

STUDIES OF ESSENTIAL AND NON-ESSENTIAL ELEMENTS IN CEREAL-BASED WEANING FOODS FOR INFANTS COMMERCIALY AVAILABLE ON THE GHANAIAN MARKET

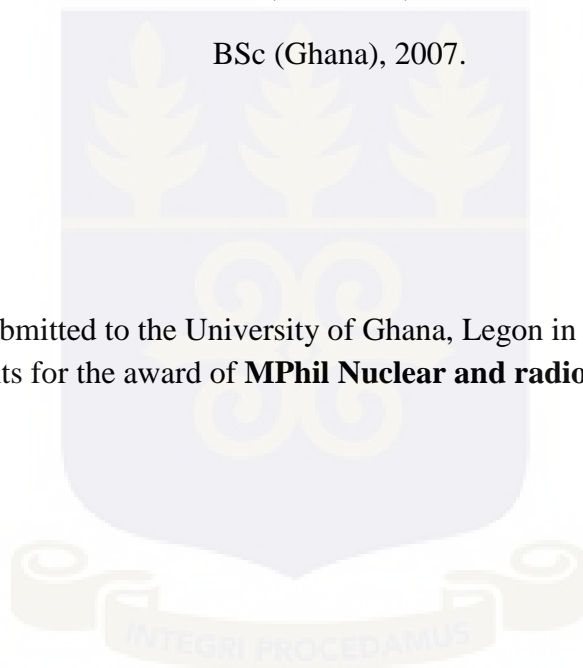
By

Bawol Stephen

(10165401)

BSc (Ghana), 2007.

This thesis is submitted to the University of Ghana, Legon in partial fulfilment of the requirements for the award of **MPhil Nuclear and radiochemistry** degree.



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DECLARATION

This thesis is the result of research work undertaken by Stephen Bawol in the Department of Nuclear Sciences and Applications, School of Nuclear and Allied Sciences, University of Ghana, under the supervision of Dr. Dennis K. Adotey and Prof. Y. Serfor-Armah. I affirm that to the best of my knowledge, this thesis has not been presented anywhere else for the award of a similar or other degree.



Sign _____

BAWOL STEPHEN
(STUDENT)

DATE.....

Sign _____

DR. D. K. ADOTEY
(SUPERVISOR)

DATE.....

Sign _____

PROF. Y. SERFOR-ARMAH
(SUPERVISOR)

DATE.....

DEDICATION

This work is dedicated to the Glory of God



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“If I have seen further than others, it is by standing upon the shoulders of giants”.

-Isaac Newton.

This work would not have been completed without the express benevolence of God Almighty, who in His infinite mercy granted me the strength, both physically and mentally, and good health to see it through. For this I am most grateful and give Him the glory.

A number of individuals also carried me on their shoulders throughout the work. Chief among them are my supervisors, Dr. Dennis K. Adotey and Prof. Y. Serfor-Armah, whose contribution to this work in diverse ways I cannot measure in monetary terms. I thank them most sincerely for their fatherly guidance, and the patience exhibited even under trying moments, just to ensure that this work saw the light of day in its finest state.

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May God’s abundant blessings be with them all.

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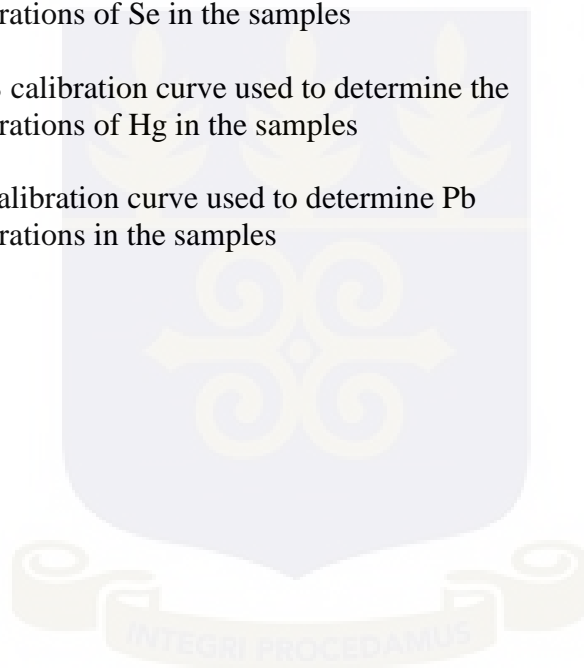
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LIST OF ABBREVIATIONS AND ACRONYMS

AAP	American Academy of Paediatrics
AAS	Atomic Absorption Spectrometry
AES	Atomic Emission Spectrometry
AI	Adequate Intake
AIDS	Acquired Immune Deficiency Syndrome
ATSDR	Agency for Toxic Substances and Disease Registry
BDA	British Dietetics Association
BDH	British Drug House
CDC	Centres for Disease Control and Prevention
CINAA	Cyclic Instrumental Neutron Activation Analysis
CNS	Central Nervous System
CONTAM	EFSA Panel on Contaminants in the Food Chain
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
CVAAS	Cold Vapour Atomic Absorption Spectrometry
DEFRA	Department for Environment, Food and Rural Affairs
DGE	Society for Nutrition of Germany
DNA	Deoxyribonucleic acid
DNAA	Derivative Neutron Activation Analysis
DP-CSV	Differential Pulse Cathode Stripping Voltammetry
EDL	Electrodeless Discharge Lamp
EFSA	European Food Safety Authority
EINAA	Epithermal Instrumental Neutron Activation Analysis
ENHIS	European Environment and Health Information Systems
EU	European Union
FAAS	Flame Atomic Absorption Spectrometry
FAO	Food and Agriculture Organisation
FINAA	Fast Instrumental Neutron Activation Analysis
FNB	Food and Nutrition Board
FSA	Food Standards Agency
FSAI	Food Safety Authority of Ireland
GAEC	Ghana Atomic Energy Commission

GFAAS	Graphite Furnace Atomic Absorption Spectrometry
GFDA	Ghana Food And Drugs Authority
GHARR-1	Ghana Research Reactor-1
GMF	The George Mateljan Foundation
HCL	Hollow Cathode Lamp
HGAAS	Hydride Generation Atomic Absorption Spectrometry
HPGe	High Purity Germanium gamma ray detector
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research into Cancer
ICC	International Cadmium Conference
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IDPH	Illinois Department of Public Health
IG	Institute of Geology
INAA	Instrumental Neutron Activation Analysis
IQ	Intelligence Quotient
JECFA	Joint FAO/WHO Expert Committee on Contaminants and Food Additives
LPH	Lead and Public Health
MQV	Manufacturer's Quoted Value
NAA	Neutron Activation Analysis
NBS	National Bureau of Standards
NIH	National Institute of Health
NIST	National Institute of Standards and Technology
ODHS	Oregon Department of Human Services
ÖGE	Society for Nutrition of Austria
PNAA	Preconcentration Neutron Activation Analysis
PTFE	Polytetraflouroethylene
PTMI	Provisional Tolerable Monthly Intake
PTWI	Provisional Tolerable Weekly Intake
RDA	Recommended Dietary Allowance
RDI	Recommended Daily Intake
RNAA	Radiochemical Neutron Activation Analysis

SAM	S-Adenosyl methionine
SCF	Scientific Committee for Food
SGE	Society for Nutrition of Switzerland
SNAA	Speciation Neutron Activation Analysis
SRM	Standard Reference Material
TINAA	Thermal Instrumental Neutron Activation Analysis
UAE	Ultrasonic Assisted Digestion
UK	United Kingdom
UL	Upper Limit of intake
UMMC	University of Maryland Medical Centre
USEPA	United States Environmental Protection Agency
VGA	Vapour Generation Accessory
WHO	World Health Organisation



LIST OF SYMBOLS

$\mu\text{g/kg bw/week}$	Microgram per kilogram body weight per week
Bw	Body weight
E_n	Energy of neutron
Kev	kilo electron volts
kPa	kilo Pascal
kW	kilo Watt
mg/kg bw/week	Milligram per kilogram body weight per week



ABSTRACT

Cereal-based weaning foods, which abound on the Ghanaian market, are nutritionally vital in the growth and development of infants. Recent reports in scientific literature however indicate that rice and other cereals used in the manufacture of the weaning foods contribute significantly to the intake of arsenic (As), cadmium (Cd) and lead (Pb) by infants. In view of this, frequent monitoring of the cereal-based weaning foods has become imperative. The study assessed the beneficial health effects and risks posed by the consumption of cereal-based weaning foods commercially available on the Ghanaian market with respect to the essential elements (Ca, Cl, Cr, Cu, Fe, K, Mg, Mn, Na, Se, V and Zn) and, non-essential, potentially toxic elements (As, Br, Hg, Pb and Sn). Twenty (20) different brands of weaning foods were purchased from various retail outlets in Accra and used for the study. Instrumental neutron activation analysis (INAA) and atomic absorption spectrometry [with flame atomisation (FAAS) and hydride generation (HGAAS)] were used in analysing the samples. Determination of Pb using FAAS was done following its extraction with dithizone. The analytical methods were validated using certified reference materials NIST 1568a (Rice flour) and NIST 1547 (Peach leaves). The levels ($\mu\text{g/g}$, in ranges) obtained for the elements were: As (< 0.025), Br ($<0.17-402$), Ca (405-2002), Cl (158-5521) and Cr ($<0.03-1.23$). Others are: Cu (0.83-55.80), Fe (1.07-11.78), Hg (< 0.025), K (49-3845) Mg (20-280) and Mn (0.84-38.94). The rest include: Na (13-1588), Pb (0.025-0.093), Se (0.145-0.317), Sn (35.8-91.8), V ($<0.01-0.990$) and Zn (0.50-7.17). The daily intakes of the essential elements were also estimated, and generally found to be lower than recommended values set either by the FAO/WHO, the European Food Safety Authority (EFSA), the US Food and Nutrition Board, or the Joint Societies for Nutrition of Germany, Austria and Switzerland. The estimated

intake levels of the non-essential, potentially toxic elements As, Hg, Sn and Pb were all lower than their respective provisional tolerable weekly intake (PTWI) values established by the Joint FAO/WHO Expert Committee on Contaminants and Food Additives (JECFA). The results indicated that the analysed food samples were generally safe for consumption with respect to their content of the non-essential elements analysed. However, the contributions of the food samples to the daily essential element needs of the infant were mostly woefully inadequate, and this may not augur well for the proper growth and development of infants that consume them.



CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Infancy is a period that spans from birth to twelve (12) months of age (FAO/WHO, 1998). The term may also sometimes refer to the age period between birth and three years (Wikipedia, 2012). An individual within the age period of infancy is termed an 'infant'. Physically, biologically, immunologically and mentally, infancy is a period of rapid growth and development (Zand et al., 2011). The normal infant undergoes a three-fold increase in weight and a two-fold increase in height over the first year after birth (Heird, 2001). This indeed is the fastest growth period in the entire life of any individual (BDA, 2007). As a result, a huge demand is placed on the child's energy and nutrient requirements. Both the WHO (2010) and the American Academy of Pediatrics (AAP) [2012] strongly recommend that an infant be fed exclusively on breast milk for the first six months after birth.

After six months however, due to increased activity and growing body mass, breast milk alone cannot sustain the infant. It therefore becomes necessary to introduce other foods that will supplement the energy and other nutrients provided by breast milk. This marks the onset of weaning. Weaning is the period when foods other than human milk are provided to infants and young children who are breast-feeding (Luther and Rivera, 2007). Weaning foods are nonhuman-milk food-based sources of nutrients that are given to the baby who is still being breastfed (Luther and Rivera, 2007). Apart from complimenting the nutrient and energy needs of the infant, weaning also plays the very important role of introducing the child to different adult food tastes and textures. Weaning can therefore

affect food preferences and eating habits later in childhood (Kazi et al., 2010) and even adulthood, either positively or negatively.

Commercially, a wide range of weaning foods abound on the Ghanaian market, most of them imported, and a few ones locally manufactured. They offer the advantage of convenience, for which reason many parents and care givers are increasingly relying on these foods to feed their babies (Zand et al., 2012). A significant number of these weaning foods are prepared from cereals such as rice, wheat, corn and millet, which are usually sold dry and can readily be reconstituted, often with milk or water. Cereal-based foods are widely used as weaning foods because of the high energy content associated with them, which is appropriate for the increasing energy needs of the child. Also, various cereals have certain characteristics that make them attractive for use as first foods for infants before moving on to other foods, such as easy digestibility, low potential for allergic reactions and low fiber content among others. Additionally, they are good sources of several mineral or dietary elements that are essential for normal body function. These dietary elements take part in chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in living cells.

However, there are reports in scientific literature that rice and other cereals contribute to a high intake of arsenic (As), cadmium (Cd), lead (Pb) and other potentially toxic elements by infants (EFSA, 2009; Ljung et., 2011). It is important therefore that cereal-based weaning foods on the Ghanaian market are frequently monitored in order to ascertain the health risks associated with their consumption. It is worth noting that infants are more sensitive to the adverse effects that non-essential, potentially toxic elements may pose to humans.

Analyses of infant foods have often focused on infant formula. Little work has been done on cereal-based foods which constitute the major food for Ghanaian children undergoing weaning. It is important therefore to turn attention to cereal-based foods meant for infants in order to find out what contribution they make to the essential element requirements of the infant, as well as exposure to hazardous elements.

1.2 Health benefits and risks associated with consuming weaning foods

Mineral elements are needed in small quantities within the body, and categorized into macronutrients, micronutrients and trace elements. Trace elements can also be grouped, from a dietary perspective, into: the essential trace elements which function at low concentrations and are constituents of hormones, vitamins and enzyme systems used for metabolic activities within cells in living tissues; the possibly essential trace elements and the non-essential trace elements, made up of the toxic and non-toxic elements that have no metabolic functions in the living organism (Joseph et al., 2011).

A deficiency in an essential element can have an adverse effect, sometimes leading to a diseased condition. It is worth noting that whilst trace amounts of the micro elements are needed in the body, they become elements of toxicological concern when their amounts ingested rise above certain threshold values (Hashmi et al., 2007). This brings into sharp focus, like the proverbial double-edged sword, the danger posed to the living body at both extreme ends of the consumption spectrum, namely: a lower than normal intake results in deficiency syndromes, and a higher than normal intake brings close the threat of potential toxicity (Zand et al., 2012).

Unfortunately, food (cereals) does not only serve as a source of nutrients for humans (hence the infant), but also serve among other things, as a main route of exposure to various elements whose presence in the body yields no beneficial effect at all. Rather, they only serve as contaminants with cumulative properties, and present health risks of different kinds even at low concentrations (Zand et al., 2012). These toxic elements get absorbed by plants from the soil as a result of their natural presence, or through pollution from anthropogenic activities such as irrigation, fertilizer application, industrial and motor emissions, and even during processing throughout the supply chain (Zand et al., 2012). Consequently they end up in the food, move up the food chain and expose consumers to their harmful effects. Infants absorb many of these elements at a higher rate, with an inproportionately lower rate of excretion. Foods meant for infants should therefore not contain these non-essential elements, and every effort should be made to ensure this, or at least keep their presence to the minimum (Zand et al., 2012).

Some of these elements are in the category of heavy metals, posing a host of toxicological concerns both acute and chronic, ranging from neurological, nephrological and immunological to carcinogenic. They normally target and accumulate in soft tissues of the body such as the central nervous system, kidneys and liver (Zkowska and Bizuik, 2008). The elements As, Cd, Pb and Hg are among the most abundant and toxic elements in food (Maleki et al., 2008; Khan et al., 2009; Akan et al., 2010). According to the European Environment and Health Information Systems (ENHIS) [2009], these metals are among the most hazardous chemicals that give cause for concern with regard to children's health due to their lower safety margins. As and Cd are said to be carcinogenic. Cd is reported to cause renal, prostate and ovarian cancers upon long term

intake. Hg and Pb on the other hand are linked with the development of abnormalities in children (Maleki et al., 2008; Khan et al., 2009; Akan et al., 2010).

1.3 Problem statement

It is within the first year after birth (the period of infancy) that organ systems such as the reproductive, digestive, nervous, respiratory and immune systems develop, thereby making it a most sensitive period that makes infant food composition and consumption patterns crucial (Pendelova et al, 2012). Providing an appropriate diet for the growing infant is therefore critical in terms of ensuring healthy growth and development, since low intake or reduced bio-availability of nutrients may lead to deficiencies and cause body function impairment (Zand et al., 2011).

Infant foods should therefore adequately provide adequate essential mineral elements that can support their growth. At the same time, such foods should be free from potentially toxic elements as infants and young children are more vulnerable to potential chemical hazards (ENHIS, 2009).

Due to poverty and lack of proper regulatory control, markets in majority of African countries are often flooded with expired or substandard products that have been repackaged and sold to consumers at cheaper prices (Jonah et al., 2003). Ghana is not excepted from this canker as evidenced by frequent media reports of the presence of sub-standard products on the local market. Though all the baby foods imported into the country may have passed through official routes, there is no guarantee that all have been subjected to proper regulatory scrutiny. Also, the lack of multi-elemental tools of analysis by the country's food regulatory body could be a hindrance to a comprehensive

assessment of both the elements of nutritional significance and those elements that pose a health risk due to the large amount of samples involved (Jonah et al., 2003). Frequent and continuous monitoring of foods for such a sensitive population as infants is therefore very imperative.

1.4 Objectives of the study

Main objective

The main objective of the study is to assess the beneficial health effects and risks that infants aged 6 months to three years are exposed to due to their consumption of cereal-based weaning foods commercially available on the Ghanaian market.

Specific objectives

The specific objectives of the study are as follows:

- Determination of the concentrations of essential (Na, K, Cl, Ca, Mg, Cr, Mn, Se, Fe, Cu, V, Zn) and non-essential elements (Br, Sn, As, Hg) in the cereal-based weaning foods using instrumental neutron activation analysis (INAA) and atomic absorption spectrometry (AAS)
- Determination of the concentrations of Pb in the cereal-based weaning foods by AAS after solvent extraction using dithizone as ligand and chloroform as solvent.
- Comparison of the levels of the essential and non-essential elements with the manufacturer's quoted values as well as levels in scientific literature.
- Determination of the dietary intake of the essential and non-essential elements by infants due to the consumption of cereal-based weaning foods.

CHAPTER TWO

LITERATURE REVIEW

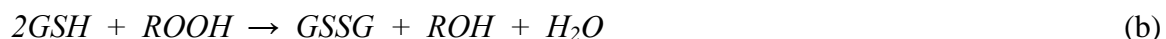
There is a steady growing interest in the assessment of both the essential and non-essential element content of foods in general, and infant foods in particular. This chapter discusses the important role played by some essential elements in human (infant) nutrition; and the potentially negative effect non-essential elements have on human health. It also gives descriptions of the theoretical principles behind the analytical methods employed in this work. A review of the most recent works on cereal-based infant weaning foods available in literature, have also been done.

2.1 Health benefits of essential elements

Essential elements are needed in the body in varying amounts to aid in the normal functioning of the body. Whilst some are needed in trace amounts, others are needed in relatively higher amounts to carry out their functions. The following sections describe the characteristics and specific roles played by some essential elements in the life of humans.

2.1.1 Selenium (Se)

Se is a trace element chemically similar to sulphur and may replace it in amino acids. It plays a role in thyroid gland activity, specifically as part of the active centre of the enzyme type I iodothyronine deiodinase, which converts the prohormone thyroxine (T₄) to the active form triiodothyronine (T₃) [Hathcock, 2004]. It is also a constituent of the antioxidant enzyme glutathione peroxidase, (GSH) for which it acts as a cofactor. This enzyme catalyses certain reactions that remove reactive oxygen species such as hydrogen peroxide (H₂O₂), organic hydroperoxides (ROOH) and free radicals in general from the body, according to the general reactions (FAO/WHO, 2002):



These actions generally protect biomolecules such as lipids, lipoproteins, DNA and membranes against oxidative stress and damage, thereby reducing significantly the risk of cancer and atherosclerosis associated with them (Rayman, 2000). Se has been known to induce DNA repair and synthesis in damaged cells, inhibit the proliferation of cancer cells, and also induces their apoptosis. Indeed, research has shown that there is an inverse relationship between the element's concentration in the body and the occurrence of cancer (GMF, 2012).

Se is also required for testosterone biosynthesis, formation and normal development of spermatozoa, processes which are essential for male fertility (Rayman, 2000). Besides, it reduces the toxicity associated with some metals by reacting with them to form their selenides [complexes that are inert] (Rayman, 2000). Perhaps more importantly, with regards to children, is the fact that Se in many studies have been suggested to be essential for the proper functioning of the immune system. Se, along with other minerals, can help build up white blood cells, which boosts the body's ability to fight illness and infection (UMMC, 2011).

2.1.2 Calcium (Ca)

Ca, as a macronutrient, is required by all body processes (Weaver, 2001). It is best known for its role in maintaining the strength and density of bones, which serve as the storehouse for more than 99 % of total body Ca (Weaver, 2001). In a process known as bone mineralization, Ca and phosphorus join to form calcium phosphate, a major

component of the mineral complex hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ which gives structure and strength to bones.

Calcium also plays a role in many physiological activities not related to bones, including blood clotting; nerve conduction; muscle contraction; regulation of enzyme activity; cell membrane function; vision; hormone release; cellular differentiation, proliferation and mobility (Weaver, 2001; GMF, 2012). Because these physiological activities are essential to life, the body utilizes complex regulatory systems to tightly control the amount of calcium in the blood so that it is available for these activities (Weaver, 2001).

2.1.3 Iron (Fe)

The main function of iron in the body is energy production through the distribution of oxygen to all parts of the body which in turn is utilized by cell mitochondria for glucose metabolism to produce energy. Its energy production role is further extended to enzyme action, as it forms part of iron catalase, iron peroxidase, and the cytochrome enzymes. It is also involved in the production of carnitine, a non-essential amino acid important for the proper utilization of fat. The function of the immune system is also dependent on sufficient iron (GMF, 2012).

In living tissues, the high redox potential exhibited by Fe is made use of by Fe-containing compounds whose activities are related to oxygen and energy metabolism (Yip, 2001). In living bodies, iron's potentially harmful reactivity and oxidative potential are carefully modulated by binding it to carrier proteins or by the presence of other molecules with antioxidant properties. If this check is not put in place, major cellular compounds such as proteins, fatty acids and nucleic acids may suffer damage due to redox reactions mediated

by iron. A classical case is the catalysis of the Fenton reaction by Iron, one of the best known means by which hydrogen peroxides and superoxides are converted to very reactive free radicals. These free radicals cause peroxidation or cross-linking of membrane lipids and intracellular compounds, thereby leading to cell aging and death.

The Fenton reaction is as follows (Yip, 2001):



2.1.4 Magnesium (Mg)

Mg (nicknamed the ‘smoothie’ mineral), is noted for its muscle relaxing ability. In conjunction with calcium, it acts to give good muscle and nerve tone. Its ability to act as a chemical gate blocker in many nerves (thereby preventing calcium from rushing in) helps prevent over excitation of the nerves, and also helps maintain a healthy blood pressure. Magnesium is also found to be part of over 300 enzymes, giving it several diverse roles in carbohydrate, protein and fat metabolism, as well as proper gene function (GMF, 2012). The cardiovascular system, digestive system, nervous system, muscles, kidneys, liver, hormone-secreting glands, and brain all rely on magnesium for their metabolic function. Hardly therefore is there any organ that is not affected by a deficiency in magnesium.

2.1.5 Zinc

Nutritionally, it is an important trace element for humans, playing diverse roles that contribute to the body’s well-being. Metabolic and blood sugar balance are controlled by zinc, through its role in the insulin response process and regulation of the thyroid

hormone. Zn is required by the proper functioning of gustin, a small protein that is needed for a normal sense of taste to prevail (GMF, 2012). The element also plays a vital role in immune response, which is particularly important in children because of their higher susceptibility to diseases. It has been shown that zinc helps in maintaining a good white blood cell count and optimal functioning (GMF, 2012).

2.2 Toxic effects of some non-essential elements

Non-essential elements have no known metabolic function in the body, but can disrupt normal cellular function when present, thereby leading to toxic effects in a number of organs. Among them are heavy metals which especially pose a health concern. These have the tendency to undergo bioaccumulation in the body, leading to the exposure of several organs and hence potential toxicity (FSAI, 2009). Of the toxic elements, arsenic (As), mercury (Hg), lead (Pb) and cadmium (Cd) have received the most attention because they occupy the topmost spot in the toxicological ladder (ATSDR, 2011). Their individual effects on human health are presented in the sub-sections that follow.

2.2.1 Arsenic (As)

Both the US Environmental Protection Agency (USEPA) and the International Agency for Research into Cancer (IARC) have classified arsenic as a Class A carcinogen. It has been associated with harmful effects on the heart and the circulatory system, cancers of the lungs, skin, colon and bladder (ODHS, 2002). Other forms of arsenic toxicity in humans have been observed in blood, bone marrow, cardiac, central nervous system, gastrointestinal, gonadal, kidney, liver, pancreatic, and skin tissues (ODHS, 2002; Wikipedia, 2013).

Ingested As undergoes a series of reactions in the body namely: reduction of inorganic pentavalent As to trivalent As in the blood by glutathione (GSH) (Thompson, 1993), and the sequential, enzyme catalysed methylation of trivalent As in which S-adenosyl methionine (SAM) serves as the methyl donor (Thomas et al., 2007; Gomez-Caminero et al., 2001).

It has been suggested that methylation is a detoxification process because the methylated products: are less toxic than the inorganic forms of As, have a lower affinity for tissue constituents (DEFRA, 2009), and also have an accelerated passage out of the body through the urine (Gedel, 2002). The contrary however seems to be emerging in recent years, with suggestions that indeed some of the methylated and intermediate products may rather be more toxic than their inorganic As precursors (Thomas, 2007; McCarty et al., 2007).

Most of the As get excreted through the urine after several days mostly as the methylated products, though some may remain for several months or even longer periods of time (Marr, 2005; Gomez-Caminero et al., 2001), thereby leading to potential toxicity. An increased burden of the element may also result when the rate of intake is higher than the rate of elimination (ODHS, 2002). For infants with still developing organs who are more sensitive to such hazards, this is particularly a matter of concern.

2.2.2 Cadmium (Cd)

Cd and all its compounds are toxic. The main source of human exposure to Cd is food (EFSA, 2009). Cd is relatively poorly absorbed into the body, but once absorbed is slowly excreted. Its main toxicity threat in the body is posed to the kidney, which serves

as its primary point of accumulation, although lung damage has also been associated with it (FSAI, 2009). Cd was classified in 1993, together with its compounds by the IARC in Group I (human carcinogens). Apart from the negative effects it has on the kidneys and lungs, it may also cause skeletal changes (fragility of bones) and reproductive deficiencies (RTSC, 2004).

2.2.3 Mercury (Hg)

Hg is a persistent heavy metal which has been used worldwide for many centuries for both commercial and medicinal purposes. Its occurrence in the environment is both natural and anthropogenic, possessing toxic properties that affect the environment and humans, developing foetuses and infants being the worst affected (Bose-O'Reilly et al., 2010). The element has three different chemical forms namely: elemental (metallic), inorganic and organic (methyl mercury and ethyl mercury). It exhibits both acute and chronic toxicity upon exposure even at low concentrations. Its toxicity effects are neurological, nephrological and immunological (Bose-O'Reilly et al., 2010).

Mercury in its methylated form is the most prevalent of organic mercury, and is the most toxic of the heavy metals. After oral consumption, it first passes into the liver, the kidneys and the brain. Accumulation takes place only temporarily (Bose-O'Reilly et al., 2010). A large portion is excreted with the faeces. Tiredness, loss of appetite and weight loss commonly are symptoms of chronic consumption of bivalent mercury salts. The end result is failure of the kidneys. Muscular weakness and paralysis are typically the other effects that occur (Bose-O'Reilly et al., 2010).

Metallic mercury is lipophilic and is stored in fatty tissues. Mercuric ions are also highly toxic, which may be explained by their high affinity to sulfurhydryl groups of amino acids, which serve as building blocks for enzymes (Bose-O'Reilly et al., 2010).

2.2.4 Lead (Pb)

Pb is a heavy metal, toxic and non-degradable. It can affect almost every organ and organ system in the body. Literature reports that as early as the second (2nd) century BC, symptoms that appeared to arise from advanced chronic Pb poisoning were documented, including: enteric distress (Pb colic), progressive motor peripheral neuropathy, myopathy, and added features of central nervous system (CNS) involvement (LPH, 2011). Early chroniclers, especially from the Greco-Roman era including Rome's Imperial period, warned against the potential for Pb poisoning associated with Pb intakes and exposures via various pathways, especially through inhalation and ingestion (LPH, 2011). Both acute and chronic effects of Pb exposure can result.

The most prominent symptoms of acute exposure are abdominal pain, constipation, nausea, vomiting and anorexia. At higher level chronic exposure, children in particular are prone to developing toxic encephalopathy. Of higher concern though is chronic exposure, for which earlier studies suggested increased mortality due to renal and cardiovascular diseases among occupationally exposed individuals (EFSA, 2010). In recent times, it has been discovered that among non-occupationally exposed individuals, even background exposure levels are causing excess mortality rates. In addition, neurotoxicity and cardiovascular effects have been discovered to be of concern (EFSA, 2010). The concern regarding chronic exposure to lead is higher in children for the reasons that: they have higher absorption rate for the element, and their developing brains

are more vulnerable. A reduced IQ has been demonstrated by low-level exposure to the metal in children (Kadir et al., 2008).

2.3 Recent studies on cereal-based infant weaning foods

Although literature is replete with data on elemental analysis of baby foods in general over the years, there is very little available on cereal-based infant weaning foods in particular. The most recent studies on cereal-based infant weaning foods that have been reported in scientific literature have been reviewed in this section.

Saracoglu et al. (2007) determined the levels of trace elements in various baby foods consumed in Turkey using the methods of flame and graphite furnace atomic absorption spectrometry (FAAS and GFAAS). The particular elements of interest included Cu, Mn, Fe, Zn, Se, Cr, Al, Ni and Co. The results showed that all the elements were present in all the 19 food samples analysed at varying concentrations. Their concentration ranges were as follows: Cu (0.52–4.38 $\mu\text{g/g}$), Mn (0.22–7.20 $\mu\text{g/g}$), Fe (1.02–67.5 $\mu\text{g/g}$), Zn (0.92–37.2 $\mu\text{g/g}$), Se (0.12–0.32 $\mu\text{g/g}$), Cr (2.02–68.8 $\mu\text{g/kg}$), Al (10.7–66.8 $\mu\text{g/kg}$), Ni (0.05–10.3 $\mu\text{g/g}$) and Co (2.67–25.4 $\mu\text{g/kg}$).

With the exception of Al, all the elements play one nutritional role or another in the body. The level of Al reported was found to be below the tolerable daily intake (TDI) value of 1 mg/kg of body weight per day (mg/kg bw/day) as established by the World Health Organisation (WHO) and the Food and Agriculture Organization (FAO). The concentrations of the nutrient elements Fe, Zn, Se and Cu were also below the reference values set by the Turkish National Food Codex regulations. The study found that the generality of the elements were below legal limits and therefore posed no health concern.

