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Total and Extractable Trace Elements in Soil Samples from Muguga, Kenya

Abdub Galgallo Huri

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature …….. Date …….

Abdub Galgallo Huri (S56/74428/2012)

This thesis has been submitted for examination with our approval as university supervisors.

Dr. Michael Gatari

Institute of Nuclear Science and Technology Signature ……

University of Nairobi Date ……..……

Mr. David Maina

Institute of Nuclear Science and Technology Signature ……

University of Nairobi Date ……..…….

Dr. Keith Shepherd

World Agroforestry Centre (ICRAF) Signature ……

Nairobi, Kenya Date ……..……

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ABSTRACT

In plant nutrition soil micronutrient concentration is classified into two; total and extractable portion. The total concentration is not related to that potentially available to plants but is used to indicate the relative abundance and potential replenishing power of a particular element in a soil. The extractable/soluble portion is the concentration as indicated by extraction methods like ammonium acetate ethylene diamine tetra-acetic acid (AAAc-EDTA) and are usually considered as plant available. Although soluble concentrations are crucial in plant nutrition, the extraction procedures involved often require the use of a sizeable amount of chemical reagents and are time consuming. Total concentrations of Mn, Fe, Ni, Cu and Zn in soil samples from Muguga were determined using Total X-Ray Fluorescence Spectroscopy (TXRF) technique while Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) was used for investigating their extractable concentration. A relation between total and extractable could mean adoption of quick, fairly nondestructive spectral methods like TXRF instead of conventional wet chemistry methods like AAAc-EDTA. Topsoil pH, topsoil carbon and variation of total elemental concentration with depth were also investigated. The soil TXRF spectra was also deconvoluted using Picofox inbuilt software and later using qualitative X-ray analysis system (QXAS) software package.

The Pearson's correlations between the extracted and total concentrations were 0.90 for Ni, 0.80 for Zn, 0.59 for Mn, 0.57 for Cu and - 0.33 for Fe. The extracted amount and pH had r values of 0.59 for Mn, 0.58 for Cu, 0.42 for Fe, 0.40 for Zn, 0.15 for Ni while that of the extracted portion and carbon were 0.76 for Zn, 0.74 for Mn, 0.66 for Ni, 0.36 for Cu and 0.09 for Fe. Topsoil to subsoil Fe, Mn, Zn, Cu and Ni concentrations had r values

of 0.87, 0.79, 0.74, 0.50 and 0.32 respectively. Copper was the only nutrient in sufficiency range while Mn and Zn were in excess. The samples were strongly acidic with an average pH of 5.4 which could have resulted in the high solubility of the micronutrients. Acid tolerant crops should be grown and the farmers should also lime their farms and use nitrate nitrogen fertilizers and avoid ammoniacal nitrogen fertilizers. The Picofox inbuilt and QXAS software did not agree on a single measurement when quantifying Fe, Mn, Zn and Cu spectra, however the two methods showed some agreements when quantifying Ni spectra. Studies involving at least a few reference soil spectra is recommended to decide on the better method of the two. The strong relation ($\alpha = 0.05$) between total and extractable concentration for Ni, Zn, Mn and Cu means total element concentration in soil could be used as an indicator of their extractable amounts. However, studies involving different regional soils, associated plants and different extraction reagents is recommended to select the most appropriate extraction method.

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LIST OF ABBREVATIONS AND ACRONYMS

AAAc-EDTA- Ammonium Acetate Ethylene Diamine Tetra-Acetic Acid

AfSIS- Africa Soil Information Services

ASCII- American Standard Cord for Information Interchange

AXIL- Analysis of X-ray Spectra by Interactive Least-Squares fitting

CNS- Carbon, Nitrogen and Sulphur

DTPA- Diethylene Triamine Penta-acetic Acid

IAEA- International Atomic Energy Agency

ICP-OES Inductively-Coupled Plasma Optical Emission Spectroscopy

ICRAF- International Centre for Research in Agroforestry

LoA- Limit of agreement

QXAS- Quantitative X-ray Analysis System

RBS- René Borghgraef Sr.

SOM- Soil Organic Matter

SSA- Sub Sahara Africa

TOC- Total Organic Carbon

TXRF- Total Reflection X-Ray Fluorescence Spectroscopy

CHAPTER ONE

INTRODUCTION

1.1 Background

Essential trace elements also known as micronutrients, include those nutrients that are required in extremely small quantities by crops, livestock and human beings. However, this does not mean they play a minor role in plant and animal nutrition. According to Uchida (2000) soil supplies 14 out of 17 essential elements required for nutrition of crop plants and 8 of them are trace elements. These are Fe, B, Cl, Mn, Zn, Cu, Mo and Ni (Osman, 2013). A reduction in their concentration in soils results in unhealthy low intake by plants and consequently domestic animals and human beings. This in turn could result in an increased risk of mineral deficiency related symptoms, diseases and malnutrition thus worsening the current food and economic situation in Africa.

Africa, with a population of over one billion people and total landmass of about 30.2 million km^2, has generally lagged behind in agricultural development. It is the only region in the world where per capita food production has been on the decline for the last two decades (Pedro, 2000; Muchena et al., 2005). It has gone from being a key exporter of agricultural commodities into being a net importer and is currently receiving most food aid. Some 30 million people require emergency food aid in any one year and yet about 60% of the World Food Program's work is in the region (Kung`u, 2007).

Even though the greatest absolute number of undernourished people is in Asia and the Pacific, the highest prevalence is in Sub-Saharan Africa (SSA), where one in every three persons experiences chronic hunger. According to Brian et al., (2012) 50% of all food-

insecure people are small-scale farmers. In Africa, over 70% of the population lives by farming and 40% of its exports is earned from agriculture yet it is still the home to the hungriest. There is also the hidden hunger issue which is deficiency of micronutrients (minerals and vitamins) in a person's diet. It is not malnutrition as classically presented as the hungry or starving individual, but malnutrition as it should properly be defined: poor overall quality of nutrition. The term "hidden" is used because often only the most severe deficiencies are clinically visible and afflicts a far greater proportion of humanity than insufficient calorie intake. Moreover, insufficient calorie consumption often goes hand-in-hand with micronutrient deficiency (Brian et al., 2012). In Kenya alone, over 10 million suffer from chronic food insecurity and poor nutrition, where mineral and vitamin deficiencies exist even among population groups with sufficient food in terms of meeting energy requirements. Children under five years are particularly affected by deficiencies in iron (73.4%) and zinc (51%). Women are among the most vulnerable with a high risk of iron deficiency (60% among pregnant woman) and an estimated 16% of adult males suffer from iron deficiency (GOK, 2011).

Among the key causes of reduction in food production are: geologically mature and highly weathered African soils being relatively nutrient deficient and prone to degradation (Kung'u, 2007); declining soil fertility (Muchena et al., 2005); insufficient information about the cause–effect relationship linking soil degradation to food production and its nutritional quality (Lal, 2009); climate change (Vermuelen et al., 2012); and farmers focusing only on macro nutrients and overlooking importance of trace elements (Ryan, 2010). In most cases farmers do not know the status of their soil and rely

on knowledge passed from generation to generation. However, that knowledge is no longer useful in the current situation of high population and reduced arable land that is over utilized. For farmers to get profitable yields and to help fight poverty and hunger, knowledge and advisories on soil micronutrient status should be improved. The present development in technology, instrumentation and organizations that are willing to provide tools and information to local farmers, experts and relevant authorities will help in providing the missing knowledge to the farmers in the perspective of addressing the problems of poverty, hunger and malnutrition.

Studies in the determination of total micronutrients in soils are limited since total concentrations are not related to that potentially available to plants (Darrell and Raymond, 1999). However, according to Gupta et al., (2008) total micronutrients indicate the relative abundance of a particular element in a soil and its potential replenishing power. With the emergence of X-ray fluorescence methods and improved instrumentations, which are fast, fairly non-destructive and capable of analyzing a wide range of elemental concentrations and sample types; total elements in soils could be easily analyzed and assessed for plant available. The extraction procedures developed to mimic plant uptake of nutrients often involve the use of a lot of chemicals, and are destructive and time consuming. In future and with a well-developed model relating total and extractable elements in soil we foresee adoption of quick and fairy non-destructive methods like TXRF, which will save the environment, time and money. In this study total and AAAc-EDTA extractable essential trace elements in soil samples from Muguga town were investigated. Muguga was selected due to its close proximity to Nairobi and also its

demographic and agro-ecological characteristics which are generally representative of the conditions found in other tropical highlands of East Africa (Makokha et al., 2001).

1.2 Problem statement

Poor soil conditions affect food security directly and indirectly. Direct effects results in overall reduction in crop yields and decline in their nutritional values. Some of the indirect effects are reduction in the efficiency of inputs and additional land area required to compensate the loss of production. The loss of household income is another indirect effect with adverse impact on access to food. Soil nutrient imbalance, caused by deficiency of some and toxicity owing to excess of others, is a principal cause of yield decline in degraded soils. Some causes of nutrient deficit is the prevalence of extractive farming practices including removal of crop residues, lack of or low rate of application of inorganic fertilizers and organic amendments. Nutrient depletion is worsened by accelerated erosion which also has strong negative impacts on crop yield and agronomic production especially in Sub Sahara Africa (Lal, 2009).

Sub-Sahara Africa is the only region in the world where food production has been on the decline for the past two decades. It has the highest prevalence of hunger while agriculture employs 60 to 90 % of the population. Unfortunately, most African soils are degraded and vulnerable to climate change. There is also insufficient information on soils, which often hinders management and investment. Depletion of soil fertility is increasingly recognized as a major biophysical cause of stagnant per capita food production in SSA (Nico, 2003). Due to rapidly rising population density, the length of fallow periods has declined and shifting cultivation is replaced by permanent cultivation systems.

Kenya with an area of approximately 583,000 km^2 and a population of about 39.5 million (KNBS, 2012) is in the same predicament as the rest of Sub Sahara Africa. Agriculture employs over two thirds of the population and is a source of raw material for local industries and foreign exchange earnings. Kenya's agriculture however, is largely rain fed making it highly sensitive to drought and other natural and man-made disasters. People living in agricultural areas are affected by insecure land tenure systems and continuous fragmentation of holdings. With 80% of its population living in rural areas and dependent on the land for their livelihood, Kenya is still consistently classified as one of the 20 most food-insecure countries on Earth (Gordon et al., 2012). This issue has to be addressed since apart from causing hunger, malnutrition and their associated problems food insecurity has been linked to national security, peace and political stability (Lal, 2009).

In the past, traditional Kenyan farming methods included shifting cultivation which allowed previously cultivated areas to recover naturally. However, with the increased land pressure due to population expansion and poverty, this practice has ceased and many farmers cultivate their land continuously. Continuous cultivation is linked to irreversible soil degradation and top soil loss (Jeff, 2003). Depletion of soil fertility on small holder farms is increasingly referred to as the root cause of diminishing per capita food production in Africa (Jeff, 2003). For farmers to produce enough food to live on they have to produce more per unit area and this means land has to be in constant production; this can only be realized if there is enough information on soil nutrient status.

With the present conditions of high population and limited resources Kenya, and indeed the whole of SSA, needs quick and affordable soil health surveillance systems to provide

information on soil nutrients to guide planning and assessment of intervention programs and safeguard the soil resource. Recent technological advances in soil-plant spectral diagnostics have created new opportunities for providing the much needed low cost information on soil constraints and plant nutritional status. The use of soil spectroscopy for soil and plant analysis is fast, cost-effective and nondestructive (Nocita et al., 2015). In addition there is no need for chemical reagents and requires minimal sample preparation. A single spectrum contains comprehensive information on several soil components and can be used to predict these simultaneously (Rossel et al., 2006, Nocita et al., 2015).

1.3 Justification

Although there are many immediate causes for food security crisis in Kenya (severe poverty, over population, drought, war, flooding, etc.), food insecurity exists as a result of these causes as well as because of the pervasive underlying condition of inadequate food production (soil infertility, poor crop selection, etc.) and poor land husbandry (Jeff, 2003). Kenya's food shortage is linked to intense land-use resulting in severe soil degradation due to soil erosion and poor soil fertility. In most cases farmers do not know the status of their soil and rely on knowledge passed from generation to generation. However, that knowledge is no longer useful in the current situation of high population and reduced arable land that is over utilized. With approximately 36% of Kenya's population classified as food insecure in and 56 % classified as living in poverty in 2007 (Gordon et al., 2012), there is an urgent need to build resilience in rural communities and increase farmers' capacity to meet challenges associated with food production.

Econutrition is another approach being proposed to enhance nutritional values of agricultural produce. Econutrition (Lal, 2009) is interrelationship among nutrition and human health, agriculture and food production, environment health and economic development. The econutrition concept is based on the realization that there exists a strong link between soil quality and human health. A reduction of nutrient concentration in soils results in an unhealthy low intake by plants and consequently domestic animals and human beings. This study focuses on trace elements in soil which is the main source for plants, then animals and human beings.

1.4 Scope

The samples were investigated for total elemental concentration using Total X-ray Fluorescence spectroscopy (TXRF) technique while Inductively Coupled Plasma Optical Emission Spectroscopy (ICP - OES) was used for investigating the extractable portion. The sampling area was 12.7 hectares and samples were collected from 32 sampling sites. Mo was not analyzed using TXRF neither was Cl using ICP-OES and B in both. Mo and B were not analyzed using the TXRF spectrometer because Mo was used as the spectrometer anode. Cl and B were also not analyzed because the AAAc-EDTA extraction reagent is not ideal for their extraction.

1.5 Study objectives

The main objective of this study was to assess total and soluble trace elements in soil samples from Muguga in Kiambu County in perspective of food security using Total X-ray Fluorescence (TXRF) and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

The following are the specific objectives:

1. Investigate soil total elemental concentration in top and sub soils.
2. Investigate AAAc-EDTA extractable micronutrients in top soils.
3. Assess relationship of AAAc-EDTA extractable micronutrients with their total concentration, pH and Carbon.
4. Assess elemental quantification from soil TXRF spectra analyzed using the inbuilt Picofox automated software and the manual Quantitative X-ray Analysis System (QXAS).
5. Develop solutions of soil management options with respect to nutrient availability to food crops.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

In geochemistry the term "trace elements" is used to refer to those elements present in rocks and soils at a concentration below 1000 mg kg^{-1}. In biological field the term is used to refer to elements occurring at low concentrations (usually < 100 mg kg^{-1}) in the dry matter of living organisms. In food and nutrition science, a trace element may be defined as an element that is of common occurrence but whose concentration rarely exceeds 20 mg kg^{-1} in the foodstuff as consumed (Blum et al., 2002). It should be noted that some of the nutritive trace elements (e.g. Mn and Zn) may often exceed this concentration (Adriano, 2001). In plant nutrition, essential trace elements are vital to plant growth but are only required in minute amounts and are generally referred to as micronutrients.

A trace element is essential either for plant or animal nutrition if it is required for normal growth and reproduction, cannot be replaced by another element and is directly involved in the nutrition of the plant (Arnon and Stout, 1939). However according to Epstein (1965) an element can also be regarded as essential for plants if it is a component of a molecule known to be an essential metabolite, even if it cannot be demonstrated that it fulfills all of the criteria proposed by Arnon and Stout (1939). The essential trace elements to plants are: boron, chlorine, copper, iron, manganese, molybdenum, nickel and zinc (Alloway, 2008). Other trace elements, which are beneficial for nutrition of some higher plants but have not been proved to be 'essential' include: sodium, cobalt, selenium, vanadium and aluminium (Alloway, 2008).

Soil is the primary source of trace elements for plants, animals and humans beings. The trace element content of a soil depends initially on the parent material from which it was formed (Mason and Moore, 1979) but subsequent leaching and nutrient cycling through plants and animal excreta creates both depletion and enrichment often in specific soil horizons. The soil profile can also gain elements through deposited dust imported from areas prone to dust storms, by adsorption from water draining into a soil from elsewhere and by pollution due to human activity (Johnson, 2005).

2.2 Bioavailability of soil micronutrients to plants

Bioavailability of an element is the amount or concentration of that element that can be absorbed by an organism thereby creating the potential for toxicity or the necessary concentration for survival (Allen, 2002). However according to Lars and Rudolph (2004) this term may include (1) the physico-chemical availability of metals in the exposure medium, (2) the actual demand of biota and (3) the toxicological behavior of metals inside the organisms' body. In this study the term "bioavailability" designates the metal species that can be taken up by plants from the soil as indicated by AAAc-EDTA method of extraction.

In plant nutrition a number of extraction methods have been suggested to indicate the plant available portion of total metal concentration is soils. Metals extracted by such extractants are usually considered as plant available. Some of the widely used extractants are: (1) complexing agents such as diethylene triamine penta-acetic acid (DTPA) and ethylene diamine tetra-acetic acid (EDTA); (2) acid extractants such as 0.1 molar hydrochloric acid and (3) neutral salt extractants such as 0.01 molar calcium chloride, 0.1

molar sodium nitrate, 1.0 molar ammonium nitrate and 1.0 molar ammonium acetate (Xu et al., 2013).

The bioavailability of trace elements in soils varies greatly due to soil properties. The primary soil factors controlling the potential bioavailability of trace elements are soil pH, the accessibility and character of sorption sites on soil surfaces, the contents of Fe and Al oxyhydroxides, soil organic matter, and clay minerals (Alvarez et al., 2008). According to Xu et al. (2013) soil pH is one of the most important factors due to its effect on mobility and speciation of trace elements in soil as a whole and particularly soil solution. In addition to pH, soil organic matter has also been reported as one of the main factors affecting metal bioavailability (Du Laing et al., 2009; Xu et al., 2013). However, availability of micronutrients changes with soil conditions and this makes generalizations extremely difficult (Johnston, 2005). Since our interest is in the analysis of selected essential trace metals, some of the main soil properties affecting their availability are briefly discussed.

2.3 Factors Affecting Availability of Trace Elements to Plants

2.3.1 Soil Reserves

In most soils the total micronutrient content is not related to that potentially available to plants (Darrell and Raymond, 1999). However total micronutrient concentrations can be used to show their abundance and potential replenishing power (Gupta et al., 2008). In addition and according to Stevenson and Michael (1999) deficiencies and toxicities of trace elements can be traced to the nature of the soil and its total content of

micronutrients. For example alluvial sands and certain organic soils often have low reserves of micronutrients.

2.3.2 Soil pH

Soil pH refers to the degree of soil acidity or alkalinity. Chemically, it is defined as the $\log_{10}$ of number of hydrogen ions (H^+) in the soil solution. It is important to note that because the scale is in logarithmic units, a change of just a few pH units can induce significant changes in the chemical environment and sensitive biological processes. Soils with pH value between 6.5 and 7.5 are classified as neutral, pH above 7.5 as alkaline, pH less than 6.5 as acidic and pH below 5.5 as strongly acidic.

Soil pH regulates the solubility of elements and compounds in soil and governs their availability to plants. When soil pH decreases below 6.5, the solubility of some elements including Fe, Al, Mn, Cu, and Zn increases while for others such as Ca, Mg, and Mo it decreases. On the other hand, when soil pH rises above 7 solubility of Ca, Mg and Mo increases and that of Fe, Al, Mn, Cu, and Zn decreases (Osman, 2013). Availability of macronutrients (Ca, Mg, K, P, N, and S) and Mo and B is restricted at low pH. While availability of most micronutrients (Fe, Mn, Zn,Cu, Co) increases at low pH (Stevenson and Michael, 1999). The most satisfactory plant nutrient levels occur at a pH range of 5.5–6.5 (Osman, 2013). Alkaline soils may have problems with deficiencies of such nutrients as zinc, copper, boron, and manganese. In acidic soils aluminum and manganese can become very soluble and toxic reducing the plant's ability to take up calcium, phosphorus, magnesium and molybdenum (Stevenson and Michael, 1999). Also soils

with an extremely alkaline pH (greater than 9) are likely to have high levels of sodium which may deteriorate soil physical properties and exert toxicity on plants.

2.3.3 Soil Carbon

Soil carbon includes total organic, total inorganic and elemental carbon constituent and is referred to as soil total carbon. Total organic carbon (TOC) is the carbon stored in soil organic matter (SOM) while total inorganic carbon is the carbon stored as part of salts. Soil organic carbon is one of the most important constituents of the soil due to its capacity to affect plant growth as both a source of energy and a trigger for nutrient availability through mineralization. According to Xu et al. (2013) the net effect of the presence of organic matter can either decrease or increase soil metal mobility. The quantity of organic matter also affects the ability of soil to store water, release greenhouse gases, modify pollutants, resist physical degradation and produce crops (Hao et al, 2003; Bronick and Lal, 2005). The elemental constituent is insignificant in most soils and the inorganic form is used as carbon source by some plant in their dissolved form under conditions of soil salinity stress, high shoot temperatures and high light intensities (Cramer and Richards, 1998).

2.4 The trace elements investigated in the study

2.4.1 Iron

Iron is commonly found in soils in amount ranging from 0.5 to 5.0% with an overall average soil content of 3.8% (Kabata-Pendias and Pendias, 1992). It occurs predominantly as oxides and hydroxides, it is present in soil solution in 3^+ and 2^+

oxidation states and as iron chelates, and is absorbed by plants mainly in the form of Fe^{2+} (Kabata-Pendias and Pendias, 1992). The solubility of Fe is affected mainly by hydrolysis and complexation (Kabata-Pendias and Pendias, 2011). Soil aeration and pH also affect the mobility of Fe. Increasing pH and oxidized conditions lead to Fe precipitation whereas acid reducing conditions result in higher solution concentration of Fe (Kabata-Pendias and Pendias, 1992). Deficiencies are generally caused by unavailability of soil iron rather than a low iron content in soil, and are likely to occur on soils which are well aerated, calcareous, alkaline or high in Mn (Haluschak et al., 1998).

Iron is considered the key metal in energy transformations needed for synthesis and other life processes of plant cells. Its deficiency affects several physiological processes and therefore retards plant growth and plant yield. The deficiency of Fe occurs in soils with AAAc-EDTA soluble content below 35mg/l (Sillanpää, 1982) and is a major worldwide problem with many crops since a large number of cultivated soils are low in its available content. The symptoms of Fe deficiency may occur at very different Fe levels in plants, and this deficiency is highly dependent on soil, plant, as well as on plant nutritional, and climatic factors. Fe uptake can produce toxic effects in plants growing in soil rich in mobile Fe fractions. Plant injury due to Fe toxicity is most likely to occur on strongly acid soils (ultisols, oxisols), on acid sulfate soils, and on flooded soils (Kabata-Pendias and Pendias, 2011).

The soluble Fe fraction is very low compared to the total Fe content. Extractable amounts of Fe determined by sequential extraction account from 0.01% to 0.1% of the total Fe (Kabata-Pendias and Pendias, 2011). In calcareous and loamy soils (pH range 7.0 to 7.8) soluble Fe contents vary from 100 to 200 μg/L, whereas in light acid sandy soils (pH

range 2.5 to 4.5), soluble Fe contents range from 1000 to 2223 μg/L (Kabata-Pendias and Pendias, 2011). The mean concentration of AAAc-EDTA soluble Fe in soils from some African countries are 197 mg/kg in Ethiopia (n=71), 72 mg/kg in Ghana (n=93), 102 mg/kg in Malawi (n=100), 65 mg/kg in Nigeria (n=103), 113 mg/kg in Sierra Leone (n=49), 91 mg/kg (n=175) in Tanzania and 53 mg/kg in Zambia (n=45) (Sillanpää, 1982).

2.4.2 Copper

The mean copper contents for uncontaminated soils world wide range from 13 to 24 ppm, but the overall range for world soils is higher (1 to 140 ppm) depending on the nature of the soil parent materials (Kabata-Pendias and Pendias, 1992). Generally, Cu is accumulated in the upper few centimeters of soils, however, due to its tendency to be adsorbed by SOM, carbonates, clay minerals, and oxyhydroxides of Mn and Fe, it may be also accumulated in deeper soil layers. Cu is a rather immobile element in soils and shows relatively little variation in total contents of soil profiles (Kabata-Pendias and Pendias, 2011). The common characteristic of Cu distribution in soil profiles is its accumulation in the top horizons. This phenomenon is an effect of various factors and mainly reflects its bioaccumulation as well as anthropogenic sources (Kabata-Pendias and Pendias, 2011).

