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Total Reflection X-Ray Fluorescence (TXRF) in Assessment of Essential Micronutrient Levels in Common Beans (*Phaseolus vulgaris)* in Kenya.

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A thesis submitted in partial fulfilment for the degree of MSc in Nuclear Science in the Institute of Nuclear Science and Technology in the University of Nairobi.

Declaration

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

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Supervisors' approval

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

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Dedication

I dedicate this work to my husband Stanley and daughter Sheena.

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List of Abbreviations

TXRF – Total reflection X-ray Fluorescence

ICP-OES – Inductively Coupled Plasma – Optical Emission Spectrometry

GDP – Gross Domestic Product

LoA – Limits of Agreement

ICRAF – International Centre for Research in Agroforestry

IAEA – International Atomic Energy Agency

GoK – Government of Kenya

KARI – Kenya Agricultural Research Institute

IELRC – International Environmental Law Research Centre

NFNSP – National Food and Nutrition Security Policy

XRF – X-ray Fluorescence

PIXE – Particle Induced X-ray Emission

ICP-MS – Inductively Coupled Plasma – Mass Spectrometry

Definition of terms

Applicability – this is the degree to which the obtained results can hold true in a particular practice.

Accuracy – this is how close the analytical/experimental result is to the ‘true’ value. The ‘true’ value in this case is the certified reference value.

Repeatability – this is how closely the results of successive measurements of the same sample carried under the same measurement conditions agree.

Reproducibility – this is how closely the measurement results of the same sample carried out under changing measurement conditions agree.

Precision – this is a measure of how close the replicates of an analysed sample agree.

ABSTRACT

In this study, essential micronutrient concentrations in common beans (*Phaseolus vulgaris*) from Muguga, Kiambu County and Kyevaluki, Machakos County were analysed using Total reflection X-ray Fluorescence (TXRF) and Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES). TXRF as a method of analysis is increasingly becoming of interest in food quality analysis since it is a fast and easy technique. This study shows the effectiveness of TXRF for fast and reliable qualitative and quantitative analysis of micronutrients in beans. Samples of both bean leaves and dry grains were collected where 32 bean leaves and 31 dry grain samples were collected. The results were then assessed for nutritional quality in comparison with sufficiency ranges for high quality yielding bean crops, that is, the comparisons were made from a plant nutrition perspective. A comparison was also made between TXRF and ICP-OES as interchangeable methods of analysis.

The TXRF analysed bean leaves from Muguga had mean concentration values of 214 ± 52, 758 ± 219, 2 ± 0.8, 9 ± 1.8 and 65 ± 8.9 mg kg^{-1} for Mn, Fe, Ni, Cu and Zn respectively. The Mn, Ni and Cu concentrations were within the sufficiency ranges while 6.2% of the samples had higher Zn concentrations. Fe concentrations were consistently high with 97% of the samples having concentrations that were higher than the sufficiency range. Out of those, 50% gave Fe concentrations that were higher than the FAO recommended toxic levels of 800 mg kg^{-1}. To compare TXRF and ICP-OES methods of analysis, the TXRF analysed samples from Muguga were further analysed by ICP-OES. To compare the results obtained by both methods, student t distribution and Analysis of Variance (ANOVA) were used. It was observed that both methods produced results that were similar. This meant that the two methods can be used interchangeably for the analysis of trace elements in plants and especially beans.

The bean leaves from Kyevaluki gave mean concentrations of 76 ± 16, 218 ± 65, 1.5 ± 0.2, 8 ± 0.8 and 27 ± 4 mg kg^{-1} for Mn, Fe, Ni, Cu and Zn respectively and all these samples were found to have concentrations that were within sufficiency ranges. In Kenya, the major form in which beans are consumed is the dry grains with less people using bean leaves as vegetables. From this study however, it was found that the analysed bean leaves had high levels of essential micronutrients and thus can be recommended to be widely used as vegetables.

Samples of dry bean grains were also analysed in which seven different local species were analysed. In the analysis of these dry bean grains, which are the main bean part consumed in Kenya, deficiencies were observed in which four bean types, that is Kyevaluki Nyayo, Muguga Red Haricot, Muguga Rosecoco and Muguga Pinto were found to have Mn concentrations lower than the sufficiency range for high quality beans. All the analysed bean species were found to be deficient in Zn. Fe concentrations were above the sufficiency ranges for all the beans except Muguga Nyayo but not to toxic levels. Samples that had Cu concentrations within the sufficiency range were 29%. Fe concentrations were highest in all the samples followed by Mn and then Zn and Cu while the Ni concentrations were the lowest. Therefore, all the samples followed the expected range of Fe>Mn>Zn>Cu>Ni.

Since dry bean grains are consumed in many households in Kenya and deficiencies were observed, interventions need to be made on how to improve their micronutrient levels. This study was done from a crop nutrition perspective and thus further studies can be carried out on the bioavailable micronutrient levels in beans. Further study can also be carried out on associated soils since plant nutrient uptake also depends on soil factors like organic matter and pH.

CHAPTER ONE

INTRODUCTION

1.1 Background

Most of the nutrients that feed the developing countries come from plant foods. These nutrients are also essential in ensuring that there is food security. This is the state in which all people can access enough, safe and nutritious food to meet their dietary needs at all times and thus leading to a healthy and active life (FAO, 2008). Therefore, where food security is achieved, all people are free from hunger at all times (WFP, 2007). Currently, there is pressure on land and food resulting from rapid population increase. Consequently, there are increased nutrient deficiencies, which more than three billion people suffer from in the world. Unfortunately, these deficiencies are more prevalent among women, children and infants especially in the developing world. Plants require nutrients for their growth and survival. These are classified into non-mineral and mineral nutrients where the former includes hydrogen, carbon and oxygen and are found in water and air. They make food for the plant through the process of photosynthesis. Mineral nutrients are classified into macro and micronutrients.

Macronutrients are further classified into primary macronutrients (nitrogen, potassium and phosphorous) and secondary macronutrients (magnesium, calcium and sulphur). The primary macronutrients are required in large amounts for the growth and survival of the plant while the secondary macronutrients are required in lesser amounts (Stevens et al., 2002). Due to their large uptake from soils, most fertilizers contain the primary macronutrients.

Micronutrients include boron, chlorine, manganese, iron, Nickel, copper, zinc and molybdenum. They usually act as activators for plant enzyme reactions and are therefore as important as the macronutrients (Mortvedt and Giordano, 1972). Despite their low requirement for plant growth, their deficiency results into abnormalities in plants like reduced growth, impaired quality of crop products and low yield. Although acute micronutrient deficiencies in food crops may be accompanied by visible symptoms, hidden deficiencies without obvious symptoms are in general more widespread (Alloway, 2008). Micronutrients are also essential to human beings and animals and are transferred from plants to them

through the food chain. According to FAO (2001), body storage for Zn is limited and therefore it (the body) depends on regular supply from the daily diet. Black et al. (2008) observed that micronutrient deficiency results in impaired physical and mental development in children, increased mortality rate, increased morbidity and decrease in work productivity among adults. Therefore, increase in availability and consumption of a diet that is nutritionally adequate is the best way to overcome micronutrient deficiencies and 'hidden hunger.'

Various studies have been carried out to determine micronutrient levels in foods in Kenya and throughout the world. A study carried out by Maina et al. (2012) on the total concentration of trace elements in beans from eastern Kenya gave concentrations of 33 – 98 mg kg^{-1}, 227 – 647 mg kg^{-1}, 17 – 28 mg kg^{-1} and 22 – 42 mg kg^{-1} for Mn, Fe, Cu and Zn respectively. Tinsley (2009) also carried out a research on micronutrient concentrations in beans in Eldoret, Kenya and gave mean concentration values of 10 mg kg^{-1} for Mn, 82 mg kg^{-1} for Fe, while Cu and Zn had the values 9.6 mg kg^{-1} and 28 mg kg^{-1} respectively.

In this study Total reflection X-Ray Flourescence (TXRF) was the preferred method of analysis. The results obtained by use of TXRF were compared with results obtained by use of Inductively Coupled Plasma – Optimal Emission Spectroscopy (ICP-OES) to determine whether there were any significant differences. ICP-OES is one of the most common analytical techniques for the analysis of trace elements. However, the organic matrix in plant samples can cause analytical bias or even cause blockage in the sample introduction systems in case the sample is not fully dissolved (Hansen, 2009). These also involve wet digestion by use of strong acids which can be a laborious process. TXRF however involves fast and less sample preparation which minimizes errors. Direct sample analysis can also be carried out in TXRF thus making it a preferred method of analysis (Klockenkamper, 1997). It is also easier to do element quantification because it involves internal standardization.

1.2 Problem Statement

Micronutrient deficiency poses a threat to the social-economic development of a nation. This is due to the associated effects, which include impaired physical and cognitive development, increased mortality rate and reduced labour productivity. In Kenya, agriculture is considered as the backbone of the economy. The agricultural sector makes a direct contribution of 24%

of the country's GDP (Gross Domestic Product) and indirectly contributes 27% of the GDP through service related sectors like distribution and manufacturing (KARI, 2012). The government derives about 45% of its revenue from agriculture and this sector also contributes a great percentage (over 50%) of the country's export earnings (KARI, 2012). The livelihoods of more than 80% of Kenya's population are depended on agriculture and related activities, especially the people living in rural areas. Despite the evident importance of agriculture in Kenya, the country struggles with severe food insecurity problems. It has been estimated that over 10 million Kenyans are food insecure and a majority of these survive on food relief (KARI, 2012). The chronic poor nutrition in Kenya was attributed to the daily diet being inadequate in terms of food quality and diversity (NFNSP, 2011). Hickey et al. (2012) reported that realizing a sustainable food production system in Kenya is a major challenge for both the government and the international community. Elimination of micronutrient deficiencies contributes to economic growth and national development (Black et al., 2008), which are part of the long-term development strategy of Kenya as outlined in the Kenya Vision 2030 (KVision, 2007). With beans being the third most important staple food and most important legume in Kenya (GoK, 1998), it is important to carry out research so as to improve knowledge on the existing problems and understanding of the required nutrient availability in common beans. In most cases, farmers do not know the current nutritional status of the foods that they eat. Farmers will mostly rely on knowledge passed from one person to another or from generation to generation with little or no regard to food quality. This however is knowledge that may not be useful in their current situations due to factors like changes in seed type and quality, farming methods and overutilization of land.

1.3 Objectives

1.3.1 General Objective

The main objective of this study was to carry out an assessment of essential micronutrient levels in common beans (*Phaseolus vulgaris*) in two small scale farming areas of Muguga, Kiambu County and Kyevaluki, Machakos County in Kenya.

1.3.2 Specific Objectives

The following were the specific objectives:

i. To determine essential micronutrient concentrations in dry bean grains and bean leaves from two small scale farming areas of Muguga and Kyevaluki in Kenya.

ii. To compare the concentrations of essential micronutrients in dry bean grains from the two small scale farming areas of Muguga and Kyevaluki in Kenya.
iii. To compare the performance of TXRF and ICP-OES techniques for the analysis of essential micronutrients in beans.

1.4 Justification

Beans are an essential food in Kenya and therefore there is great need to determine their nutritional content. Little research has been carried out on micronutrient concentration in beans in various parts of Kenya and with the continued change in seed types, seed quality, methods of farming and land utilization, it is important to continually carry out research so as to determine the nutritional quality of beans and, depending on the findings, coming up with interventions on how to ensure that this common food is micronutrient rich. The findings can also be helpful in creating awareness on the importance of common beans and therefore encouraging increased consumption. This research aimed at determining micronutrient concentrations in bean leaves, which are used as vegetables in some homesteads, and dry beans grains which is the common form in which beans are consumed in Kenya.

Although there has been various interventions aimed at alleviating malnutrition, for example food fortification, supplementation and the use of food diversities, hidden hunger is still high in the developing world. This can be attributed to the rural and urban poor population being unable to afford or even reach these interventions. Additionally, it can be due to lack of information among the general population on the nutritional status of the foods that they take. Therefore, the use of nutrient sufficient beans, which are common in the diets of the vulnerable groups, can be a major contributor towards alleviating malnutrition and hidden hunger.

For the determination of the essential micronutrients in these bean samples, two different analytical methods, that is, TXRF and ICP-OES were used and the results compared. This was to determine whether the two methods would give similar results and therefore be used interchangeably. With TXRF gaining increased use in food quality analysis this study was used to determine its performance in comparison with the conventional analytical methods of analysis.

1.5 Scope of the Study

This study provides information on the levels of essential micronutrients in common beans (*Phaseolus vulgaris*) from two areas in Kenya: Muguga area in Kiambu County and Kyevaluki area in Machakos County. For this study, analysis was done irrespective of the different bean species grown in these areas and also the seasons of growth. The essential micronutrients of interest in this study were Mn, Fe, Ni, Cu and Zn. Samples of dry grains of Rosecoco beans, Pinto beans (locally known as Mwitemania), Nyayo beans and Red Haricot beans (locally known as Wairimu) and bean leaves were collected and analysis done by Total reflection X-ray Fluorescence (TXRF). Some of the leaf samples were also analysed using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) for method comparison. This comparison was done to determine whether the two methods could be used interchangeably for the analysis of micronutrients in beans.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

To achieve food security, adequate and quality production needs to be implemented. This is achieved by ensuring that plants have the essential nutrients for their growth. The focus has mainly been on plant macronutrients and thus neglecting the importance of plant micronutrients. Although micronutrients are only required in small quantities, they are essential for growth of plants and play important roles in ensuring balanced crop nutrition. Their deficiencies can weaken or even kill a plant and although they are vital for plant metabolism, at high concentrations they can be phytotoxic. According to Malakouti (2008) micronutrient deficiencies can significantly affect crop yield and quality and subsequently the health of human beings and domestic animals.

2.2 Food Security

The world and Kenyan population is fast growing and this is accompanied by increasing needs; putting pressure on energy, water, land and biological resources. On average, the available land for crop production in the world is only 0.23 ha per capita (Pimentel and Pimentel, 2006). Population census showed that the Kenyan population was 38.6 million in 2009 (KNBS, 2010). Compared with the previous census, the Kenyan population grows at a rate of about 2.7% every year. With this growing population, more land that would otherwise have been cultivated for agriculture is being used for residential purposes. Therefore, food security is a great challenge in Kenya and it is also a threat to human health and nutrition.

The level of food security is determined by the quantity as well as the quality of food that one takes and the inadequacy of these results in deficiencies, malnutrition and 'hidden hunger'. Malnutrition and food insecurity in the developing countries is an issue of global concern (IELRC 2010). Malnutrition is caused by starvation and hunger and these are elicited by limited supply of healthy foods. The solution to hunger is more of having the right kind of food. This is food that does not just provide calories and carbohydrates but also the essential micronutrients. If people consume foods that are micronutrient and vitamins deficient, their caloric needs may be met but they will not have taken a nutritious diet and therefore lack good health (Burchi et al., 2011). Agricultural products are primarily the nutrient sources for

the Kenyan population and thus if they do not provide adequate amounts of the required nutrients, dysfunctional food systems result and these cannot support healthy lifestyles.

2.3 Micronutrients

Micronutrients are mineral plant nutrients which are required in very small quantities for the growth and survival of plants. These are B, Cl, Mn, Fe, Ni, Cu, Zn and Mo (Mortvedt and Giordano, 1972).

2.3.1 Micronutrients in Plants

Plants that have high micronutrient levels adapt better to abiotic as well as biotic stress factors like salinity, metal toxicities, bacterial and fungal diseases. Additionally, they are adaptive to photo-oxidative stress that can be caused by high irradiation, drought and low temperatures (Cakmak, 2002). The availability of micronutrients to plants is affected by soil organic matter, soil moisture levels and pH, with pH having a large effect on their availability (Truog, 1946). Soil pH regulates the mobility, solubility and concentration of ions in solution and thus plant acquisition of elements. In acidic soils (low pH), most micronutrients are at their peak availability. While high pH favours hydroxyl and carbonate complexes, low pH favours protonated anions and free metal cations (Singh et al., 2016). Therefore, the availability and solubility of micronutrients increases with increasing soil acidity since they are present as cations. Cations are held more strongly when soil pH increases from 5 to 7. However, at low soil pH, Mo is less available since it gets fixed with Fe and Al hydroxides and thus being unavailable for plant uptake (Gupta et al., 2008; Singh et al., 2016). Figure 2.1 shows the relative availability of Mn, Fe, Cu and Zn micronutrients with changes in soil pH (Truog, 1946).

