UNIVERSITY OF NAIROBI

TRACE ELEMENT ANALYSIS IN INFANT FOOD FROM SELECTED RURAL AND URBAN AREAS IN KENYA USING TXRF TECHNIQUE

SUBMITTED BY:

PAMELLA KAGELIZA KILAVI

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Declaration

This thesis is my original work and has not previously been submitted to any other University either in whole or in part for the award of a degree

Pamella Kageliza Kilavi
S56/66654/2010

Signed: ……... Date: 14/08/2014 ……

This thesis has been submitted with our approval as University Supervisors:

1. Mr. David M.Maina
   Institute of Nuclear Science and Technology
   University of Nairobi

   Signed: ……... Date: ……

2. Dr. Michael J. Gatari Gichuru
   Institute of Nuclear Science and Technology
   University of Nairobi

   Signed: ……... Date: ……

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Abstract

The need for dietary trace elements during periods of rapid growth such as infancy and early childhood is crucial. These elements are required for promotion of good growth, health and behavioural development of an infant. Inadequate dietary intake of these elements may cause delayed sexual maturation, poor growth rate, mediocre work performance, faulty immune functioning, tooth decay and altered hormonal function, ill health and even death. Strategies such as food diversification and modification of indigenous foods have been proposed as a sustainable solution to addressing trace element malnutrition in developing countries. More often, mothers in the developing countries modify the available legume-cereal food to serve as complementary infant foods.

The aim of this study was to determine selected trace elements and some toxic elements that may be present in locally produced complementary infant food from selected rural-urban areas. Ninety samples were collected from Kirwara, Bomet, Mitaboni, Turbo, Sega, Nairobi, Kericho, Kisumu, Eldoret and Thika.

Wet digestion in an open system using nitric acid was used to extract the elements from the sample matrix. This analytical procedure was validated by subjecting certified reference material NIM-GBW10017 to the same analytical procedure. For each digested sample, three aliquots were prepared and analysed using a S2-Picofox TXRF spectrometer.

The levels of Fe, Zn, Cu and Mn varied depending on the origin, type of ingredients used, and probably the proportions of these ingredients in the samples. The levels of these trace elements varied as follows: Mn, 3.31 ± 1.64 mg kg^{-1} to 329 ± 28 mg kg^{-1}; Fe, 14.6 ± 1.6 mg kg^{-1} to 376 ± 57 mg kg^{-1}; Cu, 0.72 ± 0.02 mg^{-1} to 11.0 ± 0.1 mg kg^{-1}; Zn, 11.2 ± 0.2 mg kg^{-1} to 97.1 ± 4.3 mg kg^{-1}. Concentrations of Pb ranging from 0.12 ± 0.04 mg kg^{-1} to 0.84 ± 0.16 mg kg^{-1} were found in approximately 36% of the samples. The concentrations of Fe and Zn were relatively high in cereals-legumes samples compared to samples that were based on cereals only. Finger millet flour had relatively high concentrations of Mn while the maize flour had very low levels of Mn, Fe, Cu and Zn.

This study indicates that infants who are fed maize based porridge may tend to be deficient in Zn and Fe as compared to those who are fed equal measures of cereal-legume based porridge. On the other hand, infants who are mainly fed finger millet based porridge may receive higher levels of Mn which may be detrimental to their health. Therefore proper formulation and use of cereal-legume based infant porridge flour may alleviate micronutrient malnutrition among infants in developing countries.

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Abbreviations and Acronyms

AAS	Atomic Absorption Spectrometry
FSNS	Food Security and Nutrition Strategy
GOK	Government of Kenya
FAO	Food and Agriculture Organization
FWHM	Full Width at Half Maximum
IAEA	International Atomic Energy Agency
ICP-EOS	Inductively Coupled Plasma-Atomic Emission Spectrometry
IMFNB	Institute of Medicine, Food and Nutrition Board
SD	Standard Deviation
SE	Standard Error
TXRF	Total reflection X-Ray Fluorescence
UNICEF	United Nations International Children's Emergency Fund
USAID	United State Agency for International Development
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Diet is the main source of essential nutrients for normal growth and health of a person. Insel *et al*. (2011), state that a good diet should supply the 45 essential nutrients for human growth, development and maintenance. These essential nutrients are grouped into water, macronutrients and micronutrients. The macronutrients include carbohydrates, lipids, proteins, while micronutrients include vitamins and minerals. According to World Health Organization, (WHO, 2010), a diet with adequate nutrients results in improved infant health, stronger immune system, lower risk of non-communicable diseases such as diabetes and cardiovascular disease, and longevity of life. In addition, people whose dietary intake has adequate nutrients are more healthy and productive. They are therefore able to create opportunities to gradually break cycles of poverty and hunger.

Minerals can either be macro-minerals or micro-minerals. The micro-minerals are also referred to as trace elements. Aras and Ataman (2006) classified trace elements into three groups which are of nutritional significance; essential trace elements, probably essential trace elements, and potentially toxic trace elements. Furthermore, the authors states that all the essential elements may be toxic to human if ingested at sufficiently high levels over a long period of time. Some of these essential elements include iron, selenium, iodine, zinc, copper, molybdenum, chromium, and manganese.

Human beings require essential trace elements in amounts ranging from 50 µg day^{-1} to 20 mg day^{-1} (Aras and Ataman, 2006). The essential elements are important for growth and normal functioning of the immune system. According to Insel *et al*. (2011), these trace elements are part of enzymes or serve as co-factors for enzyme-mediated reactions. They are also involved in oxidation-reduction reactions.

Inadequate dietary intake of trace elements is a global issue. WHO (2000) report identified iodine, iron, and zinc dietary intake deficiencies as being among the world's most serious factors

contributing to malnutrition related health problems. In a study of dietary essential trace elements among the general population in more than 20 countries Parr *et al*. (2006) found that iodine, iron and zinc intakes were below the dietary requirements. Yu (2009) evaluated dietary zinc and other divalent mineral intake among the population in China in which he found out that zinc intake did not meet the Chinese Recommended Nutrient Intake (RNI) requirements. In addition he observed that children and adolescents were particularly at higher risk of insufficient dietary zinc intake.

The period from birth to two years of age has been considered as the "critical window" for promotion of good growth, health and behavioural development of an infant (Dewey, 2001). Since inadequate dietary intake of essential trace elements may cause delayed sexual maturation, poor growth, mediocre work performance, faulty immune functioning, tooth decay and altered hormonal function (Insel *et al*., 2011), then their deficiencies during this period impacts negatively on the child's health. These effects may be irreversible, and can lead to poor school performance, reduced work competence and decreased productivity later in life.

Stunting, wasting and underweight in children below the age of five are indicators of micronutrient malnutrition (UNICEF, 2007). Stunting which is defined as low height for age is considered as an indicator of chronic micronutrient malnutrition whereas wasting which is low weight to height is a measure of acute micronutrient malnutrition while children whose weight is too low for their age are said to be underweight (UNICEF, 2007). Of all the children in the developing countries under the age of five years, approximately 31% of them are underweight, 38% have stunted growth and 9% show wasting (Muller and Krawinkel, 2005). A household survey done in Kenya by UNICEF (2009) showed that approximately 35% of children under the age of five were stunted, 6% of the children had wasted and 4% of the children were severely underweight as a result of micronutrient malnutrition. Epidemiological and experimental observations have proved that malnourished children are more susceptible to infectious disease (Rodríguez *et al*., 2011). Micronutrient malnutrition has therefore been linked to higher rates of morbidity and mortality among infants

Kramer and Kakuma (2002) recommends exclusive breastfeeding up to the age of six months, thereafter, complementary feeding should be introduced. WHO (2010), further recommends that

the complementary foods should adequately provide all the nutrients requirements as the infant continues to receive breast milk up to the age of 24 months or more. These complementary infants foods are needed to fill the gap between the total nutritional needs of the child and the amount provided by the breast milk. Unfortunately, reports indicate that during this period, nutrition requirements of many infants are not met, leading to the onset of micronutrient malnutrition that is prevalent in children under the age of 5 years worldwide (Gibson *et al*., 1998; Huffman *et al*., 2000; GOK, 2011). This challenge has been attributed to inappropriate complementary feeding practices, low quality of complementary foods and high cost of fortified nutritious complementary foods which is always beyond the reach of many families (Muhimbula *et al*., 2011).

Faber *et al*. (2005) proposed the use of low-cost fortified maize-meal porridge which could potentially reduce iron deficiency and improve motor development of infants from low socioeconomic status. Gibson *et al*. (2011) also showed that cereal and legume based infant complementary food fortified with micronutrients could reduce anemia and improve the iron and selenium status of infants in developing countries. Unfortunately, most of the fortified complementary infant foods, including the so called low cost fortified ones, have been considered unaffordable to many mothers in the Sub-Sahara Africa. Therefore, most households in these countries depend on inadequately processed indigenous foods consisting of un-supplemented cereal base porridges made from maize, sorghum and millet for complementary infant feeding (Tou, 2007).

In Kenya, the malnutrition levels and micronutrient deficiencies in children under the age of five remain unacceptably high (FSNS, 2008). Zinc, iron and iodine have been recognized as the micro-nutritional deficiency of public health significance in Kenya in children under the age of five (Wagah *et al.*, 2005). Therefore, as a policy, the government has scaled up the fight against these deficiencies through nutrition education, early identification of malnutrition and selective feeding programmes through health centres and schools. Occasionally, some of these feeding programmes are facilitated at social centres in the rural set-ups such as ‘Barazas’. Most of these feeding programmes are funded by donor organisation such as World Food Programs, UNICEF and USAID. These foods are mostly instant allowing them to be cooked within a very short

period of time. They are also deliberately fortified with essential trace elements such as Zn and Fe.

The use of sustainable food-based approaches for prevention and control of micronutrient deficiencies has been recognised and is being promoted (Gibson, 2011). Cultivation of cereal crops such as sorghum, amaranth and finger millet, which are rich in micro-nutrient and at the same time resistant to environmental factors such as drought have consequently been encouraged (Altieri and Koohafkhan, 2008). The nutritional importance of legumes such as soya has also been embraced by several farmers in the Sub-Sahara Africa (Odendo *et al*., 2011). These cereal crops and legumes can be roasted, milled and modified further by adding fish powder or milk powder then used as infant complementary meal in form of porridge.

In Kenya, women groups, such as in the case of Sega in this study, have been formed to foster the sustainability of food-based approaches in combating micronutrient deficiencies among infants and children. Their produce are thereafter bought and distributed among other mothers at a subsidized fee either through the health centres or social set-ups.

Complementary infant foods are either in form of semi-solid gruels or porridges prepared through a two step process (Brown *et al*., 1998). The first step involves processing of available staple foods and a major protein source, which is usually a legume or oil-seed, into intermediate flours or dough. The intermediate flours or dough can then be stored over a long period of time. The second step involves the actual preparation and cooking of the mixture from the intermediate flours or dough. This may take place at home, local health post or other community centers.

Some toxic elements such as lead, arsenic, mercury and cadmium may enter the food chain either through human activities during production or through environmental pollutants. Consequently, the infants may be exposed to these toxic elements through complementary foods. For instance, lead originating from flour milling machines has been considered as a major pathway for lead contamination in flour (Falk, 2003).

Several studies on trace element content in complementary infant food have been done in the recent past (Melø *et al*., 2008; Zard *et al*., 2011, Joseph *et al*., 2011, Ljung *et al*. (2011). However, the focus on most of these studies has been on minerals and trace elements content in

commercially produced complementary infant foods. For instance Melø *et al*. (2008) investigated the trace element content in different products of infant flour, porridges, fruit puree and dinners. The study showed that the trace element content in all the products were within the upper tolerable limit and the toxic elements were present in the products but at very low levels. On the other hand Zard *et al*. (2011) reported that the trace elements Se, Cu, Mn, Fe and Zn in commercial infant foods under investigation were below the Recommended Nutrient Intake.

1.2 Statement of the Problem

Cereal based complementary infant foods do not provide most of the essential nutrients for growth and development of children. Lartey *et al*. *(1999)* and Huffman *et al*. (2000) showed that maize flour often fail to meet the nutritional needs of an infant due to its poor nutritive values. Even though the use of cereal-legume based foods may be used to improve nutrient density and improved nutrient intake among the rural and poor urban infants (Solomon, 2005), Gibson *et al*. (1998) and Musa *et al*. (2012) studies showed that such plant based foods do not meet the total daily need of iron and zinc requirements for infants. Nevertheless, Nnam, (2001) suggested that when these legume-cereal base complementary infant foods are judiciously selected and combined in desirable pattern, then they could provide the essential nutrients including trace elements.

Investigations on the formulation on the viability of some processed and blended cereal-legume based food for complementary infant feeding in developing countries have been carried out by a number of researchers (Compaoré *et al*., 2011, Ijarotimi and Keshinro, 2013). These researchers formulated the complementary foods from particular cereals and legumes after which they evaluated protein content, carbohydrates content, some micronutrient content, and anti-nutrients content.

Huffman *et al*. (2000) conducted focused group interviews in Kenya and found that many mothers in both rural urban areas used locally made, pre-processed cereals, including brand-name and generic products for complementary infant feeding. None of the products in this study done by Huffman *et al*. had nutrition information. Information on preparation of the product and mixing to ensure adequate nutrients intake was also not provided.

Therefore, this study sought to provide information on the levels of trace element in already formulated complementary foods which were either collected from selected mothers in the rural areas or bought from the stores in selected rural and urban areas.

1.3 Justification and significance of the study

Information on the type and level of nutrients available in a given food enables a consumer to see at a glance how a particular type of food fits into a diet. This information can also enable a consumer to compare the daily dietary intake of diet to the recommended daily intake. Therefore, information on the type and level of trace elements especially in complementary infant food is very important.

Nutrition information obtained from this study forms a basis for evaluating the suitability of the complementary infants' foods in terms of what they provide and what they do not provide. This will enable the heads of households to bridge the nutrition gap. Consequently, the health and development of their infants will be boosted, reducing the infants' morbidity and mortality significantly. This will therefore contributes towards the realization of the fourth Millennium Development Goal of reducing child mortality by the year 2015.

The nutrition information obtained from this study will also be used by the Agriculture Extension Officers to advice farmers on micronutrient rich food products which can easily be adapted and modified as complementary infant food. Food industrialists will also be able to use this information for proper formulation and labelling of the infant flour products so as to strengthen trade and export. In addition the information will be used by health workers and nutritionists to plan and implement food and nutrition programmes.

1.4 Hypotheses

Null hypothesis

Formulated flours collected from mothers and stores contain all the essential trace minerals above the recommended daily intake needed for the growth and development of infants.

Alternate hypothesis

Formulated flours from mothers and stores do not contain lead as a toxic element and are safe for use for complementary infant feeding.

1.5 Objectives

1.5.1 General Objective

To determine the selected trace elements and some toxic elements that may be present in locally produced complementary infant flour from selected rural-urban areas using Total –Reflection X-Ray Fluorescence (TXRF)

1.5.2 Specific Objectives

i. To determine the essential trace elements in complementary infant flour samples from selected Kenyan rural-urban areas.
ii. To determine the level of lead in complementary infant flour samples from selected Kenyan rural-urban areas.
iii. To compare the levels of trace elements in complementary infant flour samples collected from selected rural-urban areas

CHAPTER 2

LITERATURE REVIEW

2.0 Introduction

Trace element is a term applied to those elements which are found in the body in concentrations below 10 ppm (Parr, 1983). These elements play a variety of roles. In some cases, they serve as constituents of vital biological molecules e.g. iron in haemoglobin, and iodine in thyroid hormones. In other cases, they are part of enzymes or they serve as cofactors for enzyme-mediated reactions.

The concentrations of these trace elements in the body must be maintained within narrow limits if the functional and structural integrity of the tissues is to be safe and performance of the body is to remain unimpaired (Poulsen, 2005). A high or low concentration of any of these elements in the body may induce changes in tissues and fluids adversely affecting the physiological functions and may lead to structural disorder depending on the concentration of a particular element in the body. A continued supply of adequate dietary trace element is therefore of paramount importance.

The need for dietary trace element during periods of rapid growth such as infancy and early childhood is critical (Brätter *et al*., 1998). Therefore infants tend to be at a higher risk to the impacts of inadequate dietary trace elements. Studies have shown that inadequate intake of dietary trace element during infancy is the main cause of infant morbidity and mortality in the developing countries where 50% of all infant death is related to improper diet lacking sufficient trace elements and other micronutrients (Kolsteren *et al*., 1999, Katona and Kotana-Apte, 2008). In addition, the effects, such as stunting, resulting from inadequate intake of diets lacking dietary trace elements during the infancy stages are irreversible.

Trace element absorption and bioavailability in the body is influenced by many factors, but most often it depends on the interactions with other trace metals having similar chemical characteristics and uptake process (Sandström, 2001). These interactions depend on the relative concentration of the trace element in the body. For instance, the interaction between iron, copper,

and zinc absorption has been explained by the competitive binding to the protein divalent metal ion transporter 1 (DMT1), which participates in the transport of the divalent metals (Gunshin *et al.*, 1997). A study done by Arredondo *et al*. (2006) confirmed that Cu and Zn inhibited Fe uptake, and, while Fe inhibited Cu uptake, Zn did not. Arredondo *et al*. further showed that when the three metals were given in equal measures, Fe and Cu uptake were inhibited by approximately 40%. These results show that concentration level of either of these trace elements in diet is a potential risk to the absorption and bioavailability to the other trace elements. This aspect has therefore to be considered in complementary infant food modification, supplementation and infant food fortification programs.

Other studies have shown that deficiency of dietary trace elements increases the absorption of toxic elements such as Hg, Pb, Cd and As. For instance, a study done by Turgut *et al*. (2007) showed that iron deficiency increased the absorption of lead which is a result of competition between Fe and Pb for an absorptive pathway in the small intestine. Another study done by Osman (1998) also showed that low dietary intake of Fe and Se leads to absorption of Pb and Cd in the gastrointestinal tract. Therefore, insufficient intake of some essential trace elements may facilitate the absorption of toxic elements and aggravate their toxic effects.

National and international regulatory agencies such as World Health Organisation, National Research Council of the U.S, and European Commission have made recommendations on the Dietary Reference Intake (DRI) of trace elements for infants and children, defining the intake, supplementation and toxicity of these trace elements. Where scientific data is limited, the tolerable upper limit (UL) has been defined. The level of some of these trace elements are hereby shown in table 2.1

Table 2.1: Recommended Dietary Allowance (RDA), Adequate Intake (AI) and Tolerable Upper Limit (UL) for infants and children (IMFNB, 2001)

Life stage	**Cu** (µg/d)	**Fe** (mg/d)	**Mn** (mg/d)	**Zn** (mg/d)
0-6 months	200*	0.27*	0.003*	2*(4)
7-12 months	220*	11	0.6*	3 (5)
1-3 years	340 (1000)	7	1.2*(2)	3 (7)

Adequate intakes are indicated with an asterisk
Tolerable Upper Limits are indicated in the brackets

2.2 Role of dietary trace elements in infant health

Dietary trace elements such as iron, zinc, copper and manganese play a vital role in the health of an infant. In this section these four dietary trace elements and their importance in infant health are briefly discussed.

2.2.1 Iron

Iron is a basic part of many proteins and enzymes that maintain good health in human. Almost two-thirds of iron in the body is found in hemoglobin, a protein in the red cells that transport oxygen to the tissues (Dallman, 1986). In addition, smaller amounts of iron are found in myoglobin, a protein that helps supply oxygen to the muscles. Iron deficiency will therefore reduce a person's energetic and physical competence due to limitation of oxygen delivery to the cells hence infants and children with iron deficiency will tend to feel tired all the time. It has also been noted that they are less active and attentive (Mohammed, 2008). Furthermore, they also respond and react poorly to the environment.