In a similar study on commercial infant foods in Pakistan, Kazi et al. (2010) focused on determining the concentrations of four elements that they deemed to be toxic. These included Al, Cd, Ni and Pb. The objective of the research was to determine whether the intake of the elements of interest was within permissible limits. A total of eight samples were used, all of which were commercial cereal-based baby foods in solid, dry, powdered form, including rice, wheat, oat and a cereal-mixture. The method used was ultrasonic assisted extraction (UAE) followed by atomic absorption spectrometric measurement.

The results of the study (Kazi et al., 2010) showed that all the elements under investigation were present in all the selected foods studied. The highest amounts of all four elements (Al, Cd, Ni and Pb) also occurred in the rice-based products. The estimated intake levels were mostly below tolerable limits. Those of Cd however, in some rice products were higher than the provisional tolerable weekly intake (PTWI) of 1 $\mu\text{g}/\text{kg}$ bw/day set by the FAO/WHO Expert Committee on Food Additives (JECFA).

Being generally toxic elements, the presence of Cd, Al, Ni and Pb in the food, even if below recommended limits, is a matter of concern, especially for such a sensitive population as infants. The fact that they have cumulative effects even makes it imperative for their complete absence in the food to be a primary goal of manufacturers.

Ljung et al. (2011) published, in the Journal of Food Chemistry, their findings on a study carried out on infant formula and foods available on the Swedish market. They assessed the concentrations in, and intake of essential and toxic elements from nine infant formula and nine infant foods (meant for consumption from the age of 4 months). The essential

elements assessed were Ca, Mg, Se, Cu, Mo, Fe, Zn and Mn, and the toxic ones were As, Pb, Cd, Sb and U.

In the cereal-based infant foods, the high concentrations of As in the rice-based foods (30 $\mu\text{g}/\text{kg}$) was of particular concern. The infant foods also frequently had elevated concentrations of Cd (11 $\mu\text{g}/\text{kg}$), Sb (3 $\mu\text{g}/\text{kg}$), Pb (13 $\mu\text{g}/\text{kg}$) and U (3 $\mu\text{g}/\text{kg}$). Notably, two of the rice-based foods had high concentrations of all the toxic elements as well as all the essential elements determined except Se. Of the essential elements, Mn (6 mg/kg), Fe (26 mg/kg) and Mo (146 $\mu\text{g}/\text{kg}$) exhibited higher concentrations in some foods, much higher than what would normally be derived from breast milk.

The researchers also compared intakes of the elements from breast milk and the foods studied to find out if the foods contributed more or less to intake. Compared to breast milk, the largest increases in intake with the introduction of food were found for Mn, Mo, As and Cd. Once two meals of complementary food were introduced at 4 months of age, daily Mn intake increased from 14–300 to 20–1500 times that of the exclusively breast fed infant. The intake of Mo also increased significantly with the introduction of complementary foods, from 30–170 to 40–330 times the intake of the breast fed infant. For As and Cd, daily intakes increased from 1–5 and 2–40 times, respectively, to 1–50 and 3–150 times with the introduction of food. Both the amount and variation of daily Pb intake increased with the addition of cereal based products to the infant's diet, while the intake of U decreased with regard to the median intake from the investigated foods. Intake of U showed a large variation both with and without food. The large increase in daily intakes of Mn, Mo, As, Cd and U with the introduction of foods at a few

months of age is likely a result of these elements naturally being mainly derived from cereals rather than dairy products.

There is scarce data in scientific literature on Ghana. This review could not therefore include any work from Ghana.

2.4 Methods used to analyse elements in food

Several methods have been used in literature for the determination of elemental concentrations in food. These include inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES), differential pulse cathode stripping voltammetry (DP-CSV), atomic absorption spectrometry [flame (FAAS), hydride generation (HGAAS), graphite furnace (GFAAS) and cold vapour (CVAAS)] and instrumental neutron activation analysis (INAA). The choice of a particular method for analysing particular elements in food often depend on factors such as detection capability, desired speed, accuracy, sensitivity of assay, simplicity of use and cost of analyses (Zkowska and Bizuik, 2008). In this study, HGAAS, FAAS and INAA were used for the analyses of the samples mainly because of their good detection capacities for several elements, good sensitivities, accuracy, speed and accessibility of the instruments.

2.4.1 Neutron Activation Analyses (NAA)

The possibility of using NAA as an analytical tool was first recognized and proposed by Georg Havesy and his student, Hilde Von Levi. This was in the 1930s, when they discovered that rare earth elements reacted differently to irradiation with neutrons. Realizing that dysprosium (Dy) in particular became highly radioactive after neutron

bombardment, they were able to determine sub-mg amounts of it in yttrium. This they achieved in 1936 by bombarding the sample of yttrium with neutrons obtained from a radium-beryllium source. The result was the formation of radioactive ^{165}Dy ($t_{1/2}=2.334\text{h}$) whose activity was measured. The measured activity was then used to measure the concentration of Dy (Ehmann and Vance, 1991). Understandably; NAA was not used much in the first couple of decades after its discovery chiefly because of the scarcity of neutron sources. This situation however changed dramatically from the 1950s onwards following the development of nuclear technology within the same period. It has since come of age, providing the analytical chemist with several advantages that other analytical techniques do not have (Ehmann and Vance, 1991; Soot et al., 2004).

Perhaps it should be mentioned at this point that NAA as an analytical tool forms part of a general analytical principle that relies on bombarding elements with particles (neutrons, photons or charged particles) to produce radioisotopes or excited states of the target nuclides. The resulting radioactive decay then enables both quantitative and qualitative determination of the element as the energies of the radiations produced are specific to each element. This general principle of radiologically activating an element for analytical purposes is termed nuclear activation analysis (Ehmann and Vance, 1991). Nuclear activation analysis was discovered in 1934 by I. J. Currie and F. Joliot by bombarding Al, Mg and B with alpha particles. Fermi was the first to report of the formation of a radioactive product induced by neutron bombardment, but its use as an analytical tool was recognized and developed by Havesy and Levi (Soot et al., 2004). In the specific case of NAA, the particles used for the activation process are neutrons, hence the name neutron activation analysis.

Application of NAA generally involves the exposure of a sample to a flux of neutrons, usually in a nuclear reactor. Stable nuclei in the sample undergo neutron induced nuclear reactions. The most common neutron reaction is neutron capture by a stable nucleus (A_Z) that produces a radioactive nucleus (${}^{A+1}_Z$). The “neutron rich” radioactive nucleus then decays, with a unique half-life, by the emission of a beta particle. In the vast majority of cases, gamma-rays are also emitted in the beta decay process and a high-resolution gamma-ray spectrometer is used to detect these “delayed” gamma rays from the artificially induced radioactivity in the sample for both qualitative and quantitative analysis. The diagram below illustrates the activation and decay processes that occur.

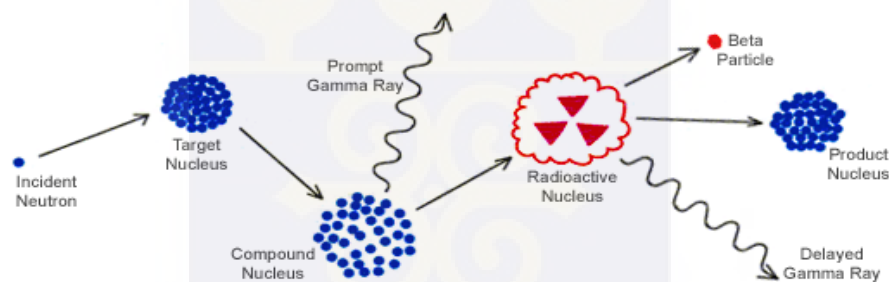


Fig 2.1: Schematic diagram showing the activation and subsequent decay of an isotope subjected to neutron bombardment.

Whilst the specific energy of a gamma ray is used to qualitatively identify an element present in the sample, the number of gamma ray occurrences (the count rate) at the specified energy is used to quantify the element. If a sample is irradiated in the whole reactor spectrum and placed on a γ -detector for measurement of the γ -activity of a specific element for a discrete period, the measured γ -activity is directly related to the mass fraction, C_m , of that element in the sample, by the equation (Kučera et al., 2000):

$$C_m = \frac{M_a \left(\frac{N_p/t_c}{S \cdot D \cdot C \cdot W} \right)_a}{N_A \cdot \theta_a \cdot \gamma_a \{ (G_{th,a} \cdot \Phi_0 \cdot \sigma_{0,a} + G_{e,a} \cdot \Phi_e \cdot I_{0,a} \cdot \alpha) \} \epsilon_{p,a} \cdot \eta_a} \quad (1)$$

Where:

M is the molar mass of target isotope;

N_A is the Avogadro's number;

θ is the relative isotopic abundance of target isotope;

γ is the absolute gamma intensity (emission probability) of the radioisotope;

N_p is the net number of counts in the full-energy peak corrected for pulse losses;

t_c is the counting time;

W is the sample mass;

G_{th} is the correction factor for thermal neutron self-shielding;

G_e is the correction factor for epithermal neutron self-shielding;

Φ_0 is the conventional thermal neutron fluence rate;

σ_0 is the activation cross section for thermal neutrons (cm^2);

Φ_e is the conventional epithermal neutron fluence rate;

I_0 is the resonance integral including the $1/v$ tail;

α is the measure of the deviation of the epithermal neutron fluence rate distribution from the $1/E^{1+\alpha}$ function;

ϵ_p is the full energy peak detection efficiency, including correction for gamma attenuation;

η_a is the chemical yield of separation (if preconcentration or radiochemical separation is carried out);

S , D , and C are saturation, decay and counting factors respectively and are given by:

$$S = 1 - e^{-\lambda t_i} \quad (2)$$

$$D = e^{-\lambda t_d} \quad (3)$$

$$C = (1 - e^{-\lambda t_i})/\lambda t_c \quad (4)$$

Where λ is the decay constant ($\lambda = \ln 2/T_{1/2}$, $T_{1/2}$ is the half-life of radioisotope); and t_i , t_d , and t_c are the irradiation, decay and counting times respectively (Kučera et al., 2000).

2.4.1.1 Types of NAA

NAA in a broad sense can be put into two main categories based on the following criteria: whether chemical separation does accompany irradiation of the sample, or sample irradiation takes place without chemical separation. When sample irradiation takes place without any chemical treatment, the technique is termed Instrumental Neutron Activation Analysis (INAA), which is the most widely used form of NAA (Ehmann and Vance, 1991). INAA may have different forms. On the bases of the energy of bombarding neutron, it has three forms: Thermal INAA (TINAA), Epithermal INAA (EINAA) and Fast INAA (FINAA). In TINAA, thermal neutrons (neutrons with most probable energy = 0.025 eV) are the bombarding particles. Similarly, epithermal neutrons (neutrons with energy = 1 eV < E_n < 1 keV) and fast neutrons (those with energy 0.5 < E_n < 2+ MeV) are used as the bombarding particles in EINAA and FINAA respectively. In situations where an element has an activated product nuclide with a very short half-life (less than 1min), cyclic INAA (CINAA) is used for its determination. This helps improve the signal to background (S/B) ratio and discriminates against short, medium and long-lived radionuclides. CINAA involves irradiation of the sample for a short period of time, followed by a quick transfer of the irradiated sample to the detector for a short counting

time (all under 1 minute). Irradiation and counting is repeated for the same sample for an optimum number of times (3 to 4 cycles) to obtain an accumulated build-up of the gamma-ray peak (Soot et al., 2004).

Sometimes, the detection limit of an element is found to be lower than that provided by INAA. It then becomes necessary that chemical separation be carried out either before or after irradiation of sample to concentrate the element of interest and/or remove interferences. If chemical separation takes place before sample irradiation, the technique is termed preconcentration or chemical NAA [PNAA or CNAA] (Soot et al., 2004). If on the other hand the chemical separation step is undertaken only after irradiation of the sample, then radiochemical NAA (RNAA) is the name. The speciation of an element is sometimes carried out before irradiation. This is termed speciation NAA (SNAA). There is also derivative NAA (DNAA). In this case, an element that has to be determined but has no characteristic gamma line is complexed or chemically exchanged with another element that is amenable to NAA (Soot et al., 2004). The NAA method that has been used in this work is INAA.

2.4.1.2 Quantifying elements in NAA

Three methods exist by which elements can be quantified in NAA. These include the absolute method, the k_0 standardisation method and the comparator method (otherwise known as the relative standardisation method). The absolute method almost exists only in theory as it is hardly used practically. This is due to large uncertainties involved in the parameters that control it. The k_0 and comparator methods are in more common use (Ehmann and Vance, 1991). The latter of these was used in this work and is described below.

2.4.1.2.1 The comparator method of quantifying elements in NAA

In this method, a standard containing a known amount of the element sought is irradiated with the samples under the same preparation and reactor conditions. The standard and sample are both subjected to the same duration of γ -activity measurement on the same γ -detector using similar geometries. Under such conditions, the mass fraction of an element whose γ -activity has been measured in both sample and standard can be calculated based on a simple comparison of the measured activities and other known parameters, given by equation 5 (Kučera et al., 2000).

$$C_{ana_sam} = \frac{A_{sam} \cdot M_{std} \cdot C_{ana_std}}{A_{std} \cdot M_{sam}} \quad (5)$$

Where;

C_{ana_sam} and C_{ana_std} are the mass fractions of the element in sample and standard respectively;

A_{sam} and A_{std} are the γ -activities of the element in irradiated sample and standard respectively; whilst M_{sam} and M_{std} are the masses of the element in sample and standard respectively.

In a situation where chemical separation has been performed for an element before or after irradiation during analysis, the above equation is multiplied by the term $1/\eta$, where η is the chemical yield of the element in the sample after chemical separation.

2.4.1.3 Advantages of NAA

NAA in general offers many advantages, including excellent sensitivity, good selectivity, inherent accuracy, high precision and low matrix effect in the analysis of many elements. (IAEA,1990). NAA in its instrumental form has the added advantage of being non-

destructive, with minimal sample handling thereby reducing greatly the possibility of contamination. Solid samples are analysed directly without having to take the extra trouble of digesting them to obtain a liquid form for analysis as required by AAS, ICP-MS, AES and other techniques. Its simultaneous multi-element detection capability also makes it very attractive.

With each element having a unique gamma-energy by which it can be identified and quantified, a single irradiation of a sample can lead to simultaneous determination of several elements. To a great extent, time and cost are reduced as a result. Most elements have more than one isotope, and an isotope can have more than one characteristic gamma energy. This situation enables verification of analytical data, thereby giving NAA a self-validation capability. In the event that there are interferences due to overlapping photo-peaks, the desired selectivity can usually be achieved with the right selection of timing parameters. Consequently, the appropriate nuclides can be individually quantified without the need to physically isolate the elements under investigation.

2.4.2 Atomic Absorption Spectrometry (AAS)

AAS is a spectroanalytical technique used for the quantitative determination of chemical elements (wikipedia, 2012). It involves measuring the absorption of light by vaporized atoms of an element in the ground state and relating the amount of light absorbed to concentration of the element. This is based on the principle that when light of just the right wavelength impinges on a free, ground state atom, the atom may absorb part of the light and go into an excited state as illustrated below.

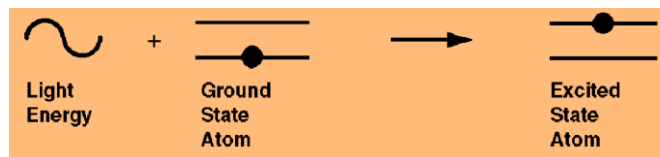


Fig 2.2: Schematic diagram of the atomic absorption process.

In AAS, an atomic ground state vapour of analyte element is generated directly in the path of a light beam of wavelength specific for that element. The atoms absorb the light as it passes through the atomic cloud. The amount of light absorbed increases with the number of atoms present in the light path. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte species present can be made (Beaty and Kerber, 1993).

The use of special light sources and careful selection of wavelength allow the specific quantitative determination of individual elements in the presence of others. The atom cloud required for atomic absorption measurements is produced by supplying enough thermal energy to the sample to dissociate the chemical compounds into free atoms. Aspirating a solution of the sample into a flame or furnace aligned in the light beam serves this purpose. Under the proper flame or furnace conditions, most of the atoms will remain in the ground state and are capable of absorbing light at the analytical wavelength from the light source. The commonest light sources used in AAS are the 'hollow cathode lamp' [HCL] and the 'electrodeless discharge lamp' [EDL] (Beaty and Kerber, 1993).

When light of initial wavelength, I_0 , passes through the atom cloud, a fraction of it would be absorbed. The light will therefore have a reduced intensity, I . A quantity called absorbance, which is directly related to the amount of light absorbed by the analyte

atoms, could then be measured by the instrument detector, from which the analyte concentration could be determined using the Beer-Lambert's equation given by:

$$A = \log I_0/I = \epsilon c l \quad (6)$$

A , ϵ , C and l are the 'absorbance', 'absorption coefficient' (a constant characteristic of the absorbing species at a specific wavelength), 'concentration of analyte species' and 'pathlength of light intercepted by analyte species in the absorption cell' respectively.

When the absorbances of standard solutions containing known concentrations of analyte are measured and the absorbance data are plotted against concentration, a calibration graph is obtained. After such a calibration is established, the absorbance of solutions of unknown concentrations may be measured and their concentrations determined from the calibration curve. In modern instrumentation, the calibration can be made within the instrument to provide a direct readout of unknown concentrations. The ease and speed at which precise and accurate determinations can be made with this technique have made atomic absorption one of the most popular methods for the determination of metals (Beaty and Kerber, 1993).

2.4.2.1 Techniques of AAS

There are various forms of AAS. The differences lie mainly in the kind of thermal energy provided for atomization of the element and/or the form in which the element is put before atomisation. These varieties have different characteristics that make them techniques of choice for various categories of elements. These include flame atomic absorption spectrometry (FAAS), hydride generation atomic absorption spectrometry, (HGAAS), cold vapour atomic absorption spectrometry (CVAAS) and graphite furnace

atomic absorption spectrometry (GFAAS). The first two of these were used in analysing the samples in this work (Beaty and Kerber, 1993).

2.4.2.1.1 Flame Atomic Absorption Spectrometry

As the name suggests, this type of AAS uses a flame as the source of thermal energy for atomizing the element under investigation. It is the most commonly used type of AAS, and the oldest, suitable for elements of higher concentrations within the sample (mg/L). It involves aspirating the sample in the form of a liquid into a nebulizer which transfers it to the flame in the form of an aerosol. The liquid evaporates, leaving behind the analyte species which are vaporised and finally atomised (Beaty and Kerber, 1993).

The flame is obtained from a fuel-oxidant combination. Flames of different temperatures are obtained with different fuel-oxidant combinations. The commonest flames used are those of acetylene-air and acetylene-nitrous oxide combinations. The element being analysed determines what kind of flame to use. The air-acetylene flame (2400-2700 °C) for instance is used for metals that are readily atomizable, and the nitrous oxide-acetylene flame (2900-3100 °C) is used for elements that do not atomise easily. Although this technique is rapid and precise, it is less sensitive compared to the other forms of AAS which can analyse elements at the µg/L levels.

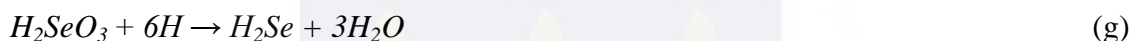
2.4.2.1.2 Hydride Generation Atomic Absorption Spectrometry

In this technique, a gaseous hydride form of the analyte is generated, which is then atomised for absorbance measurement. To generate the hydride, samples are reacted in an external system (the hydride generator) with a reducing agent, usually sodium borohydride in an acidic medium. The gaseous products formed are then swept into the

atomisation chamber in the light path of the AA spectrometer using an inert gas (mostly argon). The following equations illustrate how the reaction with borohydride leads to the formation of the gaseous hydrides, using As and Se as examples.



The H atoms thus formed then reduce the analyte compounds into hydrides:



Elements that can be analysed using HGAAS include As, Bi, Ge, Pb, Sb, Se, Sn and Te. For these elements, detection limits well below the $\mu\text{g/L}$ range are achievable. The hydride generation of an element isolates it from other elements thereby removing matrix-related interferences. Its main disadvantage is that it is limited to analysing the aforementioned elements (Beaty and Kerber, 1993).

2.5 Chemical separation

Analytical chemists are often faced with analysing elements in either complex matrices or at very low concentrations. Interferences from other elements often make determination of the element of interest very difficult. In such situations it becomes necessary to mask the interfering species, separate or preconcentrate the analyte before final measurement using a suitable method. This usually improves the detection limit by no mean degree, thereby enhancing the quality of the measurement. Different techniques have been developed over the years for separation and preconcentration of elements, among which are solid phase extraction, solvent extraction (liquid-liquid extraction), coprecipitation

and cloud point extraction (Behpour et al., 2010). In this work, solvent extraction was employed for the extraction of Pb using 1, 5-diphenylthiocarbazone (dithizone), with chloroform as extractant.

2.5.1 Solvent extraction with dithizone

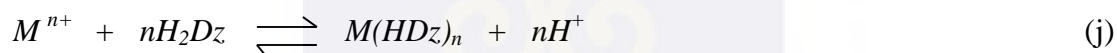
Extraction of metal ions with dithizone is a classic example of solvent extraction, which involves the transfer of a solute from one liquid phase to another, the liquids being immiscible. The commonest of this is the extraction of an aqueous phase with an organic solvent. What makes this technique attractive is its simple, rapid, clean and convenient nature, often just requiring the shaking of the mixture in a separatory funnel for a few minutes (Vogel et al., 1989). Although solvent extraction has mostly been used for the separation and preconcentration of single analytes, it can also be applied to the preconcentration of groups of metals or other chemical species (Vogel et al., 1989). Often after liquid-liquid extraction, a direct quantitative measurement of the element could be made in the solvent extract using spectrophotometry, flame photometry or AAS, the latter of which is especially advantageous as the organic solvent increases the sensitivity of the method (Vogel et al., 1989). Where a different technique need to be used that is not compatible with the organic phase, then the analyte could be stripped into a more suitable medium from the organic phase. Of many chemicals that are used as ligands for solvent extraction, dithizone has gained wide acceptance and use over many years.

Dithizone is a violet-black compound that is soluble in dilute ammonia and nonpolar organic solvents (notably chloroform and carbon tetrachloride) to produce a green solution. It is however insoluble in water below pH 7 (Vogel et al., 1989). It is widely

used in solvent extraction and for removing traces of metals from aqueous buffers, perhaps because of its ability to form hydrophobic complexes with most metals with 2 or 3 oxidation states, usually red in colour (Harris, 1991). Its ability to determine small amounts of many metals certainly has endeared it to the hearts of analytical chemists, giving it widespread use. In solution, it exists in two tautomeric forms, given by the following structures (A) and (B):



The compound acts mainly as a monoprotic acid with a pKa of 4.7 up to a pH of 12, the acid proton being that of the thiol group in (A). Dithizone (H_2Dz) reacts with any metal M with oxidation number 'n' according to the general equation (Vogel et al., 1989):



The products of this reaction are called primary metal dithizonates. With a deficiency of the reagent or at higher pH ranges, a few metals (Cu, Ag, Au, Hg, Pa and Bi) tend to form a second complex termed secondary dithizonates, given by the equation:



These secondary dithizonates however are unstable and less soluble in organic reagents, and hence are of little analytical value compared to the primary dithizonates (Vogel et al., 1989). A careful control of pH or use of masking agents is the general mode of achieving selectivity for extraction of a particular element or groups of elements.

CHAPTER THREE

METHODOLOGY

This chapter gives a detailed description of how the samples were collected and analysed. It consists of four main sections (3.1-3.4). Section 3.1 describes the collection of samples, whilst sections 3.2 and 3.3 respectively describe sample preparation and sample analyses. Section 3.3 is further divided into three sub-sections (3.3.1-3.3.3) which describe the different analytical methods used in analysing the samples for the various elements of interest. Section 3.4 describes how the daily or weekly intake amount of each element was determined.

3.1 Sample collection

Ten different brands of commercial cereal-based weaning foods meant for infants were purchased from various retail outlets within Accra, the capital city of Ghana. This was done between the months of September and October, 2012. The retail outlets from which the foods were bought were chosen because they enjoyed good patronage owing to their strategic locations and the relatively cheaper prices of their goods. The ten brands that were purchased were chosen because they were readily available, popular, relatively less costly, and hence widely used by the consuming public.

For each brand, foods made with different cereals (maize, millet, rice, wheat, sorghum) were purchased. Altogether, 20 different samples were bought. Fig 3.1 is a collection of some of the samples. A detailed description of the samples and their composition is presented in Appendix A.



Abido powdered rice

Fig. 3.1: A collection of some of the food samples.

3.2 Sample preparation

Though all the samples purchased were generally in dry powdered form, the grain sizes were not fine enough. To ensure better homogeneity, samples were further milled in a home-styled blender specially designed for milling dry substances. The blades and bowl of the blender were made of Titanium and Teflon respectively. The finely powdered samples were then placed in pre-labelled, dry 100 mL polyethylene plastic containers which had been previously washed with acid, and capped. The capped plastic containers were wrapped with aluminium foil and placed within hermitically sealed polyethylene bags. These steps were taken to prevent moisture from entering the samples. All samples thus sealed were stored in a refrigerator. Appropriate, replicate aliquots of these samples were taken for all analyses.

3.3 Analysis of samples

Two different analytical methods were employed for the analysis of the cereal-based weaning foods. The analytical techniques applied were instrumental neutron activation analysis (INAA) and atomic absorption spectrometry (AAS).

3.3.1 Instrumental Neutron Activation Analysis (INAA)

The concentrations of K, Na, Cl, Mg, Ca, Br, Mn, Cu and V were determined using INAA. The method involved irradiation of aliquots of the samples together with a known standard in a research reactor. The irradiation converted at least one stable isotope of each element of interest into a radioactive nuclide whose decay led to the emission of gamma rays. Subsequent measurement of the intensity of the resulting gamma rays of the radionuclides in both sample and standard was done using a high purity germanium (HPGe) gamma-ray detector. Based on the relative standardisation

method of quantifying elements by NAA, the concentration of each element was determined.

Instrumentation

- Sample weight was obtained using an electronic weighing balance (Mettler Toledo PM 100).
- Irradiation of samples was done using Ghana Atomic Energy Commission's 30 kW miniature neutron source research reactor [Ghana Research Reactor-1 (GHARR-1)]. It uses highly enriched Uranium (90.2%-Al alloy) as the fuel source, and beryllium metals as reflectors. All irradiations were done at a neutron flux of 5×10^{11} neutrons $\text{cm}^{-2} \text{s}^{-1}$.
- Measurement of the gamma-radiation intensity of the radioisotopes produced after irradiation of samples was done using a computer based gamma-ray spectroscopy system which had the following components:
 - a high purity Germanium (HPGe) N-type coaxial detector (model GR2518) mounted on liquid nitrogen as a coolant,
 - a high voltage power supply system (model 3103),
 - a gamma Spectroscopy amplifier (model 2020),
 - an ACCUSPEC multi-channel analyser emulation software card (all manufactured by Canberra industries Inc.), and
 - a micro-computer which was coupled to the system for data acquisition, evaluation and analysis.

The detector operates on a bias voltage of -3000 V, with a resolution of 1.8 keV at the 1332.5 keV photo-peak of ^{60}Co . Its relative efficiency is 25 % with respect to the standard NaI (Tl) detector.

- ORTEC MAESTRO-32, a gamma-ray spectroscopy software, was used for evaluation of the gamma-ray spectra obtained.

Standard Reference Materials (SRMs)

Two SRMs obtained from the National Institute of Standards (NIST), namely NIST SRM 1573a (Tomato leaves) and NIST SRM 1547 (Peach leaves), were analysed together with the samples under the same experimental conditions. The former SRM was used as a comparator standard to aid in the quantification of elemental concentrations in the samples using the relative standardisation method of NAA. The latter SRM was also used to check the validity of the INAA method used for the sample analysis.

3.3.1.1 Preparation of samples for irradiation

Triplicate aliquots (200 mg) of each sample were weighed onto clean polyethylene foils using a digital analytical balance. Each weighed sample was wrapped, labelled and heat-sealed with an electric soldering iron. SRMs used were also weighed and wrapped in a similar manner. For short-lived radionuclides, one wrapped sample each was placed in a 9.8 cm³ plastic capsule. The remaining volume of each capsule containing a wrapped sample was filled up firmly with cotton wool, capped and heat-sealed. For medium-lived radionuclides, six 'polyethylene-film-wrapped' samples were packed into one plastic capsule and heat-sealed. Irradiation of the packaged samples and SRMs then followed.

3.3.1.2 Irradiation of the samples

Samples and SRMs were irradiated for both short-lived ($25\text{s} \leq t_{1/2} \leq 5\text{ hr}$) and medium-lived radionuclides ($5\text{ hr} \leq t_{1/2} \leq 7\text{ days}$). The nuclear characteristics of the radionuclides are presented in Table 3.1.

3.3.1.2.1 Irradiation of short-lived radionuclides

The packaged samples and SRMs were transferred into the reactor one at a time, using the pneumatic sample transfer system. The transferred samples and SRMs were then irradiated with thermal neutrons for 2 minutes. After irradiation, the now activated samples and SRMs were removed from the reactor. Using two pairs of forceps, each wrapped sample or SRM was removed from its capsule. This was followed immediately by γ -radiation intensity measurement. The time (decay time) between removal of sample from reactor and measurement of the γ -radiation intensity was maintained at less than 1 minute.

3.3.1.2.2 Irradiation of medium-lived radionuclides

For the irradiation of medium-lived radionuclides, packaged samples and SRMs were transferred into the reactor using the same process as used for short-lived radionuclides. Each transferred sample was then irradiated with thermal neutrons for 2 hours. The irradiated samples were then removed from the reactor and allowed to decay for 24 hours in a lead-shield within a fume hood. Decayed samples and SRMs were then removed from their capsules and measurement of the γ -intensity of each followed.