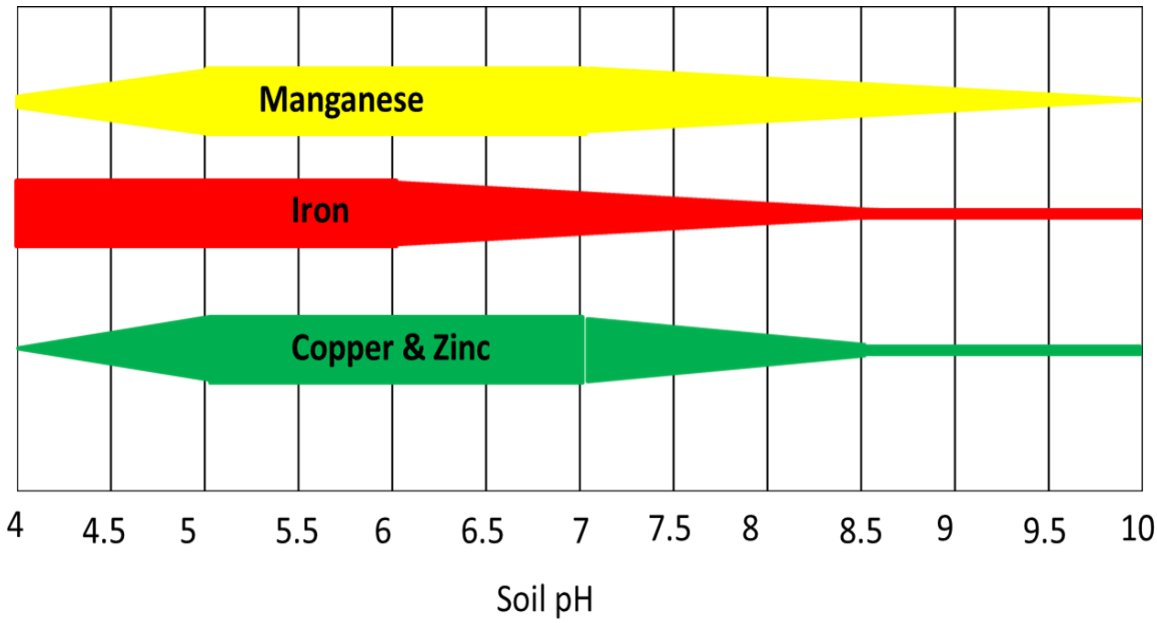

Figure 2.1: Relative availability of Mn, Fe, Cu and Zn with changes in soil pH (Truog, 1946).

2.3.2 Micronutrients in human beings

To human beings, micronutrients are important for health, growth and development. They are immune boosters since they aid the proper functioning of the immune system through repair and maintenance and also the synthesis of immune cells. Additionally, they aid in fighting infections, production of hormones, enzymes and other substances important for healthy growth and development in the body. They also improve cognitive abilities in children, labour productivity and in general the quality of life (Welch and Graham, 2000). With these evident health benefits the approach towards agriculture should be one that focuses on food crop diversity and nutritional food quality other than maximizing food production. This would ensure that the demands for human diet are met.

Research has shown that billions of people suffer from some form of micronutrient deficiency (Stein, 2010) and though this is observed in both developing and developed countries, it is more widespread in the developing countries. For instance, UNDP (2010) reported that sub-Saharan Africa was leading in the levels of poverty and hunger in the world. WHO (2002) ranked Zn and Fe micronutrient deficiencies as the 5th and 6th leading risk factors in the developing countries respectively.

2.4 Beans

Beans are significant food sources and they play a large dietary role. They are a significant supply of proteins, complex carbohydrates, fibre, essential minerals and vitamins (Gepts et al., 2008) to households both in the rural and urban areas. Because of their rich nutritional contents, quality beans can potentially alleviate malnutrition and problems associated with hunger. Approximately 12 million metric tons of beans are produced globally annually with Latin America being the largest producer. Africa produces approximately 2.5 million metric tons and this is mainly in Kenya, Burundi, Rwanda, Uganda, Congo and Tanzania (Akibode and Maredia, 2011). Beans are estimated to provide up to 50% of the dietary protein and mineral requirements in households in sub-Saharan Africa (Broughton et al., 2003; Wortman et al., 2004). Research by Beebe et al. (2013) showed that the per capita consumption of beans in Kenya, Uganda and Rwanda is 50 – 60 kg ha^{-1}. Average bean yields have been reported as 490, 540, 280, 500 and 490 kg ha^{-1} in Kenya, Democratic Republic of Congo, Angola, Uganda and Malawi respectively (Akibode and Maredia, 2011). These yields are way below the recommended 1500 – 3000 kg ha^{-1} (Hillocks et al., 2006). Although the focus of this research was nutritional quality, improving the yield of beans is an essential part of strategies for optimizing their contribution to human nutrition.

Beans can be consumed in their various forms such as green leaves, pods, green and dry grains and at different stages of growth and maturity. In Kenya, they are especially preferred and accessible to many people. The young green leaves and pods are especially taken as vegetables although not by the greater population. The most common form in which beans are consumed in Kenya is as green and dry grains which are used in various food preparations like in stews and *Githeri* (Kenyan name for a mixture of beans and maize). The average consumption of beans in Kenya has been shown to be about 68 g day^{-1} (Schoonhoven and Voysest, 1991). Among the rural and urban poor households, beans are the best alternative to the expensive animal proteins. Research by Schneider (2002) showed that beans aid in the control of non-communicable diseases like obesity and diabetes. It has also been reported that there is a high correlation between the consumption of beans and low rates of coronary heart diseases (Darmadi-Blackberry et al., 2004).

Beans are therefore referred to as the vegetarians' 'meat' of the wealthy (Schneider, 2002) and the poor man's meat. This shows that beans are an important food to all people: rich or poor, pregnant women, infants, children and adults. The Dr. Fuhrman's nutritarian food

pyramid (Figure 2.2), which is based on the principles of high nutrient eating, places beans as second among the micronutrient rich foods and these are foods which are consistently beneficial to human health.

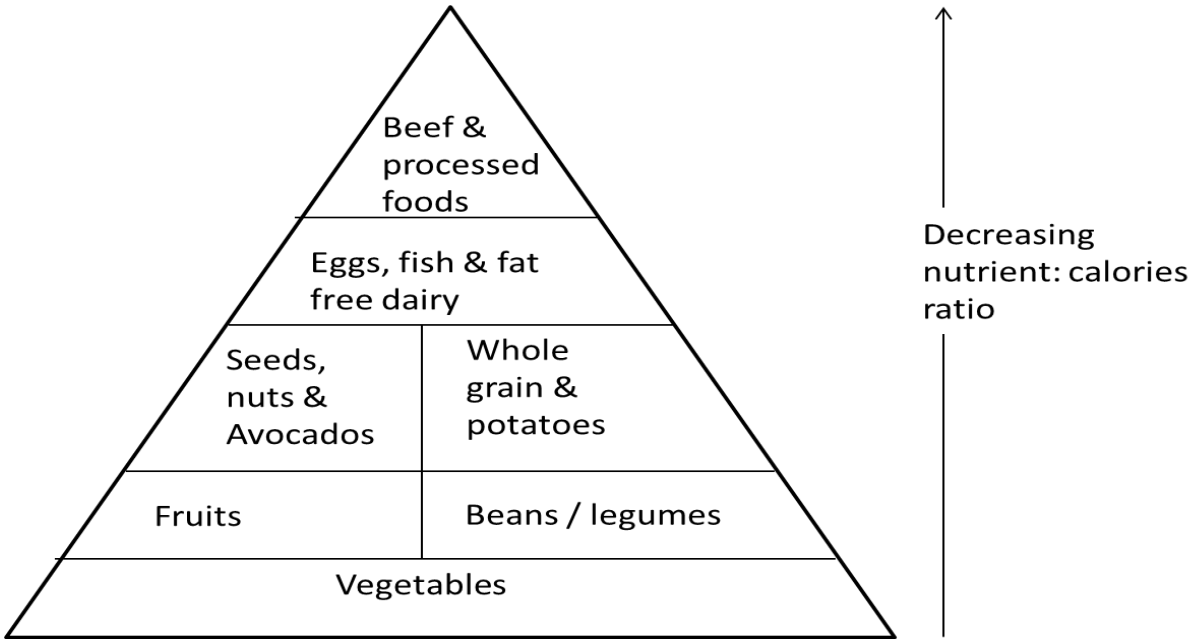

Figure 2.2: Dr. Fuhrman's nutritarian food pyramid (Fuhrman, 2012).

The micronutrient levels that have been shown to be adequate for quality yielding bean crops and high-quality beans, and WHO recommended daily dietary intakes for adults are shown in Table 2.1. The sufficiency ranges for bean leaves are based on the analysis of most recent mature leaves.

Table 2.1: Micronutrient sufficiency ranges for high quality yielding bean crops, dry grains and the range of daily dietary intake for adults

	*Sufficiency range (mg kg^{-1})		**Range of daily dietary intake for adults(mg day^{-1})
Micronutrient	Bean leaves	Dry grains	
Mn	21 – 300	27 – 35	2.2 – 8.6
Fe	50 – 350	70 – 77	8.1 – 30
Ni	0.05 – 5	Not available	0.1 – 0.4
Cu	5 – 30	2 – 6	1.1 – 2.0
Zn	21 – 80	37 – 45	8.3 – 14

(*Vitosh et al., 1994); **WHO, 1996)

2.4.1 Manganese

Mn is usually taken up by plants in the form of Mn^{2+} and is essential in nitrogen fixation, root growth, chloroplast production, energy transfer and photosynthesis. Plants grown on mineral soils with high pH and organic soils are mostly known to have Mn deficiencies.

Various researches have been carried out on Mn levels in beans in Kenya and the rest of the world. A study carried out by Maina et al. (2012) on the concentration of trace elements in beans from Eastern Kenya gave total Mn concentrations of 33 – 98 mg kg^{-1}. In 2009, Tinsley (2009) carried out a research on the micronutrient levels in beans in Eldoret, Kenya and reported a value of 10 mg kg^{-1}.A research by Adamu et al. (2015) on Nigerian beans gave concentrations of 1.0 and 8.4 mg kg^{-1} for common bean and African yam bean respectively.

2.4.2 Iron

Plants uptake of Fe from the soil is in the form of Fe^{2+}. It is essential in plants for photosynthesis and synthesis of chlorophyll. In human beings, some of the functions include carrying oxygen from lungs to other body tissues and transport of electrons within body cells. Food crops provide non-heme iron whose absorption rate is 2-10% (FAO, 2001). Although it is a micronutrient, Fe is also categorized as one of the toxic heavy metals.

Various studies have been carried out on the levels of iron in beans in Kenya and the rest of the world. For instance, in their research on beans from Eastern Kenya, Maina et al. (2012)

reported total Fe values of 227 – 647 mg kg^{-1}. Another study carried out on beans from Eldoret, Kenya gave a value of 82 mg kg^{-1} (Tinsley, 2009). Kimani et al. (2006) also carried out research on different varieties of beans in Kenya and reported Fe values ranging from 68 – 124 mg kg^{-1} mg kg^{-1}. A value of 20 mg kg^{-1} was reported from Nigerian beans by Adamu et al. (2015) while Kimani et al. (2006) reported a concentration of 89 mg kg^{-1} from Rwanda beans.

2.3.1.3 Nickel

From the soil, plants take up Ni in form of Ni^{2+}. In plants, Ni is involved in nitrogen metabolism and fixation. A study by Adriano (2001) showed that the concentrations of Ni in natural vegetation and field grown crops ranged from 0.05 – 5.0 mg kg^{-1}. Taber (2009) gives the concentration ranges of Ni in plants as 1 – 10 mg kg^{-1}.

2.3.1.4 Copper

Cu^{2+} is taken from the soil by plants and it is important in photosynthesis, synthesis of chrophyll and plant respiration. In their study of assessing the levels of micronutrients in beans in Eastern Kenya, Maina et al. (2012) reported total Cu values ranging from 17 – 28 mg kg^{-1} while Tinsley (2009) gave a value of 9.6 mg kg^{-1} in beans sampled from Eldoret, Kenya. Adamu et al. (2015) reported a value of 2.3 mg kg^{-1} on Nigerian beans.

2.3.1.5 Zinc

Plants take up Zn^{2+} from the soil and/or as $Zn(OH)_2$. Zn plays an important role in plant hormone balance. According to FAO (2001), body stores of Zn are limited and therefore it (the body) depends on regular supply from the daily diet. It is thus essential that the diet that one consumes is sufficient in Zn.

Research by Maina et al. (2012) gave total Zn concentration values of 22 – 42 mg kg^{-1} for beans from Eastern Kenya. Tinsley (2009) gave 28 mg kg^{-1} in beans from Eldoret, Kenya while Kimani et al. (2006) reported a range of 16 – 35 mg kg^{-1} for different bean varieties cultivated in Kenya. In 2015, Adamu et al. (2015) reported a value of 10.1 mg kg^{-1} in their research on Nigerian common beans while Kimani et al (2006) reported values of 31, 28 and 35 mg kg^{-1} for Zn concentrations in Uganda, Ethiopia and Rwanda beans, respectively.

2.5 Plant Analysis

Visual symptoms of micronutrient deficiency are useful indicators when used with other diagnostic tools. Some of the useful essential micronutrient deficiency symptoms are:

Mn – chlorosis, that is, the yellowing of leaves, between veins in young leaves
Fe – chlorosis especially between the veins of new leaves
Ni – leaf chlorosis
Cu – light chlorosis, twisted leaf tips and loss of turgor pressure (water pressure which prevents wilting of leaves) especially in young leaves
Zn – stunted growth

These visual symptoms alone are however not reliable in the determination of micronutrient deficiencies since they can also be a sign of other nutrient deficiencies, drought, disease, herbicide injury, soil and climatic factors which affect plant growth (Gupta et al., 2008). For instance, soil texture and pH affect the concentration of micronutrients in plants. The texture affects the ability of the soil to retain water and nutrients. For example, in sandy soils, leaching is high and as water drains, it carries nutrients and thus depriving the plant of these nutrients. When the soil pH increases, micronutrients become less available except Mo, which becomes more available with increase in pH since in acidic conditions, it is strongly held by iron and aluminium hydroxides. This makes it less available for plant uptake at low pH (Gupta et al., 2008).

Plant tissue analysis is therefore a better method of determining the nutrients that are present in the food crops and their concentrations. In plant analysis, it is assumed that there exists a relationship between plant health and the levels of chemical constituents (Mills and Jones, 1996). Slight micronutrient deficiencies may not have visual symptoms on plants but may lead to reductions in crop yields and 'unhealthy' crops and thus resulting in food insecurity, malnutrition and 'hidden hunger' (Bennett, 1993).

2.6 Instrumentation

2.6.1 Total reflection X-ray Fluorescence (TXRF)

In X-ray fluorescence analysis, the incident radiation ejects an inner shell electron from an atom. This results in the atom being in an excited state and thus unsTable. An electron from a higher energy level transits to fill the vacancy in the inner shell and the excess inner shell binding energy is emitted as characteristic radiation (IAEA, 2009). The emitted characteristic X-rays are detected and a spectrum obtained. The emitted rays have different energies specific for the different elements and thus the spectrum obtained is used for qualitative and quantitative analysis of the elements present in the sample (IAEA, 2009).

The principle of total reflection X-ray fluorescence is that an X-ray beam is generated by an air-cooled X-ray tube with Mo target (Bruker, 2015). A multi-layer monochromator then reduces the generated X-ray beam to a narrow energy range and it is this fine beam which impinges on the sample carrier (Si) at a critical angle of $<0.1^0$ thus resulting into total reflection. The sample emits characteristic fluorescence which is measured by an energy dispersive X-ray detector (Bruker, 2015) (Figure 2.3).