The main form of copper in solution is that of soluble organic chelates, but solubility of all forms of Cu decreases at pH 7 to 8 (Kabata-Pendias and Pendias, 1992). Acid leached sandy soils and calcareous sandy soils may be low in soluble copper and mobility of copper is low in reduced and neutral soils (Haluschak et al., 1998). Cu mobility may be higher under high pH because of Cu^{2+} complex formation which may increase overall Cu solubility (Haluschak et al., 1998). Concentrations of Cu in soil solution range from 0.5

to 135 μg/L; depending on extraction techniques used and on soil types (Kabata-Pendias and Pendias, 2011).

The mean concentration of AAAc-EDTA soluble Cu in soils from some African countries are 3 mg/kg in Ethiopia (n=71), 2 mg/kg in Ghana (n=93), 3 mg/kg in Malawi (n=100), 1 mg/kg in Nigeria (n=103), 1 mg/kg in Sierra Leone (n=49), 4 mg/kg (n=175) in Tanzania and 2 mg/kg in Zambia (n=45) (Sillanpää, 1982). Copper is a constituent of several enzymes and plays important functions in physiological processes, such as photosynthesis and respiration, carbohydrate and nitrate metabolism, water permeability, reproduction and disease resistance (Regis, 1998).

2.4.3 Manganese

Manganese contents of worldwide soils vary from 2 to 9200 mg/kg, with a calculated grand mean of 437 ppm and its highest levels occur in loamy and calcareous soils (Kabata-Pendias and Pendias, 1992, 2011). Higher Mn levels are often reported for soils over mafic rocks, for soils rich in Fe and/or organic matter, and for soils from arid or semiarid regions. Although Mn can be concentrated in various soil horizons, particularly in those enriched in Fe oxides or hydroxides, usually this element is also accumulated in top soils as the result of its fixation by organic matter.

Manganese functions as an activator of an enzyme that is involved in the evolution of oxygen in photosynthesis. It is a component of several enzyme systems. It also functions as part of oxidation-reduction reactions and electron transport systems and is a structural component of certain metalloproteins (Regis, 1998). Mn deficiency is common in certain crops grown on neutral and calcareous soils (Kabata-Pendias and Pendias, 2011) and

soils with DTPA soluble Mn below 5 mg/l are considered deficient. Toxicity problems are likely to be found on well drained soils with a pH <5.5, poorly drained soils with a pH of 6 or greater, or soils with a pH greater than 8 (Kabata-Pendias and Pendias, 1992).

Manganese occurs in soils and minerals mainly in the forms of Mn^{2+}, Mn^{3+}, and Mn^{4+} but only Mn^{2+} is absorbed by plants (Haluschak et al., 1998). Solubility of soil Mn is of significance since its plant supply depends mainly on the soluble Mn pool in the soil. In well-drained soils, the solubility of Mn always increases with the increase of soil acidity. However, the ability of Mn to form anionic complexes and to complex with organic ligands may contribute to increased Mn solubility in the alkaline pH range (Kabata-Pendias and kabata, 2011). The mean concentration of DTPA soluble Mn in soils from some African countries are 89 mg/kg in Ethiopia (n=71), 48 mg/kg in Ghana (n=93), 67 mg/kg in Malawi (n=100), 27 mg/kg in Nigeria (n=103), 7 mg/kg in Sierra Leone (n=49), 41 mg/kg (n=175) in Tanzania and 39 mg/kg in Zambia (n=45) (Sillanpää, 1982).

2.4.4 Nickel

The Ni status of soils is highly dependent on its contents in parent material (Kabata-Pendias and Pendias, 2011). However, the concentration of Ni in surface soils reflects the additional impact of both soil-forming processes and anthropogenic activities. Ni is present in all soil groups and its greater accumulation is observed in cambisols and calcisols. Soils throughout the world contain Ni in the very broad range, however its mean concentrations, as reported for various countries are within the range 13 to 37 mg/kg (Kabata-Pendias and Pendias, 2011).

The Ni distribution in soil profiles is related either to OM or to amorphous oxides and clay fractions, depending on soil types. Forms of Ni in soils are diverse and range from highly mobile to ones that have no reactivity. Several soil properties, particularly clay fraction, SOM, and pH control Ni behavior and phytoavailability, in particular (Kabata-Pendias and Pendias, 2011).

Ni is the most recent element to be added to the list of plant essential trace element. It is a functional constituent of seven enzymes (Walsh and Orme-Johnson, 1987). Among the seven, urease is extremely important to N metabolism in plants. As a Ni-metalloenzyme, urease assists in the hydrolysis of urea. Nickel works as a cofactor to enable urease to catalyze the conversion of urea into the ammonium ion, which plants can use as a source of N. Without the presence of Ni, urea conversion is impossible. Ni deficiency is likely to occur in soils having pH > 6.7 or soils that have received excessive applications of Zn, Cu, Mn, Fe, Ca, or Mg. High pH in soils causes Ni^{2+} to be readily oxidized and unavailable while excessive use of the mentioned metals may induce deficiency because they share a common uptake system (Guodong et al., 2011).

2.4.5 Zinc

The general values for the average total Zn contents in soils of different groups, all over the world, range between 60 and 89 mg/kg (Kabata-Pendias and Pendias, 2011). Contents of Zn are closely associated with soil texture and usually are the lowest in light sandy soil. Its elevated concentration is often observed in calcareous and organic soils (Kabata-Pendias and Pendias, 2011).

Zn is essential for plants in lipid and carbohydrate metabolism and is required at levels of 10 to 20 mg/kg (Kabata-Pendias and Pendias, 1992). It is a component of several enzymes that function as electron transfer mechanisms and in protein synthesis and degradation. Zn is part of auxin, one of the well-known enzymes regulating plant growth. It is also necessary for chlorophyll synthesis and carbohydrate formation (Regis, 1998).

Weathering of Zn minerals leads to the release of Zn^{2+}, which is the most common and mobile Zn ion in soil and the main form utilized by plants (Haluschak et al., 1998). Zn is adsorbed by clay and organic matter and may accumulate in the surface horizons of soil (Kabata-Pendias and Pendias, 1992). Zn solubility in soil is high compared to other trace elements. It is controlled by the presence of clay minerals and hydrous oxides and affected by pH (Kabata-Pendias and Pendias, 1992). At pH<7 there is less adsorption of Zn^{2+}, which may result in leaching from sandy soils (Kabata-Pendias and Pendias, 1992). At high soil pH, the formation of insoluble compounds ($Zn(OH)_2$ and $ZnCO_3$) may restrict Zn availability (Haluschak et al., 1998). As a result, deficiencies are likely to be found under the following soil conditions: strongly acid or alkaline, low organic matter, free $CaCO_3$ and high N and P (Kabata-Pendias and Pendias, 1992).

The amount of available Zn in soils is affected by soil pH, soil texture, soil phosphorus, and weather conditions (Regis, 1998). Zinc availability to plants decreases as soil pH increases and may become deficient in soils with a pH above 6.5. Soil pH affects Zn availability more than any other factor (Regis, 1998). The mean concentration of DTPA soluble Zn in soils from some African countries are 5 mg/kg in Ethiopia (n=71), 1 mg/kg in Ghana (n=93), 1 mg/kg in Malawi (n=100), 2 mg/kg in Nigeria (n=103), 1 mg/kg in

Sierra Leone (n=49), 1 mg/kg (n=175) in Tanzania and 1 mg/kg in Zambia (n=45) (Sillanpää, 1982).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sampling Location

Muguga (1.18°S 36.65°E) is a town in Kiambu County in Kenya's Central region with small scale farming as one of its major economic activities. This area is characterized by Nitisols; which is part of the more than half of approximately 200 million hectares of Nitisols worldwide found in tropical Africa, notably in the highlands (>1000m) of Kenya, Ethiopia, Zaire and Cameroon. They have dark red colored and well-developed structure that is nutty in appearance with shiny surfaces. Nitisols are much sought after because of their high productivity despite a high phosphate-fixing capacity due to their low pH.

3.2 Sampling

A 50 m by 50 m grid was constructed over the site (350 by 350 m). Using off-set grid sampling pattern (Figure 2) 32 sampling points were identified within the area. Off-set grid pattern was preferred over regular grid pattern because it provides more information at a lower cost than the latter. At each sampling point top soil (0-20 cm) and sub soil (20-50 cm) samples were randomly collected with an auger from five cores within a 3 meter radius and pooled into two buckets, one for topsoil and the other for subsoil. The samples were then thoroughly mixed in the buckets using a trowel.

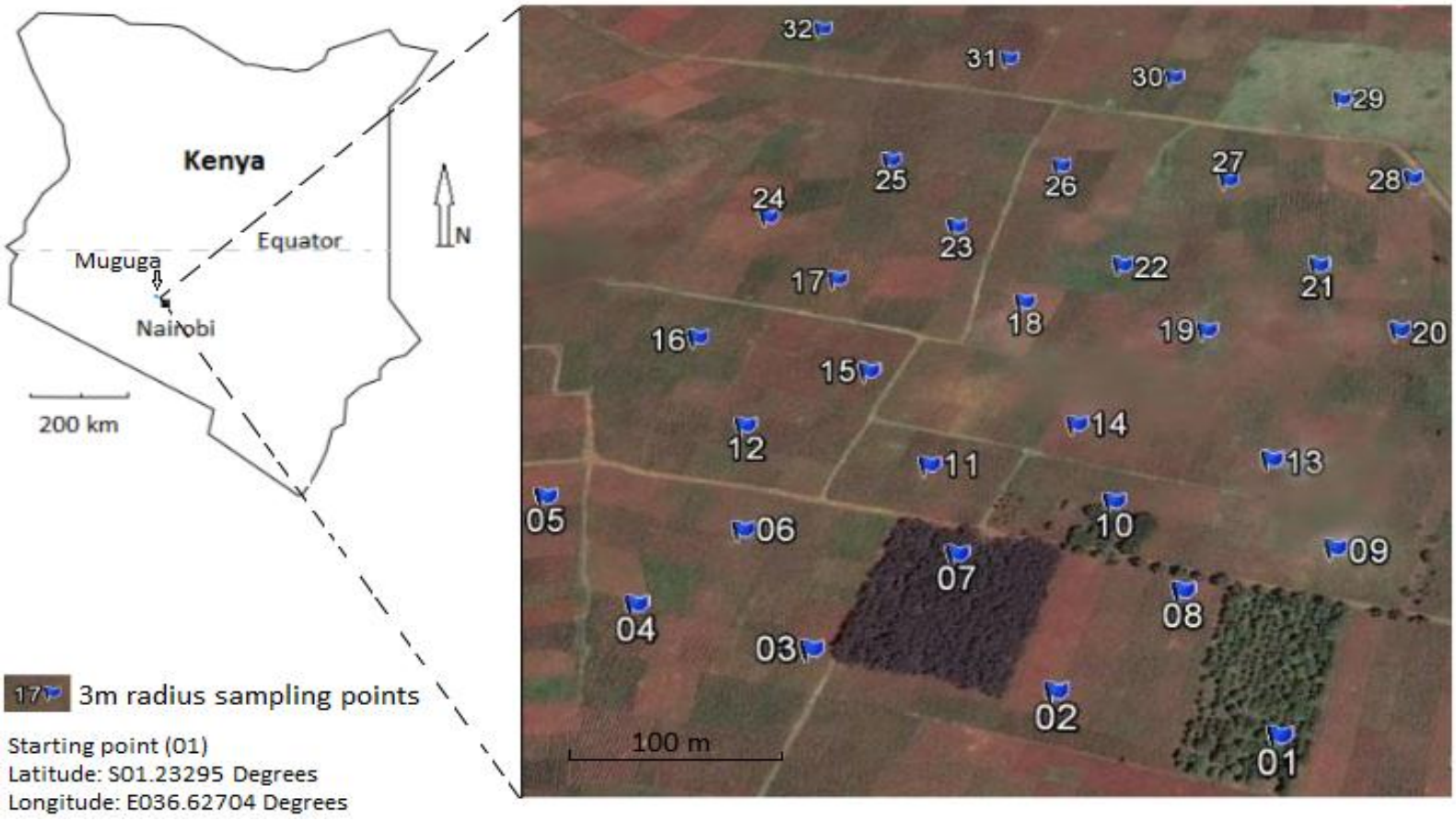


Figure 3.1: Geographical positioning of Muguga, Kenya. Numbered flags: the 32 sampling points.

About 300 g of the sample was subsampled into labeled porous bags, one for topsoil and another for subsoil. The labeled 64 soil samples were then transported to World Agroforestry laboratories in Nairobi for preparation and analysis.

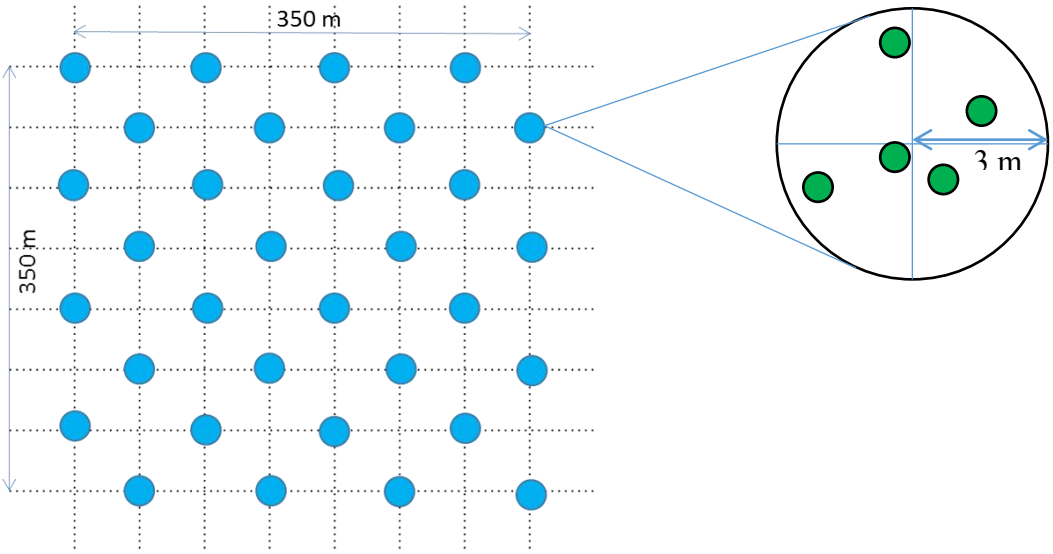


Figure 3.2: Off-set grid sampling pattern (Richard and Gary, 2009). Blue markers are grid intersections sampled, green markers represent soil cores collected about the grid for composting into one sample for analysis.

3.3 Instrumentation

3.3.1 Total X-Ray Fluorescence Spectroscopy (TXRF)

TXRF spectroscopy is a quick fairly nondestructive technique capable of analyzing a wide range of elements, concentration and sample types. It is based on measuring the X-rays emitted from the elements in a sample upon irradiation with higher energy x-rays. The incoming X-Ray removes an electron from one of the orbitals surrounding the nucleus within an atom of the material. A vacancy is then created in the orbital, resulting in a high energy/excited, unstable configuration for the atom. An electron from a higher

energy, outer orbital transits into the vacancy to restore the equilibrium. Since this is a lower energy position, the excess energy is emitted in the form of a fluorescent X-ray. The wavelength or energy of the fluorescence radiation is specific for each element. The concentration of each element can be calculated using the intensity of the characteristic radiation. Portable bench-top S2 Picofox TXRF spectrometer was used for the analysis of total element concentration in the soil. The spectrometer is independent of any cooling media and can be used for on-site analysis. The use of monochromatic radiation and total reflection optics results in a reduced background noise and consequently much higher sensitivities and a significant reduction of matrix effects compared to conventional EDXRF spectrometers (AfSIS, 2010). A summary of the technical specifications of the instrument are given in Table 3.1.

Table 3.1: Technical specifications of the S2 Picofox TXRF Spectrometer

X-ray tube	50 kV, 1 mA, Mo target
Element range	Na to U
Optics	Multilayer monochromator, 17.5 keV
Detector, area, resolution	Silicon drift, 10 mm^2, <160 eV (FWHM)
Carrier	Quartz, 30 mm diameter
Sample station	Cassette for 25 disks
Control	PC, data transfer via serial interface
Size, weight	590 x 450 x 200 mm, 37 kg
Power consumption	Max 150 W
Voltage, frequency	100-230 V ± 10%; 50-60 Hz
Manufacturer	Bruker AXS Microanalysis GmbH

The analysis was based on internal standardization; an element which is not present in the sample was added for quantification purposes. The process of analysis and quantification involved simultaneous measurement of all detectable elements. All identified peaks were then marked for further quantification and on the basis of chosen elements the spectra was deconvoluted using the software program SPECTRA 3 (Bruker AXS Microanalysis GmbH). The element concentration was calculated using the formula:

$$C_x = \frac{N_x/S_x}{N_{is}/S_{is}} * C_{is} \quad \text{.....3.1}$$

where:

N is the net intensity,

S is the relative sensitivity,

C is the concentration; each either of the analyte *x* or the internal standard *is*.

In this study 40 µl of 1000 mg/l Se was used as the internal standard due to its low concentration (0.01 to 0.3 mg/kg) in African soils (Erick et.al, 2013).

3.3.2 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

ICP-OES is one of the most powerful and popular analytical tools for the determination of trace elements in many sample types. The technique is based upon the spontaneous emission of photons from atoms and ions that have been excited. An aqueous sample is converted to aerosols via a nebulizer and transported to inductively coupled plasma which is a high temperature zone (8,000– 10,000 ºC). The analytes are heated (excited) to different (atomic and/or ionic) states and produce characteristic optical emissions. These emissions are separated based on their respective wavelengths and their intensities measured. The intensities are proportional to the concentrations of analytes in the

aqueous sample. Quantification is done by comparing the emission intensity of an unknown sample with that of a standard sample. In this study 5.00, 10.00, 20.00 mg/l Fe, 15.00, 30.00, 60.00 mg/l Mn, 0.50, 1.00, 2.00, mg/l Zn, 0.20, 0.40, 0.80 mg/l Ni and 0.15, 0.30, 0.60 mg/l Cu standard solutions were used. A summary of the technical specifications of the instrument are given in Table 3.2.

Table 3.2: Technical specifications of the PerkinElmer Optima 8300 ICP-OES

RF generator	**40 MHz solid state RF generator**
Detector	Segmented-array Charge-Coupled Device (SCD)
Polychromator	High-energy (f/6.7) echelle-based Optima polychromator
Control	PC, data transfer via serial interface
Size, weight	150 x 76 x 80 cm, 181.5 kg
Voltage, frequency	200-254 V; 50-60 Hz
Manufacturer	PerkinElmer

3.4 Sample Preparation

3.4.1 Total element analysis

3.4.1.1 Reagents

- 1% Triton solution

A 1 ml Triton solution was pipetted into a 100 ml volumetric flask. This was then topped up to volume using double distilled water.

- Cleaning solution

Five hundred ml of René Borghgraef Sr. (RBS) solution was transferred into a 5000 ml volumetric flask. The solution was mixed well and made to 5000 ml mark using double distilled water.

- 10% Nitric acid

About 300 ml of double distilled water was added into a 500 ml volumetric flask. While stirring carefully 50 ml of concentrated Nitric acid was then added into the flask. The solution was mixed well and made to 500 ml volume using double distilled water.

3.4.1.2 Cleaning and preparation of sample carriers

Sample carriers were wiped with fluff free tissue paper soaked in acetone and mounted onto the washing cassette. The washing cassette was transferred into a 1000 ml glass beaker containing hot cleaning solution (RBS 50 diluted to ratio 5:50), which had enough solution to cover the sample carriers on the washing cassette. The cleaning solution with the sample carriers was heated on a hotplate at 80^0C for five minutes. They were then rinsed with double distilled water and were immersed in 10% nitric acid in a 1000 ml glass beaker filled and heated while covered with a watch glass for two hours on a hot plate at 80^0C. After the heating they were again rinsed with double distilled water, immersed in hot distilled water in another 1000 ml glass beaker and heated for five minutes on a hot plate at 80^0C. They were then thoroughly rinsed with ultra-pure water and dried in a drying oven at 80^0C for thirty minutes. This was followed by carefully wiping them with acetone soaked tissue paper after which 10 µl silicon solution was pipetted at the center of the carriers and again dried in a drying oven at 80^0C for thirty minutes.

3.4.1.3 Quality Control

The cleaned carriers were placed in a cassette, loaded on the TXRF spectrometer where each was analyzed for 120 seconds. The cleanliness pass criterion was no elemental peak apart from Si (quartz carrier blank signal), Ar (air) and Mo (tube anode) would appear with intensity higher than the Ar Kß-line. All tasks connected to cleaning of sample carrier were done under a laboratory fume hood. The preparation of samples carriers, suspension of samples and transfer of sample materials to the carriers was done in the TXRF laboratory inside a horizontal laminar flow cabinet to ensure a contamination-free working environment. Samples were prepared as a thin layer to reduce matrix effects and disposable sample containers and pipette tips were used.

3.4.1.4 Sample preparation and analysis

The samples were air-dried and crushed to pass through a 2 mm sieve (2 mm and smaller soil particles considered as agricultural soil). Using coning and quartering 10 g of the sieved soil was subsampled and oven-dried at 40 °C. It was then ground to a fine powder using a mortar and pestle followed by milling to between 20 - 53 μm using a micronizing mill (Glen Creston McCrone Micronizing mill). An empty clean vial was weighted and about 40 to 50 mg of the milled sample was added and the weight recorded to nearest 0.01 mg. This was followed by addition of 2.5 ml of 1% aqueous Triton X100 solution and 40 µl of 1000 mg/l Se internal standard solution after which using an agitator (IKA MS 3 Vortex Mixer) the sample was homogeneously mixed and sonificated in a water bath for 15 minutes. Using a calibrated pipette 10 µl of the suspension of each sample was transferred onto the center of the siliconized sample carrier, dried for about 10 minutes on a hot plate at 50 °C and loaded on sample cassettes carrier. The loaded

samples were then analyzed in a TXRF spectrometer (Bruker S2 Picofox) and quantified using the inbuilt software and later the Quantitative X-ray Analysis System (QXAS). The data acquisition time was 1000 s per sample. All the samples were analyzed in triplicates.

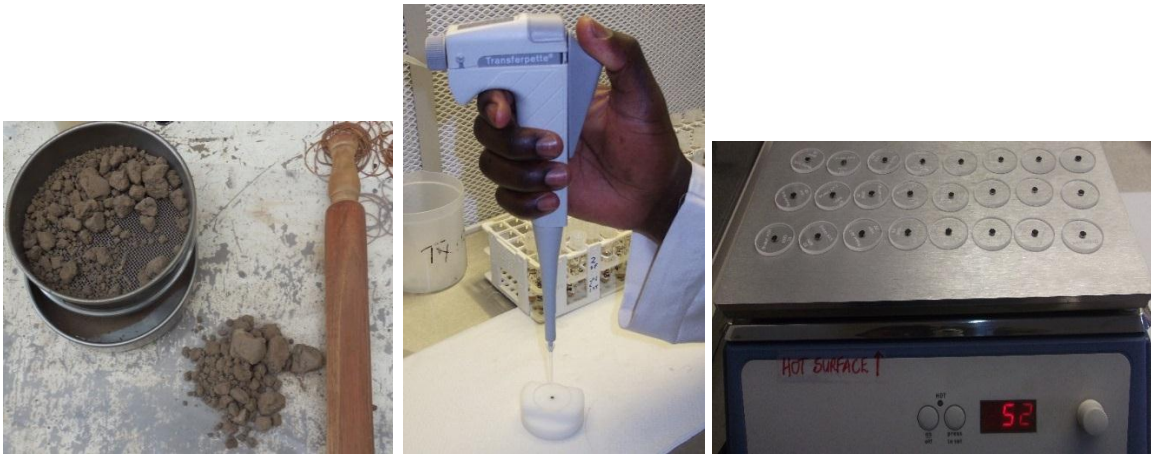


Figure 3.3: Sieving, pipetting the sample on a sample carrier and drying the sample carriers

3.4.1.5 Elemental quantification

The spectra from Picofox were de-convoluted and quantified using the inbuilt spectrometer software and later using QXAS from the International Atomic Energy Agency (IAEA). QXAS is an integrated system for quantitative evaluation of spectra measured with energy dispersive X-ray spectrometers. AXIL software package (Analysis of X-ray spectra by Iterative Least-squares fitting) is one of the main program in QXAS and it assesses the net peak areas of the characteristic lines of interest. The spectra evaluation involved three major steps; (1) Spectrum format conversion, (2) Spectrum fitting and (3) Qualitative analysis.

