EVALUATION OF SOME HEAVY METALS CONCENTRATIONS IN CHOCOLATE, CANDIES AND TOMATO PUREE IMPORTED FROM CHINA

BY

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DECLARATION

I hereby declare that the work in this Thesis entitled "Evaluation of Some Heavy Metals Concentrations in Chocolate, Candies and Tomato Puree Imported from China" was performed by me in the Department of Chemistry, under the supervision of Profs. A. Uzairu, C. E. Gimba, and Dr. O. J. Okunola. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this work has been previously presented for another degree or diploma at any institution.

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CERTIFICATION

This Thesis entitled "Evaluation of Some Heavy Metals Concentrations in Chocolate, Candies and Tomato Puree Imported from China" by Ochu, Joy Onyebuchi meets the regulations governing the award of the degree of Master of Science in Analytical Chemistry of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

This study was initiated as a follow-up information on some impounded products tomato puree (Samples A and B) chocolates and candies (Samples C, D, E and F) imported from China, and sold in Nigerian markets. This result as their failure to meet National Agency for food drug administration and control (NAFDAC) regulatory standard. Samples A, B, C, D, E and F were collected in five markets from each six states of the six geographical zones: North-West (Kano), North-East (Bauchi), North-Central (Kogi), South-West (Lagos), South-East (Abia), and South-South (Rivers) of the country. In order to evaluate the quality of the products, the levels of some heavy metals (Cu, Mn, Fe, Ni, Zn, Ti and Cr) were evaluated in the samples using X-ray fluorescence (XRF). Concentrations of metals in both tomato puree ranged 4.0 – 4.5 mg/g for Cu, 18.0 – 42.5mg/g for Mn, 26.0 – 62.5mg/g for Fe, 4.0 – 56.0mg/g for Ni, 27.0 – 35.2mg/g for Zn, ND – 25.0mg/g for Ti, and ND – 20.0mg/g for Cr while concentrations of metals in both chocolates and candies ranged 3.0 – 4.2 mg/g for Cu, 40.0 – 55.7mg/g for Mn, ND – 102.5mg/g for Fe, ND – 305.0mg/g for Ni, ND – 42.5mg/g for Zn, ND – 23.8mg/g for Ti, and ND – 10.8mg/g for Cr. The concentrations of all the metals studied were generally higher in samples A and B compared to recommended levels of these metals in vegetable crops. Also, the data showed that these metals are at higher levels in samples C, D, E and F compared to other studies in candies and chocolates in Nigeria. Correlation analysis among metals revealed positive correlations, which indicates similar sources of these metals. Also, evaluation of dietary intake of these products daily revealed that sample A (except for Cu and Fe), B, C (except for Cu), D and E are above the daily dietary recommended limit for all the metals studied in food. Thus, frequent intake of these contaminated products is likely to induce health effects arising largely from Cu, Mn, Fe, Ni and Zn.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Recently, there is an increasing concern about the quality of imported foods and food related products in several parts of the world (Maxwell and Neumann, 2009) particularly from China. This, as observed by the United States Congress, was due to the large size of shipments, the many different routes of entry, the variety of foods imported, and the large numbers of potential contaminants make effective interdiction of contaminated foods difficult (Congress Research Service (CRS) Report, 2008). Additionally, the US Congress observed that many products were brought into the United States by travelers especially residents travelling back and forth regularly to China. Among the common products imported were canned tomatoes, chocolates, candies, biscuits, bean paste, bean curd, teas and various nuts and spices (CRS Report, 2008).

According to the United States Congress Research Service Report (2008), in early 2007, evidence emerged that adulterated pet food ingredients from China had caused the deaths of many dogs and cats. However, toxicological and environmental studies have interest in the determinations of toxic elements in food. "Food safety" implies absence or acceptable and safe levels of contaminants, adulterants, naturally occurring toxins or any other substance that may make food injurious to health on an acute or chronic basis. Food quality can be considered as a complex characteristic of food that determines its value or acceptability to consumers. Besides safety, quality attributes includes nutritional value, organoleptic properties such as appearance, color, texture, taste and functional properties (World Health Organization (WHO), 1998).

Taking into consideration that products passed through different industrial processes

and are packaged to provide a means of protection, marketing or handling, and most of them have printed colour inks on the outer cover (Kim *et al*., 2008). Importantly, food products such as candies that are likely to be consumed frequently by small children are wrapped in colourful packages in order to induce them to purchase the products. Heavy metals, such as Pb, Cr, Ti, Zn and Cu can migrate from the printed surface to the food contact surface through four mechanisms: blocking, rubbing, peeling and diffusion (Bradley *et al*., 2005). Contamination of imported food products with heavy metals may cause a serious risk to human health because of the consumption of even small amount of metals can lead to considerable concentrations in human body. Metals that cannot be metabolized like cadmium, lead and mercury persist in the body and exert their toxic effects by combination with one or more reactive groups essential for normal physiological function and cellular disturbances or clinical manifestation may appear (Friberg and Elinder, 1988; Skerfving, 1988). The adverse toxic effects caused by lead (Subramanian, 1988), cadmium (Friberg *et al*., 1986), mercury (Manahan, 1989) and tin (Reilly, 1991) are widely recognized.

In spite of the hazard effects of heavy metals on public health, some of them are essential for normal physiological functions such as copper and zinc (Zaki, 1988; Hays, 1989). Also, copper sulphate exhibits clear reduction of cadmium residues in animal tissues (Ahmed *et al*., 1999), the dietary deficiencies of copper, zinc, calcium, iron, protein and an excess dietary fat cause an increase in the absorption and toxicity of lead (Goldfrank *et al*., 1990). Although there are standards set by the quality control bodies such as National Agency for Food and Drug Administration and Control (NAFDAC) and Standards Organization of Nigeria (SON) for permissible concentrations of these contaminants in imported products, the problem still exists because many Chinese manufacturers have two

or more versions of their products: one specifically designed to meet standards set by the import country, and a cheaper and poorer version for the native market (Medlin, 2004). Coupled with the fact that route of entry differs, therefore, monitoring of heavy metal content in these products is essential especially with their related health implications. The potential for exposure, though, is not completely understood due to the lack of data regarding heavy metal levels in various products and the extent of use within Nigerian communities. Canned tomatoes and candies, however, are of particular concern because of their potential for children consumption.

1.2 STATEMENT OF THE PROBLEMS

Tomatoes contribute to a healthy and well-balanced diet. They are rich in vitamins, minerals, proteins (Attia *et al*., 2000), essential amino acids (Gould, 1992), sugars and dietary fibers (Davies and Hobson, 1981). Tomatoes also contain vitamin C, vitamin E and low amounts of the water-soluble type B vitamins, thiamin, niacin and riboflavin (Beecher, 1998). Tomatoes and tomato products are used as ingredients in many traditional dishes, because of their compatibility with other food ingredients and due to the concentration and availability of several nutrients in these products and to their wide spread consumption by humans all over the world (Anonymous, 2008). However, heavy metals are one of a range of important types of contaminants that can be found on the surface and in the tissue of fresh tomatoes as well as processed tomato products. As a result, heavy metal accumulation in tomato and processed tomato products may pose a direct threat to human health.

Potentially toxic heavy metals including lead, cadmium, and arsenic have been found in chocolate products from a variety of origins (Mounicou *et al*., 2003; Rankin *et al*., 2005; Dahiya *et al*., 2005). These metals can be absorbed directly by the *Theobroma*

cacao tree or be introduced during the manufacturing process. But either way, they can still exist in the final product. Although the levels of these metals are often within acceptable ranges, it's important to realize that they accumulate in the body and can become quite toxic over an extended period of exposure to small amounts. Many chocolate manufacturers claim that the amounts are small enough to not be a concern (Anonymous, 2002), but the American Environmental Safety Institute (AESI) considered it a large enough to file suit against a number of manufacturers in 2002 and petition for required contaminant labeling (AESI, 2002). The concentration of heavy metals may very well be too low to be of significant concern, but it's definitely a legitimate reason to avoid eating sizable portions of chocolate on a regular basis. Furthermore, because the cacao beans used to make chocolate come from all over the world, the amounts of heavy metals that they contain can vary widely. Although there's no guarantee, certified organic chocolate is less likely to be produced from cacao beans that have been grown in a contaminated environment or exposed to contaminants during processing (Carter-Pokras *et al*., 2007).

Also, essential elements are present in the industrial processes and agrotechnical operations, so disregarding their nutritive essence, their contents should be determined because they can often reach toxic level (Kim *et al*., 2008). Copper intoxication can be caused by its many chemical compounds which are components of fungicide used in agriculture and veterinary practice. Using vegetables and fruits grown on high copper soil (near smelters, industrial factories) is also a cause of copper intoxication. Zinc and iron used in the form of alloys in industry and in some inorganic fertilizer components, could lead to high concentration of these metals in soil and then in plants grown in the soils.

Canned foods offer a shortcut in meal preparation which is most favored by those

who are stretched for time. Canned fish, milk, tomatoes products manufactured either locally or imported, are very popular in supermarkets and small grocery retail outlets.

