ANALYSIS OF ESSENTIAL ELEMENTS IN SELECTED INFANT FOOD FORMULATIONS SOLD IN NAIROBI COUNTY, KENYA

KENYATTA UNIVERSITY
P.O. BOX 43844 00100
NAIROBI KENYA
25 JUL 2014
RECEIVED
GRADUATE SCHOOL

By

ROPKOI JOEL K. (B. Ed Sc.)

REG NO I56/CE/11164/2006

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

JULY 2014

DECLARATION

I hereby declare that this is my original work and has not been presented for the award of a degree in any other university.

Signed.....Date.....23-07-2014.....
Ropkoi Joel K.
Kenyatta University

This thesis has been submitted with our approval as University supervisors.

Signed.....Date.....24/07/2014.....
Dr. Ruth N. Wanjau
Department of Chemistry
Kenyatta University

Signed.....Date.....24/07/2014.....
Prof. Jane I. Murungi
Department of Chemistry
Kenyatta University

DEDICATION

I dedicate this work to my loving wife Agnes and our children, Beatrica, Enock, Tabitha and Caleb.

ACKNOWLEDGEMENT

I wish to express my sincere thanks to my supervisors, Dr. Ruth Wanjau and Prof Jane Murungi for their guidance and encouragement throughout the period of my study, which have brought this thesis into a reality.

I am grateful to Kenyatta University, the Department of Chemistry for the support of my study. Thanks to the technical staff of Department of Chemistry led by the chief technician, Mr Elius Maina. Much thanks go to the management and staff of the Department of Geology and Mines, Ministry of Environment and Natural Resources, for allowing me to use their atomic absorption spectroscopy instrument (AAS).

I sincerely thank my colleagues Tonui Mathew and Ngeno Kipyegon for encouragement and support that they gave me during my research period. Thanks also to other colleages who supported me in one way or another.

May I also acknowledge my family for the support they gave me during my study period. Thanks a lot to Bishop Elijah Sabul and the entire Holy harvest church for their encouragement and prayers. Finally, I thank the Almighty God for giving me good health and strength to undertake my studies successfully. Glory be to God.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectrometry
ADI	Accepted Daily Intake
AES	Atomic Emission Spectroscopy
AIDS	Acquired Immune Deficiency Syndrome
CDCP	Centre for Disease Control and Prevention
FAO	Food Agricultural Organization
HIV	Human Immunodeficiency Virus
IF	Infant Formula
RDI	Recommended Daily Intake
SCF	Scientific Committee on Food
SPSS	Statistical Product and Service Solutions
UNICEF	United Nations Children's Fund
WHO	World Health Organization
XRF	X- Ray Florescence

ABSTRACT

Apart from breast milk, infant food formulations have a special role to play in the diets of infants because they are the major sources of nutrients. The neonatal period is one of the most critical with respect to nutrition and specifically essential elements among others. Essential elements prevent diseases such as rickets and anemia. Both calcium and iron are needed to prevent these disorders. Copper and zinc are important as cofactors of major enzymes involved in the synthesis of collagen. Potassium is a very important mineral for the proper functioning of the cells, tissues and organs in the body. Chromium is essential for protection against cardiovascular diseases. There is need therefore to analyse infant food formulations for levels of essential elements. Selection of the infant food formulations was done through random sampling. The commercial milk and cereal based formulations selected in this study were analysed for levels of essential elements, K, Mg, Ca Fe, Zn, Cu, Mn and Cr. The analysis was done by Flame Atomic Absorption Spectroscopy (FAAS). Analysis of variance and students' t-test were used to compare the levels of essential elements in infant formulations for 0-6 months and 7-12 months age groups. Mean levels (mg/100g) of essential elements in 0-6 months' infant formulations varied with the highest being K (425.03±4.67) and Cr (0.03±0.01), the lowest. The mean levels (mg/100g) contained in the cereal based infant formulations for 7-12 months age group ranged between K (394.53±2.29) and Cu (0.31±0.48). To allow an assessment of infant exposures from the essential elements in the formulations, daily intake was calculated on the basis of information specified by the manufacturers on the labels. The results show that the infant milk and cereal formula samples analysed contain adequate amounts of essential elements with respect to WHO/UNICEF recommendations. This data can be used by dietitians to advise the public on the elemental concentrations of some of the infant food formulations available in Nairobi market.

CHAPTER ONE : INTRODUCTION

1.1 Background

There has been rapid increase in malnutrition related diseases among the Kenyan children (Neuman, 1994). Child mortality rate has also increased (Kabi, 2004). A considerable number of mothers may not breastfeed their young ones for various reasons. For most babies, breastfeeding is without question the best way to be fed, but unfortunately breastfeeding can also transmit HIV. If no antiretroviral drugs are being taken, breastfeeding, for two or more years can double the risk of the baby being infected to around 40 percent (De Cock, 2000). Replacement feeding is the most effective way to prevent mother-to-child transmission of HIV after birth. Many women prepare to try out formula food after receiving counseling on the possibilities of breast milk infecting the infant (Chatterjee, 2003). Infant food formulations therefore play a leading role in the provision of essential elements. A mineral rich infant food can provide a solution to nutrition related problems among the infants.

Almost all infant food formulations are either of plant or animal origin. It consists of carbohydrates, fats, proteins, mineral elements, vitamins and water. Mineral elements which are needed in small quantity for the metabolism of the body consist mainly of the macronutrients and trace elements. Examples of macronutrients include potassium, magnesium and calcium among others. Trace elements can be divided into three groups; the essential trace elements (micronutrients), such as zinc, iron and copper, which are constituents of hormones. In addition, vitamins and catalyst for the enzyme systems, the non-essential trace elements which are made up of the toxic and non-toxic elements,

which have no metabolic functions in the living organism. The roles of the essential trace elements in human health and disease prevention have been documented in literature (Garrison and Somer, 1995; WHO, 1996) and of particular interest is the role these elements play in human nutrition and infant nutrition (Bowen, 1979). World Health Organization report recommends exclusive breastfeeding for the first 6 months of life (WHO, 2001). However, sometimes breastfeeding is not sufficient (Monte and Gulgilani, 2004) or if the mother is taking a particular drug that could harm the baby (CDCP, 2006), or after six months feed, then the complementary feeding becomes necessary (Monte and Gulgilani, 2004). Apart from breast milk, infant food formulations have special roles to play in the diets of infants since they are a major source of nutrients (Bermejo *et al.*, 2000; Food and Drug Administration, 1997). Moreover, those infant food formulations are designed to provide the required nutrients as recommended daily intake (RDI) of minerals for infants and toddlers (WHO/UNICEF, 1998). However, reports have shown that various nutritional inadequacies have been cited in infant food formulations (CDCP, 2007).

Baby food composition may vary according to region and economical status. Different types of infant food formulations are available in supermarkets located in Nairobi. Among these are 'Infacare' and 'Gold', which are milk formulas. Cereal based formulations include 'Purity' and 'Amaranth baby'. Others composed of both milk and cereal are 'Cerelac' and 'Lactogen'. It has been documented that diet is the main source of essential elements (Dim *et al.*, 2004) and the nutritional importance of many trace elements has been established (WHO, 1994; Schrauzer, 1994). Essential trace elements

like iron and copper are needed for production of hemoglobin, while calcium is required for bone development. Zinc is involved in nucleic and bone metabolism. Selenium protects body tissues against oxidative stress and modulates growth and development. Manganese on the other hand is essential for several enzyme development. To maintain the physiological and metabolic processes of the body, the appropriate intakes of these elements are required. Deprivation can lead to diseases (Oskarsson and Sandstoerm, 1995), whereas , excessive intake of some of the essential elements may adversely affect the human biomedical function (Prasad, 1993). The importance of obtaining better information on the trace elements levels in foods and diets and the necessity to provide better knowledge-based sources of food has been highlighted (Stewart, 1980).

1.2 Statement of the problem and justification

No more than 35% of infants worldwide are exclusively breastfed during the first four months of life and complementary feeding frequently begins too early and foods are often nutritionally inadequate and unsafe (WHO, 2003). Inadequate or incorrect nutrient and energy intakes can directly affect infant growth and can have long-term consequences on organ development and function, which may result in adverse health effects in later life (SCF, 2006). Rising cases of obesity in children are also a matter of serious concern. Adequate replacement feeding is needed for infants born to HIV-positive mothers who choose not to breastfeed. It requires a suitable breast milk substitute, for example an infant formula prepared in accordance with Codex Alimentarious Standards (WHO, 2002). Recent research on infant formula composition has focused mainly on protein and energy content and a few nutrients and vitamins (Agostino and Domellof, 2005). Most

essential elements present in infant food formulations have received little attention. For example, for eight out of the eleven essential elements regulated in formula food, the data required for a science-based risk assessment of infant exposure is currently lacking (Codex Alimentarious Commission, 2007). A large number of Kenyan mothers who do not breastfeed their babies for some reasons rely heavily on infant formula feeding. However, little is known about the variation in levels of essential elements in the infant formulas available in Kenyan county. It was therefore important to determine whether the infant formula foods on sale in Nairobi market are adequate in some essential elements. Some elements may constitute potential health risk if consumed above RDI values and therefore information is needed on the levels of many metals and elements in the commercial infant formulas (WHO/UNICEF, 1998).

1.3 Hypothesis

There is no significant difference in the levels of essential elements in infant food formulations and the required daily intakes.

1.4 Objectives

1.4.1 General objective

To determine the levels of essential elements in selected infant food formulations.

1.4.2 Specific objectives

I. To determine the levels of Fe, Zn, Cu, Mn, Cr, K, Mg and Ca in six milk infant food formulations for 0-6 months and 7-12 months age groups, sold in Nairobi county.

II. To determine the levels of essential elements K, Mg, Ca, Fe, Zn, Cu, Mn and Cr in five cereal based infant food formulation brands for 7-12 months age group, sold in Nairobi county.

1.5 Significance of the study

Many infants have encountered great challenges of essential element malnutrition and poor health. Infants require sufficient supply of essential elements for cell development, metabolic processes and general growth. Sufficient supply of essential elements also helps infants as immune boosters. There is now growing evidence of the importance of essential elements in human nutrition, and there, are reports that suggest essential element deficiencies can lead to impaired growth during infancy (Mohan and Stump, 2000). Since the neonatal period is one of the most critical with respect to nutrition, there is need to know the actual intake of essential elements by those feeding on infant food formulations (Mohan and Stump, 2000). The purpose of this study was to collect and document the

information on the levels of essential elements in some commercial infant food formulations. These infant food formulations are important sources of essential elements for they are useful as replacement foods to breast milk. The results were compared with the documented RDI for infants in the literature. The study shed light on how best the infant foods may be used to meet the mineral nutritional needs of essential elements by infants. The results of this study can be used by policy makers to come up with policies and guidelines on ensuring appropriate feeding of infants. The results will also be used to sensitize the public.

1.6 Scope and limitations

The study dealt with analysis of selected essential elements namely; Fe, Zn, Cu, Mn, Cr, K, Mg and Ca in infant food formula brands. Only eleven brands of infant food formulations were considered in this study. Infant food formulations for only two age groups; 0-6 months and 7-12 months were analyzed.

CHAPTER TWO : LITERATURE REVIEW

2.1 Nutrition

Good nutrition is extremely important in the management and control of diseases. There is need for proper nourishment of infants so that they are able to resist infection and malnutrition related diseases. The human body's immune system relies on a balance diet rich in trace elements and other macro and microelements also have a role in immune system (WHO and FAO, 2002). A good diet consists of a number of nutrients, which can be derived from a balanced diet. All nutrients are important but among the key nutrients are, carbohydrates, proteins, vitamins and minerals (Whanger, 2003).

2.2 Infant food formulations

Infant food formulation is manufactured to support adequate growth of infants under twelve months of age when fed as a sole source of nutrition. Besides breast milk, infant food formula is the only other milk substitute which the medical community considers nutritionally acceptable for infants under the age of one year (WHO, 2003). The composition of infant food formulation is roughly based on mother's milk approximately one to three months postpartum. The most commonly used infant food formulations contain purified cow's milk, whey and casein as protein source, lactose as carbohydrate, vitamin-mineral mix and other ingredients depending on the manufacturer. There have been progressive attempts to bring the composition of infant formulations closer to that of human milk. Important modifications include the reduction of protein and the electrolyte content, addition of vitamins, trace elements and lactose. Presently, research is

concentrating on those substances in human milk which serve other than traditional nutritional roles. Attempts are in progress to supplement infant formulas with protective and trophic factors unique only to human milk (Annemiek and Jacques, 1994). Use of infant food formulations has been cited for numerous health risks. Studies have found infants in developed countries fed on formulations are at increased risk for acute gastroenteritis, severe respiratory tract infections, type 1 and type 2 diabetes, and autism when compared to infants who are breastfed (Riordan, 1997). This calls for continuous analysis of infant food formulations to protect infants from the ailments.

2.3 Essential elements

Essential mineral elements are categorized into major and trace minerals, depending on the amounts needed per day. Major minerals are those that are required in the amounts of 100 mg or more, while trace minerals are required in amounts less than 50 mg per day. The terms major and trace however, do not reflect the importance of the mineral in maintaining health, as deficiency of either can be harmful. Some body processes require several minerals to work together. For example, calcium, magnesium and phosphorous are all important for the formation and maintenance of healthy bones. Trace elements are mostly important as cofactors for enzymes. Trace elements are essential nutrients for metabolism, growth and neurological and immunological function (Reifen and Zlotkin, 1993; Castillo and Cassorla, 1999). Bioavailability of trace elements like iron, zinc, copper and manganese from human milk is high compared to cow's milk and infant food formulations. Trace elements play an important role as far as health is concerned. They are in fact the inorganic counterparts of essential biological organic nutrients namely,

vitamins and proteins (WHO, 1996_b). Essential micronutrients include include, Fe, Cu, Zn, Mn and Se which have a variety of biochemical functions in all living organisms. This has led scientists to examine the trace element content of human milk, the ideal food during the first month of life, in order to estimate infant requirements and establish reference values for use in manufacturing infant food formulations (Tripathi *et al.*, 1999). While Fe, Cu and Zn are essential, they can be toxic when taken in excess; both toxicity and necessity vary from element to element (Tripathi *et al.*, 1997). Several researchers have carried out work on determination of key elements in commercially available infant food formulations and baby foods. The values of essential elements reported by some of the researchers are presented in table 2.1.

