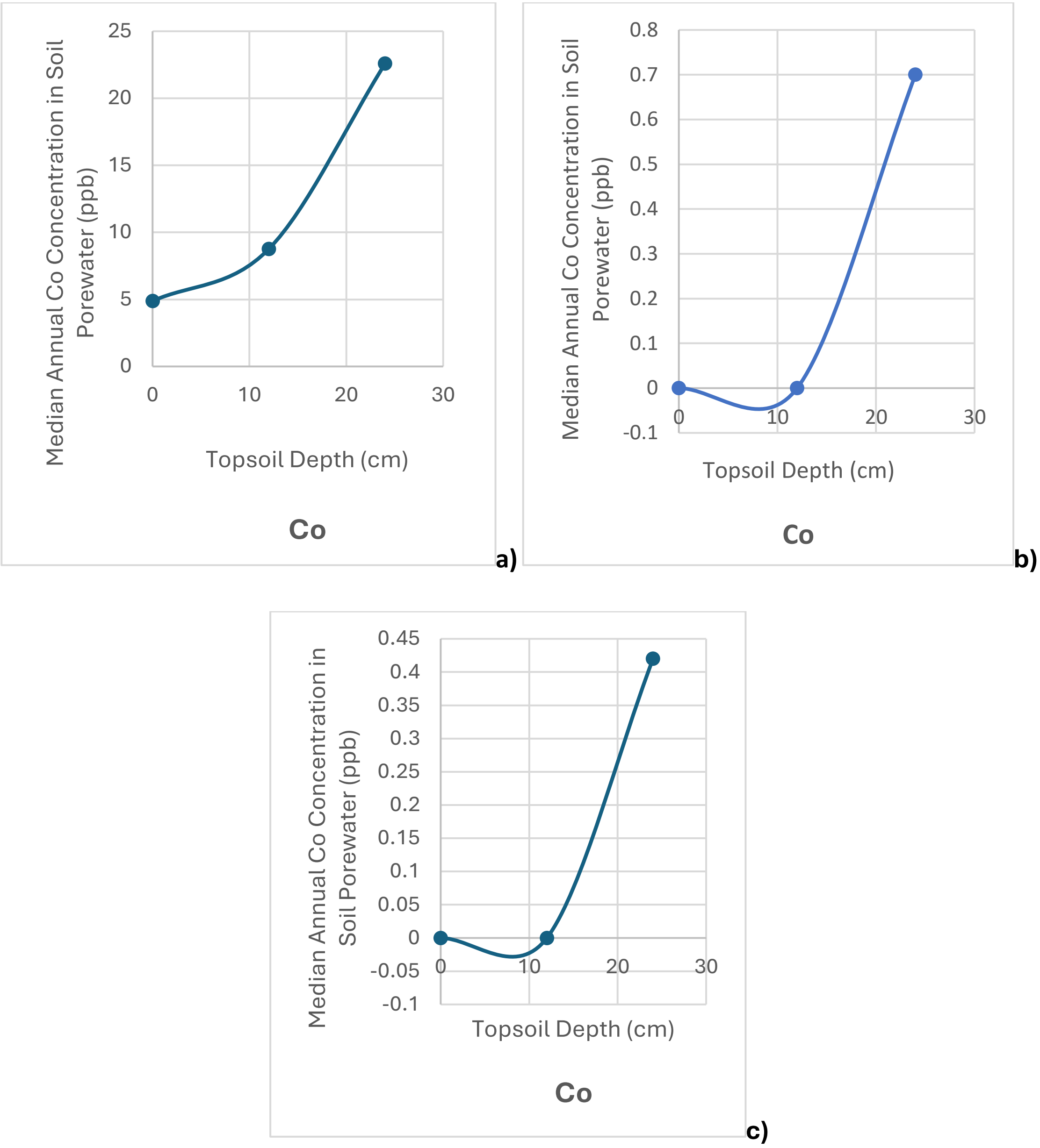


Fig 6.13: Median Annual Concentration of Co with Depth in Topsoil Porewater for Columns with OM>25% and pH<6 (control) (a), pH=6-7.5 (b) and pH >7.5 (c)

As concentrations vary depending on the pH of a topsoil with OM>25% (see Fig 6.14). When examining the temporal fluctuations of As concentration in the pore water, it is observed that the control column for the topsoil with OM>25% (pH<6) displays only a low level of As concentration in the porewater (<50ppb) over the 12-week study (see Appendix D, Fig D1). The ICP-MS analysis determined the pseudo-total to be 0.93ppm (see Table 6.7). This indicates that As is not found in significant quantities in the region and is not readily released under the conditions of the control column. Increasing the pH seems to have little impact (see Fig 6.14) with both the circumneutral pH column and pH>7.5 column showing only low levels of As concentration (<35ppb) over the 12-week study (see Appendix D, Fig D1). When examining average concentration with soil depth (see Fig 6.14), it is observed that there is an increase in the concentration of As in the porewater in all pH variations. This indicates that As is mobile in a topsoil with >25% OM irrelevant of pH range.

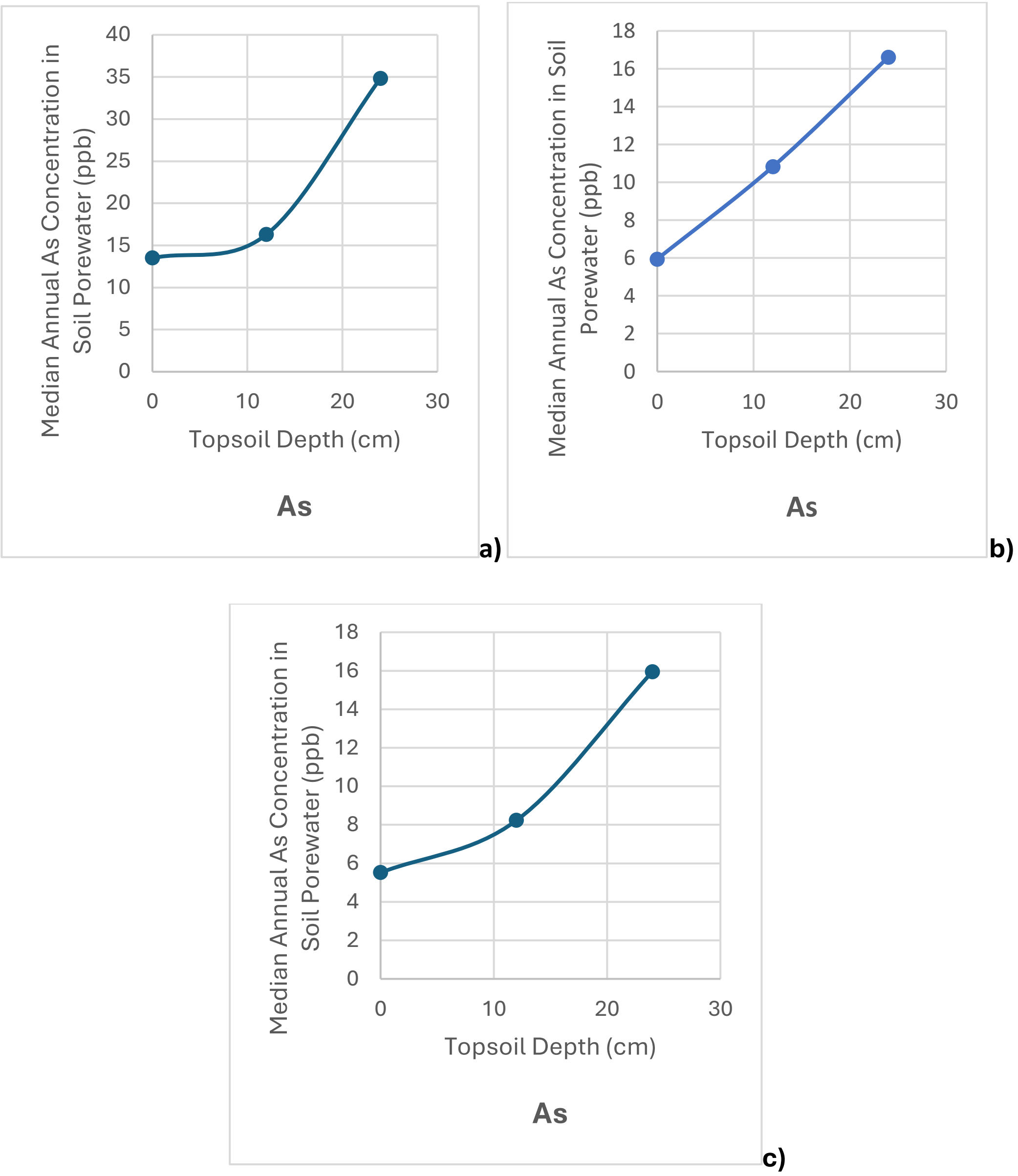


Fig 6.14: Median Annual Concentration of As with Depth in Topsoil Porewater for Columns with OM>25% and pH<6 (control) (a), pH=6-7.5 (b) and pH >7.5 (c)

Sn concentrations vary depending on the pH of a topsoil with OM>25% (see Fig 6.15). It is observed when examining the temporal fluctuations of Sn concentrations in the porewater (see Appendix D, Fig D1) that the control column for the topsoil with OM>25% (pH<6) displays a pattern that would indicate a short (during the third week of analysis) release of Sn into the porewater with the bottom sampling port providing 141ppb of Sn followed by only trace detectable levels (<10ppb) from week 5 of analysis. Pseudo-total with reverse aqua regia digestion and ICP-MS analysis showed 2.06ppm (see Table 6.7). This indicates that Sn is not found in significant quantities in the region but is mobile under the conditions of the control column. Increasing the pH seems increase Sn concentrations slightly with both the circumneutral pH column and pH>7.5 column showing only low but steady levels of Sn concentration (<50ppb) over the 12-week study (see Appendix D, Fig D1). When examining average concentration with soil depth for Sn in topsoil with high OM (see Fig 6.15), it is observed that in both the circumneutral and basic columns there is an increase of Sn in the porewater with increasing depth indicating that there is mobile Sn in the porewater under these conditions. The acidic column (control) shows only a very minor increase starting at a median of 2.81ppb in the top sampling port (0cm-2cm depth) and finishing at a median of 5.76ppb at the bottom sampling port (22-24cm depth). This indicates minor mobility of Sn under these control conditions which increases in concentration once pH reaches circumneutral.

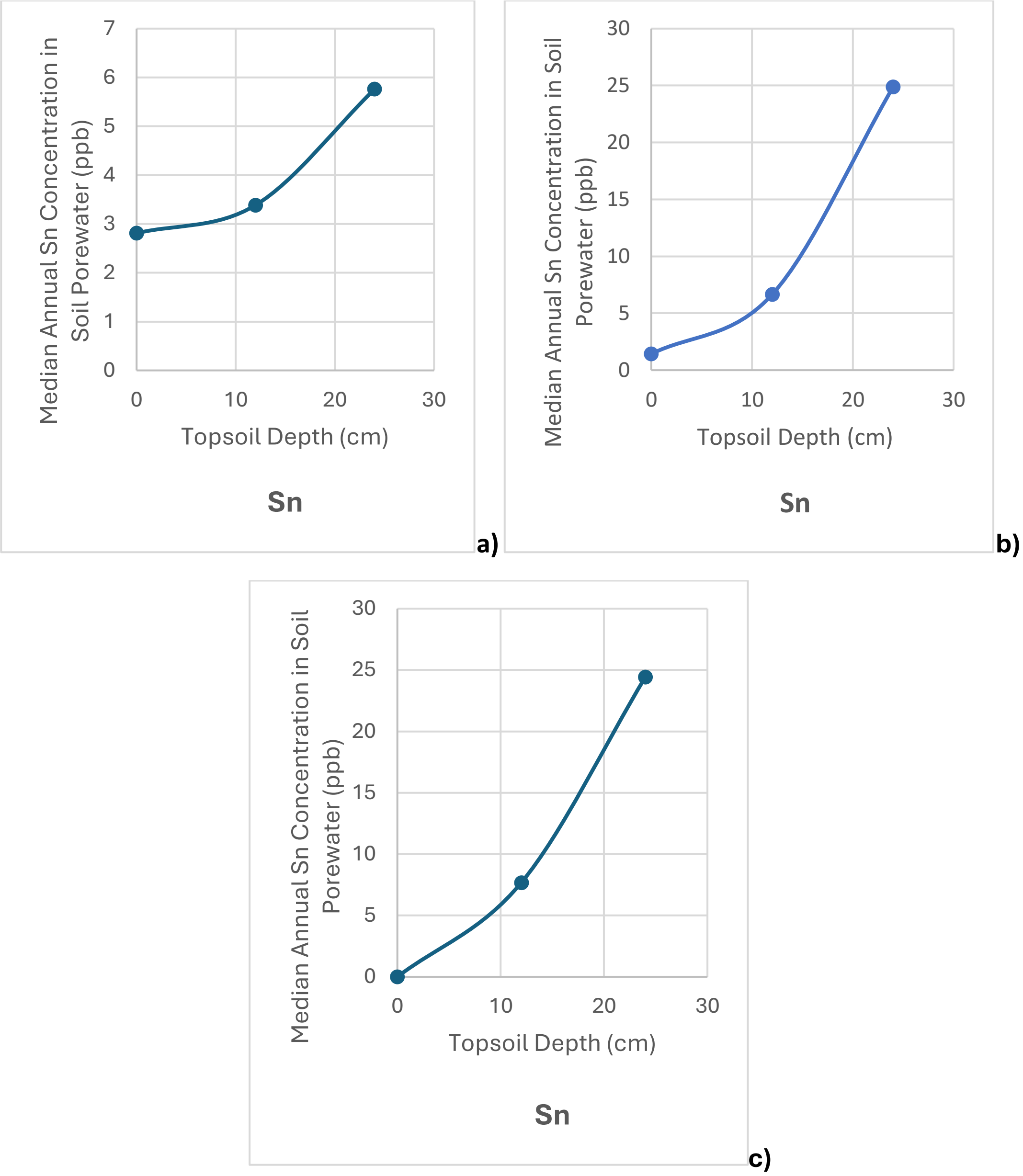


Fig 6.15: Median Annual Concentration of Sn with Depth in Topsoil Porewater for Columns with OM>25% and pH<6 (control) (a), pH=6-7.5 (b) and pH >7.5 (c)

When examining the average concentration with increasing soil depth for Ni in the porewater of a topsoil with OM>25% and pH<6 (see Fig 6.16), it is observed that Ni concentration increases with depth in the porewater. Examining the temporal fluctuations of Ni in this column (see Appendix D, Fig D1) showed a peak amount of Ni detected from all 3 sampling ports in the ninth week of analysis with 126.19ppb of Ni being detected in the porewater from the bottom sampling port (22-24cm depth). The results for Ni concentrations in the porewater of the column with circumneutral pH (6-7.5) and OM >25% show no release of Ni from any of the sampling ports on average (see Fig 6.16). When examining the temporal variations in closer detail of this pH variation (see Appendix D, Fig D1) it is observed that Ni was detected at a peak of 5.7ppb from the middle sampling port on week 2 of sampling and 21.6ppb from the bottom sampling port on week 2 of sampling with no Ni being detected for the rest of the study under these physiochemical conditions. The results for Ni concentrations in the porewater of the column with pH >7.5 and OM >25% show no detectable release of Ni from any of the sampling ports on average (see Fig 6.16). When examining the temporal variations in closer detail in this pH variation (see Appendix D, Fig D1) it is observed that Ni was detected at a peak of 11.2ppb from the top sampling port on week 2 of sampling, 12.0ppb from the middle sampling port on week 2 of sampling and 30.2ppb from the bottom sampling port on week 2 of sampling with no Ni being detected for the rest of the study under these physiochemical conditions. This indicates that Ni is considerably more mobile in the porewater of topsoil with OM>25% and pH<6 than in the circumneutral or basic pH variations.

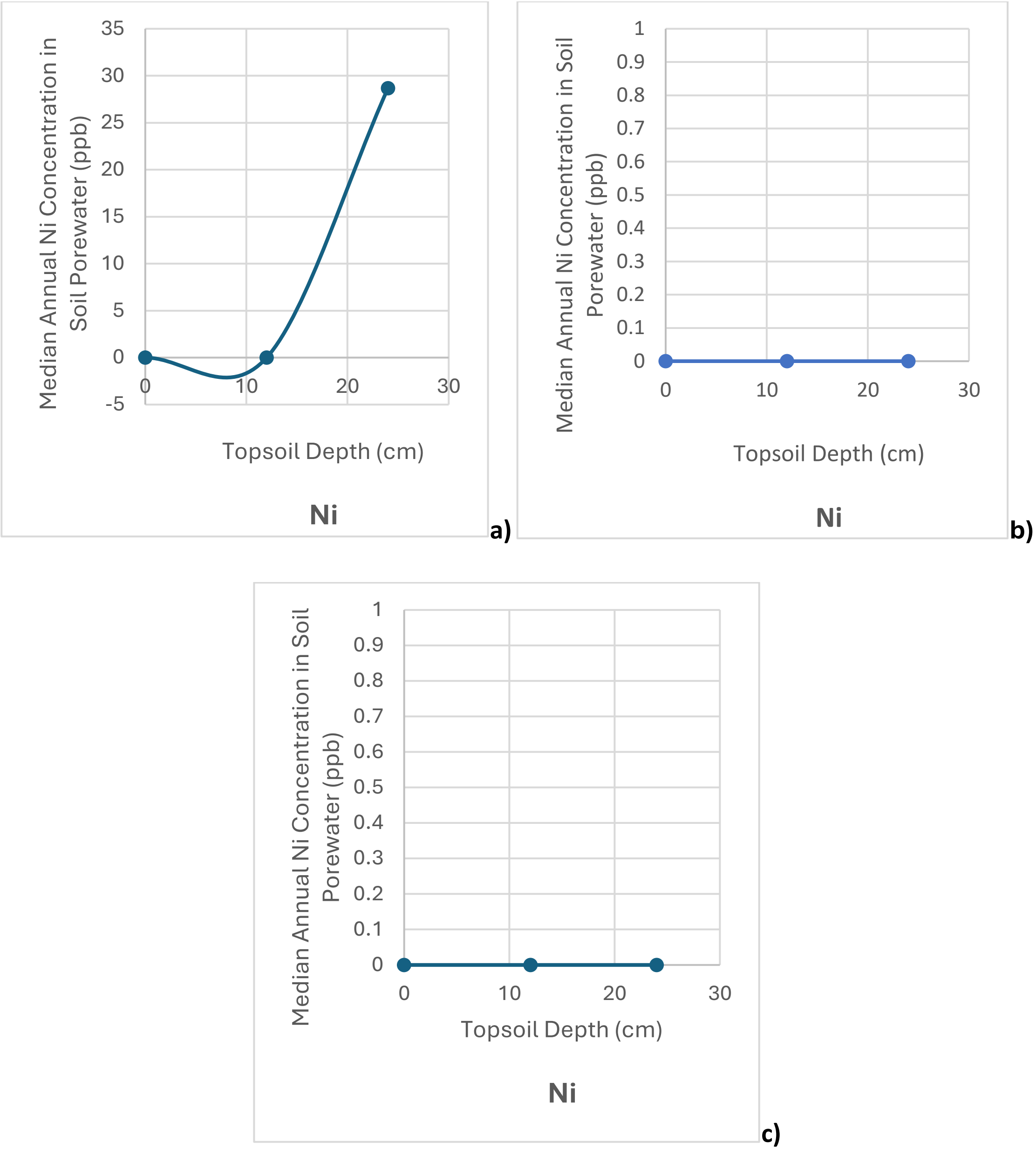


Fig 6.16: Median Annual Concentration of Ni with Depth in Topsoil Porewater for Columns with OM>25% and pH<6 (control) (a), pH=6-7.5 (b) and pH >7.5 (c)

6.3.2.2 Observations for Soil Columns with <15% Organic Matter (OM). Soil columns with <15% OM content were set-up so to have a control column with no pH adjustment (circumneutral, pH=6-7.5) as the 'no additions/adjustments control' with two further columns having their pH adjusted, one with acidic pH (<6) and one with basic pH (>7.5). These columns were run in duplicate over the course of the 12-week study (see Fig 6.6) and median results recorded to represent the central tendency of the obtained weekly results for the concentration of each trace metal element in the porewater. Average annual concentration with topsoil depth were then determined from these temporal porewater concentrations (see Figs 6.17-6.25 and Appendix D, Fig D1).

The outcomes of the mesocosm study indicates that Zn concentrations in the porewater of topsoil with low levels of organic matter (OM<15%) is influenced by the pH of the topsoil (see Fig 6.17). Lowering the pH shows significantly more Zn being detected in the porewater than in those columns with approximately circumneutral (control) pH or high pH (approximately >7.5) as Shown in Fig 6.17. In turn, raising the pH also shows reduced Zn concentrations than that of the control column which was of approximately circumneutral pH and found no detectable Zn in the porewaters, on average (see Fig 6.17). When examining the temporal variations in closer detail of the column with pH>7.5 (see Appendix D, Fig D1), it is observed that Zn was detected at trace levels (<10ppb) from all sampling ports on the second week of analysis followed by a peak of 26.28ppb on week 4 of analysis from the top sampling port (0cm-2cm depth) with all other consequent readings not showing any detectable Zn. In contrast, the acidic column (pH<6) shows consistent detection of Zn in the porewater from all sampling points across the 12-week study peaking at 57593ppb on week four (10cm-12cm depth) of the analysis (see Appendix D, Fig D1). This indicates that Zn is mobile under acidic conditions in this soil type.

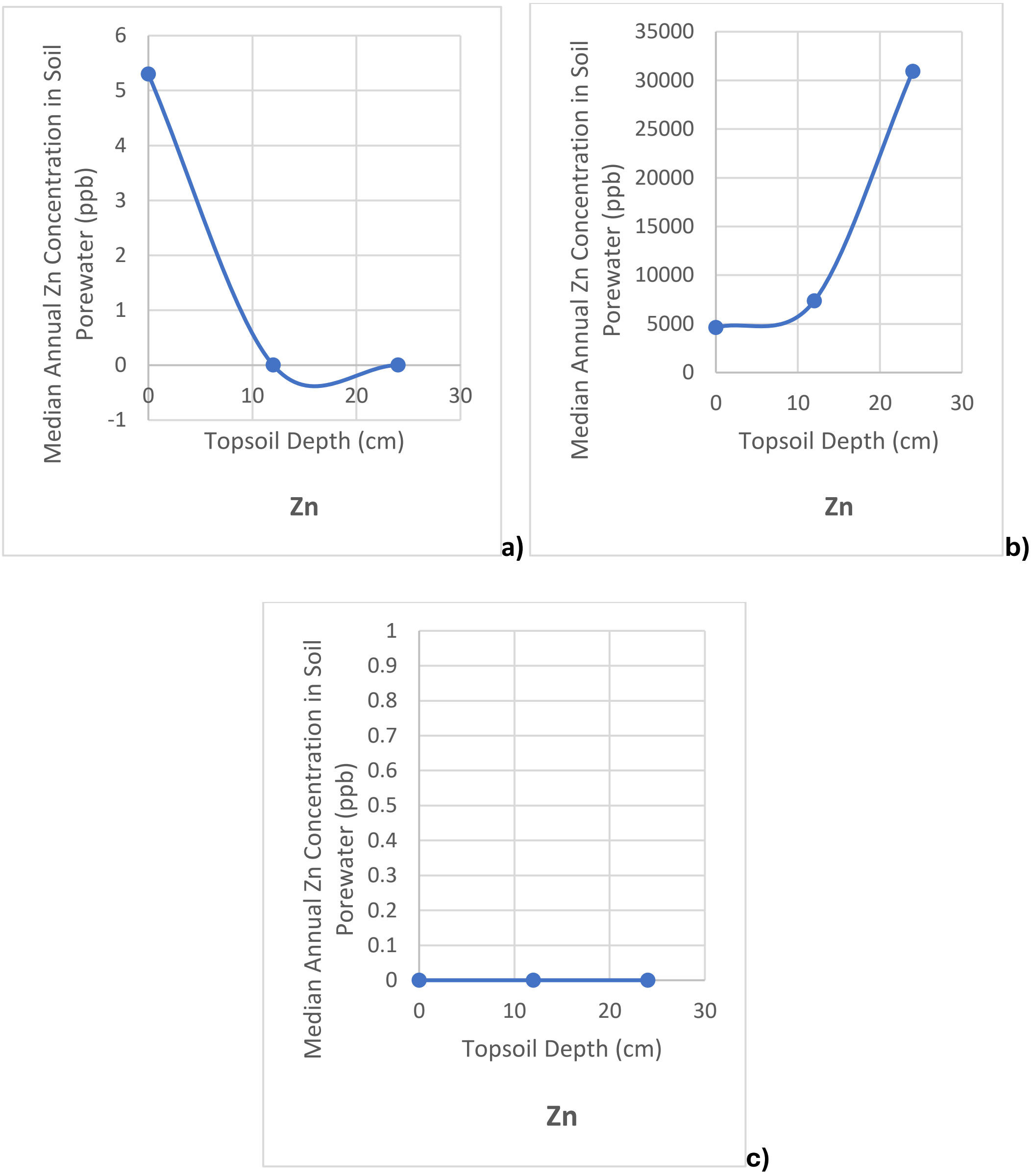


Fig 6.17: Median Annual Concentration of Zn with Depth in Topsoil Porewater for Columns with OM<15% and pH<6-7.5 (control) (a), pH=<6 (b) and pH >7.5 (c)

When examining Pb concentration in the porewater of topsoil with low levels of organic matter (OM<15%), it is found that pH has an impact on concentration of Lead (Pb) in the porewater (see Fig 6.18). Lowering the pH shows considerably more Pb than in those columns with approximately circumneutral (control) pH or high pH (>7.5). In turn, raising the pH from that of the control column shows no detectable Pb concentrations (see Fig 6.18) on average from any of the depth sampling points. When examining the temporal variations in closer detail of the column with pH>7.5 (see Appendix D, Fig D1), it is observed that Pb was detected at a peak of 27.88ppb from the bottom sampling port (22cm-24cm depth) in week 3 of the study. Trace levels of Pb (<5ppb) were detected from the middle sampling port (10cm-12cm depth) during the fifth week of analysis with all other consequent readings not showing any detectable Pb in the porewater. In contrast, the column that had its pH adjusted to acidic (pH<6) shows consistent detection of Pb in the porewater from all sampling points across the 12-week study peaking at 20957.08ppb on week three (10cm-12cm depth) of the analysis (see Appendix D, Fig D1). This indicates that Pb is mobile under acidic conditions in this soil type with an overall increase in Pb concentration in the porewater with soil depth when pH is <6.

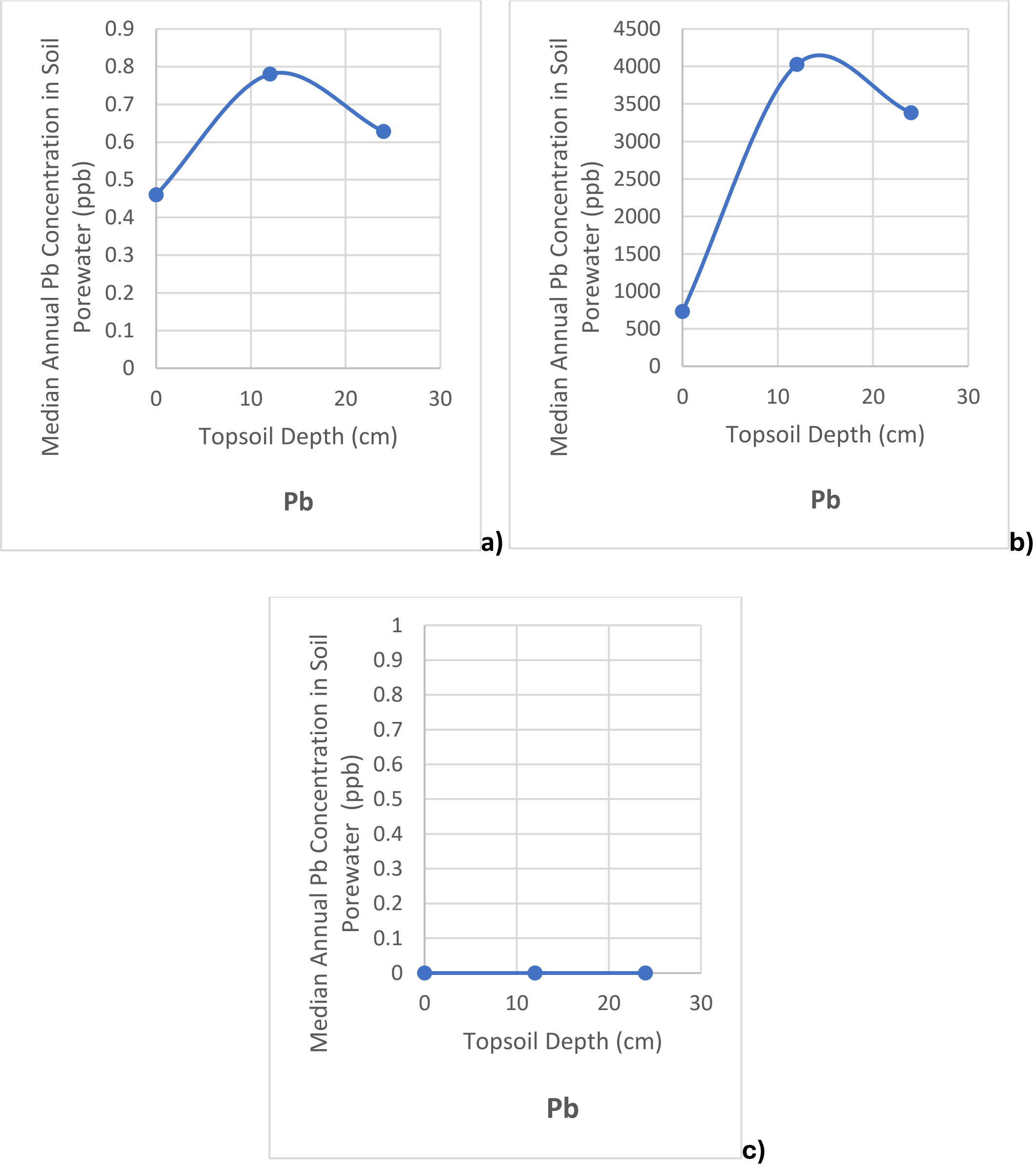


Fig 6.18: Median Annual Concentration of Pb with Depth in Topsoil Porewater for Columns with OM<15% and pH<6-7.5 (control) (a), pH=<6 (b) and pH >7.5 (c)

The outcomes of the mesocosm study show that, for V in soil with low levels of organic matter (OM<15%), that pH has an impact on concentration of Vanadium (V) in the pore water (see Fig 6.19). Lowering the pH from the control column (circumneutral to pH<6) shows considerably more V concentration in the porewater than in those columns with approximately circumneutral (control) pH or pH>7.5. Raising the pH from the control column (circumneutral to >7.5) shows little difference with only trace amounts (<10ppb) of V being found across the 12-week study period (see Appendix D, Fig D1). When examining average concentration with soil depth of the topsoil for V in the porewater of a topsoil with low OM (see Fig 6.19), it is observed that the control column (circumneutral pH) shows a decline from the top sampling point (0cm-2cm depth) to the middle sampling port (10cm-12cm depth) before levelling off at approximately 0.3ppb between the middle sampling port and the bottom sampling port (22cm-24cm depth). When this topsoil was adjusted to become more acidic (pH<6), there is a significant increase in V concentration in the porewater with the average concentration of V rising between the top sampling point (0cm-2cm depth) and the middle sampling port (10cm-12cm depth) to an average concentration of 302.06ppb at this depth annually, before declining from the middle sampling port to the bottom sampling port (22cm-24cm depth) to an average of 94.18ppb annually. The basic pH variation (pH>7.5) shows a steady decrease with very low concentrations (<0.5ppb) in average V concentration in the porewater with depth. Examining the temporal fluctuations of V in the porewater of this soil type (see Appendix D, Fig D1) shows that there is a consistent amount of V found in the porewater at 22cm-24cm depth in the column with pH<6 from the third week of the study indicating that V is mobile under these conditions annually but not mobile when pH is circumneutral or higher.