The first step involved conversion of the Picofox spectra from ASCII (American Standard Code for Information Interchange) format (.spx) to IAEA QXAS format (.spe). It was followed by spectrum fitting which involved fitting the measured spectra with suited mathematical functions using a non-linear least squares strategy. This was followed by X-ray library management, specifying parameters for spectrum analysis and spectrum fitting. The X-ray library management was done under AXIL at option, voigt peak profiles for high energy k-lines. The input model was loaded with energy calibration coefficients parameters and was followed by spectra analysis. After loading the spectrum energy calibration was done by selecting at least two known peaks and saving them after which other peaks in the spectra were identified. This was followed by choosing the region of interest, adding the identified peaks and fitting the spectrum. After the fit some information about the quality of the fit was investigated by observing the value of the chi-square for each element. Before saving the model, it was made sure that the individual chi squares were close to 1 and each spectrum's channel residual were between ±3 which indicated a well fit model.

The third step of spectrum quantification was quantitative analysis of the spectra. For elemental quantification the QXAS sub program option of Total Reflection X-ray Fluorescence (TXRF) and thin samples was used. The algorithm for the option is designed for TXRF spectrometry. Four steps were involved for quantification with this option: (1) defining calibration and standards, (2) performing calibration, (3) extrapolation of the calibration curve to elements not in the standard sample and (4) quantitative analysis of unknown samples. The calibration step involved selecting the option "element as internal standard" and specifying the internal standard element, the x-

ray line (K-α), ppm for concentration unit, excitation source (Tube excited XRF), source type (Mo) and the tube voltage (50 kV). The choice of internal standard element depends on the sample being analyzed and for our unknown samples, Se (40 µl of 1000 mg/l Se) was the internal standard (low Se concentration in African soils), and Cu for calibration standards (Merck and Craft with concentrations of 100 and 10 ppm respectively). Selenium was also used (40 µl of 1000 mg/l Se) as the standard for the reference material (river clay). A total of 192 (64 * 3 replicates) soil spectra were de-convoluted and quantified.

3.4.2 AAAc-EDTA Extractable Elements

3.4.2.1 Extraction Solution

Three litres of acetic was poured into a 50 litre vessel containing approximately 25 litre double distilled water and shaken. After shaking, 1.7 litre of ammonia and 372.24 g Na_2EDTA was added to the solution. The vessel was then filled up to 45 litre mark with double distilled water and agitated until all the Na_2EDTA dissolved. The pH of the solution was adjusted to 4.65 by addition of either acetic acid to lower or ammonia to increase which depended on the resulting solution's pH. The vessel was finally filled to 50 litre mark with double distilled water making the extraction solution containing 0.5 M ammonium acetate, 0.5 M acetic acid and 0.02 M Na_2EDTA as reported by Lakanen and Erviö (1971).

3.4.2.2 Cleaning extraction and storage bottles

The 500 ml extraction bottles were washed with tap water and rinsed with double distilled water. After addition of approximately 150 ml of 0.6 M HCl the bottles were

shaken for 1 hour, emptied and then rinsed with deionized water. Plastic storage bottles (250 ml) were washed using a dishwasher and rinsed with 0.6 M HCl acid and finally double distilled water.

3.4.2.3 Soil extraction

A 25-ml dosage cup was tared and filled with the soil (2mm sieved) sample slightly above the brim. Thereafter, the cup was tapped on its edges, after which the surface was leveled by removing the extruding soil with a plastic spatula. The samples were then weighed and poured into extraction bottles while keeping other extraction bottles in the rack capped. To each sample, 250 ml of the extraction solution was added using the Fortuna solution dispenser (the extraction ratio was 1:10). The plastic caps were then screwed tightly in place and the lid of the extraction rack was closed followed by shaking for one hour at 27 rpm. The suspensions were then filtered into 250-ml plastic storage bottles and analyzed with ICP-OES. The data acquisition time was 40 s per element.

Figure 3.4: Shaking the extraction solution with the sample and filtering the resulting suspension

3.4.2.4 Quality control

One blank sample was included in every extraction rack of ten bottles. The location of the blank sample was changed between extractions in order to conduct the blank check regularly in all of the bottles. The blanks were bottles with the extraction solution and no soil samples and were used to determine the cleanness of the extraction bottles. In every sample batch, one reference sample (F_1S) was included per 200 samples. Every tenth sample was also extracted in two replicates.

3.4.3 Soil pH

To a cup containing 5 g of air-dried 2 mm sieved soil sample, 5 ml of double distilled water was added, stirred and left to stand for 10 minutes. The electrodes of a calibrated pH meter were lowered into the solution immediately after shaking and the readings recorded after it stabilized. The meter was calibrated using buffer solutions of pH 7.0, pH 4.0 and pH 10.0.

3.4.4 Soil Carbon

The soil carbon content was analyzed using Leco CNS-2000; Carbon-Nitrogen-Sulphur Analyzer. Air-dried 2 mm sieved soil sample of 150 mg was scooped into a clean ceramic sample boat placed on a weighing balance. The samples were then transferred onto an auto loader in the carbon analyzer. In the analyzer carbon was converted to gas (CO_2) and then separated in a chromatographic column from other gases based on their molecular masses and finally detected by a highly sensitive thermal conductivity detector.

3.4.5 Comparison of Concentrations from QXAS and the Picofox Inbuilt Software

The agreement between the two methods used for elemental quantification was investigated using Bland-Altman plot. The Bland-Altman plot (Bland & Altman, 1986 and 1999), or difference plot, is a graphical method used to compare two measurements techniques. In this graphical method the differences or alternatively the ratios between the two techniques are plotted against the averages of the two techniques. According to Krouwer (2008), the differences can also be plotted against one of the two methods, if the method is a reference method. The mean of the methods was used as the best estimate of the true value. The ratio between QXAS and Picofox concentration (QXAS/Picofox) for each sample was plotted against their mean value ((QXAS + Picofox)/2). In this study we opted to use the ratio instead of differences between the two methods because a relation of the differences to the magnitude was observed.

Table 3.3: Definition of the terms used in a method comparison study

Term	Definition
Bias	The mean (overall) difference in the values obtained with two different methods of measurement
Confidence Limit	Range within which 95% of the differences from the bias are expected to be
Limits of Agreement	Confidence limits for the bias. Upper limit of agreement (Upper LOA) is computed as bias + 1.96SD, where SD is that of the bias. The lower limit of agreement (Lower LOA) is computed as bias – 1.96SD. Upper LOA to Lower LOA = Confidence limit
Standard Deviation (SD)	A measure of variability of the individual differences
Precision	The degree to which the same method produces the same results on the measurements (repeatability); the degree to which values cluster around the mean of the distribution of values (e.g., width of confidence interval)

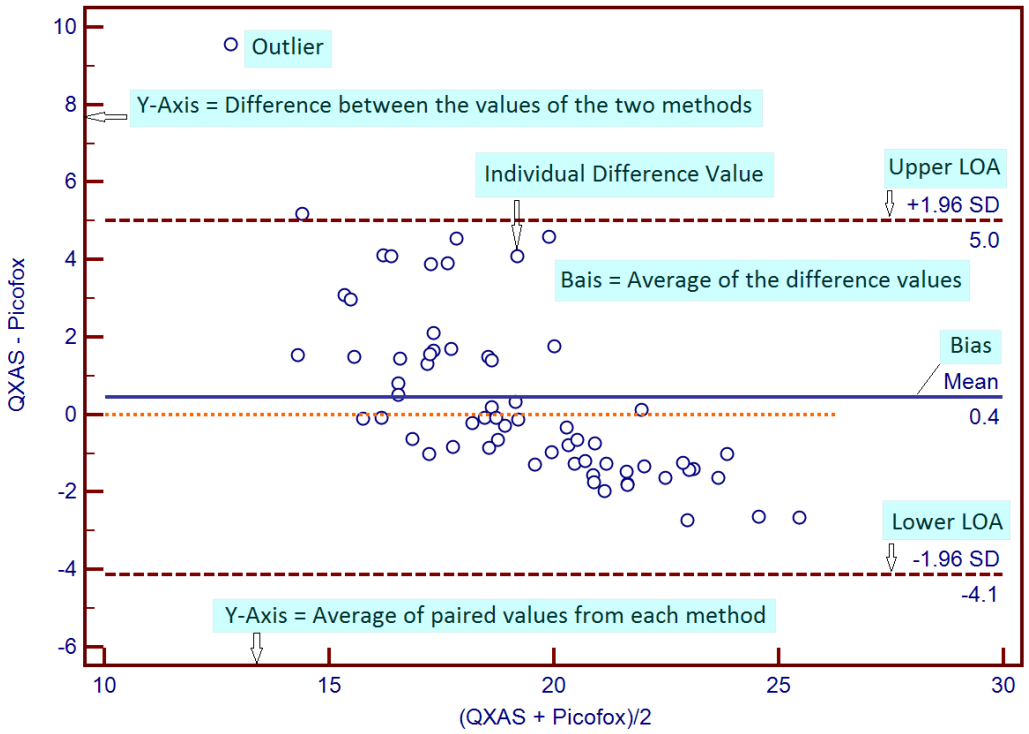


Figure 3.5: Structure of a Bland-Altman plot with explanation of elements, using a comparison of Ni concentration (ppm) obtained with the QXAS and Picofox Software from results section

The overall mean difference in values obtained with the two methods is called the bias. The plotted differences represent one method minus the other, the bias quantifies how much higher (i.e., positive bias) or lower (i.e., negative bias) values are with one method compared with the other one. The standard deviation (SD) of all the individual differences is calculated as a measure of variability (repeatability) from which the limits of agreement are determined. The 95% confidence limits of the Normal distribution are used (mean difference ± 1.96 SD). The limits of agreement represent the range of values in which agreement between methods will lie for approximately 95% of the sample.

The difference scores will be evaluated for a Normal distribution using Kolmogorov-Smirnov test for Normal distribution and histogram. The method agreement at 95% limit of agreement for each element analyzed using the QXAS and the Picofox software is described in results and discussion. With respect to using correlation for example if all the points were to lie perfectly along any straight line this would mean a perfect relation. However a perfect agreement is only observed if all the points lie along the line of equality.

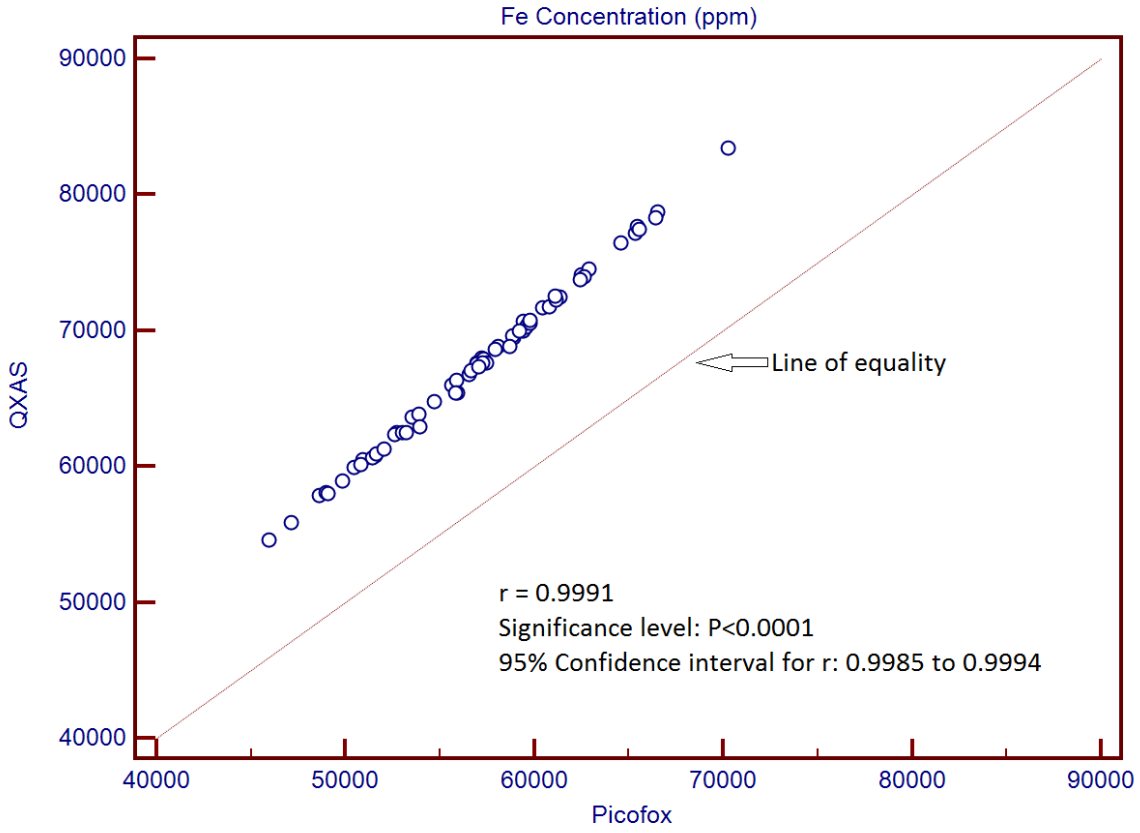


Figure 3.6: Scatter diagram, correlation coefficient (r), and 95% confidence interval (CI) of Fe concentrations (ppm) measured with the QXAS and Picofox software

In figure 3.6, example from the results of Fe concentration; the Pearson Product-moment correlation for the individual data points was $r = 0.9991$, with a significance level of $p < 0.0001$, and 95% confidence interval (CI) for r of 0.9985 to 0.9994. The correlation analysis results tells us that: (1) the concentration value obtained with the Picofox method is strongly associated with the concentration value obtained with the QXAS method; (2) the probability that this association was due to chance is less than 1 in 10,000; and (3) when these methods are used in another sample like this one and in similar conditions, we can be confident that the r will be between 0.9985 and 0.9994. However, the strong correlation between the Picofox and QXAS does not tell us about agreement between the methods. Indeed, the scatter diagram shows disagreement. If the methods resulted in perfect agreement, all the paired data points would fall on the diagonal line of equality. Also the test of significance would have been irrelevant to the question of agreement since the two software were meant to determine the same thing.

3.4.6 Statistical Analysis

All calculations, statistical analysis and plots were done using the R statistical software package version 3.0.3 (R Development Dore Team, 2014), the MedCalc software program (MedCalc®, Mariakerke, Belgium, http://www.medcalc.be/) and Excel 2013.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Soil Nutrient Status

The sampling area was 12.7 hectares and samples were collected from 32 sampling locations. Data from such a sampling density could be used for 10 to 20 years for soil organic matter, 5 to 10 years for pH and 4 to 5 years for trace elements (Richard and Gary, 2009). Out of eight essential trace elements five: Fe, Mn, Zn, Cu and Ni were investigated. Mo and B were not analyzed using the TXRF spectrometer because Mo was used as the spectrometer anode. Cl and B were also not analyzed because the AAAc-EDTA extraction reagent is not formulated for their extraction.

The minimum, maximum and mean values of the five micronutrient concentrations, carbon (%) and pH of top soil samples are presented in Table 4.1. The results show that all the samples from sites were acidic in nature (maximum pH < 6.00) with carbon ranging from 1.50 to 3.65% with a mean of 2.87%.

Table 4.1: Basic statistics of the five trace elements, pH and Carbon in topsoil

Element (mg/kg)	*Minimum*	*Mean*	*Maximum*	*Standard Deviation*
Total Fe	47091.5	56130.7	65534.5	4940.8
Total Mn	2779.1	4046.3	5232.7	544.5
Total Zn	126.7	187.1	229.0	22.0
Total Ni	11.8	20.2	26.8	3.2
Total Cu	5.3	10.3	22.9	2.9
Extracted Fe	354.1	468.2	598.3	62.1
Extracted Mn	1668.4	2573.9	3269.9	348.1
Extracted Zn	14.1	42.8	63.2	11.2
Extracted Ni	3.7	8.5	11.4	1.8
Extracted Cu	1.0	2.5	4.4	0.7
pH	5.0	5.4	5.9	0.2
% Carbon	1.5	2.9	3.7	0.4

The total concentrations of three (Mn, Ni and Zn) of the five elements investigated were within reported values for soils on a world-wide basis (Figure 4.1, Kabata-Pendias and Pendias, 1992, 2011). The average total iron content of the site was high (5.6 %) compared to concentrations established for soils on a world-wide basis of 3.8 % (Kabata-Pendias and Pendias, 1992). The high Fe content is a typical characteristic of Nitisols found in the central highlands of Kenya. The Mn content of the site ranged from 2779.1 to 5233.7 mg/kg of soil which falls within reported values for soil of 7 to 9200 mg/kg (Kabata-Pendias and Pendias, 1992). The overall Zn content of soils ranges from 10 to 300 mg/kg (Haluschak et al., 1998) while background levels of uncontaminated soils are 17 to 125 mg/kg on a world-wide basis (Kabata-Pendias and Pendias, 1992). The zinc content from the site falls within the former value with concentrations of 127-229 mg/kg. The Cu content of the soil from the site ranged from 5 to 23 mg/kg with 94% of the samples analyzed below reported values for soils of between 14 and 109 mg/kg (Kabata-Pendias and Pendias, 2011). The range of Ni content from the studied site was between 12 to 27 mg/kg with a weighted mean of 10 mg/kg which falls between the world-wide reported amounts of 1 to 450 mg/kg (Haluschak et al., 1998).

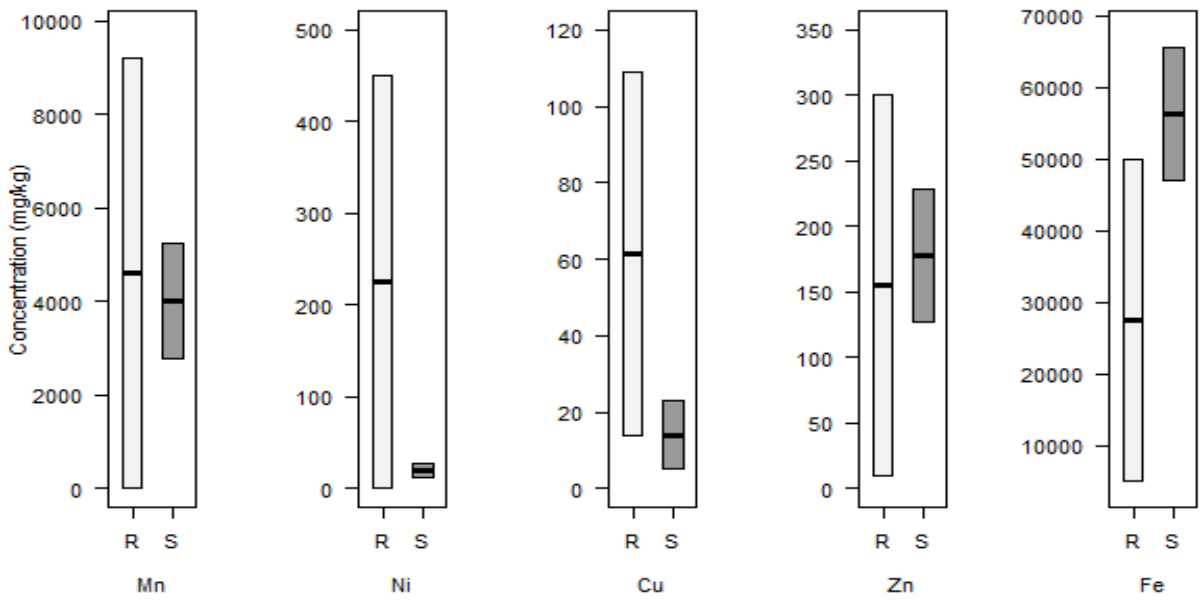


Figure 4.1: The total elemental concentration from the site (S) compared to world-wide reported values (R).

Four of the elements; Mn, Ni, Cu and Zn depicted a higher concentration in the top soil than in the sub soil, only Fe had a lower concentration in the top soil (Figure 4.2). The lower Fe concentration in topsoil could be as a result of higher weathering in top soil than in sub soil. Higher weathering of soils in top horizons involves release as well as removal of Fe from the soils (Sharma et al., 2000). The higher concentrations of Mn and Zn in top soil could be due to fixation by soil organic matter (Kabata-Pendias and Pendias, 2011). This is also supported by the fact that both Mn and Zn showed the two strongest correlations with soil carbon content compared to the other elements investigated (Table 4.3).

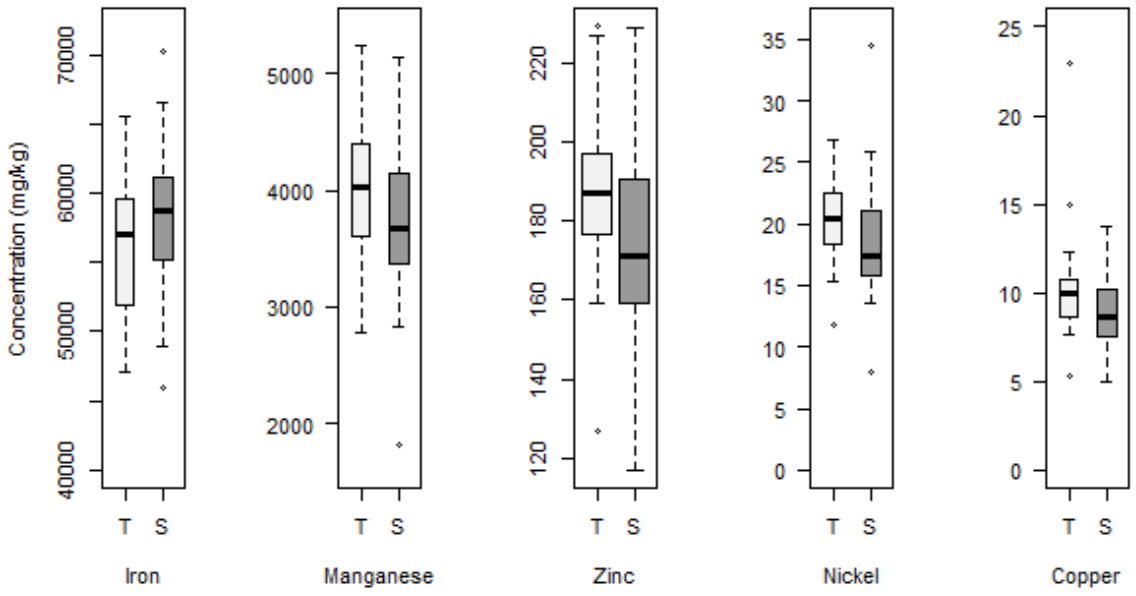


Figure 4.2: Variation of total elemental concentration with depth, T for top soil (0-20 cm) and S for sub soil (20-50 cm).