Table 3.1 Nuclear data for short-lived and medium-lived elements determined by INAA

-	Target	-	Product	-	Gamma ray
Element	isotope	Reaction	nuclide	Half-life	energy/keV
Ca	^{48}Ca	$^{48}\text{Ca}(n, \gamma) ^{49}\text{Ca}$	^{49}Ca	8.72 min	3084.5
Cl	^{37}Cl	$^{37}\text{Cl}(n, \gamma) ^{38}\text{Cl}$	^{38}Cl	37.24 min	1642.7, 2167.7
Cu	^{65}Cu	$^{65}\text{Cu}(n, \gamma) ^{66}\text{Cu}$	^{66}Cu	5.10 min	1039.2
Mg	^{27}Mg	$^{27}\text{Mg}(n, \gamma) ^{28}\text{Mg}$	^{28}Mg	9.46 min	843.8, 1014.4
Mn	^{55}Mn	$^{55}\text{Mn}(n, \gamma) ^{56}\text{Mn}$	^{56}Mn	2.58 hr	846.8, 1810.7
Br	^{81}Br	$^{81}\text{Br}(n, \gamma) ^{82}\text{Br}$	^{82}Br	35.3 hr	554.4; 776.5
K	^{41}K	$^{41}\text{K}(n, \gamma) ^{42}\text{K}$	^{42}K	12.36 hr	1524.6
Na	^{23}Na	$^{23}\text{Na}(n, \gamma) ^{24}\text{Na}$	^{24}Na	14.96 hr	1368.6; 2754.0
V	^{51}V	$^{51}\text{V}(n, \gamma) ^{52}\text{V}$	^{52}V	3.75 min	1434.1

3.3.1.3 Measurement of γ -radiation intensity

The procedure for γ -radiation intensity measurement was the same for both short and medium-lived radionuclides. Samples were placed on the high-purity germanium γ -ray semi-conductor detector and the induced γ -activity measured for 10 minutes. The ORTEC MAESTRO-32 γ -spectrum acquisition software was used to acquire the γ -spectrum obtained.

All gamma ray intensity measurements were done at a distance of 7.2 cm from the top of the detector surface. This was to ensure easy and reproducible sample positioning on the detector (De Corte, 1987). This geometry was maintained for all samples and standards. A detector dead time of less than 10 % was maintained throughout the measurement process.

3.3.1.4 Evaluation of γ -radiation spectra

The ORTEC- MAESTRO-32 γ -ray acquisition and evaluation software was used for acquisition of the γ -ray spectra and final evaluation of the net peak areas under the acquired spectra. The radioisotopes were qualitatively identified by their γ -ray energies.

3.3.1.5 Calculation of elemental concentrations in samples

After the net γ -ray peak areas of the radioisotopes were obtained for sample and comparator standard, the decay and measurement factors were also determined. By comparing the net peak areas, γ -activities and masses of sample and standard, in combination with other known parameters, the concentration of analyte in the sample was determined based on the relative standardisation method of NAA using equation

7.

$$C_{ana_sam} = \frac{A_{sam} \cdot M_{std} \cdot C_{ana_std}}{A_{std} \cdot M_{sam}} \quad (7)$$

Where:

$$A_{sam} = \left(\frac{N_p}{t_m \cdot D \cdot C_m} \right)_{sam} \quad (8)$$

$$A_{std} = \left(\frac{N_p}{t_m \cdot D \cdot C_m} \right)_{std} \quad (9)$$

$$D = e^{-\lambda t_d} \quad (10)$$

$$C_m = \frac{1 - e^{-\lambda t_m}}{\lambda t_m} \quad (11)$$

The symbols and their meanings are as follows:

- A_{sam} and A_{std} are the activities of the sample and standard respectively;
- N_p is the net γ -peak area of radioisotope produced at a specific γ -energy line;

- D is the correction factor for decay between the start of measurement of γ -activity of the sample and that of standard (the decay factor);
- C_m is the correction factor for decay during the γ -activity measurement (measurement factor);
- t_m is the time of measurement of γ -ray intensity (seconds);
- λ is the decay constant, given by $\lambda = \frac{\ln 2}{t_{1/2}}$; $t_{1/2}$ being the half-life;
- t_d is the decay time between measurement of sample and standard (seconds);
- M_{std} is the mass of the standard used for the analysis (g);
- M_{sam} is the mass of the sample used for the analysis (g);
- C_{ana_std} is the concentration of analyte in the standard ($\mu\text{g/g}$); and
- C_{ana_sam} is the concentration of the analyte being determined ($\mu\text{g/g}$).

3.3.2 Determination of As, Cr, Cd, Fe, Hg, Se, Sn and Zn using AAS

Atomic absorption spectrometry was used for determination of the concentrations of As, Cr, Cd, Fe, Hg, Se, Sn and Zn. The concentrations of Cr, Cd, Sn, Fe and Zn were determined using flame atomic absorption spectrometry (F AAS). The concentrations of Se, Hg and As were determined using hydride generation atomic absorption spectrometry (HG AAS). Prior to AAS measurement, samples were digested.

3.3.2.1 Digestion of samples

Instruments used in the digestion process and the detailed procedure for digestion is described.

Instrumentation

- Digestion of samples was carried out in 100 mL polytetrafluoroethylene (PTFE) Teflon microwave digestion bombs (Parr Instrument Company) using

a conventional microwave digester (ETHOS 900 Labstation, INST: MLS-1200 MEGA).

Sample digestion procedure

1.0 g of each sample was weighed into labelled, 100 mL polytetraflouroethylene (PTFE) Teflon microwave digestion bombs. To each weighed sample was added 12.0 mL of concentrated nitric acid (HNO₃, 35 %), followed by 2.0 mL hydrogen peroxide (H₂O₂, 30 %).

The Teflon bombs, containing the sample-acid-peroxide mixture were then capped and loaded onto a microwave parr carousel. The whole assembly was then placed in a milestone ETHOS 900 Labstation microwave digester and subjected to microwave irradiation for 25 minutes in five steps, at a constant pressure of 100 kPa and temperature range of 400-500 °C. The complete microwave programme used for the digestion is given in Table 3.2.

Table 3.2: Microwave programme used for sample digestion

Digestion		Microwave			
Step	Time (min)	Power (W)	Pressure (kPa)	Temp 1 (°C)	Temp 2(°C)
1	00:02:00	250	100	400	500
2	00:02:00	0	100	400	500
3	00:06:00	250	100	400	500
4	00:05:00	400	100	400	500
5	00:05:00	600	100	400	500

Temp 1 and 2 are the initial and final temperatures respectively

After digestion, the whole Teflon bomb-courosel assembly was cooled for 30 minutes in a water bath containing cold water. This was to reduce internal pressure and allow volatilized materials to re-stabilise. The bombs were then dismantled from the courosel and each digested sample transferred into a 25 mL volumetric flask. The

Teflon bomb was then thoroughly rinsed twice with 3 mL portions of double distilled water and the contents added to that of the volumetric flask and made to the mark with more distilled water. Standard reference materials used for the method validation were digested in the same way together with the samples. Blanks were also prepared, and digested under the same conditions as the samples.

3.3.2.2 Determination of Cr, Fe, Sn and Zn Using FAAS

The concentrations of Cr, Fe, Sn and Zn in the samples were determined using flame atomic absorption spectrometry.

Instrumentation

A Fast Sequential Atomic Absorption Spectrometer (VARIAN, AA 240 FS, Australia) equipped with a deuterium background corrector was used for all atomic absorption measurements.

Chemicals

The following chemicals, all of analytical grade, were used:

Concentrated HNO₃ (65%) [British Drug House (BDH) laboratory supplies, UK], concentrated HCl (36 %) [Sigma-Aldrich, UK] and H₂O₂ (30 %) [Fisher Scientific, UK].

Standards

The following standard stock solutions of the elements Sn, Cr, Fe and Zn were also used: 1000 ± 4 mg/L Cr in 2 % (w/w) HNO₃, 1000 ± 4 mg/L Sn in 2 % (w/w) HNO₃, 1000 ± 4 mg/L Fe in 2 % (w/w) HNO₃ and 1000 ± 4 mg/L Zn in 2 % (w/w) HNO₃ (Trace CERT[®], Fluka, Chemie, Switzerland).

Calibration standards

Standard solutions used to calibrate the F AAS system are as follows:

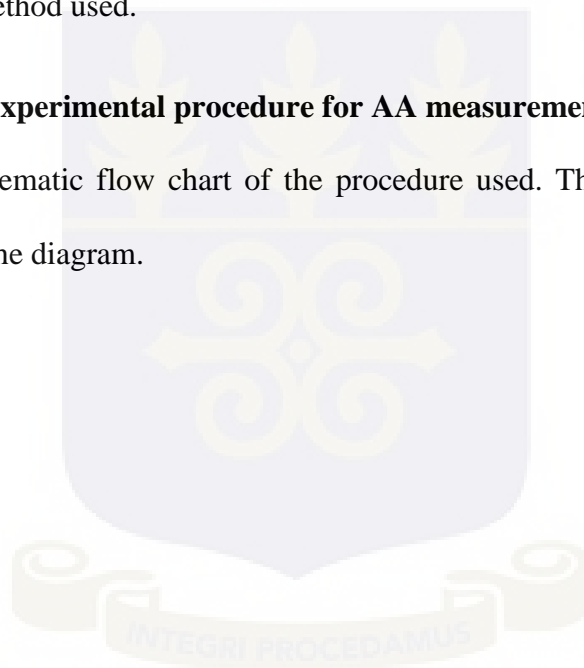
Sn standard solutions (5, 10 and 20 mg Sn L⁻¹); Cr standard solutions (0.5, 1 and 2 mg Cr L⁻¹); Fe standard solutions (2, 5 and 10 mg Fe L⁻¹) and Zn standard solutions (0.1, 0.25 and 0.5 mg Zn L⁻¹). Each standard solution was obtained by pipetting an appropriate aliquot of the stock solution into a 25 mL volumetric flask and diluting to the mark with double distilled water.

Standard Reference Material

A National Institute of Standards and Technology standard reference material, NIST SRM 1568a (Rice flour), was analysed together with the samples to check the validity of the F AAS method used.

3.3.2.2.1 Experimental procedure for AA measurements

Fig 3.2 is a schematic flow chart of the procedure used. The detailed procedure is presented after the diagram.



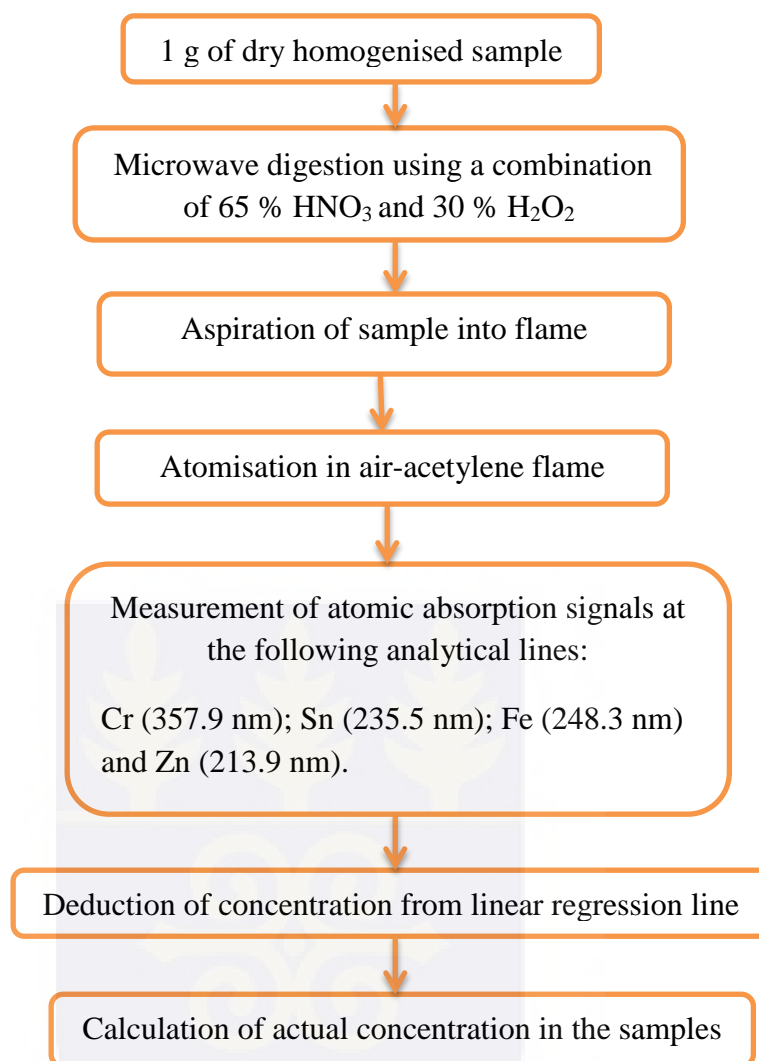


Fig. 3.2: Flow chart for determination of concentrations of Cr, Sn, Fe and Zn using FAAS.

Calibration of atomic absorption spectrometer

The atomic absorption spectrometer was calibrated using the prepared calibration standards of each element. The absorbances measured for each element's standard solutions were used to prepare a linear regression line (plot of absorbance against concentration). The linear regression lines are presented in Appendix B (Figs B1 – B4). The concentration of each element in the sample was deduced from the regression line of that element.

Atomic absorption measurement of elements in the samples

The instrumental conditions used for F AAS determination of Sn, Fe, Cr and Zn were as follows: The flame atomiser was made up of air (flow rate: 13.50 L/min) as oxidant and acetylene (flow rate: 2 L/min) as fuel. The hollow cathode lamp current, wavelength of light and spectral slit width used in the determination of each element are presented in Table 3.3.

Table 3.3: F AAS working conditions used for determination of Cr, Fe, Sn and Zn

Element	Hollow cathode lamp		
	Wavelength (nm)	Lamp current (mA)	Slit width (nm)
Sn	235.5	7	0.5
Cr	357.9	7	0.2
Fe	248.3	5	0.2
Zn	213.9	5	1.0

3.3.2.2.2 Calculation of elemental concentrations in the samples

Based on the equation of the standard calibration line obtained for each element, the concentration of each element in the digested sample and blank were determined in units of mg/L. The actual elemental concentration in the samples was calculated using equation 12.

$$C_{sam} = \frac{C_{sam_sol} \cdot D_f}{M_{sam}} \cdot V_{sam_sol} \quad (12)$$

Where:

C_{sam} is the concentration of element in sample (in $\mu\text{g/g}$);

C_{sam_sol} is the concentration of element in digested sample solution (in mg/L);

M_{sam} is the mass of sample digested (in g);

V_{sam} is the total volume of digested sample solution (in mL); and

D_f is the dilution factor (applicable only in cases where dilution was performed before absorbance measurement for an element).

3.3.2.3 Determination of concentrations of Se, As and Hg by HGAAS

The hydride generation technique of atomic absorption spectrometry was used to determine the concentrations of Se, As and Hg in the samples. The procedure used for determination of each element is described.

3.3.2.3.1 Determination of As levels

Instrumentation

The HG AAS system used for the process included a Fast Sequential Atomic Absorption Spectrometer (VARIAN, AA 240 FS, Australia) equipped with a VGA-77 vapour generation system (VARIAN, Australia) fitted with a peristaltic pump for continuous gas flow.

Chemicals

The following analytical grade chemicals were used:

- Sodium hydroxide (NaOH), Nitric acid (HNO₃, 65%) [British Drug House (BDH) laboratory supplies, UK], Hydrochloric acid (HCl, 37 %) [Sigma-Aldrich, UK], Hydrogen peroxide (H₂O₂, 30 %) [Fisher Scientific, UK], Sodium borohydride (NaBH₄) and Potassium iodide (KI).
- Standard stock solution of As included: 1000 mg As L⁻¹ in 2 % HNO₃ w/w (Trace CERT[®], Fluka, Chemie, Switzerland).

Calibration standards and reagents

- The following standard solutions of As, prepared by diluting appropriate volumes of the stock solution in 25 mL volumetric flasks with 2 % HNO₃ was used for calibration of the AA instrument: 0.2, 0.4 and 0.6 mg As L⁻¹.

- Generation of the hydride of As during the HG AAS process was done using 0.6 % (w/v) NaBH_4 in 0.5 % (w/v) NaOH and 6 M HCl . The NaBH_4 acted as a reducing agent and the 6 M HCl as a carrier solution.

Standard Reference Material

NIST SRM 1568a (Rice flour), a standard reference material supplied by the National Institute of Standards and Technology, was analysed together with the samples to check the validity of the HG AAS method used.

3.3.2.3.1.1 Experimental procedure for As determination

Fig 3.3 gives a schematic flow chart of the procedure used for As determination. The detailed procedure follows the flow chart.

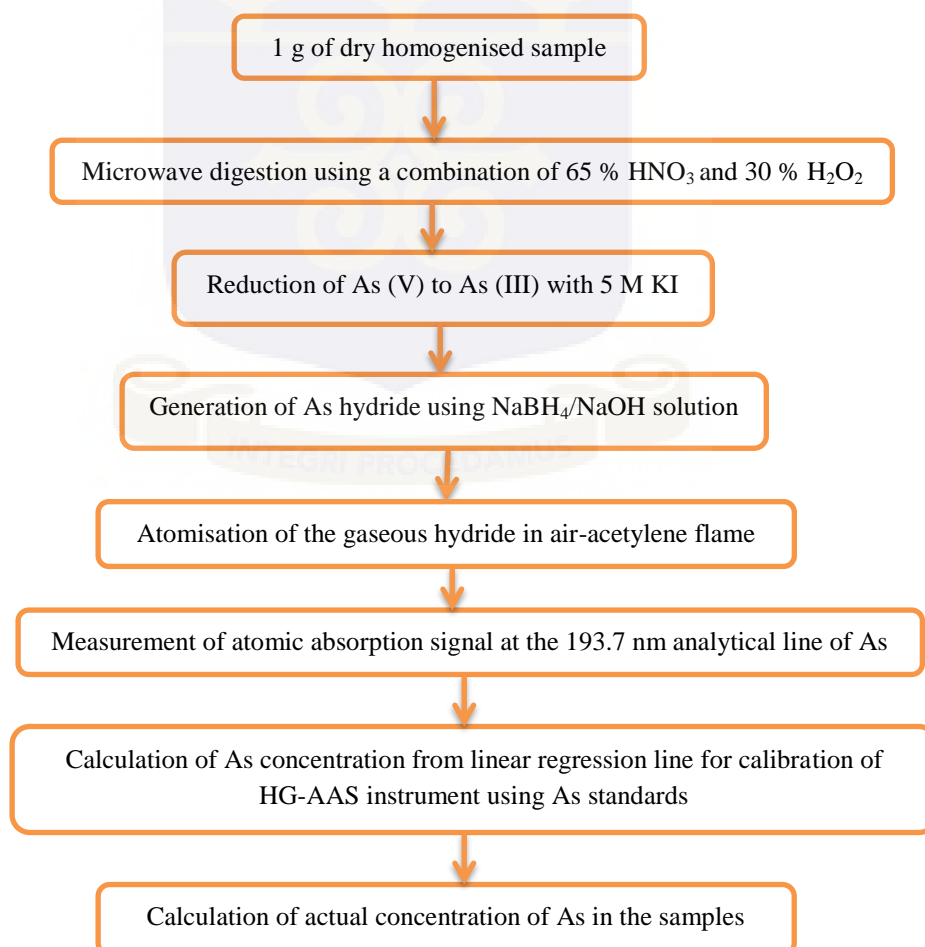


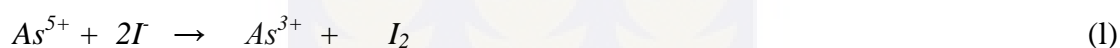
Fig. 3.3: Flow chart for the determination of As concentrations in the samples using HGAAS

Calibration of HG AAS instrument

The As calibration standards were used to calibrate the instrument. A linear regression line was prepared (Appendix B, Fig B5) using the concentrations of the calibration standards and their corresponding absorbances. Subsequent determination of the As concentration in the samples was obtained from its regression line equation after the absorbance had been measured and known.

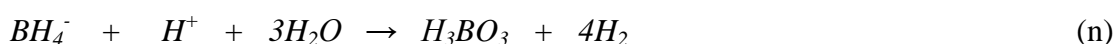
Reduction of As⁵⁺ to As³⁺

Before generating the hydride of As, all As⁵⁺ in the digested sample was converted to As³⁺ by the addition of 4 mL of 5 M KI to the sample solution. I⁻ reduced As⁵⁺ to As³⁺ as follows:



Hydride generation for As and subsequent absorbance measurement

The method of continuous flow of an HGAAS system was used to combine sample solution and reagents in the VGA. The digested sample solution (of flow rate 5 mL/45 sec) was mixed in a polyetheretherketone (PEEK) cross connector with the HCl and NaBH₄ solutions (both of flow rate 5 mL/45sec). The mixture was then pumped into the reaction coil. A reaction between tetrahydroborate ion (BH₄⁻) and As³⁺ produced the hydride of As (AsH₃) by reduction of the latter by the former. The BH₄⁻ also interacted with H⁺ in the medium to produce hydrogen gas (H₂). The following equations illustrate:



The gaseous hydride and hydrogen formed were separated from the liquid in a gas-liquid separator within the VGA system. By a flow of Argon gas, the separated gasses were carried to a Perma-pure drier for drying by a stream of nitrogen gas. The dried

hydride and hydrogen gasses were then transferred out of the VGA into the atomic absorption spectrometer with argon gas (of flow rate 13.5 mL/min). In the atomic absorption system, a flame composed of air (flow rate: 13.50 L/min) as oxidant and acetylene (flow rate: 2 L/min) as fuel was used to atomise the AsH₃ gas. In measuring the absorbance, a hollow cathode lamp current of 10 mA, light of wavelength 193.7 nm and spectral slit width of 0.5 nm were used.

3.3.2.3.1.2 Calculating the concentration of As

After the absorbance measurements, the equation of the line of regression obtained for the element after calibration was used to determine its concentration in each sample digest. This concentration, in mg/L of digest solution, was converted to µg/g of sample using equation 12.

3.3.2.3.2 Determination of Se levels

Instrumentation

Hydride generation and subsequent absorbance measurements were done using a Fast Sequential Atomic Absorption Spectrometer (VARIAN, AA 240 FS, Australia) equipped with a VGA-77 vapour generation system (VARIAN, Australia) fitted with a peristaltic pump for continuous gas flow.

Chemicals

These were the same as those used for As determination, the standard stock solution excepted. The standard stock solution used in this case was that of Se with concentration 1000 mg Se L⁻¹ in 2 % HNO₃ w/w (Trace CERT[®], Fluka, Chemie, Switzerland).

Standards and reagents

The following standard solutions of Se, prepared by diluting appropriate volumes of the stock solution in 25 mL volumetric flasks with 2 % HNO₃ was used for calibration of the AA instrument: 0.1, 0.25 and 0.5 mg Se L⁻¹.

Standard Reference Material

The validity of the HG AAS method used was checked by analysing a National Institute of Standards and Technology standard reference material, NIST SRM 1568a (Rice flour), together with the samples.

3.3.2.3.2.1 Experimental procedure for Se determination

A Flow chart of the procedure is presented in Fig 3.5, followed by a detailed description.

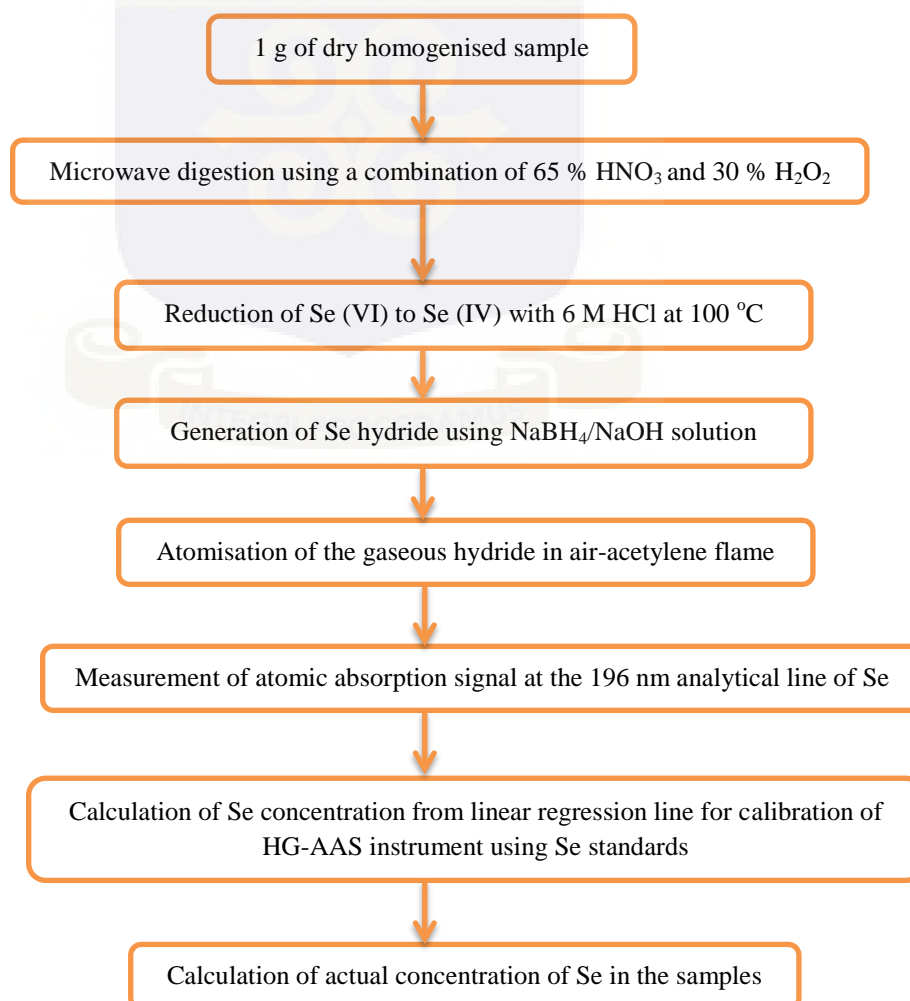


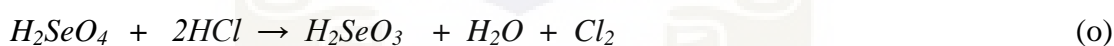
Fig. 3.4: Flow chart for the determination of Se concentration in the samples using HG AAS.

Calibration of the HG AAS instrument

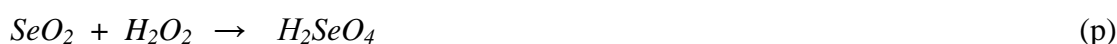
The Se calibration standards were used to calibrate the instrument. A linear regression line was prepared (Appendix B, Fig B6) using the concentrations of the calibration standards and their corresponding absorbances. Subsequent determination of the Se concentration in the sample digest was obtained from its regression line equation after the absorbance had been measured and known.

Reduction of Se⁶⁺ to Se⁴⁺

All Se present in the sample solution as Se⁶⁺ (in the form of selenic acid, H₂SeO₄) was first of all reduced to Se⁴⁺ (in the form of selenous acid, H₂SeO₃) before the generation of its hydride with NaBH₄. This was achieved by addition of 2 mL of 6 M HCl to 10 mL of the digest followed by heating on a water bath at a temperature of 100 °C for 30 minutes. In situations where the digest solution was not clear but had a turbid appearance, 0.5 mL of HCl (37 %) was used instead of the 6 M HCl, this time without heating. The HCl played the dual role of making the solution clear by breaking up all the solid matter, as well as reduction of Se⁶⁺ to Se⁴⁺ in readiness for the hydride generation step of the analysis.



The H₂SeO₄ arises from the oxidation of SeO₂ that may have been present in the sample by H₂O₂ during the digestion process;



Hydride generation for Se and subsequent absorbance measurement

The procedure for generation of the H₂Se gas and its subsequent atomisation and absorbance measurement was the same as that for As described above, and would not therefore be repeated. The reagents used were also the same. The H₂Se gas was produced by the reduction of Se⁴⁺ to Se²⁻ by the BH₄⁻ ion as follows:



Atomisation of the H₂Se gas was carried out in the atomic absorption spectrometer using an air/acetylene flame, where the air acted as oxidant and the acetylene as fuel. In the absorbance measurement for Se, a Hollow Cathode Lamp current of 10 mA, light of wavelength 196 nm and spectral slit width of 1 nm were used.

3.3.2.3.2.2 Calculating the concentration of Se

After the absorbance measurement, the equation of the line of regression obtained for the element after calibration was used to determine its concentration. This concentration, in mg/L of digest solution, was converted to µg/g of sample using equation 12.

3.3.2.3.3 Determination of Hg levels

Instrumentation

The HG AAS system used for the process included a Fast Sequential Atomic Absorption Spectrometer (VARIAN, AA 240 FS, Australia) equipped with a VGA-77 vapour generation system (VARIAN, Australia) fitted with a peristaltic pump for continuous gas flow. In place of a flame, an absorption cell was placed in the optical path of the atomic absorption spectrometer.

Chemicals

The following analytical grade chemicals were used:

- (NaOH); HNO₃, 65% (BDH, UK); HCl, 36 % (Sigma-Aldrich, UK); H₂O₂, 30 % (Fisher Scientific, UK) and NaBH₄.
- Standard Hg stock solution of concentration 999 ± 4 µg Hg L⁻¹ in 1.4 % w/w HNO₃ (Spectroscan, Teknolab AB, Sweden).

Calibration standards

Hg standard solutions used to calibrate the Hg AAS instrument had the following concentrations:

0.1, 0.25 and 0.5 mg Hg L⁻¹. These solutions were prepared from the Hg standard stock solution by diluting the required aliquots to the right volume in a 25 mL volumetric flask with double distilled water.

Standard Reference Material

The standard reference material, NIST SRM 1568a (Rice flour), supplied by the National Institute of Standards and Technology was analysed together with the samples to ascertain the reliability of the method.

3.3.2.3.3.1 Experimental procedure

Fig 3.5 is a schematic flow chart of the procedure for determination of Hg, followed by the detailed description.

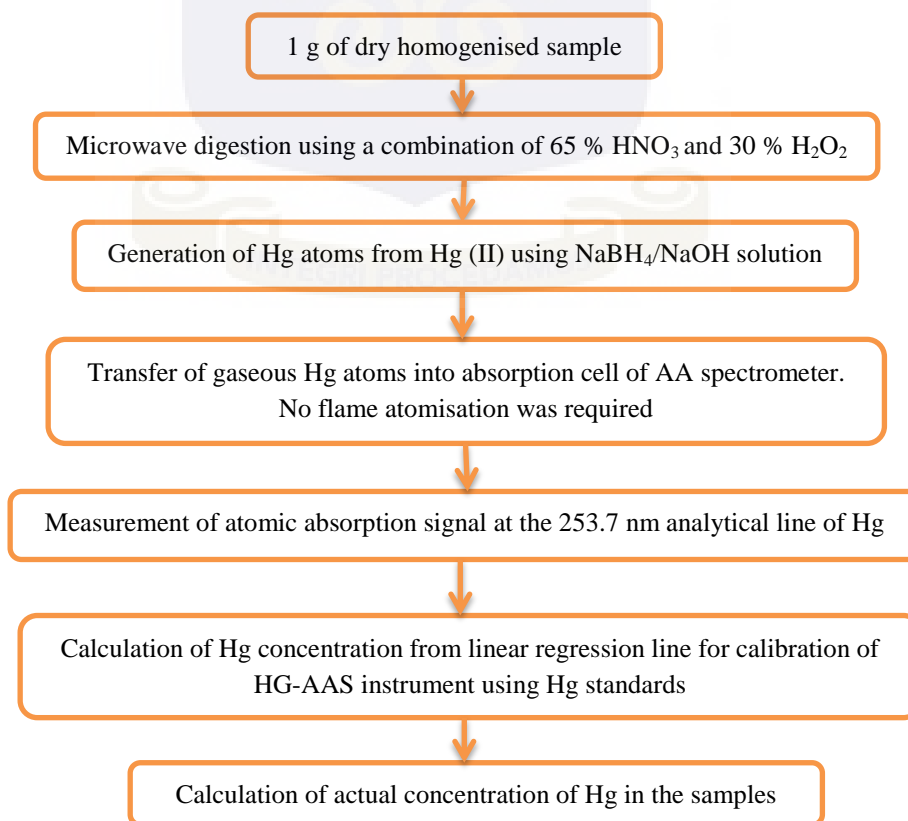


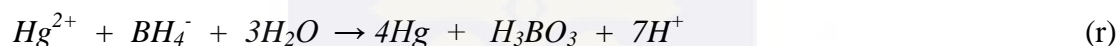
Fig. 3.5: Flow chart for the determination of Hg concentration in the samples using HG AAS.