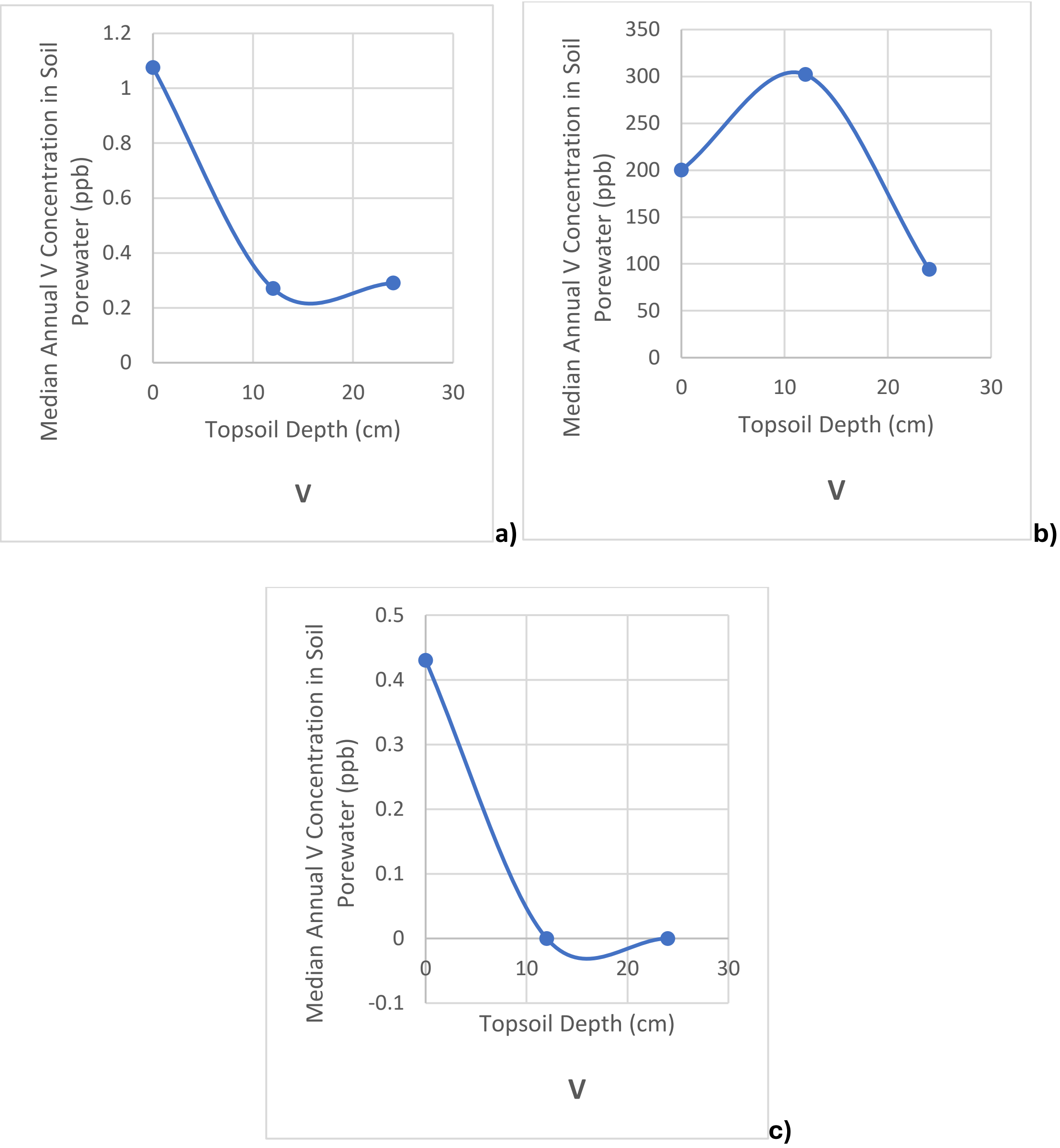
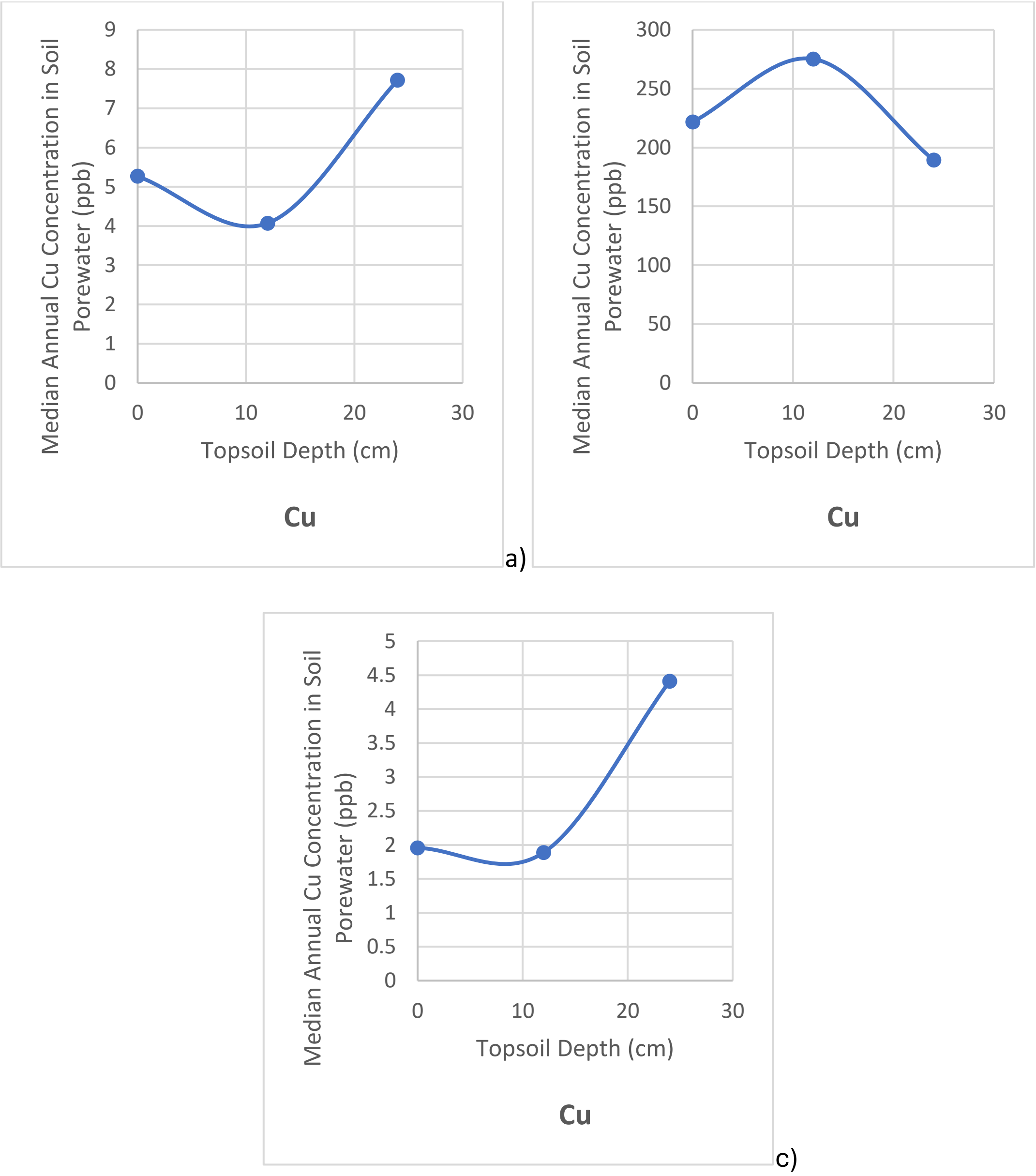


Fig 6.19: Median Annual Concentration of V with Depth in Topsoil Porewater for Columns with OM<15% and pH<6-7.5 (control) (a), pH=<6 (b) and pH >7.5 (c)

The mesocosm study found that for Cu in soil with low levels of organic matter (OM<15%), that pH has a considerable impact on concentration of Copper (Cu) in the pore water (see Fig 6.20 and Appendix D, Fig D1). When examining the average Cu concentration in the porewater with soil depth (see Fig 6.20) for the topsoil columns with low OM (<15%) and circumneutral pH (control), it is observed that, initially, there is a slight decline in Cu concentration in the porewater corresponding with depth from 0cm-2cm depth through to 10cm-12cm depth before increasing at 22cm-24cm depth whilst remaining at trace levels (<10ppb). When reducing the pH, there is observed to be a much higher concentration of Cu detected in the porewater which initially rises from the top sampling point (0cm-2cm depth) to the middle sampling port (10cm-12cm depth), peaking at 275.30ppb on average annually, before declining slightly with depth from the middle sampling port to the bottom sampling port (see Fig 20) at an average of 189.21ppb annually. When raising the pH (>7.5), a significant reduction in average concentration of Cu in the porewater is observed (see Fig 6.20) with <5ppb of Cu in the porewater being recorded on average annually (12-week study). When examining the temporal fluctuations of Cu in the porewater (see Appendix D, Fig D1) it is observed that lowering the pH from the control column (circumneutral to <6) shows significantly more Cu concentration over the 12-week course of the column study than in those columns with approximately circumneutral (control) pH or high pH (>7.5) for this soil type (OM<15%). This indicates that Cu is mobile in acidic soils, but this mobility is considerably lessened in a soil with basic or circumneutral pH properties.



a)

b)

c)

Fig 6.20: Median Annual Concentration of Cu with Depth in Topsoil Porewater for Columns with OM<15% and pH<6-7.5 (control) (a), pH=<6 (b) and pH >7.5 (c)

Observations from the mesocosm study show that, for Cr concentrations in the porewater in topsoil with low levels of organic matter (OM<15%), pH has an impact on Cr concentrations annually (see Fig 6.21 and Appendix D, Fig D1). Lowering the pH from the control column (circumneutral to <6) shows an increased Cr concentration in the porewater (see Fig 6.21), with soil depth, annually when compared to those columns with approximately circumneutral pH (control) or high pH (>7.5). The median results for Cr in the basic column (pH >7.5 and OM <15%) show no release of Cr from any of the sampling ports on average. When examining the temporal fluctuations (see Appendix D, Fig D1) for Cr concentrations in the porewater of a topsoil of this type (OM<15%), the circumneutral pH control column displays an initial concentration of Cr in the first week of analysis at 39.24ppb from the bottom sampling port (22cm-24cm depth) but displays only trace amounts thereafter (<10ppb) and showing no detectable signs of Cr after the fifth week of analysis for the bottom sampling port (22cm-24cm depth) and from the ninth week of analysis on both top (0cm-2cm depth) and middle sampling points (10cm-12cm depth). The top (0cm-2cm depth) and middle (10cm-12cm depth) sampling points detect only trace levels of Cr throughout the 12week study (<10ppb) under these conditions (circumneutral pH). Raising the pH from the control column (circumneutral to high) shows that Cr was detected at a peak of 1.51ppb on week 4 of analysis from the top sampling port with a peak of 1.12ppb from the middle port in week 5 and then a peak of 1.06ppb from the bottom port in week 6. Trace amounts (<1ppb) being detected in week 2 for all sampling ports, in week 4 for the middle and bottom sampling ports, week 5 from the bottom sampling port, week 6 from top and middle sampling ports and week 8 from the top sampling port with all other readings not showing any detectable Cr. Cr concentration in the porewater was detected consistently from all sampling ports across the 12-week study when the pH was lowered from the circumneutral control (pH<6). This indicates that Cr is mobile under acidic conditions for this soil type (OM<15%) but less so under circumneutral and basic pH conditions.

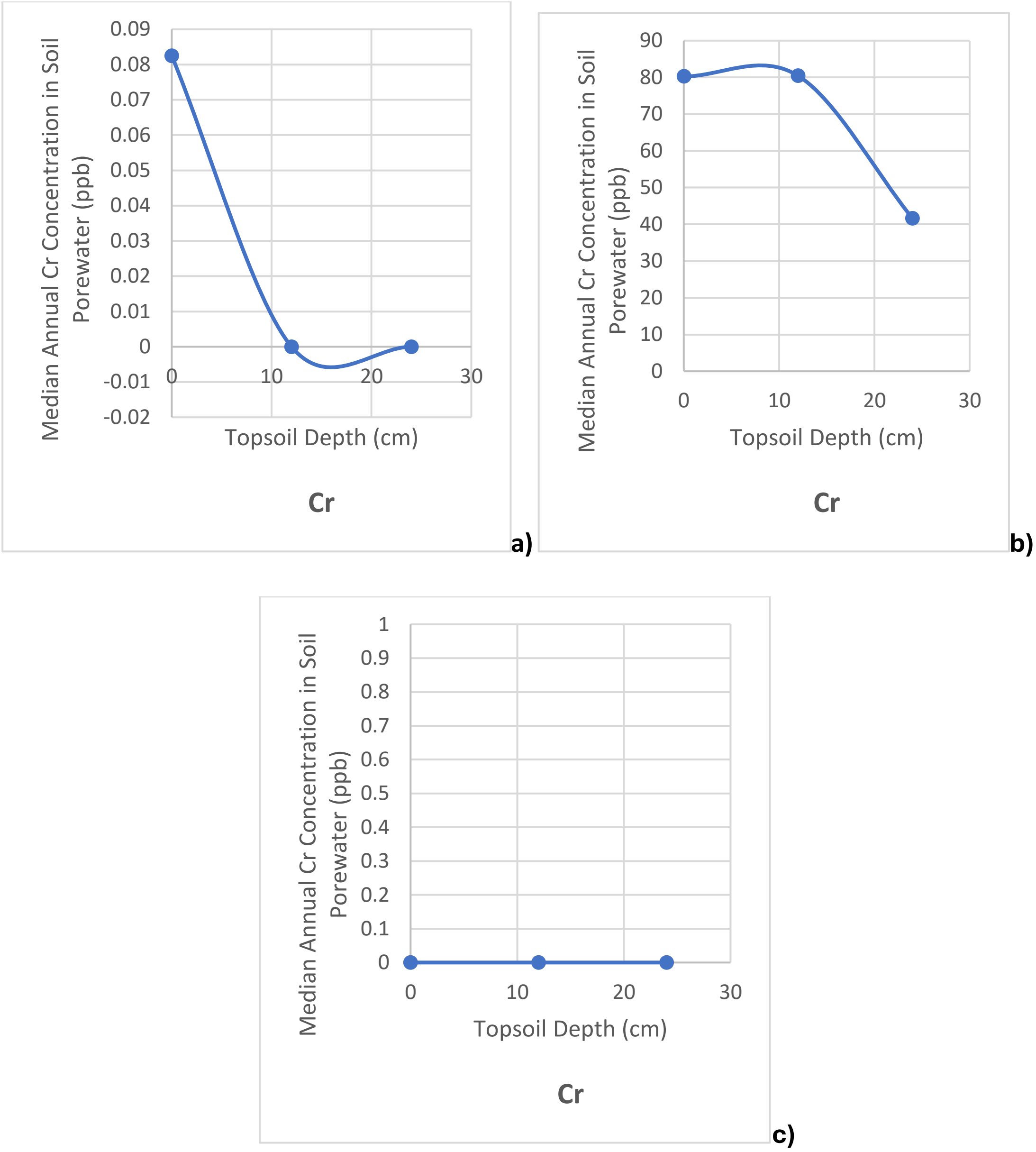


Fig 6.21: Median Annual Concentration of Cr with Depth in Topsoil Porewater for Columns with OM<15% and pH<6-7.5 (control) (a), pH=<6 (b) and pH >7.5 (c)

When examining the concentration of Co in the porewater of the topsoil with low levels of organic matter (OM<15%), it can be seen that varying pH has an impact on the concentration of Cobalt (Co) found in the pore water (see Fig 6.22 and Appendix D, Fig D1). Lowering the pH from the control column (circumneutral to <6) shows an increased Co concentration over the 12-week course of the column study when compared to those columns with approximately circumneutral (control) pH or high pH (>7.5). When examining the median Co concentration with soil depth for the topsoil columns with low OM (see Fig 6.22), it is observed that there is an overall increase of Co in the porewater corresponding with depth in the acidic pH variation (pH<6). This acidic column variation shows considerably more Co in the porewater than the other two pH variations. The circumneutral column (control) shows a very slight decline in Co in the porewater with depth, albeit at very low concentrations (<1ppb). The median results for the column with pH>7.5 and OM <15% show no detectable concentrations of Co from any of the sampling ports on average (see Fig 6.22). When examining the temporal variations in closer detail (see Appendix D, Fig D1) for this column (pH>7.5) it is observed that Co was detected at a peak of 1.1ppb on week 6 of analysis from the bottom sampling port (22cm-24cm depth). Trace amounts (<1ppb) of Co were detected in week 2 for all sampling ports/depths and in week 6 for the middle (10cm-12cm depth) and top sampling points (0cm-2cm depth) with all other readings not showing any detectable Co. This indicates very little mobility of Co is observed in the porewater under these conditions. The acidic column (pH<6) for Co with low OM (<15%) shows significantly more concentrations of Co in the porewater which increases from the top of the column through to the bottom of the column, peaking at 1126.60ppb in week 10 of the analysis (see Appendix D, Fig D1) from the bottom sampling port (22cm-24cm depth), indicating mobility of Co under these acidic conditions in a topsoil of this type (see Fig 6.22 and Appendix D, Fig D1). The circumneutral pH column (control) displays only trace amounts of Co (<5ppb) over the entire 12-week study (see Appendix D, Fig D1). Raising the pH from the control column (circumneutral to basic) shows little difference with a slightly reduced concentration to that of the circumneutral column with only trace amounts (<2ppb) of Co being found across the 12-week study period and no detectable quantifies in any sample port from week 7 of the study resulting in the central tendency indicating no detectable Co under these conditions (see Fig 6.22 and Appendix D, Fig D1). These results indicate that whilst Co is mobile in a topsoil with OM<15% and has acidic pH, it is considerably less so when the pH of the soil is circumneutral or greater for this soil type.

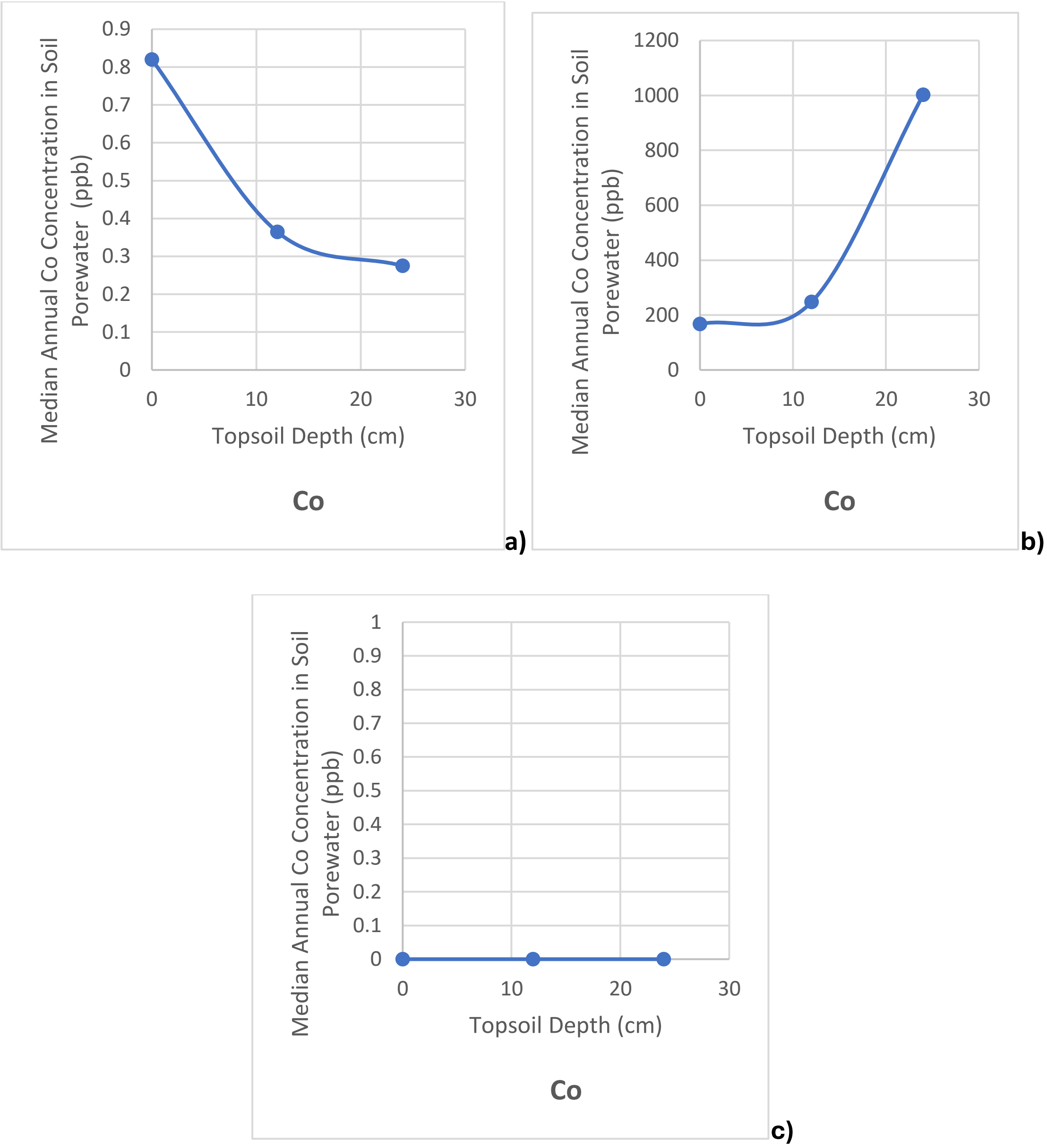


Fig 6.22: Median Annual Concentration of Co with Depth in Topsoil Porewater for Columns with OM<15% and pH<6-7.5 (control) (a), pH=<6 (b) and pH >7.5 (c)

The outcomes of the mesocosm study show that for As concentrations in the porewater of the topsoil with low levels of organic matter (OM<15%), that pH has an impact on concentration of Arsenic (As) in the pore water (see Fig 6.23 and Appendix D, Fig D1). When examining the average concentration with soil depth (see Fig 6.23), over the course of the 12-week study, for As in the porewater of a low OM topsoil (<15%), it is observed that the acidic column shows a consistent, low concentration from all sampling depths of between approximately 6ppb and 10ppb. This indicates some mobility of As under these conditions. The circumneutral (control) and basic columns show very little concentration of As (<3ppb median concentration for all sampling ports) with only a slight increase of As concentration in the porewater with depth overall, indicating limited to negligible mobility under these conditions. When examining the temporal fluctuations of As concentration in the porewater (see Appendix D, Fig D1), lowering the pH from the control column (circumneutral to <6) shows an increased As concentration over the 12 week course of the column study when compared to those columns with approximately circumneutral (control) pH or high pH (>7.5). The temporal fluctuations for As under these acidic conditions display a low but consistent concentration of As found in the porewater (approximately 4ppb-9ppb) from week 4 of analysis at 22cm-24cm depth, with a peak of 43.65ppb at the top of the sampling column (0cm-2cm depth) during week 11 of analysis. This indicates minor mobility of As under these conditions. The Circumneutral pH column displays only trace amounts of As (<6ppb) over the entire 12-week study. Raising the pH from the control column (circumneutral to >7.5) shows little difference with a similar pattern to that of the circumneutral column with only trace amounts (<6ppb) of As being found across the 12-week study period. This indicates that pH has a minor impact on As concentration in the porewater of a topsoil of this type (OM<15%).

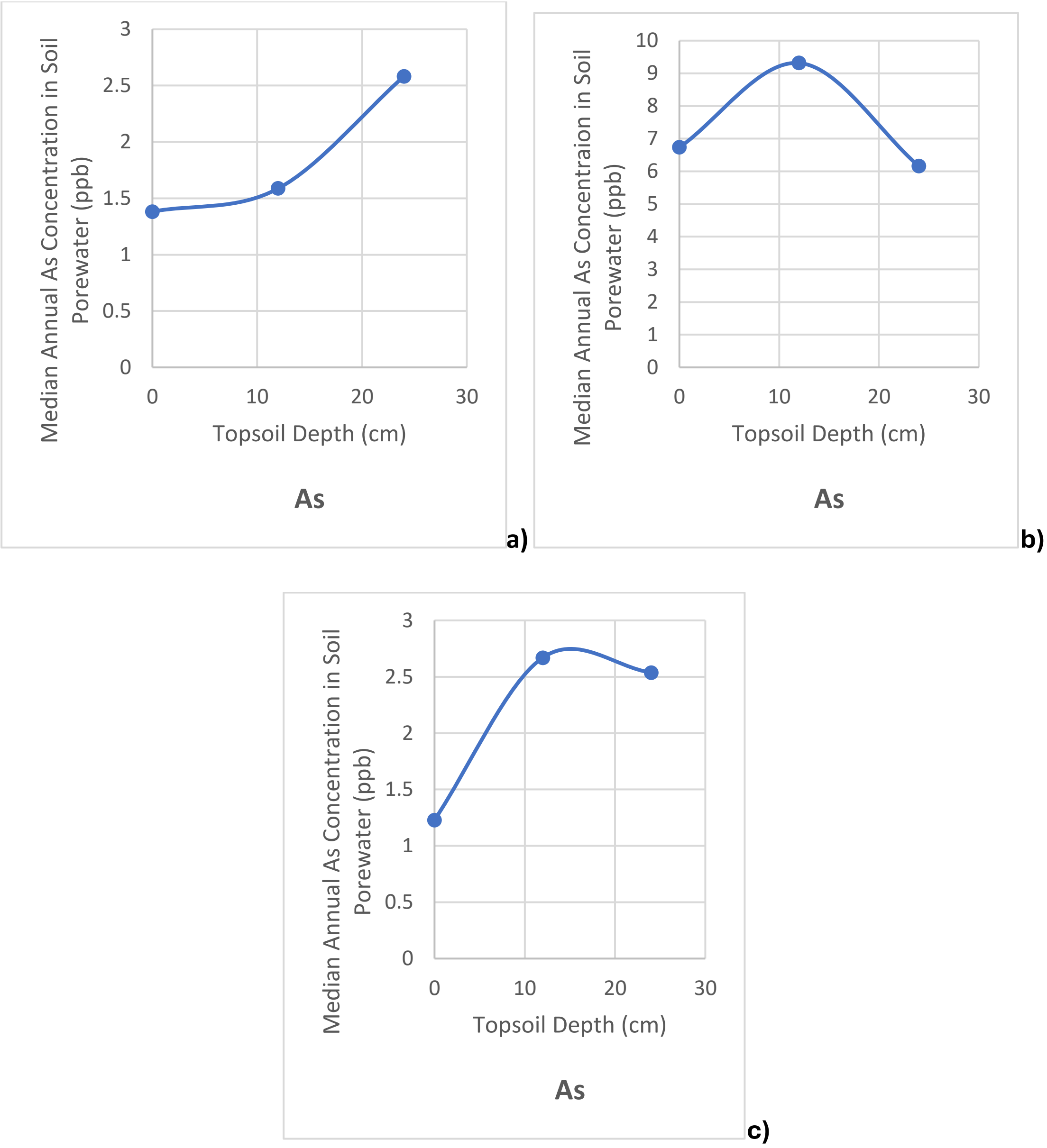
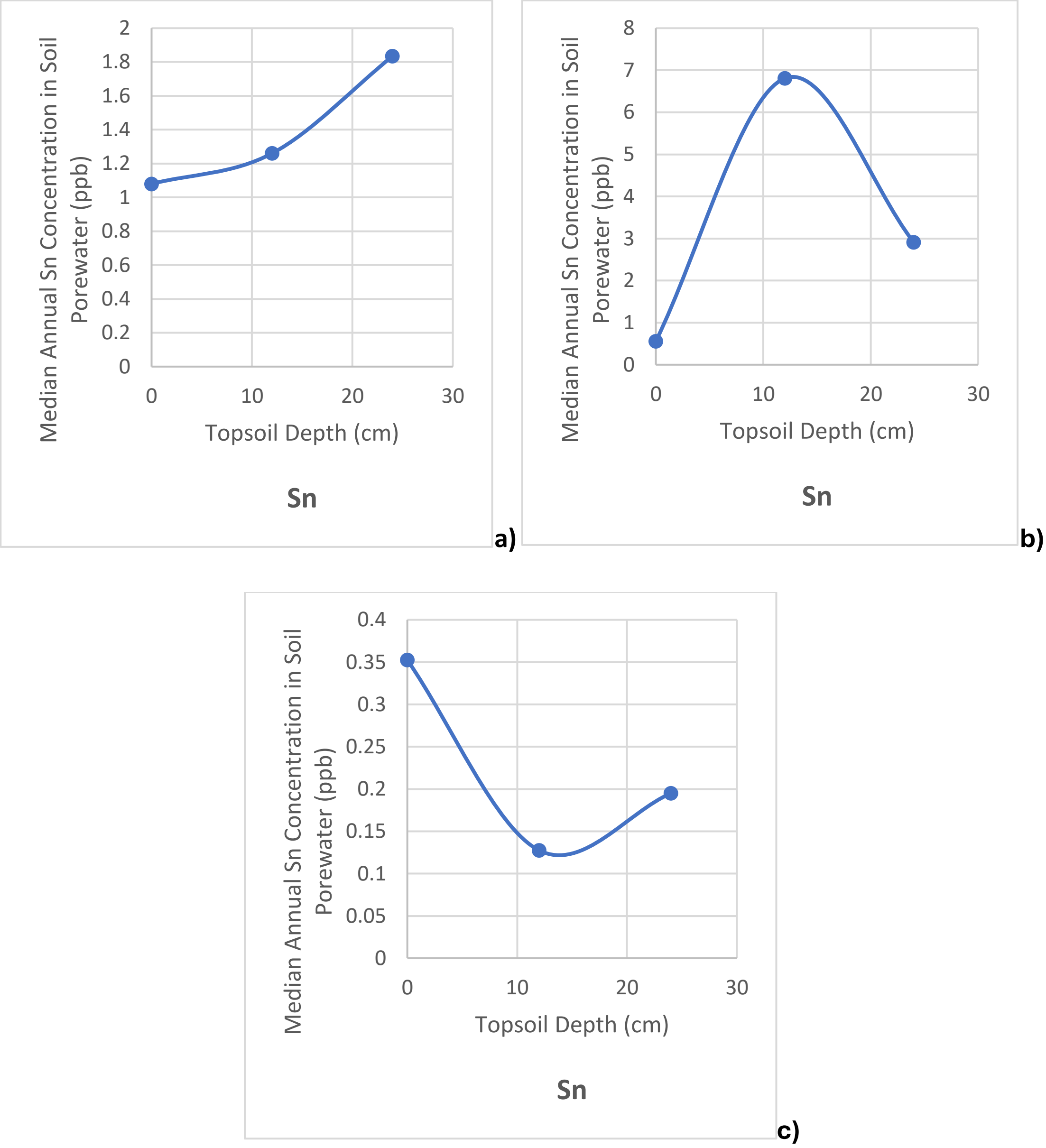