Iron is an essential trace element in the regulation of cell growth and differentiation (Beard, 2001). For instance, it is involved in the normal structure and function of the nervous systems which is important in the biochemistry of the brain and the production of the neurotransmitters (Youdim and Yehuda, 2000). Its deficiencies, especially during the infancy stage adversely affect the intellectual and cognitive ability of the child which is usually observable during the middle childhood where work and school performance is decreased. Studies have also shown that the deficiency of iron results to developmental delays and disability (Mathers *et al.*, 2009).

Iron deficiency and iron deficiency anaemia are the leading world's nutritional disorder in infant and children, with the highest percentages coming from the developing countries where Vitamin A deficiency and malnutrition often occurs (Olivares *et al*., 1999). Iron deficiency develops gradually and begins when iron intake does not meet the daily need of dietary iron. On the other hand, iron deficiency anaemia is a severe effect of iron deficiency which occurs when iron storage sites are undersupplied and the iron levels in the blood are unable to meet the daily needs.

Developed countries also have their share of iron deficiency and iron deficiency anaemia. For instance, a study done by Hay *et al*. (2004) had showed that Norwegian children aged between 6 and 24 months had a mild iron deficiency. Another study done by Brotanek *et al*., (2008) also showed that about 9 percent of 1 to 3 year old toddlers in United States, have iron deficiency and 2 to 3 percent have iron deficiency anaemia.

Studies have shown that breast milk does not supply enough iron to an infant after the age of six months (Kramer and Kakuma (2002). As a result of this the infant's iron liver storage starts diminishing (Melø, 2008). An infant who has been breastfed exclusively up to the age of six months will therefore be at risk of developing iron deficiency anaemia if the total daily iron requirements are not met. Complementary infant foods which are rich in iron should gradually be introduced at this age. These foods should be able to provide 75% to 100% of total daily iron requirements for an infant aged from 6 months and by the age of 9 months these foods should supply 97% of iron (Melø, 2008).

Storage levels of iron in the body and the type of dietary iron consumed influences absorption and bioavailability of iron in the body. For instance, the absorption of iron increases with a decrease in iron stores to help protect the body against iron overload (Fairbanks, 1999). A study done by Allen *et al*., (2006) showed that heme iron is not significantly affected by diet and its absorption and bioavailability is above 15%. However, only 1% to 9% of non-heme iron is absorbed from simple, monotonous diet based on cereals, roots or tubers with negligible amounts of heme- iron. Furthermore, nonheme iron found in legumes and cereal grains is significantly affected by the presence of phytic acid such that the iron absorption in infants is almost similar to

that in adults (Hurrell, 2003). Cereals such like sorghum and mill*et al*so have phenol compounds which inhibits absorption of Fe (Allen *et al*., 2006)

In many of the rural-urban settings of developing countries, plant based foods are often used as complementary infant foods. These foods are usually based on mixtures of cereal grains, tubers, roots and legumes and these cannot supply adequate dietary iron. For instance, a study done by Gibson *et al*. (1998) and Musa (2012) revealed that all the complementary infant foods under investigation, which were based on cereals and legumes, did not meet the total daily iron requirements.

Different strategies have been put in place to improve the bioavailability of iron. These strategies include flour fortification, dietary diversification or enhancement and iron/folic acid supplementation (Allen *et al*., 2006). For instance iron fortification of staple cereals flours has been suggested to be a sustainable solution to addressing iron deficiencies in developing countries (Andang'o *et al*., 2007).

Dietary diversification and enhancement include among others using processing, preservation and preparation practices that retain iron availability in the food. For example fermentation, germination or addition of ascorbic acid to cereal and legume based food has been proposed as a cheap and alternative solution to addressing iron deficiencies in the developing world (Hurrell, 2003).

Iron and folic acid supplementation has also been used as one of the strategy to alleviate iron deficiency in infants (Baltussen *et al*., 2004). However, iron supplementation of infants in developing countries has been found to increase the adverse effects and death due to malaria or/and other communicable diseases. For example, a study done by Sazawal *et al*. (2006) showed that 12% of the children aged between one month and 35 months who were supplemented with 2.5 mg oral iron and 50 µg of folic acid were likely to die or needed to be treated in hospital due to the adverse effects of malaria, diarrhoea, sepsis, measles and pneumonia. In addition, 11 % of these children were likely to be admitted in hospital. These adverse effects of iron supplementation on infants could be explained by the effect the excess iron has on absorption of zinc in the gastrointestinal tract (O'Brien *et al*., 2000).

Excessive iron in the body has also been associated with increase in oxidation and initiation of free radicals which are toxic to biomolecules and may initiate carcinogenesis and promote tumor cell proliferation (Zacharski *et al., 2000*). Swaminathan *et al.* (2007) noted that moderate elevation of iron stores appear to increase the risk of type 2 in otherwise healthy people. Other clinical studies have shown that excessive iron intake enhanced growth and gut colonization of pathogenic bacteria (Zimmermann *et al*., 2010; Guus *et al*., 2012). In addition, excessive iron in the body limited the bioavailability of manganese, zinc and copper, enhancing the effects associated with the deficiencies of manganese, zinc and copper.

2.2.2 Zinc

Zinc is a central part of several cellular growth and differentiation that have a rapid turnover, including those of the immune system and in the gastrointestinal tract (Allen *et al*., 2006). This element enables normal growth during pregnancy, childhood and adolescence playing a major role in skeletal development (Ganss and Jheon, 2004), hair stimulation (Nriagu, 2007) and enabling the body to carry out immune functions such as protection against infection and cancer (Perveen *et al*., 2002), and wound healing (Lansdown *et al*., 2007).

Zinc deficiency affects the structure and functions of the growth hormone (MacDonald, 2000). A study done by earlier by Ninh *et al*. (1996) showed that the circulating and serum insulin-like growth hormone factor-I, a mediator of the growth promoting action of growth hormone, was lower in Zn deficient children than controls. This therefore implies that decreased growth (stunting) in children and dwarfisms may be as a result of severe zinc deficiency. Studies reviewed by Brown *et al*. (2002) confirmed that zinc supplements administered for 8 or more weeks significantly increased the height and body weight of children aged below 12 years, thus reversing stunting and dwarfism.

Zinc deficiency has been noted as the most prevalent risk factor for nutrient related diseases and deaths in infants in the developing world. World Health Organization (2002) estimated that about 1.5% of all deaths and about 20% of prenatal mortality worldwide could be attributed to zinc deficiency. Furthermore, zinc deficiency has been recognized as a risk factor for many chronic diseases and it is believed to be responsible for 10% of diarrheal diseases, 16% of lower respiratory tract infections and 18% of malarial attacks worldwide (WHO, 2002).

Severe zinc deficiency depresses immune function (Shankar *et al*., 1998), and even mild to moderate degrees of zinc deficiency impairs macrophage and neutrophil functions (Wintergerst *et al*., 2007). This causes alterations in immune response which may be a possible predisposing risk factor to increased susceptibility to infections such as diarrhoea, malaria and pneumonia in infants and children (Bahl *et al*., 1998; Brooks *et al*., 2005; Black *et al*. 2008).

Studies done by Black (2003) showed that poor and malnourished children who received 4 to 40 mg of zinc supplements experienced shorter episodes of infectious diarrhoea. Further studies done by Bhutta *et al*. (2000) showed that zinc supplementation reduces the duration and severity of diarrhoea in zinc deficient or otherwise malnourished children. In 2003, a report reviewed by Black reported that zinc supplementation reduced the rate of diarrhoea. As a result of these studies, WHO and UNICEF (2004) have proposed the use of Zn supplementation as a therapy to reduce the severity and the episodes of diarrhoea.

Zinc has no specific "stores" in the body. Its absorption is done in the small intestine through a carrier-mediated transport process which is not saturated under normal physiological conditions (Sandström, 1992). At higher intakes, the fractional absorption of Zn decreases and intestinal excretion increases while urinary losses remain fairly constant (Coppen and Davies, 1987). At very low Zn intakes, absorption can increase by 59% to 84% as faecal and urinary losses decreases accordingly (Wada *et al*., 1985). When these primary homeostatic mechanisms are not sufficient to handle large dietary excesses of Zn, excess Zn is lost via the hair (Jackson, 1989).

Complementary infant foods rich in dietary Zn should be given to infants after the age of six months since Zn intake from breast milk is subsidiary at this age. At the age of 9 months, these foods should be able to provide 86% of dietary zinc (Dewey, 2001). However, most of the complementary foods in developing countries are based on cereals and legume which contain anti-nutritional elements such as phytic acids which may be involved in etiology of the Zn deficiency and also inhibits the re-absorption of endogenous Zn secretion that enters the lumen of the small intestine postprandailly (Hurrell, 2003). For instance, a study done by Gibson (1998) showed that complementary infant foods based on cereals with high phytic acid content could not meet the daily requirements of Zn. Another study done by Musa *et al* (2012) revealed that the staple cereals which were often used as complementary infant foods had very low levels of Zn

which could not meet the recommended daily intake. However, Gibson (1998) observed that complementary food that had dried fish, dried skim milk powder and eggs met the daily requirements of Zn.

2.2.3 Copper

Copper is widely distributed in biological tissues, occurring largely in form of organic complexes, many of which are metalloproteins and functioning as enzymes (Aras and Ataman, 2006). The copper enzymes are involved in many metabolic reactions such as utilization of oxygen during cell respiration and energy utilization in the mitochondria. Hence deficit of copper may result in impaired production of energy leading to body weakness and chronic fatigue.

Lysyl oxidase, another copper enzyme, is also required for the cross-linking of collagen and elastin, which are essential for the formation of strong and flexible connective tissue (Turnlund, 2006). This enzyme helps in maintaining the integrity of connective tissue in the heart and blood vessels. This enzyme also plays a role in the bone formation. A review done earlier by Uauy *et al*. (1998) had linked copper deficiency to osteoporosis and fractures of long bones and ribs, epophyseal separation, fraying and cupping of the metaphyses with spur formation, and subperiostal new bone formation in copper-deficient low birth weight infants and young children.

Copper is also known to play a vital role in the development and maintenance of the immune system function. Deficiency of copper has been associated with decreased production of white blood cells and impaired functions of the T and B lymphocytes (Tong *et al*., 1996). Moreover, advance effects of insufficient copper on immune function have been noticed to be more pronounced in infants. For instance, a study done by Heresi (1985) indicated that the ability of white blood cells to engulf pathogens increased significantly when malnourished infants were supplemented with copper. Another study done by Kelley *et al*. (1995) showed a decreased proliferation response on subjects that were on low Cu diet when white blood cells were isolated from their blood and presented with an immune challenge in a cell culture. Study done by Percival (1998) showed that infants with Menkes disease, a genetic disorder of copper deficiency, suffered from frequent and severe infections.

Adequate nutritional copper status is also necessary for formation of red blood cells and therefore important in normal metabolism of iron. Two of the copper enzymes, ferroxidase I and ferroxidase II, oxidize ferrous iron to ferric iron, a form of iron that can be loaded onto the protein transferin for transport to the site in the bone marrow for red blood cell formation (Turnlund, 2006). Therefore, copper deficiency has been associated with a decreased mobilization of iron from storage sites allowing iron to accumulate in these storage sites; and anemia has been identified as a late sign of Cu deficiency along with iron accumulation in various body tissues.

The levels of Fe and/or Zn in the body may have an effect on absorption of Cu. Studies have shown that high intakes of iron have been found to interfere with copper absorption in infants (IMFNB, 2001). This was confirmed by studies, among others, done by Turnlund (2006) who showed that infants who were fed on formula with high levels of iron absorbed less copper than infants fed on a low iron formula. Other studies showed that high intakes of zinc supplements over an extended period of time may results in copper deficiency. High intake of dietary Zn increases the synthesis of a copper-binding protein, metallothionein (Turnlund, 2006), which traps copper within intestinal cells preventing it from systematic absorption.

Copper can be absorbed in all segments of the gastrointestinal tract and in particular in the small intestine which plays a substantive role in copper absorption. Its absorption is dependent on the nutritional status of an individual. However metallothlonein in the epithelial cells of the intestine plays a key role in its regulation (Turnlund, 2006). Studies have shown that absorption of Cu is higher in presence of copper deficiency infants than it is when copper is adequate. For instance, infants are able to adjust to copper absorption by reducing absorption at higher intakes and increasing absorption at lower intakes (Uauy *et al*., 1998).

Full term infants have adequate stores of copper in the liver which is sufficient for the newborn infant to the first 6 months of life. However, as the child grows older, a steady decrease of stored copper has been observed as a result of increase in serum copper due to production of ceruloplasmin and mobilization of liver stores as opposed to copper absorption (Cordano, 1998). Infants may have marginal copper stores as from the age of 6 months and therefore good dietary sources of copper such as organ meat; legumes and nuts should be incorporated in the

complementary food. Complementary infant foods such as refined cereals, milk and dairy products which are often used are poor sources of dietary copper. For instance, cow's milk is relatively low in copper, and infants and children fed on cow's milk formula have been reported to be at risk of copper deficiency (Dorner *et al.*, 1989)

Copper toxicity has rarely been reported in infants and children, but it may result from diets that lack copper antagonists such as zinc, ascorbic acid, and manganese (Watts, 1989) or from copper-rich foods such as cocoa, liver seeds and nuts, seafood and foods or beverages that are stored in copper containers or copper contaminated tap water (Fitzgerald, 1998). Copper toxicity has been reported to be detrimental to the physiological processes and may lead to impairment of membrane properties, DNA functions, enzyme functions and cellular injuries (Kabata-Pandias and Mukherjee, 2007).

2.2.4 Manganese

Manganese is a vital trace element in the body for the proper development of bones and cartilage. A study done on young men fed on low-manganese diet indicated an elevation in the blood calcium, phosphorous, and alkaline levels which increased continuous turnover of bone as a consequence of insufficient dietary manganese (Friedman, *et al*., 1987). Another study done on a child receiving a long term total parenteral nutrition lacking manganese developed bone mineralization and impaired growth that was later corrected by manganese supplementation (Norose *et al*., 1992). Deficiency of manganese in the body has therefore been associated with skeletal abnormalities such as retarded bone growth with bowing, postural defects and impaired growth (Watts, 1990).

Manganese is essential in the function and development of the brain. Studies have shown that manganese is a co-factor of enzyme glutamine synthetase in the brain, which is responsible for the degree and balance of excitation inhibition critical in the development of seizure (Takeda, 2003). Deficiency of manganese is therefore one of the primary contributing factor of convulsive disorder which has been supported by studies done on both experimental animals and humans (Gaby, 2007)

The absorption of manganese is influenced by dietary factors. For instance, a study done by Hurrell (2003) showed that phytic acid inhibits absorption of manganese. This confirmed an experimental study that was done earlier by (Davidsson, *et al*., 1995) which showed that only 0.7 % of Mn was absorbed from infant soy based formula which had high phytic acid. However, when the phytic acid was enzymatically degraded from the formula, absorption of manganese increased from 0.7% to 1.6%.

The interaction between Mn and Fe may lead to either Mn deficiency or Mn toxicity. For example, a study done by Keen *et al*. (1996) showed that absorption of Mn from a meal decreased as the meal's Fe content increased. Another study done by Davis and Greger (1992) indicated that iron supplementation was associated with decreased blood Mn levels and decreased manganese superoxide dismutase activity in the white blood cells, indicating a reduction in Mn nutritional status. In addition, Malecki *et al*. (1999) observed that intestinal absorption of Mn increased in presence of Fe deficiency. However, increased Fe stores have been associated with decreased Mn absorption.

In addition to dietary factors, absorption of manganese is also influenced by age. Infants and young children tend to absorb more manganese from food and very little is excreted since the homeostatic mechanism is not yet fully developed (Gregus and Kalssen, 1986). Most of the Mn is excreted into the faeces by the way of bile. Some studies have shown that insufficient biliary excretion may result in accumulation of Mn in the brain. Kriegler *et al* (1995) study observed that accumulation of Mn in the brain had a role in the pathogenesis of disease, especially neurotoxicity. Therefore high retention of manganese in young infants combined with relatively high dietary intake of manganese has led to some concern about potential manganese toxicity (Lonnerdal, 1989).

Complementary infant food should be able to provide between 50% and 75% of absorbable manganese (Gibson *et al*. 1998). Studies have shown that whole grains and nuts are good sources of Mn. However, the high content of phytic acids in whole grains and nuts tend to inhibit absorption of Mn (Davidsson *et al*., 1995).

2.3 Dietary intake and health effect of lead

Lead is very toxic to the body. Its toxicity is attributed to the affinity of lead to thiol groups (Vallee and Ulmer, 1972) and other organic ligands in the proteins. Furthermore, Pb has the ability to substitute for calcium and sometimes zinc (Bressler and Goldstein, 1991). Lead affects the nervous system by interfering with calcium-dependent reactions and/or disrupting calcium homeostasis (Bressler *et al*. 1999). For instance the gamma-isoform, one of the several calcium ion dependent of protein kinase C which is neuron-specific, that is thought to be involved in spatial learning and memory process form, is a likely target of lead neurotoxicity (Lidsky and Schneider, 2003). The excessive influx of calcium into the mitochondria due to lead toxicity also results in the production of free radicals and in the opening of the membrane transition pores, damaging the neurons (Sidhu and Nehru, 2003).

Dabrowska-Bouta *et al*. (1999) study on rats showed that lead accumulates in brain myelin causing gross morphological alterations and composition. This effect may take place in the human brain. Furthermore a study done by Adonaylo and Oteiza (1999) on rats showed that lead may cause lipid clustering in liposomes increasing the rate of iron-initiated lipid oxidation and consequently damaging the membrane.

Infants and young children tend to be vulnerable to the harmful effects of Pb since they are undergoing a period of rapid development (WHO, 2010). Infants and young children absorb lead more easily and excrete it less efficiently than adults. Studies have shown that infants and young children appear to absorb between 40% and 50% of the ingested Pb whereas adult population absorbs only about 10% (Pfadenhauer *et al.*, 2014). In addition, absorption of Pb is strongly affected by the nutritional status of an individual with higher absorption of lead in infants and children who are iron deficient (Turgut *et al.*, 2007).

Lead poisoning has been recognized as a common childhood disease. It accounts for about 0.6% of global burden of disease in infants and children (WHO, 2009). Its neurobehavioral effects associated with it during infancy and childhood development is persistent and irreversible. For instance, early childhood exposure to lead and performance on tests of cognitive function and behavior have been found to relate inversely at 10, 15, and 20 years after the blood levels were measured (Bellinger *et al*., 1991). Early exposure to lead has also been linked to increased rate of

hyperactivity, inattentiveness, conduct disorder, juvenile delinquency, drug use and incarceration (WHO, 2010). Therefore, level of lead in infant food is of major concern. Consequently, the Codex Alimentarius Commission set up by FAO and WHO based on research done has recommended that the maximum levels of lead in cereals except bran should be 0.2 mg kg^{-1} (Codes Alimentarius Commission, 1999).

Infants may be exposed to Pb through the complementary foods which may enter the food chain either through human activities during production or environmental contamination. Studies have shown raw effluent discharge from industries and municipal sewage system, and flooding may contaminate rivers and lakes with toxic elements such as Pb (Odada *et al.*, 2004). Fish in turn bio-accumulate such toxic elements their bodies (Jezierska and Witeska, 2006) and the bioaccumulation is dependent on their age and their feeding habits (Ansari and Raissy, 2011). Therefore, consumption of contaminated fish among children below the age of five years may be a pathway of Pb and other toxic elements (Oyoo-Okoth *et al*., 2010).

Plants grown with effluent irrigation tend to accumulate higher amount of metals in their tissues and legume crops have been found to bio-accumulate heavy metals (Hussain *et al.*, 2010). Okoye *et al*., (2009) showed that much higher concentrations of these metals in the legume grains could be attributed to the fact that legume grain have high protein content, thereby easily accumulated the metals in the active sites of the proteins.

Lead concentration tends to increase in flour during grinding in a mill process as a result of excessive wear and tear of posho mill components (Wyasu *et al.*, 2010). Since most of the complementary infants foods in developing countries are based on grinding of cereals, tubers and legumes in posho mills, then there is a possibility of Pb contamination resulting from this process. For instance the Pb levels a study done in on mill processed cereals of Qalyoubia region were higher than the permissible levels (MSEI, 2008). Wyasu *et al*. (2010) also noted a higher level of Pb in mill processed cereals and legumes.