Table 2.1 Mean concentrations of essential elements in different brands of formulations by some researchers

Element	Milk formulations	Cereal based food	Reference
K	-402.47±80.96 mg/100g -5.5±0.1 mg/g	408.22±2.64 mg/100g -	Khalifa and Ahmad, 2010 Paola *et al.*, 2011
Mg	-53.53±10.47 mg/100g -	66.52±4.96 mg/100g 39±1.7 mg/Kg	Khalifa and Ahmad, 2010 Ljung *et al.*, 2011
Ca	- 445.47±17.7 mg/100g -370±63 mg/Kg	551.38±70.7 mg/100g 38±0.59 mg/Kg	Khalifa and Ahmad, 2010 Ljung *et al.*, 2011
Fe	- 6.58±3.20 mg/100g - 65±4 mg/g	13.01±7.6 mg/100g -	Khalifa and Ahmad, 2010 Paola *et al.*, 2011
Zn	- 3.57±0.85 mg/100g - 35±1 mg/g - 52±1.3 µg/g	2.46±0.65 mg/100g - 40.08±4.0 µg/g	Khalifa and Ahmad, 2010 Paola *et al.*, 2011 Joseph *et al.*, 2011
Cu	- 0.508±0.40 mg/100g - 343±3 µg/l	0.18±0.06 mg/100g -	Khalifa and Ahmad, 2010 WHO, 1989
Mn	- 0.09±0.011 mg/100g -188±2 µg/ml - 3728±5 mg/Kg	0.97±0.45 mg/100g - 3156±10 mg/Kg	Khalifa and Ahmad, 2010 WHO, 1989 Ljung et al., 2011
Cr	<0.001 - 0.037±0.05 µg/g	<0.001 0.08±0.08 µg/g	Khalifa and Ahmad, 2010 Joseph et al., 2011

The essential elements considered in this study are discussed in the following sub-sections.

2.3.1 Iron

Iron is one of the most essential elements for plants and animals. Iron plays a very important role in production of hemoglobin. Decreased availability of hemoglobin in human body leads to symptoms of anemia. Anemia has many different causes, although iron deficiency and its effects are the most common causes in the western world. As

absence of iron decreases heme synthesis, red blood cells in iron deficiency anemia are hypochromic (lacking the red hemoglobin pigment). The iron in breast milk is highly bioavailable and covers the needs for the infant until about the age of six months. The Academy of pediatricians suggests infants should be supplemented with iron-fortified cereals (Reifen and Zlotkin, 1993).

Iron absorption from infant food formulations is significantly lower (Fomon *et al.*, 1993), probably due to presence of iron in different forms. The tolerable upper intake of iron in humans is 14 mg/l, while the recommended daily intake for infants is 10 mg (Standing Committee, 1997). Some studies have found association between infant formulations and lower cognitive development, including iron supplementation in baby formula being linked to lower intelligence quotient and other neuro-developmental delays (Kerr and Desiree, 2008). Studies have also shown that Fe deficiency in infants and older children may be associated with irreversible behavioral abnormalities and abnormal functioning of the brain (Nokes *et al.*, 1998). Studies in man have confirmed that anemic individuals have greater chances of developing infectious diseases, particularly those associated with respiratory system (Mindel and Mundis, 2004).

2.3.2 Zinc

Zinc is an important micronutrient that supports normal growth (Lynne, 2004). Taking just a little zinc in the diet can greatly reduce cases of malaria, pneumonia, diarrhoea and other diseases (Fox, 1998). These diseases are the major killers of children all over the world (Muchemi, 2006). Preterm infants are especially vulnerable to zinc deficiency

(Friel *et al.*, 1985., Fuller, 1992). Deficiency of trace elements such as zinc and copper can occur in infants for different reasons. It has been reported that zinc intake by infants from breast milk is inadequate during weaning period, especially if weaning foods are introduced at an early stage (Khanghani *et al.*, 2009). Decrease of zinc causes growth stoppage (Ruel *et al.*, 1997) and its reduction damages immune system (Magnus *et al.*, 2004., Zhang *et al.*, 2008). The incidence of cytomegalovirus infection is related to zinc deficiency (Schumann *et al.*, 2002). Some researchers recommend Zn supplementation for infants on vagan diets during weaning (Allen, 1998), although high levels of dietary Zn may inhibit absorption of Pb. However, Zn supplementation is not recommended for infants with elevated blood lead levels (CDCP, 2000). The tolerable upper intake of zinc in humans is 12 mg/l required for several enzymes such as carboxypeptidase, liver alcohol dehydrogenase and carbonichydrase. The recommended daily intake of zinc for infants is 3 mg (Standing Committee, 1997). Table 2.2 shows the dietary reference values for zinc according to world health organization (WHO/FAO, 2002).

Table 2.2 Dietary reference values for zinc

Age	Zn levels (mg/day)
0-12 months	3.3-5.6
1-10 years	3.8-10
11-18 years	8.7-15
Adults	6.7-15
During pregnancy	7.3-15
During lactation	11.7-19

Source: WHO/FAO, 2002

2.3.3 Manganese

Manganese is an essential micronutrient of several enzymes including pyruvate carboxylate, mitochondrial superoxide dismutase and enzymatic systems of matrix turnover in skeletal growth (Branca and Valtuena, 2001). The deficiency of manganese has been related to bone deformation, impairment of reproductive organs and reddening of hair amongst others, while excess manganese may inhibit iron assimilation (Solomons *et al.,* 1981). Previous studies indicate that diabetics invariably have low Mn levels in blood; therefore lack of Mn in regular diets could be linked to prevalence of diabetes in man (Williams and Caliendo, 1988). Mene Zes-Filho *et al.* (2009) reviewed studies on health effects in children environmentally exposed to Mn and found that most studies reported neuropsychological effects, such as poorer cognitive outcome and hyperactivity with elevated postnatal exposure. Exposure sources ranged from industrial pollution and mine waste to drinking water and food including infant food formulations (SCF, 2006). The recommended daily intake of manganese for infants is 0.5-0.7 mg (Standing Committee, 1997). Manganese levels determined in this study will be compared to those of other researchers in table 2.2.

2.3.4 Copper

Copper is a cofactor in several metalloproteins, essential oxidation metabolism, myelination and the metabolism of several steroid hormones (Department of Health, 1994). Clinical copper deficiency is a recognized hazard among preterm infants (Lonnerdal, 1998). Copper deficiency increases the free radicals and leads to reduction of defense against oxidative stress (Schneider *et al.,* 2007). Reduction of zinc and copper in

infants are associated with iron deficiency and leads to several complications (Ebringe *et al.*, 2008 ; Leotsinidis *et al.*, 2005). Copper is a recent addition to many infant food formulations and little is known about its bioavailability (Khangani *et al.*, 2009). A study by Lynne (2007) suggested that variation in copper levels present in infant formulations and previously published values might be due to recent increase in copper content of infant milk formulations in line with the infant food formula and follow-on formula regulations. The recommended daily intake of copper for infants is 0.5-0.7 mg (Standing Committee, 1997). The levels of copper found in this study will be compared together with those of other researchers in literature.

2.3.5 Potassium

Potassium is a very important mineral for the proper functioning of the cells, tissues and organs in the human body. It is also an electrolyte, a substance which conducts electricity in the body, along with sodium, chloride, calcium and magnesium. Potassium is crucial to heart function and plays a key role in skeletal and smooth muscle contraction (He *et al.*, 2008). Potassium regulation and homeostasis during infancy are, owing to growth and development, different from later life. Infants need to retain more K^+ than adults, to avoid growth retardation (Aizman *et al.*, 1998). Many foods contain potassium, including all meats, fruits and vegetables. Dairy products are also good sources of potassium (He *et al.*, 2008). Having too much potassium in blood is called hyperkalemia while having too little is called hypokalemia (Perazzella, 2000). Studies show a positive link between a diet rich in potassium and bone health, particularly among elderly women, suggesting that increasing consumption of foods rich in potassium may play a role in osteoporosis

prevention (Zhu *et al.*, 2009). Adequate intake of potassium dietary sources are 400 mg/day and 700 mg/day for infants of 0-6 months and 7-12 months respectively (Physicians reference desk, 2001).

2.3.6 Calcium

Calcium, about which, 99% is found in the skeleton and other parts such as plasma, (Bruadus, 1993), is very important element. Calcium requirements are affected substantially by genetic variability and other dietary constituents, and the interrelation of these factors make identification of a single unique number for calcium "requirements" for all children impossible (Institute of medicine, food and nutrition board, 1997). It has been recognized that a very low calcium intake can contribute to the development of rickets in infants and children, especially those consuming very restrictive diets such as macrobiotic diets (Legius *et al.,* 1989). Premature infants have higher calcium requirements than full-term infants while in the nursery and that this may be met by using human milk fortified with additional minerals or with specifically designed formulations for premature infants (Schanler *et al.,* 1995). Although Ca and P levels of human milk are significantly lower than those of current infant formulas, bone mineralization is similar in breast and formula fed infants (Mimouni *et al.*, 1993). It is not likely that in the future both Ca and P levels in infant formulations will be further reduced to levels closer to those in human milk, provided that homeostasis and bone mineralization prove to be adequate (Mimouni *et al.*, 1993). The recommended daily intake for infants is 360 mg (Standing Committee, 1997). Calcium levels in infant food formulations analysed in this

study will compared with the required daily intakes and also those of other researchers presented in table 2.2.

2.3.7 Magnesium

Magnesium is the fourth most abundant cation in human body after sodium, potassium and calcium (Civitelli *et al.*, 1994). The human body contains about 760mg of magnesium at birth, approximately 5g at age 4-5 months and 25g when adult. An amount of 30- 40 percent magnesium in the body is found in muscles and soft tissues, 1 percent is found in intracellular fluid and the remainder is in the skeleton, where it accounts for up to 1 percent of bone ash (Webster, 1987).

Soft tissue magnesium functions as a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis and maintenance of the electrical potential of nervous tissues and cell membranes. Of particular importance with respect to the pathologic effects of magnesium depletion is the role of this element in regulating potassium fluxes and its involvement in the metabolism of calcium (Al-Ghamdi *et al.*, 1994). Pathologic effects of primary nutritional deficiency of magnesium occur infrequently in infants but are even less common in adults unless a relatively low magnesium intake is accompanied by prolonged diarrhea or excessive urinary magnesium losses (Lonnerdal, 1995). The recommended diet intake for infants is 50mg (Standing Committee, 1997). The reported levels of magnesium by other researchers in different countries will be observed with those obtained from this study.

2.3.8 Chromium

Chromium is an essential trace element which has been used in alloy steels and electroplating for a long time in chemical industries. It is important in the human body for protection against cardiovascular disease by helping to regulate fat and cholesterol synthesis in the liver (WHO, 1988a). It also plays a role in activation of vitamin C in the body. Chromium aids in Fe absorption and reduces oxidative stress and HIV viral load (WHO, 1996_b). The daily human intake of Cr ranges from 50 to 200ug (Al Durtsch, 1999). Modern refining of foods and over consumption of refined carbohydrates, remove the Cr naturally present. Chromium deficiency has been demonstrated in malnourished children with the basic disturbance being an impairment of the action of circulating insulin. The body requires chromium when producing protein and failure to get enough chromium leads to premature aging (Al Durtsch, 1999).

2.4 Analytical techniques

The most common modern methods for analysis of trace elements are: Flame atomic absorption spectroscopy (FAAS) (American Chemical Society, 2006), X-ray fluorescence (XRF) (Beckhoff *et al.*, 2006), Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (Wanjau *et al.*, 2001), Mass spectrometry (MS) (Becker *et al.*, 2008), Neutron activation analysis (NAA) (Horwitz, 2001)and Proton induced x-ray emission (PIXE) (Preoteasa *et al.*, 2008). Flame atomic absorption spectrophotometry has been discussed at length because it was the main method used in this analysis.

2.4.1 Flame Atomic absorption spectroscopy (FAAS)

The AAS works on the principle of absorption of radiation by atoms at discrete wavelengths characteristic of the absorbing species. The radiation beam is attenuated by an amount that is proportional to the concentration of the element under consideration in the atomizer (American chemical society, 2006). The concentration of an element is measured by the absorption of radiation with a characteristic frequency by free atoms of an element. The strength of this method is that atoms absorb only at a wavelength that is unique to the test element. A hollow cathode with the cathode made of the element to be determined emits spectral lines corresponding to the excitation of the element of interest. Atomization of the element can be achieved by introducing a fine spray of test solution through a nebulizer into an air/acetylene or nitrous oxide/ acetylene flame. An electrically heated graphite furnace is used when very high sensitivity is required. Before the solution enters into the flame, it is dispersed into a mist of very small droplets, which gets evaporated to give a dry salt. Part of the salt is dissociated into atoms of the element of interest, which then absorbs radiation from an external source. The unabsorbed radiation from the flame is allowed to pass through a monochromator, which isolates the existing spectral lines of the light source. The unabsorbed radiation is then led into the detector and the output of which is amplified and measured.Absorption of light is associated with the process of transition from one steady state to another. Figure 2.1 shows a schematic diagram of an atmic absorption spectrophotometer.

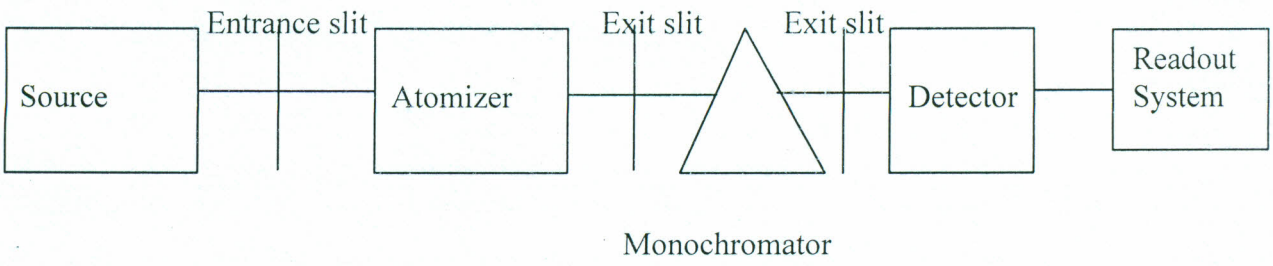


Figure 2.1: Schematic diagram of an atomic absorption spectrophotometer

The components of the instruments as shown in the Figure 2.1 are:

(a) Radiation source

There are two types of sources:

(i) Continuous source

The continuous source gives a wide range of radiation including deuterium lamp and mercury vapour lamp. Limitations of continuous source are that only a small band of radiation passed by monochromator is absorbed and a large portion of unabsorbed radiation falls on the detector and this reduces the sensitivity.