Manganese was the most soluble (64%) of the five elements investigated, it was followed by Ni at 42%, Cu 24%, Zn 23% and Fe <1% (Figure 4.3). The soluble amount of Fe of 0.83 % is higher than reported values of 0.01 to 0.1% according to Kabata-Pendias and Pendias (2011) and could be due to the high Fe total content in the soil compared to soils from other regions of the world. The AAAc-EDTA soluble Fe content ranged between 339 and 626 mg/L with a mean of 478 mg/L. The deficiency limit of AAAc-EDTA extracted Fe according to a global study by Sillanpää (1982) is <35 mg/L. From the results it is clear that AAAc-EDTA extracted Fe in Muguga is greater than the deficiency range.

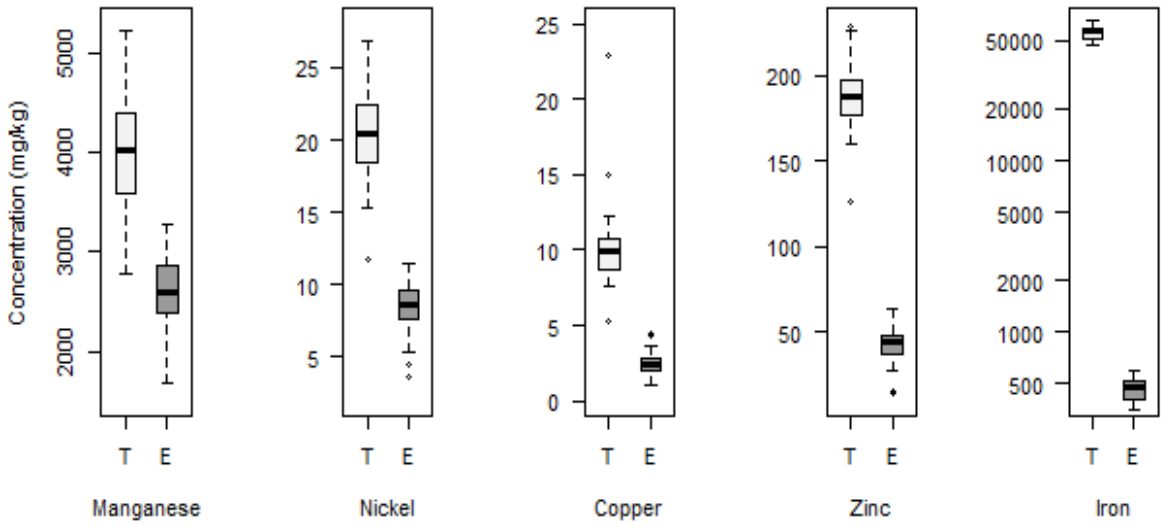


Figure 4.3: Concentration of total (T) and extracted (E) elements, starting with the most soluble to the least.

Table 4.2: Critical levels of Fe, Cu, Zn and Mn (Sillanpää, 1982) compared with the results from Muguga, (mg/L)

Trace Element	**Range of deficiency**	**Results** (Muguga)	**Range of excess**
Fe	<35.0	339-626	Not available
Cu	<1.0	1.0-4.4	>17.0
Zn	<1.5	13.5-63.3	>20.0
Mn (DTPA)	<5.0	1597-3232	>140.0

The extractable Fe of 339 to 626 mg/L was also very high compared to a study by Sillanpää (1982) of soil samples from different parts of Africa which had soluble Fe content ranging from 72 to 199 mg/L. The mean AAAc-EDTA extracted Mn concentration in our study area was 2621 mg/L which is above the Mn excess range of >140 mg/L after extraction with DTPA (Sillanpää, 1982). We compared our value to DTPA due to lack of critical values of Mn extracted by AAAc-EDTA. The mean AAAc-

EDTA extracted Zn of 44 mg/L in the study area was also higher than >20 mg/L excess range as reported by Sillanpää (1982). The AAAc-EDTA soluble Zn of 14 to 63 mg/L was also high compared to values from other parts of Africa which ranged from 1 to 5 mg/L (Sillanpää, 1982). The mean AAAc-EDTA Cu content in the study area ranged from 1 to 4 mg/L with a mean of 2.5 mg/L. These concentrations were above the deficiency range of <1.0 mg/L and below the excess range of >17 mg/L AAAc-EDTA soil Cu content (Sillanpää, 1982). Cu was the only element in the sufficiency range and the extracted amount (1 to 4 mg/L) was also within reported values of extractable Cu concentration in African soils of 1 to 4 mg/L (Sillanpää, 1982). Ni is a relatively new essential trace element and critical values for extractable Ni have not been established (Guodong et al., 2011) and were not discussed. Overall Cu was sufficient; Mn and Zn were in excess and Fe was also way above the deficiency range. The high solubility of the micronutrients could be due to the low soil pH which results into higher solubility/availability of micronutrients.

The AAAc-EDTA extraction procedure required more chemical reagents when determining extractable nutrients compared to the total nutrient concentration determination. To analyze a single soil sample 250 ml of extraction solution was used for the extractable elemental concentration while when determining total elemental content only 2.5 ml of triton solution was used. The difference in the amount of sample used also was huge; for total elemental concentration about 45 mg was required while for the extraction procedure about 25 g of soil sample was used. Adoption of TXRF technique in place of wet chemistry methods could save the environment and help utilize our limited resources efficiently by cutting down on the amount of chemical reagents required.

4.2 Correlation Studies

Simple linear correlation studies of AAAc-EDTA extractable Fe, Cu, Zn, Ni and Mn, pH and soil carbon are shown in Table 6. All the investigated micronutrients were influenced by the soil environment.

Table 4.3: Pearson's Correlation coefficients for Top soil total elemental concentration, Soil pH, Soil carbon, verses AAAc-EDTA extracted amount and Sub soil total elemental concentration

		Topsoil total elemental concentration					Other soil parameters	
		Fe	Mn	Zn	Ni	Cu	Soil pH	Soil Carbon
AAAc-EDTA extracted	Fe	-0.327					**0.419**	0.087
	Mn		**0.593**				**0.585**	**0.744**
	Zn			**0.801**			**0.395**	**0.755**
	Ni				**0.900**		0.146	**0.655**
	Cu					**0.574**	**0.584**	**0.359**
Subsoil		**0.866**	**0.785**	**0.738**	0.317	**0.504**		
In Bold, significant values at the level of significance $\alpha = 0.05$ (two-tailed test)								

Total top soil – sub soil Fe concentration (Figure 4.4) showed the strongest relation (r=0.866) compared to the other four elements. The relation of Mn in top soil and sub soil (Figure 4.5) was the second strongest with r value of 0.785. The relation of total Zn concentration in top and sub soil (Figure 4.6) was also strong with r value of 0.738. The concentration of copper in top and sub soil (Figure 4.7) showed a moderate relation (r = 0.504) while Ni (Figure 4.8) had the poorest correlation between total concentration in the top and sub soil of 0.317. The strong relation as in the case Fe, Mn and Zn could have been as a result of their high concentrations compared to that of Ni and Cu. High concentration could mean less variation in their content with depth since there will be less effects from interferences from human activities while the low amounts of Cu and Ni could be easily affected.

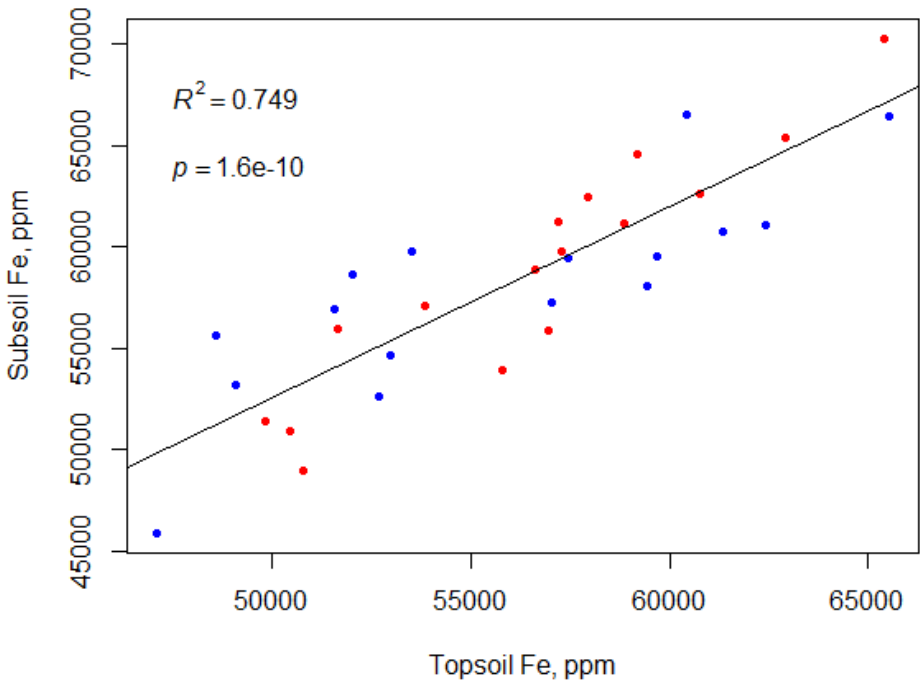


Figure 4.4: Total sub soil against total top soil Fe concentration

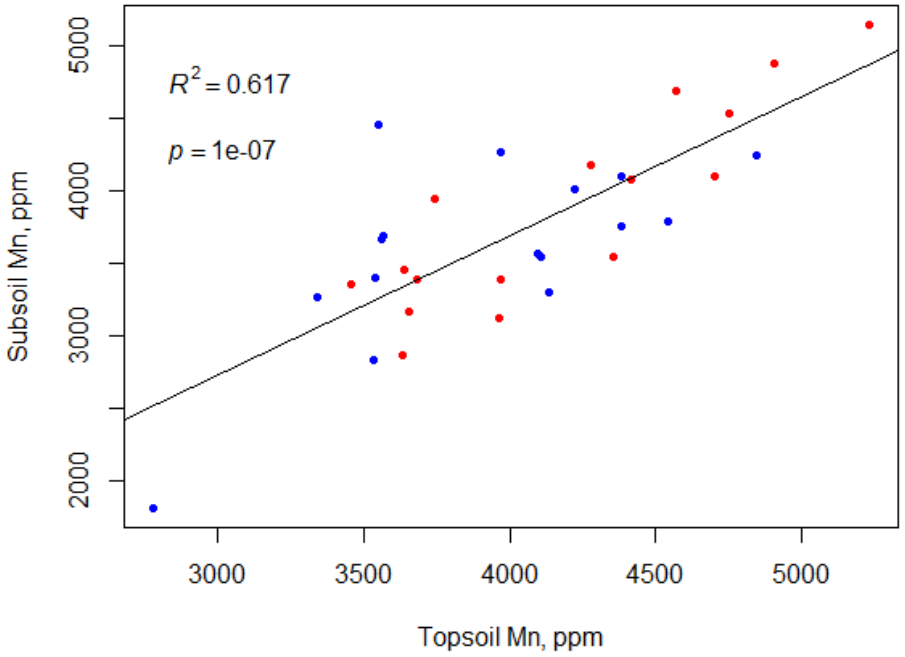


Figure 4.5: Total sub soil against total top soil Mn concentration

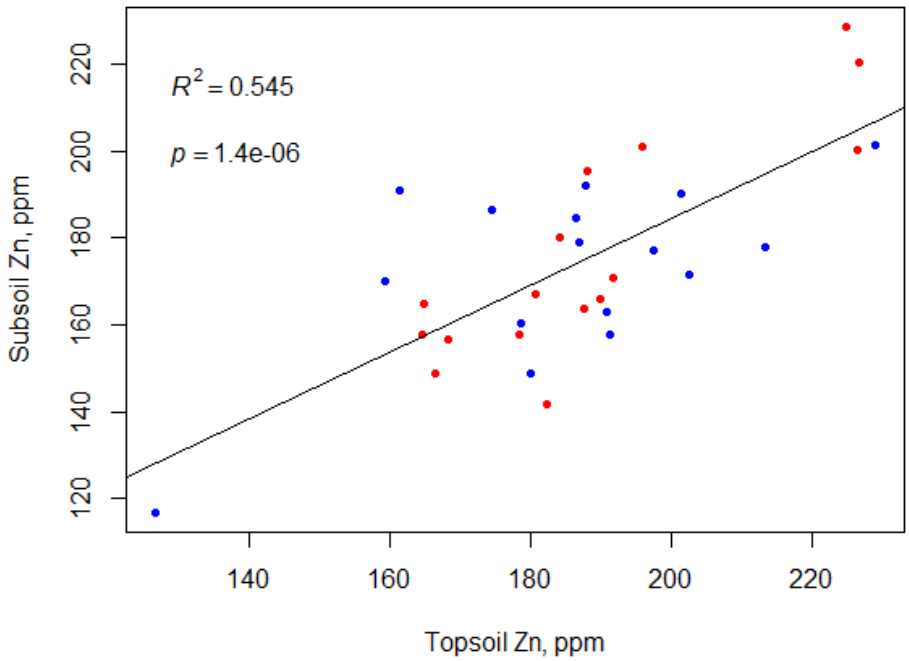


Figure 4.6: Total sub soil against total top soil Zn concentration

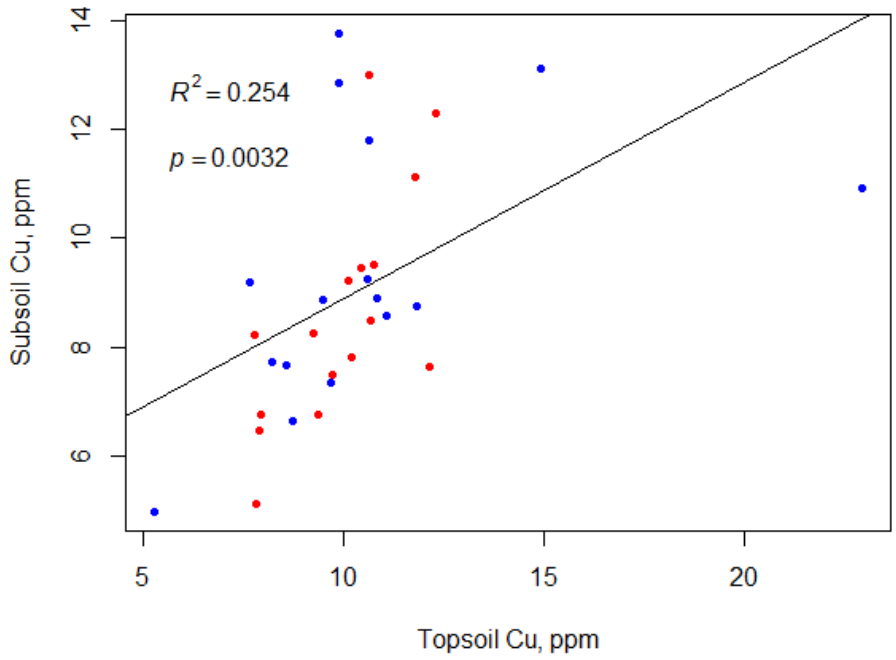
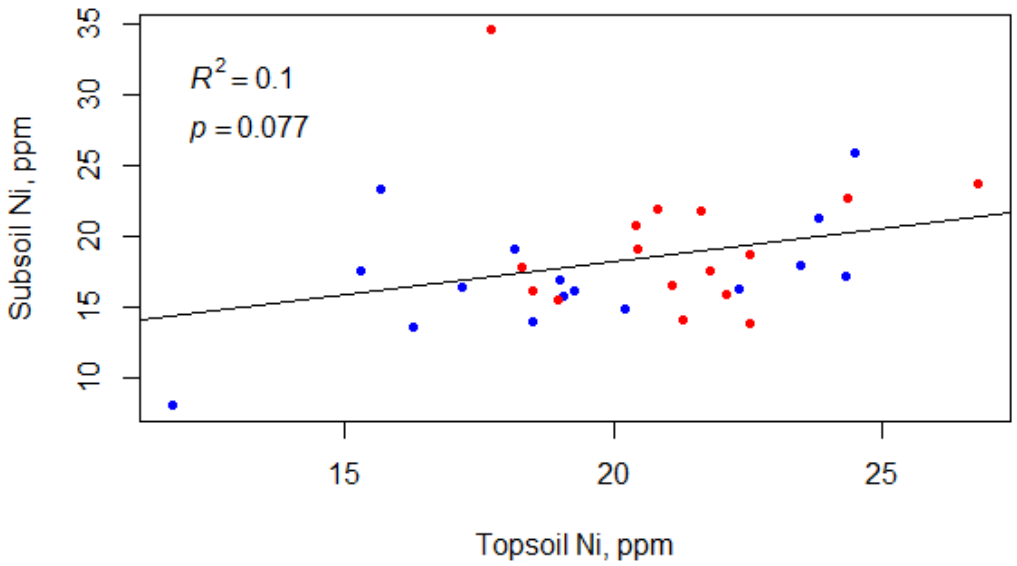
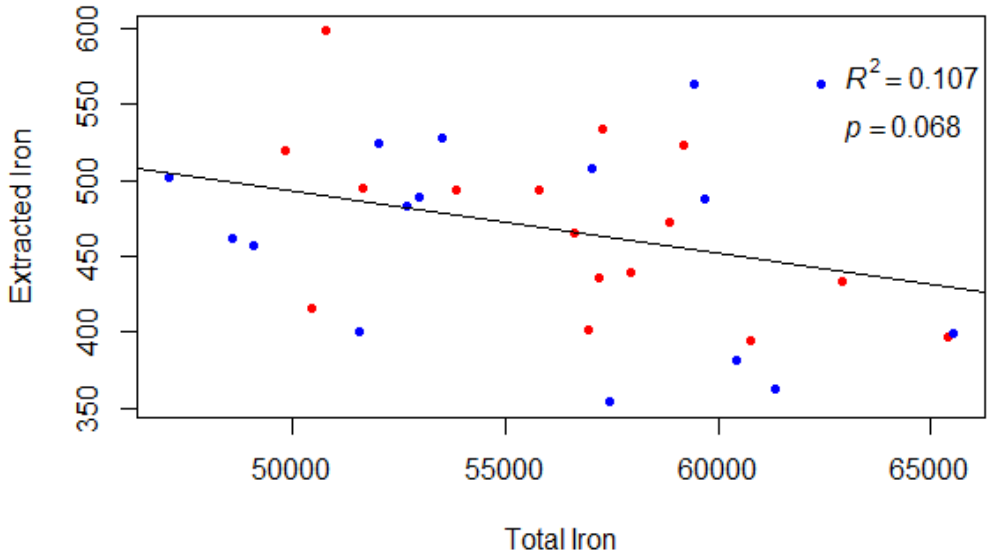


Figure 4.7: Total sub soil against total top soil Cu concentration



Figures 4.8: Total sub soil Ni against total top soil Ni concentration



Figures 4.9: AAAc-EDTA extracted iron against total Fe concentration

All the elements studied showed an increase in their extractable amounts with their total concentration in soil except for Fe which decreased with increase in its total concentration. The increase in extractable amounts of Mn, Ni, Cu and Zn with the

increase in total content in soil may be due to dependence of their availability on quantity present in the soil. The total and AAAc-EDTA soluble Fe concentration showed (Figure 4.9) the weakest correlation (r = -0.327) compared to the other four elements. The relation of AAAc-EDTA extracted Fe with carbon concentration and pH was also investigated. There was no relation between the extracted iron and carbon (Figure 4.11) concentration (r = 0.087) but a weak positive relation was observed (Figure 4.10) between the soluble iron and pH (r = 0.419). The positive correlation between the extracted Fe and pH is in agreement with the results of Sillanpää (1982) of (r = 0.300) who investigated 3538 soils samples from different regions of the world. However the low (r = 0.087) soluble Fe carbon correlation was not in agreement with other findings which showed significant relations. For example Sillanpää (1982) reported r value of 0.547 while Jetro et al., (2013) reported r value of 0.178.

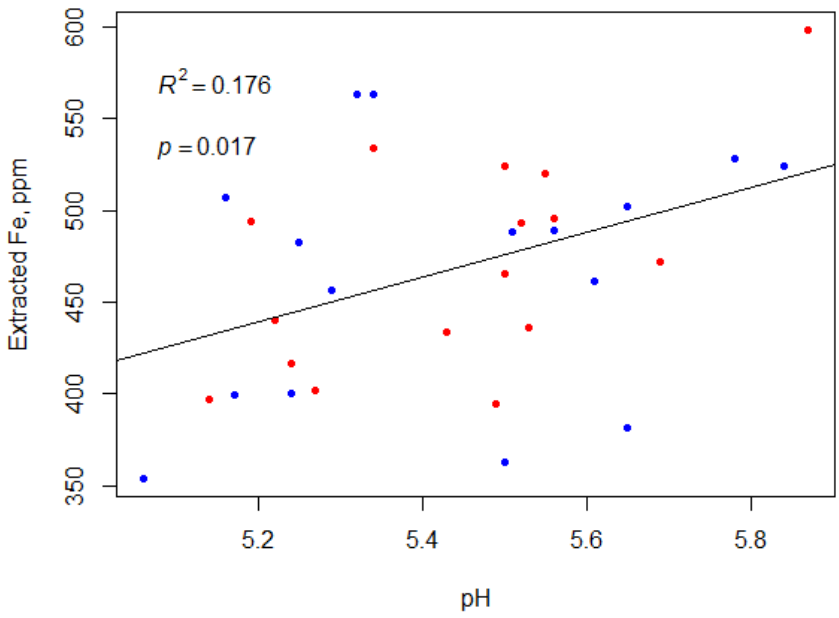


Figure 4.10: Top soil AAAc-EDTA extracted Fe against top soil pH

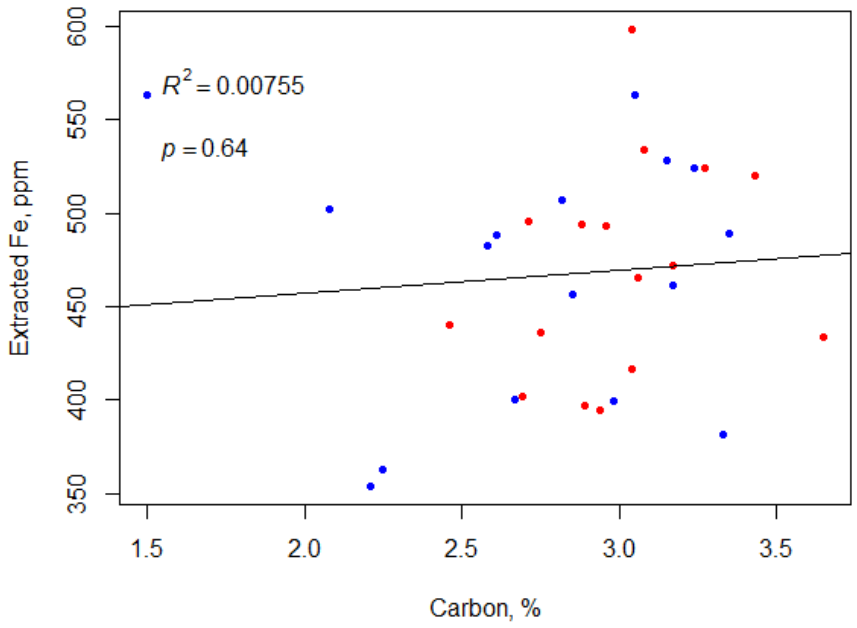


Figure 4.11: Top soil AAAc-EDTA extracted Fe against top soil carbon

The ratio of AAAc-EDTA extracted Mn to total Mn concentration showed (Figure 4.12) a moderate correlation (r = 0.593). It also showed a moderate correlation (Figure 4.13) with pH (r = 0.585) and a strong one with carbon (Figure 4.14, r = 0.744). The relation of extractable Zn to total Zn was also quite strong (Figure 4.15) with r value of 0.801. Extracted zinc also showed a strong relation to soil carbon (Figure 4.17) with r value of 0.755 and a weak one to soil pH (Figure 4.16) with r value of 0.395. A higher relation of extractable Zn to soil carbon than to soil pH has also been reported, high carbon content in soil results in a higher production of complexing agents which promote better extractability of Zn (Behera et al., 2011).