Fig 6.23: Median Annual Concentration of As with Depth in Topsoil Porewater for Columns with OM<15% and pH<6-7.5 (control) (a), pH=<6 (b) and pH >7.5 (c)

The mesocosm study results showed that Sn concentrations in the porewater of the topsoil with <15%OM is minorly influenced by the pH of the topsoil (see Fig 6.24 and Appendix D, Fig D1). When examining median Sn concentration with soil depth in the porewater of a topsoil with low OM (see Fig 6.24), it is observed that there is little change in concentration from the top of the column to the bottom of the column for the control column and the basic (pH>7.5) column with both showing very low concentrations of Sn in the porewater (<1ppb median concentration for the basic column and <2ppb for the control column) compared to the acidic pH variation (pH<6) which had a peak median concentration of 6.81ppb at 22cm-24cm soil depth. When examining the temporal fluctuations for Sn in this low OM topsoil (see Appendix D, Fig D1), it is observed that lowering the pH from the control column (circumneutral to <6) results in a very similar pattern to that of the control column over the 12-week course of the column study. Low levels of Sn are detected across the 12-week study period (<20ppb) except for in week 3 of the study in which a maximum of 129.36ppb and 104.38ppb were found in the low pH (<6) and the control/circumneutral pH columns respectively. The concentrations of Sn in the porewater were consistently lower in the control column than for the acidic column which indicates limited increased mobility of Sn in a topsoil with low OM under acidic conditions. Raising the pH from the control column (circumneutral to >7.5) shows trace amounts of Sn concentration (<4ppb) being found across the 12-week study period and at similar levels to that of the circumneutral control.



a) b) c)

Fig 6.24: Median Annual Concentration of Sn with Depth in Topsoil Porewater for Columns with OM<15% and pH<6-7.5 (control) (a), pH=<6 (b) and pH >7.5 (c)

When examining the median concentration of Ni in the porewater of a topsoil with low OM (see Fig 6.25) with soil depth, it is observed that in the acidic column there is a steady increase in Ni concentration with depth indicating that there is a presence of mobile Ni in the porewater. Concentrations of Sn in the porewater for this acidic column were found to be 1406.09ppb on average at 22cm-24cm depth (see Fig 6.25). The circumneutral and basic column pH variants showed no detectable Ni from any of the sampling points/ports on average under these pH conditions indicating that Ni is not mobile in a topsoil of this type under these pH conditions. When examining the temporal fluctuations of Ni in this topsoil type (see Appendix D, Fig D1), it was observed that a consistent concentration of Ni was found in all sampling points over the 12-week study for the variation with pH<6, peaking at 1677.74ppb at the tenth week of the study from the bottom sampling point (22cm-24cm depth). These temporal fluctuations show increasing concentrations from top sampling point (0cm-2cm depth) to the bottom sampling point (22cm depth-24cm depth) for each of the 12 weeks of analysis except for week 3 in which slightly more Ni was detected at 10cm-12cm depth than for 22cm-24cm depth. This consistent increase in Sn concentration indicates that Sn is mobile under these acidic conditions in this soil type (OM<15%). When examining the temporal variations in closer detail for the circumneutral control column (see Appendix D, Fig D1) it is observed that Ni was detected at a peak of 20.9ppb in the bottom sampling port (22cm-24cm depth) during the first week of the study. Ni was detected at 6.8ppn, 4,4ppb and 3.0ppb from the top (0cm-2cm depth), middle (10cm-12cm depth) and bottom (22cm-24cm depth) sampling points respectively during the third week of the study. During the fourth week of the study, Ni was detected at 9.7ppb, 5.5ppb and 3.9ppb from the top (0cm-2cm depth), middle (10cm-12cm depth) and bottom (22cm-24cm depth) sampling points respectively. Ni was not detected for the rest of the study under these physiochemical conditions. The basic column (pH>7.5) showed no detectable Ni from any of the sampling points/ports under basic pH conditions indicating that Ni is not mobile in a topsoil of this type under basic conditions. This indicates that pH is a significant controlling factor for the release of Ni into the porewater of these low OM mineral soils and that remediation measures such as liming would be effective in both raising the pH, if necessary, and reducing the amount of Ni in the porewater.

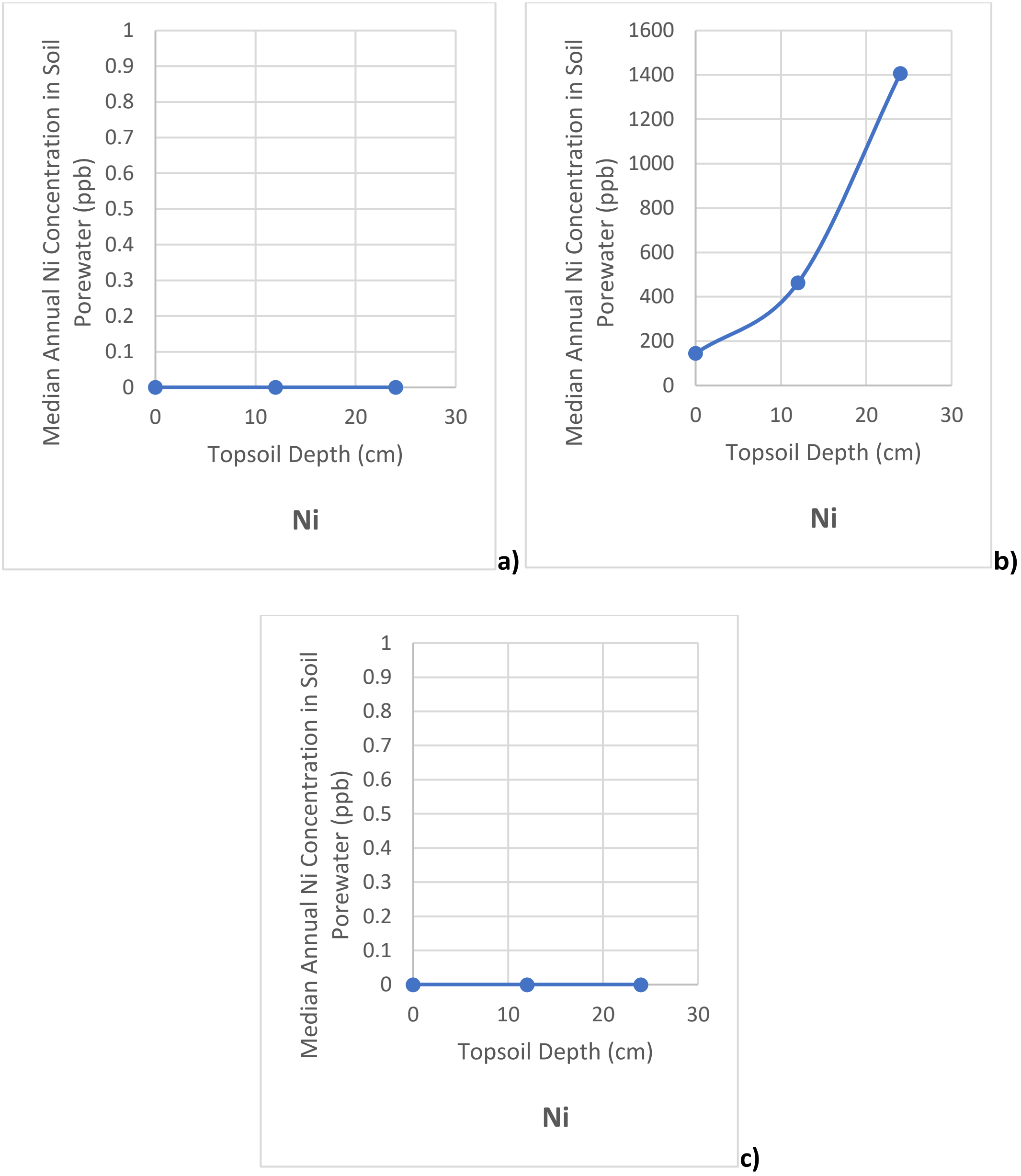


Fig 6.25: Median Annual Concentration of Ni with Depth in Topsoil Porewater for Columns with OM<15% and pH<6-7.5 (control) (a), pH=<6 (b) and pH >7.5 (c)

6.3.2.3 The Influence of pH and OM on Trace Metal Mobility in the Soil Porewater. The results of the mesocosm study show a clear influence of topsoil pH on trace metal mobility in the topsoil for many of the trace metals studied (as shown in section 6.3.2.1 and section 6.3.2.2). This influence on mobility is impacted by the soil type and, more specifically, the OM content of the topsoil (see Figs 6.8-6.25 and Appendix D, Fig D1). Examination of each soil type, the trace metal being studied, and their interactions with OM content and pH variations, aid in determining the impacts of physiochemical properties on trace metal mobility in topsoil.

Previous studies have found Zn mobility in soil to be greatly influenced by the pH of the soil and, to a lesser extent, the organic matter content (Azouzi et al, 2015; Kicinska et al, 2022; Rutkowska et al, 2015). These studies have shown that not only do these physiochemical properties influence the total Zn in the soil but also the Zn concentrations in the porewater (Rutkowska et al, 2015). This may be in part due Zn sorption favouring the labile (Exchangeable and Reducible) fractions of the soil (Azouzi et al, 2015), as covered in section 6.3.3. When examining the Zn content of the two soil types studied here, it was determined that each soil type had a similar pseudo-total concentration of Zn (see Table 6.7) following digestion with reverse aqua regia and analysis via ICP-MS (48.69ppm for the topsoil with OM>25% and 47.29ppm for the topsoil with OM<15%). The mesocosm study showed that Zn concentrations in the porewater are heavily influenced by pH in both soil types being studied (see Fig 6.8 and Fig 6.17). This influence is more pronounced in the mineral soil with low OM (<15%) in which no mobile Zn was detected in the porewater when pH was adjusted to >7.5.

Research has shown Pb concentrations in the environment resulting from anthropogenic sources to persist in and around the soil surface and the topsoil following deposition (Chaney et al, 1989). pH and soil OM content has been found to be influencing factors for the mobility within the topsoil, with these physiochemical properties being found to have an inverse correlation to Pb concentrations in the porewater (Bondar et al, 2019). Following analysis via ICP-MS with reverse aqua regia digestion on the two control samples, there was found to be similar levels of Pb in both the topsoil with OM>25% and topsoil with OM<15% (36.31ppm and 32.65 respectively). The mesocosm study determined that pH levels impact the occurrence and mobility of the Pb in the porewater of the topsoil (see section 6.3.2.1 and section 6.3.2.2). This influence of pH appears to be of a greater magnitude in the topsoil with OM<15% (see Fig 6.18) than for the topsoil with OM>25% (see Fig 6.9) indicating that topsoil OM content plays a role in Pb concentration in the porewater of a topsoil.

V is deposited on soils from a variety of anthropogenic sources, including fertiliser use in agriculture, pesticide application, and industrial activities (Chen et al, 2021). The behaviour of V in the soil is known to be linked to physiochemical properties, including pH and soil OM content, which have been found to play a key role in the retention of V in soil (Wnuk, 2023). When studying the two topsoil types in this study, there was found to be an increased amount of V in the topsoil with OM<15% (10.77ppm) when compared to the topsoil with OM>25% (2.51ppm) following ICP-MS analysis with reverse aqua regia digestion (see Table 6.7). The topsoil with OM<15% also shows to be influenced to a greater extent by soil pH than the soil type with OM>25% when studying the outcomes of the mesocosm study (see section 6.3.2.1 and section 6.3.2.2). V in the topsoil with

OM>25% is observed to mobile under all pH conditions (albeit at low concentrations), with concentrations increasing with depth for all pH varieties studied (see Fig 6.10) whereas in the topsoil with OM<15% V concentration is influenced by pH with circumneutral and basic pH levels reducing trace metal concentrations with depth in the porewater see Fig 6.19).

When studying the outcomes of the mesocosm study, it is observed that Cu concentrations in the porewater are impacted by pH in both topsoil samples (see section 6.3.2.1 and section 6.3.2.2) with the topsoil with OM<15% showing greatest change under acidic conditions when compared to other pH variations (see Fig 620). This was despite both soil samples having been found to have similar pseudo-total concentrations (see Table 6.7). This indicates that OM plays a role in the mobility of Cu in the topsoil. These findings are in alignment with previous laboratory column studies examining the impact of pH and OM on Cu mobility and studies which have found OM binding capacity to be a factor in Cu mobility within the porewater of soil (Bravin et al, 2015; Temminghoff et al, 2012).

Previous studies have found that conditions in the soil (pH, moisture content and organic matter content) are the principal drivers influencing mobility of chromium in the soil (Banks et al, 2006; Choppala et al, 2018; Richard & Bourg, 1991). When studying the outcome of the mesocosm studies for Cr concentration in the porewater, it is observed that both pH and OM influence the concentrations of mobile Cr detected during the 12-week study. The topsoil with OM>25% was found to have a pseudo-total of 5.14ppm of Cr whereas the topsoil with OM<15% was found to have a pseudo-total of 12.86ppm following ICP-MS analysis with acid digestion (see Table 6.7). The mesocosm study found that Cr is most mobile in a topsoil which is acidic and with low levels of organic matter. This is in alignment with previous studies which found that OM can have an influencing factor on Cr mobility in acidic soils (Jardine et al, 1999) and studies that have found that liming application to a circumneutral soil (as is the case with the mesocosm study examining the topsoil with OM<15% in this study) greatly reduces the mobility of Cr (more specifically, Cr(III)) in the porewater of the soil (Choppala, 2018).

pH is shown to have a considerable impact on Co concentrations in the porewater of both topsoil types (see section 6.3.2.1 and section 6.3.2.2), with the topsoil with OM<15% being influenced to a greater extent than the topsoil with OM>25%. Co is found to be present in low level concentrations in both topsoil samples (see Table 6.7) with 0.44ppm being found in the topsoil with OM>25% and 1.61ppm being found in the topsoil with OM<15%. It is observed that OM content influences the porewater concentrations of Co (see Fig 6.13 and Fig 6.22). This is in agreeance with previous studies which found that increasing OM content of soil reduced Co content in the porewater (Skarpa et al, 2011). Previous studies have also found that Co is most mobile under acidic conditions and that application of lime ($CaCO_3$) limits the release of Co into the soil porewater (Malinowska & Jankowski, 2020).

As is found in low concentrations in both of the topsoil types studied, with a pseudo-total of 0.93ppm being found in the topsoil with OM>25% and 2.89ppm being found in the topsoil with OM<15% (see Table 6.7). pH was found to have a small influence on the concentration of As found in the porewater, with the topsoil with OM>25% showing increased concentration with depth (see Fig 6.14). This indicates that OM content plays a role in As mobility in topsoil. The role of pH and OM on As mobility in soil porewater has previously been studied (Lee et al, 2015; Grafe and Sparks, 2006; Moreno-Jiminez et al, 2010; Pigna et al, 2015). As has been found to be more effectively sorbed with the soil matrices at higher pH values than for those in more acidic soils (Pigna et al, 2015). This is in alignment with the mesocosm study which found lower concentrations of As in the porewater in the circumneutral and basic/alkaline columns than for the acidic columns for both topsoil types (see Fig 6.14 and Fig 6.23). The relationship between As mobility in the porewater of soil and organic matter content has been found to be complex (Lee et al, 2015) with the concentrations of Fe, Al and Mn within the organic matter being found to play a role (Graf and Sparks, 2006; Moreno-Jimenez et al, 2010). Further investigation would aid in elucidating these influences on As mobility in the porewater of topsoil.

Previous column studies have found that Sn is relatively immobile in soil (Elbana et al, 2016; Hou et al, 2005) and that migration with depth tends to be limited (Murata et al, 2018) with some influence by physiochemical properties (Hou et al, 2005). When examining the influence of pH and OM content in topsoil on the concentrations of Sn in the soil porewater from the mesocosm study, it was determined that pH plays a role in the mobility of this trace metal element in both soil types studied (see section 6.3.2.1 and section 6.3.2.2). The concentration of Sn in these soils were determined to be 2.06ppm and 5.37ppm in the topsoil with >25% OM and the topsoil with <15% OM respectively (see Table 6.7) when undergoing ICP-MS analysis with acid digestion (see Chapter 5). Despite the relatively similar occurrence in both these soils, the behaviour was seen to differ with OM content. Sn concentration in the porewater was seen to increase with depth for all pH variations in the topsoil with OM>25% (see Fig 6.15). The concentration was seen to be at an increased level for both circumneutral and basic pH variants when compared to the acidic control. Conversely, in the topsoil with OM<15%, Sn was found at only trace levels in the basic pH variant when compared to the circumneutral control, whilst showing minorly elevated levels of Sn in the porewater under acidic conditions when compared to the circumneutral control (see Fig 6.24). This indicates that, whilst ICP-MS analysis determined that the topsoil control sample with OM<15% had the greater concentration of Sn, the topsoil sample with OM>25% was found to have more mobile Sn in the topsoil porewater following analysis of the mesocosm study, especially under basic pH conditions. This suggests that OM may play a role in the mobility of Sn in topsoil.

Studies have shown that pH is the primary factor behind the sorption, solubility and mobility of Ni in the soil (Iyaka, 2011) with organic matter content playing a secondary role (Ge et al, 2000; Iyaka, 2011; Suave, 2000), although studies have found that OM has a strong binding capability for Ni, thus restricting release into the porewater (Rinklebe & Shaheen, 2017). Results from the mesocosm study indicated that Ni concentration in the porewater of both topsoil types was found to be heavily influenced by the topsoil pH with both showing considerably more Ni in the acidic pH variation (pH<6) as shown in Fig 6.16 and Fig 6.25. Previous ICP-MS analysis had found there to be a pseudo-total of 4.7ppm of Ni in the topsoil with OM>25% and there to be a pseudo-total of 7.38ppm of Ni in the topsoil with OM<15% (see Table 6.7). pH variation was found to impact the concentrations of Ni in the topsoil porewater for both the topsoil with OM>25% and OM<15% to the extent that no detectable Ni was found in both the circumneutral and basic pH variations on both soil types (see Fig 6.16 and Fig 6.25). However, the acidic variation showed considerably more Ni in the porewater, on average, in the topsoil with OM<15% at a peak of1406.09ppb compared to a peak of 28.68ppb peak result for the topsoil with OM>25%, both at 22cm-24cm depth. This indicates that increased OM content of the topsoil may reduce Ni concentrations in the porewater at this lower pH level.

This column leaching/mesocosm study found that pH variation had a notable impact on release of certain trace metals within the mesocosms. This was most apparent when the pH was lowered from the low OM (<15%) control column (circumneutral pH) sample to the acidic low OM column variation. All of the trace metals analysed showed increased mobility when the pH was altered from approximately circumneutral (pH 6.-7.5) to mildly acidic conditions (pH <6) in this low OM topsoil, with higher concentrations of each trace metal element being found, with depth, in the porewater of the acidic column when compared to the circumneutral control. These are the pH levels in line with those found in the screening study (see section 4.3.1 of Chapter 4 of this thesis). When raising the pH of the low OM control column so to reflect the influence of anthropogenic impacts on this type of topsoil (such as liming), 7 of the 9 trace metals studied reduced in concentration further from the control column with As and Ni showing little change or slight increase. This shows that low OM topsoil, that are abundant in the region, and associated with managed agricultural land, are very much influenced by pH when studying trace metal mobility.

When examining the control column with high levels of organic matter (>25%) and its pH variations (see Figs 6.8-6.16), it is observed that this high OM soil appears more resistant to pH change, with less variability of trace metal concentrations, than in the topsoil with <15%OM (See Figs 6.17-6.25). Sn, Cr, Cu, V, and Pb increase in concentration as pH is raised from that of the acidic control, especially so in the case of Pb. The remaining trace metals being studied (Zn, Co, As and Ni) show a decrease in concentration in the porewater as pH is increased. A general observation is that the magnitude of impact of pH variation tends to be greater in the topsoil type with <15% organic matter content. It is established that the binding capacity of the organic matter content of the soil plays a role in the potential mobility of trace metals in the soil porewater (Amery et al, 2007; Benedetti et al, 1996; Koopmans and Groenenberg, 2011). This indicates that areas of naturally high organic matter, such as those organo-mineral soils found adjacent to meres, are more resistant to variations in pH in terms of trace metal release in comparison to those areas with a low OM topsoil.

Measuring trace metal concentration in the porewater is considered an important aspect in the estimation of bioavailability of the trace metals (Kim et al, 2015) and, as such, it can be said that, from the findings of this study, both OM and pH are important influencing factors in both the mobility and bioavailability of trace metals in the porewater annually in the topsoil of this meres and mosses habitat. These findings are also in alignment with previous studies that have shown that certain trace metals (such as Cu and Pb) are known to bind strongly to dissolved organic matter (DOM) and that trace metal mobility in the soil porewater is influenced by both soil pH and the OM content of the soil (Amery et al, 2007; Cances et al, 2003; Koopmans and Groenenberg, 2011).

6.3.3 BCR/SM&T Sequential Extraction Study

It is possible to determine those trace metals that may, under certain conditions, become bioavailable when studying the results of the sequential extraction procedure. This is done by examining the 3 fractions implemented in the chemical fractionation procedure.

When examining the constituents of the results of the first 3 steps of the sequential extraction procedure (see Figs 6.26 and 6.27), it can be seen that, for the control column with low organic matter (<15%), Cr, Cu, Zn and Pb are the most mobile trace metal elements with 27%, 18%, 24% and 20% respectively of these trace metals occurring in the Exchangeable Fraction with these elements being bioavailable in this form. It can also be observed that a large proportion of the results for all the trace metals occur in the Reducible Fraction which is also considered to be bioavailable. When examining the fractions that are considered to be those in which trace metals are most mobilisable and, hence, bioavailable (Exchangeable and Reducible fractions) it can be observed that, from the three-step extraction procedure, the concentration in terms of relative abundance in these bioavailable phases are in the following order:

Sn(100)>As(99.34)>Co(96.4)>Pb(91.35)>Zn(88.07)>Cu(87.59)>Cr(87.31)>Ni(83.12)>V(81.37)

The Oxidisable fraction is the organic fraction that may be degraded via oxidisation leading to a release of soluble trace metals. This fraction contains those trace metals bound to organics and sulphides and is considered the fraction with the least potential for bioavailability out of the three chemical fractions.

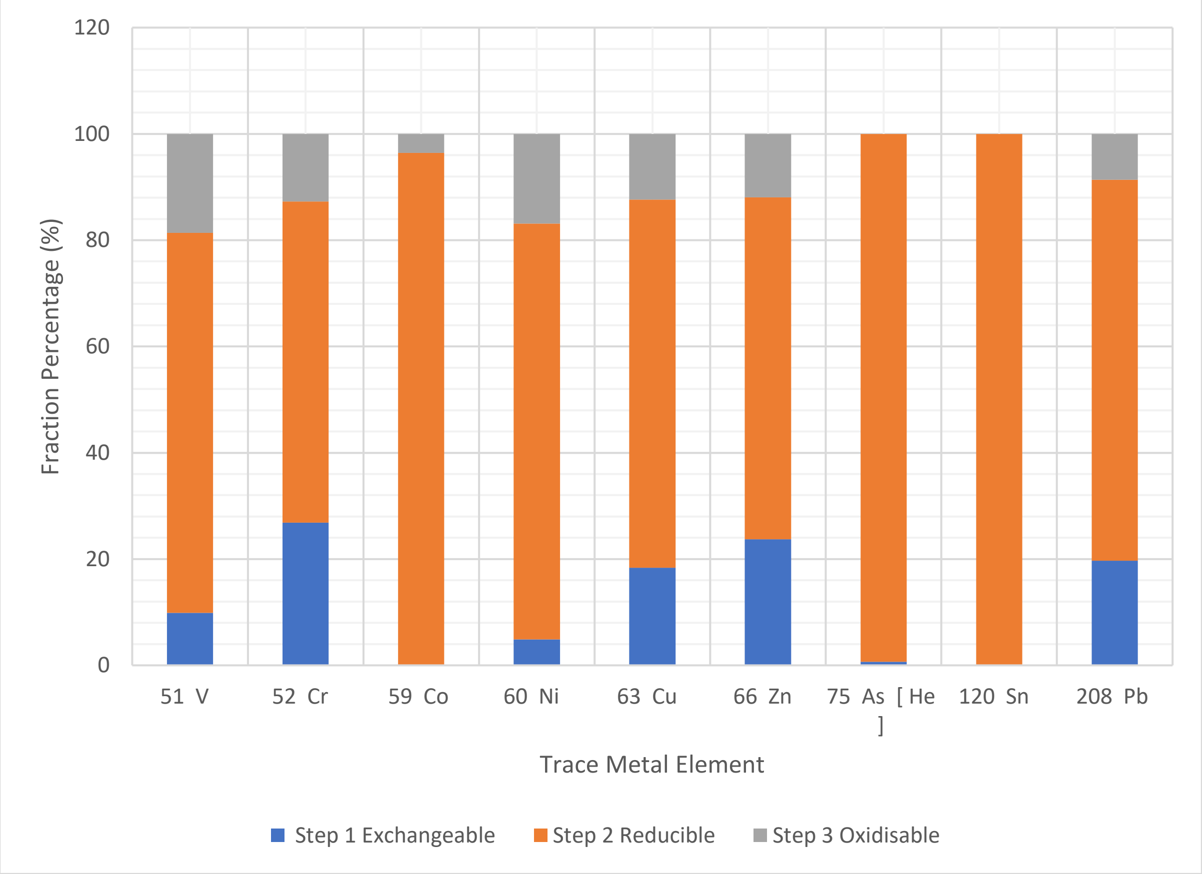


Fig 6.26: Trace Metal Chemical Fraction Percentage in Topsoil Sample with OM<15%

When examining the control column with organic matter >25% (see Fig 6.27), Cr, Ni, Zn and Pb are the most mobile trace metal elements with 18%, 13%, and 9% respectively of these trace metals occurring in the Exchangeable Fraction with these elements being bioavailable in this form. It can also be observed that a large proportion of the results for all the trace metals (except for 59 Co and 120 Sn which were not detected in the bioavailable fractions) occur in the Reducible Fraction which is also considered to be bioavailable. When examining the fractions that are considered readily bioavailable (Exchangeable and Reducible fractions) it can be observed that, from the three-step extraction procedure, the concentration in terms of relative abundance in these bioavailable phases are in the following order:

As(100)>Pb(82.88)>Zn(81.90)>Ni(74.69)>V(66.61)>Sn(57.85)>Cr(53.24)>Cu(42.57)

It is important to note that, for this sequential extraction study, no sub-samples were analysed and that the obtained results are based on a singular set of samples undergoing the sequential extraction procedure. This method may be improved by undertaking replicate analysis and establishing the average outcome with uncertainties factored into the results.

It is observed that a greater proportion of all trace metals are in the oxidisable fraction in the high organic matter (>25%) with low pH (<6) control sample when compared to the low organic matter with approximately circumneutral pH control sample except for As which is 100% in the reducible fraction for the sample with high organic matter content (see Fig 6.26 and Fig 6.27) with previous studies having shown that As is often found in the bioavailable fraction of the soil matrix (Beesley and Dickinson, 2010). This indicates that the topsoil in the low OM control column is more likely to have trace metal contents that are in the mobile fractions, and hence bioavailable, than areas with a topsoil with similar constituents as those found in the OM>25%control column.