(ii) Hollow cathode source

It is the most common source of radiation used in most FAAS instruments. The cathode is made of metal of interest. The lamp consists of a tungsten anode and a cylindrical cathode sealed in a glass tube that is filled with neon or argon at a pressure of 1-5 torr.

Figure 2.2 shows a diagram of a hollow cathode tube.

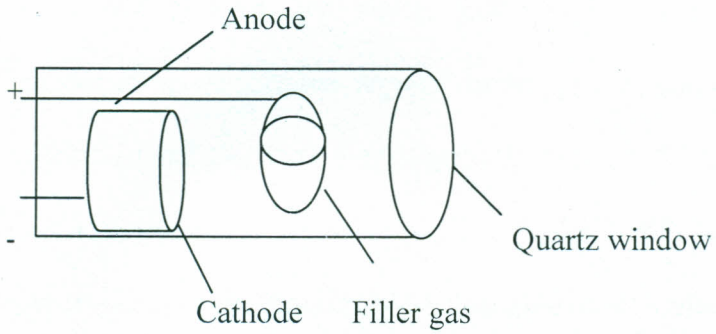


Figure 2. 2: Hollow cathode lamp

(b) Atomiser

There are two types, flame and electrothermal atomizers. In the flame the final temperature is determined by flow rate and ratio of oxidant and fuel. When the desolvation occurs in the flame, it is evaporated to produce a finely divided solid molecular aerosol. Dissociation of these molecules then leads to an atomic gas. Some of the atoms then ionize to give cations and electrons. In electrothermal atomizers, a few microlitres of sample are first evaporated at a low temperature and then ashed at a higher temperature in an electrically heated graphite. After ashing the temperature is increased to 2000-3000 °C causing atomization of the sample.

(c) Monochromators

Monochromators are filters, prisms or gratings that disperse or separate radiation so that selected wavelengths corresponding to a particular energy within the sample is transmitted to the detector.

d) Detectors

There are several kinds of detectors including, phototubes, photomultiplier tube and photodiode array detectors. These detectors convert radiant energy into electrical signal.

e) Read out systems

Read out systems are digital and coupled with microprocessors that allow the programming of various aspects bringing simplicity to separate procedures such as calibration and calculation of concentration.

The working of the atomic absorption spectrophotometer involves absorption of light that is associated with the process of transition from one steady state to another. For instance the case of steady states O and J where $E_j > E_O$, the O-J transition results in the absorption of light with frequency as shown by Maxwell-Boltzman Equations 2.1 and 2.2 below

$$V_{oj} = {}^{E_j - E_O}\!/_{h} \quad \text{----------} \quad (2.1)$$

Where h is the plank constant.

The Maxwell-Boltzmann law gives relative number of atoms in the excited state to the number of atoms in ground state as: -

$${}^{N_j}\!/_{N_o} = {}^{P_j}\!/_{P_O} \exp(-E/kt) \quad \text{----------} \quad (2.2)$$

Where k is the Bortzmann constant, T is the temperature in degrees Kelvin, and E_j is the energy difference between the excited state and the ground state. The quantities P_j and P_o are statistical factors that are determined by the number of states having equal energy at each quantum level. The relative fraction in the excited state is depended on temperature (Skoog *et al.*, 1998).

CHAPTER THREE : MATERIALS AND METHODS

3.1 Research design

The study focused on eleven types of infant formulation brands. Infant food formulations analysed were selected randomly and purchased from Nakumat, Tuskys, Ukwala and Naivas which are the major supermarkets in Nairobi County. The research experimental design, involved determination of concentration of iron, zinc, manganese, chromium, copper, potassium, magnesium and calcium in infant food formulations. The foods for age sets; 0-6 months and 7-12 months were analyzed for the levels of the essential elements. Levels of essential elements indicated on labels were noted and compared with the experimental values of this study.

3.2 Cleaning of glassware and preparation of standard solutions

All glassware were cleaned, washed with detergent and rinsed in de-ionized water. They were then soaked in 1:1 nitric acid solution. The reagent used was of high quality analytical grade. Stock solution was used to prepare working solution by serial dilution. The working solution was then used to prepare standard solution. The standard solution of metal was freshly prepared on daily basis. All the powdered infant food formulations samples were kept at room temperature. The infant formula food samples were then weighed.

3.3 Instrumentation and apparatus

Analysis of K, Ca, Mg, Fe, Zn, Cu, Mn and Cr was carried out in triplicate using a computerized atomic absorption spectrophotometer model SpectrAA-10 from Varian Techtron company, Australia. A blank solution made by using acids for the digestion process was used to zero the instrument before calibration and aspiration of samples into the flame. Standard solution for each element was prepared from a stock solution by serial dilution. By aspirating standard in the atomizer the amount of absorbed radiation was determined. Using concentration of standards, a calibration curve was obtained and used to quantify the essential element levels in the digested samples and blank. Table 3.1 given shows the operating conditions of the FAAS instrument.

Table 3.1 The FAAS operating conditions

Operating parameters / Element	Wavelength (nm)	Slit wit (nm)	Flame
K	766.5	1.0	Air/acetylene
Ca	422.7	0.5	Air/nitrous oxide
Mg	285.2	0.5	Air/acetylene
Fe	248.3	0.2	Air/acetylene
Zn	213.9	1.0	Air/acetylene
Cu	324.7	0.5	Air/acetylene
Mn	279.5	0.1	Air/acetylene
Cr	357.9	0.2	Air/nitrous oxide

3.4 Reagents

The reagents used were of high quality analytical grade. The standard solutions of the analysed essential elements were freshly prepared on the day of analysis.

3.5 Sample collection and pretreatment

The more popular infant milk and cereal formulations in the major supermarkets in Nairobi were first considered. Afterwards, eleven different branded commercial infant formulas sold in Nairobi were purchased from the supermarkets. These brands comprised of six infant milk formulations and five cereal formulations. The eleven brands show a fair representation of the types of infant food formulations in Nairobi market. The infant food formulations were collected from various supermarkets in Nairobi. The samples were kept at room temperature. The total number of samples collected based on their brand names, age group and batch numbers were 51.

3.6 Sample preparation

An amount of 2.5000 g of sample was weighed using a Mettler Toledo analytical balance model AG 204. The weighed samples were placed in a 250 ml Kjedhal digestion flask and a 27 ml mixture of HNO_3 , and $H_2 SO_4$ was added to the sample in the ratio 2:1. The mixture was heated gently on a hot mantle until brown fumes disappeared. It was then cooled and 3 ml of perchloric acid was added carefully. Digestion was continued until the solution was clear and white fumes observed. The digest was allowed to cool and filtered using Whatman No. 42 filter paper into a 50 ml volumetric flask. Finally the solution was diluted to the mark with distilled de-ionised water. The digested sample was transferred into a plastic bottle (Horwitz, 2001).

3.7 Method validation

The procedure and method were validated by use of matrix spikes. The sample was spiked with a known concentration of the analyte and allowed to pass through the procedure as the unspiked sample. Concentration of each analyte used in spiking was as presented in table 3.2.

Table 3.2 Analyte concentrations

Analyte	Volume (ml)	Concentration (ppm)
K	1	1000
Mg	1	1000
Ca	1	1000
Fe	1	100
Zn	1	100
Cu	1	10
Mn	1	10

The percentage recovery was calculated using the formula;

$$\%\text{Recovery} = \frac{\text{SSR} - \text{USR}}{\text{AS}}$$

Where, SSR – spiked sample result
USR – unspiked sample result
AS – amount spiked

3.8 Data analysis

The results obtained in this research were analyzed using statistical product and service solutions (SPSS), version 17.5. Analysis of variance (ANOVA) and students' t-test were used to compare the concentration of the essential elements in the infant food formulations. Percentage contributions were also used to compare levels obtained for each essential element and their recommended daily intake.

CHAPTER FOUR : RESULTS AND DISCUSSION

4.1 Introduction

The major essential elements, K, Mg, Ca and trace essential elements Fe, Zn, Cu, Mn and Cr were analysed in triplicates using FAAS. The results obtained varied between the different types of eleven (11) infant food formulations which were based on milk and cereals. The results, as discussed in the following sections, demonstrated a variation in the mean concentrations of most of the essential elements in the infant food formulations that were analysed.

4.2 Method validation

This was done by use of recovery analysis of the elements. The results obtained are presented in table 4.1.

Table 4.1 Percentage recoveries of analyte sample

Element	USR (mg/100 g)	SSR (mg/100 g)	AS (mg)	% RECOVERY
Potassium	230.15	457.72	255.400	89
Calcium	183.35	348.40	163.420	101
Magnesium	26.56	47.90	22.460	95
Iron	3.13	6.23	3.560	87
Zinc	2.89	5.43	3.020	84
Copper	0.16	0.35	0.155	103
Manganese	0.31	0.61	0.330	94
Chromium	0.21	0.39	0.210	86

USR, SSR and AS means unspiked sample result, spiked sample result and amount spiked respectively

From table 4.1 the percentage recoveries were in the range of 84 %- 103 %. The results obtained from these recoveries show that the method used in the analysis was reliable. The acceptable recoveries ranges from 80 %-105 % (Singh and Taneja, 2010). The lower

values of percentage recoveries could be due to random errors. Therefore the results from these recoveries confirm the reliability of the analytical method used in this study.

4.3 Linearity of FAAS calibration curves

Regression analysis was used to evaluate the linearity of the established calibration curves. The absorbance readings and concentrations of ideal standards were used to calculate correlation coefficient (R^2) values and the results are presented in table 4.2.

Table 4.2 Correlation coefficient (R2) values of AAS calibration curves

Element	Correlation values (R^2)
K	0.9970
Mg	0.9870
Ca	0.9980
Fe	0.9970
Zn	0.9990
Cu	0.9980
Mn	0.9970
Cr	0.9951

From the results presented in Table 4.2, it can be concluded that the linearity of the established calibration curves are good, hence accurate measurements guaranteed. The performance of the FAAS spectrophotometer used in this study was therefore good and reliable to warrant its use in the analysis of the selected essential elements in infant food formulations studied.

4.4 Levels of essential elements in infant milk formulations for 0 – 6 months and 7 – 12 months age groups

The results for the mean levels of each of the essential elements K, Mg, Ca, Fe, Zn, Cu, Mn and Cr determined and measured in infant milk formulations for 0 – 6 months and 7 – 12 months age groups, are discussed in the following sections.

4.4.1 Mean levels of K in infant milk formulations for 0 – 6 months and 7 – 12 months age groups

The results of the analysis of K in the milk formulas for 0 – 6 months and 7 – 12 months age group are presented in table 4.3.

Table 4.3 Mean levels of K (mg/100g) in infant milk formulations for 0 – 6 mont hs and 7 – 12 months age groups

Code	0 – 6 months	7 – 12 months
	(n=9)	(n=9)
A	464.00±6.05^{bc}	478.83±2.35^{b}
B	447.33±4.04^{b}	461.50±1.73^{b}
C	358.17±7.70^{a}	394.33±16.37^{a}
D	365.00±7.64^{a}	463.83±6.93^{b}
E	445.00±0.87^{b}	468.17±3.24^{b}
F	470.67±1.69^{c}	479.67±1.01^{b}

Mean values with the same superscript in the same column do not differ significantly

Table 4.3 presents the mean levels of K in infant food formulations for 0 – 6 months and 7 – 12 months' age groups. From the results of 0 – 6 months infant food formulations, it can be noted that F had the highest mean level of K followed by A, B, E, D and finally C

with averages of 470.67, 464.00, 447.33, 445.00, 365.00 and 358.17 mg/100g respectively. The differences in the levels of K in C and D were not statistically significant. However, there was a significant difference ($p < 0.05$) in the levels of K in E and F. Furthermore, table 4.3 present the results of 7 – 12 months infant food formulations with F having the highest concentration of K followed by A, D, E, B and C in that order. Levels of K in infant food formulations A, B, E, D and F are not significantly different. But K levels in infant formula C of 7 – 12 months was significantly different from those of A, B, D, E and F. Comparing the means of K in infant food formulations for 0 – 6 months and 7 – 12 months age groups, it was observed that infant formulations of 7 – 12 months always had the highest mean level. However, there was significant difference in the K means of A and C.

There was variation in the mean levels of K in some of the infant food formulations and this may be attributed to ingredients used to make up the several formulations. It is notable from the results presented in table 4.3 that all the analysed infant food formulations, except for C, had low standard deviations in their K levels and this can be attributed to consistency in the milling techniques used in the manufacture of infant food formulations. The high standard deviations for C, 0 – 6 months (7.7) and C, 7 – 12 months (16.37) may be due to ingredients. The formulations may be used, generally as replacement foods so as to boost potassium requirements for infants and toddlers.

The mean levels of K in infant food formulations found in this research study are comparable to K mean value reported by Paola *et al.* (2007). Other researchers, Khalifa

and Ahmad (2010) in their work on determination of key elements in commercially available infant formulations reported K levels of 402 mg/100g, which is also comparable to the values reported in this study.

4.4.2 Mean levels of Mg in infant milk formulations for 0 – 6 months and 7 -12 months age groups

The results of the analysis of Mg in infant milk formulas are presented in table 4.4.

Table 4.4: Mean levels of Mg (mg/100g) in infant milk formulations for 0 – 6 mont hs and 7 – 12 months age groups

Code	0 – 6 months	7 – 12 months	
	(n=9)	(n=9)	p-value
A	25.83±1.69	31.83±1.30	0.0480
B	34.17±3.38	27.00±0.87	0.1090
C	19.67±0.73	22.50±2.75	0.3760
D	34.00±0.00	35.17±0.88	0.2560
E	30.17±0.44	37.67±0.44	0.0003
F	32.33±3.18	29.83±1.69	0.5260

From the results in table 4.4, it can be noted that B had the highest mean level of Mg (34.17), followed by D (34.00), F (32.33), E (30.17), A (25.83) and finally C (19.67) mg/100g respectively among the infant food formulations for 0 – 6 months. On the other hand the results of 7 – 12 months infant food formulations presented mean value of Mg in E (37.67) as the highest followed by D, A , F, B and finally C with averages values of 35.17, 31,83, 29.83, 27.00 and 22.50 respectively. Comparing the results of 0 – 6 months

and 7 – 12 months infant food formulations, there was no significant difference in the average values of Mg in B, C, D and F with $P>0.05$ at 95% confidence level. However, there was significant difference in the mean values of Mg in A and E, ($P<0.05$). The highest mean value of Mg in E, 7 – 12 months, suggests that the infant food formulations can be recommended for feeding infants with magnesium deficiency disease, magnesemia. The other infant formulas may be used, generally, as replacement foods so as to boost magnesium requirements for infants.