Calibration of the HG AAS instrument

The Hg calibration standards were used to calibrate the instrument. A linear regression line was prepared (Appendix B, Fig B7) using the concentrations of the calibration standards and their corresponding absorbances. Subsequent determination of the Hg concentration in the samples was obtained from its regression line equation after the absorbance had been measured and known.

Reduction of Hg²⁺ ions to Hg atoms

The digested sample solution was mixed in a polyetheretherketone (PEEK) cross connector with HCl and NaBH₄ solutions (both of flow rate 5 mL/45sec) with the aid of the peristaltic pump. The mixture was then pumped into the reaction coil. The tetrahydroborate ions reacted with the Hg²⁺ ions present in the mixture, converting them to Hg atoms as follows:



The Hg atoms produced, which are in the vapour phase, were carried out of the VGA into an absorption cell installed in the optical path of the atomic absorption spectrometer by an argon gas flowing at the rate of 13.5 mL/min. This absorption cell was used in place of a flame which would normally have been used to atomise an element. Since the Hg was already in atomic, gaseous form, there was no need for a flame.

Atomic absorption measurement for Hg

Absorbance measurement was done for the Hg atoms in the vapour state which had been transferred to the absorption cell. No flame was used to atomise the Hg as it was already in the atomic form. The wavelength of light used was 253.7 nm, the hollow

cathode lamp operated with a current of 4 mA, and the spectral slit width used was 0.5 nm.

3.3.2.3.3.2 Calculation of Hg concentration

Using the linear regression line obtained from the calibration, the concentration of Hg was deduced and the actual concentration in the samples calculated from equation 12.

3.3.3 Determination of Pb

The levels of Pb in the samples were determined by F AAS after chemical separation by solvent extraction using 1, 5-diphenylthiocarbazone (dithizone) as ligand.

Instrumentation

- A Fast Sequential Atomic Absorption Spectrometer (VARIAN, AA 240 FS, Australia) equipped with a deuterium background corrector was used for atomic absorption measurement of Pb after solvent extraction.
- A digital pH meter (WTW pH 523, typ E 50) was used to adjust the pH of solution to 8 before solvent extraction was done.
- An electronic weighing balance (Mettler Toledo, XS603S) was used to weigh samples for digestion. It was also used to weigh appropriate masses of chemicals for preparation of the required solutions.

Chemicals

Chemicals used in the process included the following, all of which were of analytical grade:

- Sodium potassium tartarate tetrahydrate [(NaKC₄H₄O₆·4H₂O), Hopkin and Williams Ltd, Essex]; Potassium cyanide [(KCN), BDH, Poole]; Hydroxylamine hydrochloride [(NH₂OH·HCL), Qualikems Fine Chemicals

pvt Ltd]; dithizone [1, 5-diphenylthiocarbazone ($C_{13}H_{12}N_4S$), BDH, Poole]; Hydrochloric acid (HCl, 37 %, $d=1.16$); trichloromethane, otherwise known as chloroform ($CHCl_3$); and Ammonia [$(NH_3, 25 \%$, $d=0.88$), Fisher Scientific].

- Standard Pb stock solution of concentration $1000 \pm 4 \text{ mg Pb L}^{-1}$ in 2 % w/w HNO_3 (Trace CERT[®], Fluka, Chemie, Switzerland).

Calibration standards

Calibration of the AAS instrument for measurement of Pb concentration after extraction was performed using three standard solutions prepared by appropriate dilution of the Pb standard stock solution with 2 % HNO_3 in 25 mL volumetric flasks. These solutions had concentrations 2, 5 and 10 mg Pb L^{-1} respectively.

Standard Reference Material

NIST SRM 1547 (Peach leaves), a standard reference material supplied by the national institute of standards and technology (NIST) was analysed together with the samples to check the reliability of the method.

3.3.3.1 Experimental procedure for Pb extraction

The procedure used for chemical separation of Pb by solvent extraction was adopted from Marcenko and Balcerzak (2000). The procedure is summarised as a flow chart in Fig 3.6.

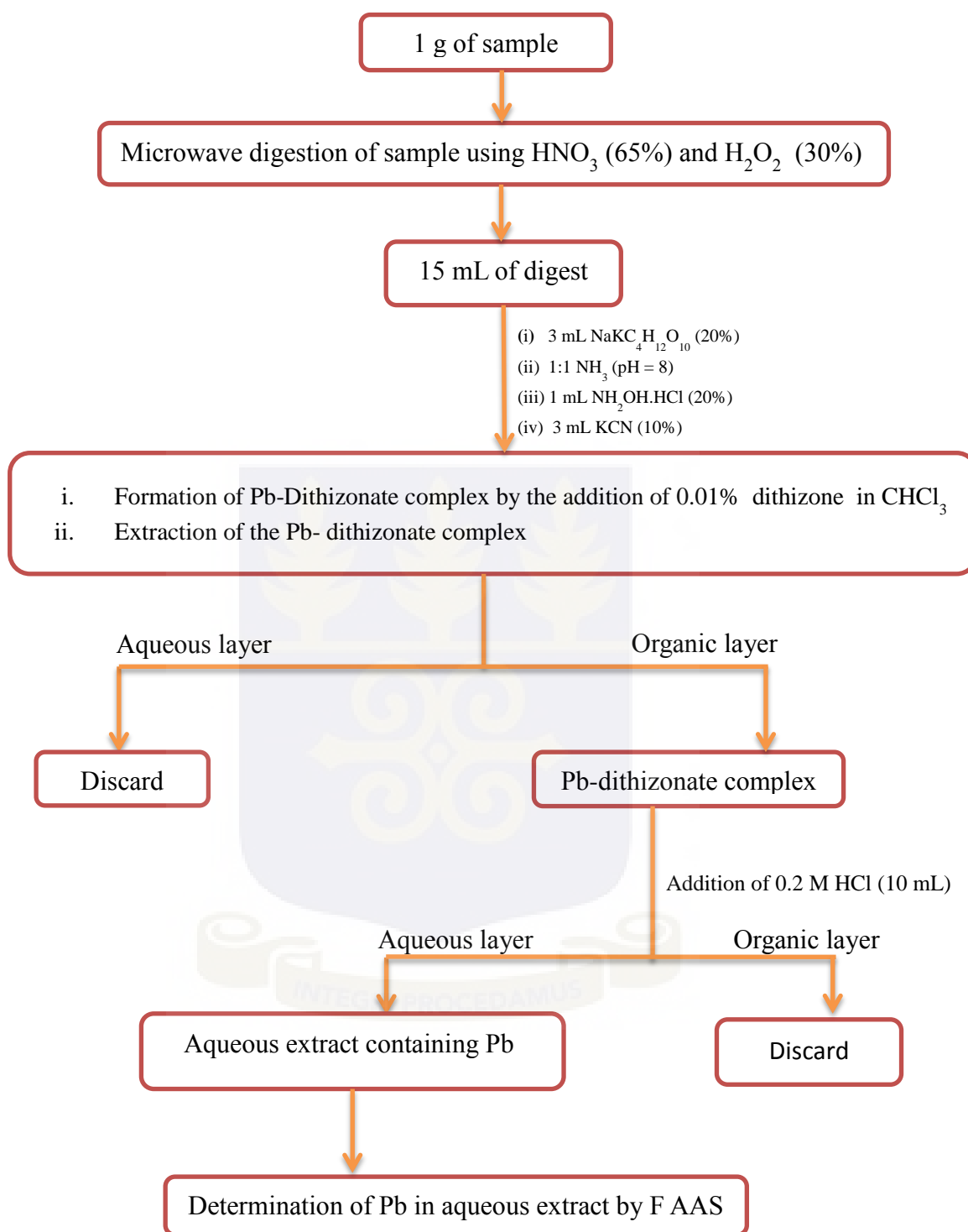
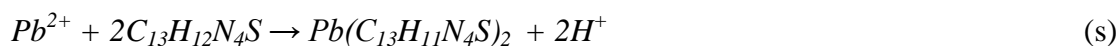


Fig 3.6: Flow chart for the determination of Pb by F AAS after solvent extraction

The detailed procedure for the extraction of Pb using 0.01% dithizone, with chloroform as extractant, is as follows.

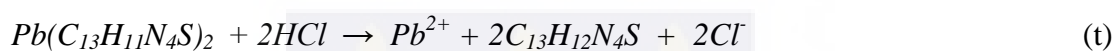
15 mL of digest solution was pipetted into a 100 mL beaker. To this was added 3 mL of 20 % (w/v) sodium potassium tartarate. The addition of this solution was to prevent the oxidation of metals such as Al, Fe²⁺ and Ti which are easily hydrolysed. Following the addition of the tartarate solution to the digest, the pH of the mixture was adjusted to 8 by the slow addition of a 1:1 aqueous ammonia solution using a dropping pipette, with intermittent cooling. A digital pH meter was used to determine that the required pH had been attained. To the now basic mixture was added 1 mL of 20 % (w/v) hydroxylamine hydrochloride. This was to prevent the oxidation of dithizone in the alkaline medium of the mixture when it was added. This was followed by the addition of 3 mL of 10 % (w/v) potassium cyanide. The KCN was to serve as a masking agent for other metals that may be present in the sample digest and could also react with dithizone, thus preventing them from interfering with the analysis. These include Ag, Hg, Pd, Au, Cu, Zn, Cd, Ni and Co.

The total mixture of reagents in the beaker was then transferred into a 100 mL separatory funnel. To the contents of the separatory funnel, 5 mL of 0.01 % dithizone solution in chloroform (this solution is green in colour) was added. The mixture was shaken for three minutes and allowed to stand for 2 minutes so the aqueous and organic phases could separate. Any Pb²⁺ present in the medium would have reacted with the dithizone to form a complex that was pink in colour:



Since dithizone is insoluble in water, the Pb-dithizonate complex formed will be in the organic (in this case chloroform) phase. The chloroform layer, which was the lower of the two layers, was separated into a second separatory funnel. Extraction of the aqueous layer in the first separatory funnel was repeated with 5 mL portions of

dithizone until the organic layer remained green (which meant that there was no longer any Pb^{2+} present, otherwise the organic layer will be pink). To the combined extracts in the second separatory funnel, 10 mL of 0.2 M HCl was added. The mixture was shaken for three minutes and allowed to stand for 2 minutes for separation of the phases. The addition of HCl was meant to strip the Pb^{2+} from the organic phase into an aqueous phase by breaking the bonds between Pb and dithizone in the Pb-dithizonate complex, thereby setting Pb^{2+} free which then moved into the aqueous phase:



Separation of the two phases clearly showed a clear upper aqueous layer and a green coloured lower organic layer (due to the liberated dithizone from the complex which is dissolved in the chloroform to form its characteristic green solution). The aqueous layer was separated into a polyethylene sample container for Pb determination using F AAS. A blank solution and standard reference material were also extracted using the same procedure. Figs 3.7-3.10 show the various colour changes encountered at different stages of the extraction process.

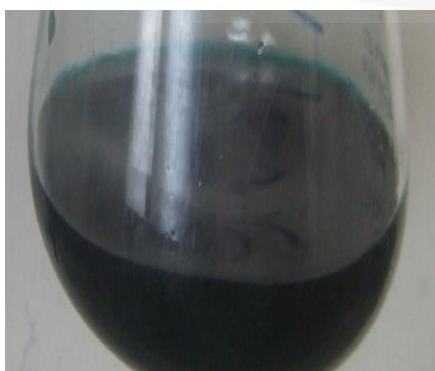


Fig. 3.7: Freshly prepared solution of dithizone in chloroform (green in colour).



Fig. 3.8: The aqueous and organic layers obtained after addition of dithizone in $CHCl_3$ solution. The pink colour of the $CHCl_3$ layer is due to the Pb-dithizonate present.

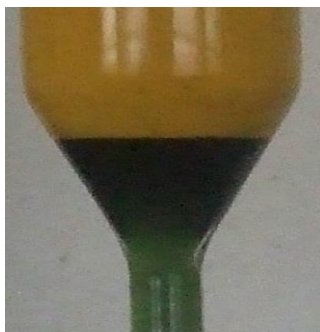


Fig. 3.9: Green colour of dithizone in CHCl_3 obtained after complete extraction of Pb^{2+} .

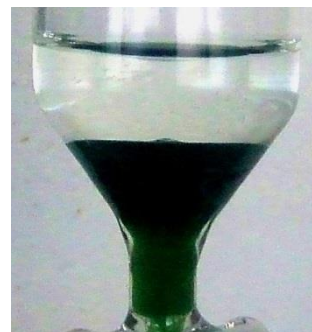


Fig. 3.10: Green colour of CHCl_3 layer obtained after addition of HCl indicating complete stripping of Pb^{2+} into the aqueous layer.

Calibration of the AAS instrument

Atomic absorbance measurements were done for each of the prepared Pb calibration standard solutions. With the known absorbances and their corresponding concentrations of Pb, a regression line was obtained (Appendix B, Fig B8) from a plot of absorbance against concentration. From this regression line was obtained the unknown concentrations of Pb in the samples and standards after the atomic absorbance measurement was done for each of them.

Absorbance measurement

Each sample, standard or blank extract was aspirated into the nebuliser of the AAS instrument. The nebuliser then transferred the aspirated sample in the form of an aerosol into the flame for atomisation. The flame was composed of air as the oxidant flowing at the rate of 13.5 L/min, and acetylene as the fuel with a flow rate of 2 L/min. Atomic absorbance measurement was performed for the atomised element using a light of wavelength 217 nm passing through a spectral slit of width 1 nm from a hollow cathode lamp source operating at a current of 5 mA.

3.3.3.2 Calculation of the concentration of Pb

Based on the absorbance obtained for Pb in each extract, the concentration of Pb in each sample extract was obtained from the standard linear regression line. The Pb concentration in each sample was then calculated using equation 12.

3.4 Estimation of the dietary intakes of the elements determined

Daily consumption data for each brand of food was obtained from recommended daily feeding guidelines provided by the manufacturer of each food brand. Based on this, the daily amount of each food brand consumed (in grams) was determined (Ljung et al., 2011; Kazi et al., 2010; COT, 2003). The daily amount of each food brand consumed, together with the concentration of each element determined were used to estimate the daily or weekly intakes of the various elements of interest due to the consumption of each food brand. Equation 13 was used to estimate the daily intake of each essential element determined.

$$DI = C_{sample} \cdot M_{sample_day} \quad 13$$

Where;

DI is the daily intake of element in a particular food sample,

C_{sample} is the concentration of the element in that food sample, and

M_{sample_day} is the daily amount of that sample consumed (in grams).

For the non-essential elements, the safety guidelines provided by the Joint FAO/WHO Expert committee on Contaminants and Food Additives (JECFA) regarding their intake were in units of either $\mu\text{g}/\text{kg bw}/\text{week}$ or $\text{mg}/\text{kg bw}/\text{week}$, taking into account their cumulative effect and hence the long term exposure. For purposes of a meaningful comparison of the intakes of the non-essential elements determined in this

work with the safety guidelines provided by JECFA, the intakes were calculated in the same units, assuming an average body weight (bw) of 7 kg for an infant (Kazi et al., 2010). Equation 14 was used.

$$WI = \frac{C_{sample} \cdot M_{sample_day} \cdot 7}{M_{infant}} \quad 14$$

Where;

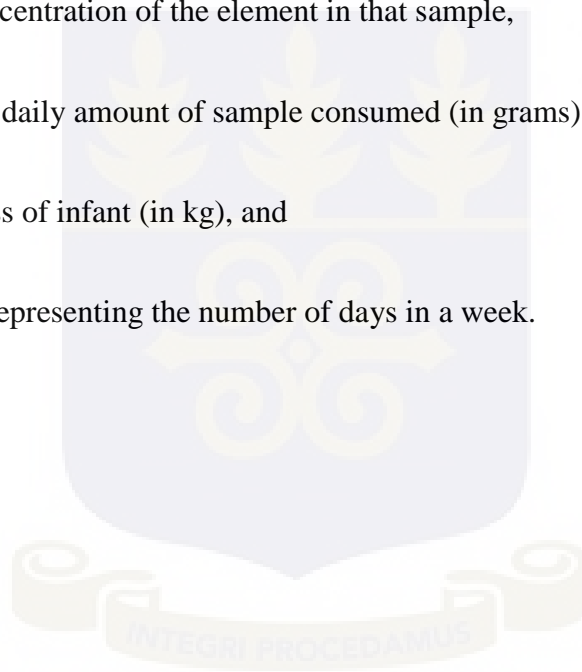
WI is the weekly intake of an element in each sample per kg body weight of infant,

C_{sample} is the concentration of the element in that sample,

M_{sample_day} is the daily amount of sample consumed (in grams),

M_{infant} is the mass of infant (in kg), and

7 is a constant, representing the number of days in a week.



CHAPTER FOUR

RESULTS AND DISCUSSION

This study seeks to determine the concentrations of some essential (Ca, Cl, Cr, Cu, Fe, K, Na, Mg, Mn, Se, V and Zn) and non-essential (As, Br, Hg, Sn and Pb) elements in cereal-based infant weaning foods commercially available on the Ghanaian market. It also seeks to determine whether the essential and non-essential elements present in the food meet allowable concentrations recommended by the World Health Organisation (WHO), the Food and Agriculture Organisation (FAO) and the European Food Safety Authority (EFSA). Two main analytical techniques were used in the work, namely instrumental neutron activation analyses (INAA) and atomic absorption spectrometry (AAS). This chapter presents the results obtained, as well as a detailed discussion of those results. Data for validation of the methods have also been presented to demonstrate and assure reliability of the experimental procedures adopted and hence the results obtained.

4.1 Validation of INAA method

The concentrations of the elements Br, Ca, Cl, Cu, K, Na, Mg, Mn and V were determined in the samples using INAA. The method was validated by analysing a standard reference material (SRM) with the samples under the same experimental conditions. The SRM used for this purpose was NIST SRM 1547 (Peach leaves). A comparison of the experimental concentrations of elements in the SRM and the certified values are provided in Table 4.1. There is excellent agreement between the experimentally obtained data and the certified values. This attests to the reliability of the INAA method used for the determination.

Table 4.1: Experimental concentrations of elements in NIST 1547 (Peach leaves) compared with certified values.

Element	Concentration ($\mu\text{g/g}$)		% Recovery
	This work*	Certified Value	
Al	225 ± 6	249 ± 8	90
Ca	14710 ± 240	15600 ± 200	94.3
Cl	322 ± 12	360 ± 19	89.4
Cu	4.1 ± 0.3	3.7 ± 0.4	111
K	23000 ± 300	24300 ± 300	95
Mg	3920 ± 50	4320 ± 80	91
Mn	87 ± 4	98 ± 3	89
Na	18 ± 3	24 ± 2	75
V	0.40 ± 0.03	0.37 ± 0.03	108

*Experimental results are presented as the mean \pm standard deviation of three measurements.

4.2 Elemental concentrations in samples by INAA method

The concentrations of elements analysed in the samples using INAA are presented in Appendix C (Table C1). The elements include: Br, Ca, Cl, Cu, K, Mg, Mn, Na and V.

The elements Ca, Mg, K, Na, Cl, Cu and Mn were detected in all the samples. In two samples (SE-MS, and TB-LO), Br was below the instrument's detection limit. V on the other hand was below the detection limit in five samples (CE-RF, CE-WF, CE-YF, MI-WF and TB-WS). It is very clear from the data that the concentrations of Ca, Mg, Cl, Na, Br and K are relatively very high compared to the rest of the analytes. These (Mg, Cl, Na, Br and K) were present in the concentration ($\mu\text{g/g}$) ranges: 405 – 2002, 20 - 280, 158 - 5521, 13 - 1588, 41 - 7402 and 49 - 3845 respectively, with their respective averages ($\mu\text{g/g}$): 774, 146, 1539, 419, 1273 and 2010. The concentration of each element in the samples is discussed in detail.

4.2.1 Calcium

Among the elements determined, Ca clearly falls in the category of those with relatively high concentrations in the samples, with an average of 774 $\mu\text{g/g}$.

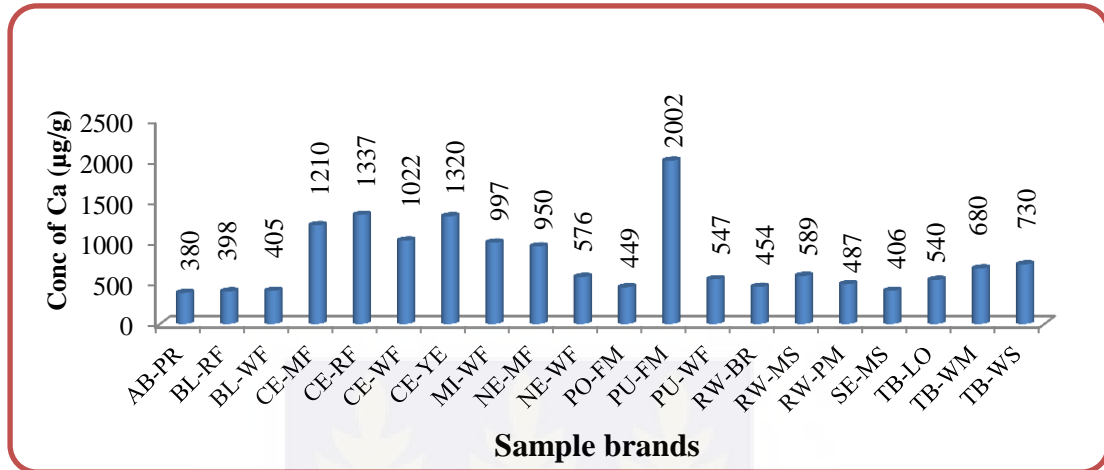


Fig. 4.1: Comparison of the concentration of Ca in the samples

The highest concentrations of Ca occurred in samples coded CE-MF, CE-WF, CE-YE, CE-RF and PU-MF with concentrations ($\mu\text{g/g}$) of 1210, 1022, 1320, 1337 and 2002 respectively. These concentrations are all higher than the remaining samples by about one order of magnitude on average. The main reason for this may be due to the calcium salts added to these samples (Appendix A), which is not the case in the other samples except for the one coded PU-WF. It is not surprising therefore that the first four of the aforementioned samples (CE-MF, CE-WF, CE-YE, CE-RF), being of the same brand, do not have their Ca concentrations differing by any significant margin. The same Ca additive was added to all four of them in the form of CaCO_3 , to serve as an acidity regulator. The general level of Ca in the samples also seem to reflect the fact that most of the foods contain some level of soya, milk or both (foods which serve as some of the best store houses of the element). As if to buttress this point, the sample with the least concentration of Ca indeed happens to be that coded AB-PR

(with concentration 380 $\mu\text{g/g}$), which has neither added Ca salt, soya or milk (Appendix A).

4.2.1.1 Calcium concentrations compared with MQVs

The concentrations of Ca obtained were compared to the Ca concentrations quoted by the manufacturers (MQVs) to ascertain the agreement between the levels of Ca obtained and the Ca levels quoted by the manufacturers (Fig 4.2). On the whole, six of the samples (NE-MF, NE-WF, PU-MF, PU-WF, MI-WF and CE-WF) had their Ca concentrations quoted by the manufacturers. The quoted Ca levels in the six samples have been compared with the Ca levels obtained in this study. The MQVs have been observed to be higher than the values obtained in this work for all the samples under consideration. The reason for this is not clear. Changes in conditions from time of manufacture to transportation, storage and retail cannot however be discounted as a possible explanation.

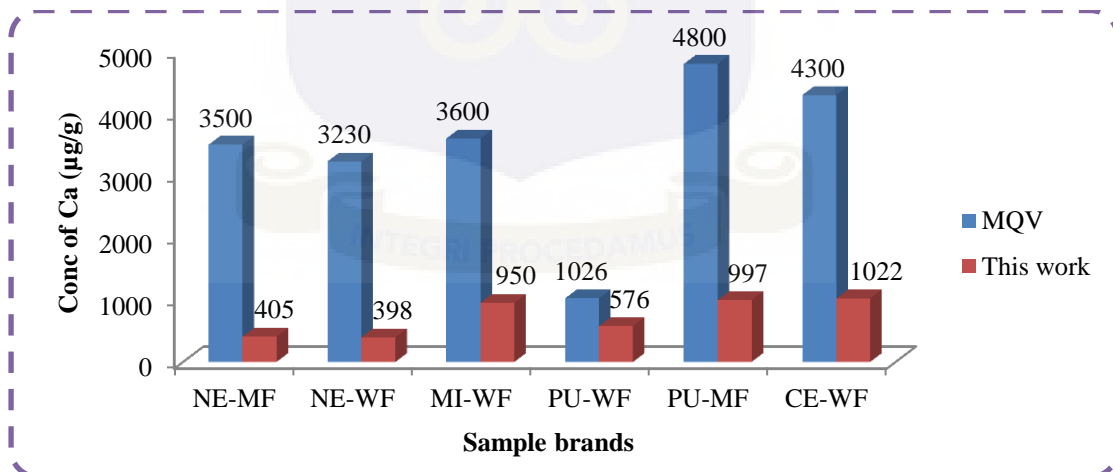


Fig. 4.2: Ca concentrations in some of the analysed samples compared with their MQVs.

4.2.1.2 Calcium levels in the samples compared with literature data

The average concentrations of Ca in the samples have also been compared with available data obtained in similar studies in other countries (published in scientific literature). These include the EU (Pandelova et al., 2012), Sweden (Ljung et al., 2011)

and Nigeria (Joseph et al., 2011; Jonah et al., 2003). The results obtained in this study compare very well with that of Sweden (Fig 4.3), slightly lower than that of the EU but much lower than those of Nigeria.

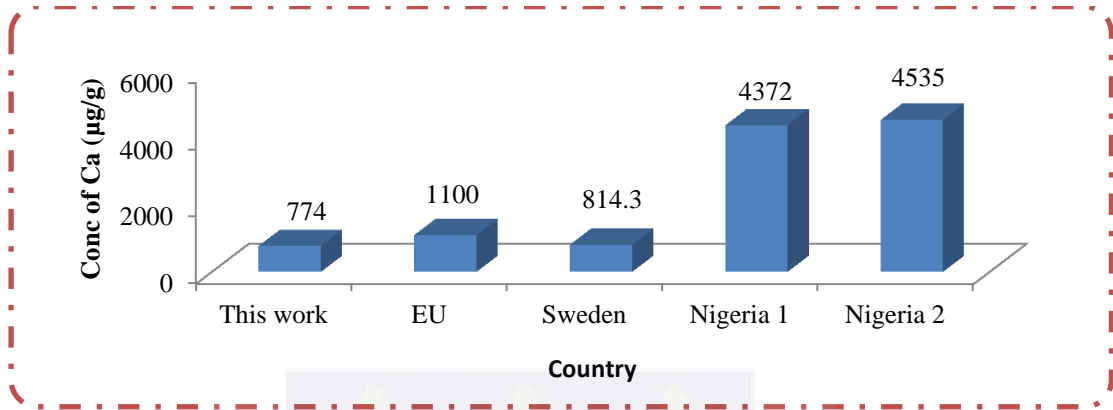


Fig. 4.3: Mean Ca concentration of the analysed samples compared with literature data.

4.2.2 Magnesium

Whole grains and legumes serve as very high sources of Mg for humans, that of fruits and dairy products being intermediate and refined foods the poorest (Fleet and Cashman, 2001). The range of Mg concentrations (20 - 280 µg/g, Fig 4.4) observed does seem to confirm this, being fairly high in most of the samples, which are composed mainly of cereals, some of which have added soya (a legume).

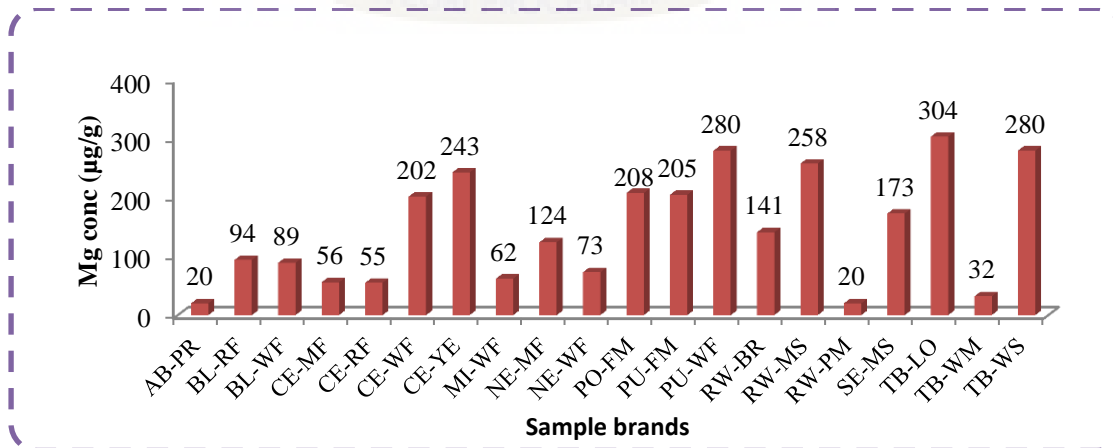


Fig. 4.4: Comparison of the concentrations of Mg in the samples analysed

Some of the samples have been seen to record Mg concentrations almost or more than twice the average of 146 $\mu\text{g/g}$. These are samples coded TB-LO (304 $\mu\text{g/g}$, which is the highest), PU-WF (280 $\mu\text{g/g}$), TB-WS (280 $\mu\text{g/g}$) and RW-MS (258 $\mu\text{g/g}$). Of these four samples, TB-LO is a local brand that is composed of unrefined maize and soya, perhaps explaining why it recorded the highest amount of Mg. The remaining three samples also contain cereal/soya mixtures, except for PU-WF, which has no soya, but do have added Mg as a supplement which may account for the elevated amount of the element in that sample.