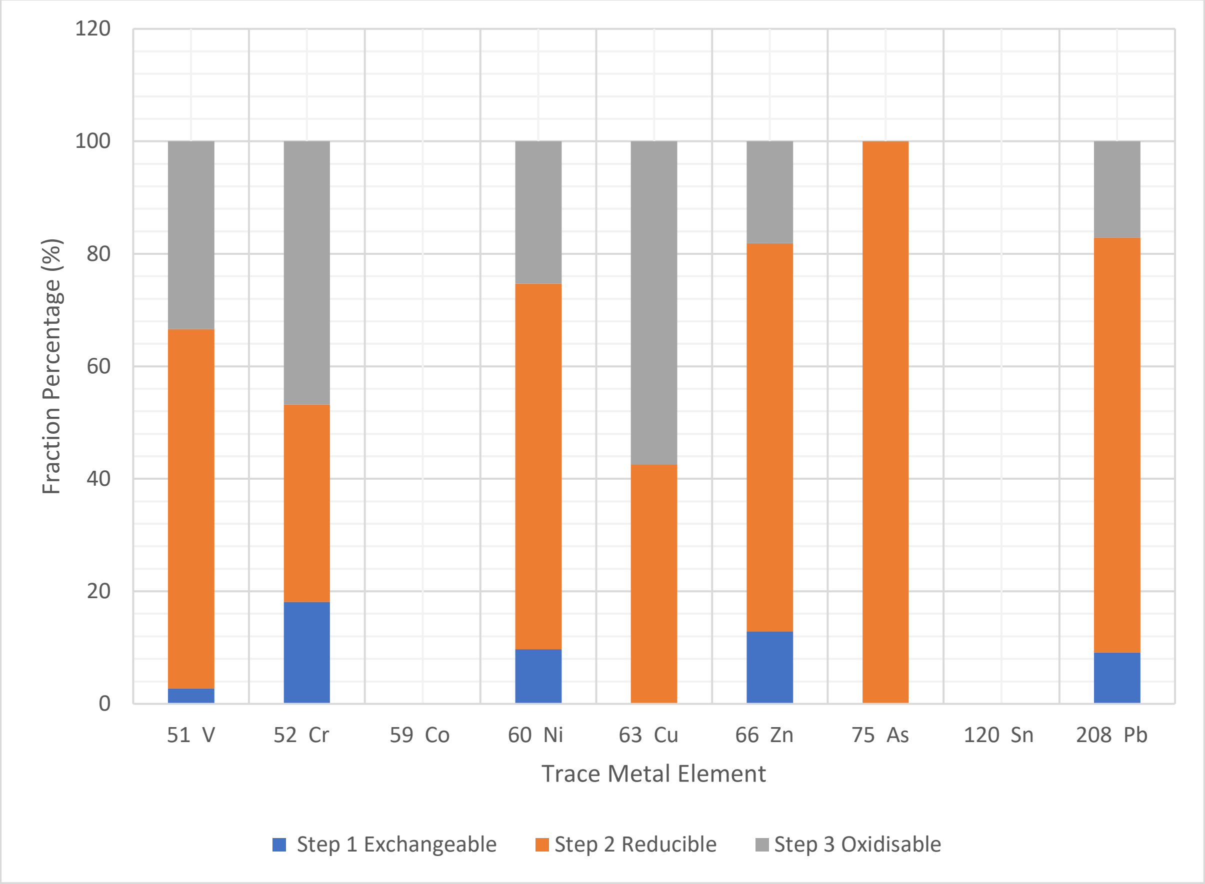


Fig 6.27: Trace Metal Chemical Fraction Percentage in Topsoil Sample with OM>25%

When studying and comparing the most mobile, and therefore the most potential for bioavailability, fractions from examining the sequential extraction results of the two control columns (see Fig 6.26 and Fig 6.27), it can be seen that the control column with organic matter content <15% and approximately circumneutral pH (6-7.5) has the greater potential for bioavailability with a greater proportion of all the trace metals analysed being found to be in the first two fractions (Exchangeable and Reducible). It can also be seen that a greater proportion of all trace metals are in the oxidisable fraction in the high organic matter with low pH control when compared to the low organic matter with approximately circumneutral pH control.

This sequential extraction study showed that, when examining the first 3 steps of the extraction study, it is observed that the trace metals most present in the 'bioavailable' phases are Sn, As and Co for the topsoil with low OM and As, Pb and Zn for the topsoil with high OM. It is determined that all of the trace metal elements in the topsoil with <15% OM have 80% or more of their content in the three-step aspect of the study in the first two, most bioavailable fractions (extractable and reducible fractions. The topsoil with high OM shows only As, Pb and Zn with over

80% in the first two extraction fractions with Cu showing less than 50% being considered readily bioavailable (extractable and reducible). This also indicates that the topsoil with high OM (>25%) is more resistant to lowering pH due to its already acidic nature (most likely due to the formation of humic acids within the soil profile) and that OM is effective at binding trace metals in this oxidisable fraction. This high OM soil was found to be low pH (4.10) prior to any adjustments in the column study with 42.1% OM (LoI) and 24.18% TOC on average. Elevated levels of organic matter can have the potential to influence trace metal mobility within the topsoil by inherent binding capacity of the organic matter and the formation of humic acids via flora and litter decay (Amery et al, 2007; Benedetti et al, 1996; Koopmans and Groenenberg, 2011). This decay can result in lower topsoil pH and the formation of complexes with trace metals (Renella et al., 2004). The resultant Increase in the amount of humic and organic acids on and within the topsoil have the potential to impact trace metal desorption, solubility, and mobility as well as influence the buffering capacity of the topsoil (Boguta & Sokolowska, 2012; Krishnamurti et al, 1997; Khodadoust et al., 2005; Schwab et al, 2005). It is observed in both control columns that because the vast majority of the trace metal concentrations are found in the residual fraction (see Table 6.5), both of these topsoil types undergo little anthropogenic influence in terms of trace metal contribution. The results of this sequential extraction study were then implemented to determine the risk to the local environment via the Risk Assessment Code (RAC). This RAC analysis determined potential risk of the trace metal constituents in the topsoil by establishing which trace metals were most present in a bioavailable fraction.

6.3.4 Risk Assessment Code (RAC) and Mass Balance Calculation

The RAC assesses the bioavailability of trace metals in porewater by applying a scale to the percentage of constituents that can reduce trace metals in the most bioavailable (exchangeable and carbonate) fractions (Singh et al, 2005). The RAC assessment system is based on the percentage of the trace metal found in the first step of the three-step extraction procedure the classification key is as follows:

Zero (RAC < 1), low (1 < RAC < 10), medium (10 < RAC < 30), high (30 < RAC < 50) or very high (RAC > 50) risk.

Examination of the residual fraction in the sequential extraction process can aid in determining the potential risk to a local environment via the Risk Assessment Code (RAC). By determining the sum of the 3 extraction steps of the BCR/SM&T protocol, plus the fourth residual step, the total metal concentration of the sample is determined (see Table 6.5).

	Cu	Pb	Zn	V	Cr	Co	Ni	As	Sn
OM>25% Control Residual Fraction (ppm)	15.16	26.12	49.75	2.96	13.48	0.22	10.17	0.24	1.29
Sum of Sequential Extraction in OM>25% Control Column (ppm)	15.47	28.24	53.69	3.07	13.56	0.218	10.33	0.242	1.29
OM<15% Control Residual Fraction (ppm)	3.99	2.98	8.37	5.83	6.38	2.16	8.49	0.80	0.28
Sum of Sequential Extraction in OM<15% Control Column (ppm)	4.57	4.36	10.996	6.03	6.51	2.33	8.94	0.878	0.328

Table 6.5: Residual Fraction Results for Control Columns and Sum Total of Fractions

To determine if the sum of the fractions was representative of the pseudo-total obtained from acid digestion methodology, statistical analysis was undertaken on the two datasets. The relationship between the total trace metal contents acquired by the summing of the four extraction steps in the sequential extraction process and those pseudo-total results obtained via acid digestion in Chapter 5 of this thesis were determined by examining the Pearson correlation coefficient (see Table 6.6a and Table 6.6b).

As there was found to be a very high positive correlation between the sum of the four extraction steps in the sequential extraction study and the pseudo-total determined by reverse aqua regia digestion (see Table 6.6a and Chapter 5), with significance at <.001, the sum of the extraction steps was determined to be valid when determining ‘Ct’ in implementation of the Risk Assessment Code (Soares et al, 2015; Ates et al, 2020 for the control column with >25% OM).

Correlations		Pseudo-total result for OM>25% Column (ppm)	Sum of Sequential Extraction OM>25% Column (ppm)
Pseudo-total result for OM>25% Column (ppm)	**Pearson Correlation**	1	.961**
	Sig. (2-tailed)		<.001
	N	9	9
Sum of Sequential Extraction OM>25% Column (ppm)	**Pearson Correlation**	.961**	1
	Sig. (2-tailed)	<.001	
	N	9	9

**. Correlation is significant at the 0.01 level (2-tailed).

Table 6.6a: Pearson Correlation Coefficient between the Sum of the Extraction Steps and Pseudo-Total Results for Topsoil Control Column with OM >25%

There was found to be a moderate positive relationship between the sum of the four extraction steps in the sequential extraction study and the pseudo-total determined by reverse aqua regia digestion (see Table 6.6b) for the control column with topsoil with <15% OM. At a significance of .066, this was not found to be significant at the 5% threshold. As such, a mass balance calculation was undertaken so to examine the trace metal elements in greater detail (see Table 6.7).

Correlations

		Pseudo-total result for OM<15% Column (ppm)	**Sum of Sequential Extraction OM<15% Column (ppm)**
Pseudo-total result for OM<15% Column (ppm)	**Pearson Correlation**	1	.636
	Sig. (2-tailed)		.066
	N	9	9
Sum of Sequential Extraction OM<15% Column (ppm)	**Pearson Correlation**	.636	1
	Sig. (2-tailed)	.066	
	N	9	9

Table 6.6b: Pearson Correlation Between the Sum of the Extraction Steps and Pseudo-Total Results for Topsoil Control Column with OM <15%

A mass balance calculation was performed by examining the sum of the fractions in the sequential extraction process and comparing to the obtained result from the ICP-MS analysis (pseudo-total) for the two control samplers, based on the results obtained via the methodology in Chapter 5 of this thesis, so to determine which trace metal elements best recovered in the sequential extraction procedure as shown in Table 6.7.

Trace Metal Element	Sum of Sequential Extraction Column with pH < 6 and OM>25% (ppm)	Pseudo-Total Result for Column with pH<6 and OM>25% (ppm)	% Recovery	Sum of Sequential Extraction Column with pH6-7.5 and OM<15% (ppm)	Pseudo-Total Result for Column with pH6-7.5 and OM<15% (ppm)	% Recovery
Pb	28.24	36.31	77.77	4.36	32.65	13.35
Zn	53.69	48.69	110.2	10.996	47.29	23.25
Cu	15.47	10.49	147.47	4.57	14.17	32.25
V	3.07	2.51	122.31	6.03	10.77	55.99
Cr	13.56	5.14	263.81	6.51	12.86	50.62
Co	0.218	0.44	49.55	2.33	1.61	144.72
Ni	10.33	4.7	219.79	8.94	7.38	121.14
As	0.242	0.93	26.02	0.878	2.89	30.38
Sn	1.29	2.06	62.62	0.328	5.37	6.11

Table 6.7: Mass Balance Results for the Sequential Extraction Compared to the Pseudo-total results of Two Topsoil Types.

The mass balance calculation (see Table 6.7) shows that, whilst correlation is high between the sum of the fractions and the pseudo-total (see Table 6.6a), percentage recovery is variable for both soil types. The column with OM >25% and pH <6 shows average-good recovery across all trace metal elements except for Cr, As and Ni. The column with OM <15% and pH 6-7.5 shows good recovery for Ni, average recovery for V, Cr and Co with the remaining elements showing poor recovery. This variability in recovery between the soil types indicate that the effectiveness of the outcome of the RAC may depend on the soil type being studied and the volume of sample used.

Reasons for variable recovery of trace metals in the sequential extraction procedure compared to the observed results for pseudo-total concentrations obtained have previously been studied and include soil type, extraction procedure/methodology and volume of sample used in the extraction steps. Soils that are known to be temporarily anaerobic (such as those in wetland regions) have been found to produce variable results in regard to recovery in sequential extraction studies that implement the BCR/SM&T methodology due to potential for release of trace metals bound to the organic fractions (Van Hullebusch et al, 2005). The filtration carried out in the residual fraction step has been shown to be a possible reason for under-recovery when compared to the pseudo-total result (Kryc et al, 2003). Instrumental sensitivity, differing calibration between sample runs and limits of detection (LoD) may also be a factor for topsoil samples in which only trace amounts of certain trace metals are being studied and, rather than the variability within the method, it has been found that variability of recovery is influenced by the homogeneity of the trace metal element in the topsoil sample when examining precision of outcome at low ppb levels (Hall & Pelchat, 1994) when using ICP-MS as the analytical procedure. Studies have also shown that in extractions in which <1.0g of sample is used (as is the case for the residual step undertaken in this study) can result in weaker recoveries when examining the mass balance (Ciceri et al, 2008). The potential mobility of the trace metals being studied and the physiochemical properties of the soils being studied may also result in high or low recovery (Qayum et al, 2016). This variability in recovery should be considered when making any assessment on a Risk Assessment Code exercise. As there were no subsamples analysed for this RAC study, further research would benefit from focussing on increased reproducibility of methodology and outcomes.

Trace Metal	Percentage Concentration in Exchangeable Fraction in Control Column with OM >25%	Percentage Concentration in Exchangeable Fraction in Control Column with OM <15%	Risk in Control Column with OM >25% Topsoil	Risk in Control Column with OM <15% Topsoil
Cu	0	2.37	No	Low
Pb	0.69	6.24	No	Low
Zn	0.95	5.69	No	Low
Ni	0.15	0.25	No	No
Cr	0.11	0.54	No	No
Co	0	0	No	No
As	0	0.06	No	No
Sn	0	0	No	No
V	0.10	0.32	No	No

Table 6.8: Risk Assessment Code for Topsoil Control Samples

In the above results (see Table 6.8) the RAC is calculated based on the percentage of trace metals found in the most bioavailable fractions (exchangeable and carbonate fractions), which is step 1, of the sequential extraction procedure (Liu et al, 2019). Risk is calculated by dividing the trace metal content of an exchangeable fraction (step 1) to the total content of the trace metal in all fractions (Ates et al, 2020). It is important to note that this risk index does not indicate the toxicity of the trace metal and that a ‘high risk’ result of a low toxicity trace metal element may not be as impactful as a ‘low risk’ result for a trace metal element considered to be more toxic (Davutluoglu et al, 2010; Liu et al, 2019).

When examining the Risk Assessment Code, when using all the fractions in the extraction process (including residual fraction as per Ates et al, 2020) to represent Ct, it is observed that Cu, Pb and Zn are classified as ‘low risk’ for the topsoil with OM <15% with the remaining trace metals analysed being classified as ‘no risk’. The RAC results for this control column may be underestimated, however, due to the poor recovery of some trace metals when compared to the obtained pseudo-total as displayed in the mass balance (see Table 6.7). All the trace metals analysed for the topsoil with >25% OM were classified as ‘no risk’ under this RAC methodology. This Risk Assessment Code exercise aids in the determination if a particular trace metal may be of concern to the local environment once potential for bioavailability and toxicity has been established.

6.4. Conclusion

In this study, the relationship between, and consequent risk of, OM, pH and the release of 9 trace metals (Pb, Cu, Zn, Co, Cr, V, As, Sn and Ni) in an environmentally sensitive meres and mosses habitat was evaluated using mesocosm column leaching experiments and sequential extraction procedures with consequent implementation of Risk Assessment Code (RAC). The topsoil samples chosen were two representative samples that had the pH and OM levels as would be found in a meres and mosses habitat and surrounding area and representative of the sampling area of the Meres and Mosses of Cheshire and Shropshire (within LPS/NIA boundary), as determined from physiochemical studies in Chapter 4 of this thesis. This was undertaken so to examine the mobility of in-situ trace metals within the porewater of the topsoil that is representative of this region. These two samples with differing OM content were chosen from within the sampling region and designated as ‘control’ samples within the mesocosm study. A further 4 mesocosms were set up so to display impacts of pH variability on both topsoil types and then duplicates of each column were established for replication purposes (12 mesocosms in total) with the median result being applied to represent the central tendency for trace metal concentration for the duplicate columns. Artificial Rainwater (ARW) was applied so to the mesocosms to simulate the known conditions of the sampling area from data obtained from the local climate observatory (Shawbury). The purpose of the mesocosms were to display the impacts of varying the pH on these topsoil samples (so to simulate anthropogenic influence) in terms of trace metal concentration in the porewater so to ascertain the impact of pH on topsoil with differing levels of organic matter and to determine the average mobility of trace metals through the depth of the A-horizon/column annually under the varying conditions. A sequential extraction procedure was then undertaken so to elucidate the behaviour of the various trace metals under certain, increasingly aggressive, conditions and to aid in the understanding of the outcomes of the column leachate/mesocosm study. This was done so to ascertain the levels of trace metal within the topsoil that could be considered to be bioavailable and thus, potentially, harmful to flora and fauna in the local environment as well as in these habitats more generally.

The column leaching study found that pH variation had a significant impact on the release of certain trace metals within the porewater of the topsoil. Lowering the pH of a circumneutral pH (6-7.5), low OM (<15%) topsoil increased both the concentration of many of the trace metals being analysed within the porewater when compared to the control sample which also increased the mobility with the depth of the soil profile in many cases. Conversely, raising the pH reduced trace metal concentration within the porewater for some trace metals and mobility was hindered by increasing pH in many cases with this low OM(<15%) topsoil type. The high OM (>25%) topsoil type showed more resilience to pH change in terms of changes in trace metal mobility. A characteristic that has been observed to possibly prevent the transfer of trace metals to the environment (Padoan et al, 2020) and in accordance with studies in these habitat types (Borgulat et al, 2017;Lidman et al, 2014). This resilience was also observed in the sequential extraction study, in which displayed lower trace metal concentration proportions in the bioavailable fractions for the high OM (>25%) topsoil than for the low OM (<15%) topsoil. This sequential extraction study provided evidence of trace metal binding with the organic matter (oxidisable fraction) which could explain the behavioural characteristics observed for the trace metal mobility in the porewater of the topsoil being studied.

The RAC analysis, when using the sum of all 4 extraction steps as the determinant for ‘total’ metal content (Ct), determined that Cu, Pb and Zn were classified as a low risk in the mineral topsoil with OM <15% with no further risks being found in the remaining trace metals of this topsoil. The organo-mineral topsoil with OM >25% was found to have no trace metals classified as a risk under this RAC methodology. When considering the RAC methodology, it is important to note that whilst this risk index is based on individual trace metal mobility, it does not account for the toxicity of the trace metal (Davutluoglu et al, 2010; Liu et al, 2019). These RAC results also reinforce the findings found in the mesocosm study that the topsoil with OM >25% (control sample) show less risk/mobility than the topsoil with OM <15% (control sample). This is an indication to, as per the hypothesis of this study, that OM levels are an influencing factor for trace metal mobility as well as pH, thus reinforcing the outcome of the mesocosm study which displayed the impact of acidic conditions on trace metal concentrations in the porewater of a low OM topsoil. These RAC results should be treated with caution, however, as the mass balance calculation displayed poor recovery for some trace metals, especially in the control column sample with OM <15% (including for Cu, PB and Zn) which means that the results obtained in the RAC for these particular trace metals may be underestimated. It is important to note that, due to logistical constraints, no subsampling was undertaken for this aspect of the study (sequential extraction and consequent RAC). Further research would benefit from subsampling to aid in replicability, reproducibility and repeatability so that uncertainties of the dataset can be ascertained. The results obtained from the studies in this chapter not only display the impact that changes in pH have on trace metal concentrations in the porewater of topsoil and their potential mobility through the a-horizon, but also elucidate the potential risks to the flora and fauna in the local environment. With this information, local landowners, conservation and remediation organisations and interested parties can implement measures for mitigation and remediation, based on the physiochemical make-up of the topsoil involved, to help minimise potential impacts of trace metal mobility in the topsoil of this meres and mosses habitat and this type of habitat more widely. This is important as increasingly stringent soil quality guidelines and environmental protections (as outlined in Chapter 2, Chapter 4 and Chapter 5 of this thesis) for habitats of this type within agricultural regions will require effective measures in both assessment and the most appropriate mitigation of trace metal pollution.

Chapter 7

Conclusion and Final Discussion

7.0 Introduction

Trace metal pollution is one of the most important of anthropogenic impacts on meres and mosses wetland habitats due to potential bioaccumulation in the terrestrial environment and, consequently, the food chain (Fiałkiewicz-Kozieł et al, 2011; Nkwunonwo et al, 2020). Trace metals such as copper (Cu) and lead (Pb) are persistent and potentially toxic in these environmentally sensitive habitats (Tang et al, 2014). The topsoil of these habitats "serves as the transfer interface for the accumulation of trace metals in the air, plant, and water" (Sun and Chen, 2016). The results of an environmental screening study, followed by a focussed mesocosm study, are presented within this thesis with the aim of determining and elucidating the presence and spatial mobility of trace metals in the topsoil of a meres and mosses habitat and consequent potential for their bioavailability. The validity of these results are bolstered by a method development study so to enable a comparison of analytical techniques implemented in the determination of trace metal concentration and their adherence to soil quality guidelines. This conclusion chapter will discuss the findings from the studies within this thesis and place context in the wider issues around trace metal contamination in topsoil and the consequent pressures on protected and environmentally sensitive habitats.

7.1 Summary of Thesis Aims

The thesis sets out to determine the spatial occurrence of in-situ trace metals in the topsoil of a meres and mosses habitat, the relationships between and within the trace metals and physiochemical properties and the consequent risk to the local environment. It does this by initially establishing the current situation regarding in-situ trace metal concentration in the Meres and Mosses of the Marches which is a meres and mosses habitat in Cheshire and Shropshire in the United Kingdom. This area has previously been identified as being environmentally sensitive and contains various protective designations (see section 7.3.1). The sampling area was chosen so to represent the boundaries of previous environmental protection schemes (NIA and LPS) as displayed in Chapter 2 and Chapter 4 of this thesis. A screening study of in-situ trace metal concentrations in the topsoil of the region with additional determination of physio-chemical properties of the topsoil was undertaken so to establish a geochemical profile of the region within the designated boundaries. Field Portable X-Ray Fluorescence Spectroscopy (FPXRF) methodology was implemented for the purpose of determining trace metal concentration during the screening study due to its methodological advantages of being portable, rapid and non-destructive to the topsoil samples in a protected environment meaning that the samples may be re-used for alternate analysis without requirement for further sample collection. The FPXRF methodology does have limitations. The FPXRF is not as sensitive as alternative analytical procedures as it determines the trace metal concentration of the soil matrix with detection limits in the ppm range. FPXRF analytical methodology for trace metal concentration is also not a good indicator of the bioavailability of the trace metals being examined. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) with acid digestion analysis for trace metals is advantageous as it is a sensitive analytical method with limits of detection in the parts per billion. This spectroscopic methodology for determination of trace metal concentrations is desirable when examining habitats that are not heavily influenced by anthropogenic input and only trace amounts of trace metals are expected to be found. This analytical methodology also provides an indication of the bioavailability of the trace metals being studied. The disadvantages of the ICP-MS methodology are that it is destructive to the sample due to the sample preparation procedure and is more costly, requires considerable laboratory space and is time consuming compared to FPXRF. Certain environmental quality guidelines also implement the ICP-MS method (acid extraction) of determining concentration of trace metal in soils so, as well as creating a screening profile for the region via the FPXRF, a comparison study was performed, with a method development study, so to ascertain relationships between the two analytical procedures. Determining the viability and appropriate implementation of the analytical methodologies meant that, along with data obtained from the local climate centre for the region, a focussed mesocosm study could be established that would monitor the movement of trace metals through the a-horizon in a simulated calendar year. This would aid in elucidating the impact that the physio-chemical properties found within the region had on the concentrations of trace metals within the porewater of the topsoil and their potential for bioavailability. The methodology implemented in this thesis would fulfil the thesis aims of determining the present situation in terms of trace metal spatial occurrence in a meres and mosses habitat, the relationships between the physiochemical properties, and how these relationships influence the mobility of trace metals in the topsoil. The fulfilment of these aims will aid in both the understanding of trace metal behaviour in these environmentally sensitive habitats and in potential management, mitigation and remediation for trace metal contamination and, consequently, limiting the mobility of trace metals of concern so to adhere to various soil quality guidelines.

Within this overall aim, there were several key objectives:

1. To undertake a survey/screening study of the topsoil of an environmentally sensitive agricultural meres and mosses landscape via FPXRF so to determine trace metal concentrations and geophysical/geochemical factors in order to obtain a dataset that Identifies the distribution and variation of trace metals in the region sampled along with determining their source of origin, be it anthropogenic or natural/geogenic by implementation of multivariate statistical analyses.

2. Validate an appropriate analytical procedure for the determination of the concentration of multiple trace metal elements simultaneously in a soil matrix via ICP-MS with reverse aqua regia acid digestion by means of a method development study.

3. Elucidate how these differing analytical methodologies for the determination of trace metal concentrations in a soil matrix can potentially impact the outcome of soil quality assessments (by implementation of soil quality guidelines) by means of a comparison study between FPXRF and ICP-MS.

4. Determine the link between changes in geochemical and geophysical processes with trace metal mobility and bioavailability by means of a mesocosm study and chemical fractionation analysis.

Each chapter of the thesis covers these key aims by determining how each aspect play a key role in elucidating the current status of this agricultural meres and mosses habitat in regard to trace metal concentrations, distribution and variation in the topsoil of this region and how physiochemical factors influence the spatial occurrence and potential for bioavailability of these trace metals within the porewater of this topsoil. This determination of topsoil quality was then placed into context against soil quality guidelines for trace metals and can be used as an aid for conservation, mitigation and remediation in the important task of maintaining the health of these environmentally sensitive habitats.

7.2 Main Findings and Significance

7.2.1 Summary and Significance of the Screening Study.

The screening study undertaken in Chapter 4 of this thesis was an essential profiling of the sampling area so to elucidate the trace metal content and physiochemical properties of the topsoil in a meres and mosses agricultural habitat and the relationships therein. This profiling determined not only the presence and concentration of 17 trace metals in the topsoil (see Chapter 4) but also how physiochemical factors (such as pH and soil organic carbon) and other properties can be an influencing factor on the presence of these trace metals. Spatial mapping allowed these relationships to be visually determined and to ascertain patterns of incidence for the trace metals and these physio-chemical properties. Statistical analyses allowed the determination of the strength of these relationships and how one factor may influence another.

By adopting a multivariate statistical approach, these statistical analyses (Principal Component Analysis and Cluster Analysis) alongside methods to determine the presence and intensity of trace metals (such as Enrichment Factor) following analytical determination of concentrations via FPXRF can aid in determining the origin of the trace metals present, be it anthropogenic or geogenic. Principal Component Analysis (PCA) was implemented for the interpretation and elaboration of the datasets. Shan et al (2013) state that, “PCA is a multivariate statistical technique that reduces the original multidimensional space to a new lower dimensional space (principal components, PCs) which is then used to explain relationships and associations among objects and variables” (Wold et al. 1987; Jolliffe 2002). Cluster Analysis (CA) was implemented in conjunction with PCA to aid in the grouping of individual parameters and variables (Facchinelli et al., 2001). Correlation distance was studied so to measure the distance between clusters of similar trace metal contents in the cluster analysis. The outcome of this screening study determined that Pb, Zn, V, Cr, Co, As, Rb, Nb, Ga and Sn showed levels of enrichment that indicated anthropogenic influence. The statistical correlation studies determined significant correlations between certain trace metals with organic matter levels (OM) and pH. Whilst the presence of some of these trace metals are easily explained by the industrial background of the region (including steel fabrication, such as in the case of Ga and Nb) further study of some of these trace metals was undertaken so to elucidate the behaviour of these in-situ trace metals within the topsoil and how their presence may impact the region when examining environmental quality guidelines. This included a comparison study with alternative analytical methodology when determining the concentration of the trace metals in the topsoil (see Chapter 5). The data obtained from correlation analysis displayed significant positive relationships between Pb, Cu and Nb with the organic content analysis (LoI and TOC) that were moderate to very strong, as well as moderate interaction with pH for some metals, including Pb and Nb which had a significant and moderate negative correlation. This indicated that these trace metals share a common source and that the level of organic matter and acidity of the soil may be a controlling factor. Spatial mapping via GIS mapping has been specifically used in soil quality assessments (Adamus and Bergman, 1995; Meinardi et al 1995) and can aid in building a profile which highlights the influence of anthropogenic activities on topsoil health (Shan et al, 2013). The spatial data obtained from the mapping process, in conjunction Kriging interpolation (see Chapter 4), showed that some of the trace metals shared similar spatial patterns (Pb, Cu, Nb) and that some of these elements are those that have been found to display some enrichment (Pb, Zn, Nb). The results of the PCA indicated that Cu and Pb, shared a common source and that organic matter and pH are controlling factors in both their presence and mobility being in the same component. The Cluster Analysis was also found to be in close agreement with the PCA reinforcing the observed relationship between pH, OM and the trace metals. A mesocosm study was therefore established so to further investigate the impact of these relationships on topsoil deemed representative of the region (see section 7.2.4 and Chapter 6).

The results of the trace metal content from the screening study were then placed into context against widely cited environmental soil quality guidelines (Chapter 4). Dutch Target and Intervention Values, 2000 (sometimes known as the Netherlands Target and Intervention Values) of a standardised soil (see Chapter 1, Table 1.1) were adapted so to represent the sampling area being studied (see Table 7.1, Chapter 4 and Appendix F) and then used when examining the trace metal concentration results from the screening study. These are derived from the Netherlands Soil Contamination Guidelines (VROM, Department of Soil Protection, 1994) and are used as environmental pollutant reference values in soil quality assessment. These guidelines set targets and/or thresholds for those metals most associated with anthropogenic input (see Table 7.1). The ‘Target values’ referenced are considered background values as acknowledged in the Soil Remediation Circular 2013 of the Dutch authorities (Rijkswaterstaat Environment, 2013).

Some guidelines are attributable to the management of the habitat in question with The EU Sludge to Land Directive (EEC Directive, 86/278/EEC) which sets limits for trace metals in agricultural soils that receive sewage sludge due to its widely used application as a fertiliser (see Table 7.1). EC directive 86/278/EEC is implemented in England via the Sludge (Use in Agriculture) Regulations, 1989

(DEFRA, 2018). Under these regulations, the pH of the soil and land-use are also taken into consideration (see Chapter 4, Tables 4.1a-4.1c).

Trace Metal Element	**Adapted Netherlands Target/Background Value Limits* (ppm)**	**Adapted Netherlands Intervention Value Limits* (ppm)**	**EC Directive 86/278 Limits (ppm)**
Cu	19.78	104.37	100
Pb	57.96	361.40	300
Zn	63.46	326.34	250
V	15.03	89.46	-
Cr	52.02	197.68	400
Co	2.28	60.87	-
As	18.18	34.49	-
Sn	-	218.18	

- Denotes no available data
- * Adapted using median clay and organic matter content of soil samples from all soil samples.