1.3 JUSTIFICATION

According to Dahiya *et al* (2005), the future of any nation depends on the health, prosperity and progress of the forthcoming generation. Presently, in the era of industrialization, development and inter-boundry trades, one concern should be the health of the future generation. Children are the most vulnerable age group to any kind of contamination in the food chain (Arreola *et al*., 1996). Lollipop, chocolate, biscuits and milk candy are some of the favourite items of children and are often presented to them as token of love and affection from their parents, relatives and friends (Dahiya *et al*., 2005). Many brands of these kinds of child-consumed favourite items are available in the market at variable prices, among them are those imported from Peoples's Republic of China which are sold at a low price. Most of the packaging is so poorly designed that the inner coatings do not maintain structural integrity, allowing ink component in the outer packages layer to migrate into the products (Kim *et al*., 2008). This could be a possible route of heavy metals into the children body system.

Also, it is a common phenomenon to list ingredients on the wrappers. The most common ingredients listed are sugar, liquid glucose, milk solids, cocoa solids, hydrogenated vegetable oil, vegetable fats, cocoa butter, wheat flour, edible starches, added flavor, yeast and flavor improvers, buffering agents and permitted stabilizer. Out of the listed ingredients, hydrogenated vegetable oil, vegetable fats, permitted emulsifier, buffering agent and permitted stabilizer may be the source of trace metal contamination (Dahiya *et al*., 2005).

In September, 2008, at least 12 countries had banned Chinese dairy products and these products were even recalled from shelves throughout Asia and in some African countries (CRS Report, 2008). Food products such as biscuits, chocolates and milk candies could be made from contaminated milk powder and children who consumed such food products could potentially exceed the tolerable daily intake (TDI) by more than threefold (European Food Safety Authority (EFSA), 2008). It is therefore necessary to monitor the level of heavy metals in these products especially its health risk implications.

In Nigeria, tomato paste, which constitutes a significant volume among canned vegetable products are mainly imported. The processing plants for the tomato paste are depending on their farmland, most of which are irrigated, for the supply of raw materials (Jones, 1987). The plants use fertilizer and agricultural chemicals for the production of raw materials. Hence, the need to determine the levels of essential and toxic heavy metals in canned tomato paste imported into the country become essential due to their beneficial or damaging effects. Moreover, it has becomes necessary to know about the concentration of essential and toxic heavy metals in canned tomato paste to ensure the quality of the product and to comply with quality and specification standards developed by the Codex Committee on Processed Fruits and Vegetable (CCPFV).

Increase of metal contamination in food and food products over the allowed limits (either concerning non essential or essential metals in surplus), may cause toxic effects to the consumers of the products. The gravity of toxic effects depends on the nature, quantity and chemical form of metals and synergetic or antagonistic effects of other chemical contaminants. Children are mostly exposed to the toxic metal effects through food and some of the favourite gifts. Metal genoxic effects are more often in the children because

their organs grow faster, the metabolism is more intense and the cells are divided more quickly (Vitosevic *et al*., 2007).

Studies on the assessment and evaluation of safety of food products are mainly carried out in developed countries. Literature information on such studies in developing country like Nigeria where such "junks" are constantly circulated in the markets and consumed by families is scarce. Thus, this study is necessitated to identify and characterize imported candies and canned tomatoes paste from China into the country. This is because the safety of our food products supply is a shared responsibility, from the fork of the food producing industry, regulatory authorities, scientists and consumers. As part of this responsibility, this study was conceived to assess the potential risks posed by food ingredients (canned tomato paste) and candies imported from China.

1.4 HYPOTHESIS

The purpose of this study was to identify and determine the level of heavy metals in different imported canned tomato pastes and candies from China into the country, considering that these products are supplied to every market in the country. Therefore, the study is based on the following null hypotheses:

i. Mean metal concentrations of candies and canned tomato pastes purchased from different zones of the country do not differ, and their heavy metal levels are within acceptable or safe limits.
ii. Candies and canned tomato paste of different types do not have equal metal concentrations.

1.5 AIM AND OBJECTIVES

The aim of this study was to determine some heavy metals (Cu, Mn, Fe, Ni, Zn, Ti and Cr) which might be essential at low concentrations in some canned tomato paste (testi tomato paste and merysa concentrates de tomato paste) chocolates and candies (lollipop with pencil, milk candy, royal glucose and biscuits) imported from China and to provide data that may be used as the basis for preventive measures. This was set to be achieved through the following objectives:

i. To determine the metal contents of canned tomato pastes, lollipop, biscuits, milk candy and chocolates from China sold in Nigeria major markets.
ii. To compare the levels of the metals in canned tomato paste, lollipop, biscuits, milk candy and chocolates with international standards.
iii. To determine the risk associated with the consumption of these products.

1.6 LIMITATIONS OF THE STUDY

The study was limited to these brands of canned tomato pastes and candies due to their availability in all the selected markets from the six geographical zones of the country. Also, the sampling sites per zone were limited due to financial constraint as at the time of study.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ENVIRONMENTAL CONTAMINANTS

Environmental contaminants are substances that originate from diffuse sources and may appear in food based on their ubiquitous presence in the environment (Klaus *et al*., 2011). In recent years, it has been emphasized that food safety and transparency of the food-related regulatory process is of pivotal importance to enhance consumer's confidence in food (Horton, 2001). Compared to other contaminants, heavy metals (HMs) which are widespread and potentially harmful, are not biodegradable and tend to accumulate in living organisms through the food and chain becoming persistent organic contaminants (Fan *et al*., 2002; Buccolieri *et al*., 2006). At certain levels, they may cause toxic effect to the organisms (Bryan and Langston, 1992), and are serious threat to the ecological safety and human health.

One of the prices paid by humans in the course of development is pollution in one form or the other. Though not conspicuously alarming, the slow and steady pollution by heavy metals in the environment is quite hazardous (Baskar *et al*., 1999). Heavy metals arise from unorganised industrial growth and are considered as major pollutants of natural water bodies. Anthropogenic activities like industrial processes, unsafe disposal of industrial wastes, agricultural wastes, and the like release heavy metals into the environment. Once released they contaminate water, soil, plants and enter the food chain, posing health problems to human beings. These recalcitrant pollutants received considerable attention because of their inherent toxicity to living organisms. When they reach the aquatic environment and, being non-degradable remain suspended or partially dissolved in water and subsequently accumulate in aquatic lives (Sharma and Pande, 1998).

Considering the significance of heavy metals and consumption of canned food ingredient and candies, it is necessary to monitor the levels of heavy metals in these commodities.

2.2 HEAVY METALS

Increasing industrialization has been accompanied throughout the world by the extraction and distribution of mineral substances from their natural deposits. Following concentration, many of these have undergone chemical changes through technical processes and finally pass, finely dispersed and in solutions, by way of effluent, sewage, dumps and dust, into the water, the earth and the air and thus into the food chain. Heavy metals are in the water we drink, the foods we eat, the air we breathe, our daily household cleaners, our cookware and our other daily tools. A heavy metal has a density of at least 5 times that of water and cannot be metabolized, therefore accumulate in the body (Sindiku and Osibanjo, 2011).

According to Spiegel (2002), some heavy metals may be important trace elements in the nutrition of plants, animals or humans (e.g. Zn, Cu, Mn, Cr, Ni and V) in very low concentrations while others are known to have negative nutritional effects (e.g. Pb, Cd and Hg). All of these metals may cause toxic effects if they occur excessively. The presence of heavy metals in foods and food products except for accidental or criminal actions reflects the environment (Iwegbue, 2010). Heavy metals in food and food products may originate from natural sources such as soil and water; environmental contamination including fertilizers and pesticides; industrial processing and containers, such as aluminum or tin cans (Coultate, 1992; Vela *et al*., 1998; Orghêda *et al*., 2005). The bioaccumulations of these metals over large territories and long time periods, which may result in the gradual damage of living humans necessitate careful assessment of these metals in food.

2.3 HEAVY METALS IN FOOD SOURCE

The unbridled growth of population has spawned many industries to cater for the ever - growing needs of the humans. With lack of concern for environment, we have mindlessly polluted rivers, lakes, and oceans with mostly untreated effluents from cities and industries. Moreover, we have blindly polluted our environment without knowing the impact of our action on the overall ecosystem. Science started to unravel the symptoms of various unknown diseases to humankind by tracing the source to presence of heavy metals in the food we eat. Indeed, the heavy metals have reached us through the food chain. Our own action has resulted in accumulation of toxic metals in our body affecting vital human organs of the population in the end (H_2O Magazine, 2011).

Heavy metals are an important source of food contamination and health hazard. The main threats to human health are associated with exposure to arsenic, cadmium, lead, mercury and copper (National Food Policy Capacity Strengthening Programme, 2010). Sources of food contamination include environmental, industrial pollution, agricultural practices, food processing and packaging. Absorption of heavy metals through food has been shown to have serious consequences on health – and thereby economic development associated with a decline in labour productivity as well as the direct costs of treating illnesses – such as kidney disease, damage to the nervous system, diminished intellectual capacity, heart disease, gastrointestinal diseases, bone fracture, cancer and death (Jarup, 2003).

Many metals exist in our environment that we deal with every day. However, three metals are more toxic than others; they are cadmium, mercury, and lead. Cadmium is naturally present in cigarette smoke, food, and water. Also, cadmium is found in processed materials such as metal coating, plastics, fertilizers and many other manmade substances.