1.5 Trace element analysis using TXRF

Total-reflection X-ray fluorescence provides multi elemental analysis with high sensitivity and precision and it takes a very short time to analyse samples. Consequently, it has been used extensively in trace element analysis in different areas (Varga *et al.*, 2000; Magalhaes *et al.*, 2008; Borgese *et al.*, 2010). Borgese *et al.*, (2010) used TXRF to evaluate heavy metal poisoning in ayuvedic drug and found that the drug contained Pb at levels of 17.2 µg g^{-1}, Cu at levels of 4.7 µg g^{-1} and Zn levels at 63 µg g^{-1}. On the other hand Magalhaes *et al.*, (2008) used TXRF to quantify the levels of Fe, Cu and Zn in both carcinoma and normal breast tissues.

Liquid samples may be pipetted directly on the sample carrier for analysis. However, solid sample need to either be crashed into fine powder then mixed with a liquid to produce a suspension or it has to be digested before pipetting it onto the sample carrier for analysis. Digestion of biological and environmental samples just like applied for AAS or Inductively ICP-EOS have been proposed for TXRF analysis (Aras and Ataman, 2006). Sample digestion increases the homogeneity of the sample thus reducing the matrix effects and the background to noise ratios. Furthermore, sample decomposition improves the detections limits of the elements. This, in addition to other advantages of multi elemental analysis offered by TXRF, such as capabilities of analysing small samples and the use of an inert sample carrier makes it a more superior analytical tool as compared to AAS and ICP-OES (Holynska *et al.*, 1996).

Dry and wet sample digestion methods have been used in the decomposition of solids especially foods and diet samples (Aras and Ataman, 2006). Of interest is the wet digestion method in an open system, which was used in this study. Different acids such as HCI, HNO_3, H_2SO_4, $HClO_4$, and HF or a mixture of two of these acids are usually used as reagent in wet digestion method. Nitric acid which is a strong oxidizing agent is often used for organic matter and is efficient for metals, alloys and inorganic matter (Aras and Ataman, 2006). Therefore it has been recommended for decomposition of biological samples (IAEA, 1997).

CHAPTER 3

METHODOLOGY

3.0 Introduction

In this section, the study areas, the sampling methods, sample preparation and digestion procedures, experimental set up and elemental quantification methods are described.

3.1 Study areas

The study areas selected covered rural and urban areas. The selection of these areas was dependent on the proximity of the urban centres to the rural areas. The rural areas included Bomet, Kirwara, Turbo, Mitaboni and Sega; while the urban areas were Kericho, Thika, Eldoret, Nairobi and Kisumu as shown in figure 3.1.

3.2 Sampling method

Samples of complementary porridge flour for weaning infants between the ages of 6 months to 24 months were obtained from mothers in selected rural areas. A community health worker known by the locals was assigned to the researcher by the Officer In-charge of a local health centre to assist in identifying mothers who had children between the ages of 6 months and 24 months. The ages were verified by looking at the births certificates or the baptismal cards. The sampling of the infant flour from the identified mothers depended on the willingness of the mothers to give, at a small fee, part of the readymade flour to the researcher. Other samples were bought from the local shops in these rural areas.

Ten samples were collected from Bomet region. Two of the samples were bought from the stores while seven of the samples were obtained from mothers. The other one sample was obtained from the health centre. Maize, beans, finger millet, sorghum and potatoes are planted on large scales in this region with cultivation of finger millet ranking third after maize and beans (Nafuma *et al*, 2010).

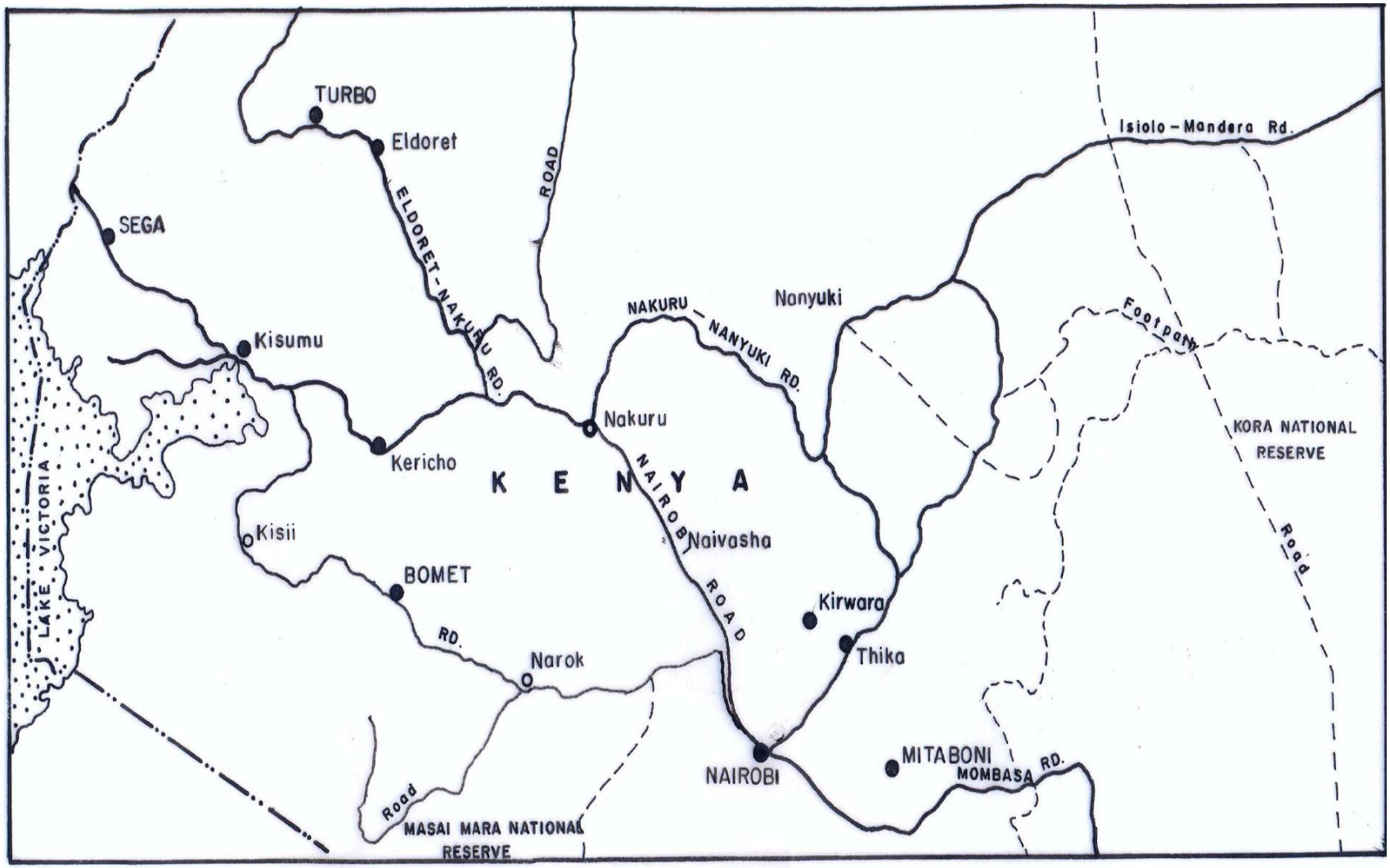

Fig 3.1: A section of map Kenya showing the study areas

Therefore most mothers preferred to modify the available food for use as complementary infant food. Maize and finger millet were the main ingredients in the samples collected from this region.

Seven samples were obtained from Kirwara. The numbers of samples collected from this region were limited given that the number of mothers who had infants between six months and 24 months were few due to a low population growth in this region which is at 0.2%, far much below the national population growth of 2.4% (http://www.ustawi.info.ke, 2013). In addition, 90% of households depend on small scale farming with food crops such as maize, beans and, finger millet being cultivated on a very small scale (http://softkenya.com/constituency/Gatanga-constituency, 2013). Most of the mothers in this area bought the complementary infant flour from the stores. Five of the samples from Kirwara region were a mixture of two or more ingredients while the other two samples were cornflour.

Turbo region is inhabited by people from different tribes, with different culture and preferences in regard to complementary infant feeding (http://softkenya.com/constituency/Turbo-constituency, 2013). Ten samples were collected from Turbo and the types of ingredients varied depending on the mothers preferences. Three of the samples were bought from the stores while the rest of the samples were collected from the mothers. It was noted that maize, millet and sorghum were the main types of ingredients used by the mothers to prepare the complementary infant flour.

Ten samples of complementary infant flour were collected from the Mitaboni region. This region has irregular weather patterns and farming activities is usually unpredictable. Therefore, the region is associated with food insecurity and disturbing malnutrition levels (http://fts.unocha.org, November 2012). Eight of the samples were obtained from the mothers and most of them had bought the flour from the stores. The types of ingredients used to prepare the flour were unknown to most of the mothers. However, millet was one of the ingredients but the mothers were unable to provide information on the type of millet used. The other two samples were bought from the stores.

Nine samples were obtained from Sega region. This region has malnutrition levels estimated to range between 2.5% and 5% in children less than 36 months of age (http://medicine.unm.edu/globalhealth/facilities/siaya.html, accessed in April, 2013). In this region, women groups have been formed to foster a sustainable food based approach in combating micronutrient deficiencies. Crops such as soya, cassava, finger millet, maize and groundnuts are being cultivated, then modified and used for complementary infant feeding. Therefore, the types of ingredients used in these nine samples depended on the availability of these food crops.

Samples of commercialized flour were bought from selected stores in selected urban areas, which included Nairobi, Kericho, Kisumu, Eldoret and Thika. The samples were randomly bought from these stores. A total of twelve samples were bought from Nairobi; five from Kirinyanga millers and seven from different stores in the central business centre. In Thika, fourteen samples were bought from different stores along Mama Ngina Drive; ten samples were bought from different stores and the Municipal Council market in Eldoret; while in Kisumu and Kericho, five samples from each of these areas were bought. Although many of the major stores stocked similar branded infant porridge flour, only one of each kind was bought from the stores. The ingredients of the branded flour were indicated on the package. Sweet potatoes, pumpkin, carrots and corn which are not commonly used in preparation of complementary were used as ingredients in some of the branded porridge flour. Other branded flour had unspecified minerals added in them. However, the relative proportions of the ingredients in both generic and branded complementary infant flour are not indicated as observed in a study done by Huffman (2000). On the other hand, the size, ingredients and packaging of the generic flour varied depending on the need of the buyer.

The samples were then packed in polythene bags, marked and transported to the Institute of Nuclear Science and Technology laboratory at the University of Nairobi.

3.3 Sample preparation

3.3.1 Cleaning of glassware and sample carriers

The glassware were thoroughly washed with 0.2% Devo-Clean® solution. They were then rinsed with double distilled water and soaked in 5% nitric acid solution for an overnight. Finally the glasswares were thoroughly rinsed with double distilled water.

The quartz sample carriers were gently pre-cleaned with serviette (Fay™) soaked in acetone to remove any visible dirt and then mounted on a washing cassette. The cassette was transferred into a Pyrex glass beaker containing 0.2% Devo-Clean® detergent solution and heated on a hot plate at 100^0 C and stirred at 9 rev/ minutes for 5 minutes.

The carriers were then thoroughly rinsed in double distilled water and afterwards transferred into a Pyrex glass beaker filled with 10% ANALAR nitric acid which was then heated using a hot plate at 100 ^{0}C and stirred at 9 rev/ minutes for 2 hours. The carriers were finally transferred to a Pyrex beaker with double distilled water and heated at 100 ^{0}C and stirred 9 rev/ minutes for another 5 minutes.

The sample carriers were then removed from the cassette and directly dried on a hot plate at 60 ^{0}C for 5 minutes. Finally, they were wiped with a serviette (Fay™) soaked in acetone. Ten µl of silicon solution in isopropanol (Serva ™) was pipetted at the centre of the samples carriers. The carriers were dried on a hot plate at 60 ^{0}C for one minute. The cleanliness of the sample carriers were checked on the S2-Picofox TXRF machine and stored in a dessicator for use.

3.3.2 Sample digestion

A sub sample weighing between 1.00 g and 1.05 g was transferred into pre-cleaned flat bottomed flasks. Wet digestion in an open system using ANALAR nitric acid was used to extract the trace elements from the samples matrix (Korn, *et al.*, 2008).

Thirty millilitres (30) of the nitric acid and 15 ml of double distilled water were added to the sub sample and left for an overnight for cold open digestion. The solution was then transferred to a round bottomed flask and heated at a temperature of between 60 ^{0}C to 100 ^{0}C. More double

distilled water was added to the solution and heated at this temperature until the solution was clear. The final solution was then transferred to a well labeled vial. The round bottomed flask was rinsed three times with double distilled water and solution added into the vial. The volume was finally brought to 15 ml with double distilled water.

This analytical procedure was validated by digesting a standard reference material - New NIM-GBW10017, a milk powder, in triplicates.

For each of the digested samples, three aliquots were prepared. 100 µl of internal standard (2 ng μl^{-1} of gallium) was added to 100 µl of each aliquot and the standard reference material and stirred. 10 µl of each was then pipetted onto a quartz carrier and dried on a hot plate at 60 ^{0}C for approximately 3 minutes. The quartz carriers were subsequently stored in a Petri dish for analyses.

3.4 Experimental set up

S2-Picofox TXRF (figure 4.2) spectrometer was used. The spectrometer operated at 50 kV, 1 mA and it was fitted with a Mo anode and Ni/C multilayer monochromator. The monochromatic beam produced impinged the sample holder placed at an angle less than 0.1^0 in the direction of the incident beam (Kregsamer *et al*, 2002).

The energies of the fluorescent X-rays emitted are detected by Peltier cooled Silicon drift detector (Tiwari, *et al*. 2002) with a resolution of 146 eV at Mn K_α FWHM. The intensities of the X-ray fluorescence are then measured by an amplifier coupled to a multichannel analyser installed in a personal computer.

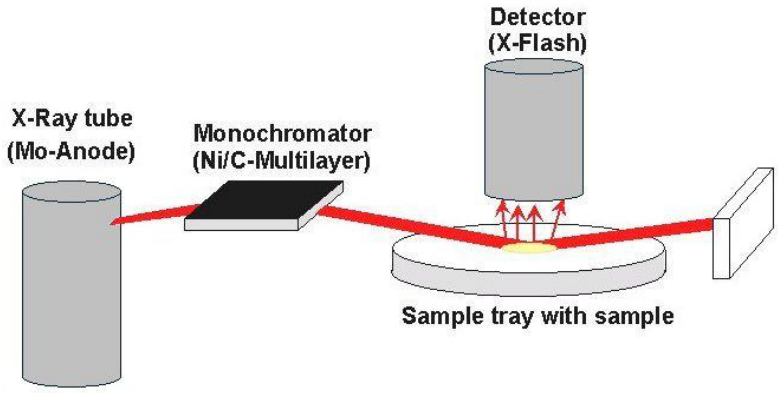

Fig. 3.2: Schematic working principle of the S2 PICOFOX spectrometer

3.5 Sensitivity of S2-Picofox instrument

The sensitivity of the instrument was assessed by analysing Merck XVI, a multi-elemental standard pre-deposited on a quartz carrier, for 1000 seconds.

3.6 Quantification of the elements

Spectra software was used to deconvolute the spectra and calculate the net intensities of the peaks using internal standardization. The internal standard was used to correct for any variations in sample deposition on the sample-reflector surface that could produce difference in regard to the resulting absolute sensitivity (Alvarez *et al*., 2007). Since the sample was a thin film whose absorption and enhancement effects are negligible (Klockenkämper and Bohlen, 1989), with gallium as an internal standard, the concentration of the respective elements was calculated using equation 3.1

$$C_i = \frac{C_{Ga} \times N_i \times S_{Ga}}{N_{Ga} \times S_i} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 3.1$$

where

C_i is the concentration of element to be analyzed,

C_{Ga} is the concentration of Ga,

N_i is the net peak area within the measurement spectrum of the element to be analyzed,

N_{Ga} is the net peak within the measurement spectrum of Ga,

S_{Ga} is the relative sensitivity of Ga, and

S_i is the relative sensitivity of element to be analyzed.

Equation 3.2 was then used to convert the elemental concentrations from mg l^{-1} to mg kg^{-1},

$$C_s = \frac{C_i \times V \times D}{W} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 3.2$$

where

C_s is the sample concentration in mg kg^{-1},

C_i is the concentration of extract in mg l^{-1},

V is the volume of extract,

D is the dilution factor, and

W is the weight of the sample in the extract.

3.7 Lowest Limits of detection (LLD)

Borgese *et al*. (2011) defines lowest limits of detection as the smallest amount of an element in a given sample that can be detected by an instrument based on statistical inspection of the peak area and the subjacent spectral background. In addition, the element is detectable if the peak area is three times larger than the counting statistics of the background and is calculated using either equation 3.3 or equation 3.4

$$LLD = \frac{3 . C_i . \sqrt{N_{BG}}}{N_i} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.3$$

$$LLD = \frac{3 . \sqrt{I_B}}{S} . \frac{1}{t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.4$$

Where

LLD is the lowest detection limit.

C_i is the concentration of element i,

N_i is the area of the fluorescence peak in counts,

N_{BG} is the background area subjacent the fluorescence peak,

I_B is the background intensity,

S is the sensitivity equal to net intensity divided by the sample mass, and

t is the counting time.

From the two equations, the detections limits could therefore be increased by either reducing the background intensity or increasing on the measuring time. In this study, 1000 counting seconds was used.

3.8 Statistical analyses

The results obtained in this study were subjected to both t-test and analysis of variance (ANOVA) using Origin pro and Sigmaplot. The experimental and certified values of the certified reference material GBW 10017 were compared using paired t-test while the trace element levels in any two of the samples were compared using unpaired t-test. For multiple comparisons of means, ANOVA was used. In all the case p-values less than 95% confidence level ($\alpha = 0.05$) were considered significantly different.

CHAPTER 4

RESULTS AND DISCUSSION

4.0 Introduction

In this chapter, results obtained after the analysis of a multi-elemental standard to check the sensitivity of the spectrometer and the results of the analysis of reference material are presented and discussed. The selected trace elements levels and lead levels in the samples from both rural and urban areas are also presented and discussed. The selected trace elements included Mn, Fe, Cu and Zn.

4.1 Sensitivity of the TXRF spectrometer

The measured mean concentration values for each of the K and L series of the multi-elemental standard, Merck XVI are presented in table 4.1. The bias defined as the difference between the mean value determined for the analyte of interest and the accepted true value (Taverniers *et al.*, 2004) is also presented in this table.

Table 4.1: Measured mean values and Bias of MERCK XVI

N = 10

	Certified values (mg l^{-1})	Measured mean value and one std deviation (mg l^{-1})	Bias (%)
Ca	100	100.8 ± 3.0	0.8
Ti	100	97.7 ± 2.5	2.3
V	99	100.5 ± 2.7	1.5
Cr	99	99.4 ± 2.5	0.4
Mn	100	100.4 ± 2.5	0.4
Fe	101	103.4 ± 2.7	2.4
Co	100	100.2 ± 3.8	0.2
Ni	101	98.4 ± 7.7	2.6
Cu	101	101.0	Set as internal standard
Zn	99	99.2 ± 1.9	0.2
As	100	99.2 ± 2.6	0.8
Se	101	93.3 ± 2.2	7.6
Sr	100	98.0 ± 3.9	2
Sb	100	108.7 ± 3.6	8.7
Tl	102	96.6 ± 9.2	5.3

Pb	101	82.7 ± 11.2	18.1

These results show that the errors of margin of all the K- series elements were below 10%. Only lead among the L-series elements had an error of margin above 10%. This is similar to an observation by Koreleff and Kremling (1999) which showed that the errors of margin for Pb could be as high as 30% or more.