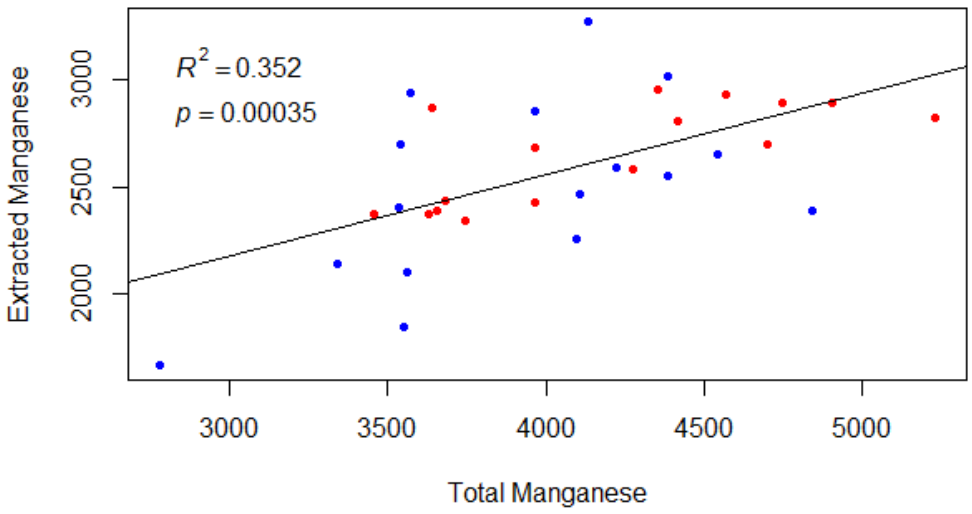


Figure 4.12: Top soil AAAc-EDTA extracted Mn against total Mn concentration

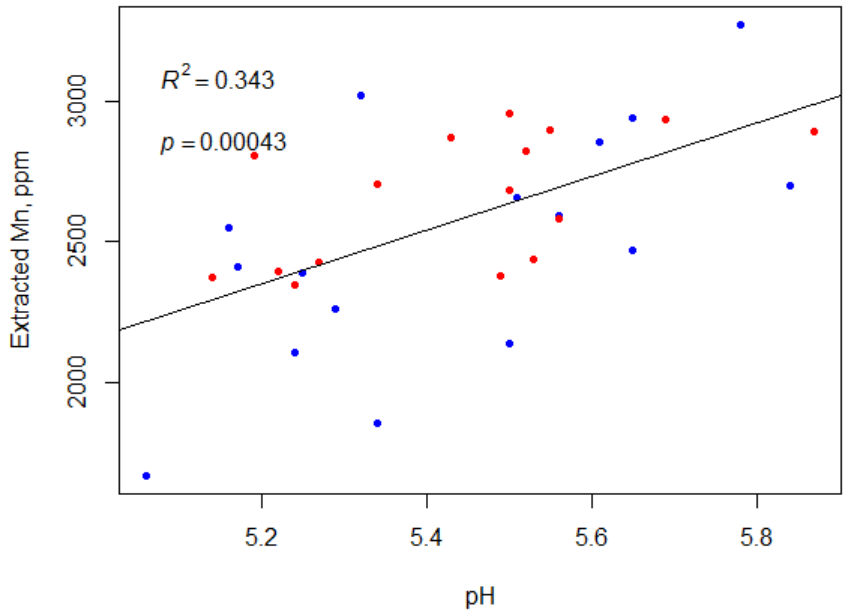


Figure 4.13: Top soil AAAc-EDTA extracted Mn against top soil pH

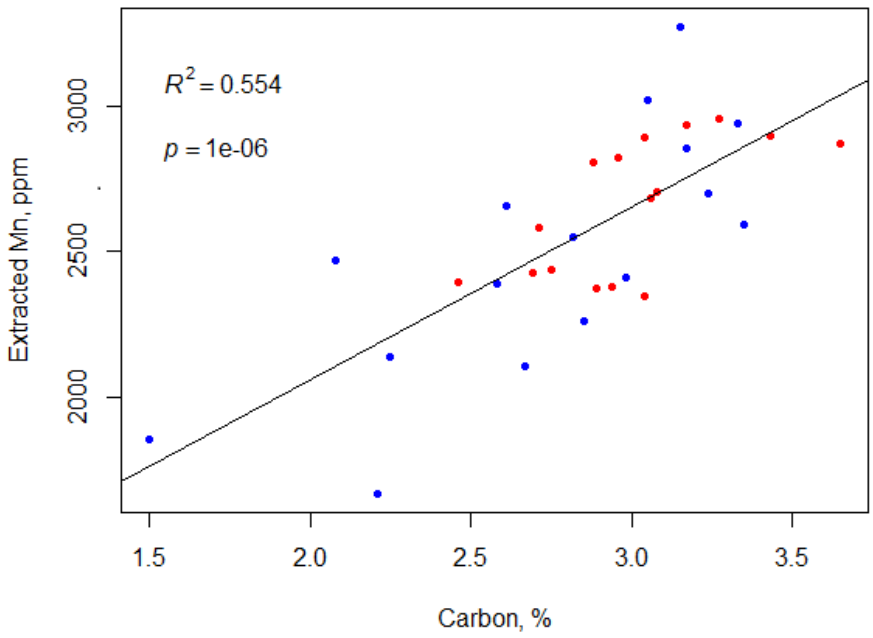


Figure 4.14: Top soil AAAc-EDTA extracted Mn against top soil carbon

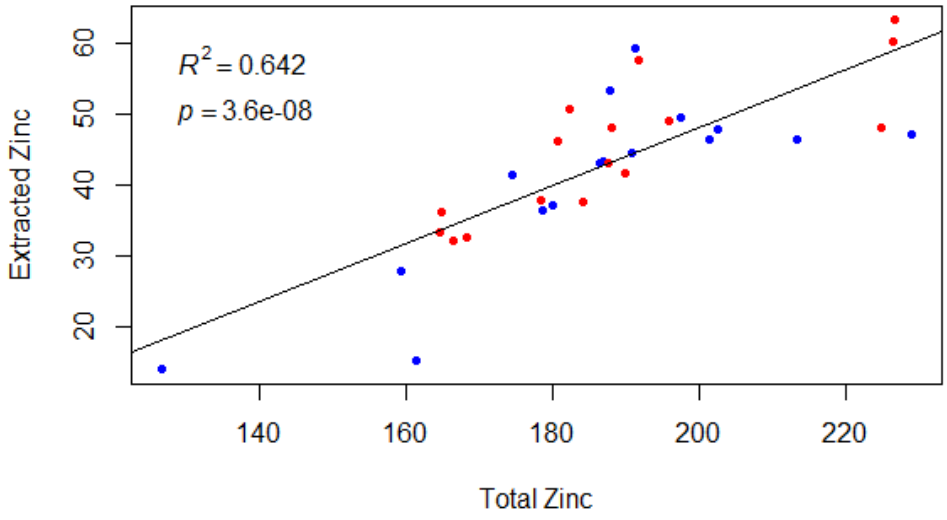


Figure 4.15: Top soil AAAc-EDTA Zn against top soil total Zn concentration

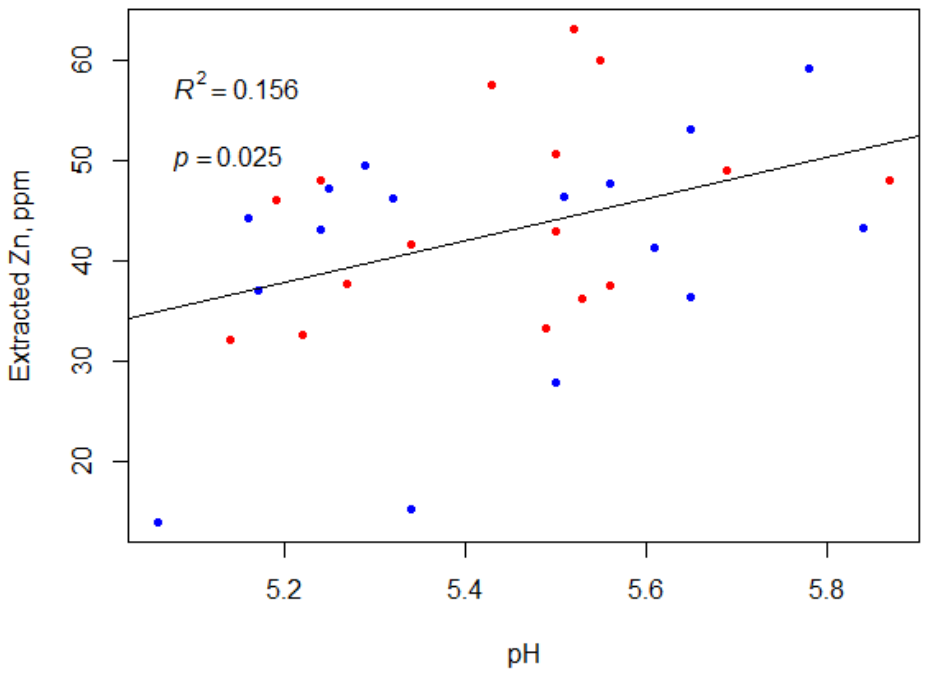


Figure 4.16: Top soil AAAc-EDTA extracted Zn against top soil pH

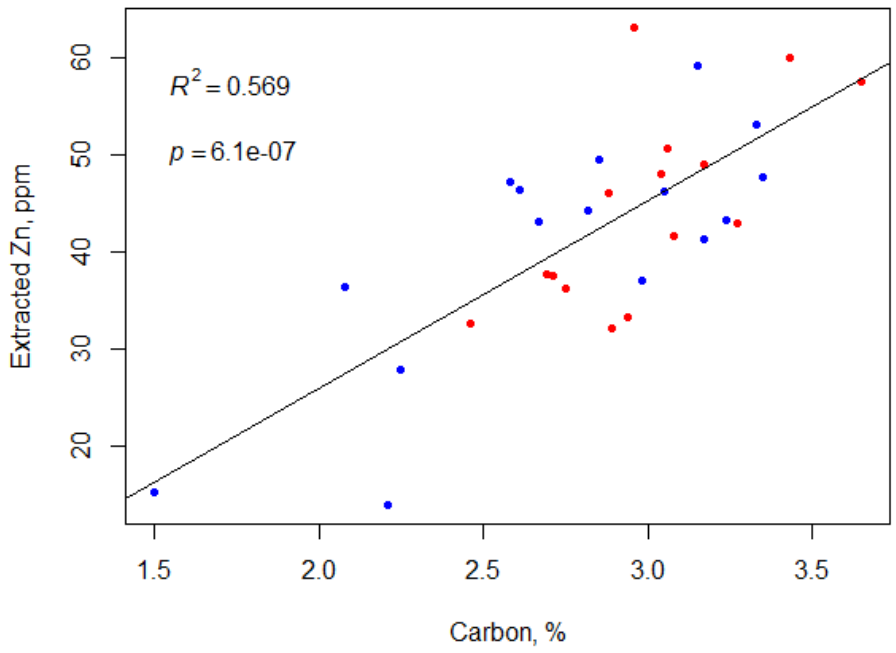


Figure 4.17: Top soil AAAc-EDTA extracted Zn against top soil carbon

Ni showed the strongest relation between the extractable and total concentration (Figure 4.18) with r value of 0.900. The relation of the extracted amount to soil carbon (Figure 4.20) was moderate (r = 0.655) while that of extracted amount to pH (Figure 4.19) was the weakest compared to those of other elements with r value of 0.146. AAAc-EDTA extractable Cu also depicted a moderate correlation to total Cu (Figure 4.21, r = 0.574). The relation of AAAc-EDTA extracted Cu to soil carbon (Figure 4.23) was weak (r = 0.359) while its relation to pH (4.22) was moderate with r value of 0.584. The increase in extractable Cu with increasing soil carbon can be attributed to the formation of highly stable Cu-humate complexes (mobilization), which are dissolved to a large degree in soils with higher organic matter level (Jetro et al., 2013).

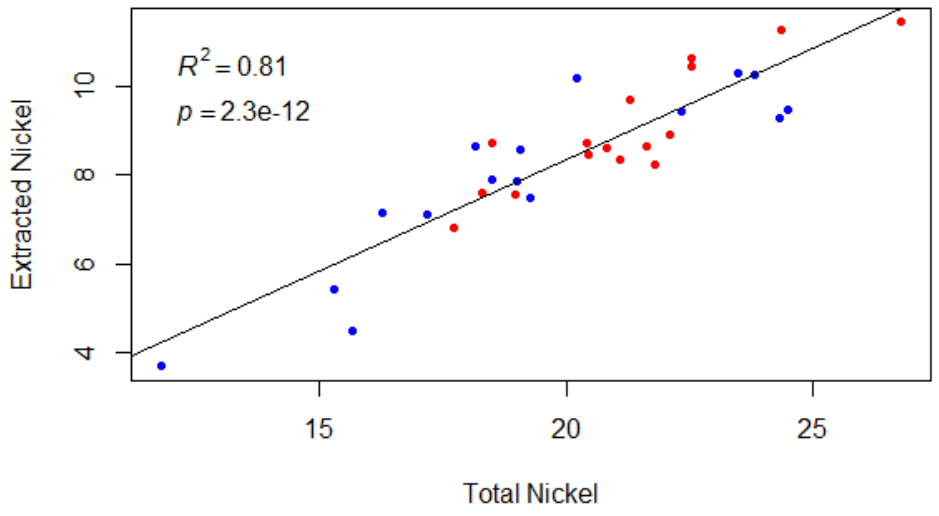


Figure 4.18: Tops soil AAAc-EDTA extracted Ni against top soil total Ni concentration

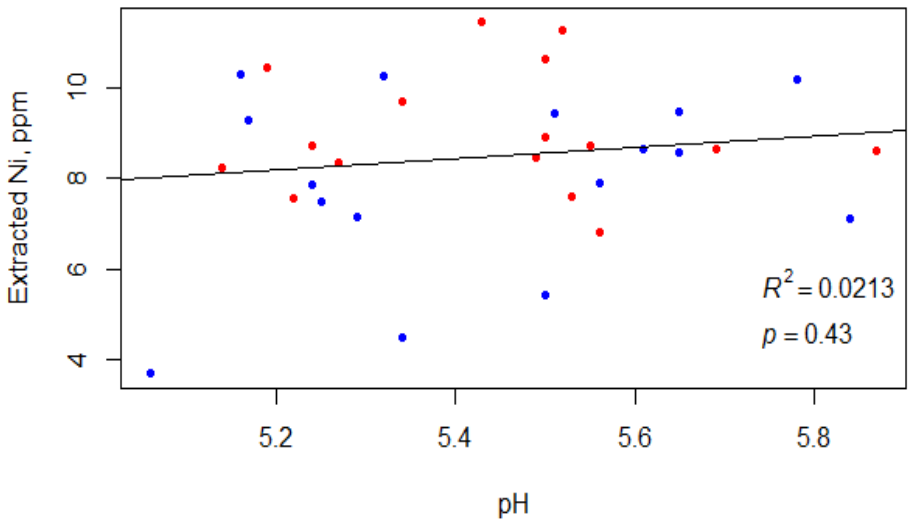
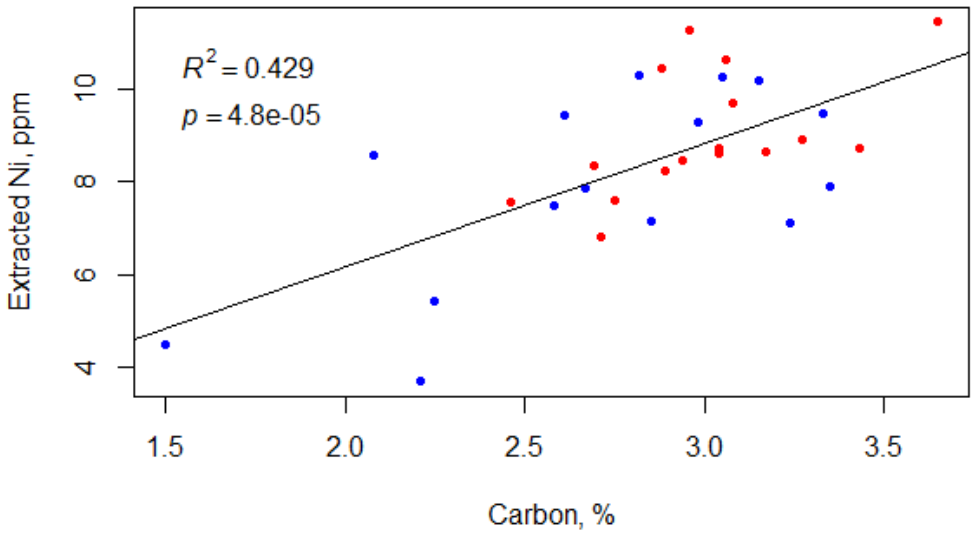


Figure 4.19: Top soil AAAc-EDTA extracted Ni against top soil pH



Figures 4.20: Top soil AAAc-EDTA extracted Ni against top soil carbon

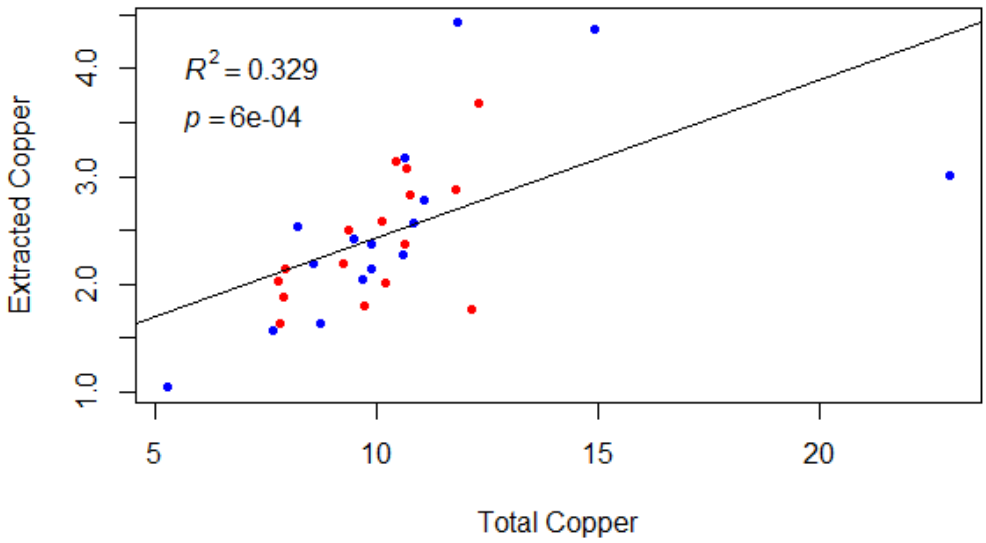


Figure 4.21: Top soil AAAc-EDTA extracted Cu against top soil total Cu concentration

The positive correlation between soil cations and soil carbon shows that the micronutrients become more available with an increase in organic matter content. This might be ascribed to the greater availability of chelating agents generated from organic matter. The organic chelating agents extract micronutrient cations from pools and make them more bioavailable. For instance organic matter improves Fe availability by combining with Fe, thereby reducing chemical fixation or precipitation of Fe as ferric hydroxide (Jetro et al., 2013). Extractable Fe, Mn, Zn and Cu showed significant positive correlations (Table 4.3) to soil pH which is in agreement with earlier findings (Jetro et al., 2013). Though their availability in general slowly decreases with increasing pH, no decrease was observed in this study probably because the pH range of the soils were within the acid region. Four (Mn, Zn, Cu and Ni) of the five elements investigated showed significant moderate to strong relation between the total concentration and their extracted amounts. This could mean that with data that cuts across different soil types and extraction methods, extractable trace elements could be estimated from their total soil

concentration. Building on spectra methods like TXRF can serve as a stepping stone towards forgoing conventional wet chemistry methods like AAAc-EDTA.

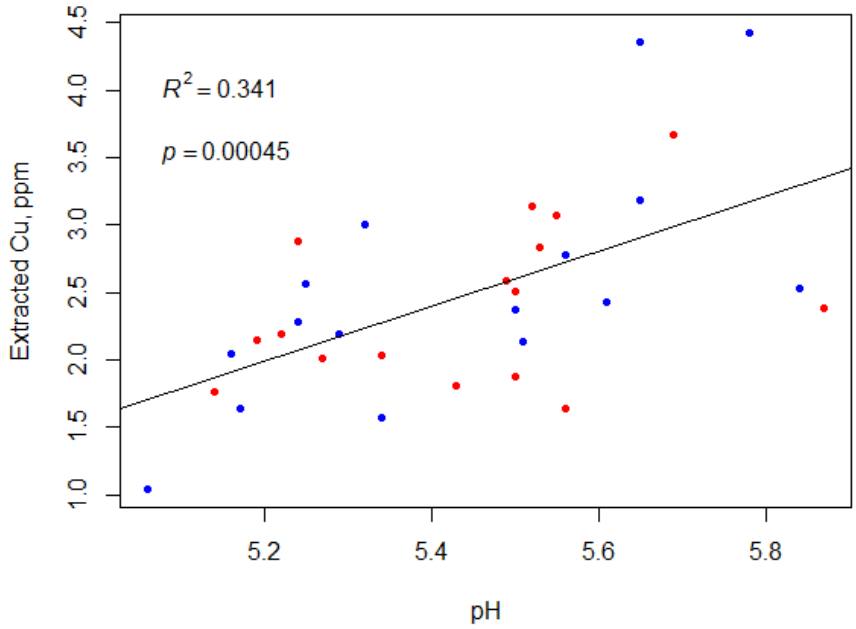


Figure 4.22: Top soil AAAc-EDTA extracted Cu against top soil pH

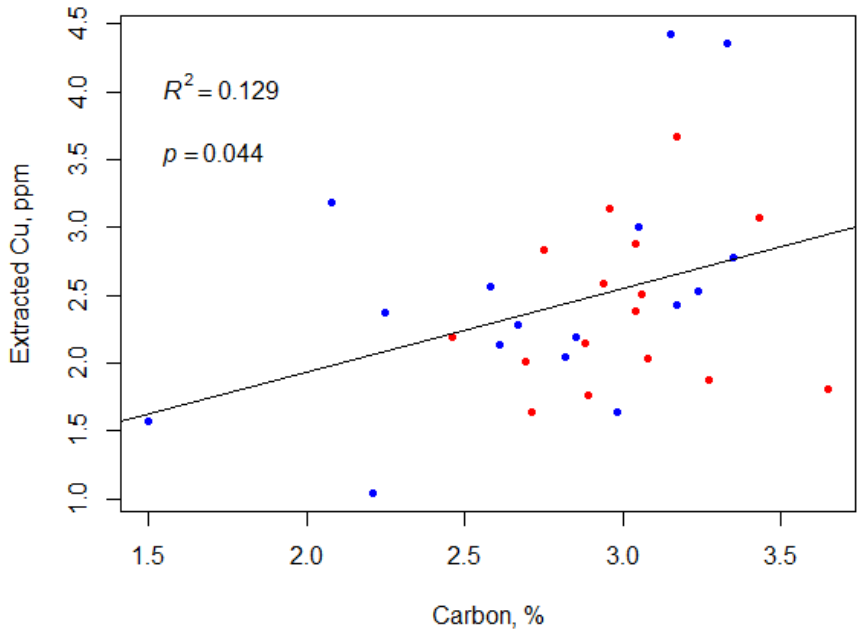


Figure 4.23: Top soil AAAc-EDTA extracted Cu against top soil carbon

4.3 Comparison of Concentrations from QXAS and the Picofox Inbuilt Software

4.3.1 Kolmogorov-Smirnov test for normal distribution

The difference scores (between QXAS and Picofox software) for every element were evaluated for a normal distribution using Kolmogorov-Smirnov test for normal distribution and histograms. The Kolmogorov-Smirnov test evaluates the extent of discrepancy between the sample distribution and the normal distribution (i.e., bell-shaped curve). A p value ≥ 0.05 indicates no significant difference between the two distributions and that the sample distribution is approximately normal; thus, the sample data can be described by mean ± SD and subjected to parametric statistical tests. A p value < 0.05 indicates a significant difference between the two distributions (i.e., the difference scores are not normally distributed), and the data would not have been subjected to parametric testing. The p values of Kolmogorov-Smirnov test for normal distribution of the differences in the elemental concentration as given by the two methods is shown in table 4.4.