Mercury is present in mushrooms and fish. Concentration of mercury has been rising steadily due to industrial pollution. The release of untreated effluent with mercury into rivers has increased the mercury concentration in river and sea fish. Unaware, we consume such fish thus accumulating mercury in our system. Moreover, the dental material, amalgam, contains mercury. In addition, mercury exists in home products such as glass, thermostats, fluorescent bulbs, thermometers, to name a few. High levels of lead found in mini-blinds, hair dyes, and some calcium supplements and lead crystal. Gasoline is also a major source of lead. The vehicular exhaust, for example, releases lead into atmosphere (H_2O Magazine, 2011).

2.4 HEAVY METALS IN CHOCOLATE AND CANDIES

Although the effects of acute heavy metal poisoning from food and industrial exposure have been described for over four thousand years, the first reports of chronic lead poisoning weren't observed until 1892, in Brisbane, Australia. These original reports were met with pervasive disbelief that lead toxicity could cause chronic damage when ingested by children (Gibson, 1982). As evidence continued to mount, this misconception was finally discarded in 1943 with the first long-term study of children who had recovered from acute lead poisonings. Survivors of childhood exposure to lead were found to exhibit ongoing behavioral disorders, learning difficulties, and difficulty in school (Byers and Lord, 1943).

As protective policies regarding industrial lead exposure in the United States improve, the occupational exposure was reduced to lesser chronic exposures, especially for those in children (Needleman, 2004). In 2002, the Centers for Disease Control (CDC), United States, reported that 890,000 American children had elevated blood lead levels,

most likely resulting from low-level chronic exposure to lead-contaminated products (CDC, 2006). Although lead-based paint is the major contributor, research also indicates several other possible contributing culprits for lead poisonings. One of these factors is lead-contaminated candies, imported into the United States from countries all over the world (CDC, 2006).

There are two possible sources of lead contamination in imported candies which can include either the candy itself, or the packaging it is housed in. Some examples of where lead has been previously found include tamarind candy in lead-glazed pottery, printed cellophane candy wrappers, and chili powder (Carter-Pokras *et al*., 2007). Contamination can occur through different phases of manufacturing. For instance, several potential contamination sources of the chili powder have been reported to originate from soil residue from fields, air-drying or storage where the chilies can accumulate dust from exhaust emissions, metal particles accumulated during the grinding process, and drying over open petrochemical fires (Carter-Pokras *et al*., 2007).

In addition, candy wrappers are often printed with leaded paint and although not consumed, there are four mechanisms by which substances used in printing inks can migrate from the printed surface to the food contact surface: blocking, rubbing, peeling, and diffusion (Bradley *et al*., 2005; Kim *et al*., 2008). If the packaging is poorly designed, the inner coating will not maintain structural integrity thereby allowing ink components from the outer layer to migrate into the candy. Candies in general are sticky and can adhere to its wrapper which suggests that children come into contact with the wrapper either through licking or sucking the wrapper (CDC, 2002). Furthermore, candy contact surfaces of the packages have a potential for contamination because finished packaging films are frequently distributed to manufacturers in reel form in which the outer printed surface and

food contact surfaces of the packages are in contact with each other (Kim *et al*., 2008). As a result, candy wrappers cannot be ignored when dealing with contaminated candies.

The popularity and consumption of a specific candy grows day by day and an acquired taste for them develops in the children (Duran *et al*., 2009). Accordingly, cases of elevated blood lead levels in children following the consumption of contaminated candies have been documented by the Center for Disease Control and Prevention (CDC, 2002). In 2000, a Hispanic child born in Los Angeles County, California, was identified through a routine screening program of having a blood lead level of 22µg/dL (CDC, 2002). Tests of the boy's home showed no significant dust, soil, or paint lead levels. There was no reported use of home remedies or imported cooking pottery but it was reported that the child had been eating Mexican candies repeatedly for three years (CDC, 2002). After suggesting that the child stop consumption of the candies, the boy's blood lead levels was reduced to 11µg/dL the following year (CDC, 2002).

In another example of the same year, a Hispanic boy aged 2 years in Orange County was identified through routine screening with a blood lead level of 26 µg/dL (CDC, 2002). The family's house was built in 1963 but tests on soil, paint, and dust on the home did not reveal high lead levels (CDC, 2002). It was noted that the child had been given greta and azarcon (folk remedies that usually contains lead) and had eaten various imported tamarind fruit candies from Mexico (CDC, 2002). Testing of a Dulmex-brand Bolirindo lollipop the child had eaten revealed 404 parts per million (ppm) and 21,000ppm of lead in the stick and wrapper, respectively, and 0.2ppm and 0.3ppm in the candy and seed, respectively (CDC, 2002). Verification tests by the Food and Drug Administration (FDA) confirmed high lead levels in the wrapper of products and issued a public health warning (CDC, 2002).

The southwest is abundant with Hispanic communities due to its close proximity to Mexico. The city of Las Vegas alone (Las Vegas and North Las Vegas) has roughly 250,000 residents who are of Hispanic or Latino origin (U.S. Census Bureau, 2008). This makes these areas especially at risk for elevated blood lead levels through the consumption of imported candies. In fact, from May, 2001 to January, 2002, approximately 1000 cases of elevated blood lead levels among children in California, 150 of them were linked to possible exposures to candies produced in Mexico (CDC, 2002). The dangers of consuming a single contaminated piece of candy is intensified with the fact that a single piece of candy can exceed the FDA's provisional tolerable daily intake level for lead of 6 µg in a typical 30 gram serving (CDC, 2002). This becomes more dangerous as a child consumes additional pieces of candy.

An elevated blood lead level is classified as having a blood lead level of 10 µg/dL or greater (CDC, 2002). Yet deficits in cognitive and academic skills have been reported in children with blood lead concentrations lower than 5µg/dL (Pokras and Kneeland, 2008). This suggests that there is no safe level of lead in a child's body. Furthermore, with lead's ability to accumulate in the body, eating contaminated candies continuously can lead to dangerously excessive levels. In a study done on contaminated tamarind candies in Oklahoma by Lynch *et al* (2000), consuming the candy containing the mean concentration of lead at the rate of once per day was predicted to result in a more than five-fold increase in the prevalence of blood lead levels among those that consumed the candy. Due to the known adverse health effects of lead, the rates of consumption of candies are just as an important measure as the lead content itself. However, other than Pb, other essential metals such as Cu, Ni, Mn, Fe, Cr and Ti should be investigated, since they are known to cause lethal effect at higher concentrations. Therefore, all measurements of these metals, whether

large or small in this study, will be vital to assess the current status of imported candies in Nigeria.

2.5 HEAVY METALS IN SOIL AND VEGETABLES

It has been reported that sewage, industrial effluents and municipal waste water contain appreciable amount of major essential plant nutrients and therefore the fertility levels of soil are improved considerably under sewage irrigation of farmlands (Rajesh *et al*., 2004). However, studies on the water Vasai Creek, Maharashtra, revealed that the presence of toxic heavy metals like Fe, Pb and Hg reduced soil fertility and agricultural output (Lokhande and Kelkar, 1999). Treated sewage water also contain variable amounts of heavy metals such as Pb, Ni, Cd, Hg, Zn and Cr (Rajesh *et al*., 2004), which have the potential to contaminate crops growing under such irrigation.

Also, in several studies, soil Cd concentration has been reported high (Torabian and Baghuri, 1996). High Cd concentration has been reported in Taiwan in brown rice samples grown on contaminated soil (Chen *et al*., 1994). In Taiwan, more than 800acres of land have been contaminated with industrial effluent water.

Delibacak *et al*. (2002) reported the heavy metal status of vegetables (tomato, pepper, bean, purslane, cowpea), fruits (apple, plum, pomegranate, walnut, watermelon, peach, cherry, grape) and leaf samples, collected from 12 different places irrigated with Gediz River of the Aegean Region. The amount of some heavy metals (Fe, Mn, Zn, Cu, B and Co, Ni, Cr, Pb, Cd) were determined in the samples and compared with permissible limits of the metals. Significant levels of heavy metals were recorded in the vegetables, fruits and leaf samples. However, the leaves of vegetables and fruits contained more heavy metals than other edible parts.

In order to evaluate the contribution of vegetables to daily intake of Cd and Pb in Spain, Cd and Pb were determined in some horticultural species cultivated in greenhouse boarders using atomic absorption spectrophotometry (Zurera-Cosano *et al*., 2006). The study revealed that if the mean levels of Cd and Pb ($0.029 mgkg^{-1}$ and $0.0221 mgkg^{-1}$ respectively) were taken into account, the daily intake contribution of these metals would be $1.4 gday^{-1}$ for Cd and $11 gday^{-1}$ for Pb, respectively.