The net intensities for each of the element for the K series elements of the multi elemental standard Merck XVI were fitted using SigmaPlot statistical program and the fitting is shown in figure 4.1.

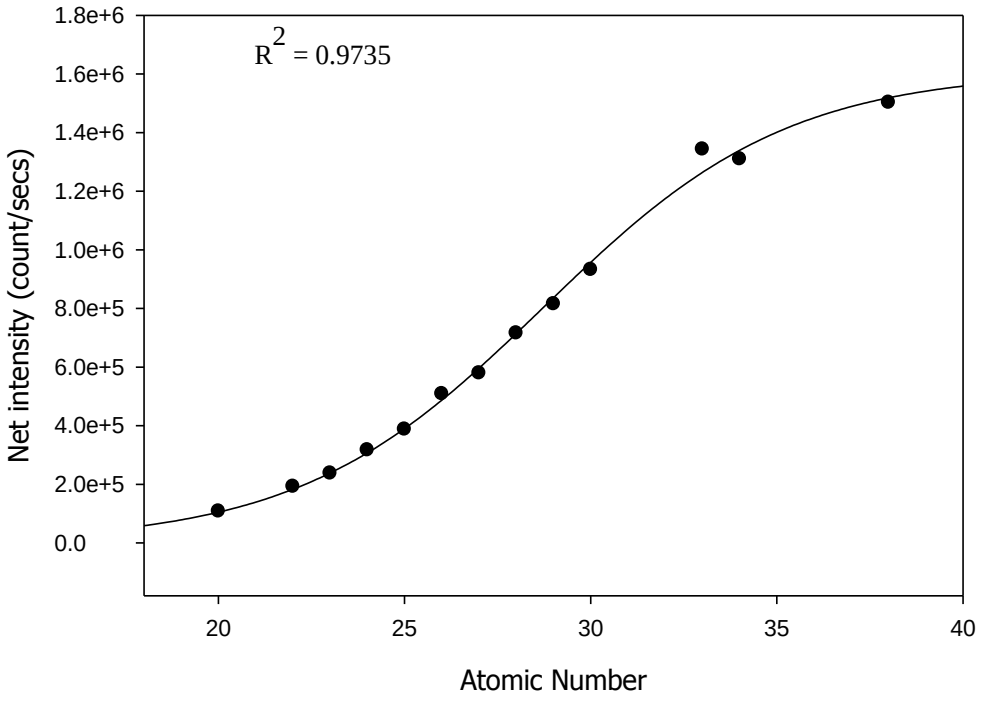

Fig 4.1: Sensitivity curve for K series elements

The fitting of the experimental values showed a strong relationship between the atomic number and the net sensitivity since $R^2 = 0.9735$ (goodness of fit).

4.2 Lowest Limit of Detection (LLD)

The experimental lowest detection limits of the elements of interest were obtained from some of the samples using equation 3.3. Furthermore, using equation 3.2, the concentrations of the elements were converted into mg kg^{-1} and are shown in table 4.3. A graph was generated from these data using SigmaPlot Statistical program and is shown in figure 4.2.

Table 4.2: Lowest detection limits of elements of interest

Element	Atomic No.	Concentration(mg kg^{-1}) N = 30
Ti	22	0.55
V	23	0.49
Cr	24	0.33
Mn	25	0.31
Fe	26	0.22
Ni	28	0.15
Cu	29	0.14
Zn	30	0.12
Pb	82	0.07

The graph indicates that the detection limits for the K-lines elements declines with increase in atomic number as expected. The trend of this curve is comparable to other detection limits curves in other studies carried out by Simabuco *et al*. (2002) and Orghêda *et al*. (2005). However, the elemental lowest detection limits in this experiment were different from these earlier studies since the detection limit of an element depends on the sensitivity of the instrument, the counting time and matrix effects of the sample (Rousseau, 2001). Therefore, this instrument could be used to detect these elements in the samples at these indicated levels.

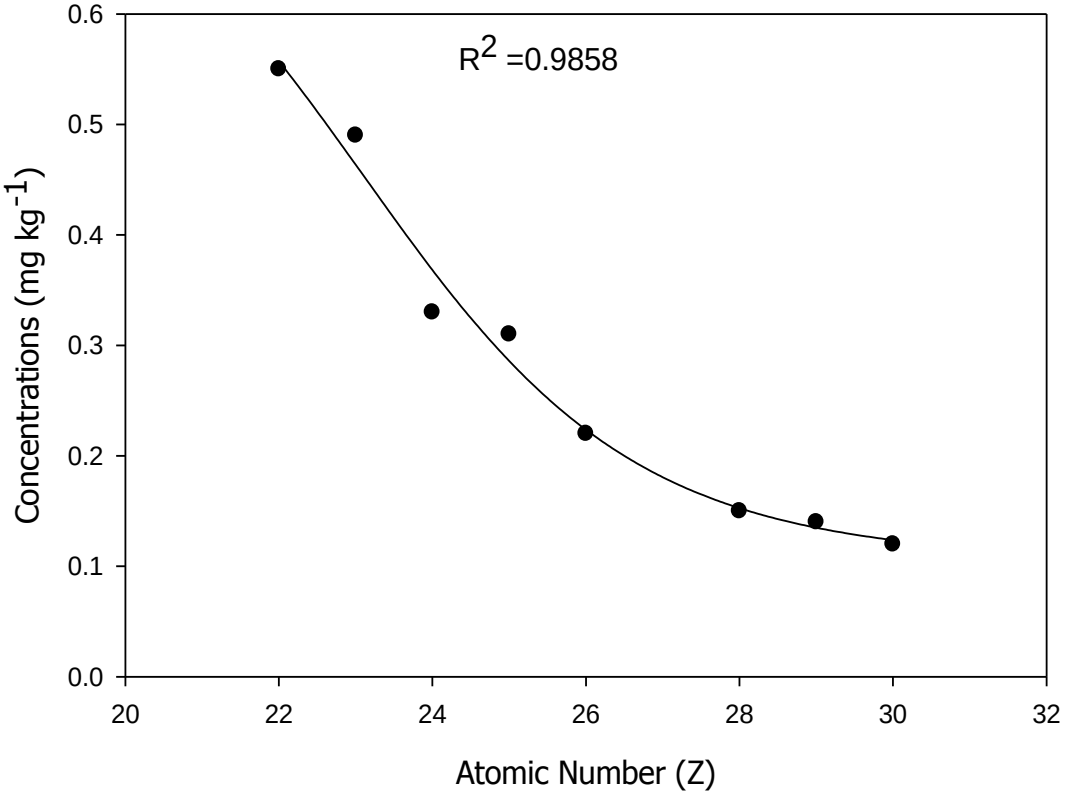

Fig 4.2: A graph showing the lowest detection limits of some K series elements

4.3 Validation of the Analytical method

To validate the analytical method, a reference material (NIM GBW 10017, milk powder) was analysed. The experimental concentrations were compared with the certified values of the reference material and subjected to a one sample t-test (IAEA, 2003). The associated p-value and bias for each element was calculated and they are shown in table 4.3

Table 4.3: Comparison of experimental and certified concentrations of NIM GBW 10017, Milk Powder

	Concentrations in mg kg^{-1}			
Element	**Experimental (N=3)**	**Certified**	**P-Value**	**Bias (%)**
Cr	0.36 ± 0.05	0.39 ± 0.04	0.483	7.7
Mn	0.45 ± 0.15	0.51 ± 0.17	0.190	6.3
Fe	7.6 ± 3.5	7.8 ± 1.3	0.945	2.6
Cu	0.48 ± 0.06	0.51 ± 0.13	0.564	5.9
Zn	31 ± 4	34 ± 2	0.307	8.8
Pb	< LDL	0.07 ± 0.02	-	-

LDL stands for lowest detection limits

The p-values at 95% confidence level for all the elements were greater than 0.05. Therefore, the experimental values of the trace elements were not significantly different from the certified values. In addition, the biases of the experimental values from the certified values were below 10% for all the elements of interest. This analytical procedure was therefore suitable for analysis of trace element in complementary infant flour from the selected rural-urban areas.

4.4 Selected trace elements levels in the samples

The samples were analysed for Mn, Fe, Cu and Zn. The results per region are presented and discussed in this section. The levels of the trace elements in the samples from the different regions are also compared and discussed.

4.4.1 Bomet Region

The mean concentrations of the trace elements in the samples varied as follows: Mn, 1.96 ± 0.39 mg kg^{-1} to 329 ± 28 mg kg^{-1}; Fe, 14.6 ± 1.6 mg kg^{-1} to 112 ± 3 mg kg^{-1}; Cu, 0.72 ± 0.02 mg kg^{-1} to 4.00 ± 0.11mg kg^{-1}; and Zn, 8.24 ± 0.26 mg kg^{-1} to 30.5 ± 0.9 mg kg^{-1} as shown in table 4.4.

It was observed that sample B9 whose ingredients were finger millet only as shown in table 4.4 had the highest level of Mn while samples B6 and B7, both maize samples had the lowest level of Mn. It is also observed that the levels of Mn in samples B2, B4, B5, B8 and B10 varied with levels of Mn ranging from 113 ± 2 mg kg^{-1} to 200 ± 42 mg kg^{-1}.

Table 4.4: Mean concentrations (mg kg^{-1} ± 1SD) of Mn, Fe, Cu and Zn in samples from Bomet region

N = 3

	Ingredients	Mn	Fe	Cu	Zn
B1	Insta flour (from the health centre)	8.12 ± 0.45	75.3 ± 3.0	3.74 ± 0.60	30.5 ± 0.9
B2	Finger Millet, maize	174 ± 59	47.8 ± 14.8	3.27 ± 0.99	19.3 ± 6.4
B3	Famila pure millet	113 ± 2	46.6 ± 5.4	3.20 ±0.12	22.1 ± 0.7
B4	Maize, finger millet and souring agent (Famila sour porridge flour)	35.7 ± 5.5	47.3 ± 2.5	1.95 ± 0.17	14.5 ± 1.3
B5	Maize, finger millet	132 ± 8	90.2 ± 7.2	1.56 ± 0.18	16.5 ± 0.5
B6	Maize	2.49 ± 0.28	15.8 ± 1.8	0.73 ± 0.05	15.7 ± 0.5
B7	Maize	1.96 ± 0.39	14.6 ± 1.6	0.72 ± 0.02	14.3 ± 0.3
B8	Maize, finger millet	167 ± 5	112 ± 3	3.41 ± 0.20	22.5 ± 0.8
B9	Finger millet	329 ± 28	31.2 ± 4.5	2.49 ± 0.18	23.2 ± 2.4
B10	Maize, finger millet	200 ± 42	37.6 ± 12.0	4.00 ± 0.25	26.37 ± 0.6

From this observation, it is evident that finger millet is the a very good source of Mn. Studies done by Shemelis *et al*. (2009) and Maina *et al*. (2012) also showed that finger millet had high concentrations of Mn. The proportions of finger millet to maize in the samples may have therefore had an effect on the concentrations of Mn in the other samples which were composed of maize and finger millet. For instance, sample B4 whose Mn levels were 35.7 ± 5.5 mg kg^{-1} may have had more proportions of maize to finger millet when compared to samples B2, B5, B8 and B10 whose proportions of finger millet to maize also seemed to have varied. This observation concurs with a study that was carried out by Gibson *et al*. (1998) which showed that the levels of trace elements in formulated complementary infant foods varied depending on the proportions of ingredients in the foods.

Studies have also shown that land preparation methods have an effect on the levels of Mn in cereals. For instance, a study done by Nafuma *et al*. (2010) showed that the high levels of Mn in finger millet was attributed to the indigenous technical method of land preparation. This farming mode involves heating of soil. According to Kitur and Frye (1983), heating of soil at certain temperatures increases the PH of the soil, consequently improving the bioavailability of Mn in

the soil hence boosting uptake of Mn by the plants (Kabata-Pandias and Mukherjee, 2007). A study done by Nafuma *et al*. (2010) reported that Mn in finger millet from Bomet region was high when the soils were burnt at 220^{o}C.

Sample B8 (maize and finger millet) had the highest level of Fe while samples B6 and B7 (maize) had the lowest levels of Fe. The levels of Fe were comparable in samples B2, B4 both composed of finger millet and maize, and B3 (Famila finger millet). The levels of Fe were also comparable in samples B9 (finger millet) and B10 (finger millet and maize). Samples B1 (Insta flour) and B5 (maize and finger millet), also had comparable levels of Fe.

High levels of Fe in some of the samples may have been as a result of either fortification of the flour with Fe or as a result of the preparation method of the flour. The type of ingredients in the samples could have also contributed to the high levels of Fe in the samples. For instance B1 which collected from the health centre was fortified with Fe; its Fe level was 75.3 mg kg^{-1}. Likewise, samples B5 and B9 which were both a mixture of finger millet and maize and they were unfortified with Fe had very high levels of Fe: B5, 90.2 mg kg^{-1} of Fe; and B8, 112 mg kg^{-1} of Fe. A study done by Icard-Verniere *et al*. *(*2012) also showed that high levels in flour may be as a result of contamination of Fe from grinding equipments during the grinding processes. In another study done by Andersen *et al*. (2011) soils residues and dust with high content of Fe may settle on cereals during the drying processes. Therefore, the high levels of Fe in samples B5 and B8 may be as a result of contamination from the grinding equipments or soil residues and dust that during the drying process.

The levels of Zn in the samples also varied. Sample B1 which was fortified with Zn had the highest level of Zn while samples B4 and B7 had the lowest levels of Zn and these levels were within a closer range with the levels of Zn in samples B2, B5 and B6. The levels of Zn in samples B3, B8 and B9 were also within a closer range.

Studies have shown that cereal grains have inherently very low concentrations of Zn and this may be aggravated by growing cereal crops on potentially low Zn deficient soils leading to further decrease in the level of Zn in the cereal grains (Musa *et al*, 2012). Removing of the outer layer of the cereals during the milling process also greatly reduces the level of Zn in cereal grains. A study done by Maina (2005) and Bauernfeind (1991) showed that milling process

reduced the level of Zn in maize by over 70%. Therefore the low levels of Zn in some of the samples from this region may probably be as a result of either cultivation of the cereal grains on Zn deficient soils or loss of Zn during the milling process.

Indigenous technical mode of land preparation has also been associated with soil degradation (Nafuma *et al.,* 2010) resulting to Zn deficiencies in soils especially after finger millet cultivation. This may lead to low yields of major cereal crops. A study done by Kabata-Pandias and Mukherjee (2007) and Wang *et al*., (2006) showed that bioavailability of Zn in plants decreased with increase in soil PH which in this case may be due to the heating of soils at certain temperatures (Nafuma, *et al.*, 2010). Therefore the land preparation method could have contributed to the low concentration of Zn in some of the samples from this region.

The concentration of Cu in the samples from this region was very low compared to the other trace elements. However sample B10 had the highest level of Cu while samples B6 and B7 had the lowest levels of Cu. Low levels of copper in plants are closely related to copper deficiency in soils (Baker and Sneft, 1995). The level of copper in plants depends on the soil type, climatic conditions and the crop grown (Kabata-Pandias and Mukherjee, 2007). High soil pH decreases the uptake of Cu by plants. Furthermore, cereal plants tend to be highly sensitive to the effect of Cu deficiencies in soils (Maina *et al*. 2012). Therefore, some of these factors may have contributed to the low level of Cu in the samples from this region.

The variations of these trace elements in the samples from Bomet region are shown in figure 4.3. It can be observed that the levels of Mn in the samples stand out in most of the samples as compared to the levels of Cu.

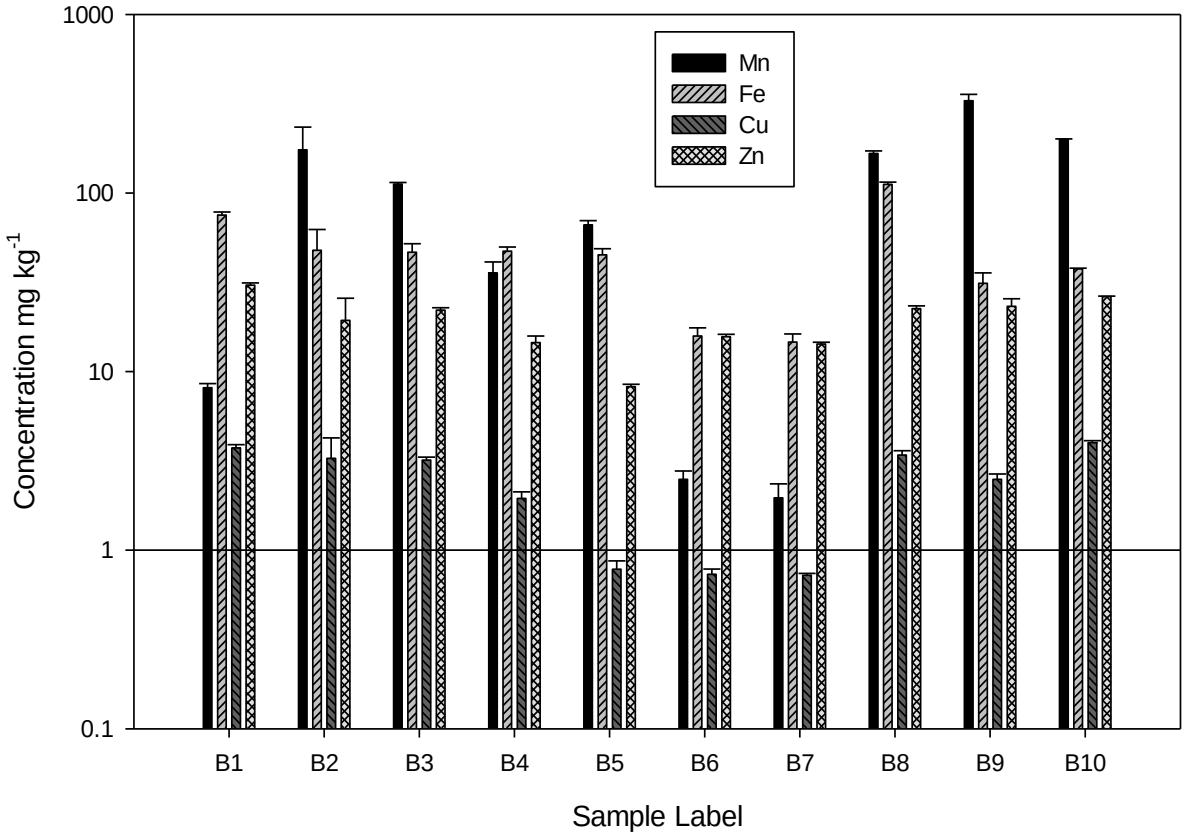

Figure 4.3: A graph showing the concentration of Mn, Fe, Cu and Zn in samples from Bomet

4.4.2 Kirwara Region

The concentrations of Mn, Zn, Cu and Fe in the samples varied as follows: Mn, 3.32 ± 0.23 mg kg^{-1} to 9.33 ± 1.84 mg kg^{-1}; Fe, 35.1 ± 3.8 mg kg^{-1} to 98.6 ± 9.0 mg kg^{-1}; Cu, 0.86 ± 0.02 to 1.74 ± 0.19 mg kg^{-1}; and Zn, 14.6 ± 1.1 mg kg^{-1} to 45.3 ± 1.5 mg kg^{-1}. These concentrations of the trace elements are shown in table 4.5.

The number and types of ingredients used in the preparation of samples from this region were different as shown in table 4.5.

The level of Mn in K1 and K7 were higher when compared to the level of Mn in other samples. In this study, it is observed that finger millet is a good source of Mn and this was one of the ingredients in these two samples. Therefore, the high but varying levels of Mn in samples K1 and

K7 may probably have been contributed by the presence of finger millet but were in different proportions in the two samples. Furthermore, it is observed that sample K6 also had finger millet as one of its ingredient. However, the level of Mn in this sample was not as high as to the levels of Mn in sample K1 and K2. This indicates that the proportions of finger millet in the samples played a major role in the levels of Mn in these samples. The ratios of finger millet to sorghum in sample K6 may have been a fraction.