The variation in the mean levels of Mg in infant food formulations for 0 – 6 months and 7 – 12 months may be attributed to the several ingredients from which the infant food formulations are manufactured. The maturity stage of the infant, 0 – 6 months or 7 – 12 months may be another factor considered when formulating.

The mean levels of Mg found in this research study are lower than Mg value of 53.53 mg/100g reported by Khalifa and Ahmad (2010). This may be due to geographical factors of ingredients used to manufacture the infant food formulations.

4.4.3 Mean levels of Ca in infant milk formulations for 0 – 6 months and 7 -12 months age groups

Table 4.5 presents the results for Ca levels in infant milk formulations.

Table 4.5: Mean levels of Ca (mg/100g) in infant milk formulations for 0 – 6 months and 7 – 12 months age groups

Code	0 – 6 months	7 – 12 months
	(n=9)	(n=9)
A	334.17±1.93^{bc}	433.00±2.47^{f}
B	163.00±1.44^{a}	261.83±1.17^{a}
C	433.67±2.89^{d}	422.83±2.17^{e}
D	366.00±9.26^{c}	408.33±2.19^{d}
E	318.33±2.62^{bc}	333.50±5.35^{c}
F	286.33±5.37^{b}	314.33±2.49^{b}
p-value	<0.001	<0.001

Mean values with the same superscript in the same column do not differ significantly

From the results of 0 – 6 months age group infant food formulations in table 4.5, C had the highest mean value of Ca, 433.67 mg/100g followed by D, A, E, F and B in that order with means of 366.00, 334.17, 318.33, 286.33 and 163.00 mg/100g respectively. The difference in the means of Ca in A, D, E and F was not statistically significant. Infant formula C can be recommended for feeding infants suffering from Ca deficiency disease, osteoporosis and those who are not breastfeeding due to its high Ca content. For 7 – 12 months infant food formulations the mean Ca levels (mg/100g) in A, 433.00, was the highest followed by C (422.83), D (408.33), E (333.50), F (314.33) and finally B (261.83). The mean of Ca in infant formula C for 0 – 6 months and 7 – 12 months is a small difference with both means, 433.67 and 422.83, well over 400 mg/100g. Therefore C is an infant food formulation specially designed for infants with high Ca requirements.

The variability in the mean values of Ca in all the infant food formulations and for both age groups may be attributed to the ingredients and the manufactures formulation techniques. The mean values of Ca in the milk formulations C of 433.67 mg/100g and 422.83 mg/100g compare well with mean level of 445.47 mg/100g reported by Khalifa and Ahmad (2010).

4.4.4 Mean levels of Fe in infant milk formulations for 0 – 6 months and 7 -12 months age groups

The levels of Fe in the infant food formulations for two maturity ages are presented in table 4.6.

Table 4.6: Mean levels of Fe (mg/100g) in infant milk formulations for 0 – 6 months and 7 – 12 months age groups

Code	0 – 6 months (n=9)	7 – 12 months (n=9)	p-value
A	3.94±0.21^{b}	4.77±0.08^{bc}	0.020
B	4.53±0.09^{b}	4.87±0.11^{bc}	0.070
C	2.87±0.15^{a}	4.08±0.35^{b}	0.040
D	3.49±0.28^{ab}	5.22±0.45^{c}	0.030
E	3.59±0.33^{ab}	5.33±0.19^{c}	0.010
F	2.73±0.34^{a}	3.17±0.11^{a}	0.295

Mean values with the same superscript in the same column do not differ significantly

From table 4.6, the highest level of Fe in infant food formulations for 0 – 6 months age group was found in B with a mean of 4.53 mg/100g followed by A, E, D, C and lastly F

with mean values of 3.94, 3.59, 3.49, 2.87 and 2.73 mg/100g respectively. There was no significant difference in Fe levels found in A, B, D and E. The mean levels of Fe in C and F, 0 – 6 months, infant food formulations were not significantly different from each other. However, the mean levels of Fe in C and F were significantly different from those found in A and B. The difference may be attributed to ingredients used by manufacturers to fortify the infant food formulations with Fe, since at this stage, 0- 6 months, infants require high amount of Fe for the making of blood. The low bioavailability of Fe from infant food formulations may be another factor that necessitates Fe fortification.

From the results in table 4.6, for 7 – 12 infant food formulation age group, the highest concentration of Fe detected is in E with a mean of 5.33mg/100g followed by D (5.22 mg/100g), B (4.87 mg/100g), A, (4.77 mg/100g), C (4.08 mg/100g) and lastly F (3.17 mg/100g). The mean levels of Fe recorded in 7 – 12 month infant F was significantly different from those recorded in A, B, C, D and E. Infant food formulation F had low mean levels of Fe and could be a food intended to be used as a substitute to breast milk. Comparing the mean levels of Fe, using t-test, in infant food formulations for 0 – 6 months and 7 – 12 months age groups, it was found that 7 – 12 months infant food formulations contain relatively higher values of Fe than the 0 – 6 months age group formulations but the difference was not statistically significant except for A, C, D and E at $P<0.05$. This may be attributed to the manufacturer's design of infant food formulations since at this age of 7 – 12 months, a child's growth rate is very fast and requires high amounts of Fe for blood development.

The mean levels of Fe in table 4.6 were higher than those reported by Ljung *et al.*, (2011), who reported concentrations of Fe as 5.7 mg/Kg in milk formulations and 1.2 mg/Kg in cereal rice based formulations.

4.4.5 Mean levels of Zn in infant milk formulations for 0 – 6 months and 7 -12 months age groups

Table 4.7 presents the results of the levels of Zn in the analysed milk formulations

Table 4.7: Mean levels of Zn (mg/100g) in infant milk formulations for 0 – 6 months and 7 – 12 months age groups

Code	0 – 6 months (n=9)	7 – 12 months (n=9)	p-value
A	2.78±0.15	3.26±0.11	0.060
B	3.83±0.05	4.09±0.03	0.010
C	2.93±0.10	3.25±0.05	0.103
D	2.15±0.05	4.34±0.06	0.00001
E	3.29±0.08	4.53±0.39	0.037
F	2.79±0.02	2.82±0.10	0.756

With reference to the results presented in table 4.7, it can be noted that among the infant food formulations for 0 – 6 months age group B had the highest mean level of Zn as 3.83 mg/100g followed by E, C, F, A and finally D with average values of 3.29, 2.93, 2.79, 2.78 and 2.15 mg/100g respectively. Considering the mean Zn levels in the 7 – 12 months infant formulas, it was observed that A had the highest value of 4.53 mg/100g, then

followed by D (4.34 mg/100g), B (4.09 mg/100g), A (3.26 mg/100g), C (3.25 mg/100g) and finally F (2.82 mg/100g). There was significant difference in the mean levels of Zn in A, C and F, 7 – 12 months infant food formulations at 95% confidence level. Furthermore, there was no significant difference in the Zn levels of B, D and E. However, a significant difference was observed in the Zn levels of A, C and F when compared to those of B, D and E, 7 – 12 months infant formulas. The difference may be due to ingredients and losses during manufacturers' milling procedures.

When the Zn mean levels in corresponding infant food formulations for 0 – 6 months and 7 – 12 months age groups were compared at 95% confidence level, the mean levels were significantly different except for those of A, C and F. This showed that the manufacturers of infant food formulations recognize that different age groups require varying amounts of zinc. The Zn mean levels for 7 – 12 months age group are slightly higher in all the infant food formulations A, B, C, D, E and F, than in all the infant formulations for 0 – 6 months age group. For this reason more informed selection of infant food formulations should be done basing on the child's stage of growth. The mean levels of zinc (mg/100g) reported in this study were lower than in previous report on trace element pattern in some Nigerian formulations which gave zinc levels as ranging between 32.00 ug/g and 89.89 ug/g (Joseph *et al.*, 2011).

4.4.6 Mean levels of Cu in infant milk formulations for 0 – 6 months and age 7 – 12 months groups

The results for the analysis of copper in the infant milk formulations analysed were presented in table 4.8.

Table 4.8: Mean levels of Cu (mg/100g) in infant milk formulations for 0 – 6 months and 7 – 12 months age groups

Code	0 – 6 months (n=9)	7 – 12 months (n=9)	p-value
A	0.32±0.05	0.39±0.04	0.313
B	0.23±0.04	0.36±0.02	0.040
C	0.25±0.03	0.33±0.03	0.034
D	0.20±0.03	0.40±0.05	0.026
E	0.35±0.02	0.42±0.02	0.083
F	0.25±0.03	0.32±0.01	0.079

The results (table 4.8) showed that the concentration of Cu in the infant food formulations, 0 – 6 months, had E (0.35 mg/100g) being the highest. This was followed by A, C, F, B and finally D having average values of 0.32, 0.25, 0.25, 0.23 and 0.20 mg/100g respectively. There was a significant difference in the Cu mean levels in A from those in B, C, D, E and F at 95% confidence level. This showed that there is consistency in the levels of copper in the infant food formulations. On the same note, it can be concluded that a good number of infant formula manufacturers comply with guidelines for nutrient levels in the formulas, for example, Codex Alimentarious Code and WHO/FAO recommendations. The mean levels of Cu in infant food formulations for 7 – 12 months was the highest in E (0.42 mg/100g), followed by D, A, B, C and F having mean values as 0.40, 0.39, 0.36, 0.33 and 0.32 in that order. The mean value of Cu in D is

significantly different from those of A, B, C, E and F (P=0.05). This confirms that Cu requirements by infants of both age groups are almost the same. Comparing the average values of Cu in 0 – 6 months and 7 – 12 months, infant food formulations, there was a significant difference in those of B, C and D with P<0.05.

The levels of Cu in the milk based infant food formulations analysed in this study were lower than Cu levels of between 0.53 mg/L and 0.7 mg/L reported in infant food formulations by Khaghani *et al.* (2009).

4.4.7 Mean levels of Mn in infant milk formulations for 0 – 6 months and 7 -12 months age groups

The results for the analysis of Mn in infant milk formulations were presented in Table 4.10.

Table 4.9: Mean levels of Mn (mg/100g) in infant milk formulations for 0 – 6 months and 7 – 12 months age groups

Code	0 – 6 months (n=9)	7 – 12 months (n=9)	p-value
A	0.15±0.02	0.33±0.04	0.014
B	0.31±0.01	0.41±0.03	0.043
C	0.34±0.02	0.44±0.02	0.038
D	0.32±0.04	0.65±0.07	0.014
E	0.26±0.02	0.52±0.02	0.001
F	0.10±0.04	0.45±0.04	0.003

From the results presented in table 4.9, the mean levels of Mn determined in the 0 – 6 months infant food formulations was highest in C with a value of 0.34 mg/100g, followed by D, B, E, A and F with average values of 0.32, 0.31, 0.26, 0.15 and 0.10 respectively. Table 4.10 presents the Mn mean levels in infant food formulations for 7 – 12 months age group with D having the highest mean level of 0.65 mg/100g followed by E, F, C, B and A, with average values of 0.52, 0.45, 0.44, 0.41 and 0.33 in that order. Comparing the mean levels of Mn in infant food formulations for 0 – 6 months and 7 – 12 months age groups showed that infant food formulations for 7 – 12 months age group contained higher Mn levels than their corresponding formulations. For example, A had Mn (0.33mg/100g) in A, 7- 12 months, higher than Mn (0.15mg/100g) in A, 0 – 6 months. The results of Mn, in D and F, 7 – 12 months age group were significantly different ($P<0.05$), from those of the corresponding 0 – 6 months age group.

4.4.8 Mean levels of Cr in infant milk formulations for 0 – 6 months and 7 -12 months age groups

The results presented in table 4.10 were obtained from the analysis of Cr in infant milk formulations

Table 4.10: Mean levels of Cr (mg/100g) in infant milk formulations for 0 – 6 months and 7 – 12 months age groups

Code	0 – 6 months (n=9)	7 – 12 months (n=9)	p-value
A	0.03±0.01	0.07±0.02	0.206
B	<DL	<DL	
C	0.02±0.00	0.03±0.01	0.116
D	0.03±0.01	0.04±0.01	0.725
E	0.02±0.00	0.02±0.00	0.130
F	<DL	<DL	

DL – Detection limit

It can be noted in table 4.10, that the mean levels of Cr in infant formulation B and F for both age groups were below detection limit. However, in the other infant food formulations for 0 – 6 months and 7 – 12 months age groups, Cr was detected and measured. The mean level of Cr determined was higher in infant formulations A (0.03mg/100g) and D (0.03mg/100g) than in C (0.02 mg/100g) and E (0.02). The low levels of Cr, though detected in some of the infant milk investigated suggests the manufacturers quest to formulate a product that can supply all the essential minerals to the infant. Infant food formulation A, for 7 – 12 months, had the highest mean level of Cr, 0.07mg/100g followed by C, D, and E with mean values of 0.04, 0.03 and 0.02mg/100g respectively. The results showed that infant's needs for Cr increased with the growth stage. There was no significant difference in the mean levels of Cr ($P>0.05$),

detected in all the infant food formulations for 0 – 6 months and 7 – 12 months. The difference can be attributed to ingredients used manufacture the infant milk formulations.

4.5.0 Levels of major essential elements in cereal based infant formulations

The mean levels of major essential elements in cereal based infant formulations (IF) were determined and the results shown in table 4.11.

Table 4.11: Levels of major essential elements (mg/100g) in cereal based infant formulations for 7 – 12 months age group

IF / Element	G	H	I	J	L
K	271.3±23.0^{c}	399.1±8.7^{d}	481.67±1.17^{f}	344.67±8.80^{d}	475.833±0.73^{e}
Mg	89.83^{b}	34.67^{b}	87.00^{d}	28.17^{b}	38.00^{c}
Ca	439.17^{d}	251.67^{c}	387.17^{e}	253.00^{c}	443.00^{d}

-Mean values with same superscript in the same row do not differ significantly
-IF represents infant formula

From the results (table 4.11), the mean value of K was highest in infant formulation I with a mean of 481.67 mg/100g and lowest in cereal formulation G with a mean of 271.33 mg/100g. The values obtained from this study are comparable to those obtained by Joseph *et al.* (2011).

The mean amount of K in cereal formulas H and J were not significantly different at 95% confidence level. However, the mean values of K in G, I and L were significantly different. This may be attributed processing practices by the manufacturers so as to produce various formulation brands.

From table 4.11, the mean amount of Mg in cereal formula G (89.83 mg/100) was the highest while that obtained from J (28.17 mg/100g) was the lowest. It can be noted that there was no significant difference in the mean values of Mg in G, H and J. But the mean values of Mg in I (87.00 mg/100g) and L (38.00 mg/100g) were significantly different. The variation may be attributed to ingredients. The mean value of Mg in cereal formulation I was significantly different from the mean values of G, H, J and L.