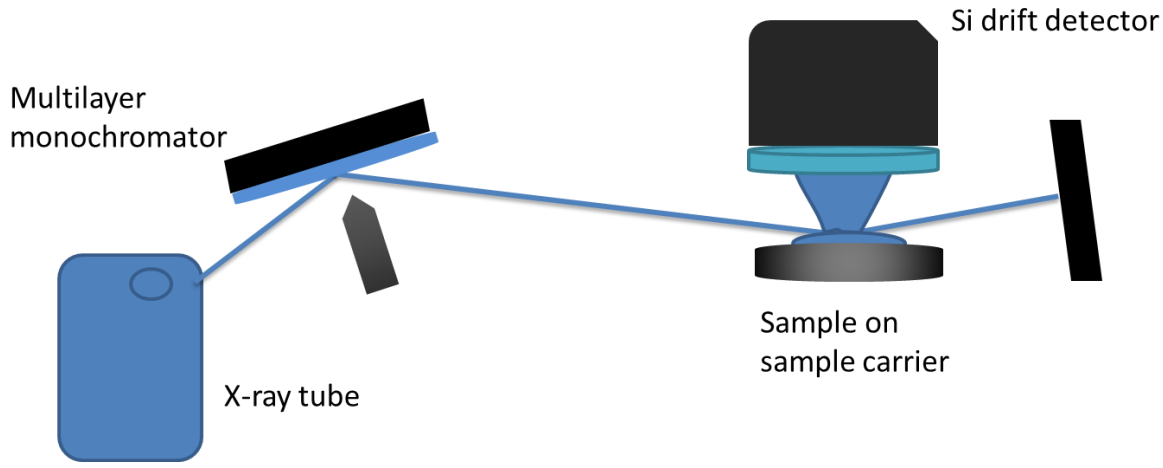

Figure 2.3: Total reflection X-ray Fluorescence (TXRF) setup adopted from Bruker (2015)

TXRF is advantageous over conventional XRF spectrometers in that the sample is very small and it is illuminated by a totally reflected beam. Therefore the probability of the sample matrix absorbing and scattering the beam is reduced (Bruker, 2015). Background noise is also significantly reduced and thus there is increased sensitivity (Bruker, 2015; IAEA, 2009). Additionally, the distance between the detector and the sample is small leading to a large solid angle, which allows maximum detection of fluorescence from the sample.

TXRF as an elemental analysis technique is increasingly becoming of interest in food quality analysis since it is a fast and easy technique. The measurement time is short (100 – 1000 s), uses small amounts of sample and the required sample preparation is fairly easy and fast. The ability to conduct direct analysis (Klockenkamper, 1997) makes TXRF a preferred method of analysis.

2.6.2 Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES)

This technique is based on the spontaneous emission of photons from atoms and ions, which are excited in a radiofrequency (RF) discharge. A liquid sample is injected into the RF-induced Ar plasma where it is converted to an aerosol and directed to the plasma's central channel. The core of the plasma is at high temperatures (up to 10,000 K) and the aerosol is vaporised resulting in liberation of the analyte elements as free atoms in their gaseous states. There is also collisional excitation in the plasma, which imparts additional energy on the atoms promoting them to excited states. The energy in the plasma is enough to ionize the atoms and the resulting ions are also promoted to excited states (Hou and Jones, 2000). Both excited atoms and ions undergo transition to the ground state by photon emission. The emitted photons are focused by a concave mirror or lens forming an image of the ICP on a monochromator/polychromator (Hou and Jones, 2000). The specific wavelength that exits the monochromator/polychromator is converted to an electric signal by a photo detector. The detector electronics amplify and process the signal, which is displayed and stored in a personal computer. The emitted photons are of elemental characteristic wavelengths/energies, which are used for the determination of sample constituent elements. The total number of emitted photons of a specific energy is proportional to the relevant element concentration in the sample.

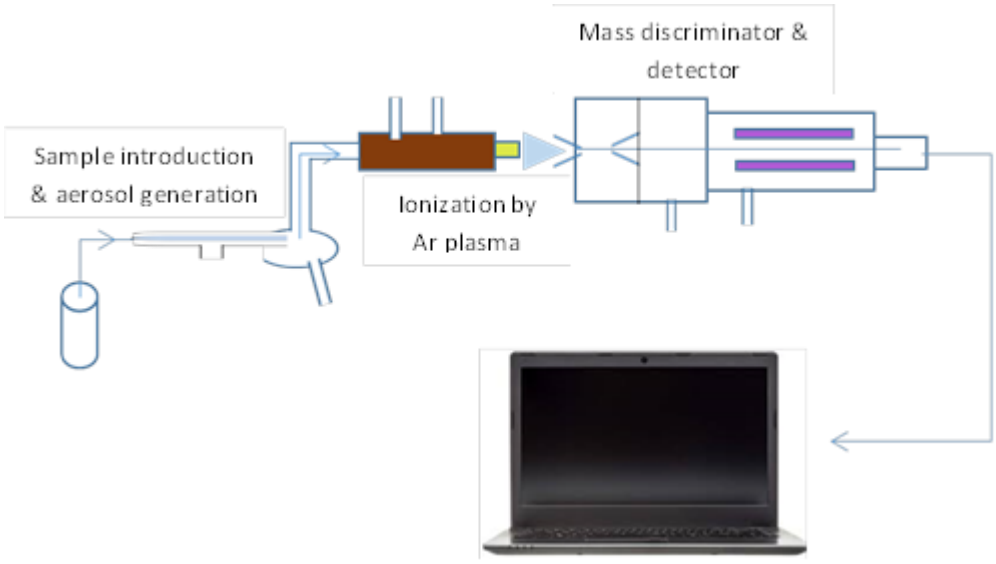

Figure 2.5: Inductively Coupled Plasma – Optical Emission Spectrometry **(**ICP-OES) working principle (Hou and Jones, 2000)

2.7 Validation of the Analytical Method

For the validation of the analytical method, IAEA certified reference samples were analysed and the experimental values compared with certified values. The *Student t distribution* is used for the comparison of the experimental values with the certified reference values to determine the applicability, accuracy, repeatability and reproducibility of the methods. In method validation, samples are analysed in replicates to ensure precision.

t is calculated using the equation:

$$t = \frac{\dot{x} - \mu}{\frac{s}{sqrt(n)}}$$

Where $\dot{x}$ - mean of the analysed reference values

μ - the certified reference value

s – standard deviation of the analysed reference values

n – number of measurements

In t-test if $t_{calc} < t_{tab}$, there is no significant difference between the experimental and reference values (IAEA, 2003).

2.8 Method Comparison

For TXRF and ICP-OES methods comparison, the student t distribution and Analysis of Variance (ANOVA) were used.

2.8.1 Student t distribution

In student t distribution, the pooled standard deviation is first calculated using the equation:

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

The t value is calculated by:

$$t = \frac{|x1 - x2|}{s_p \sqrt{\frac{1}{n1} + \frac{1}{n2}}}$$

Where: s_p – pooled standard deviation

s_1 – element standard deviation by TXRF

s_2 – element standard deviation by ICP-OES

n_1 – number of measurements by TXRF

n_2 – number of measurements by ICP-OES

x_1 – mean value from TXRF

x_2 – mean value from ICP-OES

At 95% confidence level, the calculated t_{calc} value is then compared with the tabulated t_{tab} value at $t_{(0.05,\ n1\ +\ n2\ -\ 2)}$. If $t_{calc} < t_{tab}$, it means that there is no significant difference between the two means thus no significant difference between the two methods.

2.8.2 Analysis of Variance (ANOVA)

Analysis of variance is used for testing significant differences between means. It is based on the fact that variances can be partitioned (IAEA, 2003). The variance is calculated as the sum of the squared deviations from the mean, divided by n-1 (n-1 is sample size minus 1). The test is based on a comparison of the mean square effect (variance due to between-groups variability) and the mean square error (variance due to within-group variability). To decide whether there are significant differences in variances, the one-tailed F-test (Fisher test) is used. If $F < F_{\text{Table}}$, there is no significant difference at the given probability, for example at 95% probability. The ANOVA Table also gives the *p-value* which is used to determine the relationship between means. At 95% confidence level, $\alpha = 0.05$, therefore if the p-value is greater than or equal to 0.05, there is no significant difference between the means (IAEA, 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The research was carried out in Muguga area (1.25° S, 36.66° E) in Kiambu County and Kyevaluki area (1.33° S, 36.91° E) in Machakos County. In both areas, the residents practise small scale farming both for domestic consumption and as an economic activity. The main food crops grown in these areas are maize, beans and peas and the farming is rain dependent.

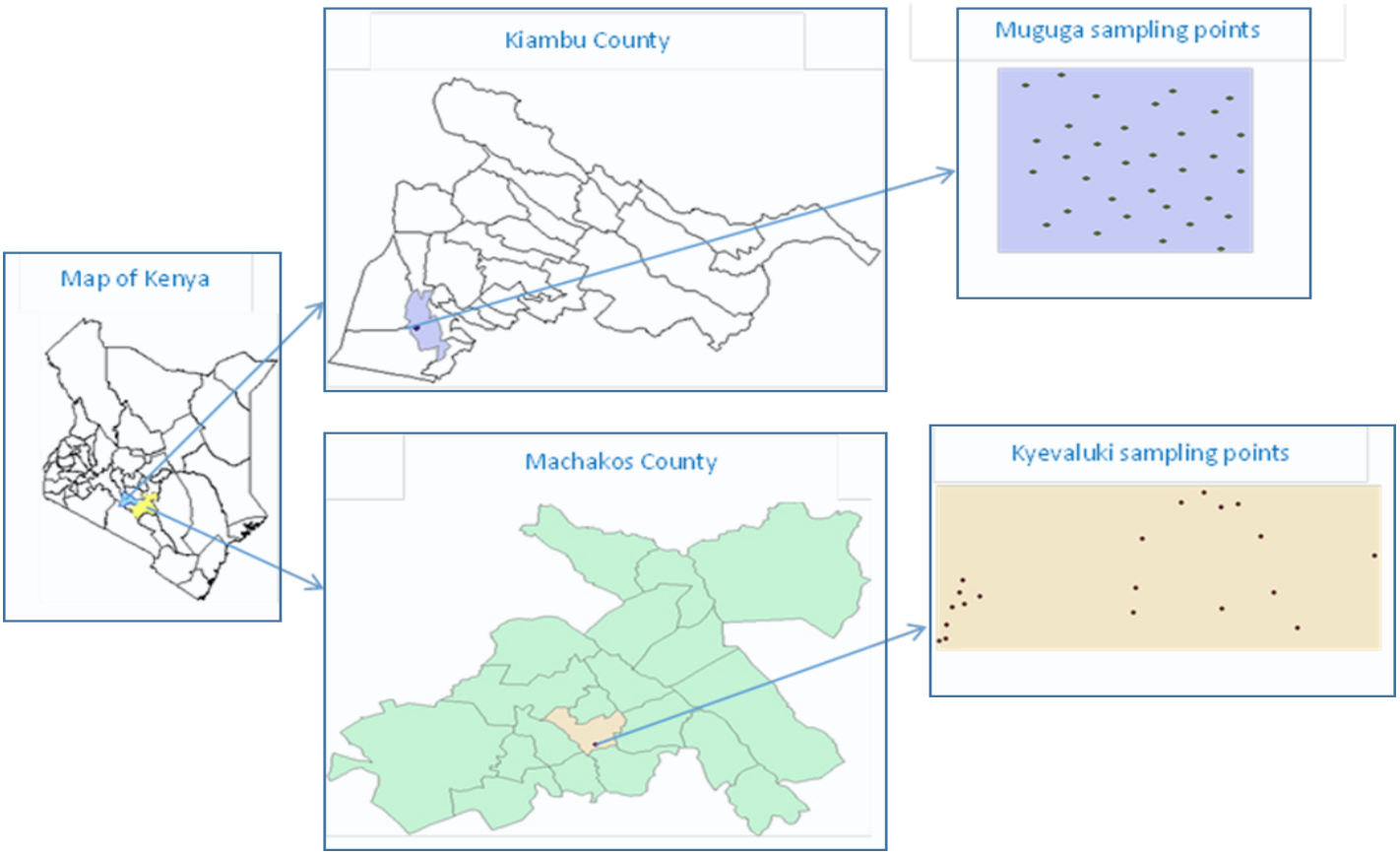

Figure 3.1: Map of Kenya showing the study areas

3.2 Sample Collection

3.2.1 Sampling of dry bean grains and bean-leaves

In sampling dry grains, random sampling was followed in which samples were purchased from individual farmers. The bean species sampled were the ones which are most commonly grown in each of the two sampling areas. From Kyevaluki, the samples collected were Rosecoco, Pinto beans (locally known as Mwitemania) and Nyayo species while Red Haricot

(locally referred to as Wairimu), Pinto beans, Nyayo and Rosecoco species were sampled from Muguga. Thirty one samples of dry bean grains were collected and of these, 1 kg of each of the seven species was transported for laboratory analysis.

For bean leaves sampling, a random sampling design with offset grid sampling pattern was used. This involved marking out plots of 100*100 meters (Figure 3.2).

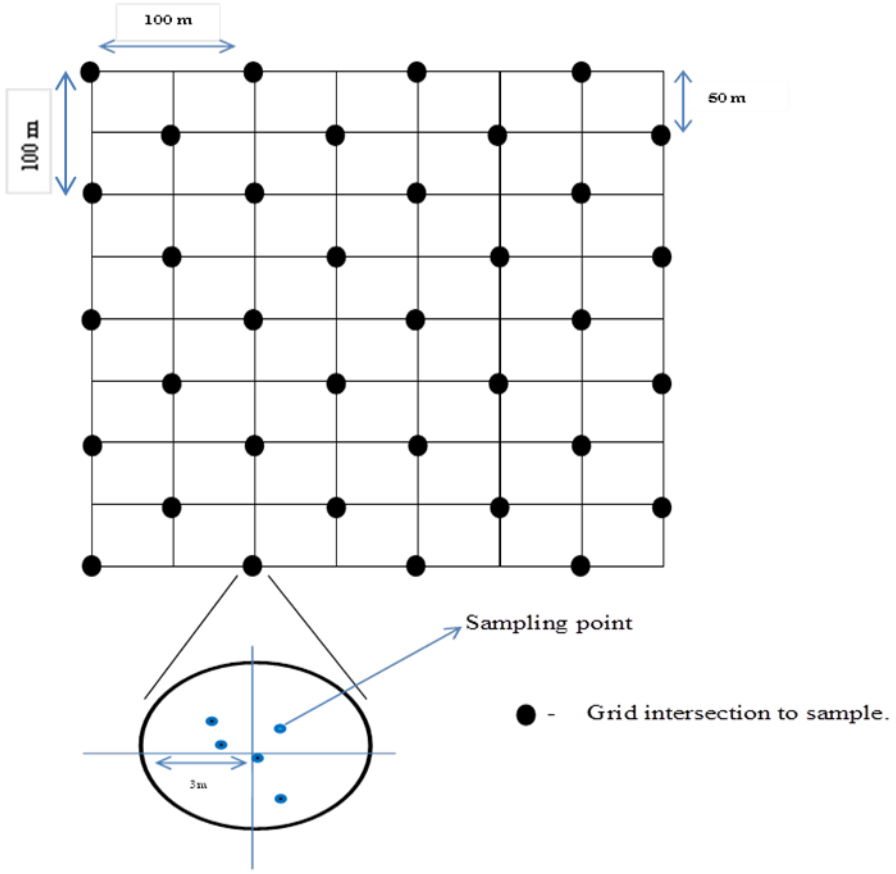

Figure 3.2: Sampling Pattern for bean leaves (Birch, Oom and Beecham, 2007).

Samples were randomly collected within a radius of 3 meters from the grid intersection and composited. A total of 32 bean leaf samples from each sampling area were collected into porous bags and labelled. Sampling was carried out during initial bloom and the most recent mature leaves (MRML) were collected which included sampling of 20 – 30 leaves at each grid point. At each of the sampling points, spatial coordinates were taken. All the samples were then transported to the World Agroforestry Centre (ICRAF) laboratories for preparation and laboratory analysis by use of TXRF and ICP-OES.

3.3 Sample Preparation

The collected bean and leave samples were cleaned with deionized water to remove any traces of dust, fertilizer residues, pesticides and any other foreign particles. The bean leave samples were then oven dried at 60 ^{0}C for 48 hours while the dry grains were oven dried at 100 ^{0}C for 48 hours. After drying the samples were ground to between 20-53 µm particle sizes using a micronizing mill (Glen Creston McCrone micronizing mill) and placed in well-labelled zip-lock polythene bags.