Table 7.1: Soil Quality Guidelines

The guidelines state that, “The ‘Target’ value is the baseline concentration value below which compounds and/or elements are known or assumed not to affect the natural properties of the soil whereas the ‘Intervention’ value is the maximum tolerable concentration above which remediation is required” (Dutch Target and Intervention Values, 2000). The results of the screening study found that Pb, Zn, V, Cr and Co were seen to exceed the Netherlands Target Value limits (see Chapter 4) when using FPXRF as the analytical procedure for trace metal concentration. This aligns with the Enrichment Factor (EF) results which found significant enrichment for Pb and minimum to moderate enrichment for Zn, V, Cr and Co. Implementation and assessment of these Dutch Target Values have previously been used in many studies in which FPXRF or XRF is the analytical method for determination of trace metal concentration (Venvik and Boogaard, 2020; Cappuyns and Slabbinck, 2012; Dao et al, 2013). The results from the screening study also showed that the median results for all the relevant trace metals analysed in screening study relevant to the EC directive limits were compliant but there were a number of samples within the dataset that were found to exceed these limits for Cu, Pb and Zn when studying the descriptive results (see Chapter 4, Table 4.2). There are also soil quality guidelines with some widely cited methodologies using specific analytical protocols when determining trace metal concentration in soils. The Finnish government sets out 'threshold' and 'guide' values from the Finnish legislation for contaminated soil (Finnish Government Decree 214/2007) based on aqua regia extractable analytical techniques which is the recommended analytical method by the Decree (see section 7.2.3, Table 7.3). So that comparison to the Finnish guidelines could be undertaken, an alternative analytical procedure was required. This meant utilising a methodology that adhered to the Finnish guidelines and that was capable of multi-element analysis. The results of the method development study (see section 7.2.2) meant that a reverse aqua regia digestion methodology could be validated for use on ICP-MS instrumentation (see Chapter 3 and Chapter 5 of this thesis) in adherence with Finnish Soil Quality Guideline protocols.

The statistical and laboratory-based methods implemented in this screening study, alongside spatial mapping (via GIS software) aided in the visualisation of spatial relationships within the environmental dataset and helped identify potential sources. This screening study also enhances the currently held data for soil quality in the Meres and Mosses region. The British Geological Survey (BGS) have undertaken analysis of deep soils (below 36cm depth) for many aspects, including trace metals, soil profile and chemistry in the Meres and Mosses of the Marches region. The BGS currently have no data available for topsoil (A-horizon) for trace metals in this environmentally sensitive region of Cheshire and Shropshire. The screening study of this thesis provides vital information which may be adopted when implementing further research in this region with the results obtained being implemented in the mesocosm study which aided in the understanding of the spatial behaviour of trace metals in topsoil porewater when examining meres and mosses habitats widely (see section 7.2.4 and Chapter 6), leading to potential mitigation and remediation measures.

7.2.2 Summary and Significance of the Method Development Study

A method development study was undertaken for the digestion of trace metals in a topsoil matrix using reverse aqua regia as the digestion medium. This was undertaken so to confirm the validity of the obtained results from the reverse aqua regia digestion process when using ICP-MS as the analytical instrumentation and a certified reference loam soil (ERM-CC141) representing the soil type found in the meres and mosses region. The method development study ascertained the parameters required in the reverse aqua regia methodology so to obtain acceptable recovery (80%-120%) following the digestion procedure (see Chapter 3) when analysing multiple trace metals simultaneously in a single run of analysis. This meant that a comparison study between the analysis undertaken in the screening study via FPXRF, which analysed the total trace metal concentration of multiple trace metals simultaneously, and a multi-element approach for ICP-MS with reverse aqua regia as the digestion medium was viable. Reverse aqua regia has been found to have an overall similar recovery capability of trace metal elements when compared to conventional aqua regia, and/or superior recovery capability of trace metal elements when compared to conventional aqua regia for certain soil types, and produces reproducible results without the requirement for harsh and aggressive acids that have traditionally been implemented in acid digestion methodology (Szymczycha-Madeja et al, 2021; Lu et al, 2007). This method development process results in increased confidence in obtained results from this analytical methodology. The pseudo-total obtained from the reverse aqua regia digestion is also considered to give a good indicator of the bioavailability of the trace metals analysed (Andersen and Kisser, 2004; Al-Hwaiti et al, 2010). Bioavailability is an important consideration which may have a potential impact on land management and remediation (Naidu et al, 2015). This method of digestion (see Table 7.2) was used alongside the implementation of Collision Cell Technology (CCT) so to reduce the impact of spectral interference in the analytical procedure (see Chapter 5, Table 5.2) and these parameters passed the method development requirements with and without CCT mode activated (see Chapters 3 and 5) for all the trace metals present in the reference material that were implemented in this thesis (Cu, Co, Cr, Ni, Pb, Mn and Zn).

Digestion Method	Ramp Time (mins)	Hold Time (mins)	Max Temperature (°C)	Power (W)
Reverse aqua regia	20	5	200	1300

Table 7.2: Microwave Parameters for Digestion of Topsoil Samples with Reverse Aqua Regia.

Obtaining satisfactory recovery data in a multiple trace metal approach, in accordance with a certified soil reference material, means that confidence can be placed in the validated results and a comparison study to alternative methodologies, including FPXRF, could be undertaken so to ascertain if relationships/correlations can be determined between these analytical techniques (see section 7.2.3 and Chapter 5).

7.2.3 Summary and Significance of the Analytical Comparison Study

A comparison study was undertaken, following method development and validation, so to establish relationships between the two analytical procedures implemented in this study and how this may impact adherence to soil quality guidelines. With both methodologies having their own advantages and disadvantages depending on the analytical requirement and logistical feasibility (see Chapter 5). This study used statistical correlation of the two datasets to determine if the pseudo-total obtained via reverse aqua regia methodology on ICP-MS instrumentation could also be used as an indicator for the total result obtained from the FPXRF methodology and, as such, be a viable option for screening studies. Nine trace metals were chosen for analysis in this comparison study. These were Cu, Pb, Zn, Co, Cr, V, As, Sn and Ni with the first eight of these being chosen from the outcomes of the screening study (see Chapter 4) and Ni also being latterly chosen for assessment due to its potential impact from anthropogenic sources (see Chapter 5). A certified reference material (the same as in the method development study in Chapter 3) was implemented to validate the results obtained from the ICP-MS instrument when using this method of acid extraction (see Chapters 3 and 5). This reference material was used so to reflect the type of soil found in the Meres and Mosses region (ERM-CC141 Loam Soil) as determined in the screening study (see Chapter 4). The results from the comparison study found that the pseudo-total obtained by reverse aqua regia digestion and consequent analysis on ICP-MS does correlate strongly with the results obtained from FPXRF for many trace metals with all trace metals analysed other than V and As showing significant relationships at either the 0.05 level (2-tailed) or 0.01 level (2-tailed) following statistical analysis. This means that, for select trace metals (Cu, Pb, Zn, Co, Cr, Ni and Sn), this reverse aqua regia methodology is a viable methodology when undertaking a multi-element screening in estimating a total concentration result as well as being an approved methodology for the implementation of the Dutch Soil Quality Standards (VROM, 2000b) unlike the FPXRF methodology. A Wilcoxon Signed Ranks Test was undertaken on both datasets so to observe the potential magnitude of difference between each methodology for a given trace metal element. This found that there was a significant difference between FPXRF analysis and ICP-MS analysis for V, Cr, Co, Zn, As, Sn and Pb with the most pronounced differences being found in V, Cr and Co. The results of this statistical procedure can aid in the estimation of the total result for trace metal concentration obtained from FPXRF when implementing the pseudo-total obtained from the ICP-MS analysis via reverse aqua region digestion.

When examining the results obtained by ICP-MS against soil quality guidelines, the results show that all the results obtained from ICP-MS analysis fall below any of the outlined values set in the Dutch standards (2000). The disparity between the outcomes of the two analytical procedures (FPXRF and ICP-MS with reverse aqua regia digestion) when implementing the Dutch Soil Quality

Standards is most likely due to the ICP-MS methodology only providing a pseudo-total result which tends to be lower than a total result obtained from the FPXRF methodology or spectroscopic methods with strong acid digestion (such as hydrofluoric acid (HF)) that provide a total trace metal concentration as demonstrated in the certification of the certified reference material used in the ICP-MS study (see Appendix G, Table G1).

The same applies when examining the pseudo-total results to the limits set out by EC Directive 86/278. When examining the ICP-MS results to the Finnish guidelines (see Table 7.3), which are specifically produced to be used with aqua regia digestion methodology, it was found that Pb exceeded the 'Threshold' value indicating that Pb exceeds the value at which the risk to both soil eco-systems and humans can be considered 'negligible'. As such, under these guidelines, an assessment for soil contamination for Pb would be triggered. This concurs with the FPXRF result in which Pb also exceeded the Dutch 'target value' for this trace metal. This indicates that further investigation would be beneficial in identifying potential sources of this Pb input in the topsoil so that consequent mitigation/remediation measures could be applied. When comparing to the results of the screening study, it highlights that whilst Co and Zn exceeded the trigger values when implementing a total trace metal concentration methodology, these same two trace metals were seen to be compliant when using an approved methodology that implements a pseudo-total for trace metal concentration. Despite many studies (Venvik and Boogaard, 2020; Cappuyns and Slabbinck, 2012; Dao et al, 2013) implementing the use of FPXRF data when examining the Dutch Soil Quality Standards (VROM, 2000b), it is important to acknowledge that FPXRF methods are not approved for use under the Dutch Soil Quality Standards (VROM, 2000b) in which ICP or AAS methods are approved for these standards. Therefore, when comparing these two methodologies, only the ICP-MS method, and not the FPXRF method, has any validity in relation to the Dutch standards when adherence to these standards is the remit of the assessment. This is despite the FPXRF methodology's usefulness as a rapid screening tool (Burlakovs et al, 2015). This emphasises the significance of implementing the correct methodology for trace metal analysis in soil when applying particular soil quality guidelines and the potential impact that these two differing analytical methods (FPXRF and ICP-MS) may have on the outcome of investigative mitigation measures when examining compliance to soil quality guidelines and when determining if the results for trace metal content in topsoil via FPXRF methodology can be a useful indicator of the results obtained via ICP-MS (as per the comparison study in Chapter 5 of this thesis). It is also important to acknowledge that, for the trace metal concentration data contained within this thesis, a more robust validation technique was applied to the ICP-MS methodology (implementation of a soil certified reference material, ERM-CC141 Loam Soil) than in the FPXRF dataset which used a single element silver (Ag) standard reference material for both calibration and determination of the accuracy of the results (see Chapters 2, 4 and 5 of this thesis).

Trace Metal Element	Finnish Threshold Value (ppm)	Finnish Lower Guide Value (ppm)	Finnish Higher Guide Value (ppm)
Cu	100	150	200
Pb	60	200	750
Zn	200	250	400
V	100	150	250
Cr	100	200	300
Co	20	100	250
As	5	50	100
Sn	-	-	-

- Denotes no available data

Table 7.3: Finnish Soil Quality Guidelines for Trace Metal Content.

7.2.4 Summary and Significance of Mesocosm Study and Chemical Fractionation Analysis

Whilst a screening study is useful in obtaining a wide range of geo-chemical and physiochemical data for a given sampling region, it only provides a snapshot in time on any given date. A column mesocosm study can aid in elucidating the mechanisms of trace metal mobility as well as attempting to simulate field conditions over time. A mesocosm study was devised so to obtain an understanding of how trace metals behave in terms of mobility over the course of a calendar year (simulated under laboratory conditions) and the elucidation of potential influencing factors. As a mesocosm study examines trace metal content of the porewater, it gives a vital indication of trace metal bioavailability. This focussed study examined the impact of variation on pH levels (as determined in the screening study) on two types of topsoil with differing OM levels considered to be representative of the sampling area (Padoan et al, 2020) as shown in Chapters 4 and 6. Brown Moss was the chosen location to obtain these two topsoil samples. Brown Moss is an area consisting of heathland, bogs and marsh as well as smaller ponds located in north Shropshire close to the town of Whitchurch (see Chapter 6). Brown Moss is part of the much wider RAMSAR designated Meres and Mosses landscape (see Chapters 2 and 4). Characterised by glacial retreat during the last ice age, Brown Moss is considered to be one of the most ecologically important sites in the UK for flora with over 200 species of plants recorded which resulted in the site gaining Special Area of Conservation (SAC) designation (Freshwater Habitats Trust, n.d.).

The mesocosm study used these two soil types (one with low levels of organic matter and one with high levels of organic matter) as control samples and placed them both in a designated column representing the topsoil in its natural state without further influence other than rainfall addition via artificial rainwater (ARW). This ARW was added at the rate determined by the climate data obtained from the local (Shawbury) climate station so to emulate real-world precipitation. The study lasted 12 weeks with each week representing a calendar month. pH variation was applied to each soil type in a manner which represented the results obtained from the screening study. Each pH variation was allocated a column and labelled accordingly. This pH was maintained over the course of the 12-week study using either HNO_3 when lowering the pH (Chotpantarat et al, 2011) or $CaCO_3$ when representing soils with alkaline qualities and the impacts of agricultural liming (Janus et al, 2018). Trace metal mobility with depth in the a-horizon was determined by having 3 sampling points on the mesocosm representing top, middle and bottom of the soil depth. Readings from these three sampling points were taken each week/month for each trace metal analysed so to garner information on how trace metal concentration in the porewater changes with depth over the course of a calendar year under differing pH levels and OM content. This study found that those topsoil with low levels of OM (<15%) show increased trace metal concentration and mobility in the porewater than when compared to those topsoil with high (>25%) levels of OM (see Chapter 6). This low OM soil type was found to be greatly influenced by pH variation when examining the trace metal concentration in the porewater. This study found that the high OM topsoil was resistant to the impacts of pH variation when compared to the low OM type soil which is in agreeance with previous studies of this type (Borgulat et al, 2017; Lidman et al, 2014). The results showed that many of the trace metals studied, their concentrations in the porewater and in relation to occurrence with soil depth, were heavily influenced by the pH variations in both soil types which reinforced the findings from the screening study. As a result, the mesocosm study determined that OM and pH are influencing factors in the annual bioavailability of in-situ trace metal in the sampling region in the meres and mosses habitat.

A chemical fractionation sequential extraction procedure was undertaken on the two topsoil samples implemented in the mesocosm study so to examine the behaviour of these topsoil under increasingly acidic conditions and to aid in the determination of bioavailability of the trace metals being studied and consequent risk to the local environment via Risk Assessment Code (RAC). It was determined that the majority of the trace metal concentrations are found in the residual fraction for both of these topsoil types and, as such, undergo little anthropogenic influence in terms of trace metal contribution and that OM content of the topsoil plays a role in determining the bioavailability of certain trace metals. Out of the nine trace metals analysed, Cu, Pb and Zn in the topsoil with low levels of organic matter reached the ‘low-risk’ threshold for the RAC with the remainder being classified as ‘zero-risk’ for both topsoil types. This was in agreeance with the mesocosm study which highlighted the impact of pH variation on Cu, Pb and Zn in terms of both concentration and spatial mobility within the porewater of the topsoil, especially on topsoil with low organic matter that are associated with managed agricultural land. The RAC results should be treated with caution, however, as variable recovery of some trace metals was observed when compared to the obtained pseudo-total, as displayed in the mass balance calculation (see Chapter 6). This was especially evident in the mineral soil type with low organic matter content (<15%). This may result in the RAC being underestimated for certain trace metal elements.

The information obtained from the mesocosm study and consequent sequential extraction procedure can, therefore, be implemented in aiding remediation, mitigation and land management for potential trace metal contamination in topsoil and provides greater understanding of how physiochemical and geochemical properties impact trace metal mobility with depth within the topsoil profile.

7.3 Environmental and Habitat Management Implications.

7.3.1 Importance of Meres and Mosses Habitats

The Meres and Mosses of Cheshire and Shropshire (also known as the Meres and Mosses of the Marches) contain approximately 200 meres and mosses with peat soils being extensive in the region (RSPB, n.d.). These habitats contain significant valuable biodiversity (Whild, 2007). The importance of this habitat is recognised by its various environmental designations, including a large proportion being assigned as Sites of Special Scientific Interest (SSSI), (Natural England, 2020; Woodland Trust, 2019). This region also includes more than 20 square kilometres designated as Ramsar sites of international importance as well as UK national environmental protection designations including Landscape Partnership Scheme (LPS) and Nature Improvement Area (NIA) designated boundaries (RSPB, n.d.), as discussed in Chapter 2 of this thesis. The region has been considered a National Nature Reserve (NNR) for over thirty years. The Meres and Mosses region is ranked as an internationally important habitat by the Network of European Wetlands. A small number of these meres in the region are also candidate Special Areas of Conservation (SAC) under the European Habitats Directive (European Commission Council Directive 92/43/EEC). These peatlands habitat types cover less than three per cent of the earth's surface but store one third of global soil carbon (Harenda et al, 2018). It is estimated that over 96% of meres and mosses habitats have been destroyed globally, despite playing a vital role in carbon sorting and aiding in the reduction of the climate crisis (Harenda et al, 2018). The raised peatbogs within these habitats are also known to soak up rainwater and can play a role in flood prevention (Verhoeven, 2014). The Meres and Mosses of Cheshire and Shropshire have experienced a loss of biodiversity across the area due to increased environmental pressures resulting in reduction in habitat quality (Whild, 2007). There has been a sharp decrease in the extent of lowland raised peatbogs in the UK since the 19th century with there presently being approximately 6000ha of this habitat type compared to an estimated 95000ha at their peak during this timeframe, which represents a 94% decline (BRIG, 2011).

Lowland raised bogs characteristic of the region are now considered to be a priority habitat in the UK Biodiversity Programme (BRIG, 2011). These include raised bogs and quaking bogs (schwingmoors) which, as Natural England (2022) point out, "are very rare habitats in Britain and Europe" (see Chapter 2). The Shropshire Biodiversity Partnership, whose funding partners consist of Shropshire Council, Telford Council, Natural England and the Shropshire Wildlife Trust, identify multiple benefits from the conservation of these peat bogs and the habitats within this sensitive region. These benefits include increased support for the local biodiversity, use as a vital historical educational tool, the preservation of unique landscape formations and implementation of historical data obtained from peat cores (such as for climate information). The rich ecological history of the region has led to the formation of multiple educational establishments within the Meres and Mosses region to aid in espousing these aforementioned benefits and the importance of these sensitive habitat types to interested parties. These include the facility established at Wood Lane Nature Reserve operated by Shropshire Wildlife Trust as a resource for large and diverse school groups and the facility at Bickley Hall Farm, which is overseen by Cheshire Wildlife Trust so to act as a base for schools, universities, community and other groups from which to explore the immediate and wider area (Hayek, 2018). The increased knowledge of the physiochemical status of the topsoil in the region garnered from the data obtained within this thesis (and the interactions with the trace metals therein) will aid in building a more expansive understanding of the environmental status of the Meres and Mosses region as a whole with implications on how these habitat types should be managed. This data will assist in the maintenance, conservation and potential mitigation and remediation of topsoil status that may influence the occurrence and dynamics of trace metals (see section 7.3.2) within these important habitats. This increased knowledge can then be disseminated to these groups and interested parties so to emphasise the importance of these habitat types locally, nationally and globally.

In conclusion, the Meres and Mosses habitats are a crucial area for biodiversity, climate change mitigation, water management, and offer significant cultural and well-being benefits. Their continued conservation and restoration are of paramount importance. As such, maintaining topsoil quality health is therefore vitally important in a meres and mosses habitat (see section 7.3.2). The findings of this thesis aid in the ability to limit the impact of trace metal contamination in the topsoil of this sensitive region, as well as similar meres and mosses regions, by way of elucidation of the current spatial occurrence of select trace metals, the relationships determined with physiochemical properties and other trace metals with resultant behaviour of trace metal mobility over time being established.

7.3.2 Importance of Topsoil Quality and Soil pH adjustment

Soil quality and soil health are of vital importance to the environment at both the local and global scale (Rengel, 2011). Topsoil is subject to short-term fluctuations due to being prone to erosion, weathering and anthropogenic influence (Darmody et al, 2009; Fox et al, 2010). These fluctuations impact moisture status, pH and organic content as well as undergoing “gradual alterations in response to changes in management and environmental factors” (chao, 1998), mean that the soil is considered to be a ‘dynamic system’ (Alloway, 1995). As Ball (2005) points out, These variations in “soil properties can affect the form and bioavailability of metals and need to be considered in decisions on management of the management of polluted soil” (Alloway et al, 1995). This study has displayed the current situation in terms of the in-situ trace metal concentration in the topsoil of a meres and mosses habitat and the relationships of the geochemical properties therein by means of a screening study. This screening study was followed up by a focussed mesocosm study to aid in elucidation of trace metal concentration and spatial mobility in the porewater of the topsoil under varying conditions. These studies showed the impact that pH and OM have on these topsoil properties. Soil acidity has direct influence and impact on crop production (Holland et al, 2019). Soil acidification is a natural process that is accelerated by agricultural practices (Anderson et al, 2013) as well as being influenced by anthropogenic input (Alloway and Steinnes, 1999). Managing soil pH, for example through liming in acidic soils, can be a strategy to improve soil health, enhance agricultural productivity, and potentially mitigate certain greenhouse gas emissions (Rengel, 2011). Liming is a long-established mitigation procedure in the UK and globally by amending topsoil pH so to improve the availability of plant nutrients for agricultural productivity (Xu et al, 2020). This study has established not only the relationship between topsoil pH and trace metal concentrations within the topsoil of the region (see Chapter 4) but also how amending the pH can influence the spatial mobility of these trace metals within the porewater and, consequently, impact bioavailability of these trace metals. These topsoil amendments and mitigation/remediation measures will, therefore, have wider implications applicable to land management and agricultural productivity. The mesocosm study demonstrated that traditional liming, which is used in reducing acidity of soils in the UK, was shown to also reduce trace metal mobility in most trace metals analysed, especially in the low OM topsoil being studied. This simple remediation technique for soil acidity may, as such, be used in aiding adherence to soil quality guidelines and in reducing the potential implications of trace metal bioavailability, especially on those soils with low organic matter content associated with managed agricultural land. As such, the findings of this thesis not only ascertain the soil health of the region via a physiochemical screening study, but also how alterations to these soil physiochemical properties (such as topsoil pH) will have implications for trace metal occurrence and dynamics, as well as potential to impact soil productivity, and, thus, influence land management practices. This information and data will be a vital tool not only for future research projects in the Meres and Mosses region (see section 7.4) but for those with interest in the management of these kind of habitats at the local and wider level (such as Shropshire Wildlife Trust and Natural England) and will provide complementary data to existing data-sets (such as those held by the BGS).

7.4 Limitations of Current Study and Recommendations for Future Research

This research study highlighted multiple areas in which further research could aid in the understanding of trace metal occurrence in a meres and mosses habitat. The current study established the situation regarding in-situ trace metals, intra-metal relationships and relationships with geochemical/physiochemical properties within the topsoil of a meres and mosses habitat via a screening study. This study then examined the mobility of the trace metals within the topsoil porewater by means of a mesocosm study. These studies found a significant relationship between certain trace metal elements (in terms of presence and concentration in the porewater of the topsoil and consequent bioavailability) with soil acidity (pH) and organic matter (OM) content. The following recommendations for further research were determined from the outcomes of this thesis:

1. Further investigation of the enriched presence of trace metals, including Nb and Pb, and their potential sources, in the sampling region of the Meres and Mosses of Cheshire and Shropshire.
2. Further investigation into the annual inputs of trace metals into the topsoil of the Meres and Mosses of Cheshire and Shropshire by increasing monitoring of trace metal concentrations and physiochemical factors.
3. Sub-samples should be analysed for sequential extraction so to improve robustness of the data obtained and to ascertain the reproducibility and repeatability of the analytical methodology.
4. Continued research into the impacts of climate change on these habitat types and how resulting changes in pH and OM content may influence trace metal mobility in the topsoil of these habitats.

This study builds on previous research that have examined the spatial mobility and occurrence of trace metal content in agricultural soil (Shan et al, 2013) as well as in the assessment of trace metal occurrence when implementing differing analytical methodology and their inherent limitations when studying agricultural soils (Hu et al, 2014). Further research could aid in source identification of anthropogenic influences as indicated in Chapter 4 of this thesis. The refractory trace metal Niobium was highlighted as being enriched within the sampling region and being influenced by these geochemical factors in the screening study as well as having a significant correlation with Pb. Due to instrumental and analytical methodology limitations, Nb was not able to be further examined in this multi-element study via ICP-MS. Pb was found to be present and enriched in levels exceeding soil quality guideline values when using FPXRF as the analytical methodology. This study surmises that Nb, Pb and other anthropogenic related trace metals are associated with the industrial heritage of the region of the Meres and Mosses of the Marches. Further research could aid in both source identification and consequent appropriate land management, mitigation and remediation of these trace metal toxins. Further study on annual inputs of trace metals and consequent potential for accumulation in this sensitive region would aid in maintaining trace metal concentrations within appropriate guideline levels and suitable for agricultural landscapes. This can only be attained via regular monitoring and screening of the agricultural land in question. Monitoring of the topsoil in meres and mosses habitats and their factors which make up topsoil quality parameters (e.g. physiochemistry and trace metal content) is vital and will need to take account the potential of the spatial variability of these parameters due to external inputs from anthropogenic sources and a changing climate.

Certain limitations in relation to the current study were identified as being potentially beneficial if addressed in further research. Reproducibility is an important aspect of analytical procedure in which knowledge of uncertainty is beneficial (Plant & Hanisch, 2020). As, due to logistical constraints, no subsamples were analysed during the sequential extraction study (see Chapter 6), further and continuing research would benefit from multiple sample analysis so to determine the repeatability and reproducibility of this analytical technique.

The outcomes of this study will aid in contributing to the wider understanding of how trace metals behave in these sensitive landscapes which have an important role to play in climate change studies and the global carbon cycle (Ma et al, 2022; Moss, 2014). Research is now examining both the beneficial qualities of these meres and mosses habitats globally on climate change as well as the potential impact that climate change has on this habitat type (Ma et al, 2022). Climate change has the potential to increase annual atmospheric precipitation and increased risk of flooding in the UK (Watts et al, 2015). A decrease in the surface area of the makeup of the soil, such as OM content, during heavy seasonal rain or a flooding may “lead to an increase in the solubility of trace metals” (Pan, 2015). Trace metal mobilisation may be further amplified during these heavy rains due to the release of this organic matter and humic acids which impact the topsoil pH and, in turn, metal mobility and binding potential of the trace metals (Koopmans and Groenenberg, 2011). Further research in how this climate change may impact the pH of the topsoil, as well as the OM content, would aid in the future planning and preparation for mitigation of these potential fluxes in geochemical and physiochemical factors and, as a result, aid in the sustainability and conservation measures required to keep these rare habitats functioning and healthy.