A curious observation could also be made about the concentration variation of the element among four samples that originated from the same brand namely: CE-MF (56 $\mu\text{g/g}$), CE-RF (55 $\mu\text{g/g}$), CE-WF (202 $\mu\text{g/g}$) and CE-YF (243 $\mu\text{g/g}$). Whilst the first two samples, made of maize and rice respectively have almost the same Mg concentration, the latter two contain much elevated levels of the element in comparison. Of these latter two, the first is made from wheat, and the last contains a mixture of wheat, corn, malt extract and fruits (banana and apple). This last sample of 'cereal/fruit mixture' recorded the highest level of Mg within the group. This pattern of Mg concentrations in the four samples appear to suggest that combining different cereals with fruits may have an additive effect on the amount of the element available to the consumer.

The lowest concentrations were recorded by the samples AB-PR (20 $\mu\text{g/g}$), RW-PM (20 $\mu\text{g/g}$) and TB-WM (32 $\mu\text{g/g}$). The first two of these have concentrations that are similar, maybe because they do not have any other ingredient apart from the cereal components (rice and maize respectively). The concentration of the third one though, is a bit surprising, seeing that it has some added soya (wheat being the cereal component). Looking more closely at the data again reveals that another wheat-based

sample, NE-WF recorded a value (73 $\mu\text{g/g}$) that is about one half the magnitude of its counterpart of the same brand, NE-MF (124 $\mu\text{g/g}$). This seems to imply a certain shortcoming on the part of wheat in its contribution to the Mg content of the samples. The reason for this may lie in the level of processing involved, which might have led to some losses.

4.2.2.1 Magnesium levels compared with literature data and MQVs

The Mg levels obtained in this work have been compared with the MQV. Only one sample had its Magnesium content provided by the manufacturer on the container. This was sample coded PU-WF with a MQV of 351 $\mu\text{g/g}$ which is slightly higher but comparable with the Mg concentration obtained in this study (280 $\mu\text{g/g}$). The average Mg concentrations in available scientific literature (Ljung et al., 2011; Joseph et al., 2011; Jonah et al., 2003) have also been compared with those of this work (Fig 4.5). The average Mg content obtained in this work is similar to obtained in Sweden but lower than levels obtained in Nigeria during similar but different studies.

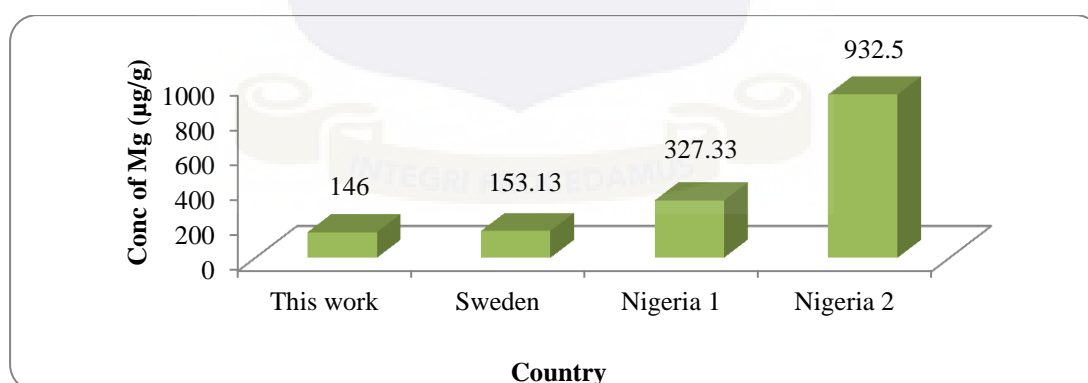


Fig. 4.5: Comparison of Mg concentrations in the analysed samples with literature data.

4.2.3 Sodium

In the case of Na, three samples, namely TB-LO, TB-WM and TB-WS (concentrations 1398, 1362 and 1588 $\mu\text{g/g}$ respectively) had concentrations almost two orders of magnitude on average above the remaining 17 samples (Fig 4.6

compares the Na levels in the various samples), contributing therefore by the biggest margin to the high average Na concentration recorded. This sharp difference is explained by the fact that NaCl is added to those samples during preparation (as indicated in Appendix A), mainly for the purpose of enhancing the taste. This situation is sharply contrasted by the relatively low concentrations (below 40 µg/g) of Na recorded in samples AB-PR, RW-PR, RW-MS and RW-BR which do not have any indication of added NaCl. Between the two extremes are samples with intermediate concentrations (between 745 and 393 µg/g). The samples to which these intermediate values are attributed also have some amount of added NaCl, but perhaps not to the same degree as the first group.

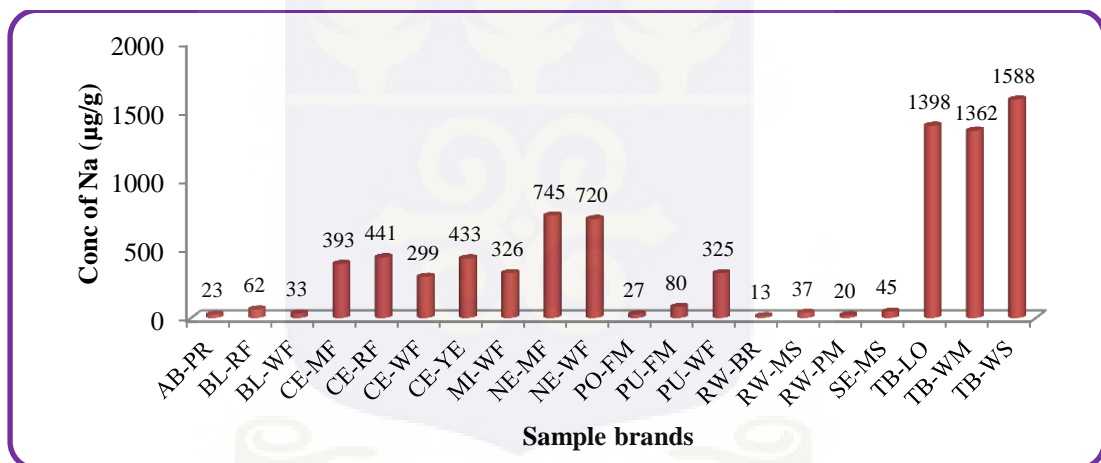


Fig. 4.6: Comparison of the concentrations of Na in the samples

4.2.3.1 Comparison of Na levels in the samples with MQVs

A comparison of the Na concentrations obtained has been made with the Na concentrations quoted by the manufacturers of eight of the samples (Fig 4.7). The eight samples are: BL-WF, BL-RF, NE-MF, NE-WF, MI-WF, PU-WF, PU-MF and CE-WF. The quoted values are indeed higher than those obtained in this work by varying degrees. What caused the differences is not very clear. It is however possible

that changes in conditions from the time of processing to transportation and storage at the point of retail led to some Na losses.

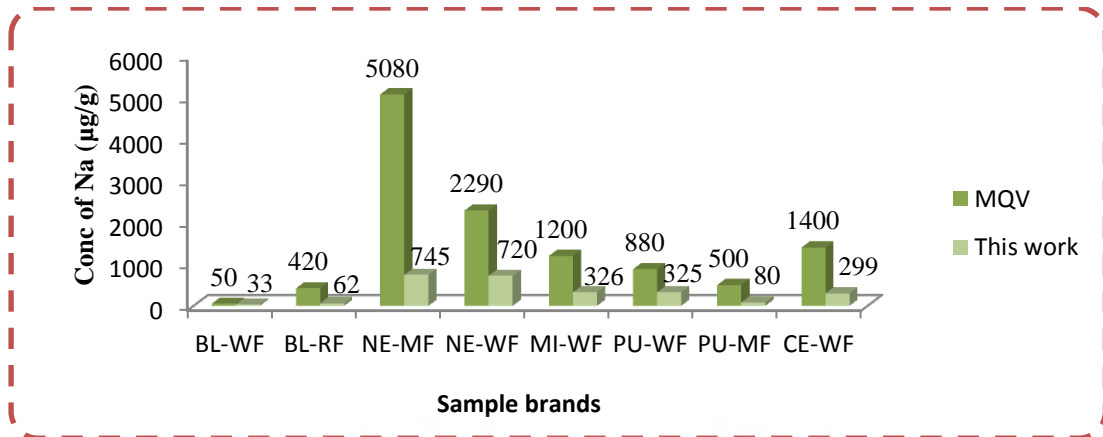


Fig. 4.7: Na concentrations in some of the samples compared with MQVs.

4.2.4 Chlorine

The Cl concentrations follow almost the same trend as Na, the highest concentrations having been recorded by samples TB-LO (4654 µg/g), TB-WM (4651 µg/g) and TB-WS (5521 µg/g). This obviously is because Cl is a constituent of NaCl which was added to these three samples (Appendix A). The overall average Cl concentration in the samples happen to be 1539 µg/g, almost 4 times that of Na (419 µg/g). The Cl content of all the samples have also been consistently higher than those of Na, in some cases by 300% or more. The only two exceptions in this case are samples NE-MF and NE-WF which display higher Na values compared to Cl. This may not be by accident, as both samples happen to be of the same brand (Nestume-the only ones of their kind), though with different cereal components. The generally higher Cl levels compared to Na may be due to the addition of KCl (which has Cl but no Na) to some

of the samples in place of NaCl (Zand et al., 2012).

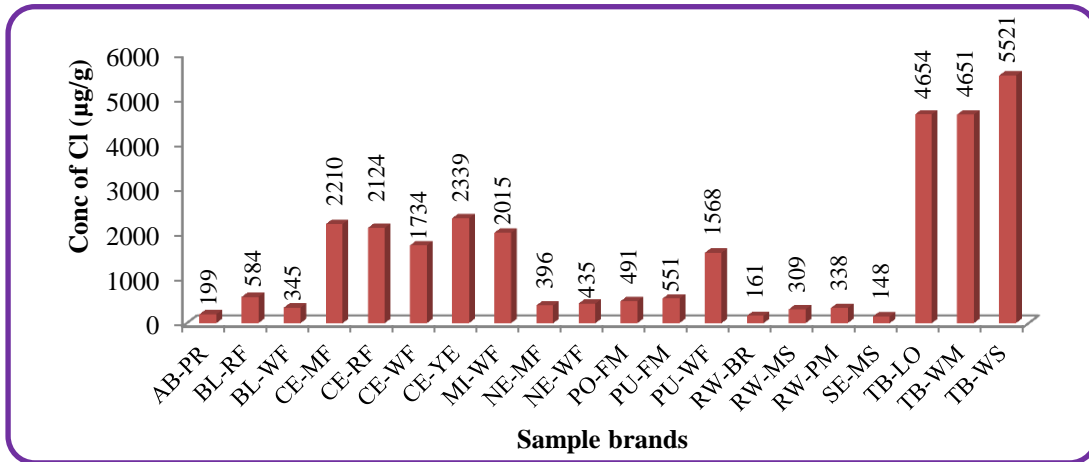


Fig. 4.8: Comparison of the concentration of Cl in the samples

4.2.5 Potassium

K, a group I metal like Na, has also been observed to have concentrations that are generally higher than that of Na, with an average of 2010 µg/g compared to that of Na (1539 µg/g). Such high K concentrations in complementary foods have also been reported elsewhere in literature (Zand et al., 2012). This may be because of the replacement of NaCl with a tasty low-sodium alternative such as KCl in the ingredients of seasoning and natural flavours used in processed foods. Manufacturers of the seasoning and flavours do this in an effort to maintain the flavour and functionality of food during processing as a response to the urge to reduce the sodium content of ready-to-eat foods (Zand et al., 2012). Also in plants, the content of K is generally higher than that of Na (deMan, 1999), a point that further accounts for the high K levels compared to Na. This notwithstanding, it has been observed that some of the samples contain KI as an ingredient, the latter ostensibly added as an additive to provide Iodine supplementation, providing additional reasons for the high levels recorded for K.

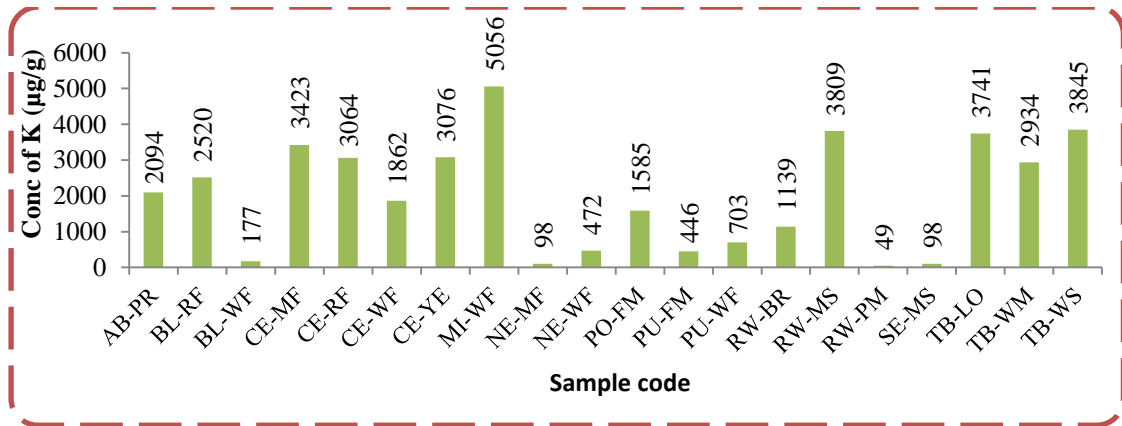


Fig. 4.9: Comparison of the concentrations of K in the samples.

Looking at individual samples as presented in Fig 4.9, the one coded MI-WF recorded the highest concentration of 5056 µg/g. A look at Appendix A (Sample composition) reveals that the major ingredients of this sample are wheat and honey, with some soya, all of which have very high K content naturally relative to the other cereals. This possibly accounts for the above observation. Three samples recorded relatively very low K concentrations. These, from the lowest, include samples RW-PM (49 µg/g), SE-MS and NE-MF (both with concentration of 98 µg/g).

4.2.5.1 Potassium concentrations in the samples compared with MQVs

Only three of the samples analysed had their K concentrations quoted on their containers by the manufacturers. Comparisons of these concentrations have been made with the values obtained in this study to ascertain their similarity or otherwise (Fig 4.10). All three samples displayed MQVs of K much higher than those obtained in this study.

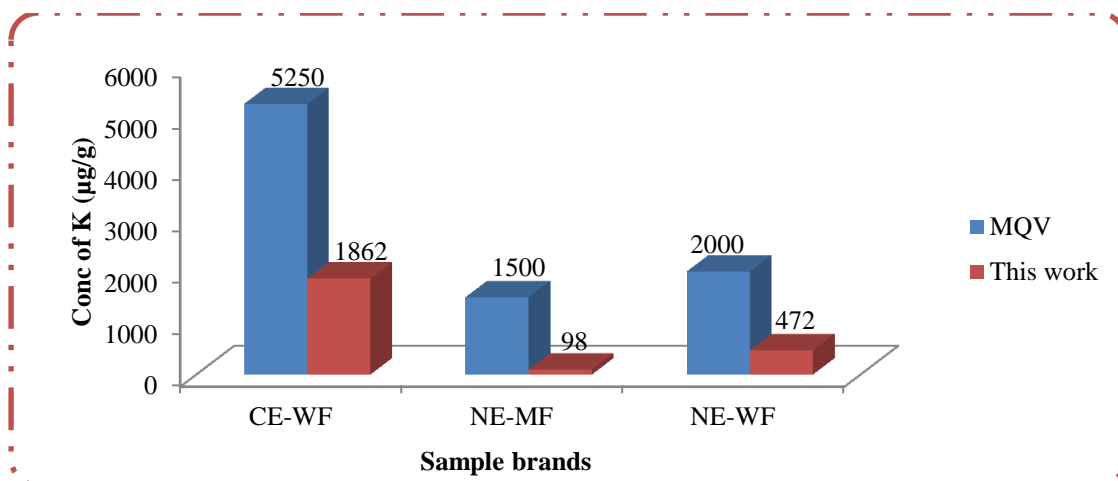


Fig. 4.10: K concentration in some of samples compared with the MQVs.

2.2.6 Bromine

Br is a non-essential element, with a high presence in cereal grains. Its main health effect occurs by a direct irritation of the skin, mucous membrane and tissues. The seriousness of poisoning depend largely on the amount, route and duration of exposure, including age of the person involved (CDC, 2006). Apart from its natural presence in the soil, it has found widespread applications in various spheres of life, especially in agriculture, thereby increasing its possibilities of contaminating food dramatically. Some of its uses in agriculture include pesticides and fumigants, the latter of which are particularly used to prevent pests from attacking stored grain. This may explain its high concentrations in the samples (range: 41 - 1992 µg/g, Fig 4.11) which are only slightly lower than those of Cl, its group VII and more nutrient-friendly counterpart. The concentration of the element also shows a wide variation between samples.

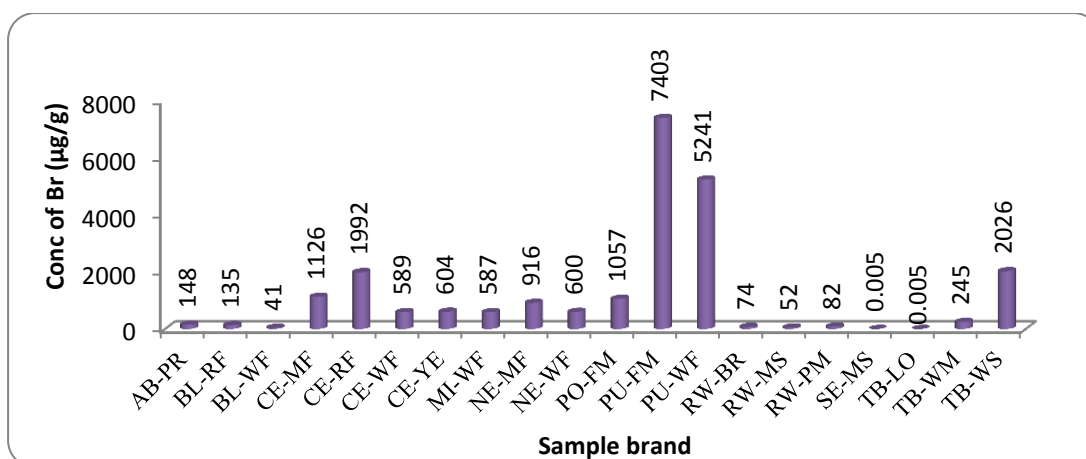


Fig. 4.11: Comparison of Br concentrations in the samples.

This observed variation may also be attributable to the extent of exposure of the grains used as raw materials in the manufacture of those foods to agricultural chemicals containing Br. It was observed for instance that samples BL-WF, RW-MS, RW-BR and RW-PM recorded the lowest concentrations of 41, 74, 52 and 82 µg/g respectively which are much lower than the average of 1273 µg/g by a factor of five. Three of these are of the same brand and local in origin. Two other samples out of the lot, namely SE-MS and TB-LO recorded Br concentrations below the detection limit. It is most likely that these samples have not been exposed to any bromine-based agricultural chemicals.

4.2.7 Manganese

Mn occurred in relatively much lower concentrations in the range 0.84 - 38.94 µg/g (Fig 4.12), with an average of 11.19 µg/g.

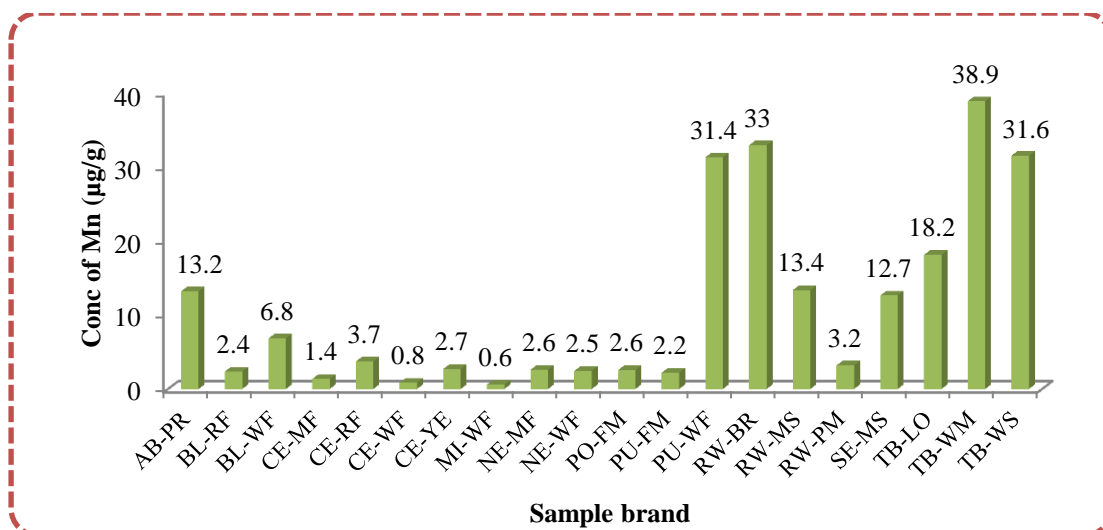


Fig. 4.12: Comparison of Mn concentrations in the samples analysed.

Samples that recorded the highest concentration of Mn were TB-WM (38.94 µg/g), TB-WS (31.56 µg/g), RW-BR (32.99 µg/g) and PU-WF at 31.36 µg/g. Of these samples, three of them have wheat as a component, and all four also contain soya in varying amounts. Wheat and soya happen to have the highest natural amount of Mn compared to the other food categories involved in this work (Wikipedia, 2013), which may be the reason those four samples recorded higher concentrations in comparison to the other samples. Processing may have a negative effect on the Mn content as oddly observed in samples MI-WF and CE-WF which both contain wheat and yet recorded the lowest concentration (0.59 µg/g and 0.84 µg/g respectively). At intermediate level (comparatively), samples TB-LO, SE-MS, RW-MS, all of which have high amounts of soya recorded concentrations of 18.16 µg/g, 12.66 µg/g and 13.39 µg/g respectively. The remaining samples generally had concentrations of Mn below 4 µg/g, with rice and maize (which generally have lower amounts of the element naturally) as the main components.

Mn, a transition metal, has some nutritional value, having been classified as a micronutrient. It is a constituent of several metalloenzymes and serves as an activator

of several other enzymes such as oxidoreductases, lyases, ligases, hydrolases, kinases, decarboxylases and transferases (Nielsen, 2001).

4.2.7.1 Mn concentrations compared with literature data

The average Mn concentration in the samples have been compared with average concentrations obtained in similar studies available in literature (Pandelova et al., 2012; Ljung et al., 2011; Joseph et al., 2011; Jonah et al., 2003). The average Mn concentration obtained in this work is within the range of the average Mn concentrations available in literature (Fig. 4.13).

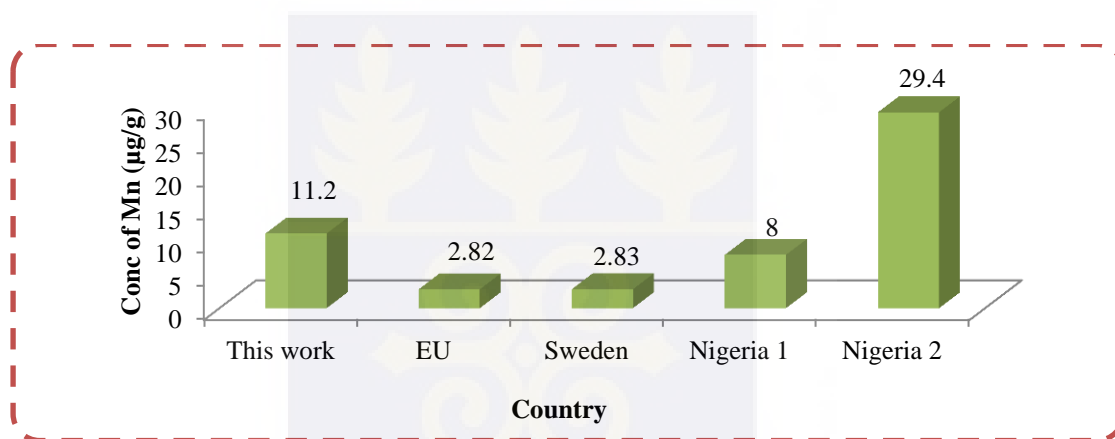


Fig. 4.13: Mean Mn concentrations in the analysed samples compared with those of literature.

4.2.8 Copper

For Cu, the average concentration is 12.0 µg/g, which incidentally compares very well with that of Mn (11.19 µg/g), with a range between 0.83 and 55.80 µg/g. Three distinct concentration categories could also be identified for Cu: high, medium and low, in comparative terms (Fig. 4.14). Samples BL-RF (55.80 µg/g) and RW-BR (41.57 µg/g), both of which are based on rice, recorded the topmost Cu values. Following these are samples BL-WF (23.74 µg/g), CE-MF (23.08 µg/g), MI-WF (22.47 µg/g) and TB-WM (15.55), three of which are based on wheat.

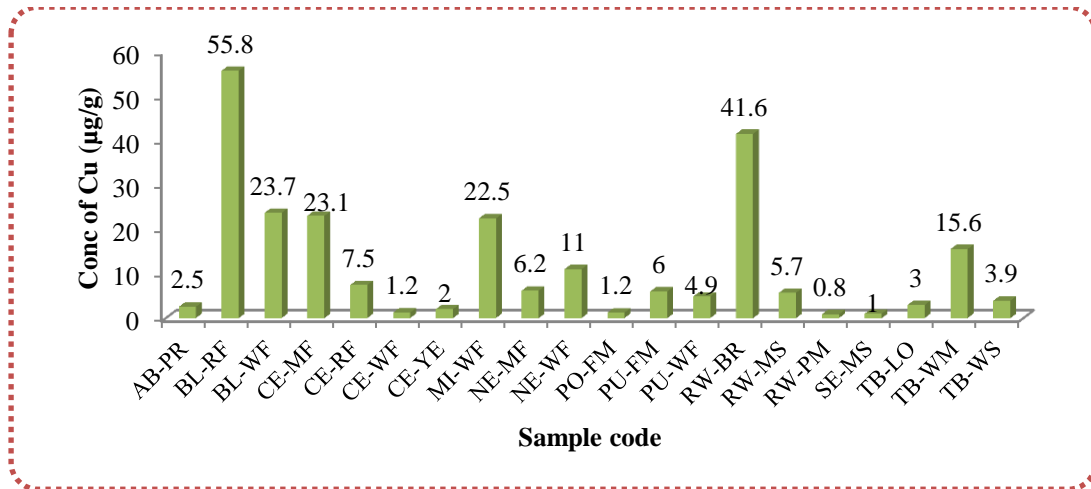


Fig. 4.14: Comparison of Cu concentrations in the samples analysed.

The remaining 14 samples generally have concentrations below 7.5 µg, the least being samples RW-PM and SE-MS (0.83 µg/g and 0.99 µg/g). This last group also have samples based on wheat and rice like the first two. This pattern shows that though the natural amount of the element in the various cereals may have played a role in the recorded amounts within samples, other contributory factors seem to have played a bigger role. Two such factors could be additions from the processing stage and agricultural chemicals to which the cereals were exposed. 15 % of the world’s Cu is said to be used in the manufacture of industrial machines, whilst a smaller percentage is used in making agricultural fungicides (Wikipedia, 2013). Different levels of ‘leakage’ from these sources into the foods may have accounted for the erratic variations observed in the element’s concentration within the samples.

Cu, like Mn is a transition metal regarded as a micronutrient needed in the body at trace amounts for specific functions. Among other things, it is essential for a normal development of the brain (Failla et al., 2001).

4.2.8.1 Copper levels in the samples compared with literature data

Fig 4.15 is a comparison of the mean concentration of Cu obtained in this work with that for other countries available in literature (Pandelova et al., 2012; Ljung et al.,

2011). Results of this work are observed to be much higher than those of the available literature quoted here.

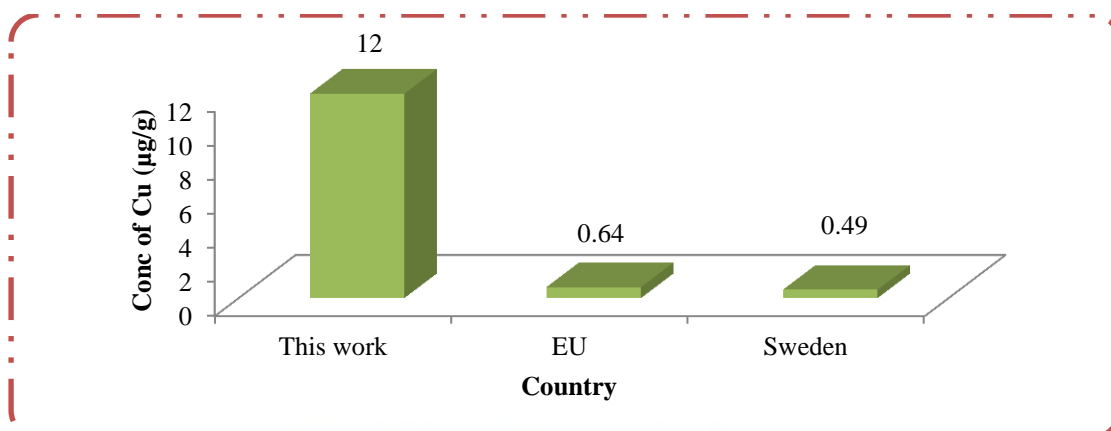


Fig 4.15: Mean concentration of Cu in the analysed samples compared with literature values.

4.2.9 Vanadium

V on the other hand occurred in the food samples with the least concentration, ranging from <10 - 990 ng/g as shown in Fig 4.16, with an average of 220 ng/g. It fell below the instrument's detection limit in five of the samples namely, CE-RF, CE-WF, CE-YF, MI-WF and TB-WS, the first three of which are of the same brand. Interestingly, V occupies the 22nd position in the ranking of elements in the earth's crust (0.015% by weight), beaten only by Cu to the last position among the elements determined (Fleischer, 1953). Its low soil content may explain the generally low concentrations recorded in the samples. However, V as a metal is also alloyed with other metals to make rust resistant, spring and high speed tool steels (ATSDR, 2011). The possibility of contamination of processed food from steel parts therefore is high, leading to possible enhancement of its natural concentration in such foods. This, together with variations in its content in different soils may account for the variation in concentration between samples.

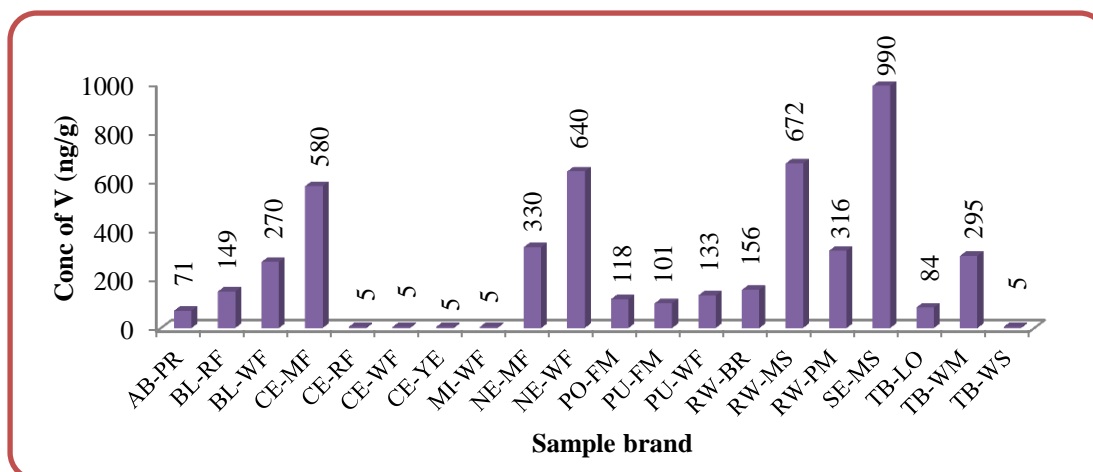


Fig. 4.16: V concentrations in the analysed samples compared.