The mean values of Ca from table 4.11 show variation with the highest value in L (443.00 mg/100g) followed by G, I, J and H with mean values of 439.17, 387.17, 253.00 and finally 251.67 mg/100g respectively. The mean values of Ca in cereal based infant formulations H and J were significantly lower than in G, I and L.

4.5.1 Levels of essential trace elements in cereal based infant formulations

Table 4.12: Levels of essential trace elements (mg/100g) in cereal based infant formulations for 7 – 12 months age group

Element/Infant Formula	G	H	I	J	L
Fe	14.87±1.53[a]	5.33±0.36[a]	7.60±0.05[c]	2.84±0.27[a]	7.47±0.20[b]
Zn	10.07±1.75[a]	2.56±0.20[a]	4.31±0.27[b]	1.18±0.05[a]	9.17±0.09[b]
Cu	0.36±0.03[a]	0.14±0.02[a]	0.51±0.02[a]	0.24±0.01[a]	0.27±0.04[a]
Mn	2.45±0.13[a]	0.62±0.04[a]	1.08±0.04[a]	0.57±0.05[a]	0.77±0.02[a]

From the results presented in table 4.12, it can be noted that infant cereal formulation G had the highest mean level of Fe followed by I, L, H and lastly J with mean values of 14.87, 7.60, 7.47, 5.33 and 2.84 mg/100g respectively. When the means of G, H and J were compared, the means were not statistically significant. However, the means of Fe in I and L were statistically significant from one another and from those of Fe in G, H and J at 95% confidence level.

Since infant cereal formulation G has the highest level of Fe (14.87 mg/100g), it should be recommended as a replacement food for infants suffering from Fe deficiency diseases such as anemia. This is because the infant is in need of a large amount of Fe at 7 – 12

months growth stage. From table 4.12, the infant formula G had the highest mean level of Zn (10.07 mg/100g), followed by L (9.17 mg/100g), I (4.31 mg/100g), H (2.56 mg/100g) and lastly J (1.18 mg/100g). The variation in the concentrations of Zn in the infant cereal formulations may be due in part to differences in sampling and analytical techniques rather than to geographic variations. Mean values of Zn were comparable with published values by Khalifa *et al.* (2010). Given the lower bioavailability of Zn from infant formulations compared with human milk, a greater quantity of Zn is required in a formulation to produce the same metabolic response as with human milk feeding. This could be the reason why some manufacturers increase Zn levels in their brands.

From the results (table 4.12), the mean levels of Cu in cereal formulation I (0.51 mg/100g) was the highest followed by G, L, J and H with average values of 0.36, 0.27, 0.24 and 0.14 mg/100g respectively. From the results, table 4.12, there was no significant difference in the mean values of Cu in all the infant formulas G, H, I, J and L. This implies that most infant cereal based formulations are consistent in the levels of Cu. However, copper is a recent addition to many infant food formulations and little is known about its bioavailability (Feely *et al.*, 1983). The difference between the mean copper levels in the cereals based infant formulas might have been due to the recent increase in copper content of infant formulations in line with the infant food formula regulations and the variations in the copper content of water supplies with geographical variations (E U S I, 1995).

It can be noted that from Table 4.12 that G had the highest Mn mean level of 2.45 mg/100g and J (0.5 mg/100g) the lowest. There was no significant difference between the

mean levels of Mn in all the infant food formulations investigated. The mean levels of Mn obtained in this study are comparable to values reported elsewhere (Khalifa *et al.*, 2010).

4.6.0 Comparison of some levels of essential elements with label values (mg/100g)

The mean levels of some elements in infant milk and cereal formulations were compared with label values and presented in table 4.13 and 4.14 respectively.

Table 4.13 Comparison of mean levels of essential elements with label values (mg/100g) in some milk based formulations

IF	A_1		B_1		C_1		D_1	
EE	This study	Label value	This study	Label value	This study	Label value	This study	Label value
K	464.00	490	447.00	520	358.00	474	365.00	470
Mg	25.83	35	34.17	36	19.67	36	34.00	42
Ca	334.17	345	163.00	336	433.67	364	366.00	415
Fe	3.94	6.3	4.53	6.4	2.87	3.9	3.49	5.6
Zn	2.78	3.6	3.83	4.8	2.93	3.6	2.15	3.4
Cu	0.32	0.35	0.23	0.27	0.25	0.28	0.20	0.36

IF – infant formula
EE- Essential element

The results in table 4.13 are the mean levels of essential elements from this study and the corresponding label values. It can be observed that the results of this study were comparably within the range of the label values. For example, the mean level and label values of K in A_1 were 464.00 and 490.00 mg/100g respectively. The results then confirm that label values can be relied upon when choosing infant food formulations for specific levels of essential elements.

Table 4.14 Comparison of mean levels of essential elements with label values (mg/100g) in some cereal based formulations

IF / EE	H		I		J		L	
	This study	Label value	This study	Label value	This study	Label value	This study	Label value
K	399.17	550	481.67	_	344.67	_	475.83	700
Mg	34.67	_	87.00	_	28.17	_	38.00	_
Ca	251.67	453	387.17	260	253.00	360	443.00	516
Fe	5.33	7.5	7.6	8.7	2.84	4.0	7.47	11.0
Zn	2.56	_	4.31	12	1.18	_	9.17	_

IF- infant formula
EE-Essential element

The mean levels of essential elements in cereal based infant formulations from this study were compared with the manufacturer's label values. The results were observed to be within the range of printed label values. For example the values of Fe in infant formulation I were 7.60 and 8.7 mg/100g respectively. Although some label values were not printed, the available ones show that adequate amounts of essential elements are present in the infant formulas. The results of this study show that cereal based infant food formulations contain substantial amounts of essential elements even though they are not printed. Since these results are comparable to most of the label values present, the consumers can use them in situations where printed labels are not available like those of Mg in all the cereal based infant food formulations.

4.7.0 Comparison of mean levels of selected essential elements in infant milk and cereal based formulations with RDI values

The mean levels of the selected essential elements (mg/100g) in infant milk and cereal formulas were compared with WHO/UNICEF (1998) recommended daily intake values. Some of the infant food formulation manufacturers advise mothers and infant caretakers on a daily consumption between 80g and 143g printed on the labels. This amount is estimated using the number of scoops that range between 3.7g to 8.3g per scoop. In this study the average daily consumption of 100g was used to work out the percentage contribution RDI value each essential element analysed. The results obtained are discussed in the following sub-sections.

Table 4.15: Comparison of mean levels of K (mg/100g) in infant milk formulations with RDI of 700 mg/day

Infant formula		Mean (mg/100g)	Contribution to RDI value(%)
Age group	Code		
0 – 6 months	A_1	464.00	66.29
	B_1	447.33	63.90
	C_1	358.17	51.17
	D_1	365.00	52.14
	E_1	445.00	63.57
	F_1	470.67	67.24
7 – 12 months	A_2	478.83	68.40
	B_2	461.50	65.93
	C_2	394.33	56.33
	D_2	463.83	66.26
	E_2	468.17	66.88
	F_2	479.67	68.52

From the results presented in table 4.15, it can be noted that daily consumption of 100g of the analysed infant milk formulations would contribute over 50% to RDI value of

700mg/day. The deficit could be offset by breastfeeding, if the infant is both breastfeeding and using infant formulation as a supplement. For infants who are fed with infant formulations only, the daily contribution can be optimized by increasing the frequency consumption. The levels of K (mg/100) in cereals based infant formulations were compared with RDI of 700mg/day and the results are presented in table 4.16.

Table 4.16: Comparison of the mean levels of K (mg/100g) in cereal infant food formulations for 7 – 12 months age group with RDI of 700 mg/day

Infant formula	Mean (mg/100g)	Contribution to RDI value (%)
G	271.33	38.76
H	399.17	57.02
I	481.67	68.81
J	344.67	49.24
K	344.67	49.24

From the results in table 4.16, it can be observed that all the cereal based infant formulations analysed, except G, contribute fairly 50% and above to RDI of 700 mg/day. Infant formulation I contribute the highest, 68.81% to RDI value of 700mg/day. The formulation may be recommended for feeding infants who are not being breastfed because it is a rich source of K.

The mean levels of Mg in the analysed infant milk samples were compared with the recommended daily intake. Table 4.17 therefore presents mean levels of Mg (mg/100g) and percentage contribution of the analysed infant formulas to RDI of 50 mg/day

Table 4.17: Comparison of mean levels of Mg (mg/100g) in infant milk formulations with RDI of 50 mg/day

Infant formula		Mean (mg/100g)	Contribution to RDI value(%)
Age group	Code		
0 – 6 months	A_1	25.83	51.66
	B_1	34.17	68.34
	C_1	19.67	39.34
	D_1	34.00	68.00
	E_1	30.17	60.34
	F_1	32.33	64.66
7 – 12 months	A_2	31.83	63.66
	B_2	27.00	54.00
	C_2	22.50	45.00
	D_2	35.17	70.34
	E_2	37.67	75.34
	F_2	29.83	59.66

From the results (table 4.17), it is evident that infant formulation E_2 contribute the highest percentage, 75.34%, to RDI value of 50 mg/day assuming a daily consumption of 100 mg of infant milk formulation and C_1 contribute the lowest percentage of 34.34%. E_2 therefore is a rich source of Mg and can be recommended for feeding infants suffering from Mg deficiency disease, magnesemia. E_2 also contribute a higher percentage, 75.34%, to infants of 7 – 12 months age group than E_1 which contributes a lower percentage, 60.34%, to infants of 0 – 6 months age group. This implies that Mg requirements for infants of 7 – 12 months age group are higher the requirements for those of 0 – 6 months age group. Most of the milk infant formulations supply above 60% of Mg to RDI of 50 mg/day. For this reason the infant milk formulas sold in Nairobi market are a reliable source of Mg. Infants who are breastfeeding can meet the deficit through consumption of breast milk.

Table 4.18: Comparison of the mean levels of Mg (mg/100g) in cereal based infant formulations for 7 – 12 months age group with RDI of 50 mg/day

Infant formula	Mean (mg/100g)	Contribution to RDI value (%)
G	89.83	179.66
H	39.67	69.34
I	87.00	174.00
J	28.17	56.34
K	38.00	76.00

With reference to the results presented in table 4.18, it can be observed that infant cereal formulation G and I contributed values above RDI of 50 mg/day of 179.66% and 174.00% respectively. These values are relatively higher than the percentage contributions by all the milk formulations in table 4.18. The variation therefore could be due to ingredients. All the cereal based formulations contribute well above 50% to RDI of 50 mg/day and are therefore good sources of Mg to infants of 7 -12 months age group. The mean levels of Ca (mg/100g) in the milk infant formulations analysed were compared with the recommended daily intake. The results were therefore presented in table 4.19.

Table 4.19: Comparison of mean levels of Ca (mg/100g) in infant milk formulations with RDI of 360 mg/day

Infant formula		Mean (mg/100g)	Contribution to RDI value(%)
Age group	Code		
0 – 6 months	A_1	334.17	92.83
	B_1	163.00	45.28
	C_1	433.67	120.46
	D_1	366.00	101.67
	E_1	318.33	88.43
	F_1	286.33	79.50
7 – 12 months	A_2	433.00	120.28
	B_2	261.83	72.73
	C_2	422.83	117.45
	D_2	408.33	113.33
	E_2	333.50	92.64
	F_2	314.33	87.31

From the results (table 4.19), it can be noted that milk based infant formulations C_1, D_1, A_2, C_2 and D_2 had percentage contributions 120.46, 101.67, 120.8 117.45 and 113.33 respectively. These values were all above the RDI. The slightly high contributions to RDI are intended to ensure adequate supply of calcium in general most formulations contain larger amount of some minerals than does human milk. However, more important than concentration of minerals in the formulation is their degree of absorption and utilization. Minerals that are not sufficiently bioavailable must be supplemented to meet the nutritional needs of the infant. In some commercial infant food formulations like C_1, D_1, A_2, C_2 and D_2, limited bioavailability and low mineral density of certain minerals have led the manufacturers to add inorganic salts of those minerals. The high percentage contribution of Ca in table 4.19 emphasises the importance of Ca in child growth and therefore the need to enrich infant food formulations with Ca.

Table 4.20: comparison of the mean levels of Ca (mg/100g) in cereal based infant food formulations for 7- 12 month age group with RDI of 360 mg/day

Infant formula	Mean (mg/100g)	Contribution to RDI value (%)
G	439.17	121.99
H	251.67	69.91
I	387.17	107.55
J	253.00	70.28
L	443.00	123.06

The percentage contributions to RDI value of 360 mg/day by the cereal based infant formulas (table 4.20), are fairly high with G (121.99%), I (107.55) and L (123.06) contributions above 100%. The cereal based formulations had percentage contributions of Ca, to RDI, that are comparable to those of milk based formulas. This shows that infant formulas sold in Nairobi market are adequate in calcium levels. Calcium level has been increased in infant food formulations to ensure better retention due to its low bioavailability. Cereal based infant formulas G, I, and L with over 100% contributions to RDI may be recommended for feeding premature infant and those with compromised bone health since Ca contribute largely to bone development.

Table 4.21: Comparison of mean levels of Fe (mg/100g) in infant milk formulations with RDI of 10 mg/day

Infant formula		Mean (mg/100g)	Contribution to RDI value(%)
Age group	Code		
0 – 6 months	A_1	3.94	39.40
	B_1	4.53	45.30
	C_1	2.87	28.70
	D_1	3.49	34.90
	E_1	3.59	35.90
	F_1	2.73	27.30
7 – 12 months	A_2	4.77	47.70
	B_2	4.87	48.70
	C_2	4.08	40.80
	D_2	5.22	52.20
	E_2	5.33	53.30
	F_2	3.17	31.70

From the results presented in tables 4.21, it can be observed that all the analyzed infant milk formula cannot meet the RDI value of 10 mg of Fe singly, if the amount consumed daily is 100mg. considering that infants are feed several times a day, the RDI for Fe may be met by giving the infant a mixed diet of either A_1, (39.40%), F_1, (27.30%) or B, (45.30%). All the milk infant formulations for age 7 – 12 months had their percentage contributions slightly higher than the corresponding infant food formulations for age 0 – 6 months. For example A_1, had 39.40% while A_2, had 47.70% and infant milk formulation E_2 gave the highest contribution of 53.30%. Since Fe deficiency and Fe deficiency anemia are common among infant and young children, then the Fe rich formulations like E_2 (53.30%), D_2 (53.20%) and B2 (48.70%) should be recommended for them.