3.3.1 Sample preparation and analysis by use of Total reflection X-Ray Fluorescence (TXRF)

Total reflection X-Ray Fluorescence (TXRF) analysis was carried out as per the procedure described by ICRAF (2011) in which approximately 45 mg of the sample was weighed into a clean-labelled vial. The actual weight of the sample was recorded to the nearest 0.01 mg. To this sample, 2.5 ml of aqueous Triton X100 solution was added to make a slurry. Internal standards of 40 µL of 1000 ppm Sc and 10 µL of 1000 ppm Y were then added. The sample was mixed using an agitator and placed in a water bath with sonics applied for 15 minutes to ensure thorough homogenization. After sonication, mixing was done using a vortex mixer and 10 µl of the suspension pipetted to the center of a silicon sample carrier. The loaded sample carrier was dried at 50 ^{0}C on a hot plate for about 10 minutes resulting in formation of a thin layer of the sample. Multi-element analysis was then carried out using TXRF, where each sample was analysed for 600 s.

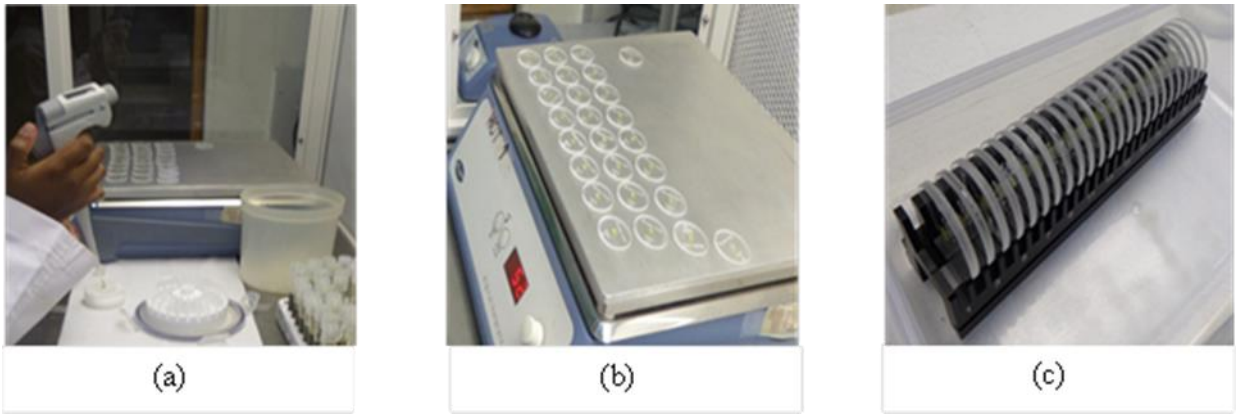

Figure 3.3: Pictures showing (a) suspension of sample pipetting onto a sample carrier, (b) drying on hot plate and (c) cassette with sample carriers ready for loading onto a TXRF instrument.

The S2 Picofox bench top TXRF was used for this study and its specifications are tabulated in Table 3.1.

Table 3.1: Technical specifications of the S2 Picofox TXRF spectrometer

Element range	**Na to U**
Sample carrier	Quartz, 30 mm diameter
Detector	Silicon drift detector
X-ray tube	50 kV, 1mA air-cooled Mo target
X-ray optics	Multilayer monochromator
Sample station	25 disk cassette
Voltage, frequency,	100 – 240 V, 50 – 60 Hz
Manufacturer	Bruker

Figure 3.4: Bench top TXRF for sample analysis at ICRAF.

3.3.2 Sample preparation and analysis by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES)

Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) analysis was carried out as per the procedure described by NRF (2010). Approximately 0.5 g of the sample and 0.5 g of the reference material were weighed into glass tubes. The plant reference material used was the IAEA certified 'ARC / CL-PP' reference. To each of these samples 5 ml of concentrated HNO_3 was added and the samples left to stand overnight in a thermolyne-heating block at 50 ^{0}C. The temperature was then increased to 90 ^{0}C for 2 hours to ensure

complete digestion. The samples were then diluted with deionized water to 50 ml, mixed using a vortex mixer and 10 ml of each pipetted into plastic vials and multi element analysis done on ICP-OES as shown in Figure 3.5.

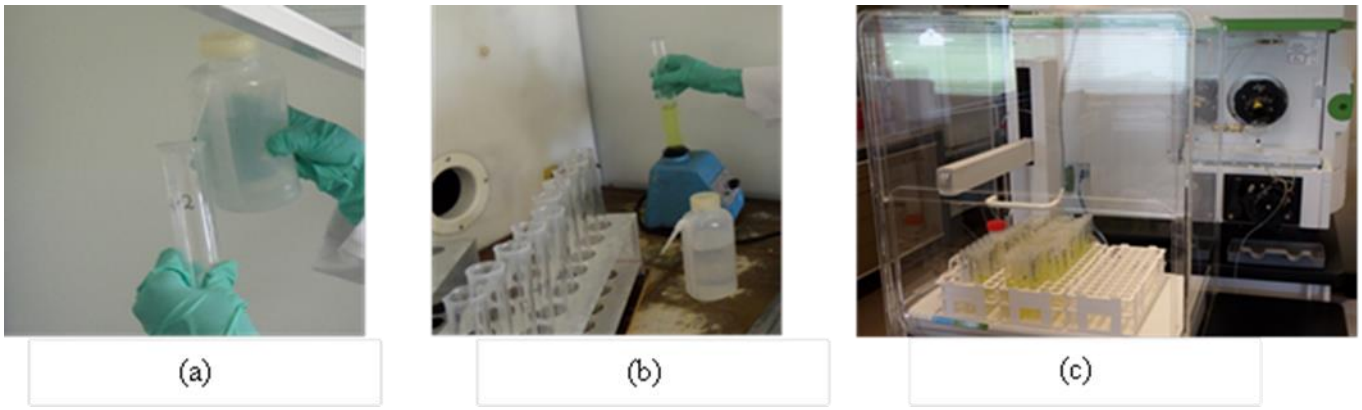

Figure 3.5: Pictures showing; (a) sample dilution with deionized water, (b) homogenization using a vortex mixer and (c) sample analysis by ICP-OES.

The ICP-OES used for this study was the PerkinElmer Optima 8300 bench top spectrometer (Figure 3.6) and its technical specifications are given in Table 3.2.

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

Detector	**Segmented-array Charge-coupled Device (SCD) detector**
RF generator	40 MHz solid state RF generator
Polychromator	High energy (f/6.7) echelle-based optima polychromator
Cooling	Water-recirculating cooling system @4 L/min
Manufacturer	PerkinElmer

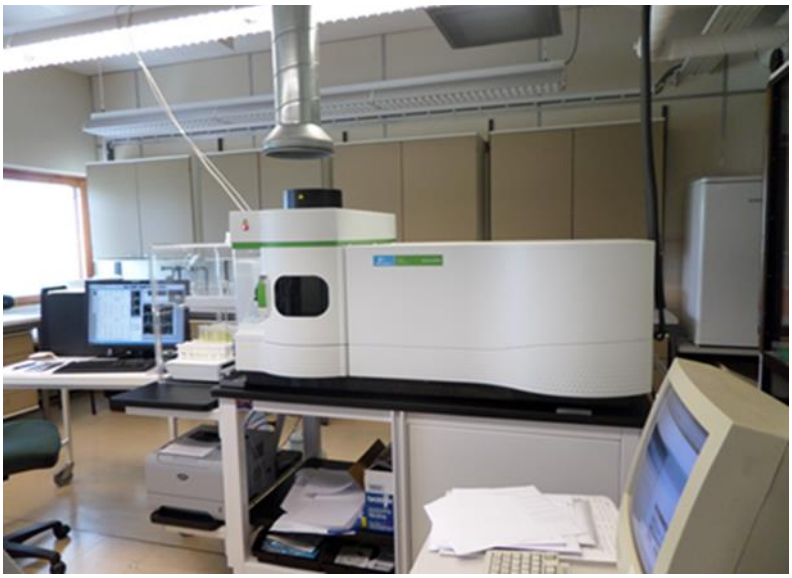

Figure 3.6: PerkinElmer 8300 bench top ICP-OES for sample analysis at MTT Finland.

3.4. Method Validation

For validation of the analytical method, reference samples were analysed and the experimental values compared with certified values. Each of the reference samples were analysed in three replicates to ensure precision. The testing was done at 95% confidence level using n-1 degrees of freedom. ARC / CL-PP (potato powder) biological reference material was used.

3.5 Method Comparison

To compare TXRF and ICP-OES methods of analysis, the bean leaf samples from Muguga were prepared and analysed by both TXRF and ICP-OES as per the procedures described in sections 3.3.1 and 3.3.2. Student t distribution and analysis of variance (ANOVA) statistical methods were then used to make comparisons between the results obtained from the use of both methods.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Method Validation

Table 4.1: Certified, experimental and t-values for ARC / CL-PP certified reference material

	Concentrations in mg kg^{-1}	**n = 3**	
Element	Experimental values	Reference values	t value
Mn	8.4 ± 0.5	8.0 ±0.7	1.0
Fe	25 ± 1.3	22 ± 1.8	3.1
Cu	3.8 ± 0.0	3.6 ± 0.4	0.9
Zn	9.0 ± 0.5	8.8 ± 1.1	0.5

t_{tab} ($_{0.05, 2}$) = 4.3. From the obtained results t_{calc} was less than 4.3 (t_{tab}) and this showed that the method was suitable and thus results obtained were accurate and applicable.

4.2 Micronutrient concentration in bean leaves from Muguga, Kiambu County (TXRF results)

The minimum, maximum, mean and standard deviation values for the bean laves from Muguga are shown in Table 4.2. Fe had the highest concentrations for all the samples followed by Mn, and then Zn, Cu, while Ni had the lowest concentrations.

Table 4.2: Micronutrient concentration in bean leaves from Muguga – TXRF results

	Concentration (mg kg^{-1})				
	Mn	Fe	Ni	Cu	Zn
Minimum	118	292	1.1	6.9	54
Maximum	296	1277	4.1	14	81
Mean	214	759	2.3	9.3	65
Standard deviation	52	220	0.8	1.8	8.9

In statistical analysis, some outlier values were observed in the obtained results for Fe, Ni and Cu and these were not included in the calculation of the statistical values shown in Table 4.2. The observed outlier values for Fe were 1648 mg kg^{-1} and 2070 mg kg^{-1} while the outlier values for Ni and Cu were 5.1 mg kg^{-1} and 19 mg kg^{-1} respectively. The concentration ranges and the observed outlier values for the five micronutrients were represented by use of box plots (Figure 4.1). Since these values significantly differed from other data points, it was concluded that they were random variations and thus not included in the calculations.

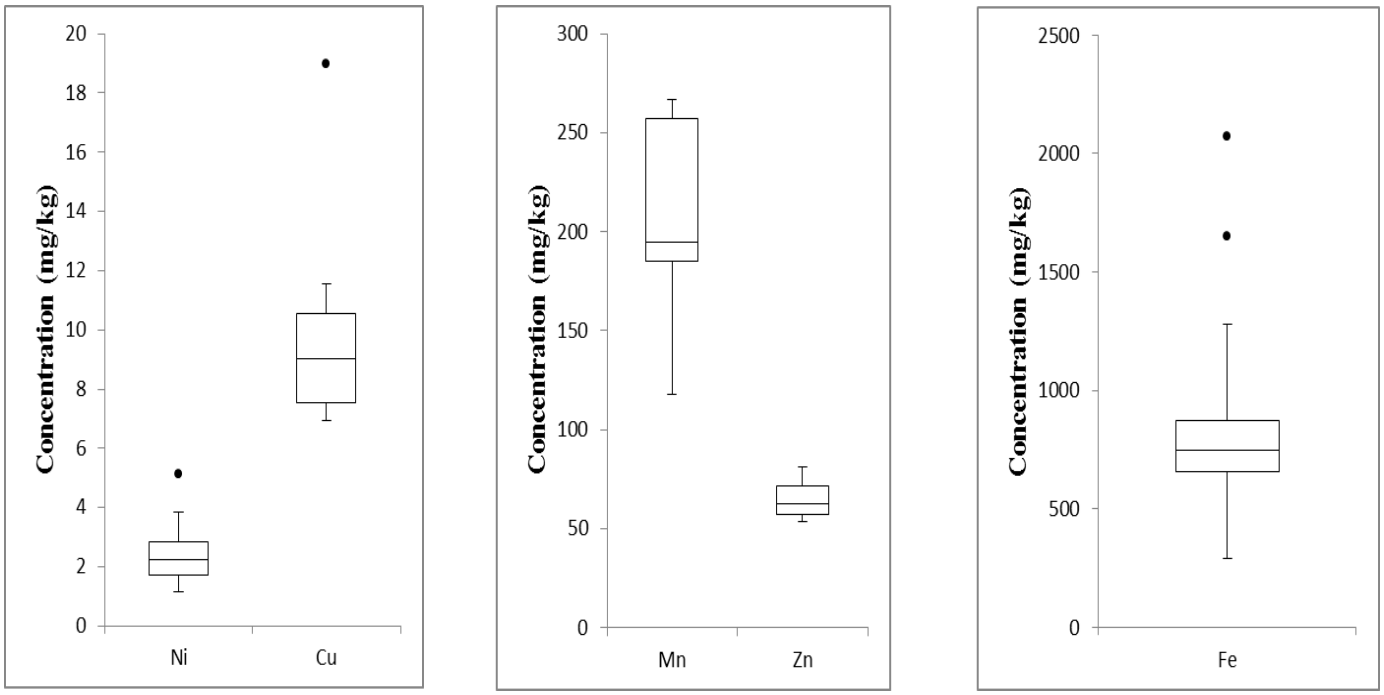

Figure 4.1: Observed micronutrient concentration ranges for bean leaves from Muguga (TXRF results).

The Mn, Ni and Cu concentration ranges observed in this study were 118 - 296, 1.1 – 4.1 and 6.9 - 14 mg kg^{-1} respectively (Figure 4.2). These were the three of the five studied elements that had all the analysed samples being within the sufficiency ranges obtained from literature. Vitosh et al. (1994) gives 21 – 300 mg kg^{-1}, 0.05 – 5 mg kg^{-1} and 5 – 30 mg kg^{-1} as the Mn, Ni and Cu (respectively) sufficiency ranges for high quality yielding bean crops. The comparisons of observed values and the sufficiency ranges are given in Figure 4.2.

From this study, Zn concentration ranged from 54 - 81 mg kg^{-1} with 6.3% of the analysed samples having concentrations above the general sufficiency range of 21 – 80 mg kg^{-1}. Comparing these levels to a previous study (Wangila et al., 2014) on micronutrient levels in bean leaves in Kenya, the Zn levels obtained were higher than the reported Zn mean of 17 mg kg^{-1}.