Table 4.5: Mean concentration (mg kg $^{-1}$ ± 1 SD) of Mn, Fe, Cu and Zn in samples from Kirwara region

N=3

	Ingredients	**Mn**	**Fe**	**Cu**	**Zn**
K1	Soya, pearl millet, sorghum, finger millet, groundnuts, stinging nettle, refined maize, fish, amaranth	9.33 ± 1.84	66.9 ± 9.1	1.74 ± 0.19	21.9 ±1.3
K2	Sorghum, pearl millet	5.36 ± 1.30	98.6 ± 9.0	1.74 ± 0.05	14.6 ± 1.1
K3	Sorghum, maize, soya, stinging nestle	5.65 ± 0.37	88.3 ± 12.5	1.20 ± 0.15	18.0 ± 0.4
K4	Cornflour	3.32 ± 0.23	71.2 ± 4.4	1.69 ± 0.13	39.7 ± 0.7
K5	Cornflour (Health centre)	3.61 ± 0.28	64.3 ± 4.8	1.77 ± 0.05	45.3 ± 1.5
K6	Finger millet, Sorghum	3.31 ± 1.65	35.1 ± 3.8	0.86 ± 0.02	27.7 ± 0.0
K7	Sorghum, refined maize, pearl millet, finger millet	15.6 ± 0.9	122 ± 26	2.21 ± 0.03	24.1 ± 0.6

The level of Mn in samples K2 and K3 were comparable. Sorghum was a common ingredient in these two samples. A study done by Rooney *et al. (2010*) indicated that sorghum was a good source of Mn and its levels were 16.3 mg kg^{-1}. Thus, sorghum could probably have contributed to the levels of Mn in K2 and K3. On the contrary, very low levels of Mn were observed in sample K6 whose ingredients were finger millet and sorghum. The level of Mn in B6 was in the same range with the levels of Mn in samples K4 and K5 which were corn flour.

The levels of Fe in samples K1, K4 and K5 were comparable. The levels of Fe in samples K2 and K3 were within a close range. Sample K7 had the highest level of Fe, while sample K6 had the lowest level of Fe.

Samples K4 and K5 were corn flour collected from the health centre and were fortified with Fe, while samples K1, K2, K3 and K7 had sorghum and millet which considered to be good sources of Fe (Musa *et al.*, 2012). In addition, sample K1 and K3 had soya, another good source of Fe (Andersen *et al.*, 2011). Sample K1 had more ingredients than K7 and most of these ingredients are considered to be good sources of Fe. However, the level of Fe in this sample was not as high as expected; sample K7 had higher levels of Fe than K1. The proportions of these ingredients in sample K1 could probably have been miniature as compared to refined maize which especially when milled provide very low levels of Fe (Maina, 2005).

The ingredients, millet and sorghum, in sample K6 were also good sources of Fe. However, the levels of Fe in this sample were not comparable to the rest of the samples in this study. A study done by Kayodé *et al.* (2006) showed that genetic factors as well environmental factors could significantly contribute to the uptake and accumulation of Fe in sorghum and millet grains. Most of the samples collected from Kirwara were bought from the stores. The source of the flour was unknown to most of the mothers. Therefore, these factors may have contributed to the different levels of Fe.

The levels of Zn in samples K4 and K5 were higher as compared to the other samples. These samples were fortified with Zn. Samples K2 and K3 had the lower levels of Zn while the levels of Zn in samples K1, K6 and K7 were comparable.

The levels of Cu in the samples from this region were lower than other trace elements as shown in fig 4.4. Sample K7 had the highest level of Cu while sample K6 had the least level of Cu. The levels of Cu in the other five samples were within a very close range.

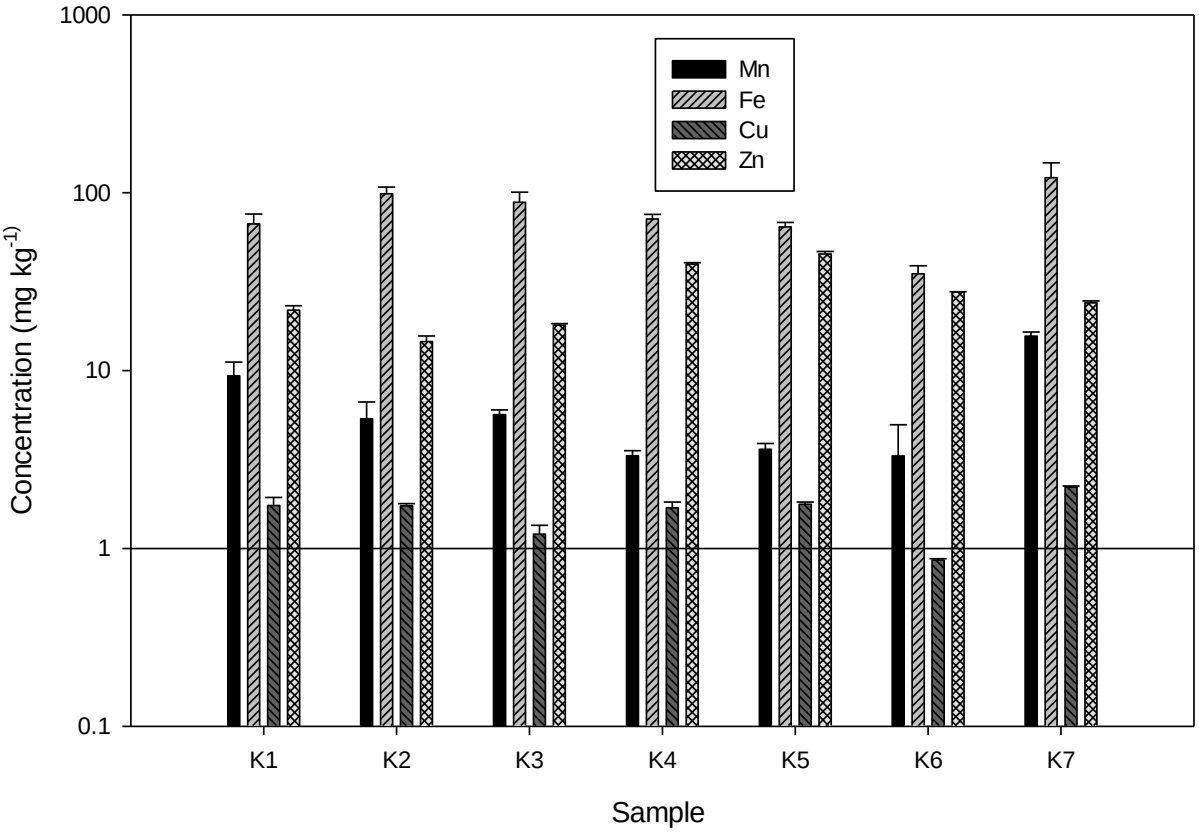

Fig. 4.4: A graph showing the concentration of Mn, Fe, u and Zn in samples from Kirwara

4.4.3 Turbo region

The concentrations of Mn, Zn, Fe, and Cu in the sample flour varied as follows: Mn, 2.46 ± 0.15 mg kg^{-1} to 78.1±2.0 mg kg^{-1}; Fe, 17.7 ± 1.0 mg kg^{-1} to 178 ± 20 mg kg^{-1}; Cu, 0.95±0.02 mg kg^{-1} to 4.08 ± 0.25; and Zn, 16.7 ± 0.5 mg kg^{-1} to 81.2 ± 0.4 mg kg^{-1}.

Samples TRB4 and TRB9 had the lowest levels of Mn compared to sample TRB7 whose levels of Mn were comparable to those in samples TRB3. Samples TRB6 and TRB8 had the highest levels of Mn while samples TRB5 and TRB10 had moderate levels of Mn. Samples TRB8 and TRB9 were composed of maize and finger millet; while samples TRB5 and TRB6 were composed of maize, finger millet and sorghum as shown in table 4.6.

In this study, it has been observed that finger millet from Bomet region had the highest levels of Mn while maize from the same region had the lowest levels of Mn. The high level of Mn in finger millet was attributed to the land preparation method which involved heating of soils before

planting finger millet. Turbo is a multi-ethnic region and one of the tribe in this region practice the same culture as the inhabitants of Bomet. Therefore, the varying levels of Mn in samples TRB5, TRB6, TRB8 and TRB10 may have been as a result of varying proportions of finger millet in the samples.

Table 4.6: Mean concentrations (mg kg^{-1}+ 1SD) of Mn, Fe, Cu and Zn in samples from Turbo region

N=3

	Ingredients	Mn	Fe	Cu	Zn
TRB1	Millet, soya, sorghum, maize, sugar	6.67 ± 0.03	158 ± 35	1.77 ± 0.05	18.5 ± 0.3
TRB2	Sorghum, soya, fine maize meal, sugar	6.83 ± 0.14	126 ± 7	2.15 ± 0.19	20.1 ± 0.4
TRB3	Millet, sorghum, soya, cassava, groundnuts, fine maize, fish powder, milk powder, green grams	13.0 ± 4.3	150 ± 12	1.85 ± 0.10	29.6 ± 1.3
TRB4	Maize	2.47 ± 0.14	28.8 ± 4.9	1.82 ± 0.13	36.2 ± 1.7
TRB5	Sorghum, finger millet, maize	46.5 ± 0.8	22.8 ± 3.2	1.53 ± 0.02	21.0 ± 0.6
TRB6	Sorghum, finger millet, maize	78.1 ± 2.0	74.4 ± 1.8	2.17 ± 0.07	22.0 ± 0.2
TRB7	Maize	11.2 ± 1.2	61.5 ± 5.6	4.08 ± 0.25	81.2 ± 0.4
TRB8	Finger millet, maize	73.2 ± 2.1	58.7 ± 4.5	2.39 ± 0.09	16.7 ± 0.5
TRB9	Maize	2.46 ± 0.15	17.7 ± 1.0	0.95 ± 0.02	17.2 ± 0.3
TRB10	Maize, finger millet	46.2 ± 0.7	178 ± 20	2.17 ± 0.03	18.5 ± 0.2

Samples TRB1, TRB2, TRB3 and TRB10 had higher levels of Fe and within a close range of 126 mg kg^{-1} to 178 mg kg^{-1} compared to the other samples. Samples TRB1, TRB2 and TRB3 were bought from the stores while sample TRB10 was obtained from one of the mothers. The types of ingredients in these samples were different even though all the samples bought from the stores contained finger millet, soya and sorghum. Finger millet, sorghum and soya have been considered to be good sources of Fe (Musa *et al.*, 2012). Furthermore sample TRB3 had groundnuts, fish powder and green grams which are also considered to be a good source of Fe (Andersen *et al.*, 2011; Saunders *et* al, 2012). However, sample TRB10 whose ingredients were maize and millet only had the highest level of Fe.

The preparation and storage methods of the flour may have also contributed to the variability of Fe in the samples. For instance, if the flour was stored in an iron container, then the levels of Fe in the flour could possibly be higher. Environmental factors such as humidity may lead to leaching of Fe from the metallic container into the flour increasing the Fe levels (Rafique *et al.*, 2004). High levels of Fe in flour may be as a result of contamination of Fe during the milling process (Andersen *et al*, 2011; Icard-Verniere *et al*, 2012). Therefore the higher levels of Fe in sample TRB10 may be resulting from the leaching of Fe into the flour or contamination of Fe during the milling process.

These effects of flour preparation and storage methods may have also contributed to the observation made in the levels of Fe in samples TRB4, TRB7 and TRB9. The level of Fe was higher in sample TRB7 than in samples TRB4 and TRB9.

It is also worth noting that two of the maize samples, TRB4 and TRB9, had the highest levels of Zn: TRB, 81.2 ± 0.4 mg kg^{-1}; and TRB4, 36.2 ± 1.7 mg kg^{-1}. However, sample TRB9, another maize sample, had the lower level of Zn of 17.7 ± 1.0 mg kg^{-1} and were comparable to those in samples TRB1, TRB2, TRB5 TRB6, TRB8 and TRB10. Sample TRB3 whose ingredients were fish powder, soya, groundnuts, green grams among others and are considered to good sources of Zn (Andersen *et al.*, 2011; Saunders *et al.*, 2012) had Zn levels of 29.6 ± 1.3 mg kg^{-1}.

Maize is one of the main cash crops in this region and therefore it is cultivated on large scale and inorganic fertilizer is often used. A study done by Alloway (2008) showed that maize is most vulnerable to zinc deficiency and it receives its highest proportion of Zn from application of Zn fertilizer that fixes Zn in the soils. This is observed in samples TRB4 and, TRB7 which had comparably high levels of Zn as compared to sample TRB9. Therefore sample TRB9 could most likely have been grown on Zn deficient soils while samples TRB4 and TRB7 were grown on varying Zn rich soils or inorganic fertilizer that richly fixed Zn in the soils could have been used. In another study carried out by Musa *et al.* (2012), a certain variety of maize concentrated more Zn in the grains. These could be a major factor in the variation of Zn in the three maize samples.

The levels of Cu in the samples from this region were lower than other trace elements as shown in fig 4.5. Sample TRB7 had the highest level of Cu while sample TRB9 had the least level of

Cu. Both samples were maize. However, the levels of Cu in the other samples were comparable low.

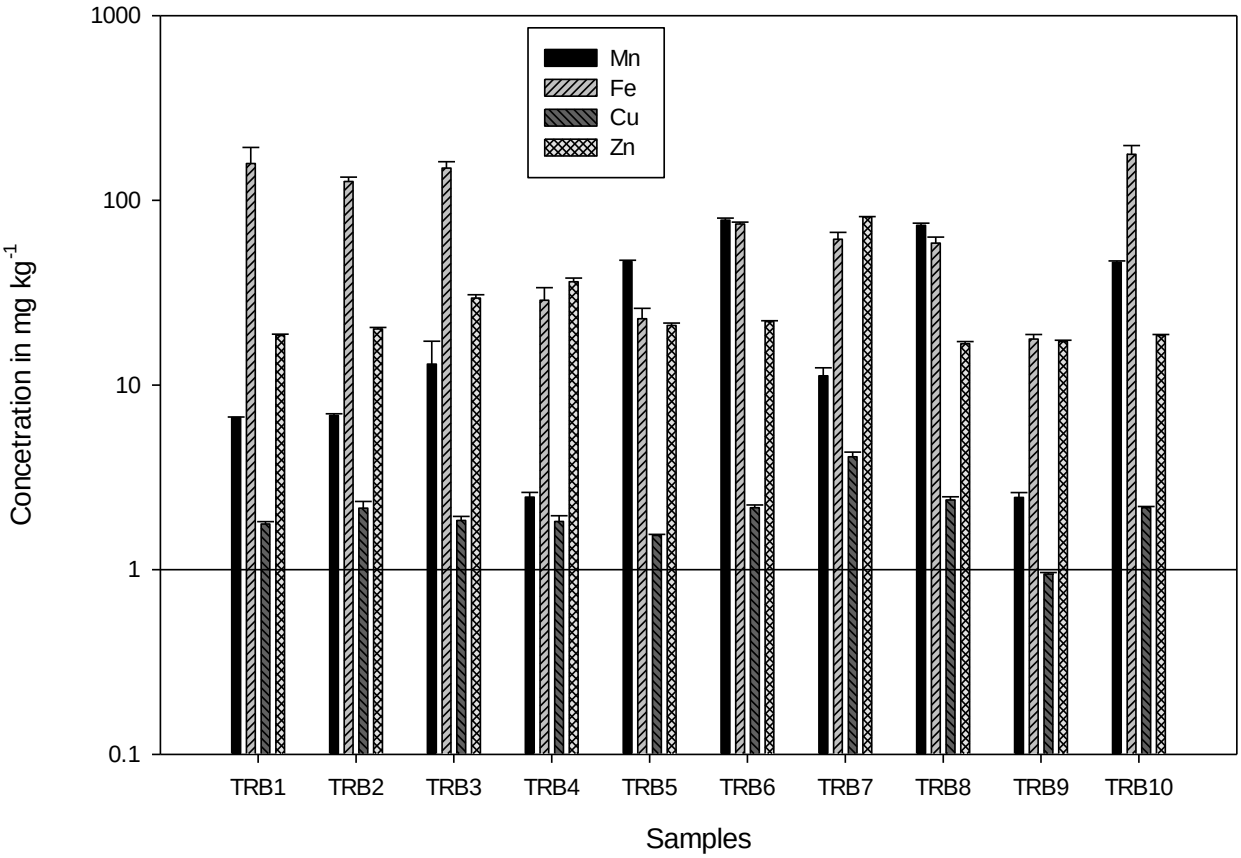

Fig 4.5: A graph showing the concentration of Mn, Fe, Cu and Zn in samples from Turbo

4.4.4 Mitaboni region

The concentrations of Mn, Fe, Zn and Cu in the samples varied as follows: Mn, 6.02 ± 0.33mg kg^{-1} and 24.8 ± 1.5 mg kg^{-1}; Fe, 48.0 ± 7.5 mg kg^{-1} and 431 ± 12 mg kg^{-1}; Cu, 1.99 ± 0.00 mg kg^{-1} and 6.43 ± 3.0 mg kg^{-1}; and Zn 18.9 ± 0.3 mg kg^{-1} and 96.8 ± 3.5 mg kg^{-1}as shown in table 4.7.

Sample MT1 had the highest levels of Mn while the levels of Mn in the rest of the samples were within a very close range of 6.09 ± 0.33 mg kg^{-1} to11.0 ± 0.2 mg kg^{-1}. Finger millet was one of the ingredients in this sample as shown in table 4.7.

In this study, it has been observed that finger millet contributed to more levels of Mn in a sample than the other ingredients in the sample. Comparing the level of Mn in this sample with the level of Mn in sample MT10 which also had finger millet as one of the ingredients then the ratio of finger millet to other ingredients in sample MTI may have been higher than this ratio in sample MT10.

Table 4.7: Mean concentrations (mg kg^{-1} ± 1SD) of Mn, Fe, Cu and Zn in samples from Mitaboni region

N=3

	Ingredients	Mn	Fe	Cu	Zn
MT1	Omena (fish), groundnuts, soya, finger millet	24.8 ± 1.5	269 ± 51	2.98 ± 0.29	29.8 ± 2.6
MT2	Other ingredients were unknown but had millet as one of the ingredients.	9.85 ± 0.55	89.8 ± 5.1	2.12 ± 0.13	25.6 ± 1.4
MT3	Unknown	6.02 ± 0.33	48.0 ± 7.5	6.43 ± 3.00	22.8 ± 1.5
MT4	Maize flour, wheat flour, soya flour, sugar, mineral and vitamin (famila infant flour)	9.43 ± 0.44	51.1 ± 9.0	3.67± 0.17	23.8 ± 0.8
MT5	Maize	9.18 ± 0.08	72.3 ± 12.7	5.06 ± 0.56	96.8 ± 3.5
MT6	Sorghum	10.5 ± 0.2	44.2 ± 0.3	2.50 ± 0.06	19.0 ± 0.1
MT7	Millet	11.0 ± 0.2	106 ± 15	2.04 ± 0.06	18.9 ± 0.3
MT8	Millet, sorghum	10.3 ± 0.2	227 ± 14	1.99 ± 0.00	25.2 ± 0.2
MT9	Other ingredients were unknown but had millet as one of the ingredients.	9.69 ± 0.16	431 ± 12	2.15 ± 0.04	22.4 ± 0.7
MT10	Sorghum, finger millet, soya, milk, maize meal	9.31 ± 1.49	195 ± 19	2.58 ± 1.09	11.2 ± 1.1

The levels of Fe in the samples varied enormously: Samples MT3, MT4 and MT6 had lower levels of Fe ranging from 44.2 to 51.1 mg kg^{-1}; the levels of Fe in samples MT2, MT5 and MT7 ranged from 72.3 to 106 mg kg^{-1}; the levels of Fe in samples MT1, MT8 and MT10 ranged from 195 to 269 mg kg^{-1}; while sample MT9 had the highest level of Fe of 431 mg kg^{-1}.