Table 4.22: comparison of the mean levels of Fe (mg/100g) in cereal based infant formulations for 7- 12 month age group with RDI of 10 mg/day

Infant formula	Mean (mg/100g)	Contribution to RDI value (%)
G	147.87	148.7
H	5.33	53.30
I	7.60	76.00
J	2.84	28.4
L	7.47	74.7

From the results (table 4.22), the percentage contributions for cereal based formulations were fairly higher than these of the infant milk formulas in Table 4.22. The cereal based formulations are, therefore, goods source of Fe. Infant formulation G, with the highest contribution of 148.7% well above RDI of 10 mg, is iron fortified and should be recommended for infants suffering from Fe deficiency disease.

The mean levels of Zn (mg/100g) in the milk based infant food formulations for 0 – 6 months and 7 – 12 months age groups were compared with the recommended daily intakes of 3 mg. The results were presented in table 4.23.

Table 4.23: Comparison of mean levels of Zn (mg/100g) in infant milk formulations with RDI of 3 mg/day

Infant formula		Mean (mg/100g)	Contribution to RDI value(%)
Age group	Code		
0 – 6 months	A_1	2.78	92.67
	B_1	3.83	127.67
	C_1	2.93	97.67
	D_1	2.15	71.67
	E_1	3.29	109.67
	F_1	2.79	93.00
7 – 12 months	A_2	3.26	108.67
	B_2	4.09	136.33
	C_2	3.25	108.33
	D_2	4.34	144.67
	E_2	4.53	151.00
	F_2	2.82	94.00

From the results presented in table 4.23 it is evident that E_2 provide the highest percentage of Zn to the RDI value followed by D_2, B_2, B_1, E_1, A_2, C_2, C_1, F_2, F_1, A_1, and finally D_1 with percentage contributions of 151.00%, 144.6%, 136.33%, 127.67%, 109.67%, 108.67%, 108.33%, 97.67%, 94.00%, 93.00%, 92.67%, and 71.67% respectively . Most of the percentage contributions were above RDI of 3mg/day Zinc. Since deficiency, marked by severe growth retardation and arrested sexual development is common with Zinc. To check the low bioavailability and inhibition of Zinc absorption by other elements, it has necessitated that many manufactures add more Zinc in the formulas. However, too high amounts of Zinc in the formulations can inhibit copper absorption, hence leading to copper deficiency. The infant formulas A_2, B_2, C_2, D_2 and E_2, 7 – 12 month infant milk formulas all provide percentage contribution above RDI value. This then shows that the manufacturers increase zinc requirements in infant formulas according to the child's growth age.

Table 4.24: Comparison of the mean levels of Zn (mg/100g) in cereal based infant formulations for 7- 12 month age group with RDI of 3 mg/day

Infant formula	Mean (mg/100g)	Contribution to RDI value (%)
G	10.07	335.67
H	2.56	85.33
I	4.34	144.67
J	1.18	39.33
L	9.17	305.67

From the results (table 4.24) cereal based infant formula G, Provided the highest percentage (335.67%) to RDI value followed by L (305.67%), I (144.67%) H (85.33%) and finally J (39.33%). The high percentage contributions suggest the great role played by Zinc in the body. For example, Iron and Zinc support immune function, while chromium and Zinc aid insulin action. Zinc is also an essential for many other bodily functions such as growth, development of sexual organs and reproduction. The mean levels of Cu (mg/100g) in the infant formulations were compared with the recommended daily intake of 0.7 mg/day and results presented in table 4.25.

Table 4.25: Comparison of mean levels of Cu (mg/100g) in infant milk formulations with RDI of 0.5 mg/day

Infant formula		Mean (mg/100g)	Contribution to RDI value(%)
Age group	Code		
0 – 6 months	A_1	0.32	64.00
	B_1	0.23	46.00
	C_1	0.25	50.00
	D_1	0.20	40.00
	E_1	0.35	70.00
	F_1	0.25	50.00
7 – 12 months	A_2	0.39	78.00
	B_2	0.36	72.00
	C_2	0.33	66.00
	D_2	0.40	80.00
	E_2	0.42	84.00
	F_2	0.32	64.00

From the results presented in table 4.27, it is evident that no single infant food formulation would meet the RDI of 0.5 mg, if the amount consumed daily is 100g. A mixed diet would be recommended, for example, infant may be fed with A_1, (64 %) and D_1,(40%) or A_2 (78%) and C_2 (66%) so as to meet the RDI. Infant food formulations A_2, B_2, C_2, D_2, and E_2 with percentage contributions of 78%, 72%, 66%, 80% and 84% are good sources of Cu for infants aged 7 -12 months and should be recommended for infants suffering from anaemia, skeletal defects and those with compromised immune system. Copper is essential for normal red blood cell formulation. It helps store and release Fe that is necessary for haemoglobin formation. It also works with Vitamin C to keep blood vessels elastic and flexible as well as in boosting body's immunity. Copper can lower LDL and raise HDL cholesterol in the blood, and also participate in the collagen synthesis and connective tissues (Adams and Keen, 2005).

Table 4.26: Comparison of the mean levels of Cu (mg/100g) in cereal based infant food formulations for 7- 12 month age group with RDI of 0.5 mg/day

Infant formula	Mean (mg/100g)	Contribution to RDI value (%)
G	0.36	72.00
H	0.14	28.00
I	0.51	102.00
J	0.24	48.00
L	0.27	54.00

From the results (table 4.26), formula I provided the highest, 102%, to RDI of 0.5 mg/day while it provided the lowest percentage, 28%. The abnormally high contribution of Cu to RDI by I suggests that the variation could be due to ingredients or that I is an infant formulation specially designed to provide a better amount of Cu in order to meet the

needs of certain group of infants. The mean levels of Mn (mg/100g) in infants milk and cereal based formulations were compared with RDI value of 0.5 mg/day and the results presented in table 4.27.

Table 4.27: Comparison of mean levels of Mn (mg/100g) in infant milk formulations with RDI of 0.5 mg/day

Infant formula		Mean (mg/100g)	Contribution to RDI value(%)
Age group	Code		
0 – 6 months	A_1	0.15	30
	B_1	0.31	62
	C_1	0.34	68
	D_1	0.32	64
	E_1	0.26	52
	F_1	0.10	20
7 – 12 months	A_2	0.33	66
	B_2	0.41	82
	C_2	0.44	88
	D_2	0.65	130
	E_2	0.52	104
	F_2	0.45	90

From the results presented in table 4.27, it can be observed that C_1 with the highest contribution of 68% among the infant formulations of 0 – 6 months is a better source of Mn. Generally infant milk formulations for 7 – 12 months provided fairly high percentage contributions to RDI of 0.5mg/day singly, if a 100g is fed to the infant daily. The other infant food formulations can meet the RDI of 0.5mg when they are fed as a mixed diet to infants or if more than 100g is given daily.

Table 4.28: The mean levels of Mn (mg/100g) in cereal based infant food formulations for 7- 12 month age group with RDI of 0.5 mg/day

Infant formula	Mean (mg/100g)	Contribution to RDI value (%)
G	2.45	490
H	0.62	124
I	1.08	216
J	0.5	100
L	0.77	154

From the result presented in Table 4.28, it can be noted that G, could provided the highest percentage of Mn to the RDI value followed by I, L, H and finally J with percentage contributions of 490%, 216%, 154% and 100% respectively. All the cereal based infant formulations provided percentage contributions that can meet the RDI by consuming 100g of each of them. The cereal based formulations are, therefore good sources of Mn. Considering that bioavailability of Mn in most cereal foods is low, the high percentage contribution of Mn to RDI value by all the analysed cereal formulas will help control gross deficiency and toxicity of Mn in the infants body.

The mean levels of Cr (µg/100g) in the analysed infant milk formulas were compared with the recommended daily intake of 0.5 µg/day. Table 4.29 therefore presents mean levels of Cr (µg/100g) and percentage contribution of the analyzed formulations to RDI of Cr.

Table 4.29: Comparison of mean levels of Cr (µg/100g) in infant milk formulations with RDI of 50 µg/day

Infant formula		Mean (µg/100g)	Contribution to RDI value(%)
Age group	Code		
0 – 6 Months	A_1	30	60
	C_1	20	40
	D_1	30	60
	E_1	20	40
7 – 12 Months	A_2	70	140
	C_2	30	60
	D_2	40	80
	E_2	20	40

From the results in table 4.29 it can be observed that infant milk formulation A_2 in which Cr was detected and measured contribute highly to the required daily intake of 50 µg. The high percentage contributions imply that the milk formulations contained excess of Cr. For this reason the manufacturers should strive and bring down Cr levels because high levels above RDI can lead to toxicity.

The mean levels of Cr in this study are higher than those reported by Khalifa *et al.* (2010) of between 0.02 µg and 0.05 µg in infant milk formulations since birth and follow on formulas.

CHAPTER FIVE : CONLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The results of the study to determine the levels of major and trace essential elements in six milk and five cereal based infant formulas showed that all the infant milk formulations analysed in this study, except B_1, B_2, F_1 and F_2, were found to contain the selected essential elements but in varying concentrations. On the other hand all the cereal based infant food formulations analysed in this study were found to contain the selected essential elements except Cr. Generally, infant formulations for 7 – 12 months age group contained significantly higher levels of the selected essential elements than infant formulations for 0 – 6 months age group. Cereal based formulations had the highest mean levels of Ca compared to milk based formulations. The results of this study show that infant formulations found in Nairobi market, contain adequate levels of essential elements selected, except the levels of iron in some formulations.

5.2 RECOMMENDATIONS

5.2.1 RECOMMENDATIONS FROM THE STUDY

(i) Infant milk formulations sold in Nairobi county are rich in essential elements except iron and hence they are important for feeding infants.

(ii) Since infant formulations analysed contain varying amounts of the selected essential elements, Kenyan mothers and health care providers may be encouraged to choose infant formulations in the light of essential element content.

(iii) Infant formula food companies are encouraged to look for other sources of chromium origin in order to enrich cereal based formulations.

(iv) Infants aged between 7 – 12 months need to be fed using both milk and cereal based formulations so as to meet the RDI of the selected essential elements.

5.2.2 SUGGESTIONS FOR FURTHER STUDY

(i) Due to influx of imported infant milk and cereal based formulations into Kenya, it is recommended that constant work be done on this infant food formulation brands that are on the Kenyan market to monitor their essential element contents.

(ii) Essential elements other than the ones determined in this study need to be investigated in the analysed infant milk and cereal formulations.

(iii) Levels of non-essential elements and vitamins, be determined in the analysed infant milk and cereal formulations.

(iv) Essential elements should be determined in other types of infant formulations, for example, Soy-based infant formulas.

(v) Processing factors that may influence levels of essential elements, for example, modification of cow's milk in the production of infant milk formulations that may lead to inadvertent loss of essential minerals require investigation.

(vi) Bioavailability of essential elements in infant milk formulations on sale in Kenya need to be investigated.

(vii) Protein, carbohydrate and vitamin levels of the infant food formulations in Kenyan market be investigated.

REFERENCES

Adams, J. Y. and Keen C. L. (2005). Copper, Oxidative Stress and Human Health. *Molecular Aspects of Medicine.* **26** (4 – 5): 268 – 298.

Agostino, C. and Domellof, M. (2005). Infant Formulae: From ESPGAN Recommendations Towards ESPGHAN-Coordinated Global Standards. *Journal of Pediatric Gastroenterology and Nutrition*, **41** (5): 580-583.

Aizman, R., Grahnquist, L. and Celsi, G. (1998). Potassium Homeostasis: Ontogenic Aspects. Department of Human Physiology. *Acta Paediatrica,* **87**: 609-617.

Al Durtsch (1999). Chromium Deficiency Diseases and Good Nutrition., Nutrition Basic Home . Walton Feed Inc., pp 1-2.

Al-Ghamdi, S. M., Cameron, E. C. and Sutton, R. A. (1994). Magnesium Deficiency: Pathophysiological and Clinical Overview. *American Journal of Kidney Diseases*, **24**: 737-754.

Allen, L. H. (1998). Zinc and Micronutrient Supplements For Children. *American Journal of Clinical Nutrition*, **68**: 4955-4985.

American Chemical Society (2006). Reagent Chemical Specification and Procedures. Oxford University Press, London. pp 60.

Annemiek, C. G. and Jacques, G. B. (1994). The Composition of Human Milk as a Model for the Design of Infant Formulas: Recent Findings and Possible Applications. *Nutritional Research Reviews*, **7**: 1-23.

Ayivor, J. E., Debrah S., Forson, A., Nuviadenu C., Buah K, A. and Denutsui D. (2011). Trace Elements in Some Imported Commercial Infant Cereal Formulas on the Ghanaian Market by INAA. *Der Pharma Chemica*, **3** (5): 94-101.

Becker, J. S., Dobrowoiska, J., Miroslav, Z. and Becker, J. S. (2008). Trends in Analytical Chemistry. *International Journal of Mass Spectroscopy*, **270**: 1-7.

Beckhoff, B., Kanngiefer, B., Wedell and Wolff, H. (2006). Handbook of Practical X-ray Fluorescence Analysis. Springer Publisher, USA. PP 37.

Bermejo, A., Dominguez, R., Frega, J. M. and Cocho, J. A. (2000). *Talanta*, **50**: 1211-1222.

Bowen, H. J. M. (1979). Environmental Chemistry of Elements, Academic Press, New York.

Branca, F. and Valtuena, S. (2001). Calcium, Physical Activity and Bone Health-Building Bones for a Stronger Future. *Public Health Nutrition,* **4**: 117-23.

Braudus, A. E., (1993). Physiological Function of Calcium, Magnesium and Phosphorus and Mineral Ion Balance. Inc: Favus M. J., ed. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 2nd ed. New York, NY: Raven Press. pp 41-46.

Castillo-Duran, C. and Cassorla, F. (1999). Trace Minerals in Human Growth and Development. *Journal of Pediatric Endocrinology and Metabolism.* **12**: 589-601.

Centers for Disease Control and Prevention (CDCP) (2006). When Should a Mother Avoid Breastfeeding? pp 5-10.

Chatterjee, (2003). Mother-to-Child HIV Transmission in India. *Lancet Infectious Diseases*, **3** (12): 111-121.

Centers for Disease Control and Prevention (CDCP) (2000). Managing Elevated Blood Lead Levels Among Children, pp 13-17.

Centers for Disease Control and Prevention (CDCP) (2007). Breastfeeding Frequently Asked Questions Olver. pp 7-9.