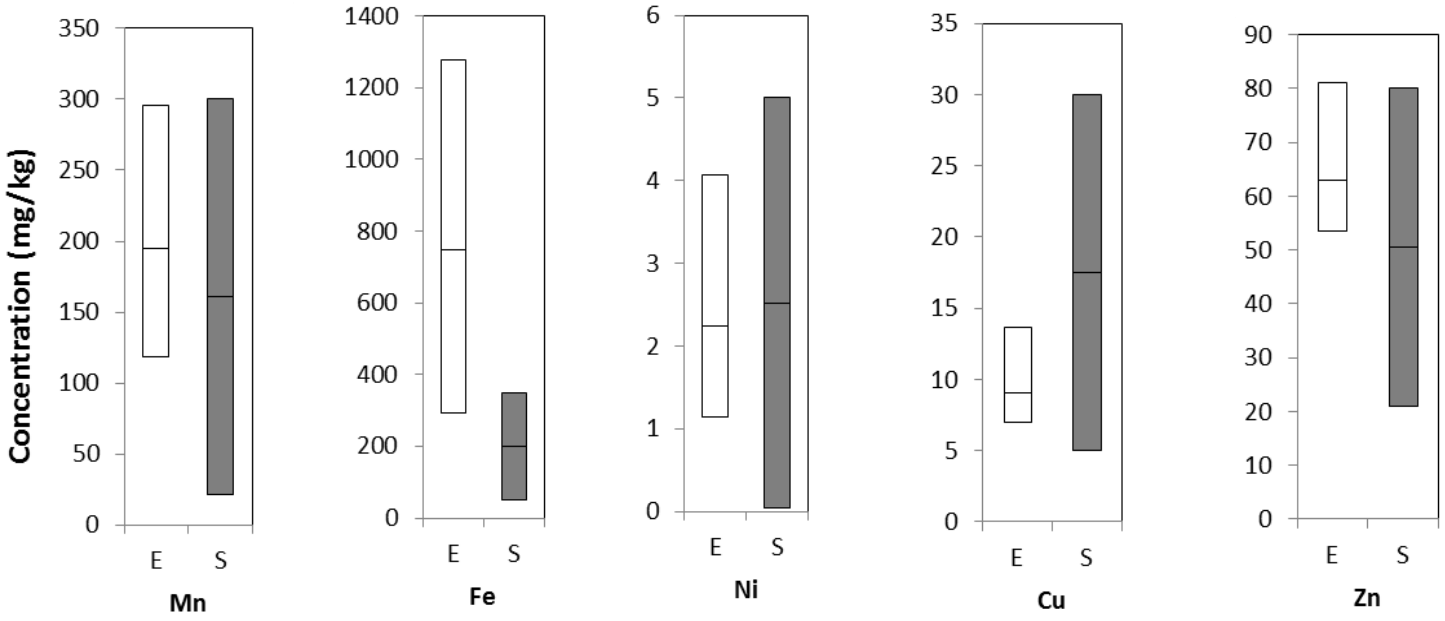

Figure 4.2: Comparison of observed elemental ranges (E) with sufficiency ranges for high quality yielding bean crops (S) – TXRF results

The concentrations of Fe were within the range of 292 – 1277 mg kg^{-1} (Figure 4.2). Iron was the only element which was observed to be consistently high in concentration with 97% of the analysed samples being above the sufficiency range of 50 – 350 mg kg^{-1} given by Vitosh et al. (1994) as shown in Figure 4.2. In their study on the Fe levels in bean leaves in Kenya, Wangila et al. (2014) reported a mean value of 213 mg kg^{-1} and this meant that the Fe levels obtained from this study were higher.

Fe levels greater than 800 mg kg^{-1} are considered toxic (FAO, 2006) and in this study, 50% of the samples had values greater than 800 mg kg^{-1}. The primary causes of iron toxicity in plants are soil pH, and soil moisture (soils being very wet or flooded). In acid soils, soil saturation with water results to poor aeration and thus too much iron may become available for plant uptake (Rout and Sahoo, 2015). This could be the reason for the high Fe levels but it is not possible to conclusively prove since this study did not include soil analysis. The observed high levels of iron could also be as a result of contamination by dust especially during grinding of the samples. Fe absorption from beans is low due to inhibitory compounds like phytic acid and polyphenols with phytic acid being the major inhibitor (Petry et al.,

2015). Therefore, since this study gave total Fe concentrations, the high concentrations do not translate to high bioavailable levels.

The potential of bean leaves as a source of micronutrients has not been studied much. Since bean leaves are not widely consumed in Kenya, little research has been carried out to determine their nutrient composition and thus in this study, a comparison with previous studies was only done for Fe and Zn.

4.3 Micronutrient concentration in bean leaves from Muguga, Kiambu County (ICP-OES results)

For method comparison between TXRF and ICP-OES, 18 out of the 32 samples analysed using TXRF were analysed using ICP-OES. The concentration values from the highest to the lowest were Fe>Mn>Zn>Cu>Ni. This was similar to the trend that was observed by use of TXRF. Table 4.3 shows the minimum, maximum, mean and standard deviation values for the analysed samples while Figure 4.3 is a boxplot representation of the experimental concentration ranges for each of the elements with the observed outlier values. Outlier values were observed in Fe, Ni, Cu and Zn and these values were 1902 mg kg^{-1} and 5.6 mg kg^{-1} for Fe and Ni respectively. The outlier value in the statistical analysis of Cu levels was 14 mg kg^{-1} while Zn gave outlier values of 44 mg kg^{-1}, 81 mg kg^{-1} and 77 mg kg^{-1}. These values were not included in the statistical analysis shown in Table 4.3.

Table 4.3: Micronutrient concentration in bean leaves from Muguga – ICP-OES results

	Concentration (mg kg^{-1})				
	Mn	Fe	Ni	Cu	Zn
Minimum	107	319	1.2	5.0	51
Maximum	291	1148	4.2	11	68
Mean	201	662	2.3	7.6	58
Standard deviation	51	203	0.9	1.93	4.8

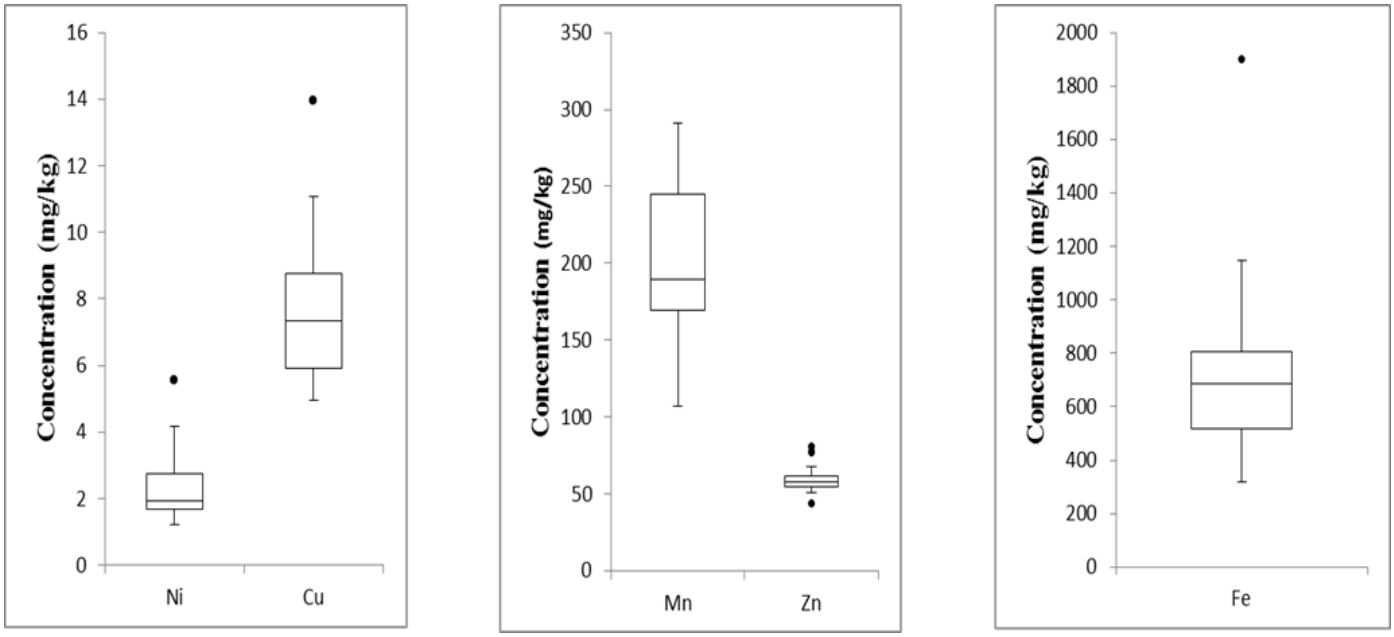

Figure 4.3: Observed micronutrient concentration ranges for bean leaves from Muguga (ICP-OES results)

The observed micronutrient levels were compared with literature sufficiency ranges for high quality yielding bean crops and this was represented in box plots as shown in Figure 4.4. The literature sufficiency ranges are Mn: 21 – 300 mg kg^{-1}, Fe: 50 – 350 mg kg^{-1}, Ni: 0.05 – 5 mg kg^{-1}, Cu: 5 – 30 mg kg^{-1} and Zn: 21 – 80 mg kg^{-1} (Vitosh et al., 1994). From this study, Mn concentrations ranged from 107 – 291 mg kg^{-1} and this was within the sufficiency range of 21 – 300 mg kg^{-1} as shown in Figure 4.4. Fe concentration range was found to be 319 – 1148 and of these, 94% of the analysed samples had concentration values higher than the sufficiency range of 50 – 350 mg kg^{-1} (Figure 4.4). In this study, 50% of the samples had values greater than 800 mg kg^{-1}, FAO (2006) toxic value limit. Ni and Zn gave concentration values of 1.2 – 4.2 and 51 – 68 mg kg^{-1} respectively. These concentrations were within the sufficiency ranges of 0.1 – 5 and 21 – 80 mg kg^{-1} respectively. The sufficiency range for Cu in bean leaves is 5 – 30 mg kg^{-1} (Vitosh et al., 1994) and from the study, the concentration range was 5.0 – 11 mg kg^{-1}. However, 6% of the samples were below the minimum concentration of 5 mg kg^{-1} while 29% of the samples gave concentrations close to the lower limit of 5 mg kg^{-1} (Figure 4.4). No deficiencies were observed for Mn, Fe, Ni and Zn.

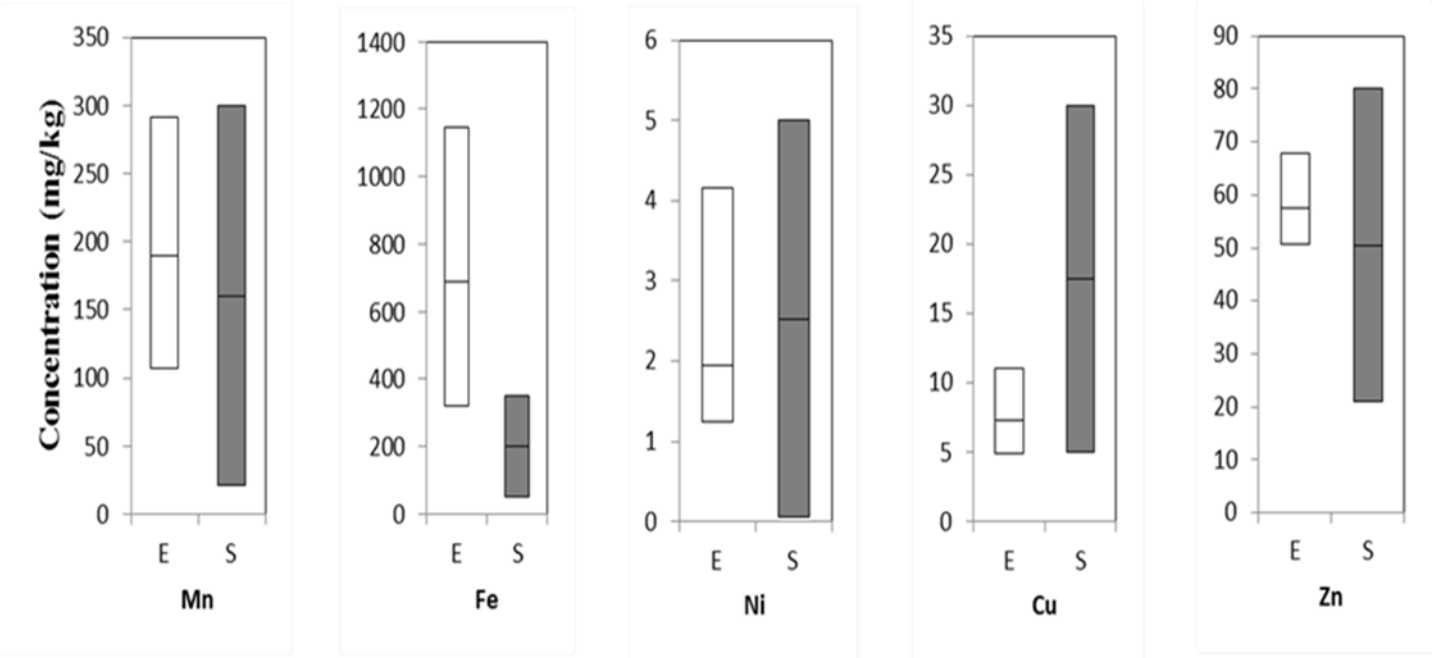

Figure 4.4: Comparison of experimental elemental ranges (E) with sufficiency ranges for high quality yielding bean crops (S) – ICP-OES results.

4.4 Comparison of TXRF and ICP-OES results

4.4.1 Student t distribution

The *Student t distribution* method of testing two means was used to compare the two methods. The testing was done at 95% confidence level. The calculated t value was then compared with the tabulated t_{tab} value at $t_{(0.05, (n1 + n2 - 2))}$. An F test was first carried out to determine whether the variances were equal and this is shown in Table 4.4. F was calculated by: $F = \frac{S_1^2}{S_2^2}$

Table 4.4: *F* test data for determining whether the variances are equal

F-Test Two-Sample for Variances										
	Mn		Fe		Ni		Cu		Zn	
	TXRF	ICP-OES	TXRF	ICP-OES	TXRF	ICP-OES	TXRF	ICP-OES	TXRF	ICP-OES
Mean	224	201	747	632	2.3	2.3	8.7	7.6	62	58
Variance	2803	2605	36651	27358	0.9	0.8	2.3	3.7	47	23
Observations	18	18	16	16	17	17	17	17	15	15
Df	17	17	15	15	16	16	16	16	14	14
F calculated	**1.1**		**1.3**		**1.1**		**1.6**		**2.1**	
F Critical	**2.3**		**2.4**		**2.3**		**2.3**		**2.5**	

If *F* calculated is less than *F* critical, the assumption that the variances are equal is made and thus t is calculated using the formula $t = \frac{|X_1 - X_2|}{s_p\sqrt{\frac{1}{n1} + \frac{1}{n2}}}$ where s_p, which is the pooled standard deviation is calculated by $s_p = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)\, s_2^2}{(n1 + n2 - 2)}}$

From Table 4.4, *F* calculated was less than *F* critical for all the five analysed elements and this implied that the variances obtained by analysis using TXRF and ICP-OES were equal for each of the elements. *t* test was then done to determine whether the two methods of analysis gave similar results or significantly different results.

The comparison between *t* calculated values and *t* tabulated values is given in Table 4.5.

Table 4.5: Comparison between t_{calc} and t_{tab}

	Mn	Fe	Ni	Cu	Zn
Df (n1+n2-2)	34	30	32	30	28
t_{calc}	1.349	1.819	0.083	1.811	1.537
t_{tab}	2.032	2.042	2.037	2.042	2.048

Df – Degrees of freedom, t_{calc} – t calculated values, t_{tab} – t tabulated values

From the analysis, t_{calc} was found to be less than t_{tab} for all the five elements as shown in Table 4.5. This meant that the two methods produced similar results. Further comparison of the two methods was carried out by analysis of variance (ANOVA).

4.4.2 Analysis of Variance (ANOVA)

The results obtained by analysis of variance are given in Table 4.6.

Table 4.6: ANOVA test results for comparing TXRF and ICP-OES

	Mn	Fe	Ni	Cu	Zn
F_{calc}	1.8	3.3	0.01	3.3	2.4
***P*-value**	0.2	0.1	0.9	0.1	0.1
F_{tab}	4.1	4.2	4.1	4.1	4.2

The testing was done at 95% confidence level and from the obtained results, the calculated F values (F_{calc}) were less than tabulated F values (F_{tab}) for the five elements. This meant that there were no significant differences between the means at 95% probability. The ANOVA test also gives the *p-value* and this provides additional information on the relationship between the means being compared. At 95% confidence level, p = 0.05 and if the obtained *p-value* is greater than or equal to 0.05, statistically, there is no significant difference between the means.

The obtained p-values in this study were greater than 0.05 as shown in Table 4.6. This further showed that for each of the five elements, TXRF and ICP-OES gave similar concentration levels. The results obtained in this study were in agreement with literature reports for similar studies. In their research on the application of TXRF in foodstuff analysis, Dalipi et al. (2017) compared results obtained from analysis of honey samples using TXRF, AAS and ICP-MS and found that TXRF gave results that were comparable to both AAS and ICP-MS.

Marguí et al. (2014) also compared the use of TXRF and ICP techniques for the determination of trace elements in edible clams and observed that there were no significant differences between TXRF and ICP results for concentrations above 5 mg kg^{-1} and thus TXRF was suiTable for analysis of trace elements in food. They however concluded that for concentrations lower than 5 mg kg^{-1}, ICP techniques were more promising. These results are similar to the findings of Elzain et al. (2016) in their comparison of XRF, PIXE and ICP-OES methods of analysis for analysis of medicinal plants. Elzain et al. (2016) concluded that XRF, PIXE and ICP-OES gave similar results for Fe and Zn. They also concluded that ICP-OES was the more preferable method for the determination of low concentration elements like Ni (Elzain et al., 2016).