Chapter 8

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Chapter 9

Appendices

Appendix A: Correlation Matrices:

Correlations

		pH	Magnetic Susceptibility (x10-8 m3kg-1)	TOC	LOI
pH	Pearson Correlation	1	.319**	-.658**	-.675**
	Sig. (2-tailed)		0.002	0.000	0.000
	N	89	89	89	89
Magnetic Susceptibility (x10-8 m3kg-1)	Pearson Correlation	.319**	1	-0.142	-0.166
	Sig. (2-tailed)	0.002		0.185	0.119
	N	89	89	89	89
TOC	Pearson Correlation	-.658**	-0.142	1	.995**
	Sig. (2-tailed)	0.000	0.185		0.000
	N	89	89	89	89
LOI	Pearson Correlation	-.675**	-0.166	.995**	1
	Sig. (2-tailed)	0.000	0.119	0.000	
	N	89	89	89	89

**. Correlation is significant at the 0.01 level (2-tailed)
N=Number of Samples.

Table A1: Correlations between topsoil geochemical factors in the meres and mosses region

		pH	Magnetic Susceptibility (x10-8 m3kg-1)	TOC	LOI
Cu	Pearson Correlation	-0.194	.443**	.558**	.550**
	Sig. (2-tailed)	0.069	0.000	0.000	0.000
	N	89	89	89	89
Pb	Pearson Correlation	-.521**	0.132	.819**	.823**
	Sig. (2-tailed)	0.000	0.217	0.000	0.000
	N	89	89	89	89
Zn	Pearson Correlation	0.092	.449**	0.188	0.175
	Sig. (2-tailed)	0.392	0.000	0.078	0.101
	N	89	89	89	89
K	Pearson Correlation	.482**	-0.076	-.493**	-.498**
	Sig. (2-tailed)	0.000	0.477	0.000	0.000
	N	89	89	89	89
Ca	Pearson Correlation	0.203	.219*	0.158	0.141
	Sig. (2-tailed)	0.057	0.039	0.140	0.187
	N	89	89	89	89
Ti	Pearson Correlation	.414**	.256*	-.515**	-.516**
	Sig. (2-tailed)	0.000	0.015	0.000	0.000
	N	89	89	89	89
V	Pearson Correlation	.456**	0.023	-.441**	-.453**
	Sig. (2-tailed)	0.000	0.830	0.000	0.000
	N	89	89	89	89
Cr	Pearson Correlation	-0.068	.330**	0.076	0.084
	Sig. (2-tailed)	0.525	0.002	0.479	0.435
	N	89	89	89	89
Mn	Pearson Correlation	.508**	.454**	-.403**	-.409**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000
	N	89	89	89	89

		pH	Magnetic Susceptibility (x10-8 m3kg-1)	TOC	LOI
Fe	Pearson Correlation	.350**	.480**	-.247*	-.252*
	Sig. (2-tailed)	0.001	0.000	0.020	0.017
	N	89	89	89	89
Co	Pearson Correlation	.256*	.490**	-0.170	-0.170
	Sig. (2-tailed)	0.015	0.000	0.112	0.112
	N	89	89	89	89
As	Pearson Correlation	0.006	-0.070	-0.075	-0.072
	Sig. (2-tailed)	0.954	0.515	0.487	0.502
	N	89	89	89	89
Rb	Pearson Correlation	.420**	-0.037	-.623**	-.625**
	Sig. (2-tailed)	0.000	0.734	0.000	0.000
	N	89	89	89	89
Sr	Pearson Correlation	.486**	.642**	-.228*	-.250*
	Sig. (2-tailed)	0.000	0.000	0.032	0.018
	N	89	89	89	89
Nb	Pearson Correlation	-.618**	-.221*	.902**	.911**
	Sig. (2-tailed)	0.000	0.037	0.000	0.000
	N	89	89	89	89
Sn	Pearson Correlation	0.121	.322**	-0.126	-0.130
	Sig. (2-tailed)	0.258	0.002	0.240	0.226
	N	89	89	89	89
Ga	Pearson Correlation	0.106	-.278**	-0.145	-0.156
	Sig. (2-tailed)	0.322	0.008	0.176	0.145
	N	89	89	89	89

**. Correlation is significant at the 0.01 level (2-tailed)

*. Correlation is significant at the 0.05 level (2-tailed)

Table A2: Correlation between metals and geochemical properties

Correlations

		Cu	Pb	Zn	K	Ca	Ti	V	Cr	Mn	Fe	Co	As	Rb	Sr	Nb	Sn	Ga
Cu	Pearson Correlation	1	.676**	.412**	-0.182	.421**	0.004	-.233*	.341**	0.167	.255*	.253*	-0.068	-.313**	.339**	.478**	0.123	-0.191
	Sig. (2-tailed)		0.000	0.000	0.088	0.000	0.972	0.028	0.001	0.118	0.016	0.017	0.529	0.003	0.001	0.000	0.250	0.073
Pb	Pearson Correlation	.676**	1	.288**	-.512**	0.180	-.258*	-.483**	.337**	-0.149	0.035	0.137	-0.082	-.547**	-0.036	.742**	.230*	-.277**
	Sig. (2-tailed)	0.000		0.006	0.000	0.092	0.014	0.000	0.001	0.163	0.746	0.201	0.446	0.000	0.740	0.000	0.030	0.008
Zn	Pearson Correlation	.412**	.288**	1	-0.078	0.208	0.046	0.006	0.114	.273**	.353**	.447**	-0.029	-0.135	.459**	0.067	0.110	-.313**
	Sig. (2-tailed)	0.000	0.006		0.469	0.051	0.668	0.956	0.287	0.010	0.001	0.000	0.784	0.206	0.000	0.532	0.304	0.003
K	Pearson Correlation	-0.182	-.512**	-0.078	1	-0.034	.647**	.624**	-.264*	.394**	.391**	.295**	0.077	.820**	.314**	-.439**	0.007	.509**
	Sig. (2-tailed)	0.088	0.000	0.469		0.749	0.000	0.000	0.012	0.000	0.000	0.005	0.473	0.000	0.003	0.000	0.950	0.000
Ca	Pearson Correlation	.421**	0.180	0.208	-0.034	1	-0.064	-0.040	0.131	0.137	0.093	0.115	-0.051	-0.181	.494**	0.105	0.028	-0.141
	Sig. (2-tailed)	0.000	0.092	0.051	0.749		0.552	0.710	0.222	0.200	0.385	0.282	0.633	0.089	0.000	0.327	0.791	0.187
Ti	Pearson Correlation	0.004	-.258*	0.046	.647**	-0.064	1	.418**	0.130	.608**	.694**	.608**	0.036	.766**	.457**	-.493**	.236*	.229*
	Sig. (2-tailed)	0.972	0.014	0.668	0.000	0.552		0.000	0.226	0.000	0.000	0.000	0.739	0.000	0.000	0.000	0.026	0.031
V	Pearson Correlation	-.233*	-.483**	0.006	.624**	-0.040	.418**	1	-.670**	.398**	.408**	.237*	0.077	.555**	.287**	-.370**	-0.087	.772**
	Sig. (2-tailed)	0.028	0.000	0.956	0.000	0.710	0.000		0.000	0.000	0.000	0.025	0.476	0.000	0.006	0.000	0.419	0.000
Cr	Pearson Correlation	.341**	.337**	0.114	-.264*	0.131	0.130	-.670**	1	0.099	.210*	.330**	-0.070	-0.179	0.147	-0.031	.308**	-.716**
	Sig. (2-tailed)	0.001	0.001	0.287	0.012	0.222	0.226	0.000		0.355	0.049	0.002	0.514	0.093	0.168	0.771	0.003	0.000
Mn	Pearson Correlation	0.167	-0.149	.273**	.394**	0.137	.608**	.398**	0.099	1	.699**	.591**	0.017	.432**	.588**	-.394**	.248*	0.065
	Sig. (2-tailed)	0.118	0.163	0.010	0.000	0.200	0.000	0.000	0.355		0.000	0.000	0.877	0.000	0.000	0.000	0.019	0.547
Fe	Pearson Correlation	.255*	0.035	.353**	.391**	0.093	.694**	.408**	.210*	.699**	1	.885**	0.081	.480**	.704**	-.262*	.248*	0.151
	Sig. (2-tailed)	0.016	0.746	0.001	0.000	0.385	0.000	0.000	0.049	0.000		0.000	0.449	0.000	0.000	0.013	0.019	0.157
Co	Pearson Correlation	.253*	0.137	.447**	.295**	0.115	.608**	.237*	.330**	.591**	.885**	1	0.044	.367**	.695**	-.247*	.296**	-0.052
	Sig. (2-tailed)	0.017	0.201	0.000	0.005	0.282	0.000	0.025	0.002	0.000	0.000		0.682	0.000	0.000	0.019	0.005	0.626
As	Pearson Correlation	-0.068	-0.082	-0.029	0.077	-0.051	0.036	0.077	-0.070	0.017	0.081	0.044	1	0.121	-0.003	0.017	-0.048	0.111
	Sig. (2-tailed)	0.529	0.446	0.784	0.473	0.633	0.739	0.476	0.514	0.877	0.449	0.682		0.259	0.981	0.876	0.654	0.302
Rb	Pearson Correlation	-.313**	-.547**	-0.135	.820**	-0.181	.766**	.555**	-0.179	.432**	.480**	.367**	0.121	1	.314**	-.533**	0.017	.488**
	Sig. (2-tailed)	0.003	0.000	0.206	0.000	0.089	0.000	0.000	0.093	0.000	0.000	0.000	0.259		0.003	0.000	0.874	0.000
Sr	Pearson Correlation	.339**	-0.036	.459**	.314**	.494**	.457**	.287**	0.147	.588**	.704**	.695**	-0.003	.314**	1	-.304**	0.196	-0.059
	Sig. (2-tailed)	0.001	0.740	0.000	0.003	0.000	0.000	0.006	0.168	0.000	0.000	0.000	0.981	0.003		0.004	0.065	0.584
Nb	Pearson Correlation	.478**	.742**	0.067	-.439**	0.105	-.493**	-.370**	-0.031	-.394**	-.262*	-.247*	0.017	-.533**	-.304**	1	-0.127	-0.032
	Sig. (2-tailed)	0.000	0.000	0.532	0.000	0.327	0.000	0.000	0.771	0.000	0.013	0.019	0.876	0.000	0.004		0.237	0.768
Sn	Pearson Correlation	0.123	.230*	0.110	0.007	0.028	.236*	-0.087	.308**	.248*	.248*	.296**	-0.048	0.017	0.196	-0.127	1	-.238*
	Sig. (2-tailed)	0.250	0.030	0.304	0.950	0.791	0.026	0.419	0.003	0.019	0.019	0.005	0.654	0.874	0.065	0.237		0.025
Ga	Pearson Correlation	-0.191	-.277**	-.313**	.509**	-0.141	.229*	.772**	-.716**	0.065	0.151	-0.052	0.111	.488**	-0.059	-0.032	-.238*	1
	Sig. (2-tailed)	0.073	0.008	0.003	0.000	0.187	0.031	0.000	0.000	0.547	0.157	0.626	0.302	0.000	0.584	0.768	0.025	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed)

Table A3: Metal to Metal Correlations in the meres and mosses region

Appendix B: Principal Component and Cluster Analysis

KMO and Bartlett's Test

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.789
Bartlett's Test of Sphericity	Approx. Chi-Square	2007.811
	df	210
	Sig.	.000

Table B1: KMO and Bartlett's Test

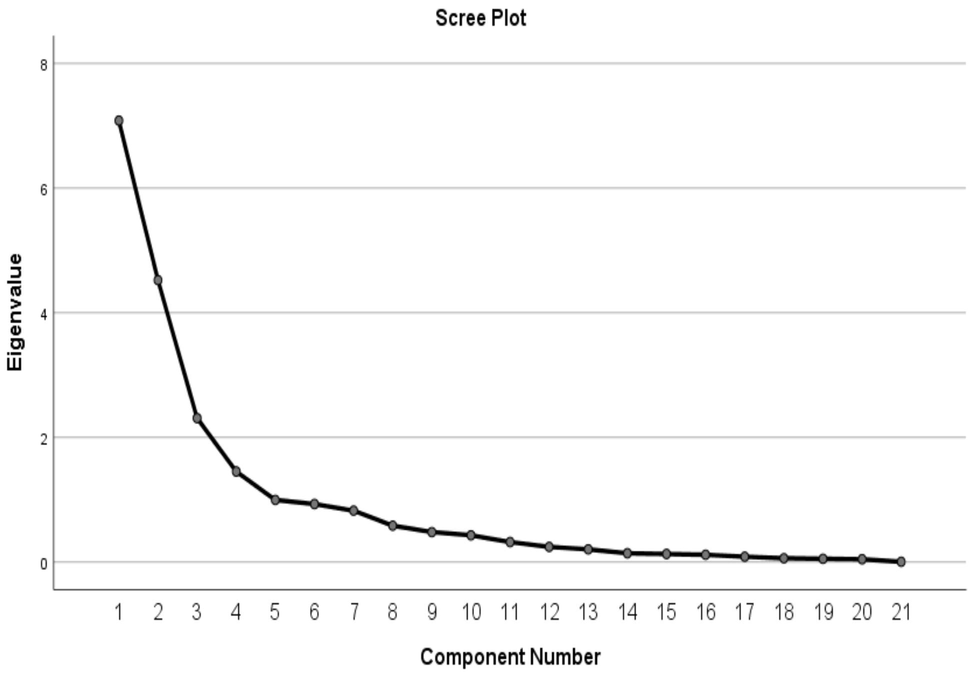


Fig B1. Chart of Eigenvalues (screes)

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	7.082	33.725	33.725	7.082	33.725	33.725	5.108	24.324	24.324
2	4.522	21.534	55.259	4.522	21.534	55.259	4.614	21.969	46.293
3	2.309	10.995	66.255	2.309	10.995	66.255	3.018	14.369	60.662
4	1.452	6.915	73.170	1.452	6.915	73.170	2.627	12.508	73.170
5	.995	4.740	77.910						
6	.930	4.427	82.337						
7	.824	3.922	86.259						
8	.582	2.773	89.032						
9	.479	2.283	91.315						
10	.429	2.043	93.357						
11	.320	1.525	94.883						
12	.243	1.156	96.038						
13	.203	.966	97.004						
14	.140	.667	97.672						
15	.128	.612	98.283						
16	.116	.551	98.834						
17	.085	.403	99.237						
18	.061	.289	99.526						
19	.052	.245	99.772						
20	.044	.212	99.983						
21	.004	.017	100.000						

Extraction Method: Principal Component Analysis.

Table B2: Total Variance

Component Matrix[a]

	Component			
	1	2	3	4
LOI	-.848		.406	
TOC	-.839		.414	
Rb	.820			.305
Nb	-.794		.469	
Ti	.774			.391
K	.752		.322	
pH	.734			-.399
V	.690		.545	
Mn	.665	.454		
Pb	-.665	.584		
Fe	.624	.603		
Cu	-.306	.744	.306	
Magnetic Susceptibility (x10-8 m3kg-1)		.703		
Co	.510	.684		
Sr	.544	.664		-.300
Zn		.637		
Cr		.584	-.579	.339
Sn		.401		
Ga	.357	-.456	.727	
Ca		.433		-.610
As				

Extraction Method: Principal Component Analysis.
a. 4 components extracted.

Table B3: Extraction Method: Principal Component Analysis.

Appendix C: Sample Site Locations

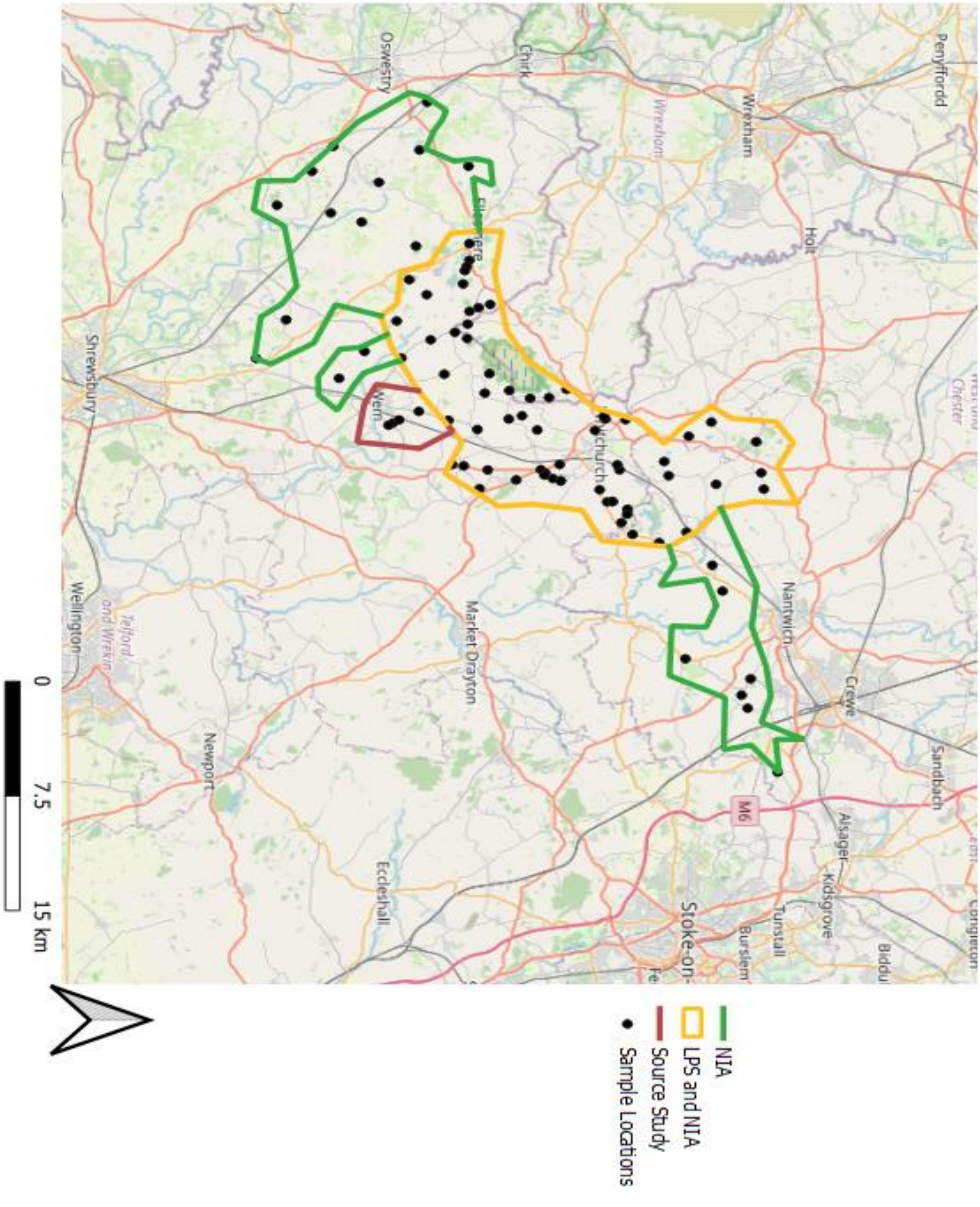


Fig C1: Map showing sampling locations for trace metals in meres and mosses Nature Improvement Area (NIA)/Landscape Partnership Scheme (LPS) Boundary

Appendix D: Temporal Median Weekly/Monthly Trace Metal Concentration in Porewater Data in parts per billion (ppb) from the Top, Middle and Bottom Sampling Points/Ports of Columns and their Duplicates.

Chart Elements Key:

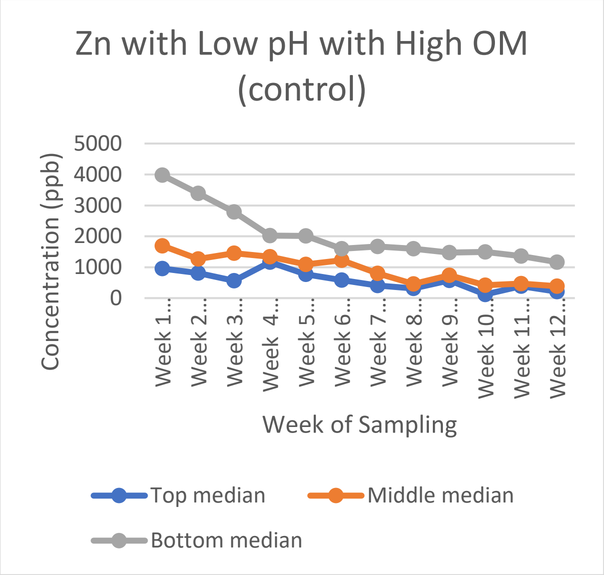
Low pH =<6, Circumneutral pH =6-7.5, High pH=>7.5

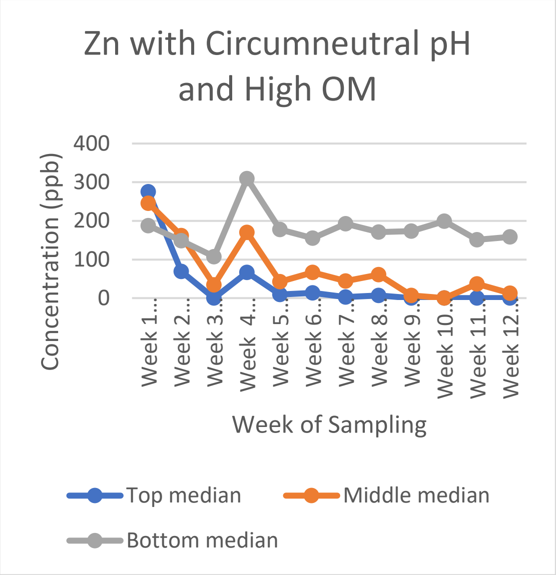
Low OM=<15%, High OM=>25%

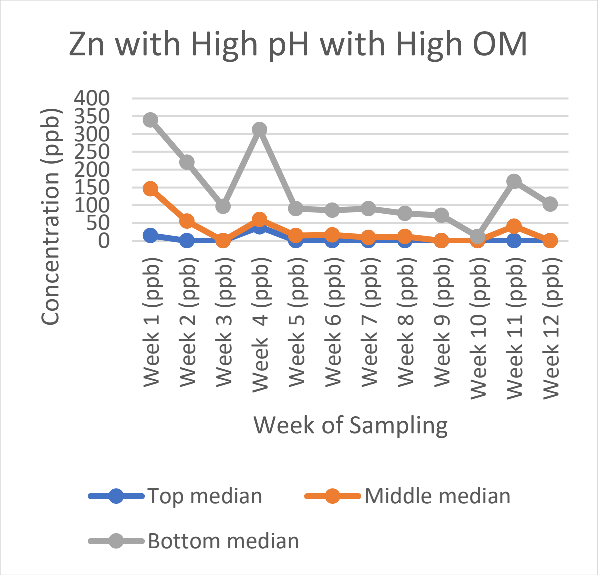
Top Sampling Port = 0cm-2cm depth,

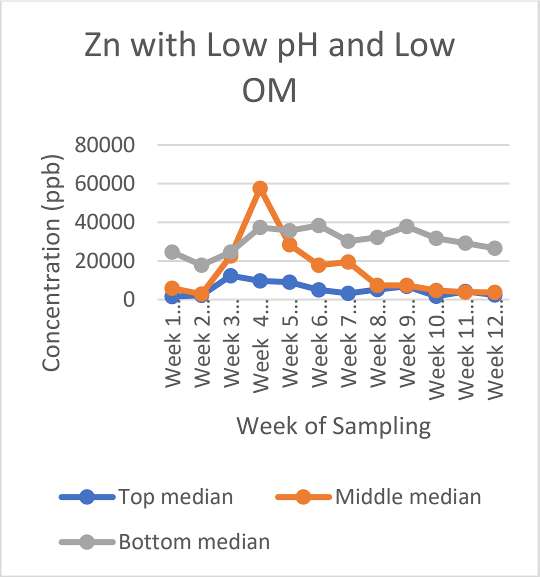
Middle Sampling Port = 10cm-12cm depth,

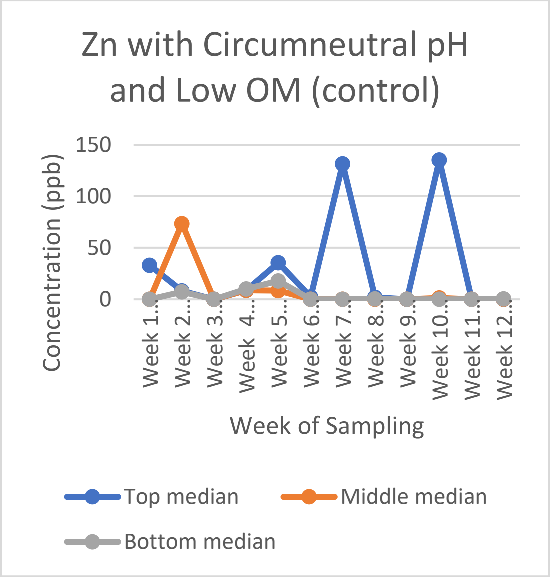
Bottom Sampling Port = 22cm-24cm depth

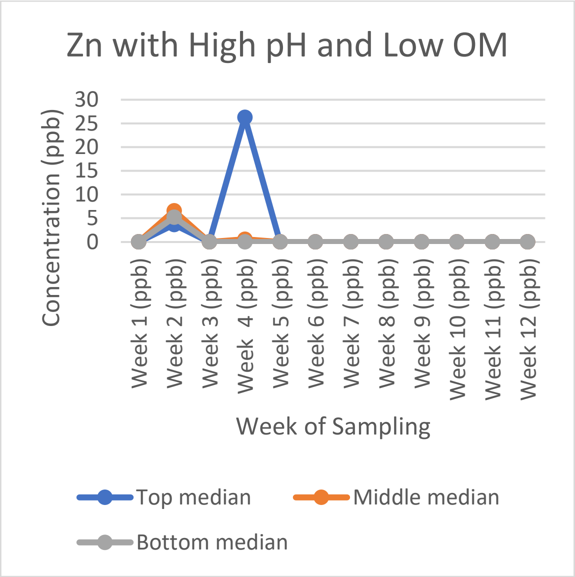


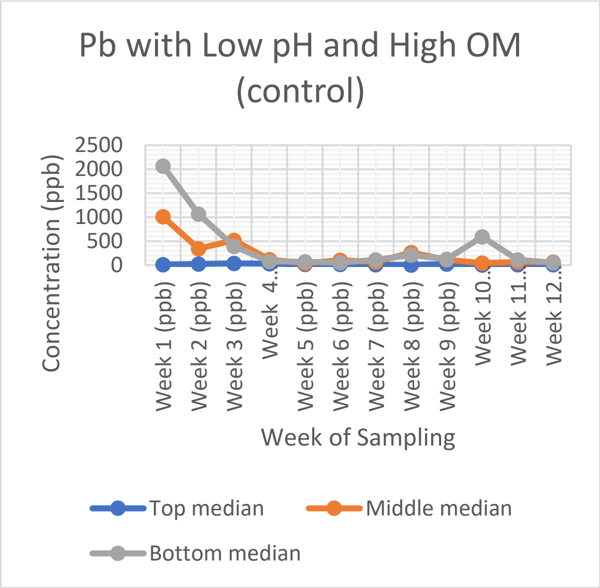


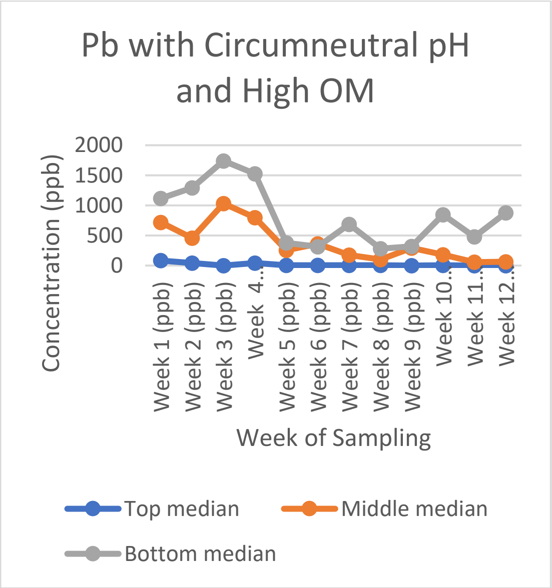


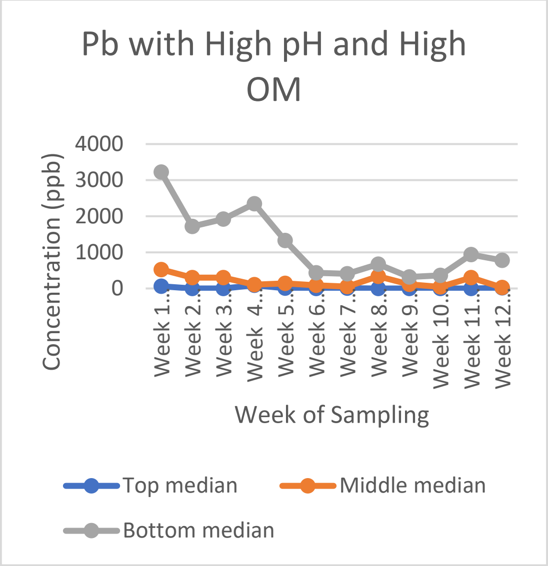

Zn with Low pH and Low OM

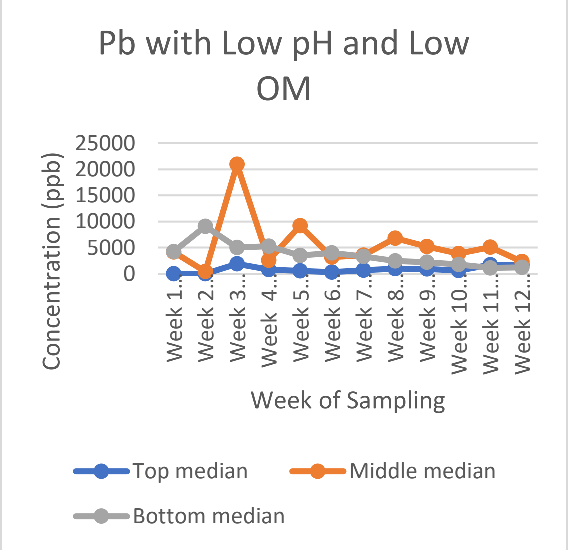

Zn with Circumneutral pH and Low OM (control)

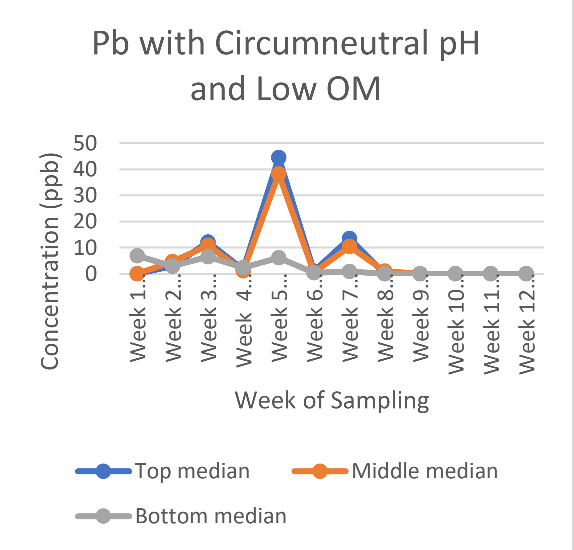

Zn with High pH and Low OM

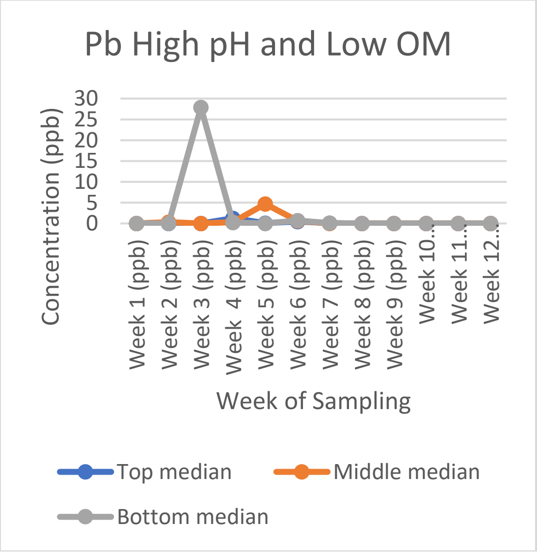

Pb with Low pH and High OM (control)

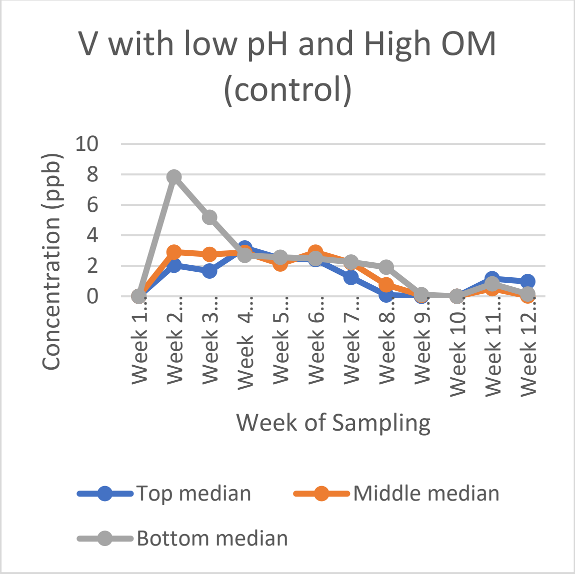

Pb with Circumneutral pH and High OM

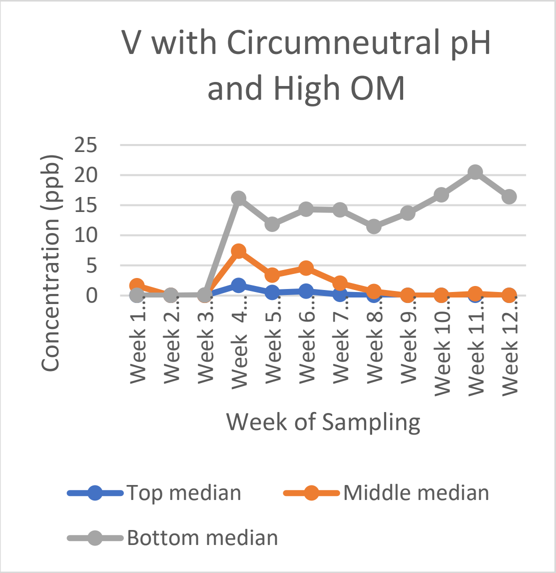

Pb with High pH and High OM
Concentration (ppb)
4000
3000
2000
1000
0
Week 1.…
Week 2.…
Week 3.…
Week 4.…
Week 5.…
Week 6.…
Week 7.…
Week 8.…
Week 9.…
Week 10.…
Week 11.…
Week 12.…
Week of Sampling
Top median
Middle median
Bottom median