Similarly, in a study of Cd and other heavy metals (Fe, Cu, Mn, Zn) in spices commonly consumed in Turkey (Faruk *et al*., 2006), 15 samples of all spices (*Pimenta dioica* (L.) Merrill), blackpepper (*Piper nigrum* L.), cinnamon (*Cinnamomum zeylanicum* L.), nutmeg (*Myristica fragrans* Houtten), clove (*Szygium aromaticum* L. Merrill), galangal (*Alpina officinarum* Hance), cardamom (*Elettaria cardamomum* (L.) Maton), ginger (*Zingiber officinale* Roscoe) and turmeric (*Curcuma longa* L.) were analysed. The results showed that Cd in the samples ranged from $13 mgkg^{-1}$ in clove to $206 mgkg^{-1}$ in blackpepper; Fe varied between $28 mgkg^{-1}$ in nutmeg to $374 mgkg^{-1}$ in blackpepper; Cu ranged from 3.0 – $11 mgkg^{-1}$, Mn ranged from $10 mgkg^{-1}$ in turmeric to $355 mgkg^{-1}$ in clove samples and zinc of the samples ranged from 4 – $25 mgkg^{-1}$ with cardamom containing the highest. Their results showed that the value of all metals studied except Cd were below the Food and Agriculture Organization (FAO)/WHO permissible limits.

Lokeshwari and Chandrappa (2006) also assessed the extent of Fe, Zn, Cu, Ni, Cr, Pb and Cd contamination in vegetation due to irrigation with sewage-fed Bellandur lake water in India on agricultural land. The results showed presence of some heavy metals in rice and vegetables, beyond the India permissible limits. Metals transfer factors from soil to vegetation were also reported significant for Zn, Cu, Pb and Cd. Comparing the results of heavy metals concentration in water, soil and vegetation with their respective natural levels,

it was observed that impact of lake water on vegetation was more than the soil.

Presently, there is a growing public concern in Zimbabwe over the cultivation of vegetables on soils amended with sewage sludge or irrigated with admixtures of sewage and sewage sludge. Excessive accumulation of heavy metals in agricultural soils may not only result in environmental contamination, but lead to elevated heavy metal uptake by crops, which may affect food quality and safety. Based on this fear, Muchuweti *et al* (2006) investigated heavy metal concentrations in crops irrigated with sewage sludge and sewage/sewage sludge admixtures at Firle Municipal Farm in Harare. The results showed that Tsunga leaves contained 3.68 mg kg^{-1} Cd, over 18 times the permissible level by the EU standards (0.2 mg kg^{-1}); Cu was 111 mg kg^{-1}, 5 times the EU Standard (20 mg kg^{-1}); concentrations of Pb was 6.77 mg kg^{-1}, over 22 times the permissible levels allowed by both EU standards and UK guidelines (0.3 mg kg^{-1}); Zn concentration was 221 mg kg^{-1}, over 4 times the guideline value (50 mg kg^{-1}). The other plants (beans, maize, peppers and sugarcane) also contained concentrations of heavy metals above the permissible levels.

The impact of wastewater irrigation on heavy metal content of *Beta vulgaris* (Palak), a highly nutritious leaf that is widely cultivated and consumed in urban India, was investigated by Rajesh *et al* (2007). Samples of wastewater, irrigated soil and the edible portion of Palak (*Beta Vulgaris L. var All green HI*) were collected and analysed for Cd, Cu, Zn, Pb, Cr, Mn and Ni. Heavy metals concentrations in water and soil were below the internationally recommended (WHO) maximum permissible limits set for agricultural use for all heavy metals except Cd at all sites. Furthermore, the edible portion of B. *vulgaris,* contained Cd levels that was higher than permissible limits during summer, whereas Pb and Ni concentrations were higher in both summer and winter seasons. The study concluded that the use of treated and untreated wastewater for irrigation had increased the

contamination of Cd, Pb and Ni in edible portion of vegetables causing potential health risk in the long term for consumers.

Similarly, in a field experiment carried out by Jamali *et al* (2007) to determine the uptake of Cd, Cu, Cr, Ni, Pb and Zn by different vegetables grown on agricultural soil irrigated with untreated domestic wastewater and sewage sludge revealed high levels of heavy metals. The study concluded that continuous irrigation of agricultural land with wastewater and solid sewage sludge increased consumer health risk in the long term.

2.6 HEAVY METAL CONTAMINATION OF CANNED FOOD

Canned foods are valuable commodity worldwide. Canning is a method of preserving food by first sealing it in air-tight jars, cans or pouches, and then heating it to a temperature that destroys contaminating microorganisms that can either be of health or spoilage concern because of the danger posed by several spore-forming thermo-resistant microorganisms, such as *clostridium botulinum* (Azlan, 2009). Many vegetables begin losing some of their vitamins when harvested. Nearly half the vitamins may be lost within a few days unless the fresh produce is cooled or preserved so canning can be a safe and economical way to preserve the food quality although there may be additional losses of sensitive vitamins in food structure during heating process in manufacturing (United State Development Agency (USDA), 2002).

Food is a major source of metal intake for people (WHO, 2001). Heavy metals can be absorbed by plant grown on contaminated soils and subsequently find its way into the food chain. Processing or packaging is another route of entry of metals (Edward, 1986). Fresh food may be contaminated by small amounts of metal from airborne fallout,

particularly noted in vegetables such as lettuce, tomatoes, onions and pepper which, in addition, may be contaminated during irrigation farming (Awode *et al*., 2008; Abdullahi *et al*., 2008). Vegetables grown in soils with high lead contents can also contain traces (Thornton *et al*., 2001).

Metals used in food cans could cause contamination, particularly in foods containing acidic fruits, such as tomatoes. The use of such cans was prohibited in the early 1980s (Thornton, 2001). Majority of food cans are sealed with a solder that is 98% lead and 2% tin (Edward, 1986). Tin-lead solders (a type of soft solder) are the most widely used, because they are cheaper than alternative solder alloys. A study of lead contents of canned foods in the UK in 1984 revealed elevated lead levels (Thornton, 2001).

In a study by Melaku (2009) on levels of essential metals (Zn, Fe, Cu and Mn) and toxic metals (Pb and Cd) in canned tomato paste sold in Addis Ababa, Ethiopia using atomic absorption spectrometry, the results showed that Zn, Fe, Cu, Mn and Pb were within the FAO/WHO standard limits. However, the Cd content obtained showed that the level was higher than the FAO/WHO limits. According to him, the high concentrations of Cd in canned tomato paste examined might be taken from soils through the roots of tomato plants or the deposition on the surface of tomato plant leaves from airborne particulates or contaminated water might be used for irrigation and food processing or food processing operations might add heavy metal contaminants into tomato paste.

Due to absence and scanty literatures on canned tomato paste, it becomes difficult to make reference to other studies, hence leading to scanty literature survey.

2.7 TOXICITY OF HEAVY METALS

The presence of toxic trace metals in the environment continues to generate a lot of

concern (Awofolu, 2005). This is due to the health implications of their presence since they are non-essential metals that are not required for any function either by plants or animals and are usually monitored for health purposes. This category of metals is not required by man even in small amount (Tyler, 1981; Borgmann, 1983). However, metals such as Cu and Zn are classified as essential to life due to their involvement in certain physiological processes, although elevated levels of these metals were found to be toxic (Spear, 1981).

Copper is one of the heavy metals that is essential to both plants and animals but could be toxic at elevated levels. It is toxic at low concentration and is known to cause brain damage in animals (Awofolu, 2005). However, symptoms of acute copper toxicity in man include hypertension, anaemia and cardiovascular collapse while chronic toxicity leads to sporadic fever, vomiting, diarrhoea and jaundice. It is also suspected that copper is carcinogenic due to high incidence of cancer among coppersmith (Luckey and Venugopal, 1977).

Chromium is present in human tissues in variable concentrations and its deficiency is characterised by disturbance in glucose, lipid and protein metabolism (Anthony *et al*., 2007). It also plays an essential role in enhancing the activity of insulin in human body. However, its compounds are well known toxins particularly Cr^{6+} which, due to its oxidising potentials, easily permeates biological membranes and can also cause renal damage and various forms of cancer (Bae *et al*., 2000).

Nickel is a naturally occurring element found in a number of mineral ores including nickel phosphates, oxides and silicates. It is present in the enzyme urease and as such is considered to be essential to plants and some domestic animals (Awofolu *et al*., 2005). However, the essentiality of Ni to health has not been demonstrated, but Ni related health effects such as renal, cardiovascular, reproductive and immunological effects have been

reported in animals. Also, its toxic effects in man are related to dermal, lung and nasal sinus cancer (Holynska *et al*., 1996).

Zinc is an essential element to plants and animals. Although, Zn has been found to have low toxicity to man, prolonged consumption of large doses can result in some health complications such as fatigue, dizziness, and neutroeria (Awofolu, 2005). Similarly, Luckey and Venugopal (1977) reported that acute Zn toxicity in man causes diarrhoea, depression of central nervous system leading to tremous and paralysis, while chronic Zn toxicity causes growth retardation, faulty reproduction and anaemia in man. Audu and Lawal (2005) also noted that Zn is an essential element that is involved in metabolic functions, and is important for both animal and plant health growth. Zn content of normal plant tissues varies according to species, but it is usually within the range of 5 – 300mg/kg.

Zinc is an element found in virtually every cell of the human body and plays vital roles in the development and healthy growth of human body and has a recognised action on more than 300 enzymes by participating in their structure or in their catalytic and regulatory action. According to Nnorom *et al* (2007), zinc rich food tend to be expensive, and so Zn fortification is an important consideration especially because daily intakes appear to be more useful physiologically than in intermittent doses. Zinc deficiency can result in poor growth, difficulty in wound healing, and loss of appetite, undesirable skin changes, and adverse effects on the immune system. Also zinc deficiency is associated with sexual maturation, fertility, immunity, tastes and appetite. The current Reference Daily Intake for Zn is 15mg/day. Overload of Zn (> 100mg/day) can also be dangerous. It can depress the immune system, or cause anaemia (Nnorom *et al*., 2007).