The highest concentration, it is observed, occurred in sample SE-MS (990 ng/g), followed by RW-MS (672 ng/g), which are about 5 and 3 times higher respectively than the average. Interestingly, these are the only two samples that contain millet; soya being the only other component they have in common. It may be because soils used for millet cultivation are relatively richer in V, leading to relatively higher amounts of it in the millet. It is also possible the elevated amounts of the element in those two samples arose as a result of contamination during processing.

V has been suggested as a nutrient element at micro levels, albeit without a known specific function (UMMC, 2011), and is controversially used as a dietary supplement mainly for increasing insulin sensitivity and body building (Wikipedia, 2013). Though scientists are not sure about the exact role V may play in the human body, one thing they do know for sure and agree on is that high doses of the element could be detrimental, and giving supplements of it to children should be avoided (UMMC, 2011).

4.3 Validation of F AAS method

The elemental concentrations of Sn, Fe, Cr and Zn were determined in the samples using F AAS. The method was validated using NIST SRM 1568a (Rice flour). The

experimental results obtained by analysis of the SRM in comparison with the certified values are provided in Table 4.2. The data in Table 4.2 indicate that the experimental concentrations compare favourably with the certified values. This affirms the reliability of the FAAS method used for the analysis.

Table 4.2: Experimental concentrations of elements in NIST 1568a (Rice flour) compared with certified values.

Element	Concentration ($\mu\text{g/g}$)		% Recovery
	This work*	Certified Value	
Ca	135 ± 14	118 ± 6	114.4
Cu	1.86 ± 0.2	2.4 ± 0.3	77.5
Fe	7.1 ± 0.7	7.4 ± 0.9	96
Mn	18.4 ± 1.2	20 ± 1.6	92
Zn	18.3 ± 1	19.4 ± 0.5	94.3

*Values for this work are presented as the mean \pm standard deviation of three measurements.

4.4 Elemental concentrations in samples determined by FAAS

The results obtained for the elements analysed in the samples using FAAS are presented in Appendix C, Table C2. These are the elements Sn, Fe, Cr and Zn.

All the elements were detected in at least one sample. Details on individual elements are given in the sections that follow.

4.4.1 Tin

The concentrations of Sn in the samples are quite high (the highest among this group of elements), ranging between $<0.03\mu\text{g/g}$ and $92 \mu\text{g/g}$ (Fig 4.17), with an average of $60 \mu\text{g/g}$. It was not detected in one sample, namely RW-BR. For samples in which it was detected, the highest and lowest concentrations occurred in samples TB-WM ($92 \mu\text{g}$) and TB-LO ($36 \mu\text{g/g}$) respectively.

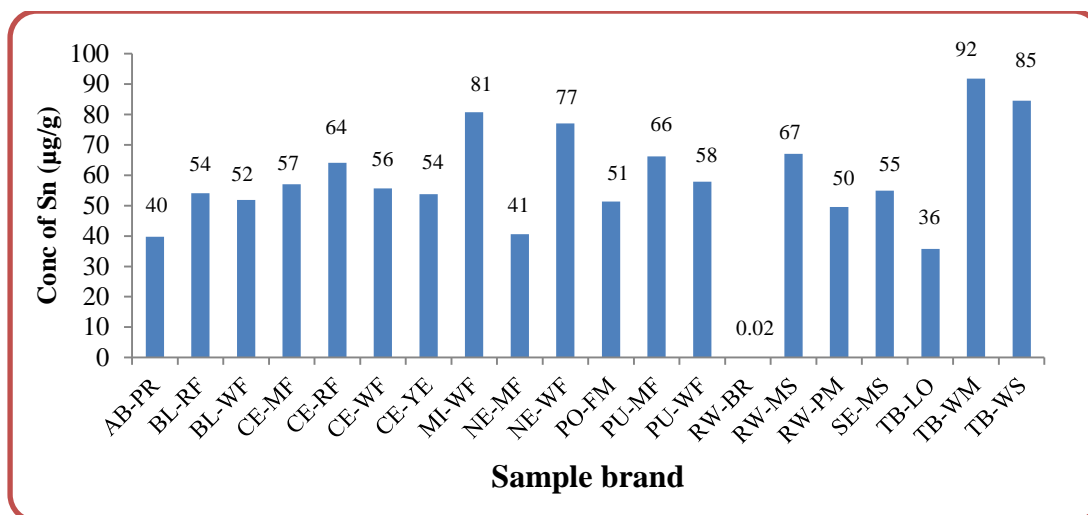


Fig. 4.17: Sn concentrations in the analysed samples compared.

Other samples that recorded relatively high concentrations include TB-WS, MI-WS and NE-WF, with concentrations of 85, 81 and 77 µg/g respectively. Coincidentally, these three samples, together with TB-WM which recorded the highest concentration, all have wheat as the major cereal component. It is also observed that most samples that belong to the same brand displayed Sn concentrations that compared quite well with each other without any significant difference (BL, CE, PU and TB brands). The brands that seem to deviate from this trend are NE and RW. This is evidenced by the sharp difference between the concentrations recorded by samples NE-MF and NE-WF (41 µg/g and 77 µg/g respectively) of brand NE, as well as those between samples RW-BR, RW-MS and RW-PM (<0.03, 67 and 50 respectively) of brand RW.

Though the average amount of Sn in soil is about 2-3 mg/kg (WHO, 2005), some soils could contain as much as 200 mg/kg of the element (ATSDR). Some variations could therefore be expected in the foods on the bases of the Sn content of the soil in which they were grown. Beyond its natural presence in the soil, another avenue by which Sn may find its way into the food is through the use of organotin compounds in materials such as PVC pipes (which could be used for irrigation), polyurethane and plastic

polymers (used for food packaging) and in biocides and pesticides. About 25-30% of commercial tin is used as a coating for other metals, especially food containers (WHO, 2005). These containers contribute by far more to elevated Sn content of foods packaged in such containers.

Sn is largely considered a non-essential element, though some studies with animals seem to suggest possible essentiality (Nielson, 2001; WHO, 2005; ATSDR). Most ingested Sn leaves the body through the faeces and urine within a short time and is therefore not considered to be of serious health concern, though studies have shown that ingestion of large doses may lead to anaemia, stomach aches, kidney and liver problems (ATSDR). Cereals and dairy products have been cited as being very good sources of Sn, which further explains its high concentration levels in the samples (ACU-CELL, 2013).

4.4.2 Iron

Fe, a trace but essential element on the other hand was detected in all the samples at relatively low concentrations in the range 1.07 - 11.78 $\mu\text{g/g}$, with an average of 6.05 $\mu\text{g/g}$. Its concentrations in the samples are compared in Fig 4.18. Sample TB-LO recorded the highest concentration, whilst sample RW-BR recorded the lowest. Out of the 20 samples, 12 of them recorded concentrations below the average stated above, four of which are rice-based. Half of this number has Fe stated as a supplement (Appendix A), either in the form of ferric diphosphate, ferric pyrophosphate or ferrous fumarate.

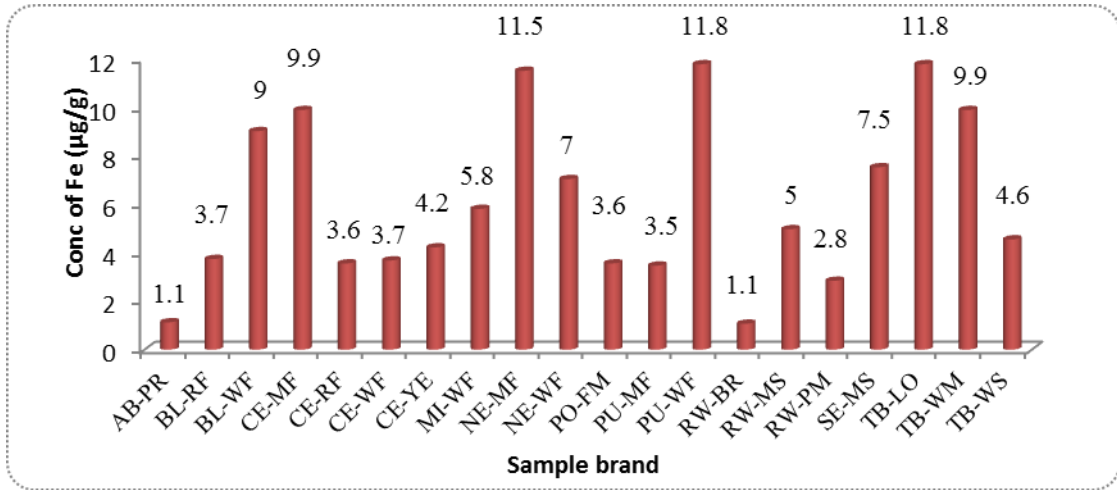


Fig. 4.18: Concentrations of Fe in the analysed samples compared with each other.

Of the other 8 samples whose concentrations are above the average, 5 out of the lot have Fe as a supplement as well. All 8 such samples are also either composed of wheat (four samples), maize (two samples) or millet (two samples), with rice conspicuously missing in this category. Interestingly, TB-LO which recorded the highest concentration does not have any added Fe as a supplement. Thus, whilst the varying levels of Fe supplementation by some of the brand manufacturers may have influenced the concentration levels found in the samples to some extent; it was not the overriding factor accounting for the variations in Fe content of the samples. The natural amounts of the element in different cereals and cereal combinations seem to have played a bigger role. All the four rice-based samples fell below the average Fe concentration, with two of them recording the lowest concentrations among all samples (RW-BR at 1.07 µg/g and AB-PR at 1.13 µg/g). This is notwithstanding the fact that the other two samples have Fe supplementation. This observation seems to suggest that the natural contribution of rice to Fe intake is lower in comparison to the other cereals involved in the analysis.

Soya, a rich source of Fe, appears to have played a significant role in the concentration patterns observed. TB-LO for instance recorded the highest Fe

concentration (11.78 $\mu\text{g/g}$). It however has only three ingredients: maize, soya and salt. In sharp contrast, samples RW-PM (ingredient: only maize) and PO-FM (ingredients: maize, salt) which have no soya recorded such paltry concentrations as 2.84 $\mu\text{g/g}$ and 3.56 $\mu\text{g/g}$ respectively. Indeed, among samples that had no indication of Fe supplementation, those containing soya generally displayed higher Fe concentrations than the rest. Most of the other samples contain traces of soya, which could have influenced the Fe concentrations in those samples as well. Intra brand comparisons do show that the Fe concentration of PU-WF (wheat-based) is almost thrice that of its maize-based counterpart, PU-MF. Also, wheat-based sample BL-WF displayed a concentration that is twice that of its rice-based colleague, BL-RF; and among the four CE brand samples, the maize-based CE-MF is the only odd one, with a concentration that is more than twice that of the other three, which are almost the same.

4.4.2.1 Comparison of Fe concentrations with MQVs

Samples whose Fe concentrations had been provided by the manufacturers on their containers have been compared with results obtained in this study (Fig 4.19). The MQVs are generally observed to be higher than the experimental results. This might have resulted from losses from time of manufacture to the time of retail.

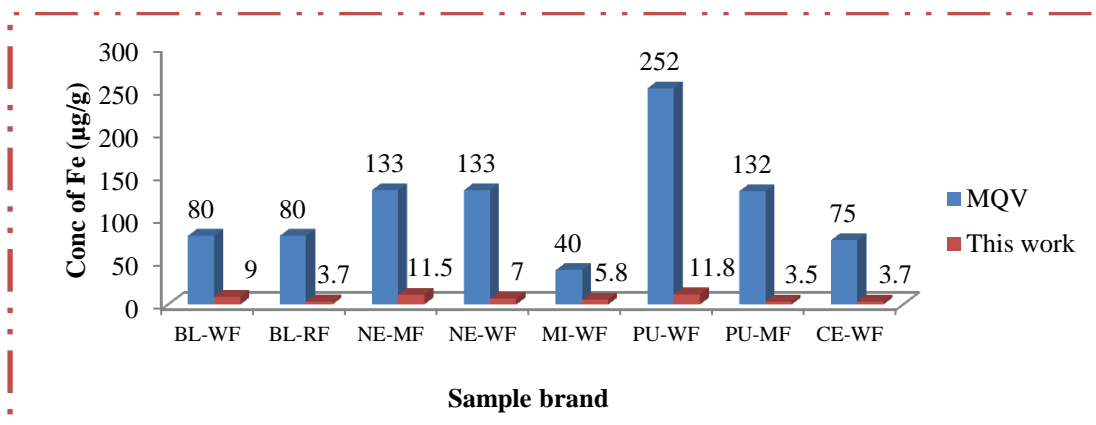


Fig. 4.19: Fe concentrations in some of the samples compared with the MQVs.

4.4.2.2 Fe concentrations compared with literature values

The mean Fe concentration obtained in this work was compared with available literature data from other countries (Pandelova et al., 2012; Ljung et al., 2011; Jonah et al., 2003) [Fig 4.20]. Whilst the results are very close to those of Sweden, they are much lower than those obtained in Nigeria and the EU.

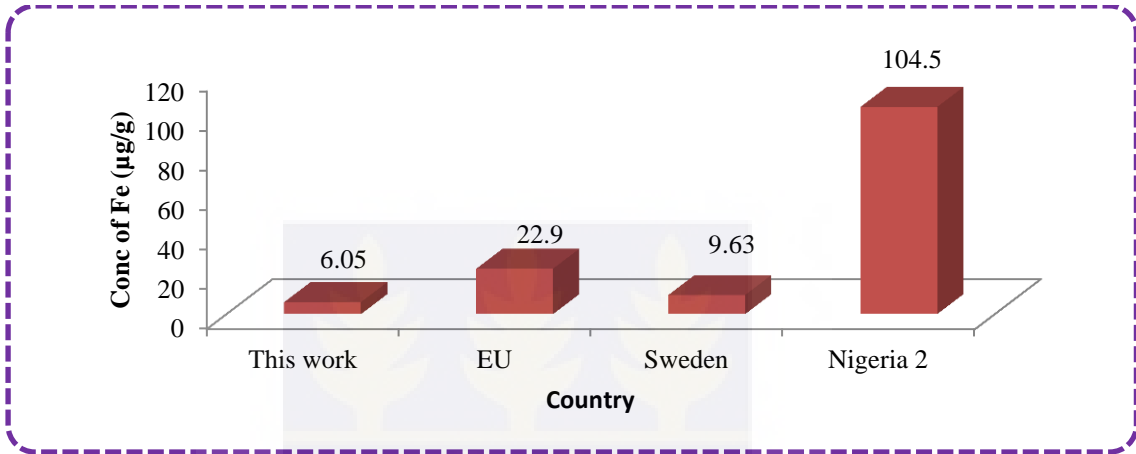


Fig. 4.20: The mean Fe concentration in the analysed samples compared with literature data.

4.4.3 Zinc

Zn, another trace but essential element, was detected in all the samples. Its concentration in the samples averaged 3.20 µg/g, ranging between 0.50 µg/g in sample RW-PM and 7.17 µg/g in sample NE-WF (as reflected in Fig 4.21).

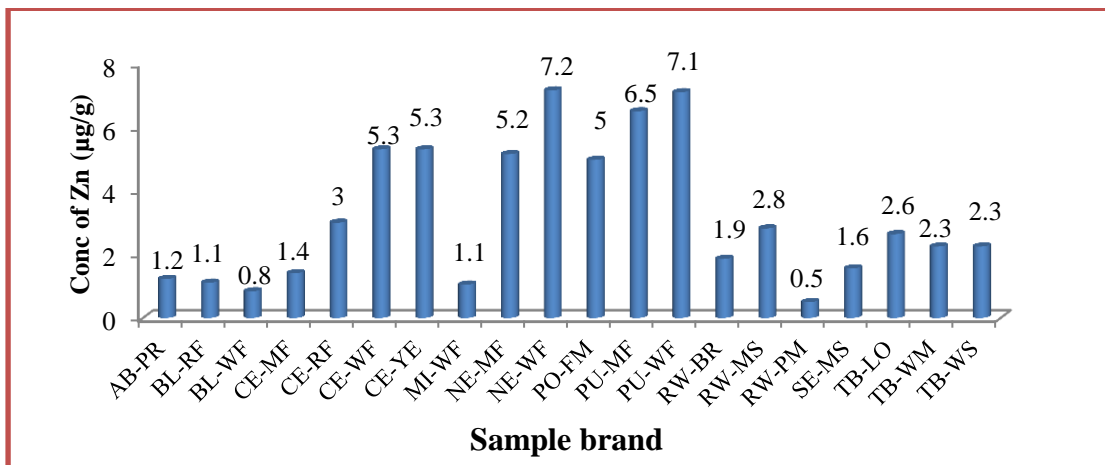


Fig. 4.21: Concentrations of Zn in the analysed samples compared with each other.

Only seven of the samples, namely CE-WF, CE-YF, NE-MF, NE-WF, PU-MF PU-WF and PO-FM had their Zn concentrations above the average. The first six of these samples have Zn supplementation which seems to explain their relatively higher concentrations. Only two other samples have an indication of Zn as added supplement, yet fell below the average concentration, possibly due to higher losses from their natural content during processing. These are samples CE-MF and CE-RF. PO-FM is the only sample without Zn supplementation that has Zn content above the average.

Of the other eleven samples that do not have Zn supplementation, though their Zn concentrations are below the average, it is observed that those with soya as an ingredient displayed values that are a notch higher than other samples of similar cereal composition. Samples TB-WS and TB-WM for instance, which both have wheat and soya as major components, have the same concentration of 2.25 $\mu\text{g/g}$, which is about three times higher than that of samples BL-WF (0.84 $\mu\text{g/g}$) and MI-WF (1.05 $\mu\text{g/g}$), which also have wheat but only a trace of soy lecithin and no Zn supplement. Sample TB-LO (2.64 $\mu\text{g/g}$) is also about five times higher than that of sample RW-PM (0.50 $\mu\text{g/g}$) in Zn content. Whilst the former is made of maize and soya, the later has only maize, but no soya. In the same vein, both samples AB-PR and BL-RF are also based on rice, without soya or Zn supplement, and their Zn concentrations fall below that of RW-BR, also rice-based, but with soya. The same could be said for samples SE-MS and RW-MS, both having millet and soya as their components and only a small difference between their Zn contents.

It is clear from the forgoing that the soya content of the samples had an influence on the Zn content. Intra brand comparisons show that most of the samples that belong to the same brand displayed no significant difference in the element's concentration.

4.4.3.1 Zinc concentrations in the samples compared with MQVs

A comparison between the experimental results obtained for Zn and the MQVs is presented in Fig 4.22. The MQVs are generally observed to be higher than the experimental results.

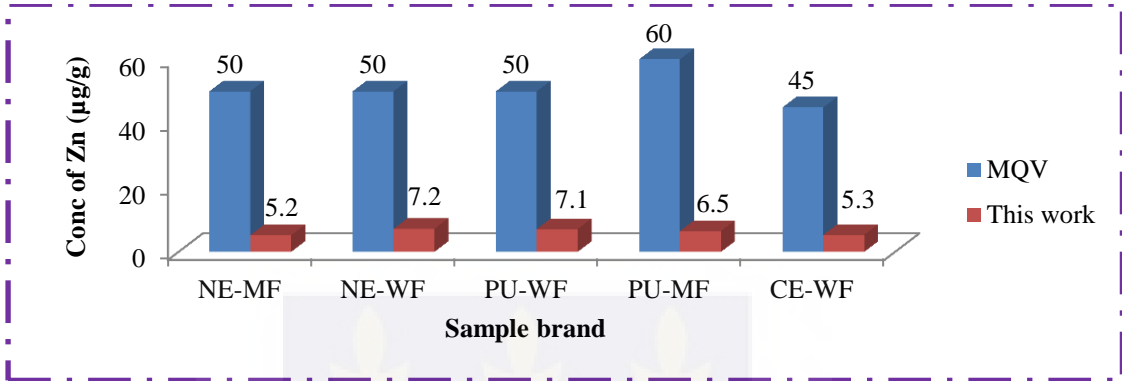


Fig. 4.22: The Zn concentration of some of the analysed samples compared with MQVs.

4.4.4 Chromium

The average concentration of Cr in the samples is 0.60 µg/g, ranging from <0.03 to 1.23 µg/g. It was below the detection limit in three samples. These are samples coded CE-RF, MI-WF and PU-MF. The concentrations are displayed in Fig 4.25 by way of comparison between the various samples analysed. The 17 samples with detectable Cr varied in their Cr levels by a factor of 10.

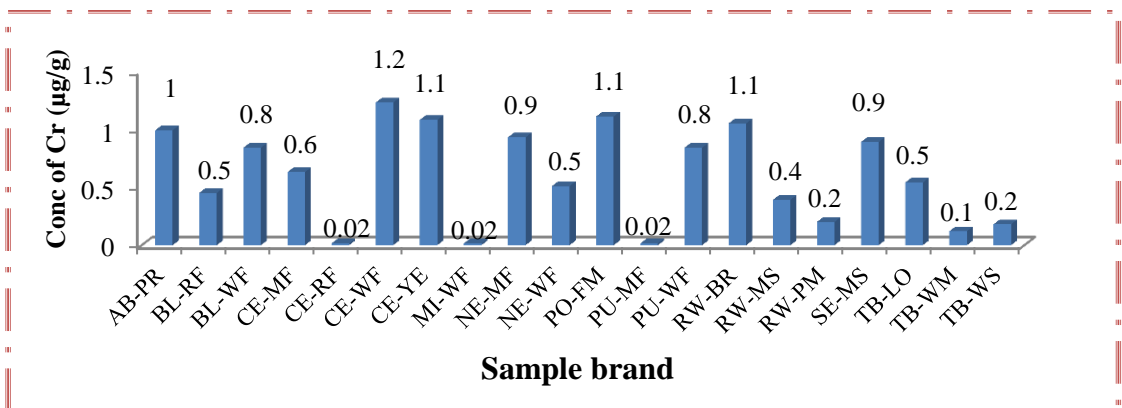


Fig. 4.23: Cr concentrations in the analysed samples compared with each other.

Ten samples had concentrations above the average, and an equal number were below the average. This together with the high variation factor of 10 further stresses the wide spectrum of concentrations displayed by the samples. No clear cut pattern is observed as different brands and cereal components are seen to swing from one end of the concentration spectrum to the other. The highest concentration of 1.23 $\mu\text{g/g}$ for instance was recorded by sample CE-WF (wheat-based), followed by a maize-based sample PO-FM (1.11 $\mu\text{g/g}$), another wheat-based sample CE-YE (1.08 $\mu\text{g/g}$) and then a rice-based sample RW-BR (1.05). These concentrations do not show any significant differences. Samples that recorded Cr concentrations below the limit of detection of the instrument were also based on these aforementioned cereals. The lowest concentrations of the element were also recorded in samples that were wheat-based, namely TB-WS (0.18 $\mu\text{g/g}$) and TB-WM (0.12 $\mu\text{g/g}$). So, whilst the highest Cr concentration occurred in a wheat based sample, the lowest concentration at the opposite end also occurred in a wheat-based sample.

Vegetables, fruits and grains are generally said to be good sources of Cr, albeit at varying levels, with whole grains tending to supply more of the element than refined products (Stoecker, 2001). Whilst the loss of Cr in the process of refining sugar has been reported, Processing on the other hand may lead to addition of Cr to the supply chain (Stoecker, 2001). Its content in foods is further affected substantially by agricultural and manufacturing processes, thereby making its dietary intake assessment quite difficult (NIH, 2005). The element's soil content also show a wide variation (1-3000 $\mu\text{g/g}$), which can be reflected in its natural content in foods depending on the Cr richness level of the soil in which they are grown. These combination of factors may be the reason behind the haphazard nature of the Cr concentrations within the samples analysed and the wide variation factor observed.

Cr is considered a micro mineral that plays a vital role in fat, carbohydrate and protein metabolism and storage by increasing tremendously, the effectiveness of insulin, the hormone responsible for the regulation of blood sugar levels (Stoecker, 2001; NIH, 2005). Studies have also shown that Cr has a positive effect on the growth of children and the repair of glucose intolerance (Stoecker, 2001).

Only a small amount of ingested Cr is absorbed from the intestinal tract (about 0.4% to 2.5%), the remainder leaving the body via egestion in the faeces. Some nutrients, including vitamin C and Niacin (a B complex vitamin) tend to enhance its absorption by the body. Absorbed Cr is typically stored in the liver, spleen, soft tissue and bone (NIH, 2005).

4.5 Validation of the HGAAS method used to determine As, Se and Hg.

The HG AAS method, which was used to determine the concentrations of Se, As and Hg in the samples was also validated using the standard reference material NIST SRM 1568a (Rice flour). Certified concentration values of the two elements As and Se in the SRM are compared with those obtained in this work in Table 4.3 below. The two sets of values are in good agreement, thereby certifying the validity and reliability of the method.

Table 4.3: Concentrations of Se and As in NIST 1568a (Rice flour) compared with certified values

Element	Concentration ($\mu\text{g/g}$)		
	This work*	Certified Value	% Recovery
Se ($\mu\text{g/g}$)	0.41 ± 0.06	0.38 ± 0.04	108
As ($\mu\text{g/g}$)	0.26 ± 0.03	0.29 ± 0.03	89.7

*Values for this work are presented as mean \pm standard deviation of three measurements.

4.6 Concentrations of Se, As and Hg in the samples

The elemental concentrations obtained for the three elements in the samples are presented in Appendix C, Table C2. Whilst Se occurred in all the samples, in sharp contrast, As and Hg were not found at detectable levels in any of the samples.

4.6.1 Selenium

Se, though appearing in all the samples, generally had concentrations below 0.5 $\mu\text{g/g}$ (Fig 4.21). Sample to sample variation mostly does not present anything of significance, the highest value (0.32 $\mu\text{g/g}$) and the lowest value (0.15 $\mu\text{g/g}$) differing only by a variation factor of about 2, the average being 0.20 $\mu\text{g/g}$. Whatever differences that are observed in the Se concentrations of samples could largely be because of variations in the Se content of the different soils in which the foods were grown. A closer look at the data shows that the wheat-based and maize-based samples generally recorded concentrations slightly higher than the millet and rice-based ones. This is reflected in the concentrations of samples BL-WF, BL-MF, CE-WF, CE-YE, MI-WF, PO-FM,

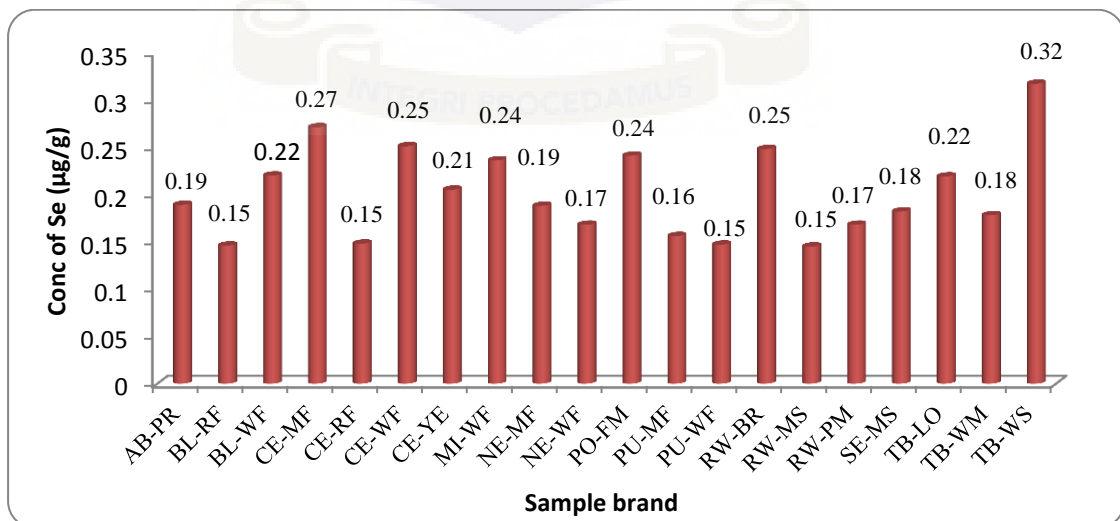


Fig. 4.24: Se concentrations in the samples compared.

TB-LO and TB-WS, recorded concentrations above the average and are either wheat or maize-based. Conversely, only one rice-based sample, RW-BR recorded a concentration above the average, whilst all the remaining rice-based and millet-based samples fell below the average in their Se content. The poorest performers in this category also recorded the lowest concentrations overall among all samples, namely RW-MS (0.15 µg/g) and BL-RF (0.15 µg/g). Again, in brands that involved at least two different cereal foods, the rice-based ones have been observed to lag behind their maize and wheat-based counterparts. The presence of soya in some of the foods, though is expected to contribute some Se as well, does not appear to be the determining factor in the observed differences.

Literature reveals that wheat is a much richer source of Se by several folds above rice, maize, millet and soya. Second to wheat, though far behind, is rice. That the highest Se concentration occurred in a wheat-based sample, and that wheat-based samples generally outweigh the others in Se content as reflected in the above discussion seems to be in order. Rice however presents something of a surprise by falling behind maize in the Se concentrations determined for the samples, something that is unexpected. The explanation for this seems to lay in how refined the rice is. The only rice-based sample (RW-BR) that recorded a concentration above the average for instance is a local product made from unpolished rice, whilst the others are foreign products made from polished rice, which, together with processing process might have led to some Se losses.

Se as a micro nutrient element is said to possess such exciting health benefits as: anticarcinogenic activity, protection against oxidative damage and aging, good effect on reproduction, protection against nutritional forms of muscular dystrophy, antitoxic effect on some toxic elements, and even considered a possible nutrient for treatment

strategies for acquired immune deficiency syndrome (AIDS) (Sunde, 2001; NIH, 2005). In addition, the numerous unique nutritional, biochemical and molecular biological properties make it a continuous target for nutrition research (Sunde, 2001; NIH, 2005).