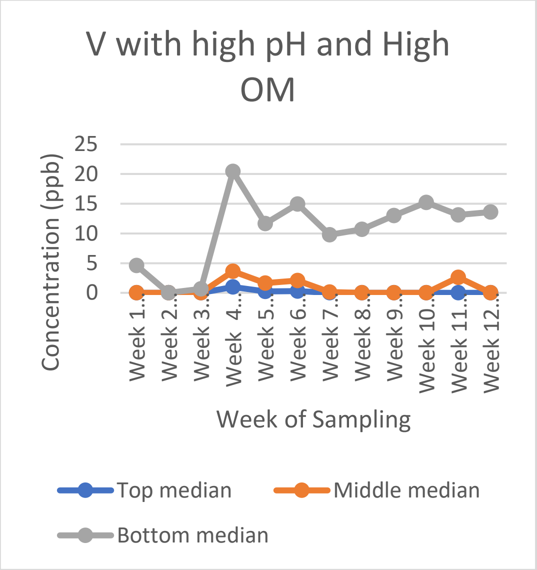

Pb with Low pH and Low OM
Concentration (ppb)
25000
20000
15000
10000
5000
0
Week 1.…
Week 2.…
Week 3.…
Week 4.…
Week 5.…
Week 6.…
Week 7.…
Week 8.…
Week 9.…
Week 10.…
Week 11.…
Week 12.…
Week of Sampling
Top median
Middle median
Bottom median

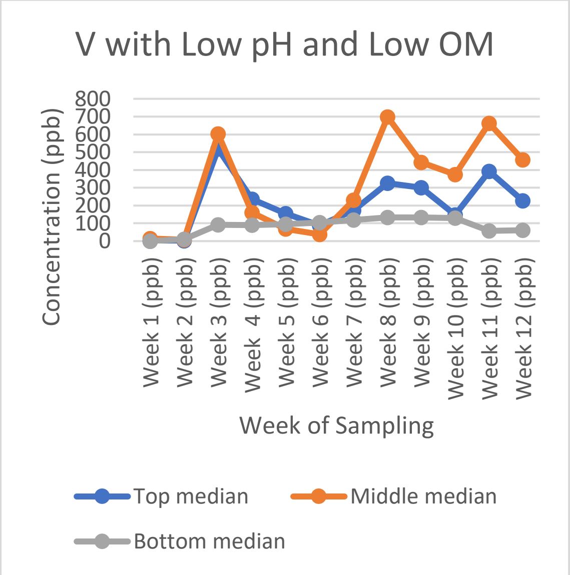

Pb with Circumneutral pH and Low OM
Concentration (ppb)
50
40
30
20
10
0
Week 1.…
Week 2.…
Week 3.…
Week 4.…
Week 5.…
Week 6.…
Week 7.…
Week 8.…
Week 9.…
Week 10.…
Week 11.…
Week 12.…
Week of Sampling
Top median
Middle median
Bottom median

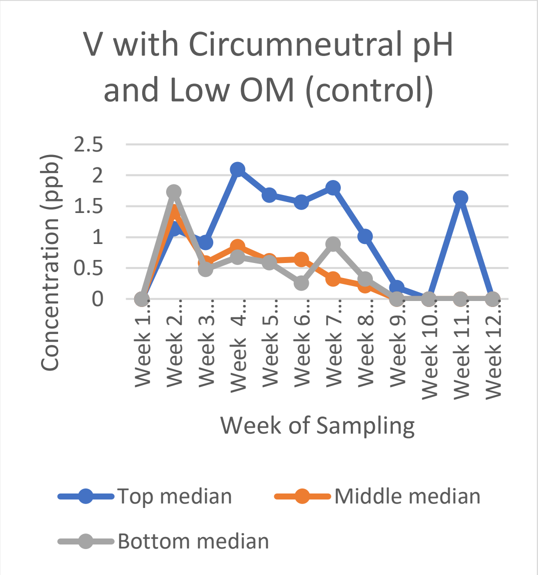

Pb High pH and Low OM
Concentration (ppb)
30
25
20
15
10
5
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10.…
Week 11.…
Week 12.…
Week of Sampling
Top median
Middle median
Bottom median

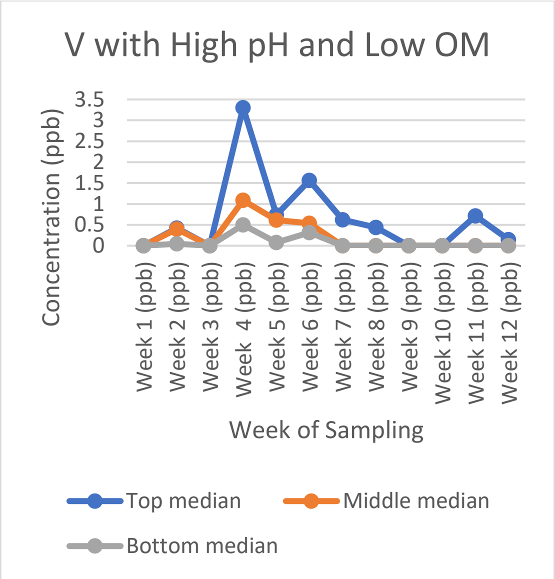

V with low pH and High OM (control)
Concentration (ppb)
10
8
6
4
2
0
Week 1…
Week 2…
Week 3…
Week 4…
Week 5…
Week 6…
Week 7…
Week 8…
Week 9…
Week 10…
Week 11…
Week 12…
Week of Sampling
Top median
Middle median
Bottom median

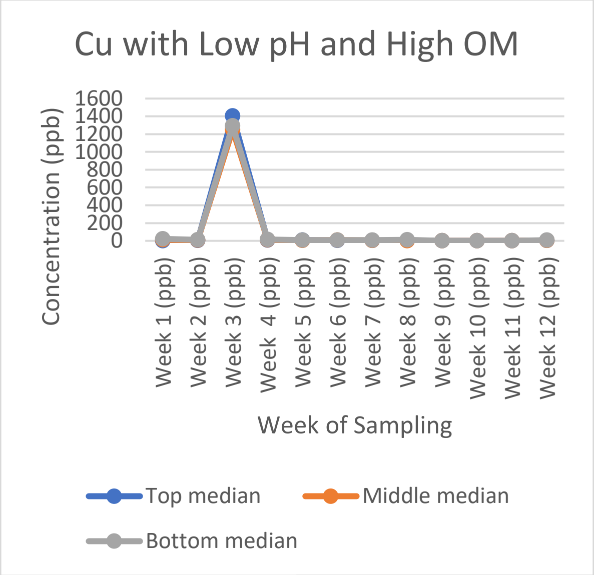

V with Circumneutral pH and High OM
Concentration (ppb)
25
20
15
10
5
0
Week 1…
Week 2…
Week 3…
Week 4…
Week 5…
Week 6…
Week 7…
Week 8…
Week 9…
Week 10…
Week 11…
Week 12…
Week of Sampling
Top median
Middle median
Bottom median

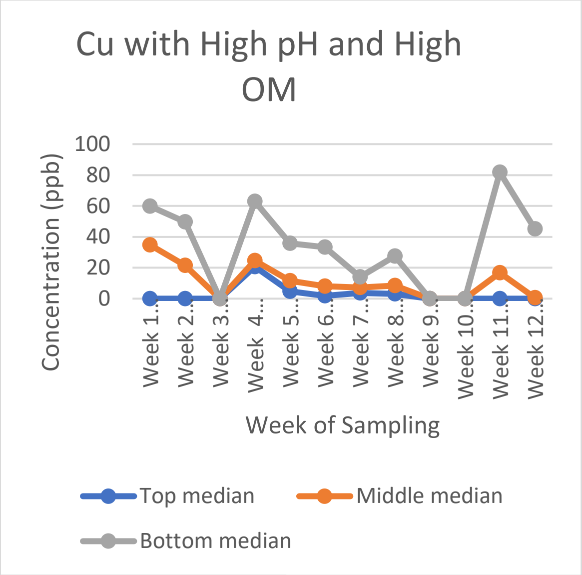

V with high pH and High OM
Concentration (ppb)
25
20
15
10
5
0
Week 1…
Week 2…
Week 3…
Week 4…
Week 5…
Week 6…
Week 7…
Week 8…
Week 9…
Week 10…
Week 11…
Week 12…
Week of Sampling
Top median
Middle median
Bottom median

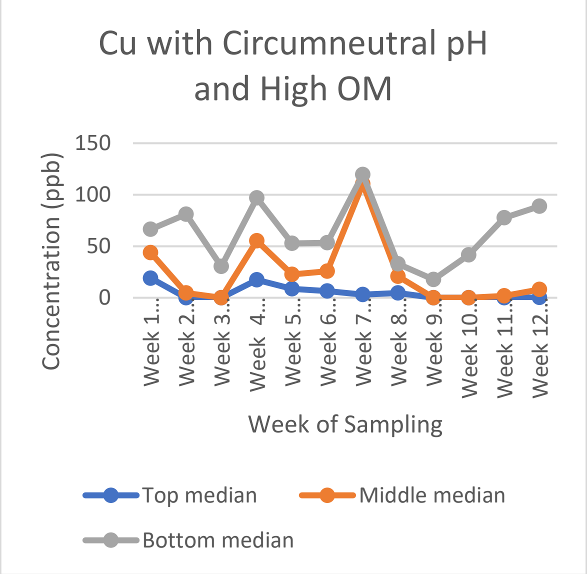

V with Low pH and Low OM
Concentration (ppb)
800
700
600
500
400
300
200
100
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

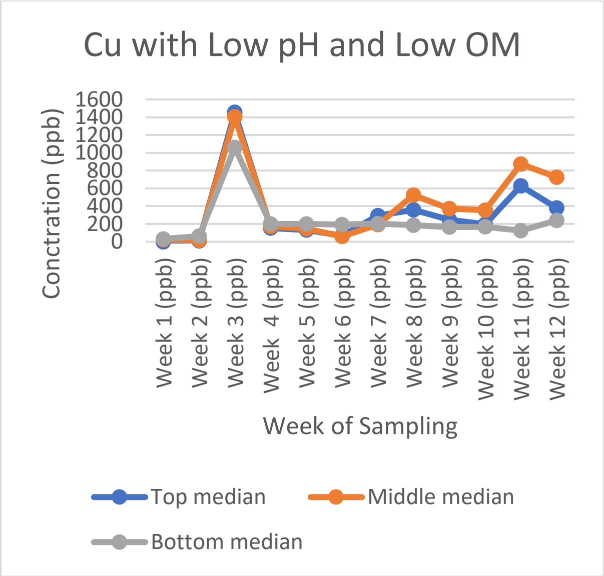

V with Circumneutral pH and Low OM (control)
Concentration (ppb)
2.5
2
1.5
1
0.5
0
Week 1…
Week 2…
Week 3…
Week 4…
Week 5…
Week 6…
Week 7…
Week 8…
Week 9…
Week 10…
Week 11…
Week 12…
Week of Sampling
Top median
Middle median
Bottom median

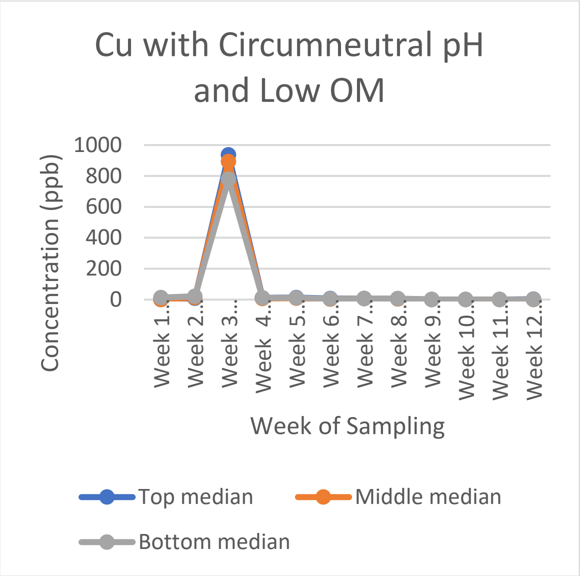

V with High pH and Low OM
Concentration (ppb)
3.5
3
2.5
2
1.5
1
0.5
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

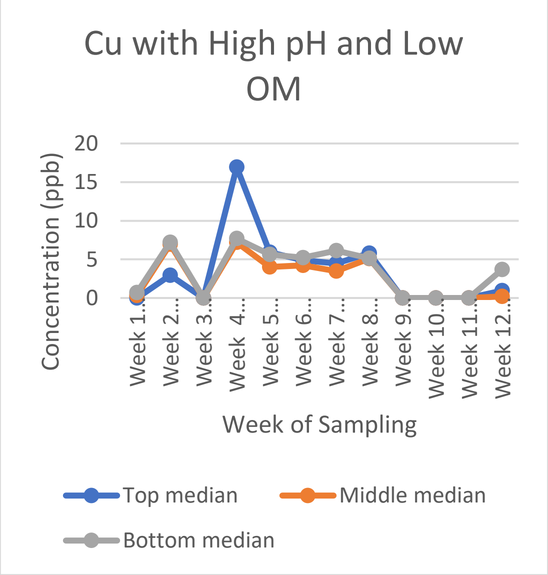

Cu with Low pH and High OM
Concentration (ppb)
1600
1400
1200
1000
800
600
400
200
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

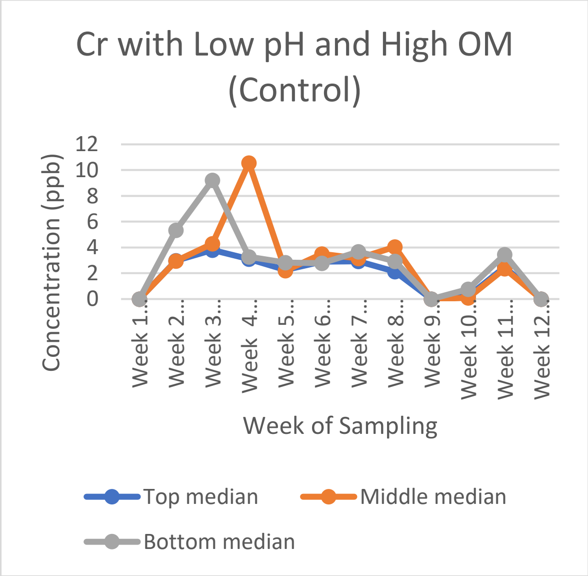

Cu with High pH and High OM
Concentration (ppb)
100
80
60
40
20
0
Week 1…
Week 2…
Week 3…
Week 4…
Week 5…
Week 6…
Week 7…
Week 8…
Week 9…
Week 10…
Week 11…
Week 12…
Week of Sampling
Top median
Middle median
Bottom median

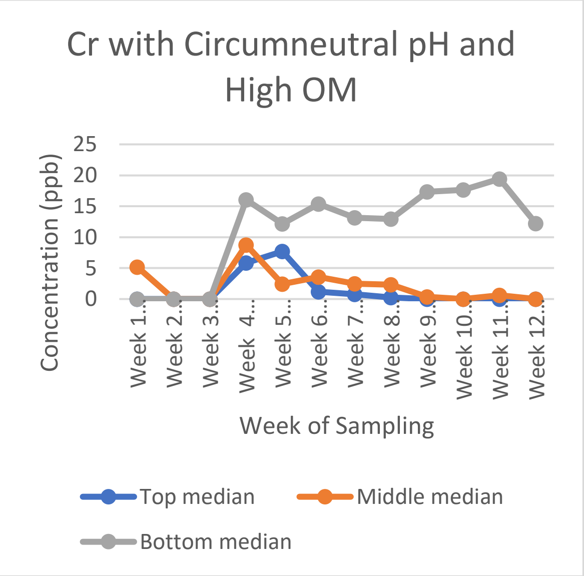

Cu with Circumneutral pH and High OM
Concentration (ppb)
150
100
50
0
Week 1…
Week 2…
Week 3…
Week 4…
Week 5…
Week 6…
Week 7…
Week 8…
Week 9…
Week 10…
Week 11…
Week 12…
Week of Sampling
Top median
Middle median
Bottom median

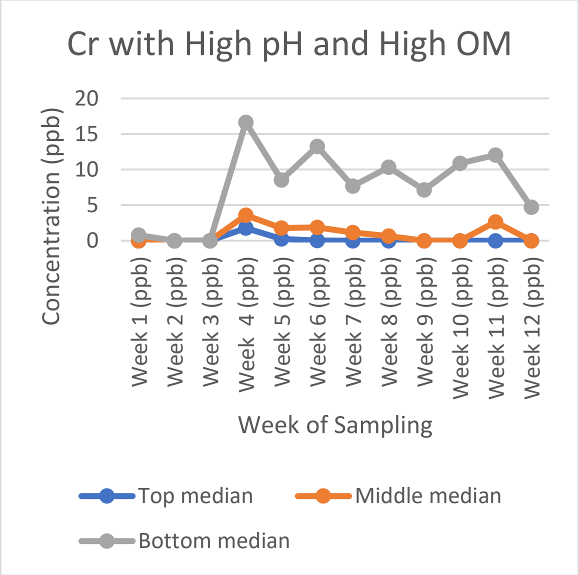

Cu with Low pH and Low OM
Conctration (ppb)
1600
1400
1200
1000
800
600
400
200
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

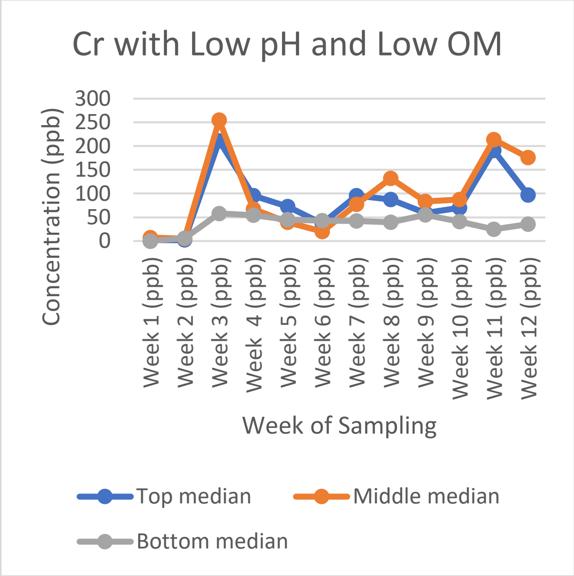

Cu with Circumneutral pH and Low OM
Concentration (ppb)
1000
800
600
400
200
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

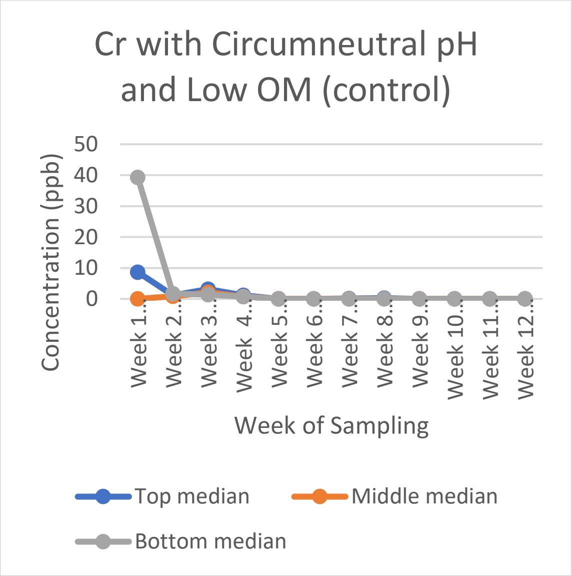

Cu with High pH and Low OM
Concentration (ppb)
20
15
10
5
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

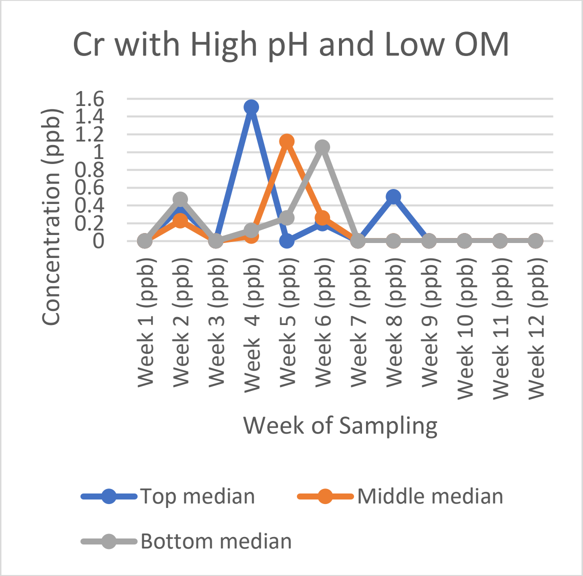

Cr with Low pH and High OM (Control)
Concentration (ppb)
12
10
8
6
4
2
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

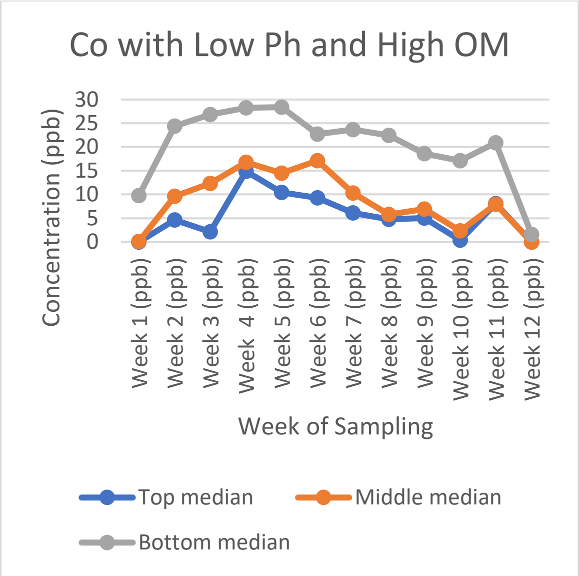

Cr with Circumneutral pH and High OM
Concentration (ppb)
25
20
15
10
5
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

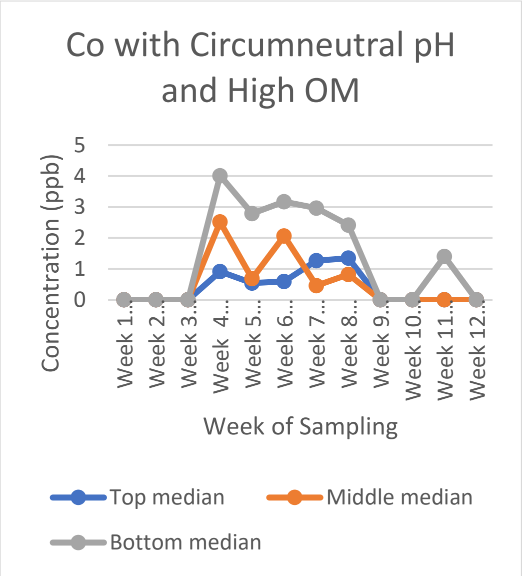

Cr with High pH and High OM
Concentration (ppb)
20
15
10
5
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

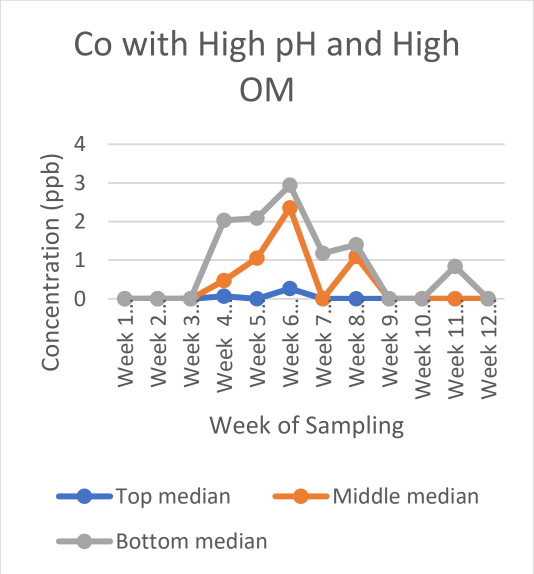

Cr with Low pH and Low OM
Concentration (ppb)
300
250
200
150
100
50
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

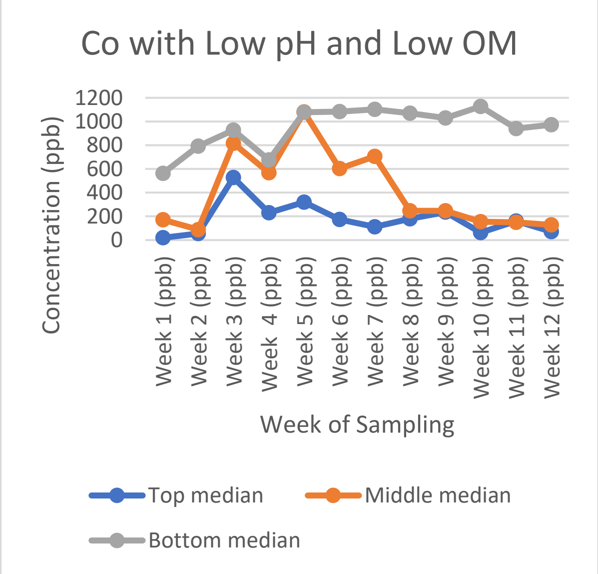

Cr with Circumneutral pH and Low OM (control)
Concentration (ppb)
50
40
30
20
10
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

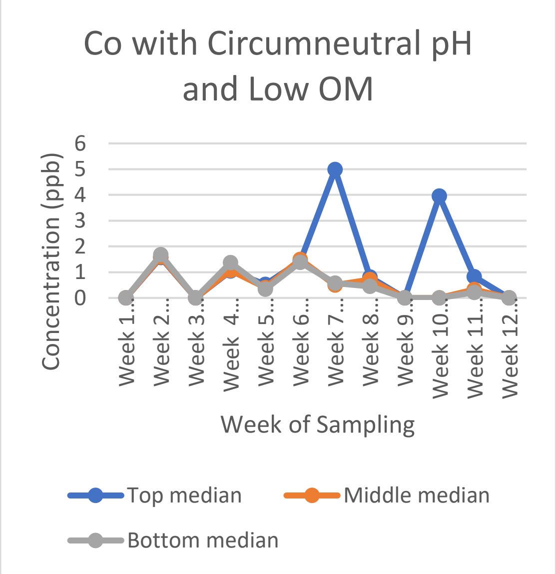

Cr with High pH and Low OM
Concentration (ppb)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

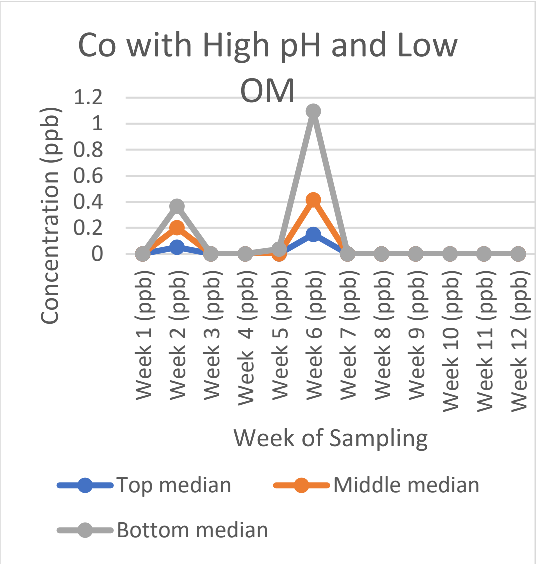

Co with Low Ph and High OM
Concentration (ppb)
30
25
20
15
10
5
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

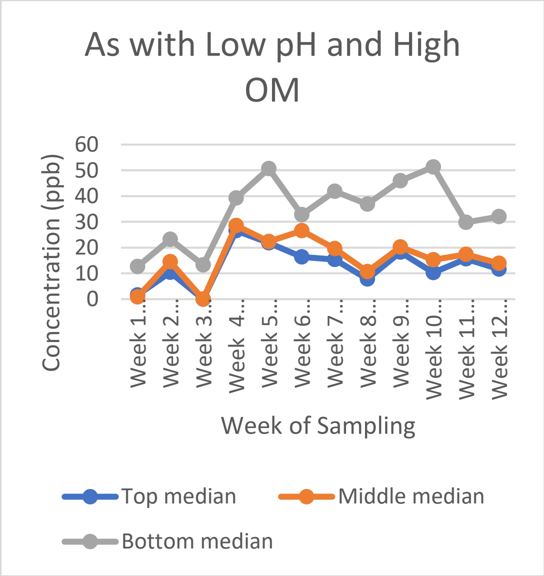

Co with Circumneutral pH and High OM
Concentration (ppb)
5
4
3
2
1
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

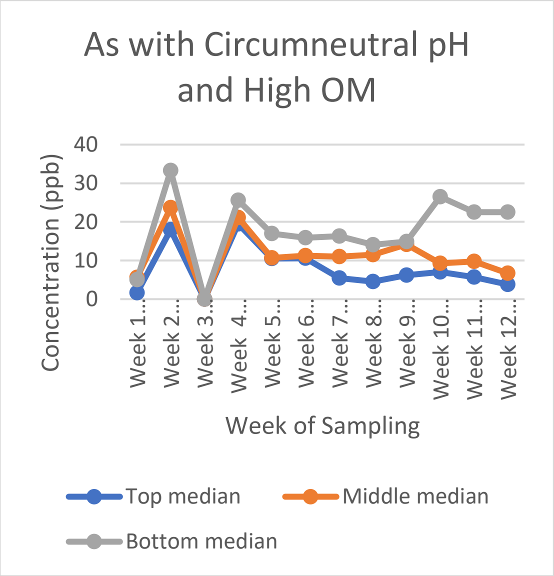

Co with High pH and High OM
Concentration (ppb)
4
3
2
1
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