Manganese is an essential nutrient, functioning in the tissues as a co-factor for arginase and the phosphotransferases (Stanley *et al*., 1975), and has its source from

industrial effluents and at certain level, it is toxic to a number of crops. However, there is no available data for human toxicity (Cornish *et al*., 1999). Cornish, *et al* (1999) noted a summary of the effects of heavy metals on plants and human health and are represented in Table 2.1.

Table 2.1: Heavy metals and their effects on plants and human health

Metal	**Sources**	**Agronomic effects**	**Effect on human health**
Cr	Leather tanneries (about) 40mg/L in surface discharges)	Not generally recognised as essential growth element. Lack of knowledge on its toxicity to plants.	Carcinogenic, dermatitis, painful chrome ulcers. Food main source of intake
Cu	Plumbing, animal wastes, pesticides, earth crust	Toxic to number of plants at 0.1 mg/L in nutrient solutions.	Liver cirrhosis. Food main source of intake, uncertain toxicity to humans
Mn	Industrial effluents	Toxic to a number of crops at a few tenths to a few mg/L	No convincing data of human toxicity
Ni	Industrial effluents	Toxic to a number of plants at 0.5mg/L to 1mg/L	Carcinogenic, lack of data on carcinogenic by the oral route
Zn	Plumbing, animal wastes, pesticides	Toxicity to many plants at widely varying concentrations	Not a major hazard to health

2.8 CHOICE OF ANALYTICAL TECHNIQUES

X-ray fluorescence (XRF) is suitable for this kind of work, because of its advantageous features, namely, multi-elemental capability, high sensitivity, precision and short time of analysis (Holynska *et al*., 1996). Other analytical techniques such as atomic absorption spectrometry (AAS) and neutron activation analysis (NAA) also can be used for heavy metal determination, however, they all need extensive sample preparation, which is usually troublesome, time consuming and prevents *insitu* analysis (Tölg and Klockenkämper, 1993).

2.8.1 PRINCIPLE OF X-RAY FLUORESCENCE

The XRF is able to identify elements because each atom fluorescence at a specific energy when excited by an X-ray. X-ray photons from the XRF create inner shell vacancies within an atom. An outer shell electron fills that space as the atom relaxes to ground states. This process releases photons with energy in the X-ray regions of the electromagnetic spectrum equal to the energy difference. Detectors on the XRF machine are able to identify the element due to its characteristic emitted X-ray. By comparing the observed intensities of the X-ray to a known standard, the machine can quickly quantify the amount of metals (Kalnicky and Singhvi, 2001).

Detection capabilities of the XRF improve as time increases, as background noise decreases, and as sensitivity increases. Detection limits are dependent on both the elements and the matrix, and most elements are still detectable below typical site action levels. Qualitative results depend largely on the application and intended use of the data, as well as the calibration of the XRF using a sample matrix.A sample matrix is designed through

laboratory procedures to minimize interfering elements that are commonly found in a specific environmental application (Kalnicky and Singhvi, 2001)

The limit of detection for the XRF can be found in multiple ways, but the most commonly applied method states that the detection limit (DL) is that amount that gives a net intensity equal to three times the standard counting error of the background intensity. The DL may also be defined in terms of the precision of repeat measurements on a standard sample. In order to determine the DL, the USEPA recommends the measurement of a sample that has a concentration of analyte close to the expected DL.

The EPA defines the detection limits as the minimum concentration of a substance that can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero. To estimate the detection limit, the standard deviation of non-consecutive replicates measurements must be multiplied by the rounded Student's t-factor, as seen in the equation below:

$MDL = 3\sigma$

Where σ is the standard deviation for the replicates and the Student's t-factor is approximately equal to 3 (Lee, 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Study Area

The study areas include six states across the six geographical zones of Nigeria. One state from each zone was used for the study. The choice of these States was based on availability of the impounded products reported in Thisday 29 September, 2009. The Zones and States for the study were as follows:

North-West	-	Kano	-	**Site 1**
North-East	-	Bauchi	-	**Site 2**
North-Central	-	Kogi	-	**Site 3**
South-West	-	Lagos	-	**Site 4**
South-East	-	Abia	-	**Site 5**
South-South	-	Rivers	-	**Site 6**

In each of the states, five markets were selected for the study.

3.1.2 Sample Collection

The sampling was carried out in December 2009 based on information from NAFDAC impoundment of unregistered Chinese products in Kaduna (Thisday Newspaper, 21-09-2009). A total of 2 different brands of tomato paste: Testi tomato paste (coded A) and merysa concentrates de tomato paste (coded B), and candies (royal glucose biscuits – coded C; lollipops with pencils – coded D; Paravo chocolate cherry – coded E; and Martino milk candy – coded F) were collected from five major markets each from a state in the geographical zones of Nigeria: North-West (Bauchi), North-East (Kano), North-Central

(Kogi), South-West (Lagos), South-East (Aba) and South-South (PortHarcourt). Before the choice of the sampling markets in each zone, a survey was carried out to ensure that these products were sold across the length and breadth of Nigeria.

For each sample product, 12 samples were purchased randomly from five major markets in each sampling states in a geographical zone. The samples were composite to form a representative sample for the zone. The composite sample was achieved by grinding samples from each zones into powder, then pooled together to give composite. Same batch of the products were purchased directly from the vendors. Each representative was also assigned an identified code as mentioned above and securely stored at room temperature until it was sent to the laboratory for Cu, Mn, Fe, Ni, Zn, Ti analysis using XRF.

3.2 METHODS

3.2.1 Sample Preparation and Analysis

The X-Ray Florescence Spectroscopy (XRF) analysis was conducted at the X-ray fluorescence analytical Research Laboratory of Centre for Energy Research and Training of the Obafemi Awolowo University, Ife, Nigeria. The procedure used was reported by Funtua (1996), and Hassan and Umar (2004). 0.5g of the pulverized samples each was homogenized with 3 drops of an organic binder (Polystyrene dissolved in toluene). This was pressed at 10 tons with a hydraulic press (SPECAC) to form a pellet of 19mm diameter die. The pellets were introduced into the energy dispersive spectrophotometer and consist of an annular 25mci Cd–109 isotope as the excitation source emitting Ag-K X-ray (221 keV) and a Mo X-ray tube (50KV, 5MA).

3.2.2 Statistical Analysis

The results of this study were analyzed using Pearson Correlation analysis and ANOVA single factor analysis of variance. Pearson correlation coefficient, r is an index without dimension that has a range -1.0 to +1.0 inclusive. The index indicates the extent of linear relationship between two variables X and Y. The coefficient is always 1 when there is a perfect linear relationship which gives a positive slope between the two variables. A coefficient of -1 is obtained when there is a perfect linear relationship which gives a negative slope between data of the two variables. A +1 coefficient is obtained when one variable has a high value and so does the other. While a -1 coefficient is obtained when one Variable has a high value while the other has low value.

A correlations coefficient is 0 when there is no linier relationship between two variables. An ANOVA is an analysis of the variation present in a given experiment. It is a test of the hypothesis that the variation in an experiment is no greater than that due to normal variation of individual's characteristics and error in their measurement. The tests in an ANOVA are based on the F – ratio: the variation due to an experimental treatment or effect directed by the variation due to experimental error. ANOVA puts all the data into one number (F) and gives us one P for the null hypothesis. The t –test tells us if the variation between two groups is "significant" P reports the significance level.

CHAPTER FOUR

4.0 RESULTS

4.1 METALS IN CANNED TOMATO PUREE

The results of the levels of heavy metals in two brands of canned tomato puree (Samples A and B) are presented in Figs. 4.1 and 4.2. For sample A, the Cu concentration was highest in Site 3 sample (4.2mg/g), and lowest in Sites 1, 2, 5 and 6 samples (4.0mg/g). Mn concentration was highest in Site 4 sample (42.5mg/g) and lowest in Sites 2 and 6 samples (40.0mg/g). The highest and lowest of Fe were 27.0 (Sites 2 and 6) and 26.0mg/g (Site 5). Ni had highest concentration (56.0mg/g) in sample from Site 5, and lowest (55.0mg/g) in samples from Sites 1 and 4. The highest concentration of Zn (27.8mg/g) was in Site 4 sample while Site 5 sample had the lowest (26.5mg/g). Ti and Cr were not detected in any of the samples.

For Sample B, the results of the levels of the metals are presented in Fig. 4.2. Mean Cu concentration was highest in the sample from Site 4 (4.5mg/g) and lowest in Sites 1 and 3 samples (4.0mg/g). Mn concentration was highest in Site 5 sample (18.6mg/g) and lowest in Sites 1 and 6 samples (18mg/g). The highest and lowest concentrations of Fe were 62.5 and 60.0mg/g in Sites 4 and 1, respectively. Ni had highest concentration (4.5mg/g) in Site 4 sample and lowest (4.0mg/g) in Sites 1 and 3 samples. The highest concentration of Zn (35.2mg/g) was in Site 5 sample while Sites 6, 1 and 5 samples had the lowest (34.5mg/g). The highest concentration of Ti (25.0mg/g) was in Site 4 sample and the lowest (24.0mg/g) in Site 1. Cr concentration was lowest in Site 4 sample (19.0mg/g) and highest (20.0mg/g) in other samples. Analysis of variation between heavy metals in Sample A and B revealed significant differences ($p < 0.05$).