The high values of Fe in sample MT9 may be as a result of either contamination of Fe from equipments used in the grinding process (Icard-Verniere *et al.*, 2012) or contamination from soil.

Millet is usually a small seed which when not carefully prepared may contain soil particles. Alternatively soil residues and dust may settle on the surface of the cereals during drying aggravating the levels of Fe in the flour (Andersen *et al.*, 2011).

High levels of Fe are also observed in samples MT1, MT8 and MT10. Fish, groundnuts, soya and finger millet which are good source of Fe (Andersen *et* al., 2011; Musa *et al.,* 2012; Saunders *et* al, 2012) were some of the ingredients in samples MT1 and MT10, while sorghum and finger millet which are also good sources of Fe (Musa *et al.*, 2012) were the only ingredients in sample MT8. However, the levels of Fe in samples MT8 were not comparable to the level of Fe in samples which were composed of the same ingredients in this study. Therefore the probability of these samples having been contaminated with Fe during either drying or grinding processes are higher.

The level of Zn in sample MT5 (maize) was higher compared to the levels of Zn in other samples from this region. This level of Zn was comparable to that of sample TRB7 (maize). The two samples may probably have been of the same variety (Musa *et* al., 2012) or may have been grown under similar conditions (Alloway, 2008).

The levels of Zn in samples MT1, MT2, MT3, MT4, MT8 and MT9 were within a close range. Zinc levels were also comparable in samples MT6 and MT7, while sample MT10 had the lowest levels of Zn. Soya is considered to be a good source of Zn (Andersen *et al.*, 2011). In this study, the samples (MT1 and MT10) which had soya as one of the ingredients did not have high levels of Zn. The proportions of soya to other ingredients in these two samples could have been small. However, it is worth noting that sample MT1 also had fish and groundnuts which are considered to be good sources of Zn (Andersen *et al.*, 2011). The level of Zn in this sample was higher than that of sample MT10.

The levels of Cu in the samples from this region were lower than other trace elements similar to an observation made for the levels of Cu in samples from Bomet. Samples MT3 and MT5 had the higher levels of Cu while level of Cu in the other samples were comparable.

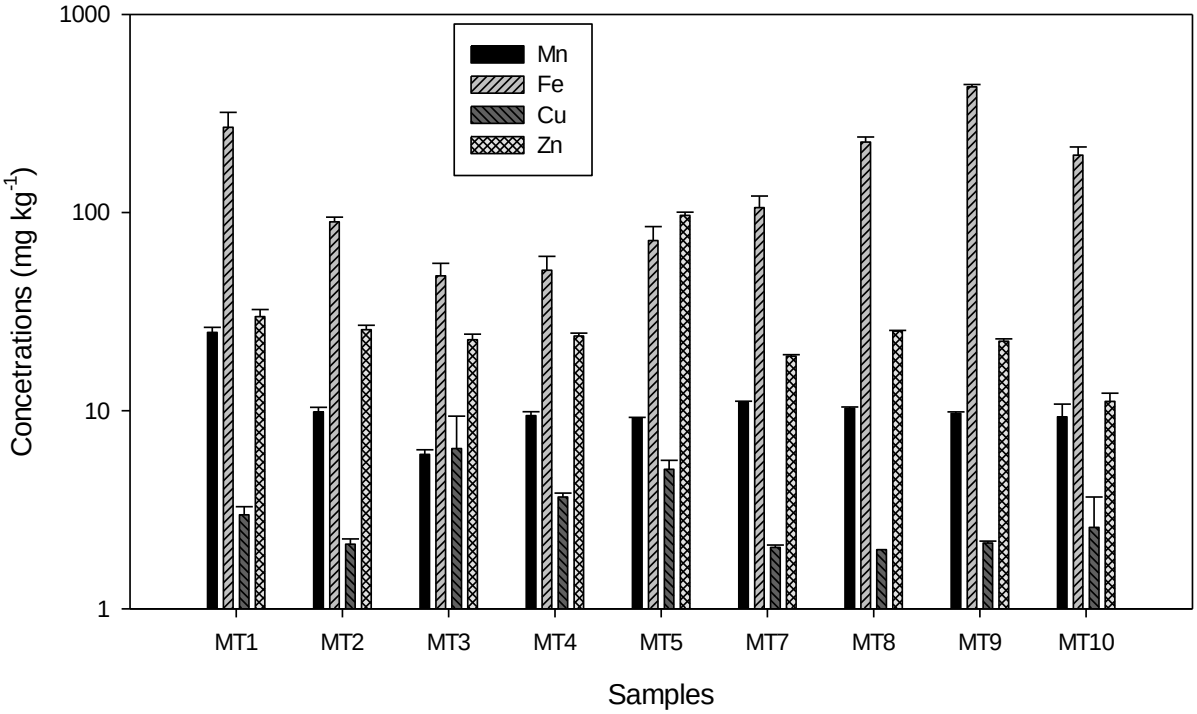

Fig 4.6: A graph showing the concentrations of Mn, Fe, Cu and Zn in samples from Mitaboni

4.4.5 Sega Region

The concentration of the trace elements in the samples varied as follows: Mn, 11.6 ± 0.3 mg kg^{-1} to 259 ± 3 mg kg^{-1}; Fe, 85.0 ± 0.8 mg kg^{-1} to 376 ± 1 mg kg^{-1}; Cu, 2.15 ± 0.06 mg kg^{-1} and 11.0 ± 0.5 mg kg^{-1}; and Zn, 11.2 ± 0.1 mg kg^{-1} to 97.1 ± 0.8 mg kg^{-1} as shown in table 4.12.

Five out of the ten samples from Sega were soya based flour as shown in table 4.13, which from the interview done the soya flour was bought from the two women groups, Ugambe and Kondiek United Progressive Association, who had been trained on how to prepare soy flour. Soya bean is a very important dietary source of proteins, fats, fibers, minerals and vitamins providing many bioactive components such as phytoestrogens with potential benefits to human beings (Messina 1999;). However anti nutritional factors in soy bean factors such as trypsin and phytates may interfere with protein digestion or chelate nutritionally essential trace elements such as Zn and Fe (Hurrell, 2003). Improper preparation will therefore result to failure of the human body to utilize some of the nutrients present in the soya bean or even make it unpalatable.

Table 4.8: Mean concentrations (mg kg^{-1} + 1SD) of Mn, Fe, Cu and Zn in samples from Sega

N=3

	Ingredient	Mn	Fe	Cu	Zn
S1	Maize, cassava, finger millet	33.8 ± 1.2	113 ± 4.8	2.15 ± 0.04	11.2 ± 0.2
S2	Maize, finger millet	19.8 ± 0.3	136 ± 5	6.16 ± 0.30	87.7 ± 1.0
S3	Groundnuts, soya, finger millet, sorghum, cassava	259 ± 1	246 ± 14	10.3 ± 0.3	49.0 ± 0.5
S4	Soya, maize, finger millet (Health centre)	43.1 ± 1.6	202 ± 18	5.56 ± 0.21	66.1 ± 2.0
S5	Soya, maize, finger millet	60.7 ± 4.6	330 ± 60	3.75 ± 0.11	21.1 ± 0.1
S6	Cassava, groundnuts, soya	38.2 ± 3.1	196 ± 34	11.0 ± 0.2	97.1 ± 4.4
S7	Soya, cassava, finger millet, sorghum	86.5 ± 0.6	376 ± 57	5.53 ± 1.04	24.6 ± 1.0
S8	Maize, sorghum, cassava	11.6 ± 0.7	85.0 ± 24.5	3.00 ± 0.39	68.5 ± 2.8
S9	Sorghum, maize, cassava	22.2 ± 1.2	290 ± 93	3.99 ± 1.75	51.0 ± 6.9

Sample S3 had the highest level of Mn while samples S8 had the lowest level of Mn. The level of Mn in sample S5 and S7 were between 60.7 mg kg^{-1} and 86.5 mg kg^{-1}. The level of Mn in samples S1, S4 and S6 were comparable. In samples S2 and S9, the levels of Mn were also comparable.

Finger millet which has been observed in this study to be a good source of Mn was one of the ingredients in samples S1, S2, S3, S4, S4 and S7. It has also been observed that the high proportion of finger millet to the other ingredients contributed more of Mn in the samples. Therefore, the proportions of finger millet in these six samples varied with its proportions to other ingredients in sample S3 being higher than the other ingredients conversely to sample S2.

The Fe levels were above 100 mg kg^{-1} in eight out of the nine samples from this region. However, the level of Fe in S8, which was below 100 mg $kg^{-1}$was not significantly different from that of S1 at 95% confidence level.

Soya, groundnuts, finger millet and sorghum which are considered to be good source of Fe and Zn were some of the ingredients used in these eight samples. Therefore, the high levels of Fe in

the eight out of the nine samples may probably be as a result of higher proportions of soya, sorghum and finger millet than the other types of ingredients in the samples.

The high levels of Zn in samples S3, S4, S6, S3, S8 and S9 may be a result of high proportions of soya or maize compared to other ingredients in the samples. It was observed that two maize samples obtained from Turbo (TRB7) and Mitaboni (MT5) had high levels of Zn. Comparing this to sample S2 and S8 which is observed to have high levels of Zn, then the proportions of maize in these two samples from Sega could have higher than the other types of ingredients. These two samples (S2 and S8) also had the lowest level of Mn therefore the proportions of finger millet which is good source of Mn may have been low.

Six out of the ten samples from this region had cassava flour as one of the ingredients. Cassava is believed to be an excellent source of energy. However it contains little Zn and Fe, at levels of approximately 3 μg/100g Zn and approximately 4 μg/100g Fe (Gegios *et al*., 2010). Infants whose diet consists largely of cassava may be at risk of inadequate Zn and Fe intake. In this study, trace element levels of Mn, Fe, Zn and Cu in samples which were a mixture of cassava and other ingredients varied. For instance, the ingredients in samples S8 and S9 were maize, sorghum and cassava and they had different levels of these trace elements. Iron levels were much lower in S8 compared to S9 while the levels of Zn were slightly higher in S8 compared to sample S9. Therefore, the proportions of the ingredients in these samples may probably have been different.

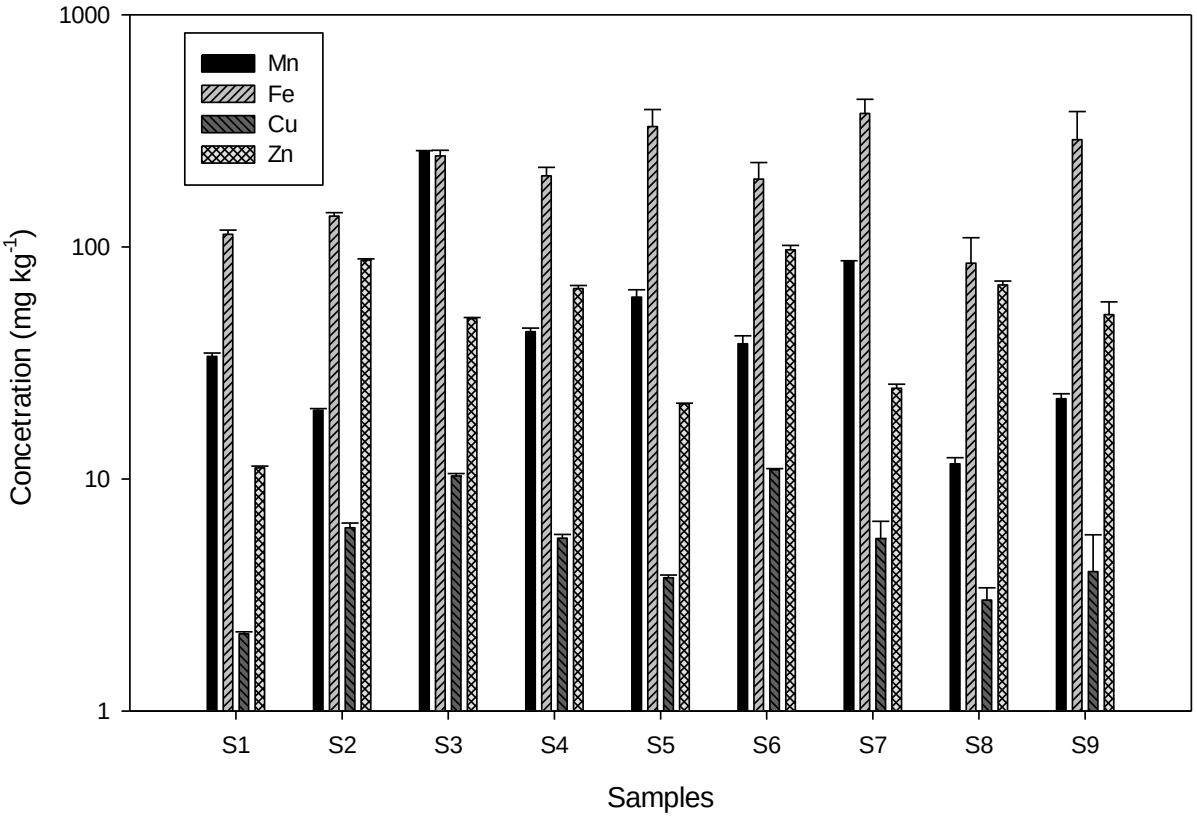

Fig 4.7: A graph showing the concentrations of Mn, Fe, Cu and Zn in samples from Sega

The levels of Cu in the samples from this region were also lower than other trace elements similar to an observation made for the levels of Cu in samples from other regions. However, the levels of Cu in samples S3 and S6 were higher and in similar range. Groundnuts which are good source of Cu was one of the ingredients in these two samples (Saunders *et al.*, 2012). The levels of Cu in sample S1 was the lowest while the levels of Cu in the rest of the samples were comparable.

4.4.6 Comparison of Zn, Cu, Fe, and Mn in the samples from the rural areas

The concentration of Zn, Cu, Fe, and Mn in the samples collected from the five rural areas showed a high variability as shown in table 5.14. The origin of the samples, land preparation techniques, preparation methods, type of ingredients used in the samples, and the proportions of these ingredients in the sample may have had an influence on the level of these trace elements in the samples.

Table 4.9: Range and mean concentrations comparison of Mn, Fe, Cu and Zn (mg kg^{-1} + 1 SE) in samples collected from the five rural areas

		Bomet (N=10)	Kirwara (N=7)	Turbo (N=10)	Mitaboni (N=10)	Sega (N=9)
Mn	Range	1.96 - 329	3.32 - 15.6	2.46 - 78.1	6.02 - 24.8	11.6 – 259
	Mean	116 ± 34	6.60 ± 1.70	28.7 ± 12.6	11.0 ± 1.6	63.8 ± 25.5
Fe	Range	14.6 - 112	35.0 - 122	17.7 - 178	44.2 - 431	85.0 – 330
	Mean	51.8 ± 11.4	78.0 ± 10.5	87.6 ± 27.7	153 ± 40	219 ± 33
Cu	Range	0.72 - 3.74	0.85 - 2.21	0.95 - 4.08	1.99 - 6.43	3.00 - 11.0
	Mean	2.51 ± 0.38	1.60 ± 0.60	2.08 ± 0.26	3.15 ± 0.47	5.71 ± 1.02
Zn	Range	14.3 - 30.5	14.8 - 45.3	17.2 - 81.2	11.2 - 96.8	11.2 - 97.1
	Mean	20.5 ± 1.6	27.3 ± 4.3	28.1± 6.3	29.6 ± 7.6	52.9 ± 9.9

Studies have shown that plants control the uptake of essential trace elements and their translocation to the seed (Welch, 1995). The concentrations levels are also influenced by the soil type, human activities and the environmental factors. For instance, a study done by Nafuma *et al.* (2010) showed that the soil PH increased when soils were burnt. This improves the bioavailability of Mn in the soil and hence boosts the uptake of Mn by the plants (Kabata-Pandias and Mukherjee, 2007). This is converse to the bioavailability of Zn and Fe in the soils and uptake of these elements by plants which decreases with increase in the pH of the soil (Kabata-Pendias and Mukherjee, 2007). Therefore, the high levels of Mn observed in samples of finger millet from Bomet region may probably be attributed to the land preparation method which involved the heating of soils which was also observed in a study carried out by Nafuma *et al*. (2010).

A direct relationship between low Zn in soil and Zn deficiency in cereal crops has been observed (Musa *et al*., 2012). It is estimated that nearly half of the soils in the world on which cereals are grown have low Zn levels (Alloway, 2008). Since cereal grains have inherently low Zn

concentrations, growing them on Zn deficient aggravates the Zn level in them. Alloway further noted that maize and sorghum cereals were highly susceptible to Zn deficiency.

Heating of soils at certain temperatures increases the PH of soils decreasing the availability of Zn in soils (Kitur and Frye, 1983). Uptake of Zn by plants is dependent on the Zn availability in the soils. Therefore, Zn levels in the soils from areas such as Bomet where these indigenous technical method of land preparation had a high impact on the bioavailability and uptake of Zn by plants grown on this soils. This is observed in low concentration levels of Zn in the maize samples, B6 and B7, from this region.

In another study carried out by Musa *et al*. (2012), a certain variety of maize concentrated more Fe and Zn in the grains. This is also observed in maize samples in this study. Samples B6, B7, TRB9 had comparably low levels of Zn and Fe while the rest of the maize samples (TRB6, TRB7 and MT5) had relatively higher levels of Fe and Zn as shown in table 4.15. It is also worth to note that the levels of the selected trace elements were not significantly different ($p > 0.05$) in samples B6, B7, and TRB 8; and were within the same ranges in studies done by Maina (2005) and Mohammed (2008).

Mixing of millet flour with maize flour in different proportions had an effect on the total concentration of the trace element in the samples as shown in figure 4.8. The level of these trace elements varied depending on the proportion of the two ingredients and the origin of the ingredients. This trend therefore tries to explain the observable variability of trace elements in the other samples collected from the different rural areas which were a mixture of two or more samples, collected from the different regions. A study done by Gibson *et al*. (1998) also showed that such variability of trace elements when two or more ingredients were mixed in different proportions.

The phytate that is localized in the outer aleurone layer of cereals such as sorghum and wheat and the germ in maize may be removed during milling. This process may enhance the extractability of some minerals although the content of other minerals of the milled cereals may be lost simultaneously (Gibson *et al*., 1998). For example, Bauernfeind (1991) reported a loss of 79% of Zn and 54% of Fe when a maize grain was milled while Maina (2005) a 70% loss of Zn and 34% Fe loss when the maize grain were milled. Therefore the variation of Zn and Fe in the

samples collected from the different regions may be as a result of preparation of ingredients in the samples.