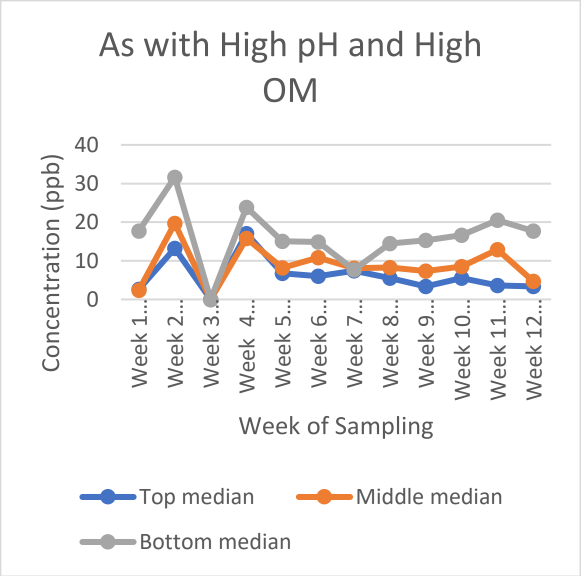

Co with Low pH and Low OM
Concentration (ppb)
1200
1000
800
600
400
200
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

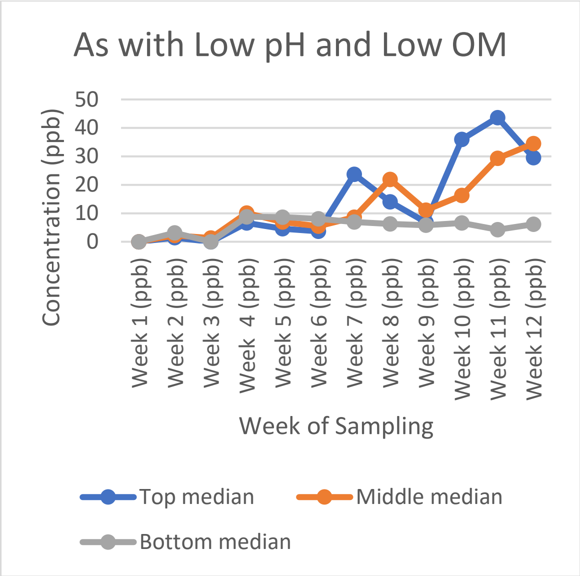

Co with Circumneutral pH and Low OM
Concentration (ppb)
6
5
4
3
2
1
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

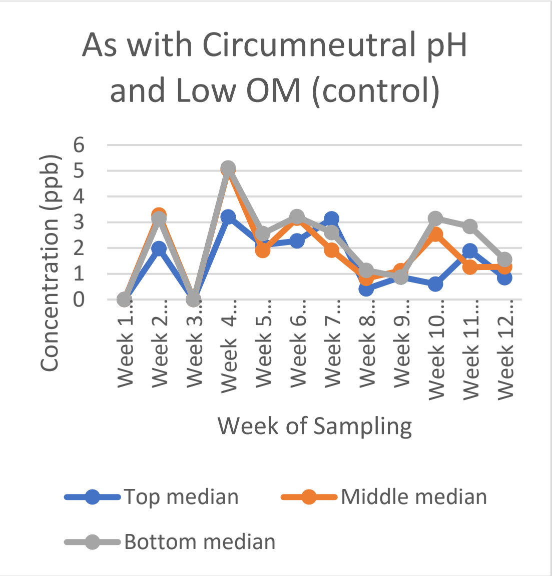

Co with High pH and Low OM
Concentration (ppb)
1.2
1
0.8
0.6
0.4
0.2
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

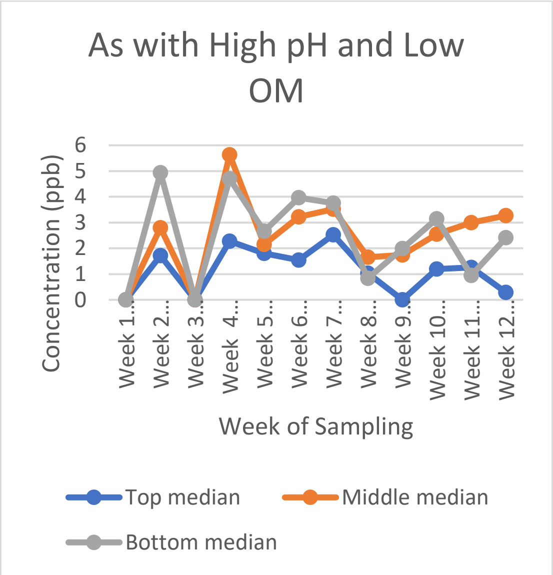

As with Low pH and High OM
Concentration (ppb)
60
50
40
30
20
10
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

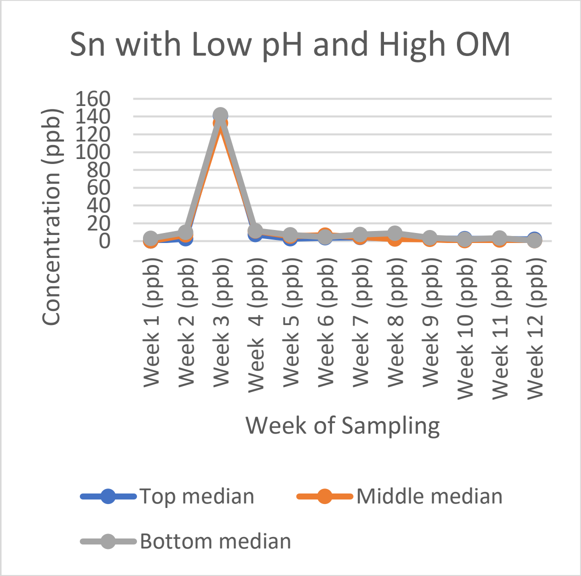

As with Circumneutral pH and High OM
Concentration (ppb)
40
30
20
10
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

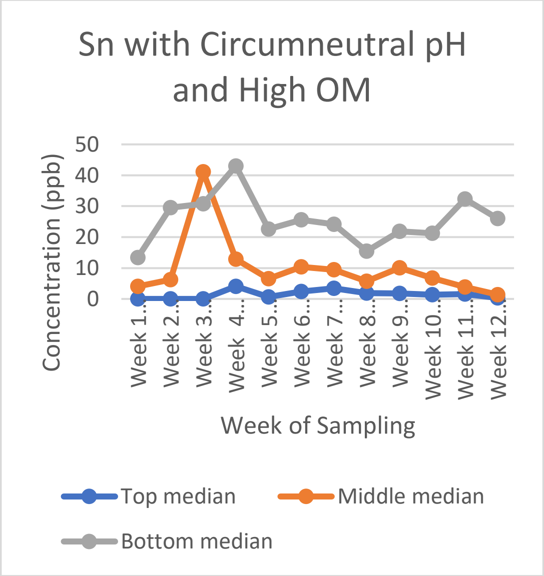

As with High pH and High OM
Concentration (ppb)
40
30
20
10
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

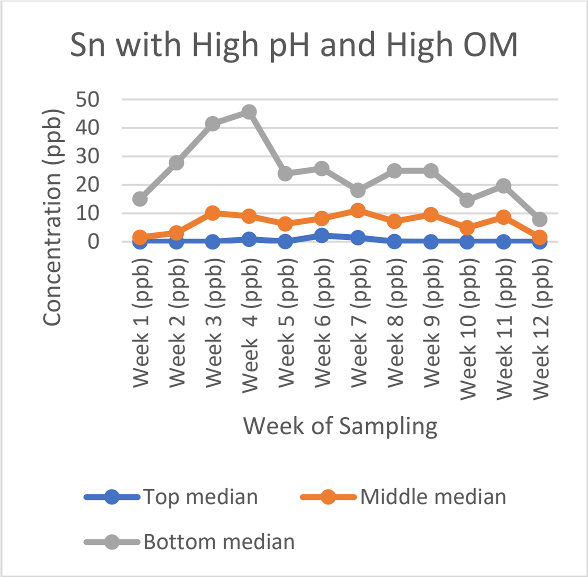

As with Low pH and Low OM
Concentration (ppb)
50
40
30
20
10
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

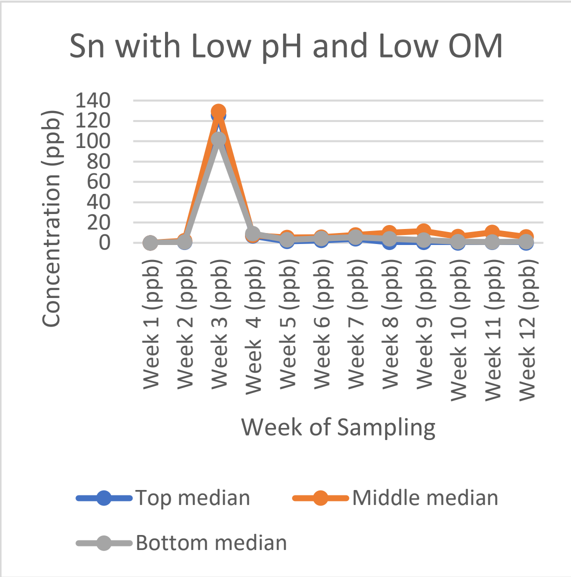

As with Circumneutral pH and Low OM (control)
Concentration (ppb)
6
5
4
3
2
1
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

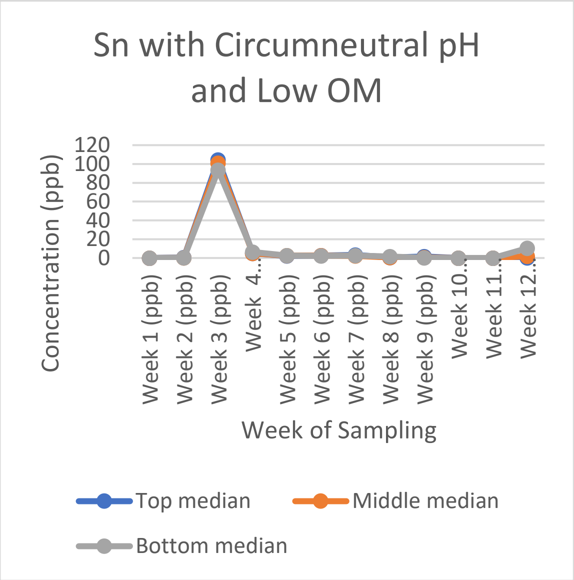

As with High pH and Low OM
Concentration (ppb)
6
5
4
3
2
1
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

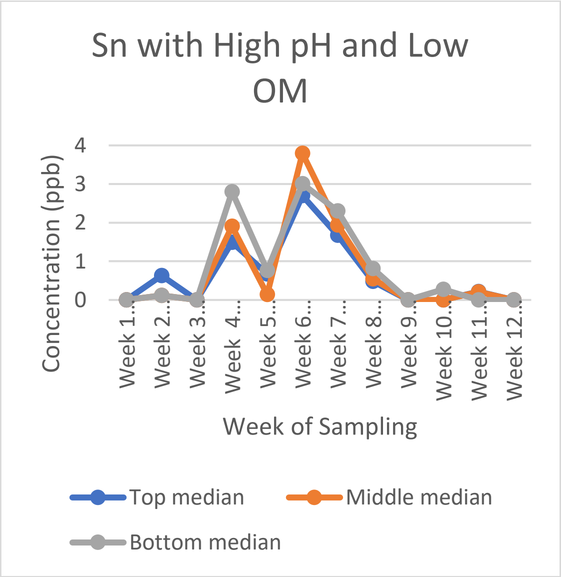

Sn with Low pH and High OM
Concentration (ppb)
160
140
120
100
80
60
40
20
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

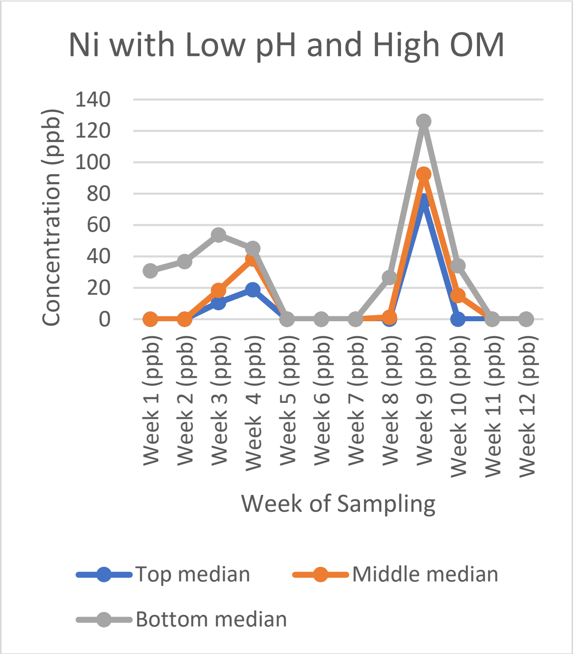

Sn with Circumneutral pH and High OM
Concentration (ppb)
50
40
30
20
10
0
Week 1...
Week 2...
Week 3...
Week 4...
Week 5...
Week 6...
Week 7...
Week 8...
Week 9...
Week 10...
Week 11...
Week 12...
Week of Sampling
Top median
Middle median
Bottom median

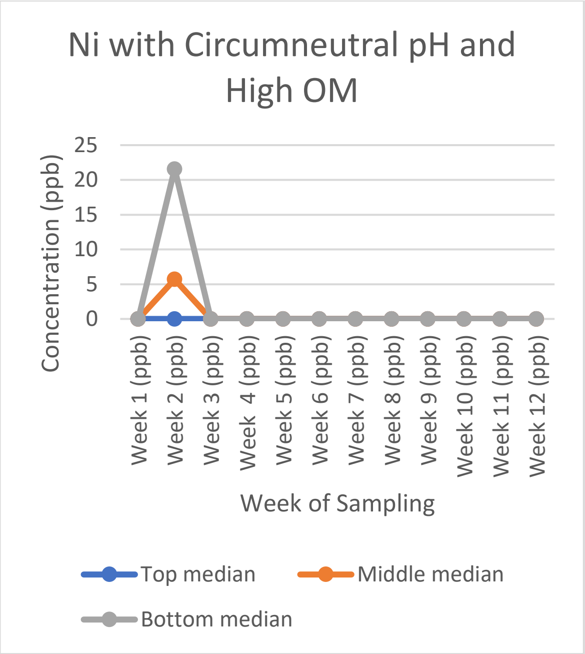

Sn with High pH and High OM
Concentration (ppb)
50
40
30
20
10
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

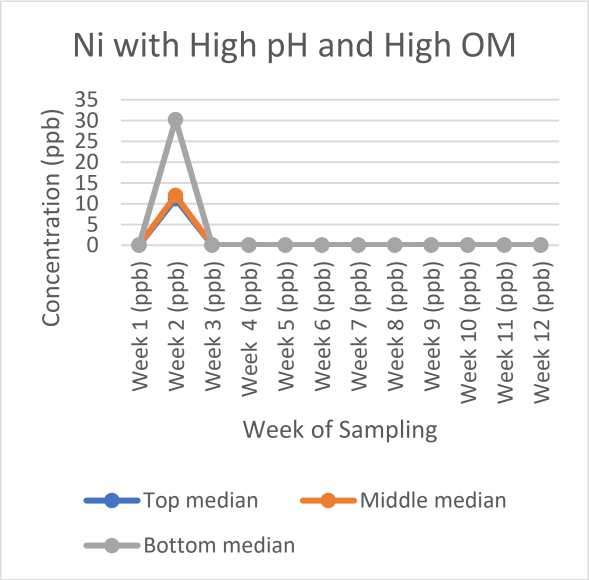

Sn with Low pH and Low OM
Concentration (ppb)
140
120
100
80
60
40
20
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median

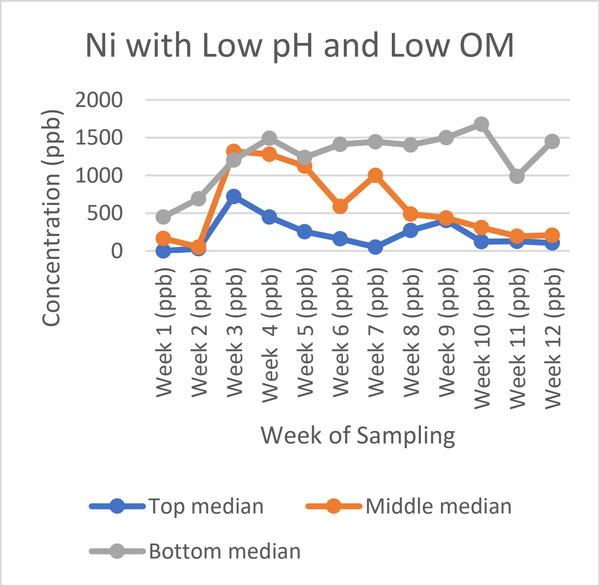

Sn with Circumneutral pH and Low OM
Concentration (ppb)
120
100
80
60
40
20
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4…
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10…
Week 11…
Week 12…
Week of Sampling
Top median
Middle median
Bottom median

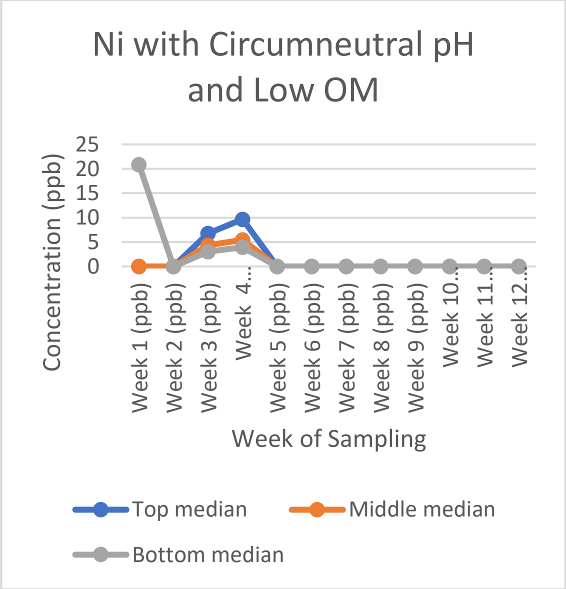

Sn with High pH and Low OM
Concentration (ppb)
4
3
2
1
0
Week 1…
Week 2…
Week 3…
Week 4…
Week 5…
Week 6…
Week 7…
Week 8…
Week 9…
Week 10…
Week 11…
Week 12…
Week of Sampling
Top median
Middle median
Bottom median


Ni with Low pH and High OM
Concentration (ppb)
140
120
100
80
60
40
20
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median


Ni with Circumneutral pH and High OM
Concentration (ppb)
25
20
15
10
5
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median


Ni with High pH and High OM
Concentration (ppb)
35
30
25
20
15
10
5
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median


Ni with Low pH and Low OM
Concentration (ppb)
2000
1500
1000
500
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4 (ppb)
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10 (ppb)
Week 11 (ppb)
Week 12 (ppb)
Week of Sampling
Top median
Middle median
Bottom median


Ni with Circumneutral pH and Low OM
Concentration (ppb)
25
20
15
10
5
0
Week 1 (ppb)
Week 2 (ppb)
Week 3 (ppb)
Week 4…
Week 5 (ppb)
Week 6 (ppb)
Week 7 (ppb)
Week 8 (ppb)
Week 9 (ppb)
Week 10…
Week 11…
Week 12…
Week of Sampling
Top median
Middle median
Bottom median

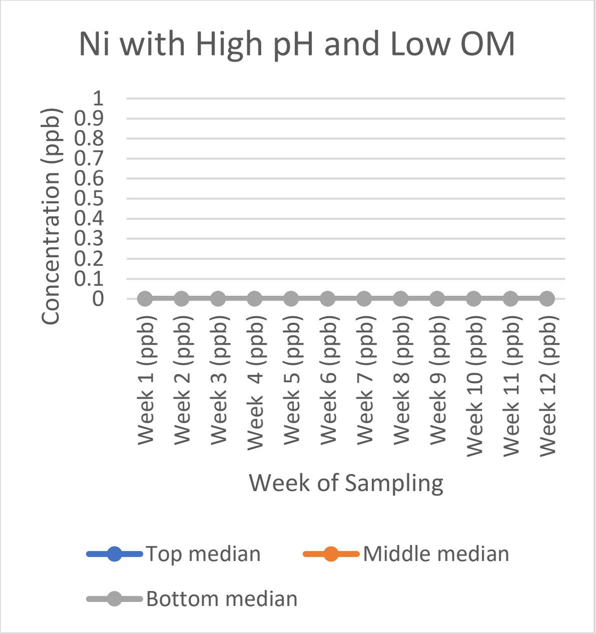


Fig D1: Temporal Median Weekly/Monthly Trace Metal Concentration in Porewater Data in parts per billion (ppb) from the Top, Middle and Bottom Sampling Points/Ports.

Appendix E: Weekly Median pH results for Mesocosm Study and Laboratory Temperature

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
pH Column 1 (control)	**4.2**	**3.9**	**4.1**	**4.2**	**3.8**	**4.1**	**4.4**	**4.2**	**4.1**	**3.7**	**4.2**	**4.2**
pH Column 2 (control)	**4.2**	**4.1**	**3.9**	**3.8**	**3.9**	**4.1**	**4.3**	**4.3**	**4.2**	**3.6**	**4.1**	**4.0**
pH Column 3	**3.5**	**3.4**	**3.8**	**3.8**	**3.6**	**3.6**	**4.2**	**4.1**	**4.3**	**3.7**	**3.8**	**3.9**
pH Column 4	**3.9**	**3.9**	**3.6**	**3.7**	**3.7**	**4.3**	**4.1**	**4.3**	**4.0**	**4.3**	**3.3**	**4.2**
pH Column 5 (control)	**6.9**	**6.6**	**7.1**	**6.6**	**6.5**	**6.9**	**7.0**	**7.0**	**6.9**	**6.7**	**6.9**	**6.5**
pH Column 6 (control)	**6.5**	**6.6**	**6.7**	**6.7**	**6.8**	**6.8**	**6.8**	**7.1**	**7.2**	**7.2**	**6.8**	**6.9**
pH Column 7	**6.8**	**6.9**	**7.2**	**7.3**	**6.9**	**6.6**	**7.1**	**6.9**	**7.1**	**7.2**	**7.2**	**7.1**
pH Column 8	**6.5**	**6.9**	**6.7**	**7.2**	**7.1**	**6.7**	**7.1**	**7.0**	**6.9**	**6.6**	**7.1**	**6.9**
pH Column 9	**8.0**	**8.1**	**8.1**	**8.4**	**8.2**	**8.1**	**7.8**	**8.1**	**7.9**	**8.0**	**8.0**	**8.0**
pH Column 10	**7.9**	**8.4**	**8.3**	**8.0**	**8.0**	**7.9**	**8.1**	**8.2**	**8.3**	**8.1**	**8.1**	**8.1**
pH Column 11	**8.4**	**8.1**	**8.0**	**8.1**	**8.1**	**8.3**	**8.1**	**8.2**	**7.9**	**8.1**	**8.0**	**8.1**
pH Column 12	**8.1**	**8.1**	**8.2**	**7.9**	**7.9**	**7.7**	**8.2**	**8.4**	**8.2**	**8.0**	**8.2**	**8.0**
Lab Temp (°C)	**19.3**	**20.1**	**21.4**	**22.1**	**22.9**	**22.7**	**24.7**	**22.8**	**22.1**	**21.8**	**21.4**	**20.7**

Table E1: Median pH for each column recorded from the 3 sampling taps and Lab Temp over time.

Column Parameters for Table E1:

pH Column 1 and pH Column 2 = pH<6, OM>25%

pH Column 3 and pH Column 4 = pH<6, OM<15%

pH Column 5 and pH Column 6 = pH = 6-7.5, OM<15%

pH Column 7 and pH Column 8 = pH = 6-7.5, OM>25%

pH Column 9 and pH Column 10 = pH>7.5, OM>25%

pH Column 11 and pH Column 12 = pH>7.5, OM<15%

Appendix F: Adaptation of Soil Quality Guidelines

The Dutch Soil Quality Guidelines (VROM, 2000a) were adapted as per the methodology set out in the annexes of the Dutch Target and Intervention Values, (VROM, 2000b):

Dutch guidelines amended from standard soil derivation.

$$(SW,IW)_b = (SW,IW)_{sb} \text{ x } [\{ A+(B\text{x}\%\text{ clay (grain size}< 2\text{ìm }^3)) + (C\text{x}\%\text{ organic matter})\}/ \{(A+(B\text{x}25) +(C\text{X}10)\}]$$

In which:

$(SW,IW)_b$ = target value or intervention value for the soil to be assessed

$(SW,IW)_{sb}$ = target value or intervention value for standard soil

% clay (grain size<2µm) = measured percentage clay (grain size <2µm) in the soil to be assessed

% organic matter = measured percentage organic matter in the soil to be assessed.

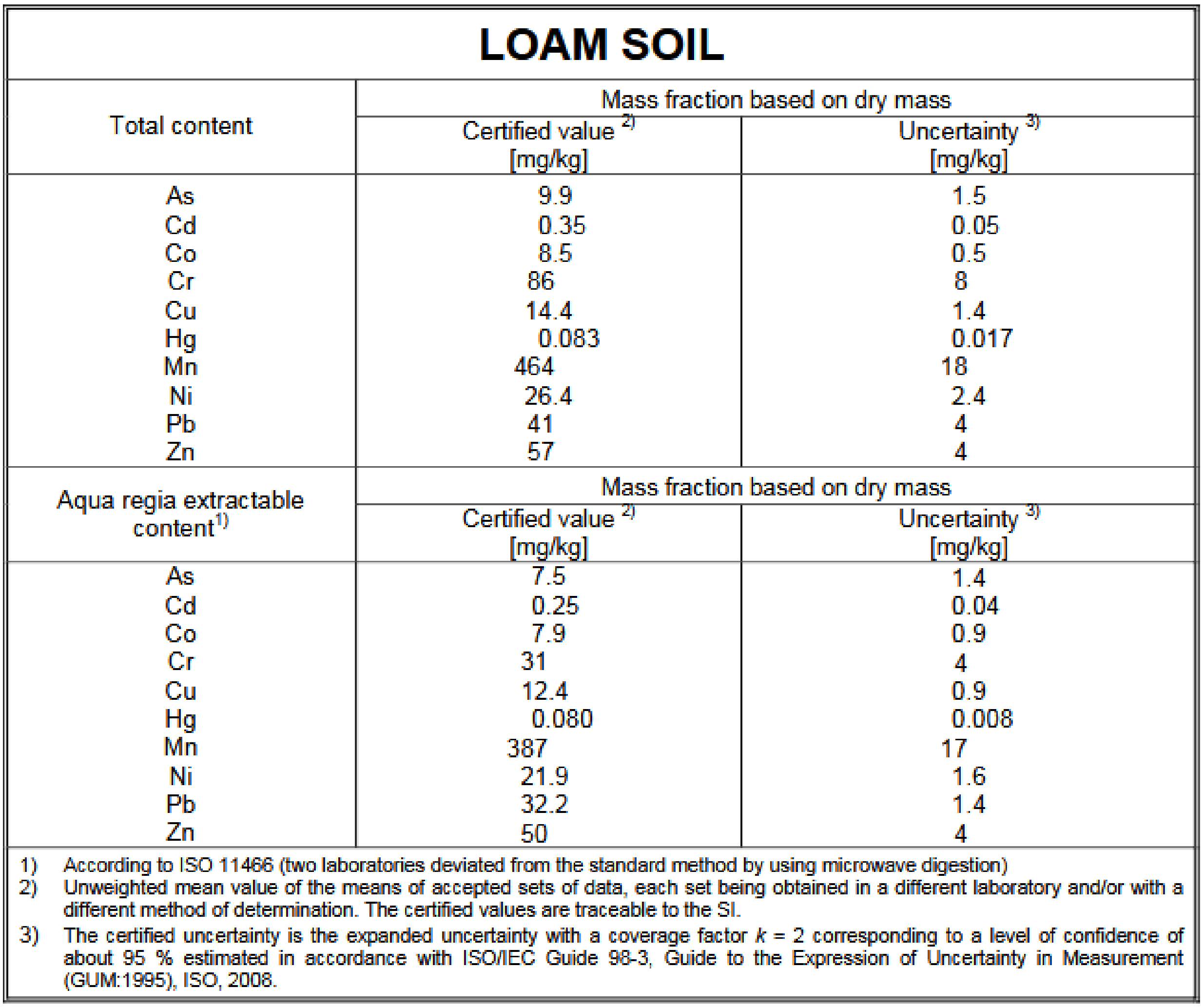
A, B, C = substance dependent constants for metals (see Table F1)

Equation F1: Calculation for Amending Dutch Soil Quality Guidelines (2000)

Trace Metal	**A**	**B**	**C**
Arsenic	15	0.4	0.4
Barium	30	5	0
Beryllium	8	0.9	0
Cadmium	0.4	0.007	0.021
Chromium	50	2	0
Cobalt	2	0.28	0
Copper	15	0.6	0.6
Mercury	0.2	0.0034	0.0017
Lead	50	1	1
Nickel	10	1	0
Tin	4	0.6	0
Vanadium	12	1.2	0
Zinc	50	3	1.5

Table F1: Substance Dependent Constant for Trace Metals

Appendix G: Certificate of Analysis ERM-CC141 Loam Soil



LOAM SOIL

Total content	Mass fraction based on dry mass	
	Certified value [2] [mg/kg]	Uncertainty [3] [mg/kg]
As	9.9	1.5
Cd	0.35	0.05
Co	8.5	0.5
Cr	86	8
Cu	14.4	1.4
Hg	0.083	0.017
Mn	464	18
Ni	26.4	2.4
Pb	41	4
Zn	57	4
Aqua regia extractable content[1]	Mass fraction based on dry mass	
	Certified value [2] [mg/kg]	Uncertainty [3] [mg/kg]
As	7.5	1.4
Cd	0.25	0.04
Co	7.9	0.9
Cr	31	4
Cu	12.4	0.9
Hg	0.080	0.008
Mn	387	17
Ni	21.9	1.6
Pb	32.2	1.4
Zn	50	4

1) According to ISO 11466 (two laboratories deviated from the standard method by using microwave digestion)
2) Unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory and/or with a different method of determination. The certified values are traceable to the SI.
3) The certified uncertainty is the expanded uncertainty with a coverage factor $k = 2$ corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

Table G1: Certificate of Analysis for Certified Soil Reference Material ERM-CC141 Loam Soil (JRC, 2024).