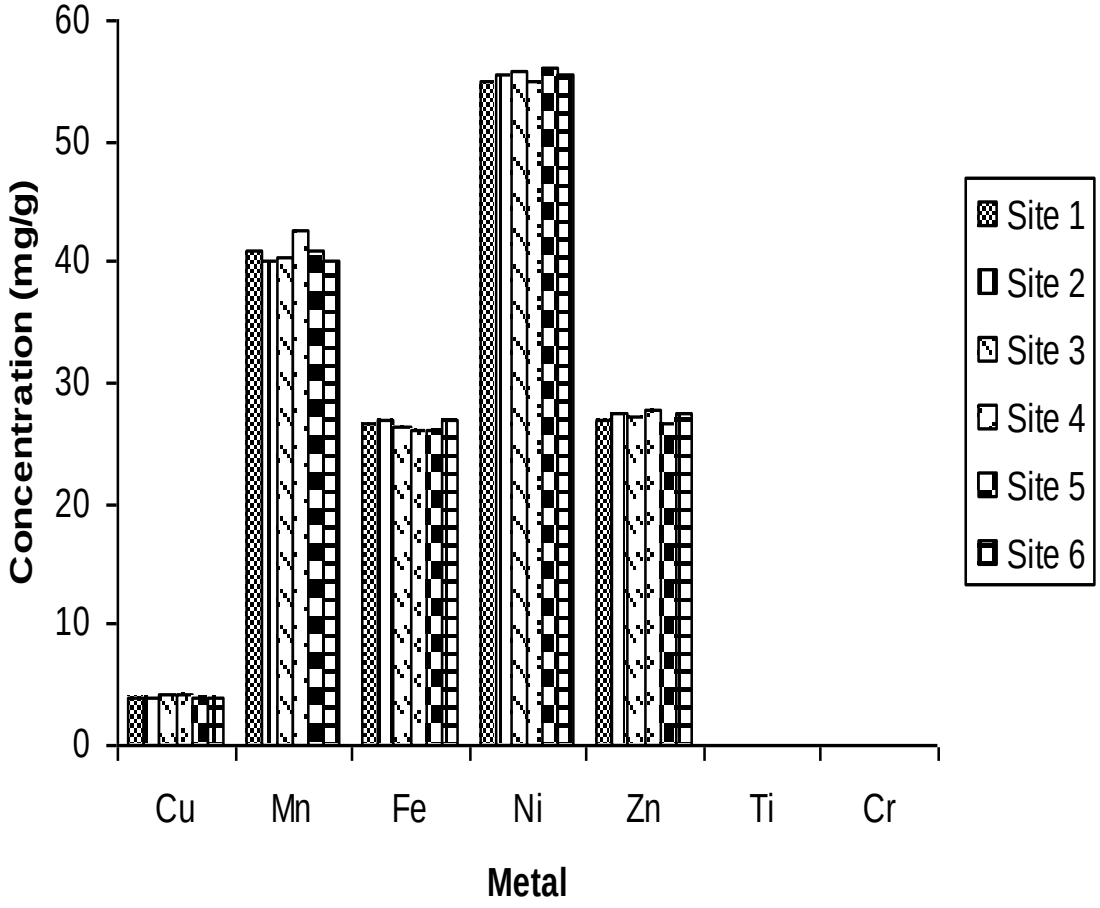


Fig. 4.1: Concentration of heavy metals in Sample A

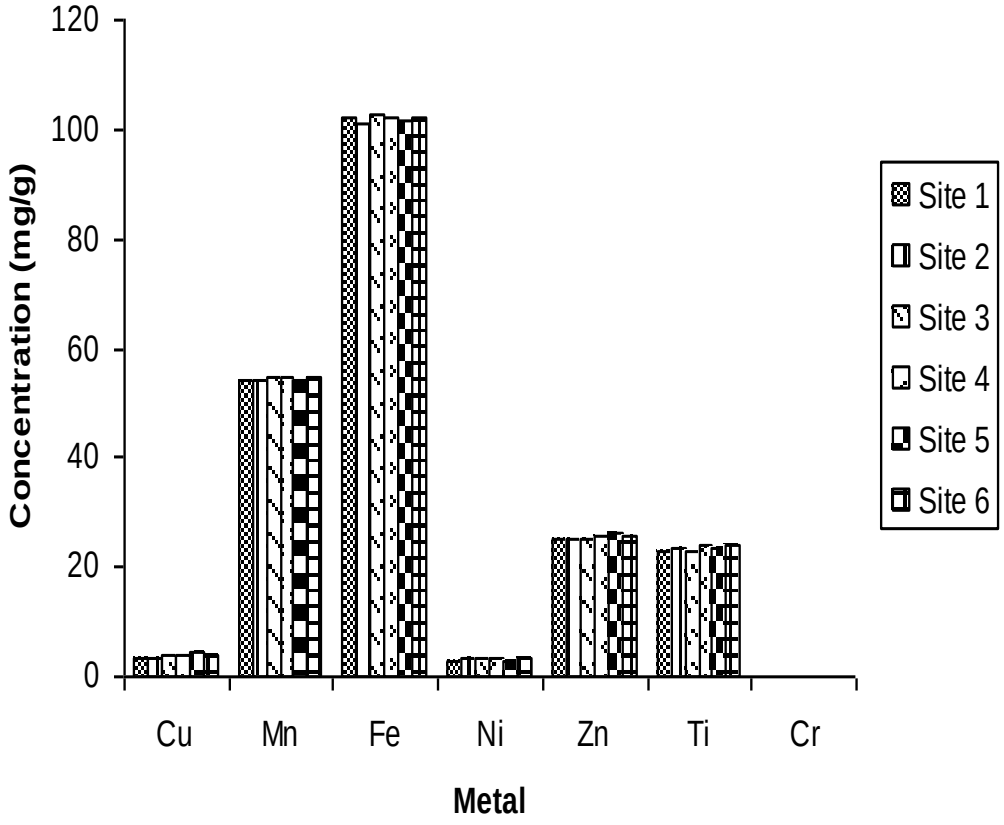


Fig. 4.2: Concentration of heavy metals in Sample B

The variations in the concentration of the metals in the samples was probably due to the differences in the sources of the metals which was brought about by difference in brands, and probably due to difference in processing of the puree in the products.

Generally, the concentration of the metals were found to be Ni > Mn > Zn > Fe > Cu > Ti = Cr and Fe > Zn > Ti > Cr > Mn > Cu = Ni for Samples A and B, respectively, in that order of decreasing magnitude. A correlation matrix for heavy metals in Sample A was calculated to see if some metals were interrelated with each other and presented in Table 4.1. Positive correlation was found between the metals: Mn Vs Cu, Ni Vs Fe and Cu, and Zn Vs Cu, Mn and Fe. However, others showed negative correlation.

Also, Correlation analysis as shown in Table 4.2 for Sample B showed positive correlation between Mn Vs Cu, Fe Vs Cu and Mn, Ni Vs Cu ($p<0.01$, Mn and Fe, Zn Vs Cu, Mn ($p<0.05$), Fe and Ni, and Ti Vs Cu ($p<0.05$), Mn, Fe, Ni ($p<0.05$) and Zn. Negative correlations were observed between Cr Vs Cu, Mn, Fe, Ni ($p<0.05$), Zn, Ti and Cr. The positive correlations indicates common sources of the metals which may include toxic waste deposited in irrigation water, soil and fertilizer, while negative correlation observed among the metals could indicate different source of the metals.

4.2 HEAVY METALS IN CHOCOLATES AND CANDIES

The concentrations of heavy metals in different candies (Sample C, D and F) and chocolates (Sample E) are presented in Figs 4.3 – 4.6. The concentrations of heavy metals in Sample C ranged from 3.2 (Site 2) – 4.2mg/g (Site 5) Cu, 54.0 (Sites 1, 2, and 5) – 54.8mg/g (Sites 4 and 6) Mn, 101.0 (Site 2) – 102.5mg/g (Site 3) Fe, 3.0 (Sites 2 and 5) – 3.5mg/g (Site 3) Ni, 2.5 (Sites 1, 2 and 3) – 26.0mg/g (Site 5) Zn, and 23.0 (Sites 1 and 3) – 23.8mg/g (Sites 4 and 6) Ti, No Cr was detected in all the sites for Sample C.

Table 4.1: Correlation analysis of metals in Sample A

Parameter	Cu	Mn	Fe	Ni	Zn	Ti	Cr
Cu	1.000						
Mn	0.257	1.000					
Fe	-0.435	-0.878*	1.000				
Ni	0.030	-0.491	0.105	1.000			
Zn	0.234	0.164	0.217	-0.592	1.000		
Ti	.a	.a	.a	.a	.a	.a	
Cr	.a	.a	.a	.a	.a	.a	.a

a. Cannot be computed because at least one of the variables is constant.
****. Correlation is significant at the 0.05 level (2-tailed).***

Table 4.2: Correlation analysis of metals in Sample B

Parameter	Cu	Mn	Fe	Ni	Zn	Ti	Cr
Cu	1.000						
Mn	0.238	1.000					
Fe	0.683	0.503	1.000				
Ni	1.000**	0.238	0.683	1.000			
Zn	0.499	0.893*	0.668	0.499	1.000		
Ti	0.852*	0.582	0.781	0.852*	0.712	1.000	
Cr	-0.877*	-0.223	-0.311	-0.877*	-0.406	-0.758	1.000

. Correlation is significant at the 0.01 level (2-tailed).