4.5 Micronutrient concentration in bean leaves from Kyevaluki, Machakos County

Table 4.7 shows the statistical values for bean leaves sampled from Kyevaluki in Machakos County. Fe concentrations were found to be the highest followed by Mn, Zn, Cu and Ni concentrations were the lowest.

Table 4.7: Micronutrient concentration in bean leaves from Kyevaluki.

	Concentration (mg kg^{-1})				
	Mn	Fe	Ni	Cu	Zn
Minimum	49	121	1.1	6.9	21
Maximum	109	321	2.0	9.8	36
Mean	76	219	1.5	8.4	27
Standard deviation	17	65	0.2	0.8	3.9

The obtained results were compared with the sufficiency ranges for high quality yielding bean crops. These sufficiency ranges are, Mn: 21 – 300 mg kg^{-1}, Fe: 50 – 350 mg kg^{-1}, Ni: 0.1 – 5 mg kg^{-1}, Cu: 5 – 30 mg kg^{-1} and Zn: 21 – 80 mg kg^{-1} (Vitosh et al., 1994). All the analysed elements were within the sufficiency ranges as shown in Figure 4.10.

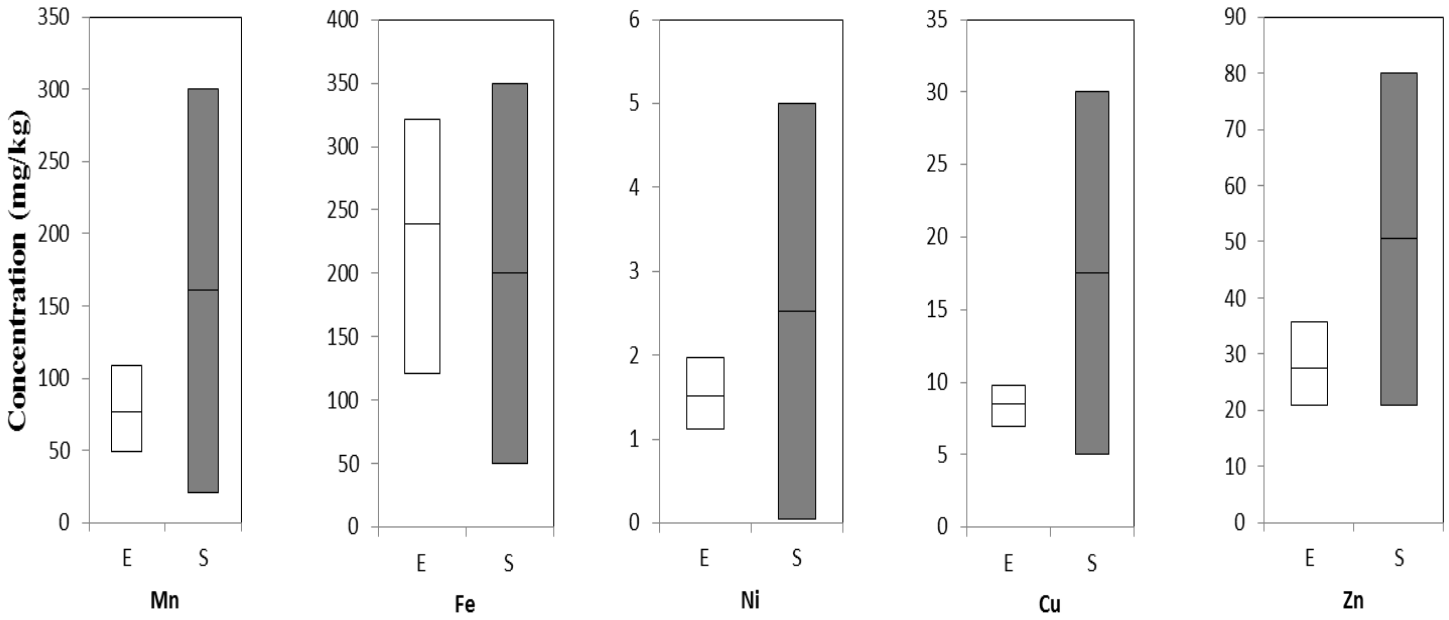

Figure 4.5: Comparison of results obtained from Kyevaluki beans (E) with sufficient ranges for high quality yielding bean crops (S)

The mean Fe concentration in bean leaves from Kyevaluki was 219 mg kg^{-1} and this was comparable to the mean Fe concentration of 213 mg kg^{-1} reported by Wangila et al. (2014). However, in comparing Zn concentrations from this study of 27 mg kg^{-1} were higher than the 17 mg kg^{-1} reported by Wangila et al. (2014).

The concentration levels of micronutrients in bean leaves from Muguga and Kyevaluki were compared to determine whether there were any differences. To statistically test the differences in the concentrations from one area to the other, analysis of variance (ANOVA) test was carried out at $\alpha = 0.05$. The results are represented in Table 4.8.

Table 4.8: Comparing micronutrient levels for bean leaves from Muguga and Kyevaluki

	Mn	Fe	Ni	Cu	Zn
P-value	2.9E-11	3.0E-10	0.001	0.1	1.6E-17

From Table 4.8, *P-values* for Mn, Fe, Ni and Zn were less than 0.05 and therefore the means were significantly different. This meant that the concentrations of Mn, Fe, Ni and Zn in bean leaves from Kyevaluki, Machakos County were different from bean leaves from Muguga, Kiambu County. Samples from Muguga were consistently high in the four micronutrients as shown in Figures 4.6 and 4.7. The mean concentration of Mn in samples from Muguga was 2.8 times higher than Mn concentration in samples from Kyevaluki.

Fe showed the greatest difference between the regions with mean concentration of Muguga samples being 3.5 times higher than Kyevaluki samples. The mean concentration of Ni in Muguga samples was 1.5 times that of Kyevaluki samples while the mean concentration of Zn in Muguga samples was 2.4 times that of Kyevaluki samples.

The only element that was found to have comparable concentrations between Muguga and Kyevaluki was Cu with a *P-value* of 0.1 (Table 4.8). The mean concentration of Cu in Muguga samples was 1.1 times the mean concentration of Cu in Kyevaluki samples but this was not a statistically significant difference.

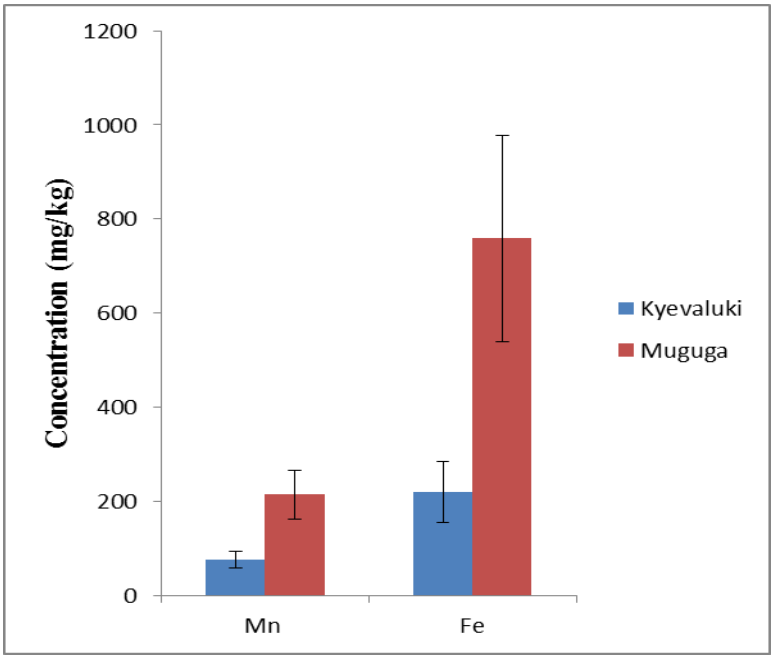

Figure 4.6: Comparison of Mn and Fe concentrations between Kyevaluki and Muguga

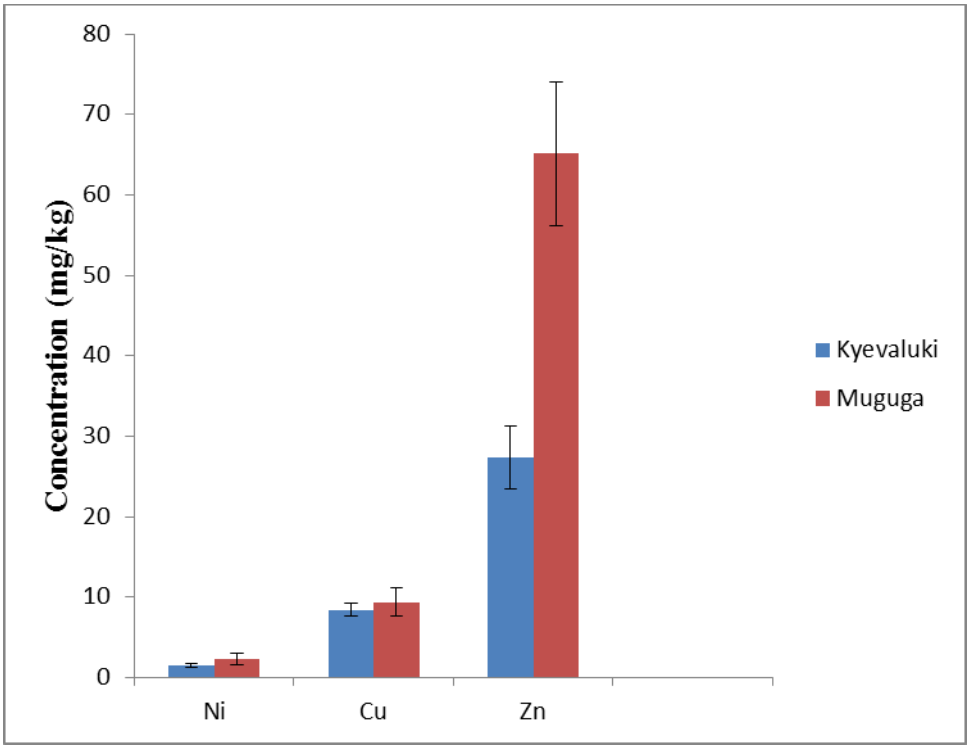

Figure 4.7: Comparison of Ni, Cu and Zn concentrations between Kyevaluki and Muguga

4.6 Micronutrient Concentration in Beans (Dry Grains)

The average concentrations of the seven bean types sampled from both Kyevaluki, Machakos County and Muguga, Kiambu County are given in Table 4.9.

Table 4.9: Elemental concentrations of the seven bean types (dry grains) from different regions of Muguga & Kyevaluki.

Bean Type	Sampling area	Concentration (**mg kg^{-1}**)				
		Mn	**Fe**	**Ni**	**Cu**	**Zn**
Rosecoco	Kyevaluki, Machakos county	27	87	0.9	6.1	23
Pinto bean		32	92	1.2	7.7	30
Nyayo		25	95	0.8	6.1	26
Red Haricot	Muguga, Kiambu county	21	89	0.9	5.3	25
Rosecoco		26	120	0.6	6.3	23
Nyayo		28	73	0.8	6.8	25
Pinto bean		24	91	0.9	6.3	25

The results were compared with sufficient ranges for high quality beans and this is shown in Figures 4.8 and 4.9. Comparison of literature sufficiency ranges and results obtained in this study were done for Mn, Fe, Cu and Zn. Ni concentration levels were not compared with literature sufficiency values since Ni sufficiency ranges for dry bean grains were not found. The sufficiency ranges were Mn: 27 – 35 mg kg^{-1}, Fe: 70 – 77 mg kg^{-1}, Cu: 2 – 6 mg kg^{-1} and Zn: 37 – 45 mg kg^{-1} (Vitosh et al., 1994).

Four of the seven bean types (57%), that is, Kyevaluki *Nyayo*, Muguga Red Haricot, Muguga Rosecoco and Muguga Pinto bean, had concentrations below the minimum sufficiency level for Mn. Although Kyevaluki Rosecoco and Muguga *Nyayo* did not show any deficiencies, their concentrations were very close to the Mn minimum level of 27 mg kg^{-1}, they gave concentrations of 27 and 28 mg kg^{-1} respectively. This could mean that with continued depletion from the soils, deficiencies could be observed in the near future. Of the different beans, only Kyevaluki Pinto bean gave a Mn concentration that could be said to be sufficient (32 mg kg^{-1}).

The Mn levels reported in this study were found to be lower than a previous study by Maina et al. (2012). They reported mean Mn concentrations of 65 mg kg^{-1}, 68 mg kg^{-1}, 46 mg kg^{-1} and 38 mg kg^{-1} in beans from Machakos, Kitui, Mwingi and Makueni districts respectively.

Tinsley (2009) reported Mn levels of 10 mg kg^{-1} in beans from Eldoret and this value was lower than those reported in this study.

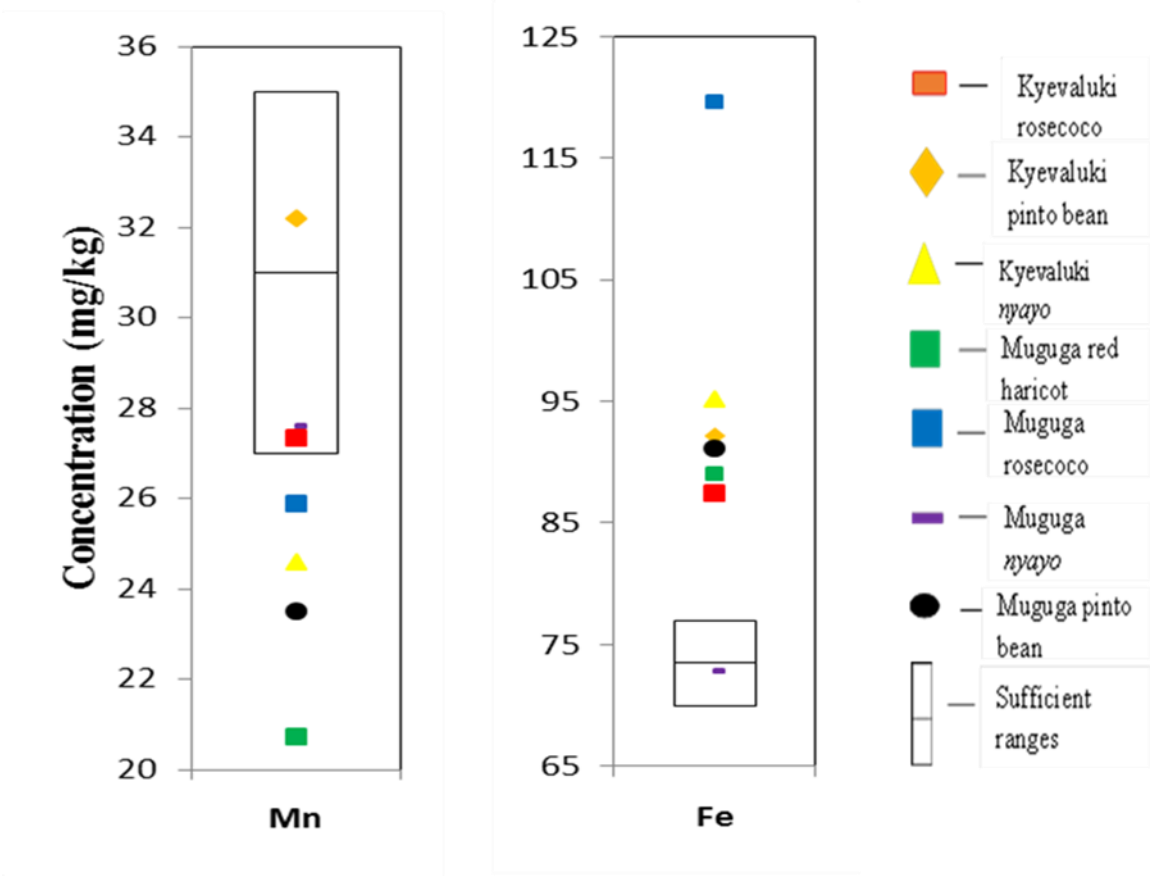

Figure 4.8: Comparison of Mn and Fe concentrations with sufficient ranges for high quality beans

All the analysed samples except Muguga *Nyayo* had Fe concentrations that were above the maximum sufficient level of 77 mg kg^{-1} (Figure 4.8). Although the Fe levels observed in the beans were above the sufficiency range, they were below the toxic levels of 800 mg kg^{-1} (FAO, 2006).