Table 4.4: The p values of Kolmogorov-Smirnov test for normal distribution of the differences in the elemental concentration as analyzed by the two methods

Differences (QXAS-Picofox)	**P value**
Fe	0.7812
Mn	0.9848
Zn	0.7256
Ni	0.0543
Cu	0.6392

The Kolmogorov-Smirnov test for the differences between QXAS and Picofox software had *p* values > 0.05 for all the elements which indicates no significant difference between the two distributions. The sample distribution is approximately normal and hence the sample data was described by mean ± SD and subjected to parametric statistical tests.

A histogram was constructed as shown in Figure 4.24 for Zn. The x-axis shows the difference scores between the QXAS and Picofox methods in increments of 5 mg/kg. Discrepancy between the sample difference scores and the normal distribution is seen at all intervals of difference scores.

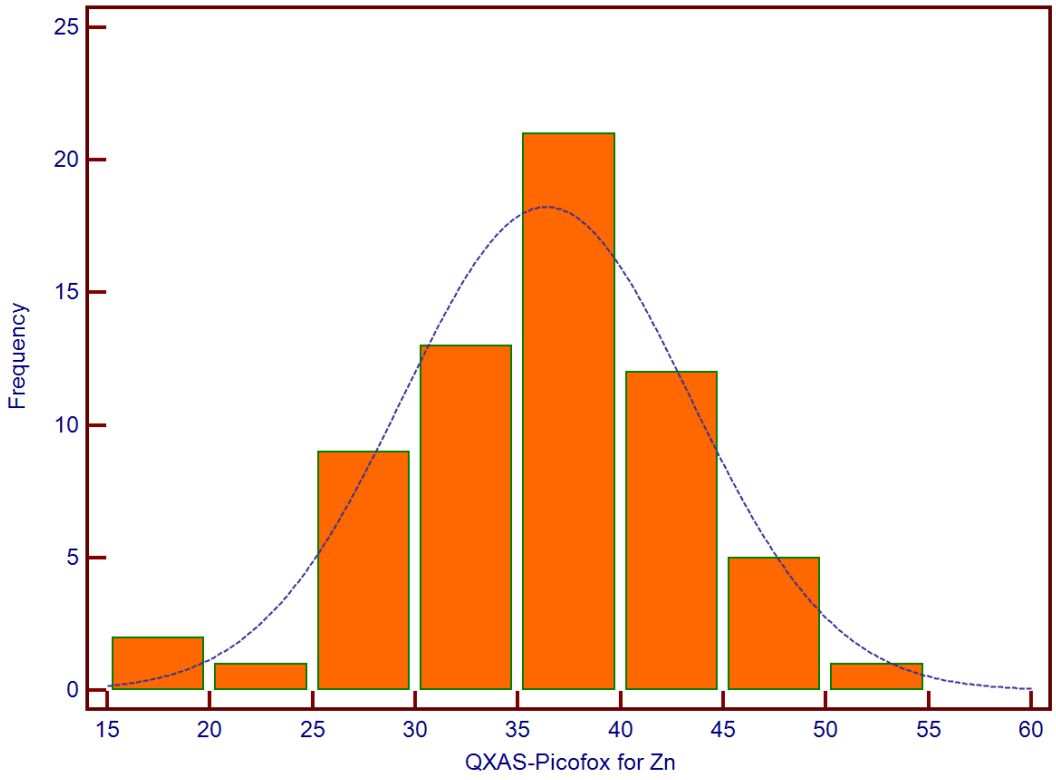


Figure 4.24: Histogram of differences in concentration (ppm) measured with QXAS and Picofox inbuilt software for Zn spectra

The data set produced more scores at the intervals of 17.5, 27.5, and 37.5; fewer scores at the intervals of 22.5, 32.5, 42.5,47.5 and 52.5; no difference scores at interval of 57.5 mg/kg than would occur if the difference scores were perfectly normally distributed. Nonetheless, the data set difference scores approximately follow the superimposed normal distribution, and, together with rejection of the hypothesis that there is a significant difference between the two distributions by the Kolmogorov-Smirnov test ($p = 0.726$), there is evidence of normal distribution. Histograms for the rest of the elements are reported in the appendices section.

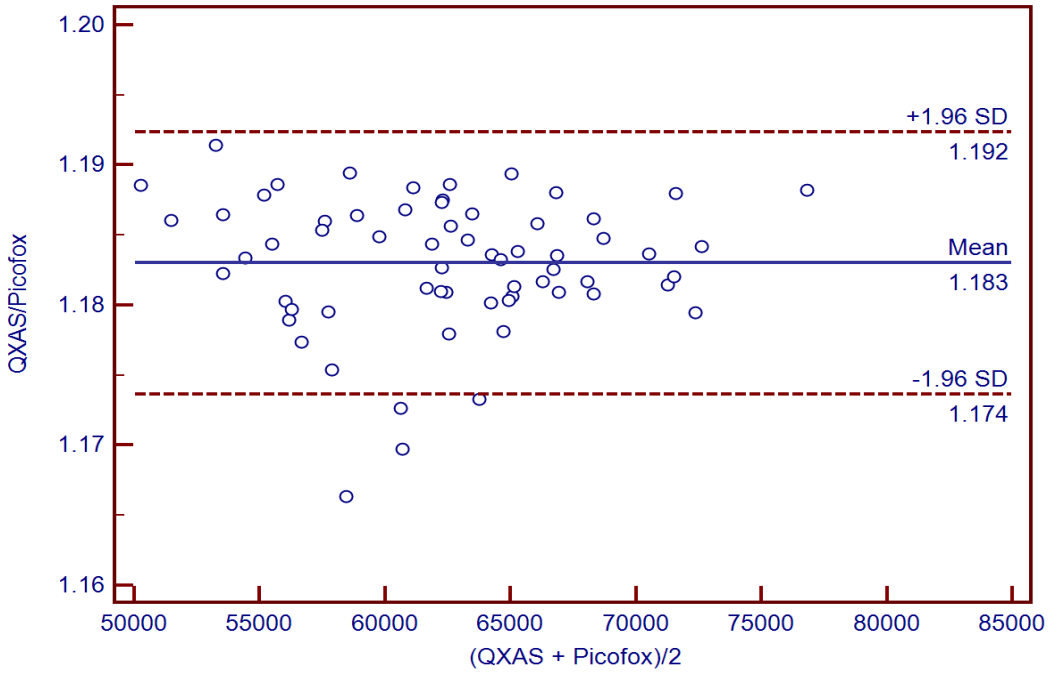
4.3.2 Method Comparison

Spectra of reference soil sample (river clay) from the International Atomic Energy Agency (IAEA) were analyzed using both Picofox inbuilt and QXAS software and compared to the certified values (Table 4.5). It was found that the Picofox software underestimated the mean concentrations of Fe by 10.17%, Mn by 19.17%, Zn by 16.92%, Cu by 29.60% and Ni by 14.51%. The QXAS software overestimated for some and underestimated for others but by a smaller margin compared to that of picofox. QXAS overestimated concentrations of Fe by 2.20%, Zn by 3.82% and Cu by 12.64%. The concentrations of Mn and Ni were underestimated by 4.16% and 5.14% respectively.

Table 4.5: The concentration (absolute values) of the elements (ppm) and percentage differences from the reference soil sample (river clay), as analyzed using Picofox inbuilt software and QXAS compared to the certified values

	Picofox		**Certified**	**QXAS**	
Fe	26678.510	-10.17%	**29700.000**	30356.080	+2.20%
Mn	808.280	-19.17%	**1000.000**	958.452	+3.82%
Zn	79.842	-16.92%	**96.100**	99.765	+12.64%
Ni	32.400	-14.51%	**37.900**	35.952	-4.16
Cu	14.153	-29.60%	**20.100**	22.640	-5.14%

The difference between the QXAS and Picofox inbuilt software were investigated using Bland and Altman plots for all the elements analyzed in the soil sample. A total of 192 (64*3 replicates) soil (Muguga) Picofox inbuilt software spectra were deconvoluted and quantified using QXAS. For method comparison/Bland Altman plots the mean of the three replicates was used; giving 64 measurements for each method per element.



Figures 4.25: Bland-Altman plot of Fe concentrations analyzed using QXAS and Picofox inbuilt software

QXAS gave values of iron concentration, which were on average 18% higher than the Picofox software values. The limits of agreement indicated that QXAS values were 17% to 19% above Picofox for 95% of the measurements (Figure 4.25). QXAS software also gave higher values for manganese which was on average 24% above the Picofox values. It also gave values, which were between 22% and 27% above Picofox for 95% of the measurements (Figure 4.26).

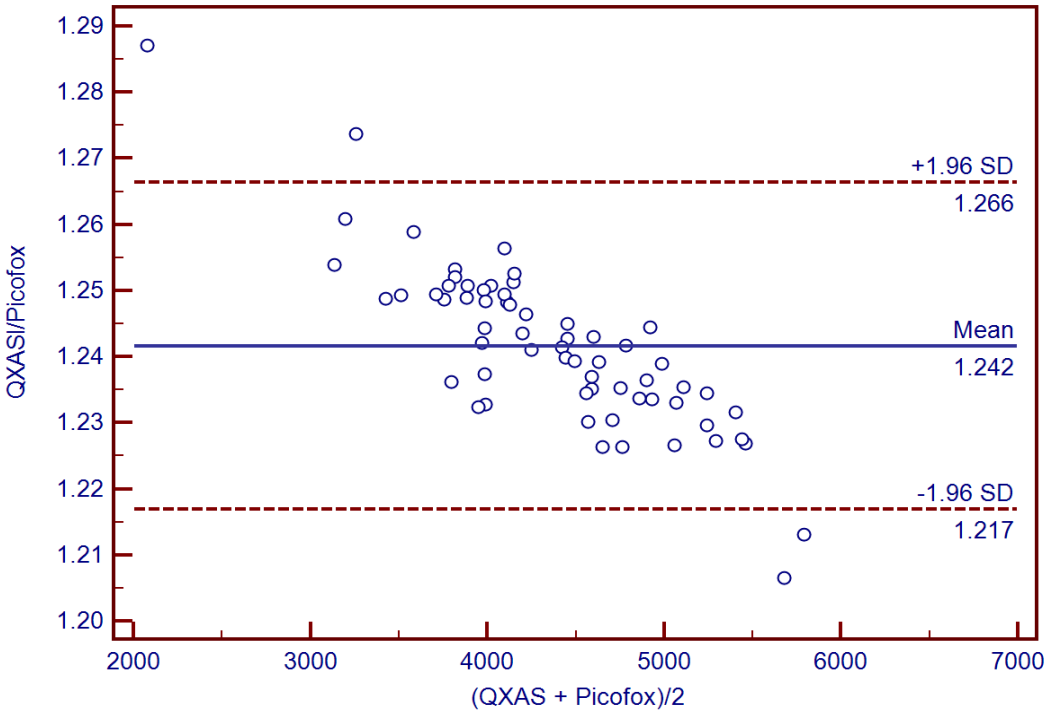


Figure 4.26: Bland-Altman plot of Mn concentrations analyzed using QXAS and Picofox inbuilt software

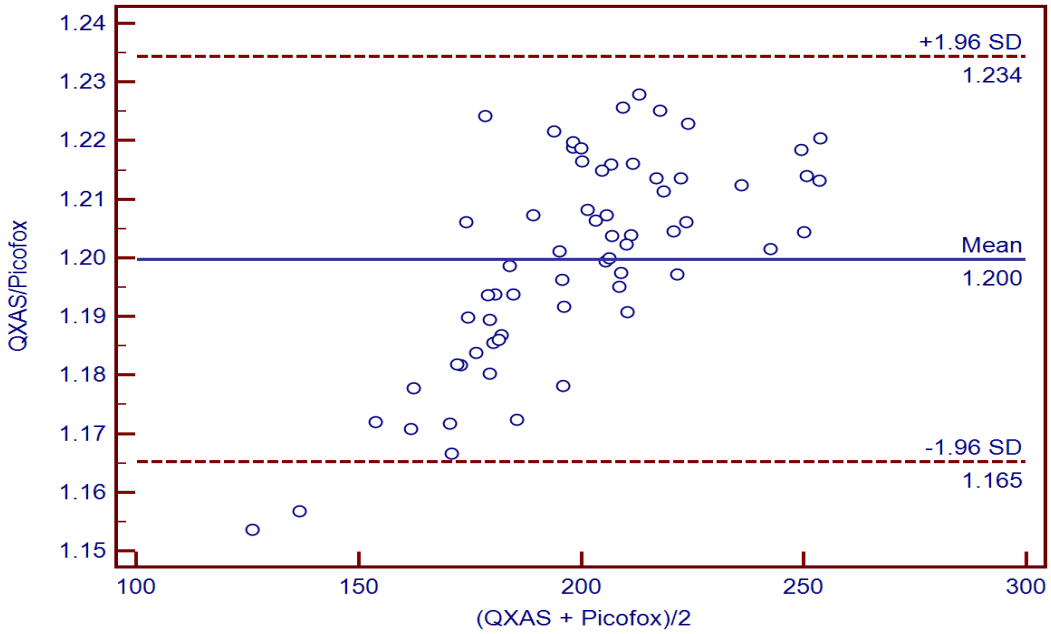


Figure 4.27: Bland-Altman plot of Zn concentration analyzed using QXAS and Picofox inbuilt software

Zn QXAS values were also higher than Picofox values by 20% on average. QXAS Zn concentrations were 17% to 23% above Picofox concentration for 95% of measurements (Figure 4.27).

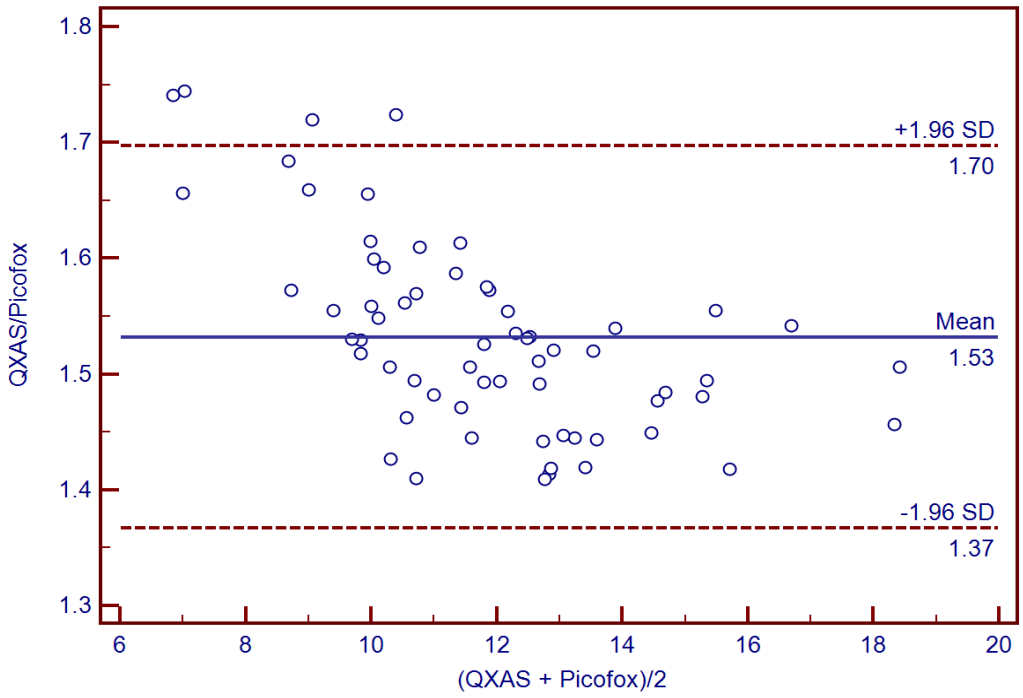


Figure 4.28: Bland-Altman plot of Cu concentration analyzed using QXAS and Picofox inbuilt software

The two methods showed the highest disagreement when used on Cu spectra. QXAS gave values, which were on average 53% higher than the Picofox values. It also gave between 37% and 70% above Picofox for 95% of the measurements (Figure 4.28). However some agreement was observed when de-convoluting Ni spectra with QXAS giving values which were on average only 5% above the Picofox software values.

The ratios between the methods did not fall on the '1' line when determining Fe, Mn, Zn and Cu concentrations. This means that the two methods did not agree on a single

measurement when analyzing these elements. However, both Axil and the Picofox software showed some agreements when used to quantify Ni (Figure 4.29).

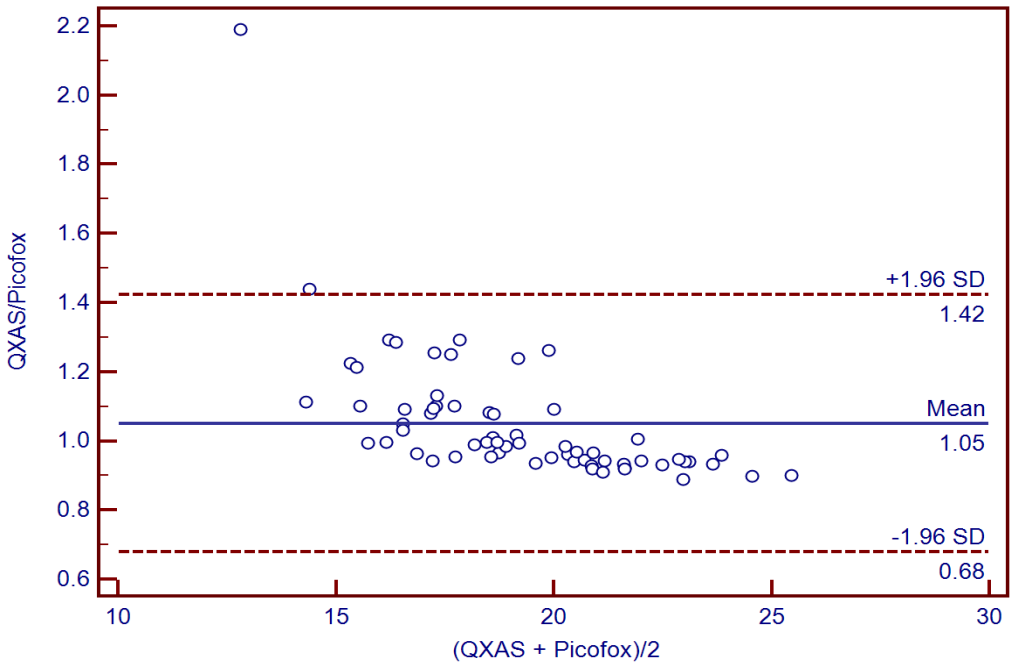


Figure 4.29: Bland-Altman plot of Ni concentration analyzed using QXAS and Picofox inbuilt software

Ninety-five percent limit of agreement quantify the range of values that can be expected to cover agreement for most of the subjects, thereby guiding the spectra analyst as to whether methods agree sufficiently for use in soil spectra assessment. It should be understood that "how small LoA should be to conclude that methods agree sufficiently" is a soil spectra/nutrient analyst's decision and not a statistical decision. The presentation of the 95% limits of agreement is for visual judgment of how well two methods of measurement agree. The smaller the range between these two limits the better the

agreement is. The question of how small is small depends on the study context: would a difference between measurement methods as extreme as that described by the 95% limits of agreement meaningfully affect the interpretation of the results? In this study, for example can QXAS Cu values which were 37 to 70% above the Picofox values used interchangeably with Picofox values?, definitely not. The question that needs consideration by laboratories using different methods for the same purpose is whether the largest likely differences are small enough for the particular purpose for which measurements are wanted. It is therefore important that soil scientists/TXRF spectra analysts come up with acceptable differences in soil spectra concentrations given by different methods within which the methods could be used interchangeably.

The consistency of both QXAS and the Picofox software was also checked for each element and sample analyzed. When comparing agreement, a method with poor repeatability will not agree even with a perfect method. It is important to note that the variation in the three repeats could be as a result of errors resulting from sample preparation. Using the three replicates to check for the consistency of the two methods was under the assumption that variations resulting from sample preparation were minimal. We used analysis of variance to test if there is equality of variances in the three replicates.

Table 4.6: Observed F, critical F and p values for the three replicates of each element's spectra as given by QXAS and Picofox inbuilt software, $\alpha = 0.05$

	QXAS			**Picofox**		
Element	F (observed)	F (critical)	P	F (observed)	F(critical)	P
Fe	1.883	3.762	0.155	1.810	3.762	0.166
Mn	1.469	3.762	0.233	1.457	3.762	0.236
Zn	1.527	3.762	0.220	1.529	3.762	0.219
Ni	0.592	3.762	0.554	0.737	3.762	0.480
Cu	1.340	3.762	0.264	1.536	3.762	0.218

At the level of significance, $\alpha = 0.05$, the decision was not to reject the null hypothesis of the equality of the variances; the inequality of variances were not significant. The observed F values were less than the critical F values for all the replicates and elements investigated using the two software. From the results it was clear that the inequality of variances between the replicates was not significant and the methods were both consistent (Table 4.6).

Student's t-test was also used to determine if there was difference in mean spectra values as given by the QXAS and Picofox inbuilt software for the five elements.

Table 4.7: Observed t, critical t and p values for the spectra as given by QXAS and Picofox inbuilt software for the five elements, $\alpha = 0.05$

Element	*t (observed)*	*t (critical)*	*P*
Fe	11.098	1.979	<0.0001
Mn	8.736	1.979	<0.0001
Zn	8.759	1.979	<0.0001
Ni	0.296	1.979	0.768
Cu	9.981	1.979	<0.0001

The observed t values were higher than the critical values for Fe, Mn, Zn and Cu, only Ni showed lower observed t than its critical t value (Table 4.7). At the level of significance,

$\alpha = 0.05$ the decision is to reject the null hypothesis of the equality of the means for Fe, Mn, Zn and Cu as deconvoluted by QXAS and the Picofox inbuilt software; the difference between the means were significant. However for Ni, at the level of significance, $\alpha = 0.05$ the decision was to not reject the null hypothesis of equality of the means; the difference between the means were not significant. The t test results are in agreement with the Bland Altman plots which showed that QXAS and Picofox software did not agree for Fe, Mn, Zn and Cu but showed some agreements when comparing Ni spectra.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Out of the five essential trace elements investigated four elements, namely Cu, Mn, Fe and Zn were above AAAc-EDTA extractable soil concentration critical values. However only Cu was in the optimum range while Zn and Mn were in the excess range. The low soil pH could be the cause of excess solubility of the micronutrients especially Mn and Zn. Fe excess and Ni critical values were not discussed due to lack of their values as indicated by the extraction reagent (AAAc-EDTA) used in the study. Comparing total elemental soil concentration to the amount that was extracted, Mn had the highest percentage of the total that dissolved followed by Ni, Cu, Zn and lastly Fe.

On comparing the spectra of reference sample as analyzed by the two methods to certified values, QXAS performed better. However this was not given much emphasis since the analysis only involved spectra from a single sample. The two methods showed no agreement when used to deconvolute Fe, Mn, Zn and Cu soil spectra, however some agreement was observed for Ni soil spectra. From the plots it was also seen that QXAS measured higher than Picofox inbuilt software for all the elements. This agreed with our observations when comparing the two when analyzing the reference material.