Despite its nutritional usefulness however, the element also can be toxic at certain high levels of intake, though the fundamental biochemical mechanism involved in this behaviour of toxicity is not known. The dietary range of Se that is adequate for good health and not toxic is said to be narrow, and modest intakes of <800 µg/day for humans are generally considered not to be toxic (Sunde, 2001). Consumption of gram amounts may however lead to serious gastrointestinal and neurological disturbances, acute respiratory distress syndrome, myocardial infarction and renal failure. Continuous intake of excess Se in the range of 3200 to 6700 µg/day may also bring to fore toxicity signs such as lesions of the skin and nervous system, mottling of the teeth, diarrhoea, nausea and weakness (Sunde, 2001).

4.6.1.1 Selenium levels compared with MQV and literature data

The Se content of only one of the samples analysed was quoted by its manufacturer. This is sample coded PU-MF with a MQV of 0.48 µg/g which is higher than the experimental result (0.16 µg/g). The mean Se concentration has also been compared with available literature values (Fig 4.27) in similar studies by Pandelova et al (2012) and Ljung et al (2011).

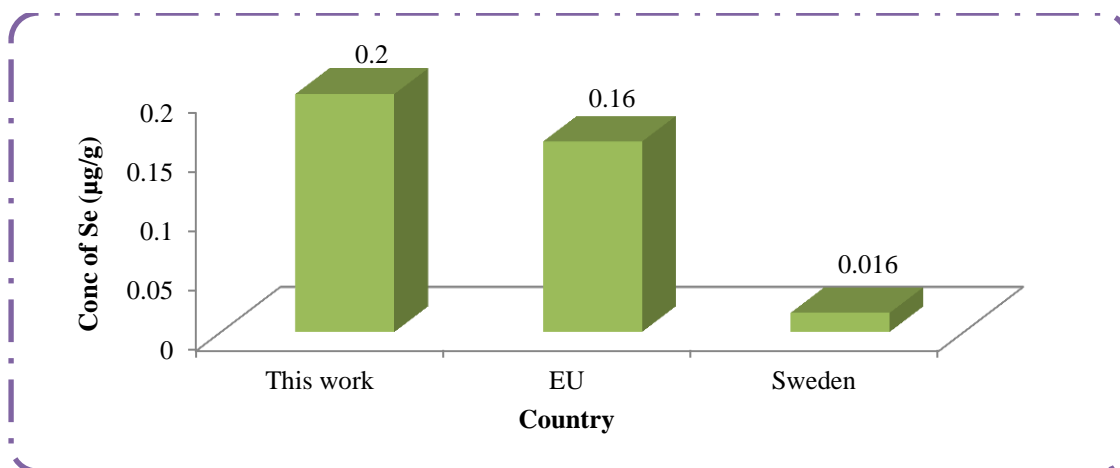


Fig. 4.25: Comparison of the mean Se concentration obtained with literature values.

4.6.2 Arsenic

The As concentration was less than 0.025 µg/g in all the samples analysed. An average As concentration of 0.0171 µg/g was obtained by Ljung et al (2011) in a similar work in Sweden. Out of over 100,000 food commodities from 15 European countries analysed for As, only 33 % contained As at detectable levels, the highest concentrations occurring in fish, sea foods and cereal and cereal-based products, especially rice (EFSA, 2009). Depending however, on the type of food processing used, temperature and time, changes on total As concentration may occur (EFSA, 2009). There are only four samples based on rice in this current study, which on the face of the results obtained do not exhibit any marked difference from the other samples. A Consumer Reports study of rice and rice products meant for consumption by all age groups in the US also found As at detectable levels in most of the products but at low concentrations and within safe limits (CNN Health, 2012).

In humans, soluble inorganic As is quickly and almost completely absorbed after ingestion. Absorption of different organic As compounds is also generally greater than 70 %. After absorption, arsenic is widely distributed to almost all organs and readily crosses the placental barrier (EFSA, 2009). Skin lesions, cancer,

developmental toxicity, neurotoxicity, cardiovascular diseases, abnormal glucose metabolism, and diabetes are the main adverse effects suffered from long term exposure to inorganic As. Evidence is further emerging of its negative impact on foetal and infant development (EFSA, 2009).

4.6.3 Mercury

The Hg concentration recorded in all the samples was below 0.025 µg/g. Pandelova et al (2012), in a similar study, obtained a Hg concentration of 0.0092 µg/g for the EU market. The paucity of data on the elemental content of cereal-based weaning foods makes it difficult for further comparisons. The concentration of Hg in earth's crust is generally low, with an average value of 0.08 µg/g (Navrátil and Minarík, IG, Prague), which invariably will reflect on food grown on the soil. Elevated levels of the element in a particular environment and hence the food may however occur through anthropogenic activities. The results obtained may be a reflection of the fact that the foods analysed have not been exposed to contamination through the latter means.

Hg is generally distributed throughout the body when absorbed, binding to sulfhydryl groups in many proteins. Methylated Hg targets mainly the brain and red blood cells (Navrátil and Minarík, IG, Prague). Hg, in particular methyl mercury can negatively affect brain development of infants and can also cause neurological changes in adults (EFSA, 2004). Hg present in foods other than fish and other seafood exist mainly in the less toxic form of inorganic mercury (EFSA, 2004).

4.7 Validation of extraction and determination of Pb using FAAS.

The concentrations of Pb in the samples were determined by FAAS following solvent extraction with dithizone. For the purposes of recovery studies and validation of the method, NIST SRM 1547 (Peach leaves) was analysed under the same experimental

conditions. The experimental concentration of Pb in the SRM is compared with the certified value in Table 4.4. The dithizone extraction-FAAS method for Pb determination yielded a good mean recovery of 91.95% (Table 4.4). This outcome provides a good assurance that the extraction procedure used, and the FAAS method with which the samples were analysed for Pb, are reliable and hence the obtained results are valid.

Table 4.4: Concentration of Pb in NIST 1547 (Peach leaves) compared with the certified value.

Element	Concentration ($\mu\text{g/g}$)		
	This work*	Certified Value	% Recovery
Pb	0.80 ± 0.06	0.87 ± 0.07	91.95

*The value for this work is presented as mean \pm standard deviation of three measurements.

4.8 Concentrations of Pb in the samples

Pb, determined by FAAS after solvent extraction with dithizone as ligand and chloroform as extractant, was detected in all the samples at low concentrations. The results are presented in Appendix C, Table C2. A comparison of the results in all samples is presented in Fig 4.26. The average concentration is 47 ng/g with a range of 93 to 25 ng/g; representing a variation factor of almost four. The highest concentrations occurred in samples RW-PM (93 ng/g), NE-WF (85 ng/g) and NE-MF (75 ng/g), the last two samples being of the same brand. The lowest concentrations also occurred in samples RW-MS, CE-WF, MI-WF, TB-LO and CE-WF; the first three had the same concentration of 25 ng/g, whilst the last two recorded slightly higher concentrations of 27 ng/g and 28 ng/g respectively.

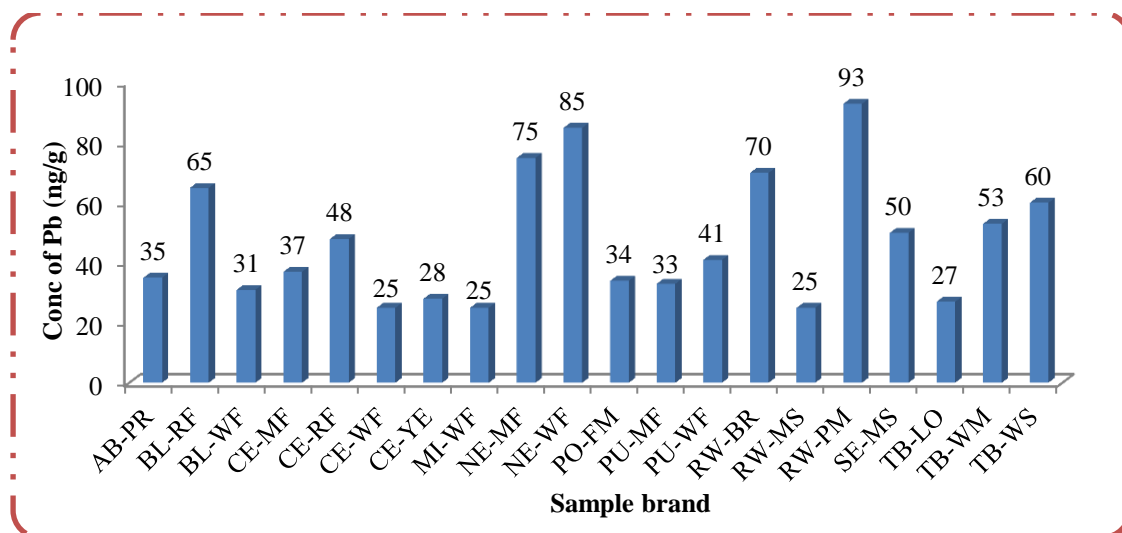


Fig. 4.26: Comparison of Pb concentrations in the various samples analysed.

Both inter-brand and intra-brand comparisons show quite wide variations in the element's concentration within the samples. This may reflect a wide variation in exposure levels of the element to the different samples. Its natural levels in the soil is low, although anthropogenic activities both past and present (fossil fuels, lead-based paint and industrial facilities and products) could enhance this natural amount and ultimately exposure level to food (USEPA, 2012).

Lead can have an adverse effect on almost every organ and organ system in the body, with children 6 years and younger being the most susceptible (USEPA, 2012). The main target for lead toxicity in children is the nervous system. Even very low levels of lead in the blood of children can result in: permanent damage to the brain and nervous system, leading to behaviour and learning problems, lower IQ, and hearing problems; slowed growth and anaemia (USEPA, 2012).

4.8.1 Comparison of Pb concentration with literature data

The mean Pb concentration obtained in this work has been compared with those available in literature for other countries. The mean Pb concentration in this work is higher than those of the EU and Sweden, but lower than that of Pakistan (Fig 4.29).

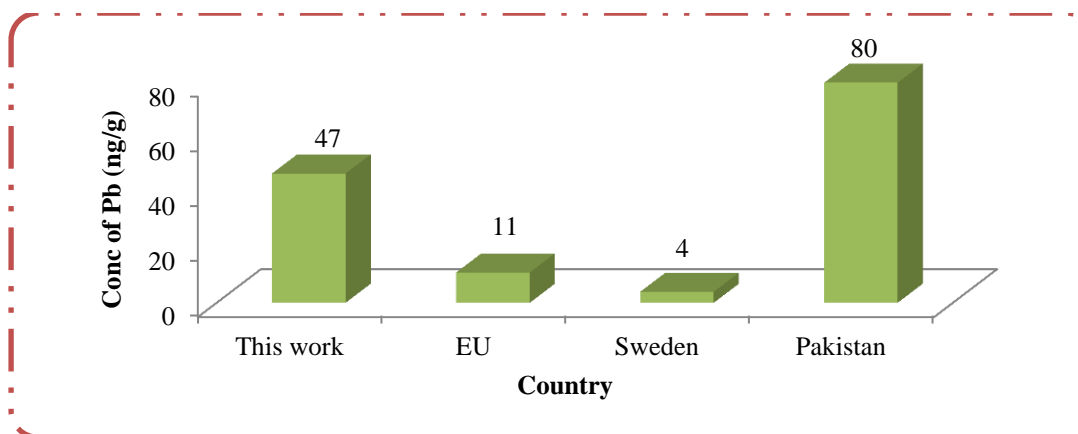


Fig. 4.27: Comparison of mean Pb concentration in this work with literature data.

4.9 Estimated dietary intake of the essential elements determined

Using the concentrations obtained and the manufacturers' recommended serving per day, an estimate has been made of the daily essential element intakes from consumption of the foods analysed (Ljung et al., 2011). Details of the calculated daily intakes of essential elements for each sample have been provided in Appendix C, Table C3. A comparison of the average daily intakes of the essential elements with recommended daily intake (RDI) values are presented in Table 4.5. With the exception of Se and Cu, all the essential elements displayed daily intake values below recommended figures for all the samples. In the case of Se and Cu, though the average daily intakes are above the recommended values, some samples do display intakes below such recommended values.

For Se, 6 samples have intake values below the RDI, and 14 samples have intake values above the RDI. However, the average intake, and the intake in some samples though above the RDI, fall way short of the tolerable upper intake level (UL) by 74 % and 58 % respectively. The UL is the intake value that is deemed safe, though it may be above normal body requirements.

What is observed for Cu however, is a reverse of that of Se. whilst 14 samples have intake levels below the RDI, only 6 samples have intakes greater than the RDI. For this element, although the average intake falls shy of the UL (1 mg/day), some of the samples overshoot it, the highest of these by 279 %. They however, fall below a revised UL of 10 mg/day set by the US Food and Nutrition Board in 2001 [which was based on a critical endpoint of liver damage] (Failla et al., 2001). Chronically, elevated intakes of Cu may predispose some groups to accumulate toxic levels of it in the liver. A case in point is the suggestion of increased risk of Cu toxicity in infants due to immaturity of the biliary excretory pathway and higher efficiency of absorption of the element (Failla et al., 2001). The use of Cu contaminated water to prepare infant formula and foods stored in brass vessels were often linked with childhood cirrhosis diagnosed in Indian children with toxic levels of Cu accumulation in the liver (Failla et al., 2001).

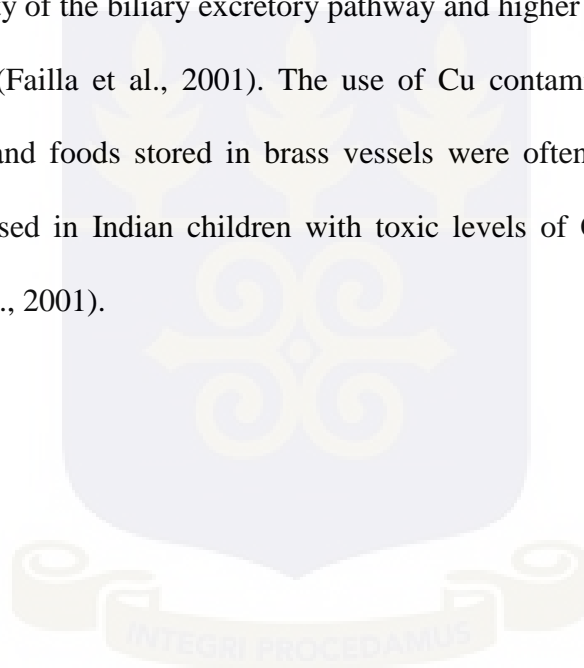


Table 4.5: Daily intake of essential elements compared with recommended values

Element	Average DI	Range of DI	RDI	Reference	UL	Reference
Na	0.032 g/day	0.001-0.121 g/day	0.6-3.5 g/day	EFSA, 2006	-	-
Cl	0.126 g/day	0.011-0.42 g/day	0.6-3.5 g/day	EFSA, 2006	-	-
Ca	62 mg/day	27-134 mg/day	400 mg/day	FAO/WHO, 2001	-	-
Mg	11.12 mg/day	1.52-24.3 mg/day	53 mg/day	FAO/WHO, 2001	250 mg/day ^(a)	EFSA, 2006
Mn	0.82 mg/day	0.06-2.96 mg/day	1-10 mg/day ^(b)	EFSA, 2006	-	-
K	0.17 g/day	0.004-0.51 g/day	4.7 g/day	EFSA, 2006	-	-
Se	15.8 µg/day	8.4-25 µg/day	10 µg/day	FAO/WHO, 2001	60 µg/day	EFSA, 2006
Fe	0.45 mg/day	0.08-0.99 mg/day	10 mg/day	FAO/WHO, 2001	-	-
Cu	0.91 mg/day	0.06-3.79 mg/day	0.9 mg/day ^(c)	EFSA, 2006	1 mg/day ^(d)	EFSA, 2006
Cr	46.2 µg/day	0.9-123 µg/day	^(e)	-	-	-
V	12.2 µg/day	0.5-75 µg/day	1.8 mg/day ^(f)	EFSA, 2006	-	-
Zn	0.235 mg/day	0.04-0.53 mg/day	5 mg/day	FAO/WHO, 2001	7 mg/day ^(g)	EFSA, 2006

^(a) For children four years and above.

^(b) No formal RDA currently; estimated acceptable range of intake by the scientific committee for food (SCF) of the EU (EFSA, 2006).

^(c) RDI set by the US food and nutrition Board for Adults.

^(d) For children 1 to 3 years of age.

^(e) Currently, there is no formal Recommended Dietary Allowance (RDA) for chromium. The US Food and Nutrition Board derived Adequate Intakes (AI) for chromium for different age groups (35 µg/day and 25 µg/day for 19 to 50 year old men and women, respectively). The Societies for Nutrition of Germany (DGE), Austria (ÖGE), and Switzerland (SGE), also jointly established an adequate daily intake of 30-100 µg/day for adults (EFSA, 2006).

^(f) No RDA provided; maximum intake recommended by the food and nutrition board (FNB) [EFSA, 2006].

^(g) For children 1 to three years of age.

DI: Daily Intake. RDI: Recommended Daily Intake. UL: Upper Limit of Intake

4.10 Estimated intake of the non-essential elements determined

Details of the estimated intakes of the determined non-essential elements by consumption of all the samples analysed have been provided in Appendix C, Table C4. The average intake of each element has also been compared with Provisional Tolerable Weekly Intake (PTWI) values recommended by the Joint FAO/WHO Expert Committee on Contaminants and Food Additives (JECFA). These are provided in Table 4.6. Since the PTWIs are provided in units of mg or $\mu\text{g}/\text{kg}$ bw/week, in order for a meaningful comparison to be made, the intakes from the analysed foods were also converted to mg or μg intake per kg bw per week as the case may be, assuming an average mass of 7 kg for an infant (Kazi et al., 2010).

All the non-essential elements determined, with the exception of Br, displayed intake values below the recommended safety guideline limits in all samples. For Br, there was no available safety limit provided to aid a meaningful comparison and subsequent determination of the safety of the foods with regards to its presence. This notwithstanding, its concentration range (41 – 1992 $\mu\text{g}/\text{g}$) should be a matter of concern as it could translate to a high intake and subsequently toxicity symptoms (irritation of the mucous membrane and tissues); especially so as these negative health effects primarily depend on the amount of it that is ingested, duration of exposure as well as the age of the individual involved, thereby making infants more vulnerable. One consolation though is that Br does not accumulate in the body as do heavy metals, and therefore has no known long term acute health effects. This is opposed to the other elements (As, Pb and Hg), which can accumulate in the body and generally have long term acute toxicity symptoms. As a result, although their concentrations are generally low and below the recommended safety guidelines, their complete absence in the food is more desirable.

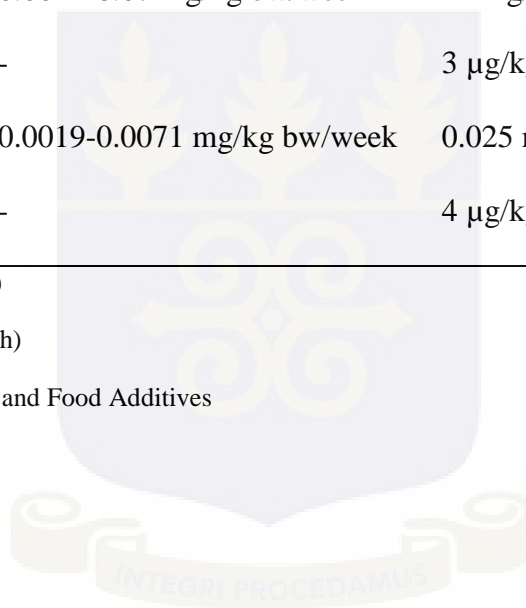
Table 4.6: Intake of non-essential/potentially toxic elements compared with recommended limits of intake.

Element	Average intake	Range of intake	PTWI/PTMI	Reference
Sn	4.39 mg/kg bw/week	0.0011-8.07 mg/kg bw/week	14 mg/kg bw/week	JECFA, 2005
As	< 1.93 µg/kg bw/week	-	3 µg/kg bw/week	JECFA, 2010
Pb	0.0035 mg/kg bw/week	0.0019-0.0071 mg/kg bw/week	0.025 mg/kg bw/week	JECFA, 2010
Hg	< 1.93 µg/kg bw/week	-	4 µg/kg bw/week	JECFA, 2010

PTWI: Provisional Tolerable Weekly Intake (mg/kg bw/week)

PTMI: Provisional Tolerable Monthly Intake (mg/kg bw/month)

JECFA: Joint FAO/WHO Expert committee on Contaminants and Food Additives



CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The main objective of the study was to assess the beneficial health effects and risks that infants aged 6 months to three years are exposed to in the consumption of cereal-based weaning foods commercially available on the Ghanaian market. This was achieved through the determination of the levels of essential elements (Ca, Cl, Cr, Cu, Fe, K, Mg, Mn, Na, Se, V and Zn) and non-essential elements (As, Br, Hg and Sn) in the cereal-based weaning foods using INAA and AAS. In addition, the levels of Pb in the weaning foods were assessed by AAS after extraction of Pb as the dithizonate with CHCl_3 . Furthermore, the study compared the levels of the elements with manufacturer's quoted values as well as literature data. The dietary intakes of the elements were compared to intakes recommended by WHO, EFSA and FAO.

The levels of the essential elements ($\mu\text{g/g}$, in ranges) were: Ca (405-2002), Cl (158-5521), Cr (<0.03 -1.23), Cu (0.83-55.80), Fe (1.07-11.78), K (49-3845), Mg (20-280), Mn (0.84-38.94), Na (13-1588), Se (0.145-0.317), V (<0.01 -0.990) and Zn (0.50-7.17). For the non-essential elements, the levels obtained ($\mu\text{g/g}$, in ranges) were: As (< 0.025 in all samples), Br (<0.17 -402), Hg (< 0.025 in all samples) and Sn (35.8-91.8).

Recovery studies conducted using certified reference material, NIST 1547 (Peach leaves) as the dithizonate with CHCl_3 followed by FAAS gave an average recovery of 91.95 %. The high recovery indicates the reliability of the determination of Pb using solvent extraction with dithizone followed by FAAS. The concentration range obtained in the samples for Pb was 0.025-0.093 $\mu\text{g/g}$.

The average Ca concentration (774 $\mu\text{g/g}$) obtained in this work compared well with Ca concentrations (814 and 1100 $\mu\text{g/g}$ respectively), obtained in similar studies in Sweden and the EU. However, the mean concentration of Ca obtained in this study was approximately six times lower than levels (4372 and 4535 $\mu\text{g/g}$) obtained in two separate studies carried out in Nigeria. The average Mg concentration obtained in this work (146 $\mu\text{g/g}$) also compared favourably with levels (153 $\mu\text{g/g}$) obtained in a study conducted in Sweden. Interestingly, the mean Mg level obtained in this study was markedly different from concentrations (327 and 933 $\mu\text{g/g}$) reported for Nigeria in two separate studies.

For Mn, the average concentration of 11.2 $\mu\text{g/g}$ obtained in this study was generally within the range (2.82 – 29.4 $\mu\text{g/g}$) reported in scientific literature from similar studies carried out in Sweden, EU and Nigeria. The average concentration of Fe obtained in this work (6.1 $\mu\text{g/g}$) was generally found to be lower than concentrations (9.63, 22.9 and 104.5 $\mu\text{g/g}$ respectively) reported from similar studies in Sweden, the EU and Nigeria. Whilst the average Se concentration in this work (0.2 $\mu\text{g/g}$) agreed well with that of a similar work in the EU (0.16 $\mu\text{g/g}$), it was however found to be much higher than that of Sweden (0.016 $\mu\text{g/g}$). For Pb, the average concentration (0.047 $\mu\text{g/g}$) obtained in this study was also generally within the range of similar Pb concentrations obtained for Sweden, the EU and Pakistan (0.004 $\mu\text{g/g}$, 0.011 $\mu\text{g/g}$ and 0.08 $\mu\text{g/g}$ respectively). The average concentration of Cu (12 $\mu\text{g/g}$) however was found to be higher than the average levels of Cu (0.64 $\mu\text{g/g}$ for the EU and 0.49 $\mu\text{g/g}$ for Sweden) reported in scientific literature by 19 and 24 times respectively.

For Ca, Fe, K, Mg, Na, Se and Zn, the concentrations obtained in this work were generally found to be much lower than the manufacturers quoted values. Their concentration ranges ($\mu\text{g/g}$) compared with the manufacturer's quoted values are as

follows [(This work); (manufacturer's quoted value)]: Ca [(398-1026); (3230-4800)], Fe [(3.47-11.78); (40-252)], K [(98-1862); (1500-5250)], K [(98-1862); (1500-5250)], K [(98-1862); (1500-5250)], Mg [(280); (351)]; Na [(33-745); (50-5080)], Se [(0.16); (0.48)] and Zn [(5.16-7.17); (45-60)].

The estimated dietary intake (in ranges) of the essential elements for all the samples were generally lower than intakes recommended by WHO, FAO, EFSA; [(intake range); (recommended value)]: Na [(0.001-0.121 g/day); (0.6-3.5 g/day)], Cl [(0.011-0.42 g/day); (0.6-3.5 g/day)], Ca [(27-134 mg/day); (400 mg/day)], Mg [(1.52-24.3 mg/day); (53 mg/day)], Mn [(0.06-2.96 mg/day); (1-10 mg/day)]. The dietary intake of the other elements compared to levels recommended by WHO, FAO and EFSA were: K [(0.004-0.51 mg/day); (4.7 g/day)], Se [(8.4-25 µg/day); (10 µg/day)], Fe [0.08-0.99 mg/day); (10 mg/day)], Cu (0.06-3.79 mg/day); (0.9 mg/day)], Cr [(0.9-123 µg/day); (30-100 µg/day)], V [(0.5-75 µg/day); (1.8 mg/day)], Zn [(0.04-0.53 mg/day); (5 mg/day)].

The estimated range of dietary intake of the non-essential elements in all the samples were generally lower than the provisional tolerable weekly intake (PTWI) values recommended by the Joint FAO/WHO Expert Committee on Contaminants and Food Additives (JECFA).{ [(intake range); (PTWI)]: Sn [0.0011-8.07 mg/kg bw/week); (14 mg/kg bw/week), Pb [(0.0019-0.0071 mg/kg bw/week); (0.025 mg/kg bw/week)], As [(<1.93 µg/kg bw/week); (3 µg/kg bw/week)] and Hg [(<1.93 µg/kg bw/week); (4 µg/kg bw/week)].

Essential elements play various roles in the body to promote normal development and functioning of body organs and systems. Since the intakes were generally below recommended levels, the infants may not acquire the optimum nutritional benefits

from the weaning foods. The shortfall in the intakes could be met from other food sources (such as breast milk which is still a part of the infant's diet). There is no significant health risk associated with the consumption of the cereal-based weaning foods available on the Ghanaian market. Consequently, all the cereal-based weaning foods on the Ghanaian market are safe for consumption by the infants with regards to the non-essential elements analysed.

5.2 Recommendations

The following recommendations are proposed based on the outcome of this study:

- There should be continuous monitoring of cereal-based weaning foods and all other categories of infant or baby foods (locally manufactured or imported) that are on sale on the Ghanaian market, to ensure that they meet all safety requirements (with respect to potentially toxic elements).
- The Ghana Foods and Drugs Authority (GFDA) should ensure that manufactured cereal-based foods meant for infants are fortified with essential elements to levels that can adequately cater for the nutritional needs of the infant.
- Due to financial constraints, this study focused only on cereal-based foods, without considering breast milk and other types of milk which are an important part of the infant's diet. Accordingly, further studies that take into account all the foods consumed in a day by the infant (including water, milk, fruits and any other food in addition to the cereal-based foods) should be undertaken. Data from such a study will help ascertain the infants' total elemental (essential or non-essential) exposure.

- A national policy that mandates the GFDA to undertake periodic national surveys of food consumption patterns of infants and children with respect to their intake of essential and non-essential elements should be formulated. Such dietary intake assessment surveys will ultimately inform policy direction on infant nutrition.



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APPENDIX A

Table A: Description of Samples, including names, ingredients and brands.

Product Name (Sample code)	M. R. A	Brand	Cereal component(s)	Ingredients
Nestum first baby Cereal (NE-WF)	from six months onwards	Nestle Nestum	Wheat flour	Wheat flour(gluten), brown sugar, caramel, Calcium carbonate (E170), Sodium phosphate (E339), Vitamin C (E300), maltodextrin, Ferrous fumarate, vanillin, Bifidus culture, Zinc sulphate, Vitamin E, niacin, alpha amylase INS1100, Calcium Panthothenate, vitamin A, vitamin B1, vitamin B2, vitamin B6, folic acid, potassium iodide, biotin, vitamin D3 and vitamin B12.
Nestum baby Cereal (NE-MF)	from six months onwards	Nestle nestum	Maize flour	Maize flour, brown sugar, caramel, Calcium carbonate (E170), Sodium phosphate (E339), Vitamin C (E300), maltodextrin, Ferrous fumarate, vanillin Bifidus culture, zinc sulphate, Vitamin E, niacin, alpha amylase INS1100, Calcium panthothenate, vitamin A, vitamin B1, vitamin B2, vitamin B6, folic acid, Potassium iodide, biotin, vitamin D3 and vitamin B12.
Baby cereal, wheat and banana flavour (PU-WF)	from 8 to 36 months	Purity	Wheat/Maize flour	Wheat flour (minimum 50%) (gluten), cane sugar, maize flour, wheat bran flour (minimum 11%) (gluten), whey powder (milk solids), Non-hydrogenated vegetable fat (palm fruit); vitamins (vitamin A, vitamin D, pantothenic acid, thiamine, riboflavin, pyridoxine, vitamin C, biotin, folic acid, cyanocobalamine); minerals (magnesium, Iodine, Iron, Zinc). Banana flavour.
Cream of maize original (PU-MF)	from 8 to 36 months	Purity	Maize flour	Maize flour, vitamins and minerals.
Golden Cereal mix (SE-MS)	from 6 months onwards	Selasie	Millet flour	Millet flour and soya beans.
Bledina Rice Flour with Carrots (BL-RF)	from 6 months onwards	Bledina	Rice flour	Rice flour (60%), sugar, dehydrated carrot powder (13.9%), maltodextrine, palm Oil; Emulsifiers: Soya lecithin; vitamins (E, C, B1, PP, B6, Folic acid, Biotin and B5); mineral contributive element: Ferric diphosphate.
Bledina multocereals (BL-WF)	from 6 months onwards	Bledina	Wheat flour/rice flour/rye flour	Cereals 71 % (wheat flour 68%, rice flour 1.1%, rye flour 1.1%), sugar, corn Starch 9.7 %, palm oil; Emulsifier: soy lecithin; vitamins (E, C, B1, PP, B6, Folic acid, boitine and B5); Mineral contributive substance: Ferric diphosphate, Traces of milk. Contains gluten.
Milumeal baby cereal (MI-WF)	from 6 months onwards	Milupa	Wheat	Wheat flour (60.3%), skimmed milk powder (25.5%), sugar, honey (8.1%) Vegetable oil (palm); Emulsifier: soya lecithin; Vitamins: A, D, E, C, B1, PP, Folic acid, Biotin and B5; Mineral: Ferric diphosphate. No colourings or added preservative.