Table 4.10: Mean concentration (mg kg^{-1} ± 1 SD) of Mn, Fe, Cu and Zn in maize samples

N = 3

	Mn	Fe	Cu	Zn
B6	2.5 ± 0.3	15.8 ± 1.8	0.8 ± 0.1	15.7 ± 0.8
B7	2.0 ± 0.4	14.6 ± 1.6	0.7 ± 0.2	14.3 ± 0.3
TRB4	2.5 ± 0.1	28.8 ± 4.9	1.8 ± 0.1	36.2 ± 0.7
TRB7	11.2 ± 1.2	61.5 ± 5.6	4.1 ± 0.3	81.2 ± 0.4
TRB9	2.5 ± 0.2	17.7 ± 1.0	0.9 ± 0.0	17.2 ± 0.3
MT5	9.2 ± 0.1	72.3 ±12.7	5.1 ± 0.6	96.8 ± 3.8
Mohammed (2008)	2.0 ± 0.1	20 ± 2	1.7 ± 0.1	10 ± 1
Maina (2005)	3.3 ± 0.3	29 ± 0.3	1.3 ± 0.4	24 ± 0.3

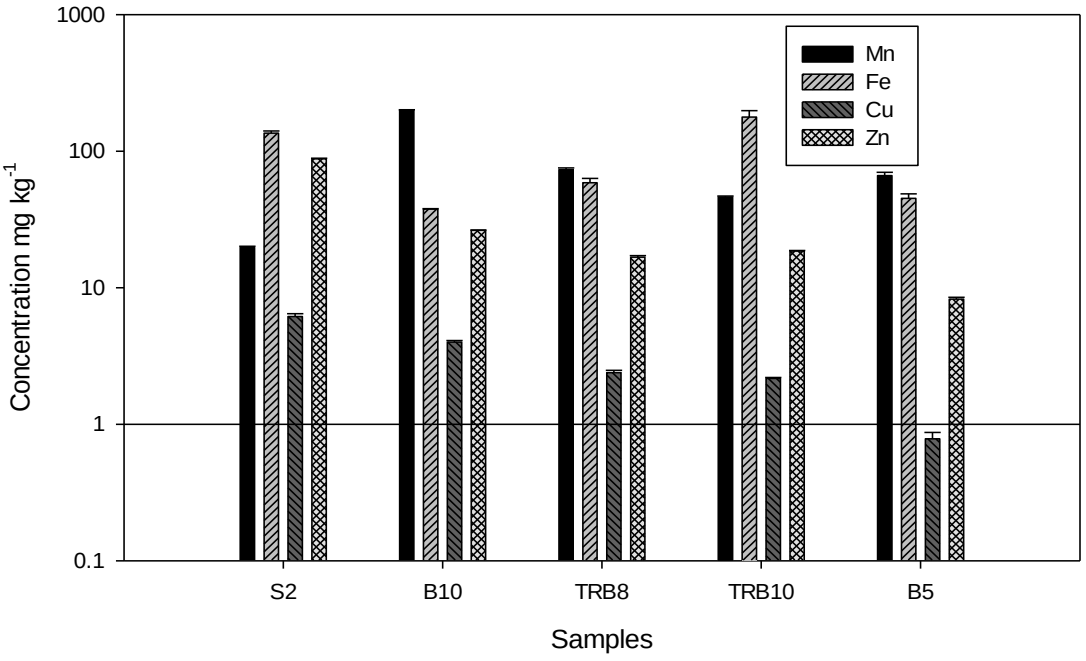

Fig 4.8: A graph showing the variation of trace element levels in samples composed of maize and millet

4.4.6 Health centers

In this study, some of the samples, B1, K4 and K5 collected from the rural health centres; Bomet and Kirwara were fortified with Zn and Fe. The sample from Bomet health centre was corn-soy infant based flour; while the sample from Kirwara were corn based infant flour. The levels of Zn and Fe in the three samples were within a closer range as shown in table 4.16.

Another sample, S4, was collected from Sega health centre and was soya-millet-maize based infant flour bought from one of the aforementioned women group. The trace element concentrations of Mn, Fe, Cu and Zn these samples were 43.1 ± 1.6 mg kg^{-1}, 202 ± 18 mg kg^{-1}, 5.56 ± 0.21 mg kg^{-1} s and 66.1 ± 2.0 mg kg^{-1} respectively. The levels of these trace elements were much higher than those in the fortified samples collected from Kirwara and Bomet.

Table 4.11: Mean concentrations of Mn, Fe, Cu and Zn (mg kg^{-1} ± 1 SD) in fortified samples from the Health centres

N=3

Region	Mn	Fe	Cu	Zn
Bomet, B1	8.12 ± 0.45	75.3 ± 3.0	3.74 ± 0.16	30.5 ± 0.9
Kirwara, K4	3.32 ± 0.23	71.2 ± 4.4	1.69 ± 0.13	39.7 ± 0.7
Kirwara, K5	3.81 ± 0.28	64.3 ± 4.8	1.77 ± 0.05	45.3 ± 1.5

The availability of Zn and Fe in home plant based complementary infant flour may be inhibited by the presence of anti-nutritional factors such as phytates and phonenols. Studies show phytates present in cereals and pulses tend to bind Zn reducing its availability (Balaji *et al*, 2000). Therefore, further studies on the bioavailability in such formulated infant complementary foods from Sega health centre need to be done since the levels of Zn and Fe were much higher than the fortified infant flours from the other health centres.

4.4.7 Urban areas

Branded infant flour

The concentration levels of Mn, Fe, Cu and Zn in the samples varied as shown in table 4.17 and figure 4.9. The levels of Mn ranged from 7.24 ± 1.11 to 256 ± 1 mg kg^{-1}, Fe levels ranged from 25.1 ± 3.1 to 194 ± 12, Cu levels ranged from 1.39 ± 0.10 to 6.84 ± 0.20 mg kg^{-1}, while the level of Zn ranged from 11.1 ± 0.6 to 93.0 ± 2.2 mg kg^{-1}.

High levels of Mn were observed in samples Y2, KS6 and E10. However sample Y2 had the highest level of Mn and were significantly different ($p < 0.05$) from Mn in sample E10 even though the two samples composed of organic finger millet and humanized milk and were of the same brand as shown in table 4.18. Sample E10 was obtained from Eldoret while sample Y2 was obtained from Nairobi. Sample KS6 composed of pure finger millet and was obtained from Kisumu. A similar trend of high levels of Mn in finger millet have been observed in finger millet samples collected from Turbo and Bomet, and they were within similar range similar to studies done by both Shemelis *et al* (2010) and Maina *et al* (2012).

The level of Mn in samples X5, Y4 and KC2 were within a closer range of 70.6 ± 11.4 to 78.6 ± 9.8 mg kg^{-1}. Sample KC2 was composed of finger millet only while in samples X5 and Y5, finger millet was one of the ingredients used. Although other samples such as Y7, T7 and E3 had finger millet as one of its ingredients, the levels of Mn in this samples were very low. Therefore, the proportions of finger millet in the samples could have played a contributed to the varying levels of Mn in the samples.

Table 4.12: Mean concentration (mg kg^{-1} ± 1 SD) of Mn, Fe, Cu and Zn in branded samples
N = 3

Study area		Brand name	Ingredients	Mn	Fe	Cu	Zn
Nairobi	X1	Kirinyanga millers	Unknown (6 month old porridge flour)	5.68 ± 0.35	25.1 ± 3.1	1.48 ± 0.13	11.2 ± 0.8
	X2	Kirinyanga millers	Rice, terere (7 month old porridge flour)	16.2± 0.87	57.2 ± 10.3	2.89 ± 0.13	27.3 ± 1.1
	X3	Kirinyanga millers	Finger millet, rice, amaranth (8months)	39.4 ± 6.4	39.5 ± 2.5	1.39 ± 0.15	11.1 ± 0.6
	X4	Kirinyanga millers	Finger millet, maize, amaranth(9-14 months)	47.3 ± 1.6	83.4 ± 17.6	2.83 ± 0.08	23.8 ± 0.3
	X5	Kirinyanga millers	Finger millet, amaranth, white millet, maize(15-24 months)	70.6 ± 11.4	94.5 ± 16.9	3.08 ± 0.02	20.7 ± 0.5
	Y1	Uji special (6 months and above)	Corn, dehulled soya, rice, sweet potatoes, milk	45.2 ± 2.8	120 ± 27	6.84 ± 0.20	93.0 ± 2.2
	Y2	Toto Afya infant flour	Organic pure millet and humanized human milk	256 ± 1	79.6 ± 3.6	6.58 ± 0.17	29.9 ± 0.9
	Y3	Famila Infant weaning	Maize, wheat, soya, sugar, minerals	10.9 ± 0.2	49.9 ± 6.0	3.78 ± 0.07	16.0 ± 0.2
	Y4	Natures Baby porridge	Amaranth, millet, oats. Kale, wheat, carrots, alfafa, corn, soya parsley, potato, sorghum	78.2 ± 2.5	155 ± 5	6.57 ± 0.19	87.7 ± 1.8
	Y5	Azuri porridge flour	Cooked beans, banana, maize, pumpkin marrow flour, carrot, amaranth leaves	22.1 ± 0.6	175 ± 17	6.25 ± 0.31	22.1 ± 0.3
	Y6	Proctor and Allan baby porridge	Maize, sorghum, soya, milk, vitamin, mineral	8.98 ± 1.34	290 ± 52	3.50 ± 0.20	36.9 ± 3.4

Study area		Brand name	Ingredients	Mn	Fe	Cu	Zn
Nairobi	Y7	Eves baby porridge	Maize, sorghum, millet, soya, vitamins, minerals	10.6 ± 2.5	142 ± 19	2.26 ± 0.42	16.3 ± 0.5
Thika	T7	Jards baby flour	Soya, groundnuts, refined maize, bulrush millet, glucose, sorghum	7.24 ± 1.11	45.0 ± 2.6	2.18 ± 0.27	21.1 ± 1.5
	T8	Baby porridge	Corn, millet, sorghum, soya	26.2 ± 4.1	63.0 ± 3.9	2.38 ± 0.30	23.6 ± 2.8
Kisumu	KS6	Tropical Pure wimbi	Millet	135 ± 54	83.6 ± 24.2	4.18 ± 0.45	25.5 ± 1.5
Kericho	KC2	Famila Pure wimbi	Millet	78.6 ± 9.8	46.1 ± 9.7	3.81 ± 0.53	16.8 ± 2.0
Eldoret	E2	Jards Junior porridge (1 year and above)	Soya, green grams, groundnuts, finger millet, sorghum, refined maize, bulrush millet	6.54 ± 0.20	31.7 ± 7.8	1.50 ± 0.24	21.6 ±1.1
	E3	Proctor and Allan baby porridge	Maize, sorghum, soya, milk, vitamin, mineral	8.28 ± 0.67	136 ± 2.5	5.36 ± 0.59	39.9 ± 3.9
	E4	Omena mix baby porridge	Millet, maize, sorghum, vitamins, minerals, amaranth, omena	16.1 ± 0.2	131 ± 7	1.75 ± 0.09	11.6 ± 0.3
	E5	Baby porridge	Finger millet, groundnuts, soya, cassava, green grams, fish powder	12.4 ± 0.6	194 ± 12	1.81 ± 0.18	21.7 ± 0.9
	E8	Famila Infant porridge	Maize, wheat, soya, sugar, minerals, vitamins	52.7 ± 0.6	94.7 ± 1.8	3.64 ± 0.28	20.7 ± 0.1
	E9	Sinda ujji bora	Finger millet, soya, groundnuts, wheat flour	56.3 ± 1.1	99.8 ± 7.0	2.01 ± 0.12	17.2 ± 0.5
	E10	Toto Afya infant flour	Organic finger millet and humanized milk	177 ± 6	49.8 ± 3.2	3.96 ± 0.32	22.4 ± 1.0

The level of Fe in samples E3, E4, E5, Y1, Y4, Y5, Y6 and Y7 were higher and ranged from 120 ± 27 mg kg^{-1} to 290 ± 52 mg kg^{-1}. Minerals were some of the components in samples E3, E4, Y6 and Y7 while samples Y1, Y4 and Y5 had ingredients that were not commonly used in preparation of complementary infant flour. For instance, sweet potatoes and carrots were some of the ingredients in samples Y1 and Y4; while pumpkin marrow was one of the ingredients in sample Y5. Sample E5 composed of finger millet, groundnuts, soya, green grams and fish powder which was good sources of Fe (Andersen *et al*., 2011; Saunders *et al*., 2012) although the same ingredients in addition to refined millet were used to prepare sample E2 which had very low levels of Fe. Therefore, the high levels of Fe in these samples may probably be as a result of the added minerals, or high proportions from sources such as sweet potatoes, carrots and pumpkin marrow.

The levels of Fe in samples E8, E10, KS 6, Y2, X5 and X2 were within a closer range and it ranged from 39.5 ± 2.5 mg kg^{-1} to 57 .2 ± 10.3 mg kg^{-1}. The samples had different types of ingredients. For example, sample Y3 had added minerals and its ingredients were similar to that of sample E8 whose Fe levels were 94.7 ± 1.8 mg kg^{-1}. Sample E10 also had similar ingredients with those of sample Y2. However, Fe levels in sample Y2 were higher than those in sample E10. On the other side, samples X1 and E2 had the lowest levels of Fe while samples E9, KS6, X5 and X4 had moderate levels of Fe.

Samples X1, X3 and E4 had the lowest levels of Zn and their values within a very close range of 11.0 ± 0.6 mg kg^{-1} to 11.6 ± 0.3 mg kg^{-1}. The levels of Zn in sample Y6 and E3 were also within a closer range. Sample Y1 and Y4 were the highest with concentrations of 93.0 ± 2.2 mg kg^{-1} and 87.7 ± 1.8 mg kg^{-1} of Zn respectively. Zinc levels in the rest of the samples were within a range of 16.0 ± 0.2 mg kg^{-1} to 29.9 ± 0.9 mg kg^{-1}. Sample Y6 and E3 were of the same brand and had similar ingredients. This is also observable in samples E8 and Y3; and samples Y2 and E10 whose levels of Zn are within a similar range. These two pairs of sample were also of the same brand and had the same ingredients.

The levels of Cu in the samples in the branded flour were lower than other trace elements similar to an observation made for the levels of Cu in other samples in this study. However, the levels of Cu in samples Y1, Y2, Y4 and Y5 were higher and they were comparable.

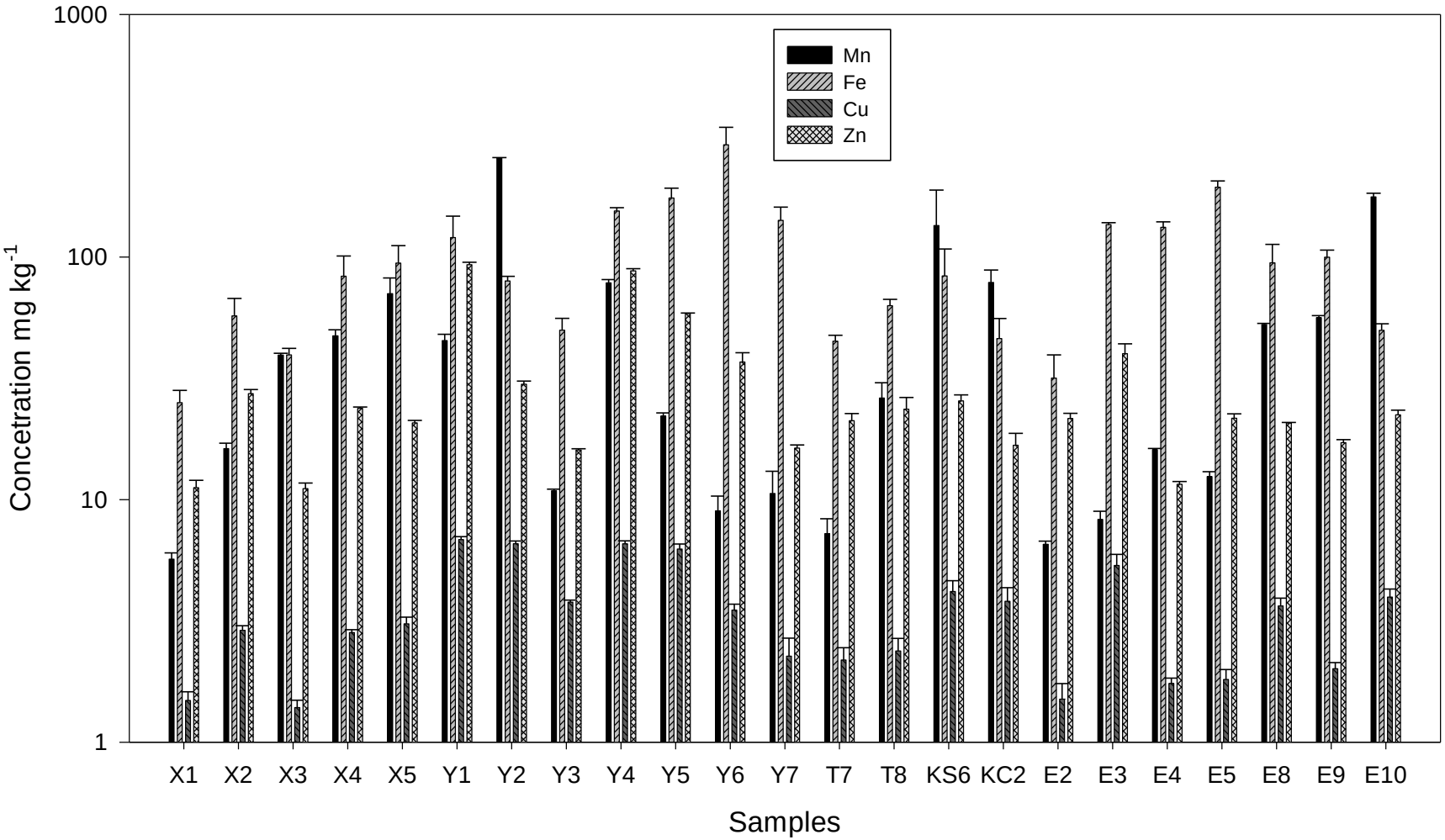

Fig 4.9: A graph showing the variation of trace element concentrations in branded infant flour

Other Samples (generic samples)

The level of Mn in the samples ranged from 11.0 ± 1.3 mg kg^{-1} to 247 ± 7 mg kg^{-1}, Fe level ranged from 46.7 ± 7.1 mg kg^{-1} to 410 ± 26 mg kg^{-1}, the level of Cu in the samples ranged from 1.56 ± 0.28 mg kg^{-1} to 8.05 ± 0.95 mg kg^{-1}, while the level of Zn in the samples ranged from 10.5 ± 5.2 mg kg^{-1} to 33.9 ± 4.3 mg kg^{-1} and are shown in table 4.19.

The level of Mn in samples E6, KC1, KC3, KS2, and KS5 were greater than 100 mg kg^{-1} with sample KC1 having the highest level of Mn of 247 ± 7 mg kg^{-1}. Sample KC1 and KC3 were composed of finger millet only; while the other three samples had finger millet as one of the ingredients as shown in table 4.20. All the other generic samples also had finger millet as one of the ingredient but there was a high variability in level of Mn in these samples.

High level of Mn has been observed in samples which were composed of finger millet only and obtained from Kericho and Bomet. Variability in the level of Mn in the other samples from these regions which had finger millet as one of the ingredients was also observed. These observations were likewise made in samples obtained from Sega and Turbo regions. Therefore it can be concluded that finger millet contributed more of Mn in samples E6, KS2 and KS5. This is contrary to the contribution of Mn in samples E1, E7, KC4, KS1, KS3 and KS5 which were also obtained from these three regions.

The levels of Mn in all the generic samples from Thika region were low and varied between 7.14 ± 0.15 mg kg^{-1} and 17.2 ± 0.17 mg kg^{-1}. Each of these samples from Thika had millet as one of its ingredients. This trend of low levels of Mn was observed in samples obtained from Kirwara and Mitaboni. Therefore, the source of finger millet or the preparation of the complementary infant flour may have been similar.

Very high levels of Fe of 477 ± 51 mg kg^{-1} and 410 ± 26 mg kg^{-1} are observed in samples T9 and KS1. Samples KSM4, T3, T5, T6, T10, T13 and T14 also had relatively high levels of Fe ranging from 117 ± 1 mg kg^{-1} to 204 ± 45 mgkg^{-1}; while the level of Fe in the rest of the samples ranged from 36.6 ± 0.5 mg kg^{-1} to 97.6 ± 6.7 mg kg^{-1}. High levels of Fe in some of these samples may have been as a result of either contamination of Fe during milling process (Icard-Verniere *et al.*, 2012) or contamination of Fe from soil during drying process (Andersen *et al.*, 2011). For

example, sample KS1 was obtained from Kibuye Market, an open air market in Kisumu town. Dust from the environs may have settled on this flour contaminating it with Fe.

Samples T5 had the lowest level of Zn of 10.5 ± 5.2 mg kg^{-1}; while the levels of Zn in samples KC4, T2, T3 and T11 were highest and within a closer range of 28.8 ± 0.2 mg kg^{-1} to 33.9 ± 4.3 mg kg^{-1}. The level of Zn in the rest of the samples varied between 14.8 ± 1.1mg kg^{-1} and 23.3 ± 0.5 mg kg^{-1}.