Overall, a strong relation (α=0.05) was observed between the extracted amount and total concentration for Ni, Zn, Cu and Mn in the samples. This means that total element concentration in soil could be used as an indicator of extractable amounts of these elements.

5.2 Recommendation

Acid tolerant crops like chili pepper, sweet potatoes, irish potatoes and pineapples should be grown in the study area instead of maize which usually does well at higher soil pH. Although some local maize cultivars like *Githigu* ((Kanyanjua et al., 2002) have been reported to have adapted to such conditions, farmers should lime their farms in order to increase maize yield which is one of the main crops grown in the area. The farmers should also use nitrate nitrogen fertilizers (potassium/calcium nitrate) instead of ammoniacal nitrogen fertilizers (urea, ammonium sulphate) which lower soil pH. Soil pH should be used as a precursor to soil nutrient analysis; for soils with high pH micronutrients should be investigated and for low pH soils macronutrients should analyzed. However, soil with different pH ranges should be analyzed for both macro and micronutrients to come to decisive conclusions.

The question of method agreement is not a statistical one and soil TXRF spectra analyst should come up with acceptable differences within which elemental concentration given by different spectra deconvolution software/methods could be used interchangeably. To assist in deciding which method to be preferably used studies involving at least a few reference soil spectra is should be done.

A combination of plant and soil analyses offers a better means of estimating the micronutrient status of soils than either alone and studies involving different regional soils, associated plants and extraction reagents are recommended. Trace elements in plants associated with these soils should also be used to determine the best method when estimating plant available portion of a given element in a particular soil. There are real chances and need to take advantage of what TXRF has to offer while narrowing the gap

that exists between total and extractable portions of plant essential elements in soil and as a region we should utilize it.

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APPENDICES

Appendix 1: Basic statistic values of top soil trace element concentration (mg/kg); AAAc-EDTA extracted, Carbon (%) and pH

	Fe	Mn	Zn	Ni	Cu	C	pH
Mean	468.2	2573.9	42.8	8.5	2.5	2.9	5.4
±s	62.1	348.1	11.2	1.8	0.7	0.4	0.2
Minimum	354.1	1668.4	14.1	3.7	1.0	1.5	5.1
Maximum	598.3	3269.9	63.2	11.4	4.4	3.7	5.9

Appendix 2: Basic statistic values of top soil total trace element concentration (mg/kg)

	Fe	Mn	Zn	Ni	Cu
Mean	56130.7	4046.3	187.1	20.2	10.3
±s	4940.8	544.5	22.0	3.2	2.9
Minimum	47091.5	2779.5	126.7	11.8	5.3
Maximum	65534.5	5232.7	229.0	26.8	22.9

Appendix 3: Basic statistic values of sub soil total trace elements concentration (mg/kg)

	Fe	Mn	Zn	Ni	Cu
Mean	58332.8	3736.0	174.7	18.3	9.0
±s	5370.9	664.5	22.9	4.7	2.3
Minimum	45916.9	1814.7	117.0	8.0	5.0
Maximum	70220.4	5145.9	228.5	34.5	13.8

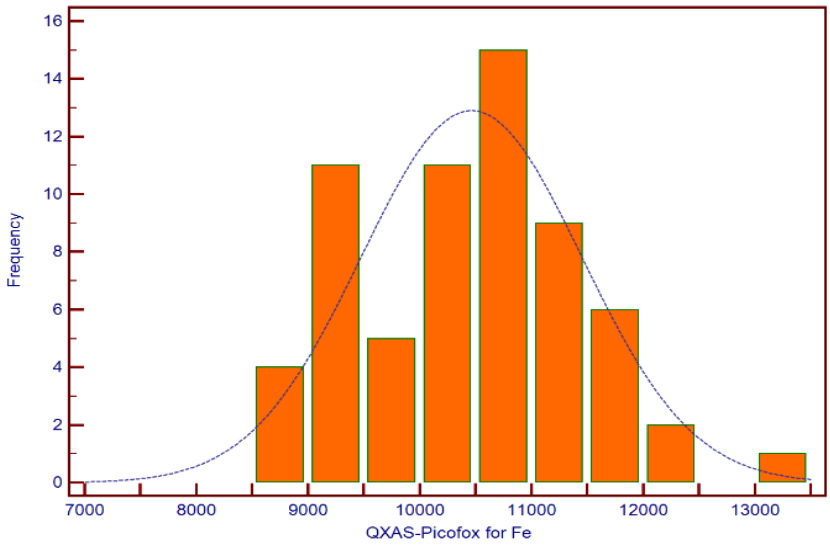
Appendix 4: Basic statistic values of the sample (top and sub soil) concentration as given by Picofox inbuilt software

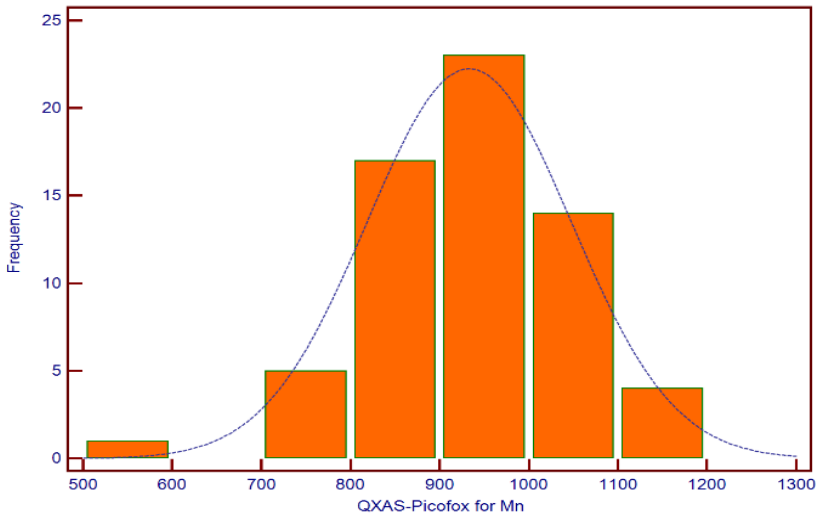
	Fe	Mn	Zn	Ni	Cu
Mean	57231.8	3891.2	180.9	19.3	9.6
±s	5238.2	622.6	23.1	4.1	2.7
Minimum	45916.9	1814.7	117.0	8.0	5.0
Maximum	70220.4	5232.7	229.0	34.5	22.9

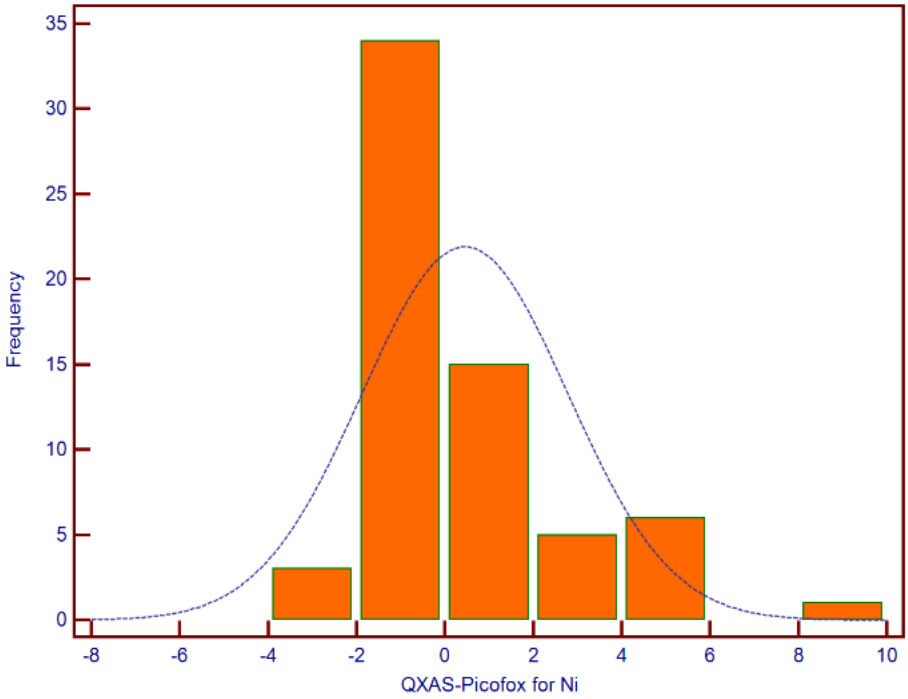
Appendix 5: Basic statistic values of the sample (top and sub soil) concentration as given by QXAS

	Fe	Mn	Zn	Ni	Cu
Mean	67555.0	4797.7	216.4	19.4	14.8
±s	5285.3	549.0	22.7	2.5	3.1
Minimum	48392.4	2989.6	150.0	14.4	9.1
Maximum	78100.5	6049.2	263.5	33.1	30.5

Appendix 6: Histogram of differences in concentration (mg/kg) measured with QXAS and Picofox inbuilt software for Fe, Mn, Ni and Cu






Frequency
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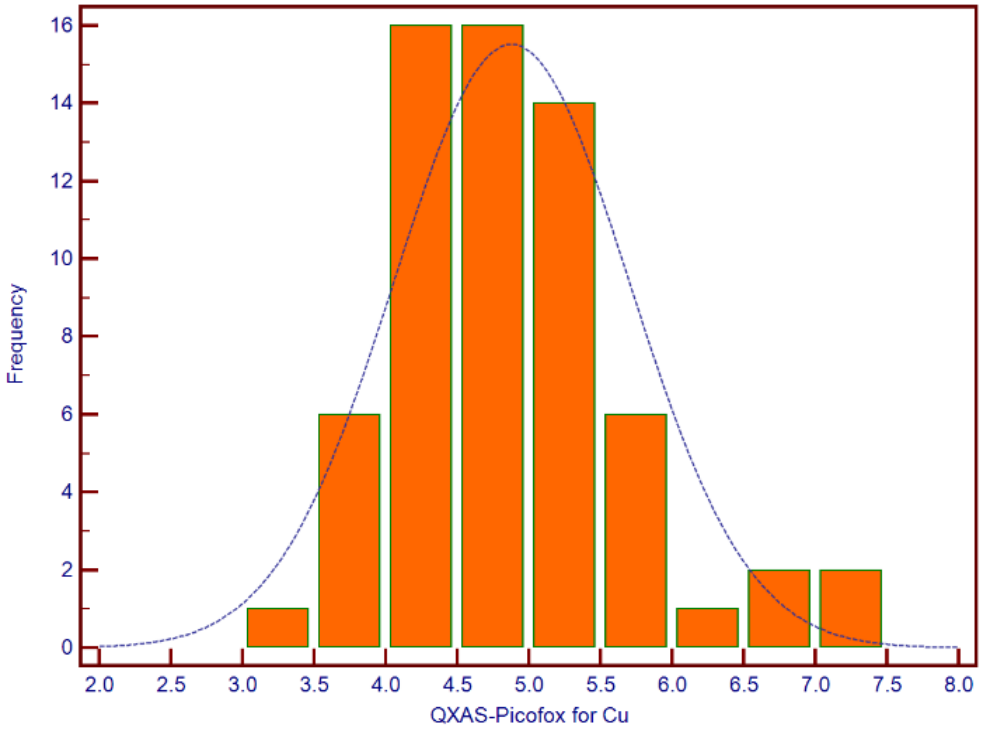
30
25
20
15
10
5
0
-8
-6
-4
-2
0
2
4
6
8
10
QXAS-Picofox for Ni


Frequency
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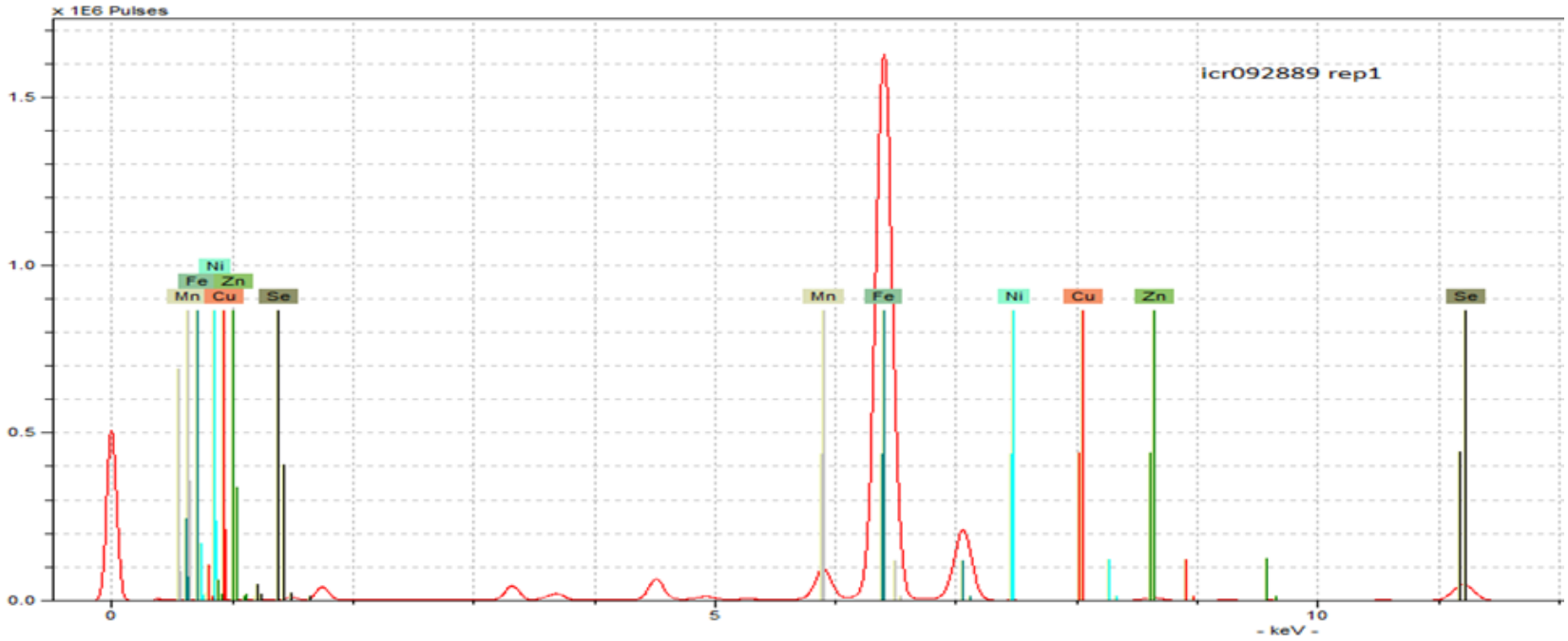
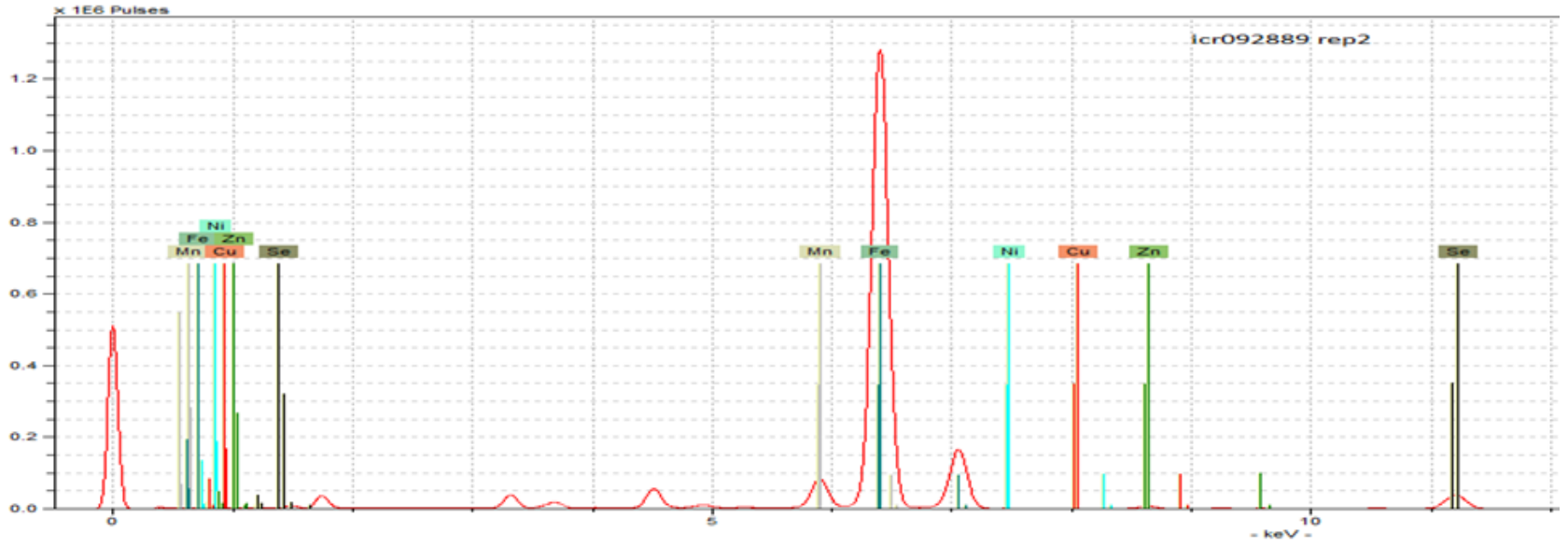
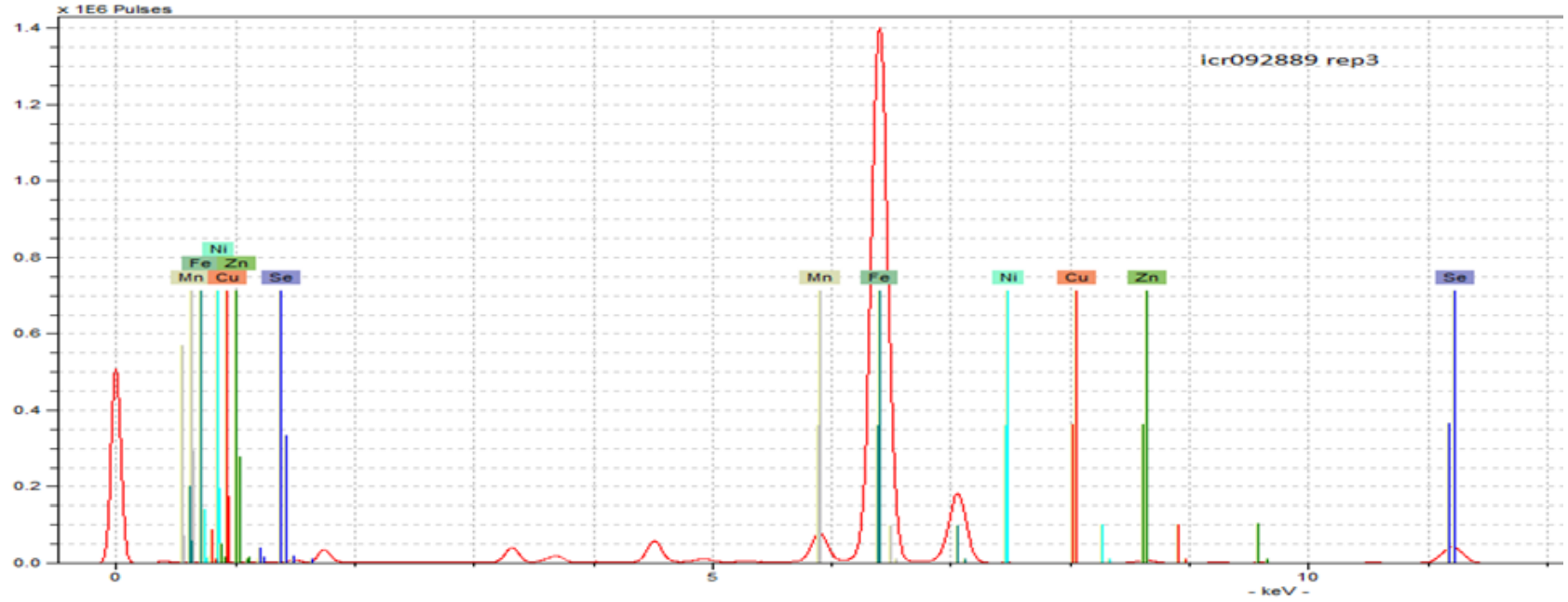
14
12
10
8
6
4
2
0
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
QXAS-Picofox for Cu

Appendix 7: TXRF Picofox spectra for a sample (SSN ICR092889) showing the elements of interest in the three replicates.




x 1E6 Pulses
icr092889 rep2
Ni
Fe Zn
Mn Cu
Se
Mn
Fe
Ni
Cu
Zn
Se
- keV -


x 1E6 Pulses
icr092889 rep3
Ni
Fe Zn
Mn Cu
Se
Mn
Fe
Ni
Cu
Zn
Se
- keV -

Appendix 8: The three replicates of the elements (mg/kg) as deconvoluted by the QXAS and Picofox inbuilt software