. Correlation is significant at the 0.05 level (2-tailed).

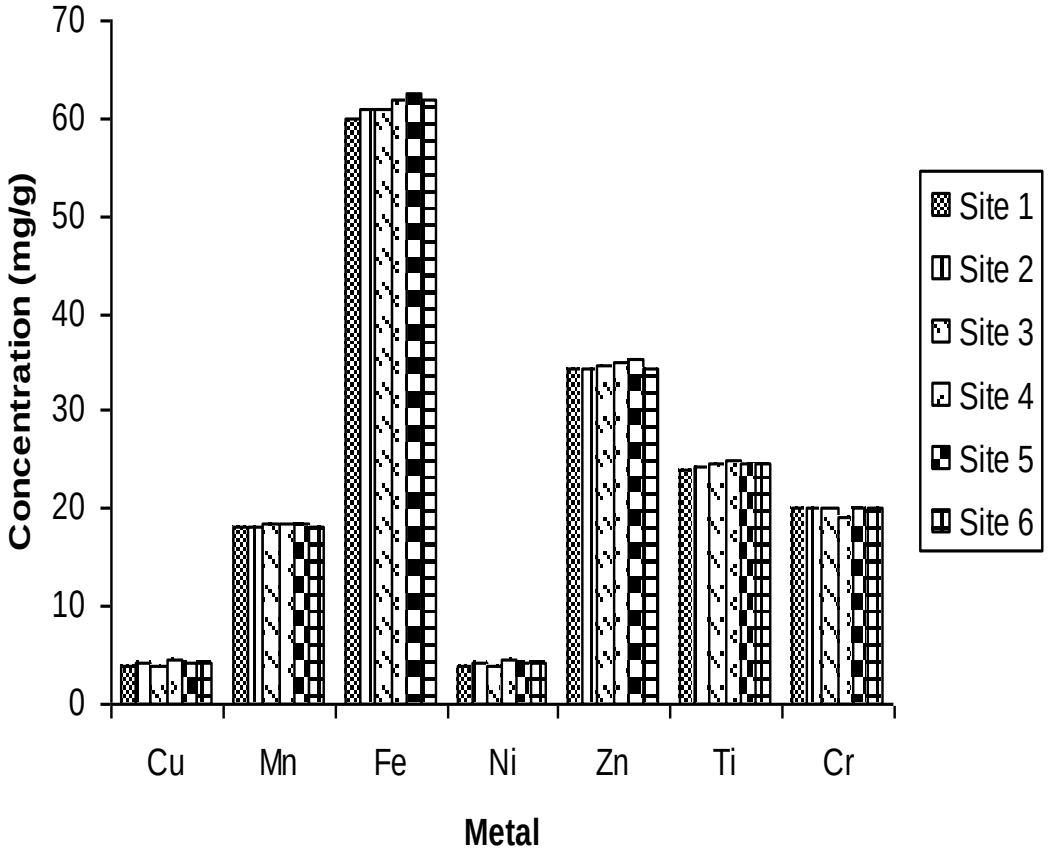


Fig. 4.3: Concentration of heavy metals in Sample C

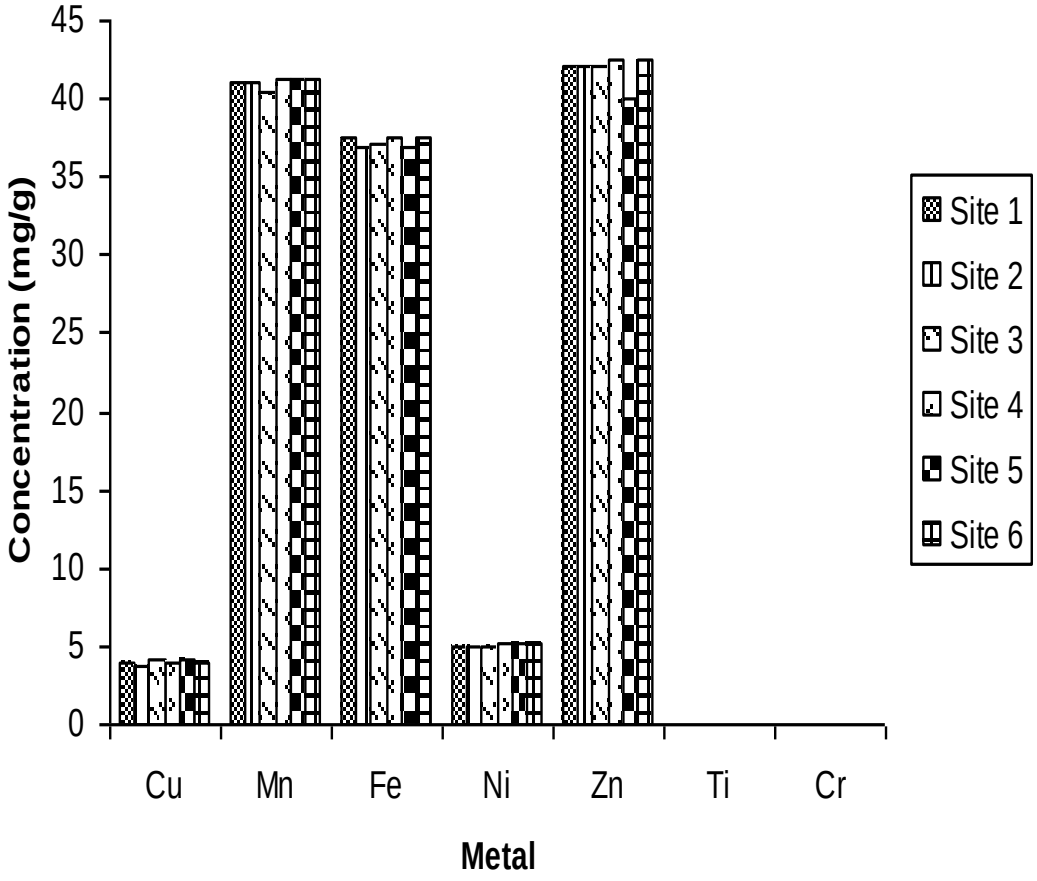


Fig. 4.4: Concentration of heavy metals in Sample D

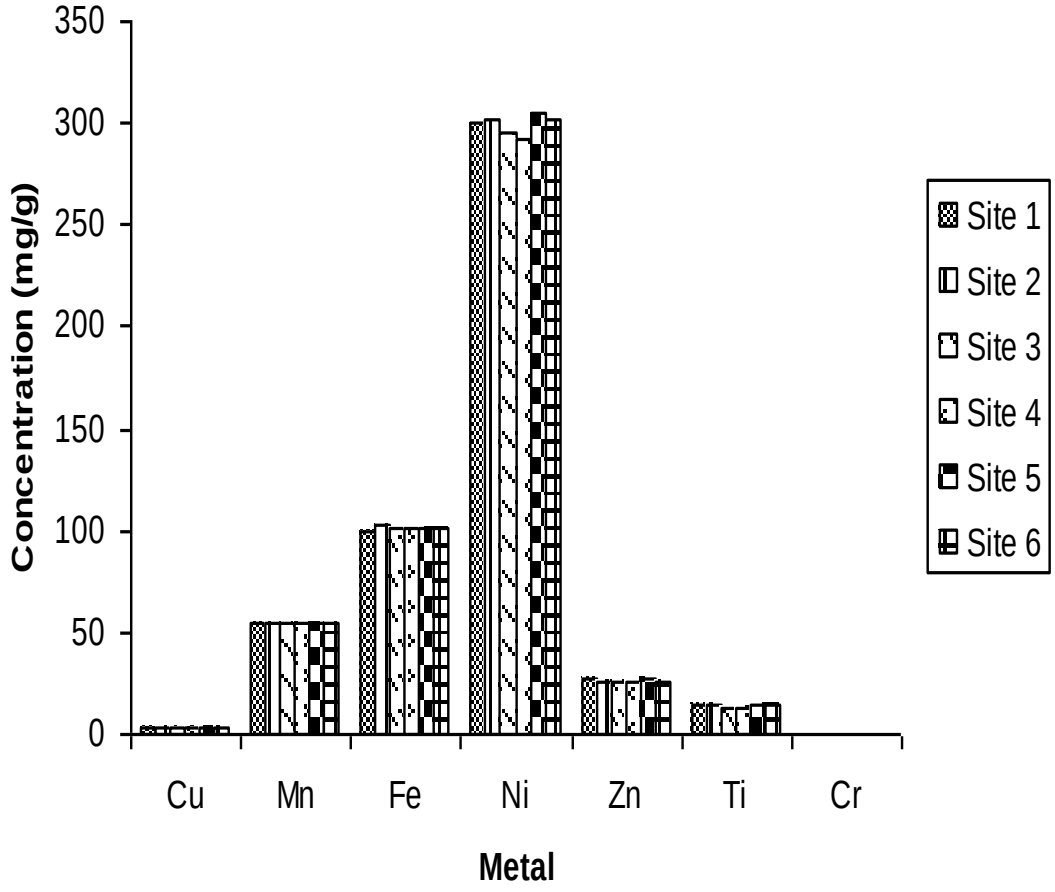


Fig. 4.5: Concentration of heavy metals in Sample E

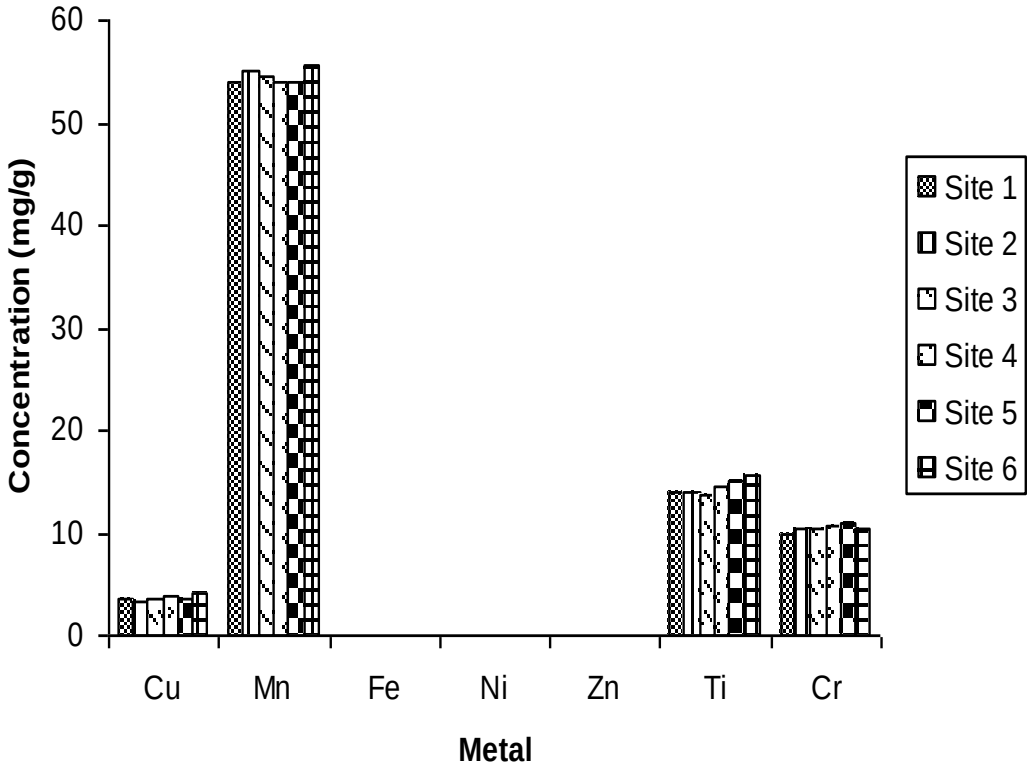


Fig. 4.6: Concentration of heavy metals in Sample F

For sample D, the range of 3.7 (Site 2) – 4.2mg/g (Sites 3 and 5) Cu, 40.5 (Site 3) – 41.3mg/g (Site 4) Mn, 37.0 (Sites 3 and 5) – 37.5mg/g (Sites 1, 4 and 6) Fe, 5.0 (Sites 1, 2 and 3) – 5.2mg/g (Sites 4 and 6) Ni, and 40.1 (Site 5) – 42.5mg/g (Sites 4 and 6) were observed. Ti and Cr were not detected in all the sites for this sample. Also, the metal concentration in Sample E ranged between 2.6 (Site 2) – 3.5mg/g (Site 5) Cu, 54.0 (Sites 1 and 3) – 55.0mg/g (Site 6) Mn, 100.0 (Site 1) – 102.0mg/g (Site 2) Fe, 292.0 (Site 4) – 305.0mg/g (Site 5) Ni, 25.0 (Sites 2 and 4) – 26.7mg/g (Site 5) Zn, and 13.0 (Site 4) – 14.7mg/g (Site 5) Ti. Cr was not detected in any of the sites. In Sample F, metal concentrations range between 3.2 (Site 2) – 4.2mg/g (Site 6) Cu, 54.0 (Sites 1, 4 and 5) – 55.7mg/g (Site 6) Mn, 13.6 (Site 3) – 15.5mg/g (Site 6) Ti, and 10.0 (Site 1) – 11.0mg/g (Site 5) Cr, Fe, Ni and Zn were not detected.

Furthermore, the profiles of metal concentration in the samples are:

Cu: D > C > F > E

Mn: F > E > C > D

Fe: C > E > D > F

Ni: E > D > C > F

Zn: D > E > C > F

Ti: C > F > E > D

Cr: F > C = D = E

Multivariate correlation analysis of heavy metals in samples as shown in Tables 4.3 – 4.6 revealed positive correlation between Mn Vs Cu, Fe Vs Cu and Mn, Ni Vs Mn and Fe, Zn Vs Cu ($p < 0.05$) and Mn, Ti Vs Cu, Mn and Zn for Sample C, Fe Vs Cu and Mn, Ni Vs Cu, Mn and Fe, and Zn Vs Fe for Sample D, Mn Vs Cu, Fe Vs Mn, Ni Vs Cu, Mn and Fe, Zn Vs Cu, Mn and Ni, and Ti Vs Cu, Mn, Fe and Ni ($p < 0.01$) for sample E, and Mn

Table 4.3: Correlation analysis of metals in Sample C

Parameter	Cu	Mn	Fe	Ni	Zn	Ti	Cr
Cu	1.000						
Mn	0.458	1.000					
Fe	0.399	0.710	1.000				
Ni	-0.287	0.255	0.349	1.000			
Zn	0.877*	0.123	-0.089	-0.524	1.000		
Ti	0.456	0.513	-0.171	-0.384	0.587	1.000	
Cr	.a	.a	.a	.a	.a	.a	.a

. Correlation is significant at the 0.05 level (2-tailed).

a. Cannot be computed because at least one of the variables is constant.

Table 4.4: Correlation analysis of metals in Sample D

Parameter	Cu	Mn	Fe	Ni	Zn	Ti	Cr
Cu	1.000						