Civitelli, R. and Avioli, L. V. (1994). Calcium, Phosphate and Magnesium Absorption. In LR(ed.): Physiology of the Gastrointestinal Tract, pp 2173-2181.

Codex Alimentarious Commission (2007). Standards for Infant Formula and Formulas for Special Medical Purposes Intended for Infants. CODEX STAN 72-1981.

De Cock, (2000). Prevention of Mother-to-Child HIV Transmission in Resource Poor Countries. *Journal of the American Medical Association,* **283** (9): 256-262.

Department of Health, Committee on Medical Aspects of Food Policy., (1994). Weaning and the weaning Diet. Report on Health and Social Subjects., London: *HMSO.*, **45**:49–50.

Dim, L. A., Funtua, I., Oyewale, A. O., Grass, F., Umar, I. M., Gworzo, U. S. and Gwozdz, R. (2004). *Journal Radioanal Nuclear Chemistry,* **261**: 225-228.

Ebringe, L., Ferencik, M. and Krajcovik, J. (2008). *Beneficial Health Effects of Milk and Fermented Dairy Products of Folia Microbiology*, **53** (5): 378-394.

European Union Statutory Instrument (1995). Infant Formula and Follow- on Formula Regulations, EUSI No. 77.

Feely, R. M., Eitenmiller R. R. and Jonnes J. B. (1983). Calcium, Phosphorous and Magnesium Contents of Human Milk During Early Lactation. *Journal Pediatric Gastroenterology and Nutrition*, **2** (2): 262-267.

Fomon, S. J., Ziegler, E. E. and Nelson S. E. (1993). Erythrocyte Incorporation of ingested Fe by 56-day-Old Breastfed and Formula-fed Infants. *Peadriatric Research*, **33**:573-576.

Food and Drug Administration., (1997). Overview of Instruments Formulas, US Food and Drug Administration Centre for Food Safety and Applied Nutrition, Office of Special Nutrition 200 C. Street SW , Washington DC, USA.

Fox, B. A. (1998). Food Science - A Chemical Approach. University of London Press Limited., pp 57.

Friel, J., Gibson R. S. and Kawash, G.F. (1985). Dietary Zinc Intake and Growth During Infancy. *Journal Pediatric Gastroenterology and Nutrition*, **4**: 746–51.

Fuller, N. J., Bates, C. J. and Evans, P.H. (1992). High Folate Intakes Related to Zinc Status in Preterm Infants. *European Journal of Pediatric*, **151**: 51–53.

Garrison, R. G. and Somer, E. (1995). The Nutrition Desk Reference, 3rd Edition, Kents.

He, F. J. and MacGregor, G. A. (2008). Beneficial Effects of Potassium on Human Health. *Physiology of Plant Journal*. **133** (4): 725-735.

Horwitz, W. (2001). Official Methods of Analysis, 17th ed., Association of Official Analytical Chemist, (AOAC) International, pp 1-19.

Institude of Medicine , Food and Nutrition Board. (1997). Dietary Supplement Intakes For Calcium, Phosphorus, Magnesium , Vitamin D and Fluoride. Washington , DC: National Academy Press.

Joseph, E., Naisirui, R. and Ahmed Y. A. (2011). Trace Elements Pattern in Some Nigerian Commercial Infant Milk and Infant Cereal Formulas. *Annals of Biological Research*, **2** (2): 351-360.

Kabi, F. (2004). Micronutrients the Hidden Hunger in HIV/AIDS. Symposium at the Kenyatta National Hospital, Kenya,12th-15th October, pp 15.

Kerr, M. and Desiree, L. (2008). Neurodevelopmental Delays Assosciated with Iron-Fortified Formula for Healthy Infants. *Medscape Psychiatry and Mental Health.* Retrieved 04-08-2008.

Khalifa, A. S and Ahmad, D. (2010). Determination of Key Elements by ICP-OES in Commercially Available Infant Formula and Baby Foods in Saudi Arabia. *African Journal of Food Science,* **4** (7): 464-468.

Khangani, S., Hamid E., Najmeh, M., Mohamed M., Givianrad , Hossein, M. and Shahi S.(2009). Zinc and Copper Concentrations in Human Milk and Infant Formulas. *Iranian Journal of Pediatric*, **20** (1): 53-57.

Legius, E., Proesmans, W., Eggermont, E., Vamdamme-Lombaerts, R., Bouillon, R. and Smet, M. (1989). *European Journal of Pediatric*, **148**:784-785.

Leotsinidis, M., Alexopoulos, A. and Kostopoulou-Farri, E. (2005). Toxic and Essential Trace Elements in Human Milk from Greek Lactating Women: Association With Dietary Habits and Other Factors. *Chemosphere*, **61** (2): 238-247.

Ljung K., Brita P., Margaretha G. and Marie V. (2011). High Concentrations in Essential and Toxic Elements in Infant Formula and Infant Foods – A Matter of Concern. pp 62.

Lonnerdal, B. (1995). Magnesium Nutrition of Infants. *Magnesium*, **8**: 99-105.

Lonnerdal, B. (1998).Copper Nutrition During Infancy and Childhood. *American Journal Clinical Nutrition,* **67**: 1046–53.

Lynne, (2004). "Food Timeline – History Notes: Baby Food". Retrieved 2006-09-16.

Magnus, D., Lonnerdal B. and Kathryn G. D. (2004). Iron, Zinc and Copper Concentrations in Breast Milk are Independent of Maternal Mineral Status. *American Journal of Clinical Nutrition*, **79** (1): 111-115.

Menezes-Filho, J. A., Borchad, M., Sarcinelli, P. N. and Moreira J. C. (2009). Manganese Exposure and Neuropsychological Effect on Children and Adolescents. A Review. *Revist a Panamerican de Salud Publica*, **26** (6): 541-548.

Mimouni, F., Campaigne, B., Neylan, M. and Tsang, R. C. (1993). Bone Mineralisation in the First Year of Life in Infants Fed Human Milk, Cow-Milk Formula or Soy- Based Formula. *Journal of Pediatric*. **122**: 348-354.

Mohan L. and Stump S. (2000). Krause's Food, Nutrition and Diet Therapy. pp 3.

Monte, C. M. and Gulgilani, E. R. (2004). *Journal of Pediatric*, **38**: 131-141.

Muchemi, G . N . (2006). Determination of some Immune Boosting Trace Elements In Selected Food Grains, Herbal Spices and Seeds. Master of Science Thesis, Kenyatta University. pp 17.

Mindel, E. and Mundis, H. (2004). Vitamin Bible; Updated Information on Nutraceuticals, Herbs, Alternative Therapies and Antiaging Supplements. Waner Book Group, New York. pp 56-71.

Neuman, C. G. (1994). Onset and Evolution of Stunting in Infants and Children. Examples from the Human Nutrition Collaborative Research Support Programme. Kenya and Egypt Studies. *European Journal of Clinical Nutrition,* **48**(1):90-102.

Nokes, C., Bosch, C. and Bundy D. A. (1998). The Effects of Iron Deficiency and Anemia on Mental and Motor Performance, Education Achievement and Behavior in Children. A Report of the International Nutritional Anemia Consultative group.

Oskarsson, A. and Sandstoerm, B. (1995). *Royal Society of Chemistry - Analyst*; **120**:911

Paola, S. S, Vera A. M and Mitiko S. (2007). Determination of Br, Ca. Na, K, Fe, Rb, Se, and Zn in Milk Formulas by INAA. PP 5.

Perazzella, M. A. (2000). Trimethoprim-induced Hyperkalemia: Clinical Data, Mechanism, Prevention and Management. *Drug Safety*, **22** (3): 227-336.

Prasad, A. S. (1993). Essential and Toxic Elements in Human Health and Disease: An update, Willey Liss. Inc., New York.

Physicians Reference Desk, 55th ed. (2001). Montvale, NJ: Medical Economics Co., Inc. pp1418-1422, 2199-2207.

Preoteasa, E. A., Preoteasa, E., Kuczumo, A., Gurban, D., Harngu, L., Grambole, D. and Hermann, F. (2008). *Journal of X-ray Spectroscopy*, **37**: 517-535.

Reifen, R. M. and Zlotkin, S. (1993). Microminerals. In: Tsang RC, Lucas A, Uauy R, eds.Nutritional Needs of the Preterm Infant. Baltimore: Williams & Wilkins, pp:195.

Riordan, J. M. (1997). The Cost of not Breastfeeding: a Commentary. *Journal of Human Lactation*, **13**(2): 93-97.

Ruel, M. T., Rivera J. A. and Santizo M. C. (1997). Impact of Zinc Supplementation on Morbidity from Diarhoae and Respiratory Infections Among Rural Guatemalan Children. *Pediatrics*, **99** (6): 808-813.

SCF (2006). Tolerable Upper Intake Levels for Vitamins and Minerals. Scientific Committee on Food. Scientific Panel on Dietric Products, Nutrition and Allergies. European Food Safety Authority.

Schanler, R. J. and Abrams, S. A., (1995). *Journal of Peditrics*, **126**:441-447.

Schneider, J. M., Fujii, M. L. and Lamp C. L. (2007). The Prevalence of Low Serum Zinc and Copper Levels and Dietary habits associated with Serum Zinc and Copper in 12-36-month-old Children from low-income families at risk for iron deficiency. *Journal of American Diet Association*, **107** (11):1924-1929.

Schumann, K., Classen, H. G. and Dieter, H. H. (2002). Hohenheim Consensus Workshop: Copper. *European Journal Clinical Nutrition*, **56** (6): 469-483.

Schrauzer, G. N. (Ed). (1994). Toxic Heavy Metals and Other Trace Elements in Foodstuffs from 12 Different Countries, Humana Press Inc. pp. 415.

Singh, K. B. and Taneja, S. K. (2010). Concentration of Zn, Cu and Mn in Vegetables and Meat Foodstuffs Commonly Available in Manipur: A North Eastern State of India. *Electronic Journal of environmental, Agricultural and Food Chemistry*, **9**: 610-616.

Skoog, D. A., Holler, F. J and Nieman, T. A. (1998). Principles of Instrumental Analysis. Saunders College Publishers, Michigan. pp 63-76.

Solomoms, N. W. and Jacob, R. A. (1981). *American Journal Clinical Nutrition,* **34**: 475.

Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (1997). Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride. Food and Nutrition Board. Institute of Medicine. National Academy Press. Washington D.C.

Stewart, K. K. (1980). In: Stewart, A. (Ed). Nutrients Analysis of Foods: the State of The Art for Routine Analytical Chemist, Washington D. C. pp 1.

Tripathi R. M., Raghunath R., Sastry V. N. and Krishnamoorthy T. M. (1999). Daily Intake of Heavy Metals by Infants Through Milk and Milk Products. *Science Total Environment,* **227**:229-235.

Tripathi R. M., Raghunath R., Sastry V. N. and Krishnamoorthy T. M. (1997). Daily Intake of Heavy Metal in Bombay City, India. *Science of Total Environment*, **208**: 149-159

Wanjau, R., Jiang, Z. C., Hu, B., Qin, Y. C., Nu, Y. L and Zhu, X. S. (2001). Simultaneous Determination of Trace Rare Earth Elements and Other Elements in High Purity Terbium Oxide (Tb_2O_7) by ICP-AES After HPLC Separation Using P507 Resin. *Wuhan University Journal of Sciences*, **61**: 110-116.

Webster, P. O. (1987). Magnesium. *American Journal of Clinical Nutrition*, **45**: 1305-1312.

Whanger, P . D . (2003). Selenium and its Relationship to Cancer, Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, 0r 97331, pp. 1-21.

WHO (1988a). International Programme on Chemical Safty. *Journal of Chromium Environmental Health Criteria*, **61:** 110-116.

WHO (1989). Minor and Trace Elements in Breast Milk. Report of a Joint WHO/IAEA Collaborative Study. Geneva.

World Health organization (1994). Report of a WHO/FAO/IAEA Expert Consultation on Trace Elements in Human and Health, WHO, Geneva.

WHO (1996_a). Trace elements in Human and Health, World Health organization, Geneva, **5**: 49-167.

WHO (1996_b) .Trace Elements In Human Nutrition and Health. Geneva, **5**: 72-103.

WHO/UNICEF (1998). Complimentary Feeding of Young Children in Developing Countries, pp. 79-108.

WHO (2001). Infant and Young Child Nutrition: Global Strategy for Infant and Young Child Feeding.

WHO (2002). Global Strategy for Infant and Young Child Feeding. pp 12

WHO (2003). Global Strategy for Infant and Young Child Feeding. pp 5.

WHO and FAO (2002). A Manual on Nutrition, Care and Support of People Living with HIV/AIDS. Rome. pp 1-50.

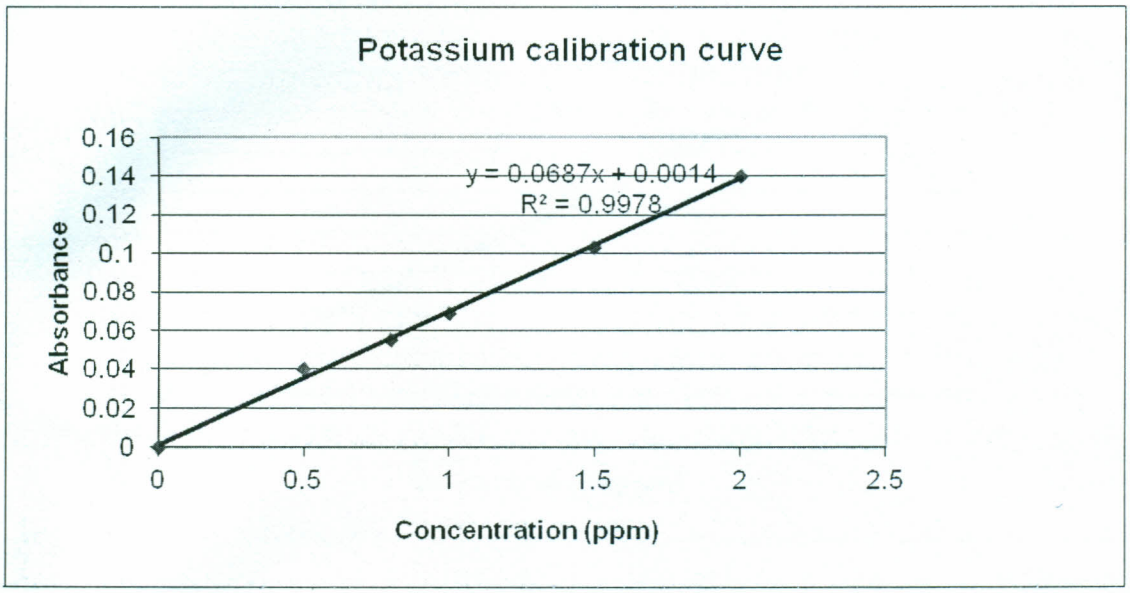
Williams, R. E. and Caliendo, M. A. (1988). Nutritional Principles, Issues and Applications. pp 23-51.