		Picofox					QXAS				
SSN	Replicate	Fe	Mn	Zn	Ni	Cu	Fe	Mn	Zn	Ni	Cu
icr092887	1	48635	3888.0	173.9	18.3	9.6	58169	4847.3	214.0	16.6	13.8
icr092887	1	50229	4265.8	180.8	19.1	10.0	63210	5037.0	218.6	17.0	13.1
icr092887	1	46940	3749.4	168.5	17.1	8.8	68650	4493.2	198.4	20.1	15.4
icr092888	2	53577	4088.1	180.3	18.8	9.0	73338	4277.0	197.8	20.0	15.5
icr092888	2	61199	4569.3	202.7	20.0	9.7	56053	5092.8	217.7	18.4	14.9
icr092888	2	52030	4136.7	176.2	18.4	7.9	56972	4550.7	204.0	16.4	17.2
icr092889	3	57797	3596.5	166.0	18.2	10.1	60422	4634.7	229.9	18.4	15.7
icr092889	3	57010	4080.3	165.4	18.9	11.3	59520	4672.1	235.2	19.5	16.4
icr092889	3	56767	3368.1	162.6	17.7	10.8	71124	4450.1	223.0	22.5	19.9
icr092890	4	62311	3438.2	167.4	18.1	9.8	72368	4260.3	211.3	20.9	18.3
icr092890	4	60513	3445.3	162.9	17.6	9.7	66147	4685.2	211.8	18.7	16.4
icr092890	4	60864	3271.4	164.5	17.6	9.1	64484	4118.8	185.6	17.3	12.1
icr092891	5	47549	4154.4	179.7	18.7	10.7	69717	5458.4	243.2	22.1	56.1
icr092891	5	49302	4176.7	185.7	19.8	11.4	69095	5005.9	229.7	19.6	16.8
icr092891	5	44424	3990.8	170.1	18.7	9.8	70252	4735.7	202.6	20.5	13.3
icr092892	6	47973	3672.7	167.0	16.6	12.1	71531	3787.6	174.8	21.0	12.9
icr092892	6	44741	3441.3	157.1	16.1	11.8	59002	5617.8	264.4	19.0	14.5
icr092892	6	45037	3508.9	156.9	14.6	11.6	57887	4937.7	225.7	16.7	11.5
icr092893	7	50580	3711.3	186.7	19.7	10.5	71780	4414.6	221.2	24.0	14.1
icr092893	7	51760	3845.5	192.4	21.8	10.6	76275	4255.8	197.5	23.6	11.9
icr092893	7	48972	3672.4	184.8	19.8	14.2	66261	5328.7	220.5	22.6	13.8
icr092894	8	49938	3756.0	191.9	20.8	11.1	66329	4479.3	187.8	20.4	10.9
icr092894	8	51129	4043.6	195.2	21.0	12.0	67750	4092.0	183.5	20.7	14.2
icr092894	8	51659	4030.5	199.4	20.2	10.3	74660	4218.3	184.9	22.4	15.3
icr092895	9	60019	3568.3	186.9	24.0	13.4	57977	4224.0	216.4	18.6	15.7

icr092895	9	60614	3493.6	183.3	24.4	14.0	64064	4492.5	215.2	17.2	12.6
icr092895	9	60690	3646.3	193.0	25.1	17.3	75859	4357.9	190.8	20.9	17.0
icr092896	10	60818	3390.7	176.7	23.3	11.7	83935	3765.6	175.6	23.5	14.0
icr092896	10	75012	4116.3	212.8	29.1	15.2	67747	3825.3	176.3	17.9	14.2
icr092896	10	63653	3552.6	186.6	25.2	12.5	68728	3869.0	191.1	18.4	15.3
icr092897	11	55590	3758.0	172.9	20.4	11.0	63495	4594.6	207.2	19.5	13.5
icr092897	11	61097	4358.4	190.7	23.7	11.0	65180	3590.5	154.2	17.9	10.6
icr092897	11	54126	3787.3	171.7	19.1	8.5	62862	3255.4	138.4	17.2	8.3
icr092898	12	54405	3303.6	152.6	15.9	7.8	71448	2403.8	146.5	18.5	8.1
icr092898	12	56899	3492.7	158.9	16.7	8.3	56029	5530.8	262.4	18.2	15.0
icr092898	12	56287	3370.6	161.9	16.9	7.3	59550	5418.9	234.1	16.3	11.4
icr092899	13	58553	4393.5	194.5	23.8	39.4	61140	5075.7	239.7	18.2	15.4
icr092899	13	61621	4554.6	211.9	24.5	16.5	63764	5021.3	205.6	16.9	12.9
icr092899	13	58065	4201.1	197.8	23.1	12.9	66006	5368.0	226.1	19.4	17.3
icr092900	14	58147	4024.1	188.6	22.0	11.0	68093	5356.3	230.8	18.4	17.5
icr092900	14	59495	4444.9	196.6	22.0	11.0	67840	5376.9	252.8	20.6	14.7
icr092900	14	56515	3834.2	184.8	19.9	10.8	67984	4441.7	199.6	18.1	12.0
icr092901	15	59383	3788.3	171.3	19.3	8.8	56260	5606.4	257.8	18.7	14.1
icr092901	15	57413	3620.2	165.6	19.8	10.3	57658	5953.2	276.6	22.0	21.0
icr092901	15	56979	3556.4	168.0	17.8	8.6	71549	4171.3	199.5	20.5	13.3
icr092902	16	60348	3008.9	150.3	15.6	8.1	81516	3742.9	182.6	22.1	11.9
icr092902	16	61897	3155.6	156.0	15.8	8.4	67111	5708.8	225.1	21.4	12.0
icr092902	16	65177	3351.9	164.2	15.3	8.2	69412	4885.4	194.0	18.3	12.5
icr092903	17	49527	4542.9	218.9	18.8	10.1	62578	4980.2	229.2	18.3	16.4
icr092903	17	55400	5189.7	237.3	19.5	11.3	70400	3865.0	182.8	18.1	13.7
icr092903	17	53094	4795.4	230.7	19.5	11.1	67725	5768.4	231.4	21.6	13.5
icr092904	18	48923	4005.6	188.6	15.4	8.0	72923	5329.1	213.7	20.6	11.6
icr092904	18	57536	4593.7	215.9	17.4	10.4	66648	5452.3	262.1	18.1	13.8
icr092904	18	51353	4156.4	200.0	15.5	8.3	76903	5198.1	250.0	21.7	13.6
icr092905	19	60922	3556.5	185.6	26.3	9.1	69866	5976.6	248.3	18.0	13.3

icr092905	19	63684	3710.0	191.1	27.3	10.1	80014	6066.6	242.2	21.8	10.1
icr092905	19	64091	3651.8	198.1	26.7	9.9	67916	4808.0	237.3	18.0	13.2
icr092906	20	64510	3400.1	170.4	23.2	6.9	85233	4877.7	262.0	23.6	14.2
icr092906	20	67800	3630.8	175.4	24.9	8.2	80082	6091.3	254.5	22.1	13.6
icr092906	20	63673	3335.3	166.3	23.0	7.4	83651	4691.9	207.3	13.3	11.7
icr092907	21	55907	4289.5	186.5	22.8	9.2	80125	4686.5	203.2	21.9	13.0
icr092907	21	60066	4572.3	198.5	24.7	10.2	77735	5671.4	239.4	21.1	14.8
icr092907	21	55142	4286.4	187.2	23.0	9.6	74106	6576.0	282.5	20.8	15.6
icr092908	22	55813	3588.4	157.8	17.7	6.9	70998	6681.1	285.9	20.0	14.6
icr092908	22	58328	3921.4	168.3	18.3	7.9	59550	5274.3	219.4	18.5	14.7
icr092908	22	57697	3749.5	163.5	17.7	7.3	72557	5675.6	245.9	19.9	14.4
icr092909	23	56905	3259.1	157.0	19.5	9.5	67955	5104.3	197.5	18.5	16.9
icr092909	23	63426	3622.7	171.7	21.5	10.8	71843	4329.4	193.8	18.8	14.7
icr092909	23	61930	3481.7	164.8	20.2	10.0	58854	5199.3	227.6	18.7	16.1
icr092910	24	63212	3375.7	159.9	19.1	9.7	53110	4268.5	192.3	15.6	16.9
icr092910	24	65329	3506.5	162.9	19.8	9.4	61059	4747.8	233.8	20.4	15.6
icr092910	24	59374	3189.9	150.4	18.5	8.6	60457	4986.6	238.6	19.6	16.6
icr092911	25	48903	3407.8	177.7	19.6	11.1	72119	4386.7	225.0	22.7	19.8
icr092911	25	59880	4076.7	212.5	20.1	12.0	88519	5136.7	253.1	25.5	23.0
icr092911	25	45859	3193.2	168.9	17.2	8.7	72567	5415.3	232.3	21.7	15.9
icr092912	26	53958	3610.5	176.9	16.8	8.8	67682	4370.0	192.9	17.8	12.5
icr092912	26	64357	4090.3	205.5	18.1	10.5	73392	5673.0	254.8	23.1	24.8
icr092912	26	52466	3315.9	172.0	15.7	8.5	70482	5499.7	233.4	21.5	17.3
icr092913	27	63966	3477.8	160.7	21.3	11.0	68418	4526.3	202.4	19.0	14.9
icr092913	27	70696	4020.3	180.3	23.3	13.1	73332	3957.2	184.2	19.4	13.7
icr092913	27	61582	3393.3	158.5	20.8	12.2	65883	6391.3	289.9	19.8	16.8
icr092914	28	70504	2955.9	148.9	18.7	8.1	68151	5658.0	262.0	18.0	14.5
icr092914	28	77903	3218.2	167.0	18.4	8.5	75602	4631.0	233.8	24.2	15.3
icr092914	28	62253	2422.5	130.9	15.6	6.3	80180	4530.2	207.7	22.1	13.3
icr092915	29	57253	3058.4	149.3	14.2	9.4	71074	5633.0	241.7	22.1	15.2

icr092915	29	68480	3568.1	173.5	16.1	10.7	69130	4877.2	199.0	19.0	12.1
icr092915	29	58332	3396.5	155.3	15.6	9.6	74370	4484.6	200.1	19.6	15.9
icr092916	30	58040	3089.6	160.9	16.5	10.2	76767	4360.2	189.8	21.3	14.6
icr092916	30	69190	3693.0	193.4	19.7	18.1	70742	5058.6	257.6	19.6	16.4
icr092916	30	55014	3004.4	156.1	16.4	10.3	76238	5100.1	249.2	21.8	15.0
icr092917	31	53812	3720.0	174.7	21.7	9.1	84153	5060.8	214.5	20.1	20.6
icr092917	31	63030	4355.5	201.3	24.6	10.2	92244	4084.1	196.5	26.1	14.5
icr092917	31	53040	3822.3	171.1	21.2	8.7	81033	4479.0	209.9	21.4	16.5
icr092918	32	55398	2884.3	131.7	12.9	6.3	85673	4847.6	259.5	7.0	37.6
icr092918	32	67393	3583.1	161.2	15.0	7.7	75089	5436.0	245.8	22.8	15.9
icr092918	32	53930	2895.8	131.9	13.5	6.3	79805	4493.9	189.3	21.0	12.8
icr092919	33	53470	2607.2	118.6	11.0	5.2	76084	3837.7	161.8	20.9	8.9
icr092919	33	65048	3072.0	142.1	13.1	5.5	76784	2532.1	144.0	20.5	11.6
icr092919	33	53792	2658.0	119.4	11.2	5.1	65121	6444.2	301.2	20.1	16.9
icr092920	34	60499	1868.4	126.5	8.8	4.8	65579	6066.2	261.9	17.6	13.2
icr092920	34	65296	1961.7	125.8	8.5	6.9	70207	5828.3	271.8	20.3	18.2
icr092920	34	52428	1614.0	98.6	6.8	3.3	71332	5793.0	228.0	19.1	13.7
icr092921	35	47273	4503.7	216.1	17.8	10.2	81372	6512.8	271.0	22.5	21.1
icr092921	35	55048	5242.5	248.4	19.9	12.1	80782	6607.2	271.7	23.0	20.2
icr092921	35	47192	4501.5	214.7	17.8	9.8	78812	6308.2	283.3	22.5	17.0
icr092922	36	50485	4426.4	195.6	15.8	8.5	76958	5269.5	226.4	20.0	14.2
icr092922	36	55651	4945.4	218.3	17.5	9.1	69371	6997.2	314.6	23.4	17.6
icr092922	36	48049	4249.1	186.4	15.3	7.9	63913	6605.9	305.1	24.1	18.9
icr092923	37	51400	4095.7	196.1	17.9	11.1	88716	4970.5	236.9	24.7	16.0
icr092923	37	59858	4755.3	227.0	20.6	12.8	81426	3690.2	179.4	22.1	12.1
icr092923	37	47701	3814.2	184.6	17.0	9.4	72278	6194.3	239.2	20.7	12.0
icr092924	38	53750	4056.1	169.3	14.4	8.8	75410	5590.5	209.5	19.6	13.4
icr092924	38	60345	4686.3	190.2	15.3	9.4	64244	5223.5	238.4	19.0	18.4
icr092924	38	49977	3298.0	154.8	12.3	7.5	72268	4229.3	190.6	18.7	14.5
icr092925	39	55359	4317.5	184.9	20.3	12.1	62917	5412.4	215.9	19.7	12.0

icr092925	39	69596	5327.8	226.5	24.9	14.3	64576	4905.0	193.8	17.3	10.2
icr092925	39	51567	4064.7	176.4	19.7	10.5	55288	5023.6	234.6	16.4	12.1
icr092926	40	57786	4357.5	189.8	20.3	12.1	56546	4121.6	197.7	15.6	11.2
icr092926	40	68379	5354.6	225.3	24.7	13.6	56072	4878.8	208.6	16.1	11.4
icr092926	40	57197	4354.7	187.7	20.2	11.3	59862	4793.3	238.0	61.7	22.3
icr092927	41	56811	4315.9	205.6	21.6	9.8	56265	4050.6	203.6	15.2	11.5
icr092927	41	67153	5144.4	236.0	25.0	11.3	59807	3768.3	186.0	16.4	11.1
icr092927	41	55063	4160.8	198.1	20.4	8.5	67513	5158.4	216.8	18.7	11.8
icr092928	42	57394	3564.4	165.4	14.7	7.7	75592	4439.1	194.0	20.0	10.5
icr092928	42	65879	4290.4	190.9	18.4	9.0	70403	4364.6	185.7	18.7	11.8
icr092928	42	55344	3517.1	177.9	15.7	24.6	71971	5618.7	234.0	22.4	14.5
icr092929	43	47624	4584.2	211.8	19.5	10.3	63771	6517.3	279.0	24.9	14.5
icr092929	43	58752	5720.3	259.3	24.4	12.4	59601	6096.8	258.0	21.8	13.6
icr092929	43	45981	4404.9	203.4	18.7	9.2	55999	4671.6	206.0	16.9	12.7
icr092930	44	48538	4844.9	225.4	21.8	14.9	62198	5174.3	215.6	18.4	11.8
icr092930	44	54097	5403.8	251.8	24.1	13.3	67335	4222.8	193.9	15.6	16.7
icr092930	44	44281	4393.7	208.4	19.7	10.8	72229	4120.4	195.0	19.0	14.2
icr092931	45	60390	3332.8	168.5	22.9	8.6	52654	4927.3	207.3	19.1	14.1
icr092931	45	75270	3984.1	202.5	27.8	9.7	53648	4375.1	192.4	15.0	17.3
icr092931	45	60942	3287.2	168.6	22.2	7.9	58256	4581.4	227.4	19.5	20.8
icr092932	46	68685	2951.9	155.3	17.2	7.2	61561	5027.8	244.8	20.7	15.2
icr092932	46	69669	2952.0	154.5	18.3	6.9	71778	4556.6	230.0	23.3	25.5
icr092932	46	60878	2581.6	137.1	15.9	5.9	75342	4456.6	221.6	23.3	19.5
icr092933	47	57044	4656.9	191.7	21.0	7.9	64129	4718.8	208.8	19.0	13.3
icr092933	47	61404	5059.8	200.0	22.7	8.2	66996	4241.3	192.4	18.4	12.0
icr092933	47	53335	4380.4	177.9	20.1	7.2	68886	5232.8	240.9	22.0	19.5
icr092934	48	58249	3911.8	162.7	14.1	8.0	67068	4787.9	219.4	19.2	16.4
icr092934	48	64514	4572.9	176.9	15.5	8.7	67200	4425.7	197.7	18.4	13.5
icr092934	48	56423	3816.9	158.1	12.9	7.9	77457	4235.5	192.4	20.0	13.3
icr092935	49	52746	4022.1	187.2	18.5	11.2	62530	5883.8	279.1	18.7	15.7

icr092935	49	53768	4204.5	192.9	21.2	12.6	61030	5161.6	236.1	16.2	11.5
icr092935	49	54035	4173.1	193.4	21.0	11.6	76180	4595.9	237.0	24.2	15.2
icr092936	50	59314	3073.7	154.0	13.8	8.6	75086	4180.0	195.3	21.2	12.0
icr092936	50	60981	3386.4	161.2	16.0	9.3	65044	5295.8	225.7	22.0	14.4
icr092936	50	58999	3438.6	157.9	14.7	8.4	68286	4677.4	197.5	18.5	11.3
icr092937	51	56908	4662.6	189.1	23.1	8.6	73260	4366.9	199.1	20.0	15.6
icr092937	51	52863	4377.5	176.8	22.0	7.8	70465	4019.1	177.3	18.9	13.6
icr092937	51	51767	4207.5	175.7	22.5	7.5	53596	3930.5	201.1	16.1	12.7
icr092938	52	62028	4328.8	177.1	19.0	7.0	62474	4155.0	209.2	16.6	13.3
icr092938	52	54474	3964.6	163.8	18.3	6.8	73125	4265.1	187.5	20.5	19.0
icr092938	52	54647	3942.7	160.5	18.9	6.6	74128	3100.2	154.0	20.9	11.0
icr092939	53	56457	4417.0	216.6	15.3	9.3	68592	4211.1	182.7	18.2	14.8
icr092939	53	46863	4104.4	194.0	17.0	8.4	64813	3741.4	183.6	17.8	15.9
icr092939	53	43888	3771.7	181.8	16.5	8.0	62624	4720.7	207.9	19.9	13.4
icr092940	54	65343	4194.9	208.0	14.1	8.6	63577	3613.1	154.5	11.7	10.3
icr092940	54	48176	3357.2	164.5	14.6	7.5	64033	3360.7	139.4	16.8	9.0
icr092940	54	46054	3165.6	159.1	11.9	7.0	61737	2071.1	114.2	16.6	6.3
icr092941	55	59602	4905.2	208.1	19.4	8.8	55777	5510.9	260.9	17.8	14.4
icr092941	55	47266	3960.7	171.3	16.9	7.6	56855	5223.4	227.1	14.5	11.8
icr092941	55	48038	3961.8	173.2	16.9	7.0	56154	4678.9	221.6	16.7	13.6
icr092942	56	68206	4952.4	203.9	15.6	6.4	59310	4108.3	187.3	14.9	11.8
icr092942	56	51889	3924.6	185.8	75.0	4.3	61552	5056.6	216.3	18.4	16.7
icr092942	56	47696	3658.8	150.8	13.1	4.7	67968	5401.7	229.0	18.6	17.1
icr092943	57	58126	3937.7	199.1	18.2	8.7	64720	5110.8	239.5	19.5	13.6
icr092943	57	47649	3273.5	170.9	16.3	7.7	65893	4401.3	213.1	17.1	35.2
icr092943	57	50329	3403.2	190.3	17.0	8.3	54818	5442.7	249.5	18.5	13.6
icr092944	58	72866	3950.2	218.4	18.4	8.2	52740	5414.4	254.9	20.0	15.3
icr092944	58	50953	3049.5	155.7	15.3	7.2	72126	4114.2	199.4	19.6	13.0
icr092944	58	52166	3188.5	162.8	15.6	7.8	72048	3265.7	161.3	19.5	10.4
icr092945	59	67956	4978.3	212.4	25.6	8.3	63476	5431.5	216.5	19.6	11.6

icr092945	59	56741	4144.9	181.6	19.4	7.3	66862	4741.9	186.9	17.0	12.6
icr092945	59	52803	3933.6	168.5	21.4	8.0	64148	5162.5	236.7	19.5	17.9
icr092946	60	71197	3797.6	173.6	15.9	6.6	69596	4273.5	185.9	12.2	13.5
icr092946	60	63604	3535.3	163.8	15.1	6.6	61005	5161.8	211.6	20.9	11.4
icr092946	60	58934	3305.2	154.3	16.5	6.3	64634	4871.9	193.4	18.0	10.1
icr092947	61	68693	3828.6	172.1	15.6	7.8	52109	4647.5	221.0	15.9	11.7
icr092947	61	59063	3475.7	156.3	15.8	7.6	54110	3892.5	191.1	14.6	10.4
icr092947	61	59438	3342.6	155.7	15.6	7.5	56810	4876.6	209.7	16.0	10.8
icr092948	62	65636	4611.6	198.0	23.4	9.0	56400	4514.1	178.5	15.0	7.8
icr092948	62	60377	4513.3	191.7	24.0	9.6	59610	4223.0	231.2	16.4	12.4
icr092948	62	57182	4238.9	182.6	22.5	9.0	61446	3949.3	191.8	14.1	11.8
icr092949	63	64293	5537.4	239.5	23.9	10.3	62436	4859.3	203.6	19.6	12.2
icr092949	63	53900	5321.6	229.7	24.3	10.4	70077	4149.3	183.5	18.6	10.5
icr092949	63	49208	4839.1	210.9	24.8	10.6	70678	4197.4	184.3	18.2	12.3
icr092950	64	62110	5672.0	242.3	23.1	9.9	67941	5266.5	222.6	21.6	14.2
icr092950	64	50499	4992.8	212.7	22.4	9.1	58431	5950.4	257.7	24.4	15.0
icr092950	64	49219	4772.9	205.7	22.4	9.3	58153	5847.7	250.1	22.2	14.2

Appendix 9: Topsoil total and AAAc-EDTA extractable elements (mg/kg), T for total and E for the extractable, Carbon %, pH and volume weight (g/ml)

SSN	FeT	FeE	MnT	MnE	ZnT	ZnE	NiT	NiE	CuT	CuE	Carbon	pH	g/ml
icr092887	48601	461.3	3967.7	2851.7	174.4	41.4	18.1	8.6	9.5	2.4	3.2	5.6	1.0
icr092889	57191	436.5	3681.6	2439.1	164.7	36.2	18.3	7.6	10.7	2.8	2.8	5.5	1.0
icr092891	47091	502.2	4107.3	2467.2	178.5	36.4	19.0	8.6	10.6	3.2	2.1	5.7	1.1
icr092893	50437	416.2	3743.1	2346.1	188.0	48.0	20.4	8.7	11.8	2.9	3.0	5.2	1.1
icr092895	60441	381.9	3569.4	2937.8	187.7	53.2	24.5	9.5	14.9	4.4	3.3	5.7	1.0
icr092897	56938	401.7	3967.9	2427.3	178.4	37.8	21.1	8.4	10.2	2.0	2.7	5.3	1.0
icr092899	59413	563.3	4383.1	3019.9	201.4	46.4	23.8	10.3	22.9	3.0	3.1	5.3	1.0

icr092901	57925	439.9	3655.0	2391.9	168.3	32.6	19.0	7.6	9.2	2.2	2.5	5.2	1.0
icr092903	52674	482.7	4842.7	2388.0	229.0	47.2	19.3	7.5	10.8	2.6	2.6	5.3	1.0
icr092905	62899	433.3	3639.4	2870.1	191.6	57.6	26.8	11.4	9.7	1.8	3.7	5.4	1.0
icr092907	57038	507.5	4382.7	2550.6	190.7	44.4	23.5	10.3	9.7	2.0	2.8	5.2	1.0
icr092909	60754	394.5	3454.5	2378.8	164.5	33.3	20.4	8.5	10.1	2.6	2.9	5.5	1.1
icr092911	51547	400.0	3559.3	2103.0	186.4	43.2	19.0	7.9	10.6	2.3	2.7	5.2	1.0
icr092913	65415	397.3	3630.5	2372.2	166.5	32.3	21.8	8.2	12.1	1.8	2.9	5.1	1.0
icr092915	61355	362.5	3341.0	2139.5	159.4	27.9	15.3	5.4	9.9	2.4	2.3	5.5	1.1
icr092917	56627	465.3	3965.9	2683.0	182.3	50.7	22.5	10.6	9.3	2.5	3.1	5.5	1.0
icr092919	57437	354.1	2779.1	1668.4	126.7	14.1	11.8	3.7	5.3	1.0	2.2	5.1	1.0
icr092921	49838	520.1	4749.2	2895.3	226.4	60.1	18.5	8.7	10.7	3.1	3.4	5.6	1.1
icr092923	52986	489.2	4221.7	2590.6	202.6	47.8	18.5	7.9	11.1	2.8	3.4	5.6	1.0
icr092925	58840	472.2	4570.0	2934.4	195.9	49.1	21.6	8.7	12.3	3.7	3.2	5.7	0.9
icr092927	59676	488.1	4540.4	2653.6	213.2	46.4	22.3	9.4	9.9	2.1	2.6	5.5	1.0
icr092929	50786	598.3	4903.1	2891.8	224.8	48.1	20.8	8.6	10.6	2.4	3.0	5.9	1.0
icr092931	65534	399.3	3534.7	2409.8	179.9	37.1	24.3	9.3	8.7	1.6	3.0	5.2	1.0
icr092933	57261	534.1	4699.0	2704.1	189.9	41.6	21.3	9.7	7.8	2.0	3.1	5.3	0.9
icr092935	53516	528.5	4133.2	3269.9	191.2	59.2	20.2	10.2	11.8	4.4	3.2	5.8	1.0
icr092937	53846	493.9	4415.9	2807.4	180.5	46.1	22.5	10.4	7.9	2.1	2.9	5.2	1.0
icr092939	49069	456.9	4097.7	2258.1	197.5	49.6	16.3	7.2	8.6	2.2	2.9	5.3	1.0
icr092941	51636	495.5	4275.9	2583.6	184.2	37.6	17.7	6.8	7.8	1.6	2.7	5.6	1.0
icr092943	52034	524.5	3538.1	2700.7	186.8	43.3	17.2	7.1	8.2	2.5	3.2	5.8	1.1
icr092945	59167	523.9	4352.2	2955.4	187.5	43.0	22.1	8.9	7.9	1.9	3.3	5.5	1.1
icr092947	62398	563.3	3549.0	1852.5	161.4	15.4	15.7	4.5	7.6	1.6	1.5	5.3	1.1
icr092949	55800	493.5	5232.7	2822.3	226.7	63.2	24.4	11.3	10.4	3.1	3.0	5.5	1.0