Maina et al. (2012) reported mean Fe levels of 366 mg kg^{-1}, 446 mg kg^{-1}, 396 mg kg^{-1} and 391 mg kg^{-1} in beans from Machakos, Kitui, Mwingi and Makueni districts respectively. These values were higher than the mean Fe levels in this study. The Fe levels reported in this study are comparable to the mean Fe concentration of 82 mg kg^{-1} reported by Tinsley (2009) in beans sampled from Eldoret. Okoth (2005) reported Fe content in beans from western province of Kenya as 184 mg kg^{-1}; these values were found to be higher than those found in this study. Kimani et al. (2006) reported Fe values in Pinto beans and Red Haricot beans

sampled from different parts of the country as 68 mg kg^{-1} and 93 mg kg^{-1} respectively. Their Red Haricot concentrations are comparable to the concentrations obtained in this study but the Pinto beans concentrations are lower than those found in this study.

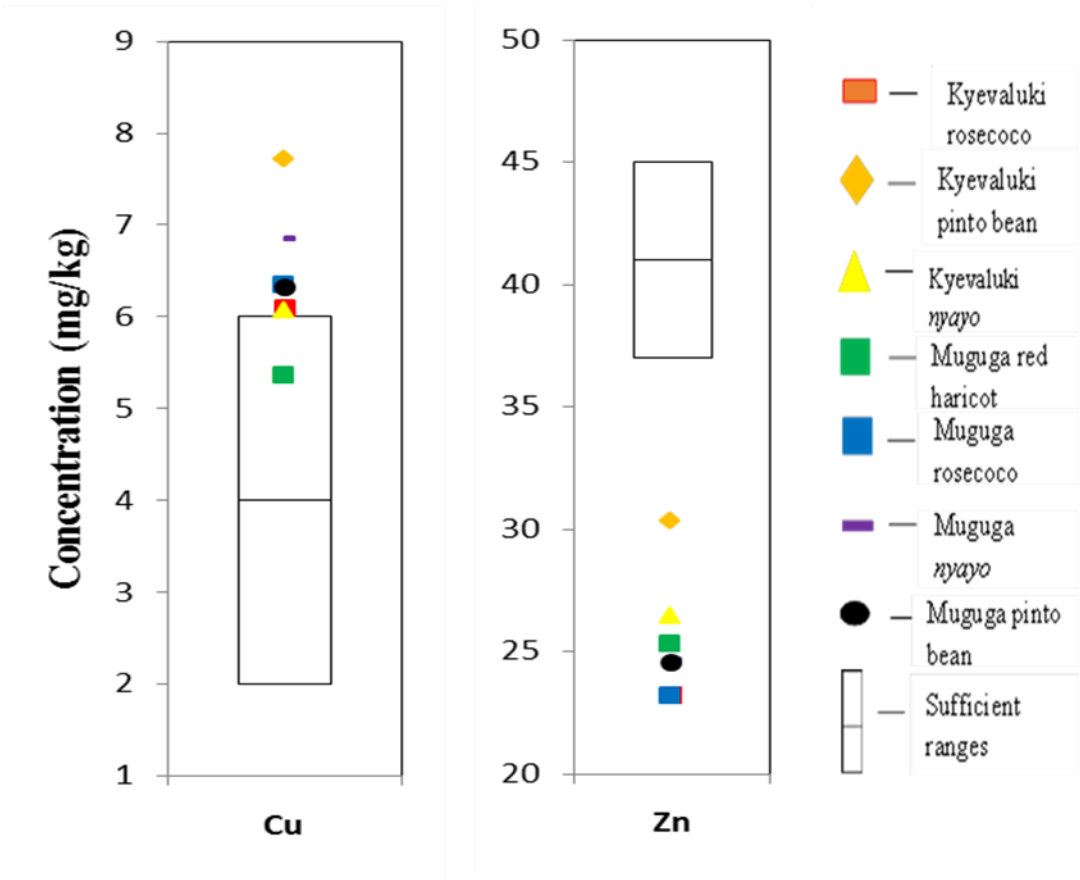

Figure 4.9: Comparison of Cu and Zn concentrations with sufficient ranges for high quality beans

Rosecoco and *Nyayo* beans from Kyevaluki and Red Haricot beans from Muguga had Cu concentrations that were within the sufficiency ranges. The Cu concentration in Rosecoco, Pinto beans and *Nyayo* beans from Muguga and Pinto beans from Kyevaluki were only slightly higher than the sufficiency range (Figure 4.9).

The Cu concentrations in this study were significantly lower than those reported by Maina et al. (2012). Maina et al. (2012) reported mean Cu concentrations of 28 mg kg^{-1}, 22 mg kg^{-1}, 18 mg kg^{-1} and 17 mg kg^{-1} in beans from Machakos, Kitui, Mwingi and Makueni districts respectively. Comparing this study to a study by Tinsley (2009), the Cu concentrations in this

study were also found to be lower than the concentration of 9.6 mg kg^{-1} reported by the same author in beans from Eldoret.

All the samples were found to have Zn concentrations lower than the minimum sufficient level as shown in Figure 4.9. However, these values were similar to the concentration of 27.9 mg kg^{-1} reported by Tinsley (2009) in beans from Eldoret. The Zn concentrations in this study were consistently lower than those reported by Maina et al. (2012). They reported Zn values of 32 mg kg^{-1}, 42 mg kg^{-1}, 34.5 mg kg^{-1}, and 34 mg kg^{-1} in beans from Machakos, Kitui, Mwingi and Makueni districts respectively. Kimani et al. (2006) reported mean Zn values of 16 mg kg^{-1} and 35 mg kg^{-1} in Pinto beans and Red Haricot beans respectively. The Red Haricot Zn concentrations were higher than those found in this study while the Pinto bean concentrations were lower.

Comparison of each of the bean types between the two areas was done using student t test was to determine whether there were any significant differences between the two sampling areas for the same bean type and these are represented in Tables 4.10, 4.11 and 4.12. Testing was done at 95% confidence level.

Table 4.10: Comparison of Rosecoco beans from Kyevaluki and Muguga using t test

	Rosecoco beans				
	Mn	Fe	Ni	Cu	Zn
t_{calc}	1.8	12	4.0	-1.7	0.6
$t_{critical}$	2.8	2.8	2.8	2.8	2.8

If $t_{calc} < t_{critical}$, there is no significant difference between the means. Mn, Cu and Zn concentrations were similar in the Rosecoco beans in the two areas. Significant differences were observed in Fe and Ni concentrations with Rosecoco beans from Muguga having higher Fe concentrations while Rosecoco beans from Kyevaluki had higher Ni concentrations. The biggest difference was in Fe concentrations.

When comparing Pinto beans from Muguga and Kyevaluki, significant differences were reported in Mn, Ni, Cu and Zn concentrations (Table 4.11). Fe concentrations were found to be comparable.

Table 4.11: Comparison of Pinto beans from Kyevaluki and Muguga using t test

	Pinto beans				
	Mn	Fe	Ni	Cu	Zn
t_{calc}	3.8	0.2	3.2	9.9	8.2
$t_{critical}$	2.8	2.8	2.8	2.8	2.8

The biggest differences in concentration were found in Zn with Pinto beans from Kyevaluki having higher concentrations than those from Muguga. In general Pinto beans sampled from Kyevaluki had higher concentrations than those sampled from Muguga although the differences in Fe concentrations were not statistically significant.

Micronutrient concentrations in Nyayo beans were also compared and this is shown in Table 4.12. Mn, Ni and Zn concentrations in *Nyayo* beans were found to be comparable. However, significant differences were observed in Fe and Cu concentrations (Table 4.12).

Table 4.12: Comparison of Nyayo beans from Kyevaluki and Muguga using t test

	Nyayo beans				
	Mn	Fe	Ni	Cu	Zn
t_{calc}	-0.7	9.1	-1.5	-4.4	2.6
$t_{critical}$	2.8	2.8	2.8	2.8	2.8

Fe concentrations were found to have the highest differences. Nyayo beans from Kyevaluki had higher Fe concentrations than those from Muguga while Nyayo bean samples from Muguga had higher Cu concentrations than those from Kyevaluki.

In general, differences in concentrations were observed when the beans were compared from one area to the other. Differences in Fe and Ni concentrations were observed in Rosecoco beans while differences in Mn, Ni, Cu and Zn concentrations were found in Pinto beans. Nyayo beans had Fe and Cu concentrations that were statistically different. No single bean type or sampling area was found to have all the micronutrient concentrations being consistently high or low. These observed differences could be due to the differences in seed quality, use of fertilizers and also differences in soil types and soil properties like pH, moisture and organic matter.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In the analysis of micronutrients in bean leaves, sufficient concentrations of Mn, Ni and Cu were found in bean leave samples from Muguga, Kiambu County. The concentrations of these three elements were within the sufficiency ranges for high yielding bean crops. Considering bean leaf samples from Muguga, 94% had Zn concentrations that were within the sufficiency range. However, Fe concentrations in bean leaves from Muguga were consistently higher than the sufficiency range with 50% of the samples having higher concentrations than the FAO (2008) reported toxic levels of 800 mg kg^{-1}. In this study however, the total element concentration levels were analysed and not the bioavailable levels. Generally, the highest concentrations were reported in Fe, followed by Mn, Zn, Cu and the lowest concentrations being Ni.

The bean leaves sampled from Kyevaluki, Machakos County were found to have Mn, Fe, Ni, Cu and Zn concentration levels that were within the sufficiency ranges for high yielding bean crops. Just like the bean leaves from Muguga, the concentration levels of the analysed micronutrients in bean leaves from Kyevaluki followed the order Fe>Mn>Zn>Cu>Ni.

When comparing micronutrient concentrations in bean leaves from the two sampling areas, at $\alpha = 0.05$, Mn, Fe, Ni and Zn concentrations were found to be significantly different. Bean leaves from Muguga had Mn, Fe, Ni and Zn concentrations that were significantly higher than the concentrations of bean leaves from Kyevaluki. However, although the Cu concentrations in bean leaves from Muguga were slightly higher than bean leaves from Kyevaluki, there were no significant differences between the two areas.

When comparing TXRF and ICP-OES methods of analysis, at 95% confidence level, there were no significant differences between results obtained by use of both methods. This was true for comparisons done by both student t distribution and ANOVA. However, TXRF was the preferred method of analysis since it did not involve time consuming sample digestion and the use of hazardous chemicals like ICP-OES which involved the use of concentrated

HNO_3. Additionally, the less sample preparation in TXRF minimizes errors and since it uses internal standardization, element quantification was easier.

The micronutrient concentration in dry bean grains followed the order Fe>Mn>Zn>Cu>Ni, which was the same order that was observed in bean leaves. Seven bean types were analysed and four of these, that is, Kyevaluki *Nyayo*, Muguga Red Haricot, Muguga Rosecoco and Muguga Pinto beans had Mn concentrations that were lower than the minimum sufficiency level for high quality beans. Although Rosecoco beans and *Nyayo* beans from Kyevaluki and Muguga respectively did not show Mn deficiency, their concentrations were only slightly above the minimum sufficiency level. Fe concentrations in all bean types except Muguga *Nyayo* were above the sufficiency range but below FAO reported toxic levels. Rosecoco and *Nyayo* beans from Kyevaluki and Red Haricot beans from Muguga had Cu concentration levels within the sufficiency range with the other four bean types having Cu concentrations slightly higher than the sufficiency range.

When the dry bean grains were compared, differences in concentrations were observed from one area to the other. Rosecoco beans were found to have significant differences in Fe and Ni concentrations while Pinto beans had significant differences in Mn, Ni, Cu and Zn concentrations. Significant differences were observed in Fe and Cu concentrations in *Nyayo* beans. No single bean type or sampling area was found to have all the micronutrient concentrations being consistently high or low.

5.2 Recommendations

This study provided useful information on the micronutrient levels in bean leaves and dry grains and the results obtained can be used in making interventions to improve the nutritional quality of beans.

In Kenya most of the households do not use bean leaves as vegetables and since they were found to have sufficient micronutrients levels, recommendations can be made for their consumption as vegetables.

This study showed that TXRF and ICP-OES give similar results in the analysis of trace elements and thus it recommends the use of TXRF since it is faster, fairly easy, does not require the use of hazardous chemicals and involves less sample preparation therefore minimizing errors.

Interventions need to be made on how to improve the micronutrient levels in beans (dry grains) since they are consumed in many households and deficiencies were observed in this study.

Since this study was on micronutrients in plants and nutrient levels are also affected by soil factors like pH and organic matter, further studies which include both soil and plant analyses should be carried out. These studies should also include analysis of different plant parts so as to determine the translocation of micronutrients from soil to roots all the way to the grains. Both soil and plant analyses will also be helpful in the determination of the plants that would be best grown in those soils and this could be helpful advice to the farmers.

Plant based diets can contain high levels of anti-oxidant compounds like phytates, polyphenols and dietary fibre and these can slow and inhibit the absorption of minerals like Mn, Fe and Zn. It is thus important to study the bioavailable levels to determine the amounts that actually end up on the meal Table.

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Appendices

Appendix 1: TXRF results for bean leaves from Kiambu County

SSN	Replicate	K12_Mn	K12_Fe	K12_Ni	K12_Cu	K12_Zn
icr096641	1	194	654	3.0	11	82
icr096642	1	167	871	2.6	8.5	57
icr096643	1	178	762	4.0	11	62
icr096644	1	303	1053	3.0	8.2	68
icr096645	1	277	582	1.7	10	59
icr096646	1	273	818	5.8	14	64
icr096647	1	202	709	2.8	11	79
icr096648	1	131	518	2.6	16	70
icr096649	1	209	882	3.1	11	60
icr096650	1	165	557	3.5	8.9	77
icr096651	1	194	568	2.3	9.0	81
icr096652	1	210	790	2.2	9.3	80
icr096653	1	204	957	2.0	11	61
icr096654	1	313	921	3.9	7.2	76
icr096655	1	262	849	1.9	7.1	81
icr096656	1	145	672	2.9	8.9	63
icr096657	1	174	667	1.1	8.4	55
icr096658	1	213	704	3.3	8.0	71
icr096659	1	286	1035	1.8	7.3	62
icr096660	1	202	781	2.3	13	56
icr096661	1	265	729	3.8	8.7	70
icr096662	1	292	1579	1.3	9.7	62
icr096663	1	239	722	2.9	7.1	75
icr096664	1	250	1125	3.2	18	76
icr096665	1	293	1285	2.7	10	60
icr096666	1	182	757	1.8	7.0	65
icr096667	1	193	379	1.3	7.5	57
icr096668	1	211	1008	3.0	10	69
icr096669	1	278	985	3.0	18	83
icr096670	1	296	2199	1.5	9.0	56
icr096671	1	122	320	1.4	8.7	56
icr096672	1	187	900	2.2	8.1	63
icr096641	2	181	689	2.2	11.6	78
icr096642	2	152	778	1.5	6.8	52

icr096643	2	152	565	3.4	9.2	53
icr096644	2	264	888	2.4	6.4	59
icr096645	2	247	631	1.5	9.2	53
icr096646	2	228	708	4.8	11	54
icr096647	2	180	623	2.7	9.6	72
icr096648	2	108	387	1.8	8.1	55
icr096649	2	186	855	3.4	9.5	55
icr096650	2	138	418	2.4	7.2	64
icr096651	2	170	474	1.9	7.6	71
icr096652	2	177	607	1.8	8.7	68
icr096653	2	171	717	1.3	9.6	51
icr096654	2	264	764	4.1	7.2	65
icr096655	2	256	829	1.8	8.1	79
icr096656	2	134	545	1.3	9.2	58
icr096657	2	173	670	1.1	8.7	54
icr096658	2	202	688	3.1	6.9	67
icr096659	2	268	991	1.4	7.4	57
icr096660	2	185	739	2.5	12	52
icr096661	2	245	608	3.5	8.7	68
icr096662	2	297	1669	1.0	9.9	64
icr096663	2	241	730	3.2	7.7	76
icr096664	2	252	1148	2.9	18	80
icr096665	2	279	1181	1.7	12	57
icr096666	2	200	869	2.0	8.0	73
icr096667	2	183	367	1.3	6.5	57
icr096668	2	198	958	2.7	10	64
icr096669	2	260	894	1.7	10	77
icr096670	2	280	1963	1.1	8.1	52
icr096671	2	115	278	1.9	9.3	56
icr096672	2	185	891	2.6	10	62
icr096641	3	184	627	2.6	11	80
icr096642	3	147	755	2.0	7.0	50
icr096643	3	165	669	3.7	9.6	56
icr096644	3	256	915	1.7	6.3	57
icr096645	3	238	518	1.6	9.2	52
icr096646	3	243	668	4.7	11	55
icr096647	3	183	638	2.6	10	72
icr096648	3	115	425	1.9	8.7	61

icr096649	3	196	883	3.7	9.4	57
icr096650	3	156	523	4.0	7.7	72
icr096651	3	183	515	2.6	9.1	76
icr096652	3	188	607	1.6	9.1	73
icr096653	3	190	841	1.8	11	56
icr096654	3	277	870	4.2	7.1	68
icr096655	3	262	780	1.6	7.1	81
icr096656	3	143	574	2.0	9.0	63
icr096657	3	182	768	1.2	8.7	56
icr096658	3	234	1017	2.7	7.4	74
icr096659	3	285	1137	1.1	7.6	64
icr096660	3	192	734	2.0	14	54
icr096661	3	258	698	2.7	11	71
icr096662	3	298	1697	1.2	12	65
icr096663	3	248	772	2.9	8.1	79
icr096664	3	256	1132	1.9	19	77
icr096665	3	292	1364	1.0	10	60
icr096666	3	196	986	1.6	7.2	70
icr096667	3	199	446	1.9	7.8	61
icr096668	3	209	1074	3.3	11	69
icr096669	3	282	1000	2.0	12	83
icr096670	3	297	2050	1.5	11	55
icr096671	3	121	277	1.3	8.6	58
icr096672	3	195	993	3.0	8.7	66