The levels of Cu in the samples in the generic flour were lowest among the than other trace elements. However, sample KC4 had the highest level of 8.05 ± 0.95 mg kg^{-1}; while many of the other samples had the level of Cu ranging from 1.56 ± 0.47 mg kg^{-1} to 3.74 ± 0.05 mg kg^{-1}, which is similar to an observation made for the levels of Cu in other samples in this study.

The variation of these trace elements in the generic samples collected from the five urban centers are illustrated in figure 4.10

Table 4.13: Mean concentration (mg kg^{-1} ± 1 SD) of Mn, Fe, Cu and Zn in generic samples
N= 3

Study area		Ingredients	Mn	Fe	Cu	Zn
Thika	E1	Sorghum, bulrush millet, refined maize	69.4 ± 3.4	81.7 ± 5.6	2.52 ± 0.16	18.6 ± 0.8
	E6	Refined maize, whole meal wheat, sorghum, finger millet, bird millet	130 ± 3	63.6 ± 0.2	2.90 ± 0.09	19.4 ± 0.4
	E7	Bulrush millet, sorghum, refined maize	45.7 ± 0.8	58.1 ± 8.5	3.38 ± 0.11	21.9 ± 0.7
	KC1	Soya, groundnuts, finger millet, sorghum, fish	247 ± 7	36.6 ± 0.5	3.10 ± 0.12	19.1 ± 0.4
	KC3	Soya, groundnuts, finger millet, sorghum, lablab (njahi), green peas	157 ± 4	58.8 ± 5.0	3.24 ± 0.27	21.5 ± 1.3
	KC4	Soya, groundnuts, finger millet, sorghum, fish	26.2 ± 2.7	51.9 ± 6.2	8.05 ± 0.95	33.9 ± 4.3
	KS1	Finger Millet, sorghum, bird millet, refined maize	41.0 ± 1.4	410 ± 26	5.22 ± 0.21	18.6 ± 1.1
	KS2	Fish, roasted soya, finger millet, sorghum, bulrush millet, maize, roasted groundnuts	103 ± 3	97.9 ± 6.7	3.47 ± 0.05	20.5 ± 0.6
	KS3	Finger millet, Sorghum, bulrush millet, maize, roasted groundnuts	51.0 ± 4.3	26.5 ± 4.6	2.54 ± 0.19	17.9 ± 1.3
	KS4	Finger millet, soya, refined maize, sorghum	57.8 ± 1.9	124 ± 22	3.71 ± 0.57	23.7 ± 2.9
	KS5	Finger millet, Sorghum	183 ± 2	60.9 ± 12.1	5.64 ± 0.22	23.3 ± 0.5
Eldoret	T2	Roasted groundnuts, roasted soya, sorghum, finger millet, cassava, bulrush millet	7.14 ± 0.15	58.4 ± 0.8	2.24 ± 0.12	30.5 ± 0.1
	T3	Sorghum, finger millet, groundnuts, soya	17.2 ± 1.0	117 ± 4	2.85 ± 0.33	31.6 ± 0.2
	T4	Cassava, sorghum, roasted groundnuts, roasted soya, finger millet	7.21 ± 0.17	81.2 ± 0.4	2.34 ± 0.08	18.0 ± 1.5
Kericho	T5	Millet	8.18 ± 0.21	257 ± 178	1.56 ± 0.47	10.5 ± 5.2
	T6	Millet	8.22 ± 0.26	204 ± 45	1.69 ± 0.07	19.5 ± 0.6
	T9	Millet, maize, fish, soya, groundnuts	18.9 ± 1.5	477 ± 51	3.91 ± 0.28	20.4 ± 0.1
	T10	Millet, sorghum	7.46 ± 0.17	117 ± 1	1.75 ± 0.09	28.8 ± 0.2
Kisumu	T11	Millet, sorghum, soya, cassava	11.0 ± 0.4	69.8 ± 24.1	2.32 ± 0.10	32.3 ± 0.2
	T12	Millet, sorghum, cassava	10.9 ± 1.5	46.7 ± 7.1	3.10 ± 0.35	27.7 ± 3.3
	T13	Millet, sorghum, cassava	11.0 ± 1.3	145 ± 7	2.37 ± 0.35	26.9 ± 4.6
	T14	Millet, sorghum, cassava	11.5 ± 0.6	159 ± 20	2.41 ± 0.38	14.8 ± 1.1

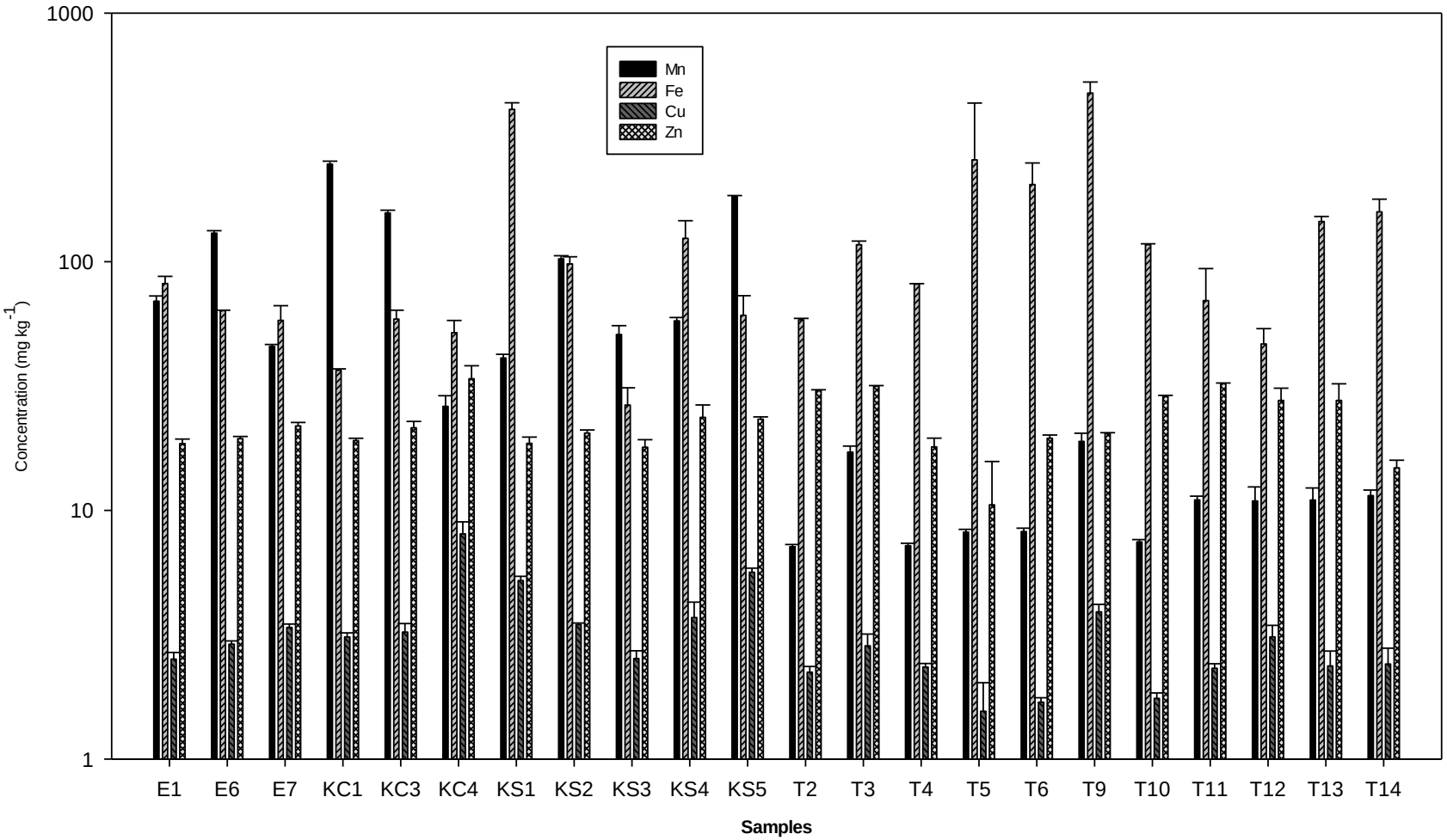

Fig 4.10: A graph showing the variation of trace element levels in generic infant flour collected from urban centres.

4.5 Lead concentrations in the flour from rural and urban areas

Some of the samples from the rural and urban areas had lead as shown in table 4.21. Seven out of the 9 samples from Sega area had lead varying from 0.13 mg kg^{-1} to 0.84 mg kg^{-1}. Only two samples out of the 9 and 10 samples from Turbo and Bomet areas respectively had lead. Six out of the 10 samples from Mitaboni had lead which varied between 0.13 mg kg^{-1} to 0.38 mg kg^{-1}.

Table 4.14: Mean Pb concentration (mg kg^{-1} ± 1 SD) in sample from rural areas

N = 3

Area of study	Sample	Pb
Bomet	B3	0.30 ± 0.08
	B8	0.38 ± 0.09
Mitaboni	MT2	0.34 ± 0.10
	MT3	0.26 ± 0.05
	MT7	0.13 ± 0.02
	MT8	0.30 ±0.07
	MT10	0.19 ± 0.06
Sega	S1	0.13 ± 0.02
	S2	0.81 ± 0.09
	S3	0.84 ± 0.16
	S5	0.28 ±0.07
	S7	0.43 ±0.14
	S8	0.33 ± 0.06
	S9	0.31 ± 0.07
Turbo	TRB4	1.77 ± 0.12
	TRB6	0.12 ± 0.04
	TRB7	0.37 ± 0.15

Both branded and generic commercial infant flour had lead. Three samples out of the 10 samples from Eldoret had Pb; one of each of the samples from Kisumu and Kericho had Pb; five out of the fourteen samples from Thika had Pb; while from Nairobi stores, four out of the twelve samples had Pb

Table 4.15: Mean Pb concentration (mg kg^{-1} ± 1 SD) in samples from the Urban areas

N = 3

Area of study	Sample	Pb
Eldoret	ELD4	0.17 ± 0.03
	ELD5	0.02 ± 0.00
	ELD9	0.10 ± 0.02
Kericho	KRC4	0.35 ± 0.24
Kisumu	KS1	0.34 ± 0.05
Thika	T3	0.13 ± 0.06
	T6	0.25 ± 0.06
	T9	0.49 ± 0.09
	T13	0.42 ± 0.06
	T14	0.24 ± 0.07
Nairobi	Y1	0.31 ± 0.11
	Y2	0.29 ± 0.07
	Y4	0.91 ± 0.21
	Y5	0.31 ± 0.07

The mean concentrations of Pb in most of the samples were higher than the permissible levels of 0.2 mg kg^{-1} (Codes Alimentarius Commission, 1999) in cereals and legumes. The level of Pb in samples MT7, S1, TRB8, T3 and the three samples from Eldoret were below the permissible levels of Pb in cereals and legumes. However, these levels were below the levels of Pb in flour in a study done in Qalyoubia region (Millennium Science and Engineering Inc., 2008) which was

above 0.5 mg kg^{-1} but higher than those obtained by Okoye *et al* (2009) which ranged between 0.007 to 0.032 mg kg^{-1}. In another study carried out by Orisakwe *et al.,* (2012), the level of Pb in some of the food crops consumed in Nigeria were higher than permissible levels and Soybean had 0.46 mg kg^{-1} of Pb.

Lead contamination of flour may result from excessive wear and tear of components during the grinding process (Millenium Science and Engineering Inc., 2008). Studies have also toxic metals accumulation in rivers and lakes may result from pollution of the water sources through enhanced raw effluent discharge from industries, flooding, municipal sewage system (Odada *et al.,* 2004). Aquatic animals tend to bioaccumulate such toxic metals in fish tissues (Jezierska and Witeska, 2006). In addition, bioaccumulation of such heavy metals was dependent on the age and feeding habits of the fish and their levels were higher in older fish. Therefore, use of fish from contaminated lakes or rivers could be a possible source of Pb in some of the samples obtained in this study.

Plants grown with effluent irrigation tend to accumulate higher amount of metals in their tissues and legume crops have been found to accumulate more heavy metals (Hussain *et al.,* 2010). Okoye *et al*., (2009) showed that much higher concentrations of these metals in the legume grains could be attributed to the fact that legume grain have high protein content, thereby easily accumulated the metals in the active sites of the proteins.

The increasing use of fertilizers and pesticides in modern agriculture as well as emissions of toxic elements from other anthropogenic sources has also been recognized as source of toxic elements in foodstuff (Moreno *et al*., 2002; Chiroma *et al*., 2007; Wuana and Okieimen, 2011). Therefore presence of lead in different concentration in the samples may be as a result of use of fertilizers and pesticides especially for crops that were cultivated on large scale.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

Conclusion

Most of the cereal-legume based samples in this study could meet the required daily intake of Mn, Fe, Cu and Zn of infants. The levels of these trace elements were far much above the recommended daily intake. However, maize based porridge could not meet the required daily intake Mn, Fe, Cu and Zn of the infants since the levels of these trace elements were far much below the daily recommended intake. The levels of Mn in samples that were composed of finger millet only were much higher than the recommended daily intake.

Thirty six percent of the samples in this study were contaminated with Pb and the levels were above the permissible levels in thirty one percent of the samples.

Therefore this study indicates that infants who are fed maize based porridge may tend to be deficient in Cu, Fe and Zn as compared to those who are fed equal measures of cereal-legume based porridge. On the other hand, infants who are mainly fed finger millet based porridge may receive higher levels of Mn which may be detrimental to their health. Therefore proper formulation and use of cereal-legume based infant porridge flour may alleviate micronutrient malnutrition among infants in developing countries.

This study shows that if the cereals and legumes available on the Kenyan market are judiciously selected, carefully prepared and combined in desirable patterns, then they could meet the required daily intake of the necessary trace elements during infancy. Mothers and care givers should therefore, be provided with the necessary nutrition education to help in alleviating micronutrient deficiencies in infants. Furthermore, they should be taught on importance of preparing and storing complementary infant flours in a clean environment to minimize contamination of the food with toxic elements such as lead.

Recommendation for future studies

Closed digestion system could be used in future to determine a wider range of trace elements in complementary infant food which are of importance of the growth and development of infants and young children. Other toxic elements could also be evaluated since they are of health concern. Trace element levels could also be evaluated in total diet of infants from selected regions in Kenya. This could be important in evaluating the micronutrient deficiency levels among Kenyan infants.

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APPENDICES

Appendix A: An example of a spectrum of a clean sample carrier

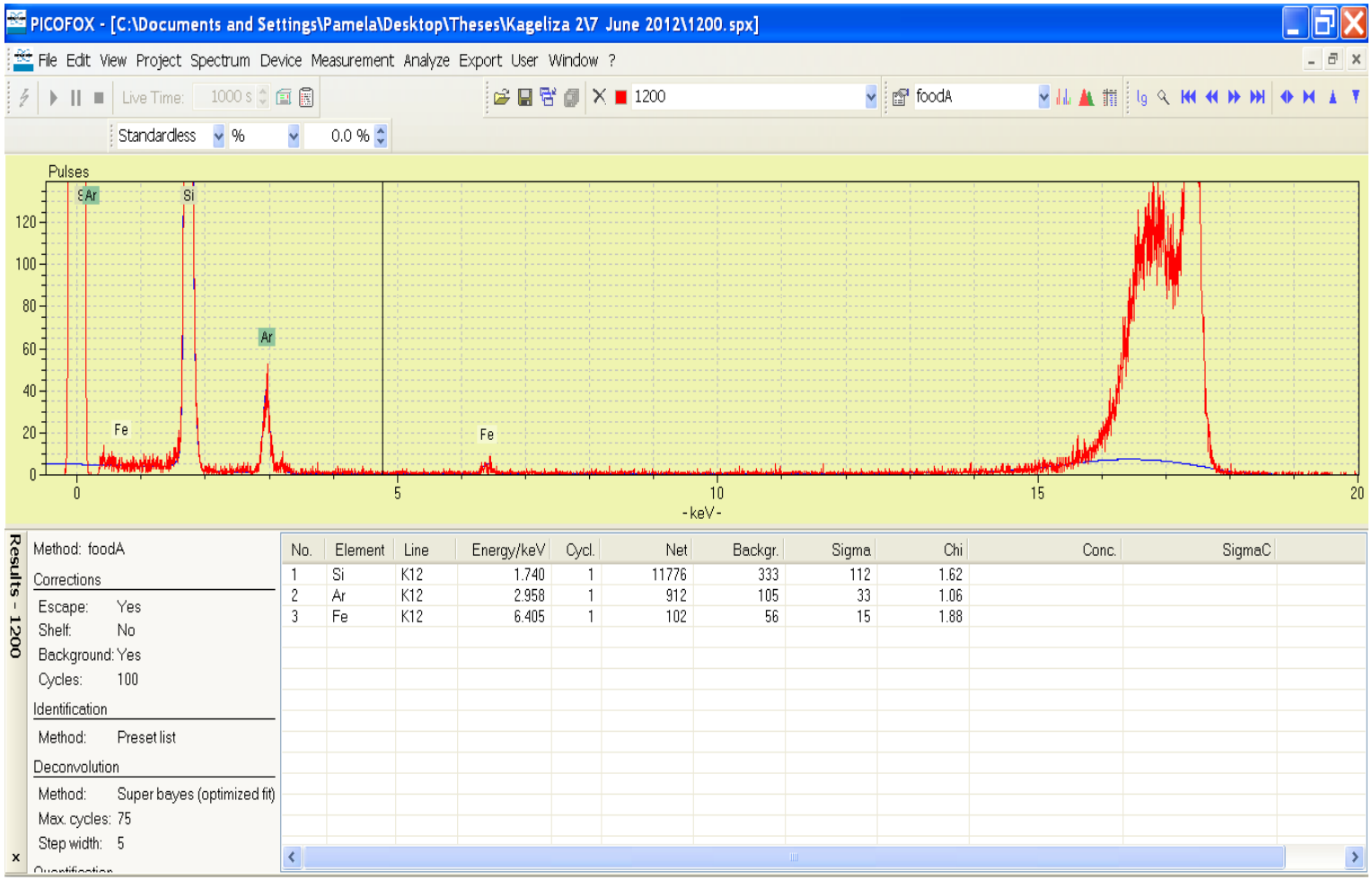

Fig 6.0: A spectrum of a clean sample carrier 1200

Appendix B: Location of the sampling sites

Rural Areas

Sega	$0^0$15′ N; $34^0$13′48″ E
Bomet	$0^0$47′6″ S; $35^0$20′6″ E
Turbo	$0^0$38′6″ N; $35^0$02′42 E
Kirwara	$0^0$55′56.64″S; $36^0$56′22.56″E
Mitaboni	$1^0$22′17.76″ S; $37^0$14′57.12″ E

Urban Areas

Thika	1^0 02′ S; $37^0$06′ E
Kisumu	$0^0$06′ S; $0^0$54′ E
Kericho	$0^0$21′54″ S; $35^0$18′18″ E
Eldoret	$0^0$30′54″ N; $35^0$176″ E
Nairobi	$1^0$17′31.44″ S; $36^0$4919.01″ E

Appendix C: Net intensities of K –Lines elements in the Merck XVI standard

Net intensities (N=8)

Element	Atomic Number(Z)	Relative Intensities
Ca	20	108074 ± 30340
Ti	22	192672 ± 55614
V	23	237970 ± 67263
Cr	24	316741± 89008
Mn	25	387413 ± 107673
Fe	26	508632.9 ± 141614
Co	27	579227 ± 160147
Ni	28	715563 ± 207195
Cu	29	815321 ± 218631
Zn	30	932264 ± 255239
As	33	1343220 ± 376917
Se	34	1309986 ± 368781
Sr	38	1502702 ± 416459

Appendix D: Calculation of Bias

$$Bias\ (\%) = \frac{experimental\ mean - certified\ value}{certified\ value} \times 100$$