N/A: Not available. M.R.A: Manufacturer's recommended age

Table A continued.

Product Name (Sample code)	M. R. A	Brand	Cereal component(s)	Ingredients
Powdered rice (AB-PR)	N/A	Abido	Rice flour	Powdered natural rice, free from preservatives and colorants.
My first rice cereal (CE-RF)	from six months onwards	Cerelac	Rice flour	Rice flour, skimmed milk, sucrose, palm olein acidity regulator: Calcium carbonate; maltodextrin, vitamins, ferrous fumarate, vanillin, Zinc sulphate, bifidus culture, Sodium chloride, Potassium iodide. May contain traces of gluten and soya.
My first wheat cereal (CE-WF)	from six months onwards	Cerelac	Wheat flour	Wheat flour, skimmed milk, sucrose, palm olein; acidity regulator: Calcium carbonate; maltodextrin, vitamins, ferrous fumarate, vanillin, Zinc sulphate, bifidus culture, sodium chloride, potassium Iodide. May contain traces of gluten and soya.
My first maize cereal (CE-MF)	from 6 months onwards	Cerelac	Maize flour	Whole Corn flour, skimmed milk, sucrose, palm olein acidity regulator Calcium carbonate; maltodextrin, vitamins, ferrous fumarate, vanillin, Zinc sulphate, bifidus culture, Sodium chloride, potassium iodide. May contain traces of gluten and soya.
Yummy....fruits (CE-YF)	from 6 months onwards	Cerelac	Wheat/Maize flour	Wheat flour, skimmed milk, sucrose, palm olein, corn flour banana and apple; Acidity regulator: Calcium carbonate, citric acid; orange, maltodextrin, Malt extract, vitamins, sodium chloride, ferric pyrophosphate, vanillin, Zinc sulphate, bifidus culture, potassium iodide. Contains gluten. May Contain traces of soya.
Tom brown (TB-WS)	N/A	Tom brown	wheat/Sorghum flour	Wheat flour, sorghum flour, soya, skimmed milk, sodium chloride.
Winimix (TB-WM)	N/A	Tom Brown	Wheat flour	Wheat flour, soya, sodium chloride.
Local Tom Brown (TB-LO)	N/A	L. Tom Brown	Maize flour	Roasted maize flour, soya, and Sodium chloride.
L. Tom Brown (PO-MF)	N/A	L. Tom Brown	Maize flour	Roasted maize flour and sodium chloride.
N/A (RW-MS)	N/A	N/A	Millet flour	Millet flour, with soya.
N/A (RW-BS)	N/A	N/A	Rice flour	Brown rice flour, with soya.
N/A (RW-MF)	N/A	N/A	Maize flour	Maize flour.

N/A: Not available. M. R. A: Manufacturer's recommended age. L. Tom Brown: Local Tom Brown

APPENDIX B

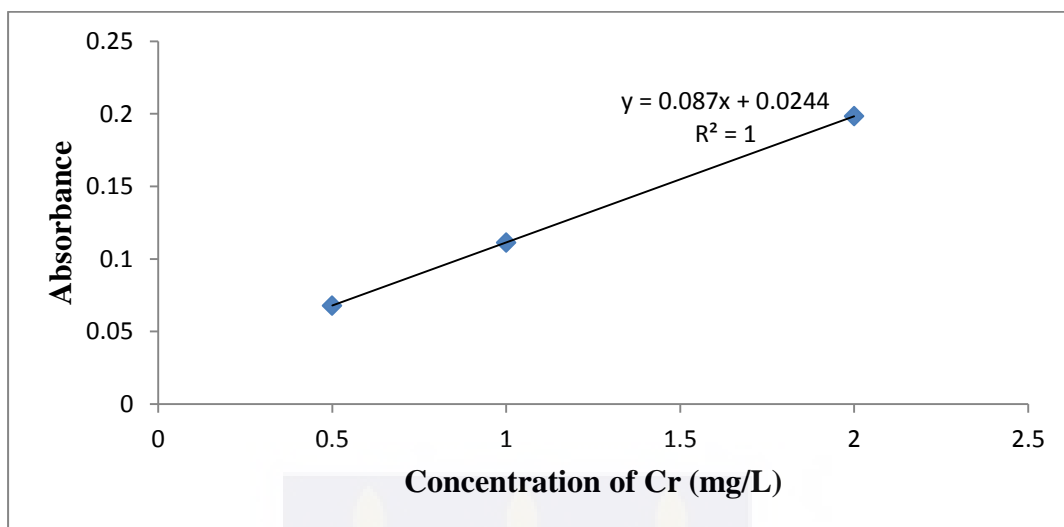


Fig. B1: F AAS calibration curve used to determine the concentrations of Cr in the samples.

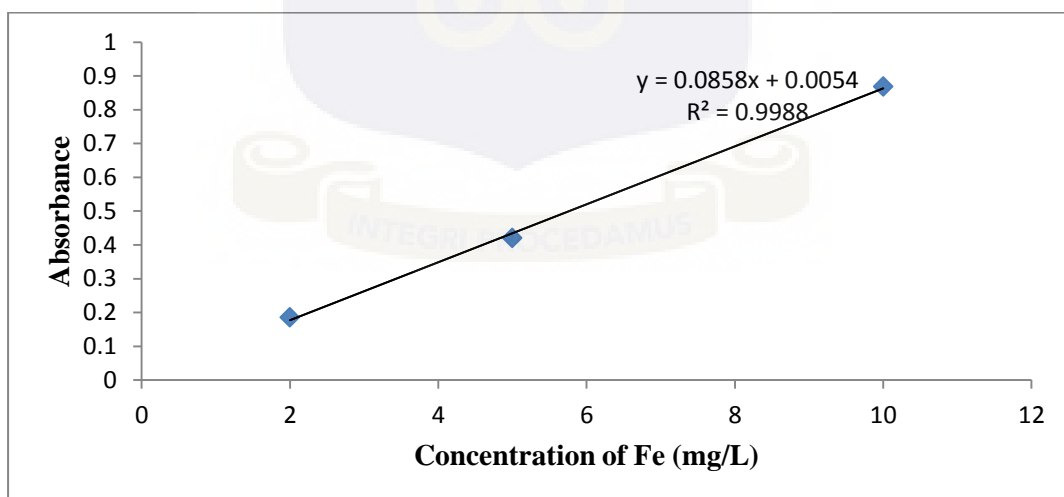


Fig. B2: F AAS calibration curve used to determine the concentrations of Fe in the samples.

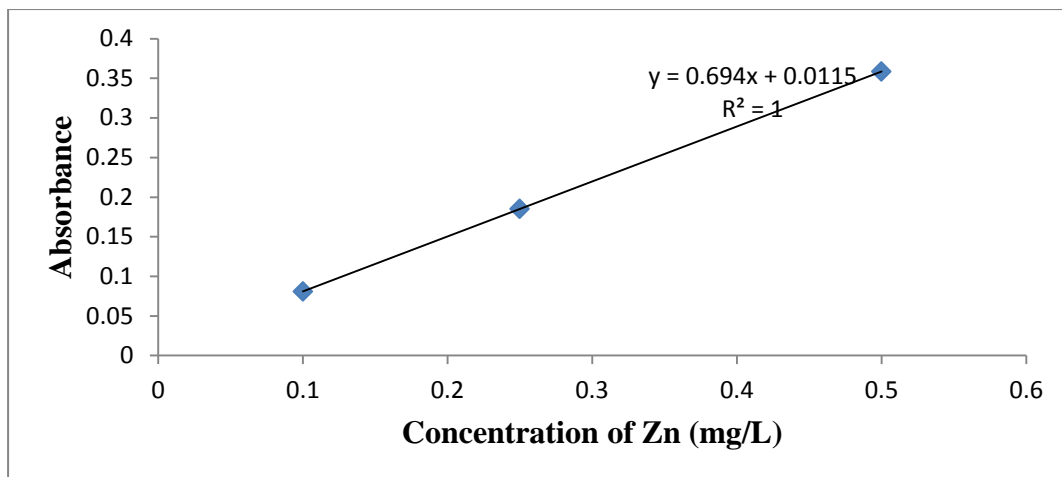


Fig. B3: F AAS calibration curve used to determine Zn concentrations in the samples.

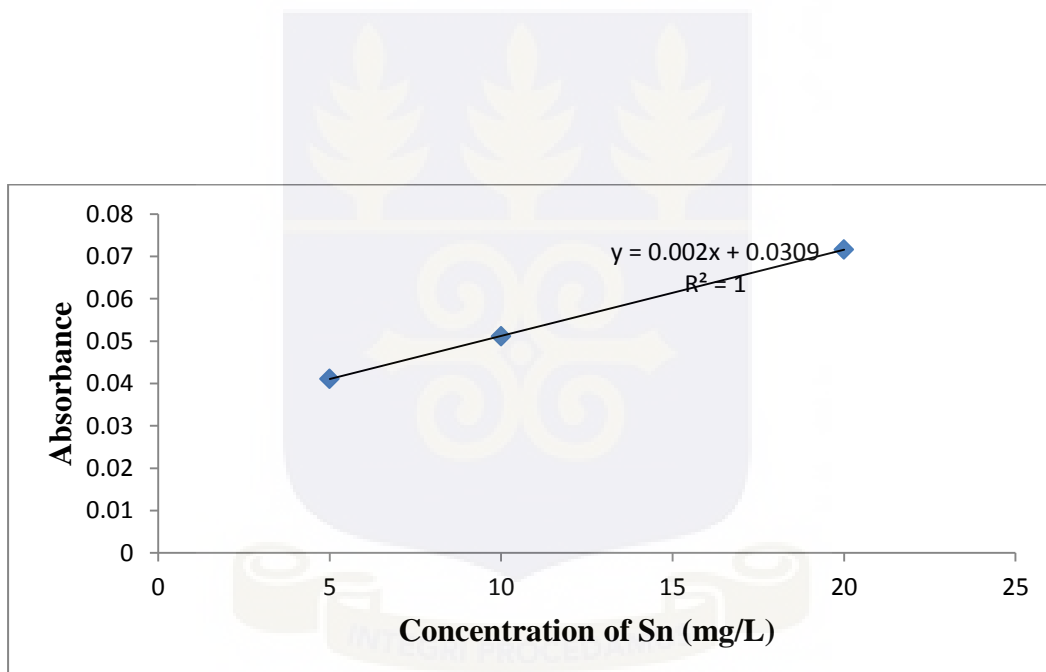


Fig. B4: F AAS calibration curve of Sn standards used to determine Sn concentrations in the samples.

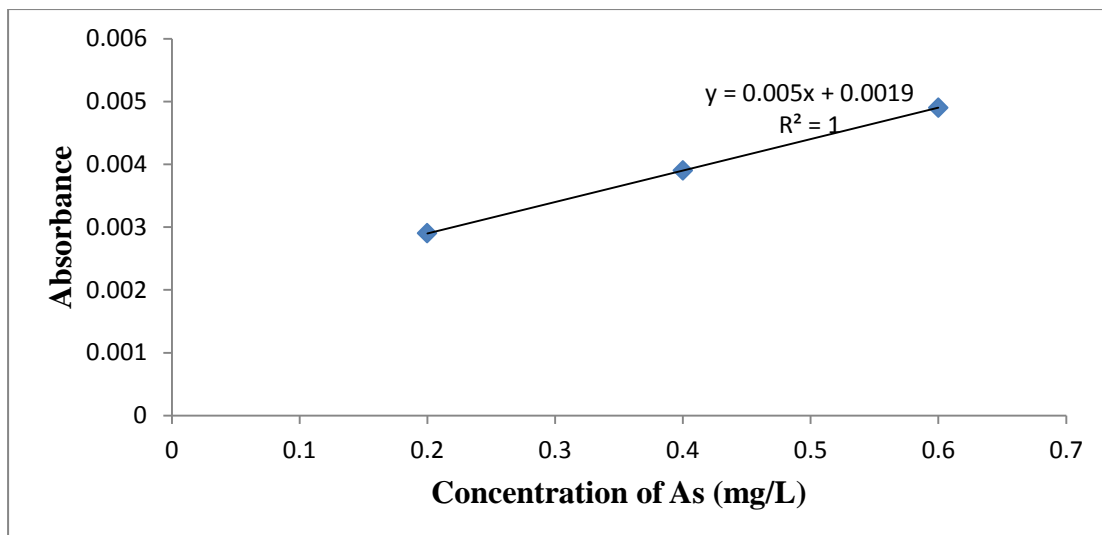


Fig. B5: HG AAS calibration curve used to determine the concentrations of As in the samples.

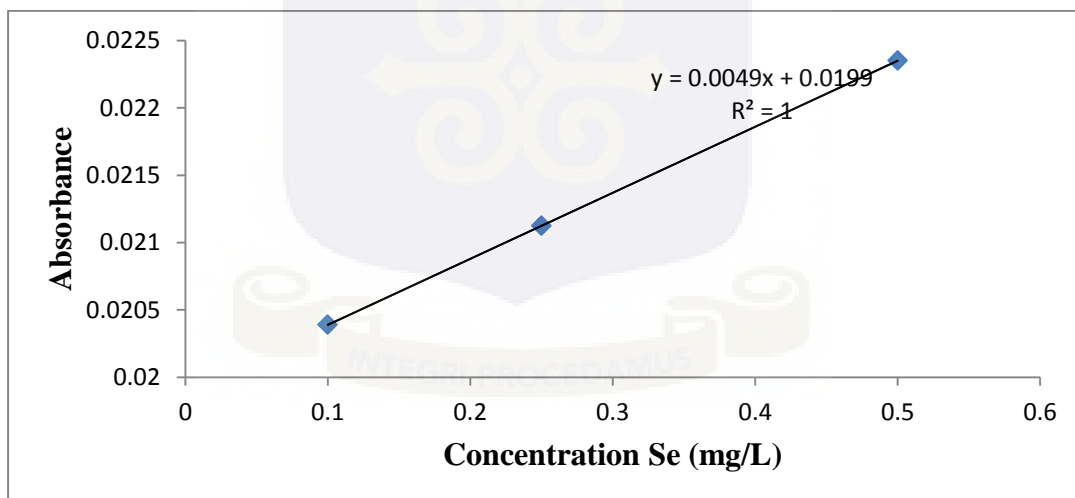


Fig. B6: HG AAS calibration curve used to determine the concentrations of Se in the samples.

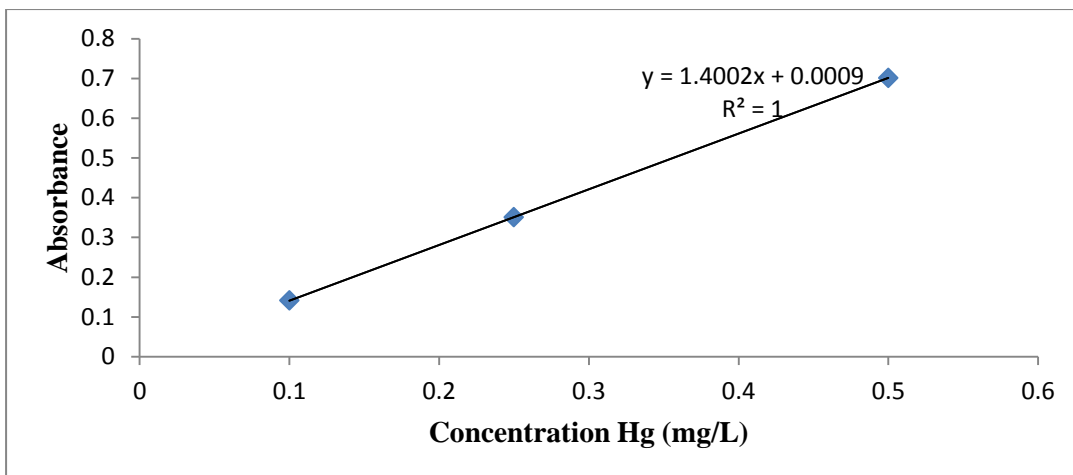


Fig. B7: HG AAS calibration curve used to determine the concentrations of Hg in the samples.

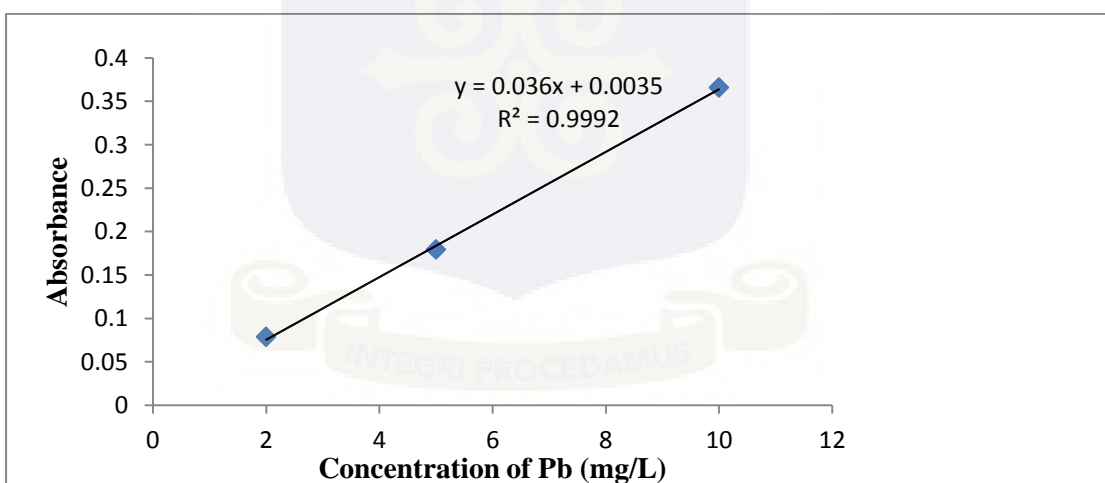


Fig. B8: F AAS calibration curve used to determine Pb concentrations in the samples.

APPENDIX C

Table C1: Elemental concentration of cereal-based infant food samples determined by INAA.

Sample Code	Elemental Concentration ($\mu\text{g/g}$)*									
	Al	Cu	Ca	Cl	Mg	Mn	K	Na	Br	V
AB-PR	26.8 \pm 1.3	2.54 \pm 0.04	380 \pm 27	199 \pm 8	20 \pm 2	13.23 \pm 0.60	2094 \pm 140	23 \pm 2	148 \pm 4	0.071 \pm 0.002
BL-RF	32.4 \pm 1.1	55.80 \pm 2.51	398 \pm 30	584 \pm 35	94 \pm 8	2.36 \pm 0.08	2520 \pm 147	62 \pm 4	135 \pm 6	0.149 \pm 0.004
BL-WF	25.0 \pm 4.2	23.74 \pm 1.42	405 \pm 53	345 \pm 12	89 \pm 10	6.84 \pm 0.38	177 \pm 11	33 \pm 2	41 \pm 2	0.027 \pm 0.001
CE-MF	38.7 \pm 2.4	23.08 \pm 1.15	1210 \pm 98	2210 \pm 97	56 \pm 6	1.36 \pm 0.04	3423 \pm 99	393 \pm 12	1126 \pm 68	0.058 \pm 0.003
CE-RF	38.3 \pm 2.1	7.47 \pm 0.19	1337 \pm 130	2124 \pm 91	55 \pm 6	3.73 \pm 0.22	3064 \pm 73	441 \pm 20	1992 \pm 60	<0.01
CE-WF	30.6 \pm 2.2	1.24 \pm 0.03	1022 \pm 111	1734 \pm 101	202 \pm 26	0.84 \pm 0.02	1862 \pm 80	299 \pm 10	589 \pm 15	<0.01
CE-YE	29.4 \pm 0.7	2.02 \pm 0.08	1320 \pm 145	2339 \pm 110	243 \pm 31	2.71 \pm 0.11	3076 \pm 92	433 \pm 13	604 \pm 24	<0.01
MI-WF	38.5 \pm 3.2	22.47 \pm 1.52	997 \pm 100	2015 \pm 85	62 \pm 7	0.59 \pm 0.02	5056 \pm 103	326 \pm 15	587 \pm 21	<0.01
NE-MF	31.9 \pm 1.8	6.18 \pm 0.37	950 \pm 86	396 \pm 23	124 \pm 12	2.61 \pm 0.08	98 \pm 3	745 \pm 28	916 \pm 45	0.033 \pm 0.001
NE-WF	34.2 \pm 2.1	11.04 \pm 0.33	576 \pm 37	435 \pm 20	73 \pm 8	2.45 \pm 0.08	472 \pm 13	720 \pm 22	600 \pm 23	0.064 \pm 0.001
PO-FM	81.9 \pm 3.1	1.23 \pm 0.07	449 \pm 11	491 \pm 13	208 \pm 19	2.55 \pm 0.13	1585 \pm 38	27 \pm 1	1057 \pm 57	0.118 \pm 0.003
PU-MF	50.6 \pm 2.3	6.01 \pm 0.18	2002 \pm 140	551 \pm 19	205 \pm 22	2.22 \pm 0.13	446 \pm 14	80 \pm 3	7403 \pm 82	0.101 \pm 0.004
PU-WF	29.0 \pm 2.7	4.87 \pm 0.23	547 \pm 58	1568 \pm 90	280 \pm 31	31.36 \pm 0.76	703 \pm 32	325 \pm 5	5241 \pm 51	0.133 \pm 0.006
RW-BR	40.2 \pm 2.2	41.57 \pm 2.66	454 \pm 32	161 \pm 10	141 \pm 18	32.99 \pm 1.81	1139 \pm 46	13 \pm 0.6	74 \pm 2	0.156 \pm 0.005
RW-MS	50.1 \pm 1.8	5.70 \pm 0.23	589 \pm 65	309 \pm 18	258 \pm 29	13.39 \pm 0.53	3809 \pm 60	37 \pm 1	52 \pm 2	0.672 \pm 0.013
RW-PM	41.0 \pm 1.8	0.83 \pm 0.02	487 \pm 57	338 \pm 25	20 \pm 3	3.20 \pm 0.11	49 \pm 2	20 \pm 0.5	82 \pm 4	0.316 \pm 0.011
SC-MS	118.3 \pm 1.8	0.99 \pm 0.07	406 \pm 48	148 \pm 7	173 \pm 25	12.66 \pm 0.32	98 \pm 3	45 \pm 1.2	<0.1	0.990 \pm 0.043
TB-LO	83.0 \pm 1.7	2.96 \pm 0.13	540 \pm 48	4654 \pm 120	304 \pm 21	18.16 \pm 0.65	3741 \pm 97	1398 \pm 25	<0.1	0.084 \pm 0.002
TB-WM	58.3 \pm 2.3	15.55 \pm 0.56	680 \pm 55	4651 \pm 111	32 \pm 4	38.94 \pm 1.68	2934 \pm 106	1362 \pm 32	245 \pm 10	0.295 \pm 0.012
TB-WS	38.0 \pm 2.7	3.93 \pm 0.22	730 \pm 72	5521 \pm 115	280 \pm 12	31.58 \pm 1.42	3845 \pm 89	1588 \pm 34	2026 \pm 54	<0.01

* Data are presented as the 'Mean of three measurements \pm standard deviation

Table C2: Elemental concentrations of cereal-based infant food samples determined by AAS.

Sample Code	Elemental Concentration ($\mu\text{g/g}$)*							
	Sn	Fe	Zn	Se	Cr	Pb	As	Hg
AB-PR	39.8±0.2	1.13±0.02	1.23±0.01	0.189±0.031	0.99±0.02	0.035±0.003	<0.025	<0.025
BL-RF	54.1±1.9	3.74±1.89	1.11±0.02	0.146±0.050	0.45±0.01	0.065±0.006	<0.025	<0.025
BL-WF	51.9±2.1	9.02±0.59	0.84±0.02	0.220±0.070	0.84±0.03	0.031±0.001	<0.025	<0.025
CE-MF	57.0±1.4	9.89±0.27	1.41±0.02	0.271±0.041	0.63±0.05	0.037±0.004	<0.025	<0.025
CE-RF	64.1±1.6	3.56±0.08	3.00±0.02	0.148±0.032	<0.03	0.048±0.007	<0.025	<0.025
CE-WF	55.7±1.1	3.68±0.11	5.31±0.06	0.251±0.080	1.23±0.04	0.025±0.003	<0.025	<0.025
CE-YE	53.8±1.9	4.22±0.03	5.31±0.12	0.205±0.031	1.08±0.02	0.028±0.002	<0.025	<0.025
MI-WF	80.7±3.6	5.81±0.19	1.05±0.04	0.236±0.070	<0.03	0.025±0.004	<0.025	<0.025
NE-MF	40.6±0.5	11.51±0.50	5.16±0.13	0.188±0.053	0.93±0.03	0.075±0.008	<0.025	<0.025
NE-WF	77.1±0.8	7.04±0.07	7.17±0.19	0.168±0.009	0.51±0.02	0.085±0.008	<0.025	<0.025
PO-FM	51.3±3.1	3.56±0.06	4.98±0.03	0.241±0.042	1.11±0.05	0.034±0.002	<0.025	<0.025
PU-MF	66.2±0.9	3.47±0.10	6.51±0.03	0.156±0.070	<0.03	0.033±0.001	<0.025	<0.025
PU-WF	57.9±0.5	11.78±0.47	7.11±0.12	0.147±0.052	0.84±0.02	0.041±0.001	<0.025	<0.025
RW-BR	<0.03	1.07±0.04	1.86±0.01	0.248±0.062	1.05±0.42	0.070±0.005	<0.025	<0.025
RW-MS	67.1±3.4	4.97±0.07	2.82±0.11	0.145±0.021	0.39±0.01	0.025±0.003	<0.025	<0.025
RW-PM	49.6±0.3	2.84±0.07	0.50±0.02	0.168±0.035	0.20±0.01	0.093±0.007	<0.025	<0.025
SE-MS	54.9±0.8	7.53±0.47	1.56±0.03	0.182±0.063	0.89±0.05	0.050±0.004	<0.025	<0.025
TB-LO	35.8±1.1	11.78±0.53	2.64±0.05	0.219±0.070	0.54±0.02	0.027±0.003	<0.025	<0.025
TB-WM	91.8±5.8	9.89±0.22	2.25±0.07	0.178±0.061	0.12±0.01	0.053±0.005	<0.025	<0.025
TB-WS	84.5±5.1	4.55±0.18	2.25±0.05	0.317±0.051	0.18±0.01	0.060±0.004	<0.025	<0.025

*Data are presented as the mean of three measurements \pm standard deviation

Table C3: Daily intake of essential elements by consumption of the samples analysed.

Sample	Daily intake of essential Elements (mg/day)*											
	Ca	Na	Cl	K	Fe	Cu	Mg	Mn	Zn	Se($\mu\text{g/day}$)	Cr($\mu\text{g/day}$)	V($\mu\text{g/day}$)
AB-PR	29	1.7	15	159	0.09	0.19	1.52	1	0.093	14.4	75	5.4
BL-RF	27	4.2	40	171	0.25	3.79	6.39	0.16	0.075	9.9	30.6	10.1
BL-WF	28	2.2	23	12	0.61	1.61	6.05	0.47	0.057	15.0	57	1.8
CE-MF	121	39	221	342	0.99	2.31	5.60	0.14	0.141	27.1	63	5.8
CE-RF	134	44	212	306	0.36	0.75	5.50	0.37	0.300	14.8	1.5	0.5
CE-WF	102	30	173	186	0.37	0.12	20.20	0.08	0.531	25.1	123	0.5
CE-YE	132	43	234	308	0.42	0.20	24.30	0.27	0.531	20.5	108	0.5
MI-WF	100	33	202	506	0.58	2.25	6.20	0.06	0.105	23.6	1.5	0.5
NE-MF	48	37	20	5	0.58	0.31	6.20	0.13	0.258	9.4	46.5	1.7
NE-WF	29	36	22	24	0.35	0.55	3.65	0.12	0.359	8.4	25.5	3.2
PO-FM	34	21	37	120	0.27	0.09	15.81	0.19	0.378	18.3	84	9
PU-MF	120	4.8	33	27	0.21	0.36	12.30	0.13	0.391	9.4	0.9	6.1
PU-WF	33	20	94	42	0.71	0.29	16.80	1.88	0.427	8.8	50.4	8
RW-BR	35	1	12	87	0.08	3.16	10.72	2.51	0.141	18.8	79.8	12
RW-MS	45	2.8	23	289	0.38	0.43	19.61	1.02	0.214	11.0	29.6	51
RW-PM	37	1.5	26	4	0.22	0.06	1.52	0.24	0.038	12.8	15.2	24
SE-MS	31	3.4	11	7.4	0.57	0.08	13.15	0.96	0.118	13.8	67.6	75
TB-LO	41	106	354	284	0.90	0.23	23.10	1.38	0.201	16.6	41	6.4
TB-WM	52	104	353	223	0.75	1.18	2.43	2.96	0.171	13.5	9.1	22.4
TB-WS	55	121	420	292	0.35	0.30	21.28	2.40	0.171	24.1	13.7	0.4
AVERAGE	62	32	126	170	0.45	0.91	11.12	0.82	0.235	15.8	46.2	12.2
RANGE	27-134	1-121	11-420	4-506	0.08-0.99	0.06-3.79	1.52-24.3	0.06-2.96	0.04-0.53	8.4-25	0.9-123	0.5-75

* Daily intakes are provided in units of mg/day unless otherwise stated

Table C4: Intake of non-essential/potentially toxic elements by consumption of the foods analysed.

Sample	Intake of non-essential/potentially toxic elements			
	Sn (mg/day)	Pb (mg/day)	As ($\mu\text{g/g}$)	Hg ($\mu\text{g/g}$)
AB-PR	3.03	0.0026	< 1.9	< 1.9
BL-RF	3.68	0.0044	< 1.7	< 1.7
BL-WF	3.53	0.0022	< 1.7	< 1.7
CE-MF	5.70	0.0037	< 2.5	< 2.5
CE-RF	6.41	0.0048	< 2.5	< 2.5
CE-WF	5.57	0.0025	< 2.5	< 2.5
CE-YE	5.38	0.0028	< 2.5	< 2.5
MI-WF	8.07	0.0025	< 2.5	< 2.5
NE-MF	2.03	0.0038	< 1.25	< 1.25
NE-WF	3.86	0.0043	< 1.25	< 1.25
PO-FM	3.90	0.0026	< 1.9	< 1.9
PU-MF	3.97	0.0020	< 1.5	< 1.5
PU-WF	3.47	0.0025	< 1.5	< 1.5
RW-BR	0.0011	0.0053	< 1.9	< 1.9
RW-MS	5.10	0.0019	< 1.9	< 1.9
RW-PM	3.77	0.0071	< 1.9	< 1.9
SE-MS	4.17	0.0038	< 1.9	< 1.9
TB-LO	2.72	0.0021	< 1.9	< 1.9
TB-WM	6.98	0.0040	< 1.9	< 1.9
TB-WS	6.42	0.0046	< 1.9	< 1.9
AVERAGE	4.39	0.0035	< 1.93	< 1.93
RANGE	0.0011-8.07	0.0019-0.0071	-	-