Zhang, J. P., Li, F. and YU, X. W. (2008). Trace Elements and Cytokine Profile in Cytomegalovirus-infected Pregnancies. *Gynecolic and Obstetric Investigation Journal.* **65** (2):128-132.

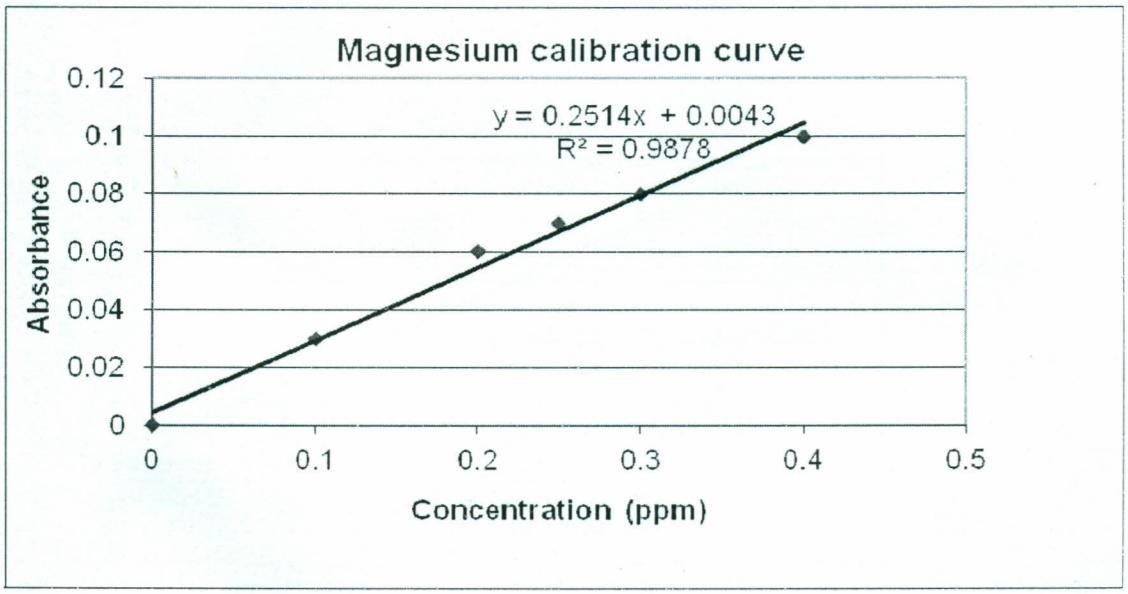
Zhu, K., Devine, A. and Prince, R. L. (2009). The effects of High Potassium Consumption on Bone Mineral Density in a Prospective Cohort Study of Elderly Postmenopausal Women. *Osteoporosis Internationál*, **20** (2):335-3

APPENDICES

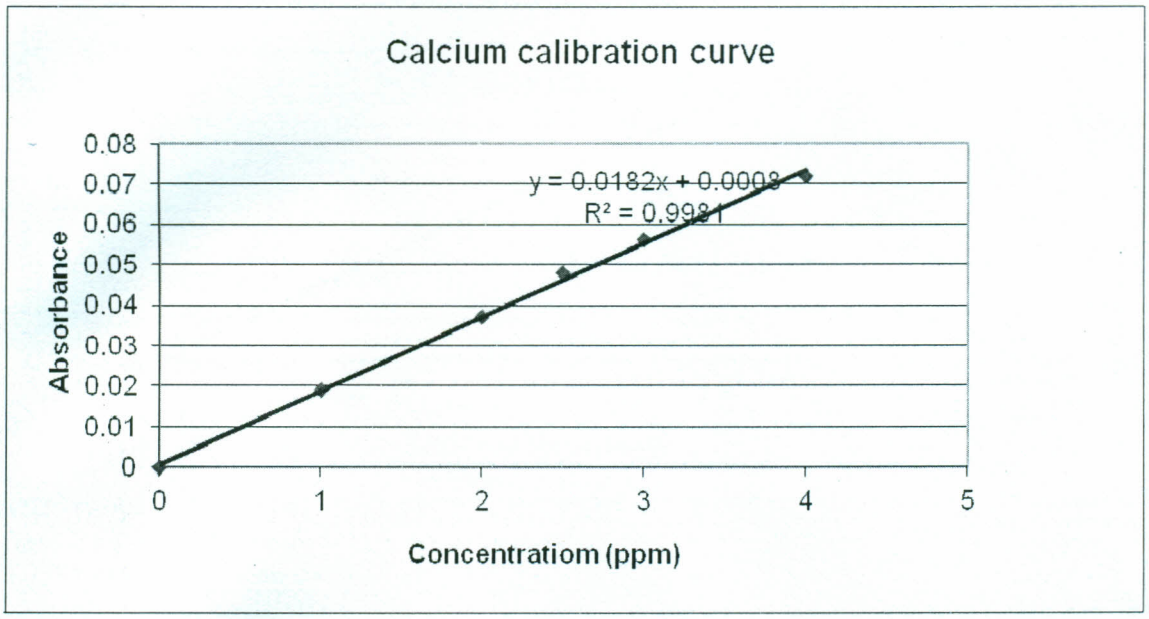
APPENDIX I: Potassium calibration curve by AAS



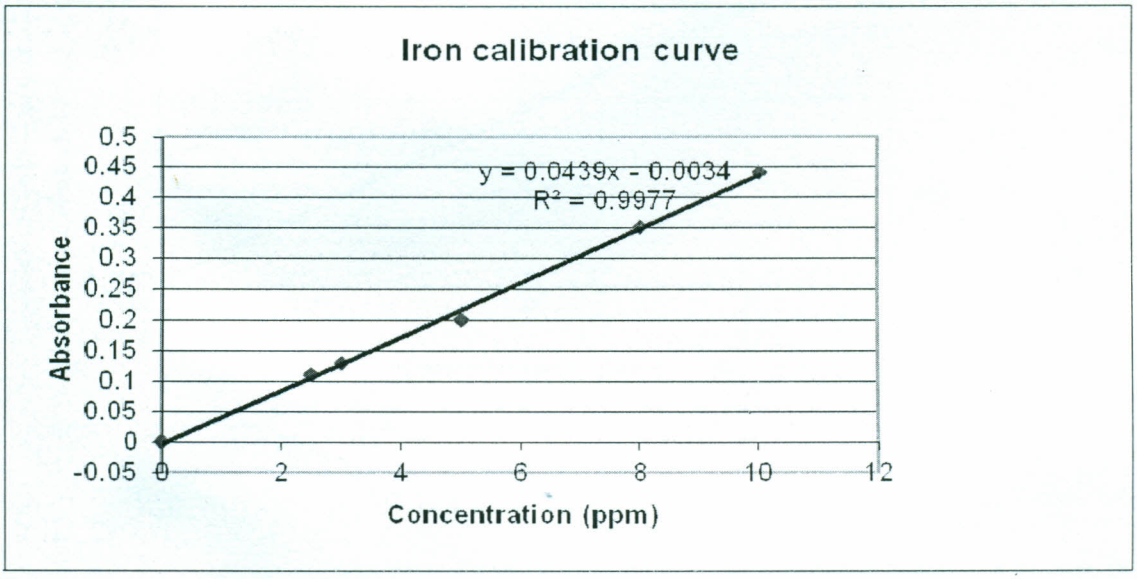
APPENDIX II: Magnesium calibration curve by AAS



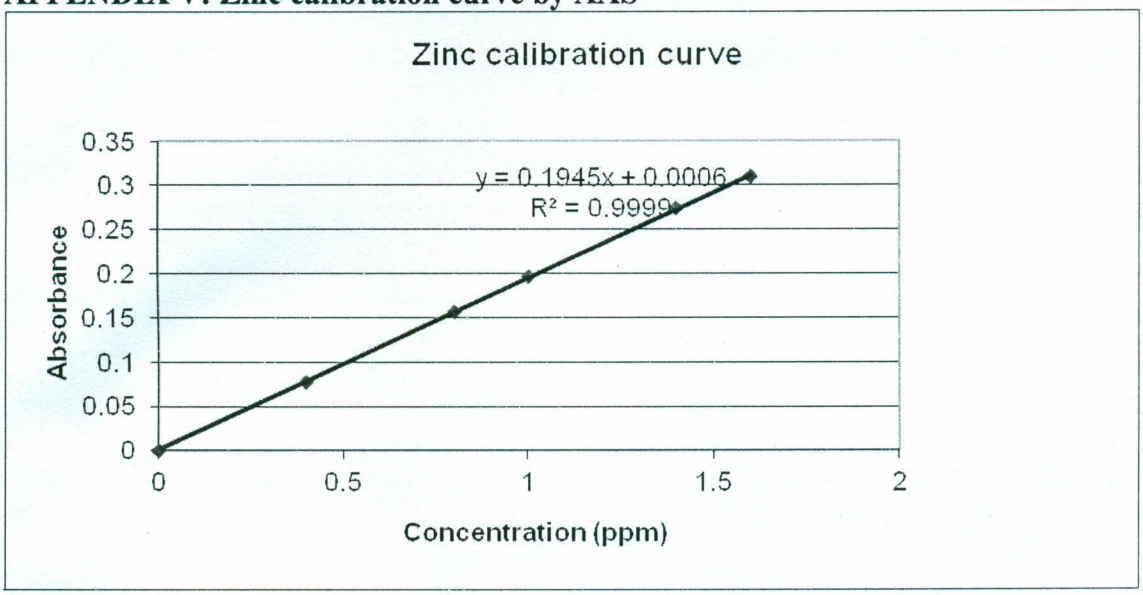
APPENDIX III: Calcium calibration curve by AAS



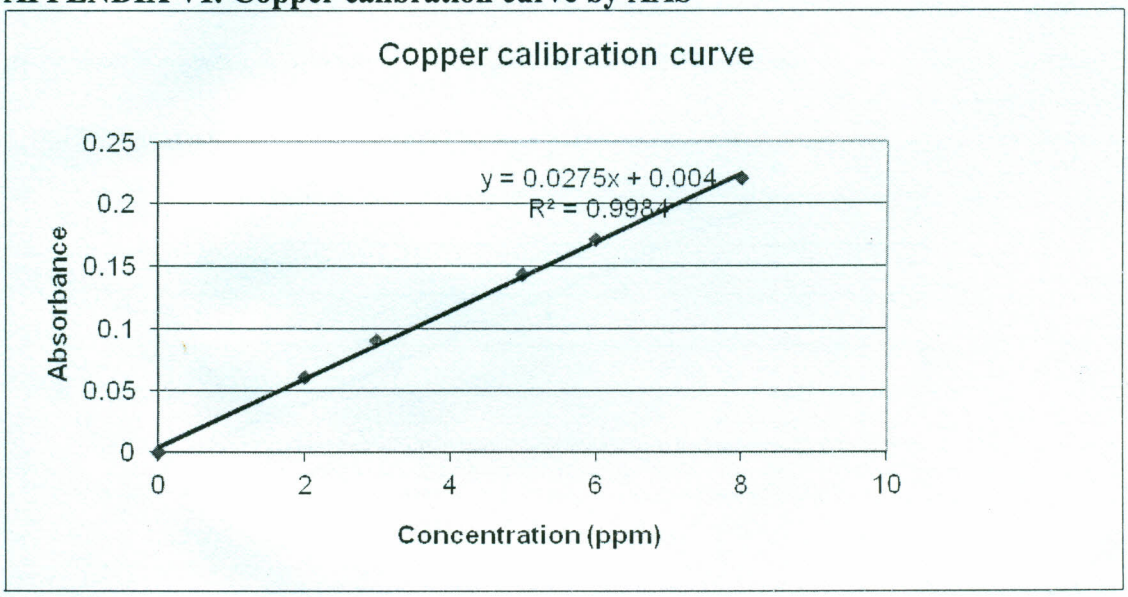
APPENDIX IV: Iron calibration curve by AAS



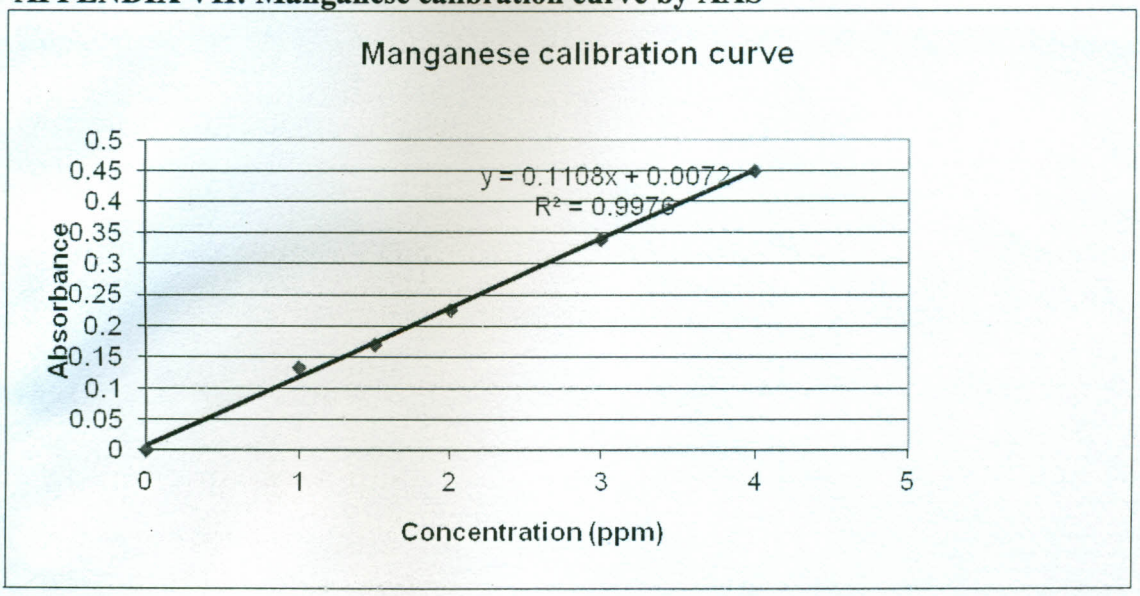
APPENDIX V: Zinc calibration curve by AAS



APPENDIX VI: Copper calibration curve by AAS



APPENDIX VII: Manganese calibration curve by AAS



APPENDIX VIII: Chromium calibration curve by AAS