Appendix 2: ICP-OES results for bean leaves from Kiambu County

	Weight/g	Mn 257		Mn 259		Mn 260	
		mg/L		mg/L		mg/L	
Calib Blank 1		[0,00]		[0,00]		[0,00]	
ST1		[1,0]		[1,0]		[1,0]	
ST2		[5,0]		[5,0]		[5,0]	
ST3		[10,0]		[10,0]		[10,0]	
Blank		0.005		-0.001		0.002	
S-1		0.005		-0.002		0.001	
S-2		0.004		-0.002		0.001	
P-1	0.5	0.1	7.7	0.1	7.7	0.1	8.0
P-2	0.5	0.1	8.3	0.1	8.5	0.1	8.7
			8.0		**8.1**		**8.4**
			0.4		**0.6**		**0.5**
173-1-1	0.5	0.5	48	0.5	51	0.5	51
173-1-2	0.5	0.5	47	0.5	49	0.5	49
			48		**50**		**50**
			0.8		**1.3**		**1.3**
42	0.5	1.3	139	1.3	146	1.3	146
43	0.5	1.3	147	1.4	155	1.4	154
44	0.5	2.2	235	2.4	255	2.3	254
45	0.5	2.2	225	2.3	242	2.3	242
46	0.5	2.2	229	2.3	247	2.3	246
48-1	0.5	1.0	102	1.0	107	1.0	107
48-2	0.5	0.9	100	1.0	108	1.0	107
			101		**107**		**107**
			1.3		**0.3**		**0.3**
49	0.5	1.7	180	1.8	192	1.8	191
51	0.5	1.6	172	1.7	183	1.7	182
54	0.5	2.3	247	2.5	263	2.5	262
55	0.5	2.1	217	2.3	236	2.3	236
Blank-1		0.004		-0.002		0	
Blank-2		0.004		-0.003		0	
57-1	0.5	1.2	122	1.3	130	1.3	130
57-2	0.6	1.3	131	1.4	142	1.4	142
			127		**136**		**136**
			6.5		**8.9**		**8.6**
58	0.5	1.4	155	1.6	170	1.6	170
59	0.5	1.8	199	2.0	219	2.0	219
62	0.5	2.2	224	2.4	252	2.4	251
63	0.5	1.5	159	1.6	170	1.6	170
67	0.5	1.5	158	1.7	170	1.7	170
68	0.5	1.6	171	1.7	188	1.7	188
70-1	0.5	2.5	275	2.7	293	2.7	291
70-2	0.5	2.5	271	2.7	294	2.6	292
			273		**293**		**291**
			3.0		**0.9**		**0.9**

	Weight/g	Fe 238			Ni 221		Ni 231		Ni 232		
		mg/L			mg/L		mg/L		mg/L		
Calib Blank 1		[0,00]			[0,00]		[0,00]		[0,00]		
ST1		[5,0]			[0,5]		[0,5]		[0,5]		
ST2		[10,0]			[1,0]		[1,0]		[1,0]		
ST3		[20,0]			[1,5]		[1,5]		[1,5]		
Blank		-0.2			-0.003		-0.003		-0.004		
S-1		-0.2			-0.004		-0.004		-0.004		
S-2		-0.2			-0.003		-0.003		-0.003		
P-1	0.5	0.1	0.2	24	-0.002		-0.003		-0.003		
P-2	0.5	0.1	0.3	26	-0.002		-0.003		-0.002		
				25							
				1.3							
42	0.5	7.0	7.2	797	0.02	1.9	0.01	1.6	0.02	0.02	2.1
43	0.5	5.2	5.4	591	0.03	3.1	0.03	2.7	0.03	0.03	3.4
44	0.5	7.3	7.5	808	0.02	2.4	0.02	1.8	0.02	0.02	2.6
45	0.5	4.8	4.9	516	0.01	1.4	0.01	0.8	0.01	0.02	1.7
46	0.5	6.5	6.7	714	0.05	5.0	0.04	4.5	0.05	0.05	5.6
48-1	0.5	3.7	3.9	407	0.02	2.5	0.02	2.0	0.02	0.03	2.8
48-2	0.5	3.6	3.7	394	0.02	2.1	0.02	1.8	0.02	0.03	2.7
				400		**2.3**		**1.9**			**2.8**
				8.7		**0.3**		**0.1**			**0.1**
49	0.5	7.6	7.7	805	0.04	3.8	0.03	3.2	0.04	0.04	4.2
51	0.5	4.7	4.8	526	0.02	2.3	0.02	1.7	0.02	0.04	4.9
54	0.5	6.8	6.9	725	0.04	3.8	0.03	3.3	0.03	0.04	3.8
55	0.5	6.6	6.8	688	0.02	1.5	0.01	1.0	0.02	0.02	1.9
Blank-1		-0.2			0.005		0.006		0.004		
Blank-2		-0.2			-0.004		-0.003		-0.004		
57-1	0.5	5.1	5.2	533	0.01	0.8	0.002	0.2	0.01	0.01	1.2
57-2	0.6	5.6	5.7	574	0.01	0.8	0.004	0.4	0.01	0.02	1.5
				554		**0.8**		**0.3**			**1.4**
				29		**0.0**		**0.1**			**0.2**
58	0.5	4.7	4.8	517	0.01	1.8	0.01	1.4	0.02	0.02	1.9
59	0.5	7.4	7.5	811	0.01	1.2	0.01	0.7	0.01	0.02	1.4
62	0.5	11	11	1148	0.01	1.2	0.01	0.9	0.01	0.02	1.2
63	0.5	4.4	4.6	476	0.02	1.7	0.01	1.0	0.02	0.02	1.8
67	0.5	3.0	3.1	319	0.01	1.0	0.01	0.6	0.01	0.02	1.3
68	0.5	7.7	7.9	856	0.02	2.5	0.02	2.1	0.02	0.03	2.4
70-1	0.5	18	17	1919	0.02	1.8	0.01	1.4	0.02	0.02	1.8
70-2	0.5	17	17	1885	0.02	1.9	0.01	1.3	0.02	0.02	1.9
				1902		**1.9**		**1.4**			**1.9**
				24		**0.03**		**0.1**			**0.03**

	Weight/g	Cu 224		Cu 327			Zn 202		Zn 206		Zn 213	
		mg/L		mg/L			mg/L		mg/L		mg/L	
Calib Blank 1		[0,00]		[0,00]			[0,00]		[0,00]		[0,00]	
ST1		[1,0]		[1,0]			[1,0]		[1,0]		[1,0]	
ST2		[2,5]		[2,5]			[2,5]		[2,5]		[2,5]	
ST3		[5,0]		[5,0]			[5,0]		[5,0]		[5,0]	
Blank		0.017		0.024			-0.004		-0.006		-0.004	
S-1		-0.003		0.003			-0.002		-0.003		-0.001	
S-2		-0.009		-0.003			-0.002		-0.003		-0.001	
P-1	0.5	0.02	2.2	0.03	0.04	4.0	0.1	8.0	0.1	8.3	0.1	8.6
P-2	0.5	0.02	2.2	0.03	0.04	4.0	0.1	8.8	0.1	9.1	0.1	9.4
			2.2			**4.0**		**8.4**		**8.7**		**9.0**
			0.04			**0.01**		**0.6**		**0.6**		**0.5**
42	0.5	0.1	7.0	0.06	0.1	7.3	0.4	49	0.5	53	0.5	54
43	0.5	0.1	7.9	0.07	0.1	8.8	0.5	53	0.5	57	0.5	57
44	0.5	0.1	7.6	0.06	0.1	8.0	0.5	55	0.5	59	0.6	61
45	0.5	0.1	9.8	0.1	0.1	11	0.5	52	0.5	55	0.5	55
46	0.5	0.1	10	0.1	0.1	11	0.5	55	0.6	59	0.6	60
48-1	0.5	0.1	11	0.1	0.1	13	0.5	57	0.6	60	0.6	62
48-2	0.5	0.1	14	0.1	0.1	15	0.5	57	0.6	61	0.6	62
			12			**14**		**57**		**61**		**62**
			1.8			**2.1**		**0.2**		**0.5**		**0.4**
49	0.5	0.1	9.5	0.1	0.1	10	0.5	57	0.6	61	0.6	62
51	0.5	0.1	7.1	0.1	0.08	8.2	0.7	74	0.7	79	0.7	81
54	0.5	0.05	5.3	0.05	0.06	5.8	0.6	63	0.6	67	0.6	68
55	0.5	0.06	6.0	0.06	0.07	6.8	0.7	70	0.7	75	0.8	77
Blank-1		-0.02		-0.01			-0.002		-0.004		-0.002	
Blank-2		-0.02		-0.02			-0.003		-0.004		-0.002	
57-1	0.5	0.05	4.8	0.05	0.06	5.9	0.4	38	0.4	39	0.4	42
57-2	0.6	0.05	5.4	0.05	0.06	6.3	0.4	41	0.4	43	0.5	45
			5.1			**6.1**		**39**		**41**		**44**
			0.4			**1.2**		**2.4**		**2.7**		**2.5**
58	0.5	0.04	4.0	0.04	0.05	4.9	0.5	51	0.5	54	0.5	57
59	0.5	0.05	5.3	0.05	0.06	5.9	0.4	45	0.5	48	0.5	51
62	0.5	0.08	8.5	0.07	0.08	8.5	0.5	49	0.5	52	0.5	55
63	0.5	0.04	3.9	0.04	0.05	5.1	0.5	49	0.5	54	0.5	54
67	0.5	0.05	4.6	0.05	0.06	5.8	0.5	50	0.5	53	0.5	55
68	0.5	0.08	8.4	0.07	0.08	8.9	0.5	58	0.6	64	0.6	66
70-1	0.5	0.09	9.6	0.06	0.07	7.8	0.5	53	0.5	56	0.5	58
70-2	0.5	0.09	9.7	0.06	0.07	6.7	0.5	52	0.5	56	0.5	58
			9.7			**7.3**		**52**		**56**		**58**
			0.1			**0.7**		**0.6**		**0.2**		**0.1**

Appendix 3: Elemental concentrations for Machakos beans

		Concentration ($mg\ kg^{-1}$)				
Sample ID		**Mn**	**Fe**	**Ni**	**Cu**	**Zn**
icr173556	1	85	120	1.3	6.8	25
icr173556	2	83	123	1.3	7.1	25
icr173556	3	87	126	1.4	7.5	23
icr173557	4	79	265	1.5	8.7	30
icr173557	5	73	232	1.5	8.0	26
icr173557	6	86	270	1.5	8.8	28
icr173558	7	49	154	1.2	7.5	24
icr173558	8	50	159	1.1	7.3	26
icr173558	9	54	157	0.8	7.0	27
icr173559	10	63	163	1.3	8.2	29
icr173559	11	65	162	1.3	8.0	29
icr173559	12	64	159	1.4	8.1	30
icr173560	13	65	138	1.4	8.8	28
icr173560	14	74	146	1.7	9.0	29
icr173560	15	74	140	1.1	9.1	27
icr173561	16	54	174	1.7	8.7	31
icr173561	17	61	172	1.9	8.9	30
icr173561	18	56	173	1.6	9.2	31
icr173562	19	84	278	1.3	8.3	27
icr173562	20	79	290	1.4	8.4	26
icr173562	21	79	261	1.5	8.0	27
icr173563	22	60	249	1.3	9.3	36
icr173563	23	66	247	1.5	10	35
icr173563	24	63	251	1.3	9.5	36
icr173564	25	82	223	1.8	8.4	28
icr173564	26	74	236	1.4	8.8	30
icr173564	27	69	213	1.4	7.9	28
icr173565	28	96	286	1.5	8.0	28
icr173565	29	100	274	1.8	8.2	26
icr173565	30	104	288	1.7	8.4	26
icr173566	31	108	318	2.0	8.9	22
icr173566	32	109	325	1.9	9.0	21
icr173566	33	108	309	1.6	8.2	22
icr173567	34	91	251	1.7	8.9	20
icr173567	35	80	267	1.5	8.7	22
icr173567	36	89	258	1.3	8.7	21
icr173568	37	28	84	1.0	5.8	23
icr173568	38	26	91	0.9	6.3	24
icr173568	39	26	89	1.1	5.9	24
icr173569	40	34	92	1.3	7.7	29
icr173569	41	30	93	1.2	7.8	31

icr173569	42	32	101	1.3	7.5	30
icr173570	43	21	96	0.8	6.0	26
icr173570	44	28	94	0.8	6.1	27
icr173570	45	27	96	0.8	5.6	26
icr173571	46	22	93	0.9	5.5	26
icr173571	47	20	85	0.8	5.2	25
icr173571	48	20	91	0.9	6.1	23
icr173572	49	26	122	0.5	6.5	24
icr173572	50	26	117	0.6	6.2	23
icr173572	51	24	117	0.7	6.3	23
icr173573	52	29	73	0.9	7.1	24
icr173573	53	26	72	0.8	6.6	25
icr173573	54	27	79	1.0	7.0	25
icr173574	55	27	91	1.0	6.1	25
icr173574	56	20	91	0.8	6.5	24
icr173574	57	25	101	1.1	6.3	25
icr173575	58	30	248	1.0	5.6	24
icr173575	59	34	250	0.9	5.9	23
icr173575	60	30	250	0.9	6.4	23

Appendix 4: Elemental concentrations for the seven analysed bean types (dry grains)

Sample ID		Element concentration ($mg\ kg^{-1}$)				
		Mn	Fe	Ni	Cu	Zn
icr173568	1	28	84	1.0	5.8	23
icr173568	2	26	91	0.9	6.3	24
icr173568	3	26	89	1.1	5.9	24
icr173569	4	34	92	1.3	7.7	29
icr173569	5	30	93	1.2	7.8	31
icr173569	6	32	101	1.3	7.5	30
icr173570	7	21	96	0.8	6.0	26
icr173570	8	28	94	0.8	6.1	27
icr173570	9	27	96	0.8	5.6	26
icr173571	10	22	93	0.9	5.5	26
icr173571	11	20	85	0.8	5.2	25
icr173571	12	20	91	0.9	6.1	23
icr173572	13	26	122	0.5	6.5	24
icr173572	14	26	117	0.6	6.2	23
icr173572	15	24	117	0.7	6.3	23
icr173573	16	29	73	0.9	7.1	24
icr173573	17	26	72	0.8	6.6	25
icr173573	18	27	79	1.0	7.0	25
icr173574	19	27	91	1.0	6.1	25
icr173574	20	20	91	0.8	6.5	24
icr173574	21	25	101	1.1	6.3	25