Mn	-0.164	1.000					
Fe	0.051	0.121	1.000				
Ni	0.129	0.660	0.478	1.000			
Zn	-0.494	-0.307	0.614	0.135	1.000		
Ti	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	
Cr	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]

a. Cannot be computed because at least one of the variables is constant.

Table 4.5: Correlation analysis of metals in Sample E

Parameter	Cu	Mn	Fe	Ni	Zn	Ti	Cr
Cu	1.000						
Mn	0.332	1.000					
Fe	-0.087	0.157	1.000				
Ni	0.378	0.318	0.421	1.000			
Zn	0.746	0.314	-0.303	0.662	1.000		
Ti	0.259	0.486	0.432	0.938**	0.538	1.000	
Cr	.a	.a	.a	.a	.a	.a	.a

a. Cannot be computed because at least one of the variables is constant.
****. Correlation is significant at the 0.01 level (2-tailed).***

Table 4.6: Correlation analysis of metals in Sample F

Parameter	Cu	Mn	Fe	Ni	Zn	Ti	Cr
Cu	1.000						
Mn	0.491	1.000					
Fe	.[a]	.[a]	.[a]				
Ni	.[a]	.[a]	.[a]	.[a]			
Zn	.[a]	.[a]	.[a]	.[a]	.[a]		
Ti	0.684	0.388	.[a]	.[a]	.[a]	1.000	
Cr	0.048	-0.124	.[a]	.[a]	.[a]	0.544	1.000

a. Cannot be computed because at least one of the variables is constant.

Vs Cu, Ti Vs Cu and Mn, and Cr Vs Cu for Sample F. However, others are either negative or no correlation value. The positive correlations indicate similar source of these metals in the sample, with contamination of raw materials such as sugar, cocoa, milk and flour, and wrappers of these products likely to be the sources of the metals.

4.3 ESTIMATED DAILY INTAKE OF METALS

To appraise the health risk associated with heavy metal contamination of the studied products, estimated daily intake of metal (DIM) was calculated as shown below according to Zhuang *et al*. (2009).

$$DIM = \frac{C_{metal} \times W_{food}}{B_w}$$

Where,

C_{metal} (mg/g) is the concentration of heavy metals in the samples

W_{food} represents the daily average weight of the sample consumed (assumed 20g of each sample is taken by an individual)

B_w is the body weight assuming that sample A and B are consumed by adult of average weight of 65kg and samples C – F consumed by children between the ages of 1 -5 years with average body weight of 20kg.

The estimated daily intake of metals (DIM) value for the six products studied as shown in Figs. 4.7 – 4.13 varied from 1.2 (sample A) – 4.0mg/day (sample D) for Cu, 5.6 (sample C) – 54.5mg/day (sample F) for Mn, ND (sample F) – 101.9mg/day (sample B), ND (sample F) – 299.3mg/day (sample E) for Ni, ND (Sample F) – 41.9mg/g (sample D) for Zn, ND (sample A and D) – 23.4mg/day (sample B) for Ti, and ND (sample A, B, D

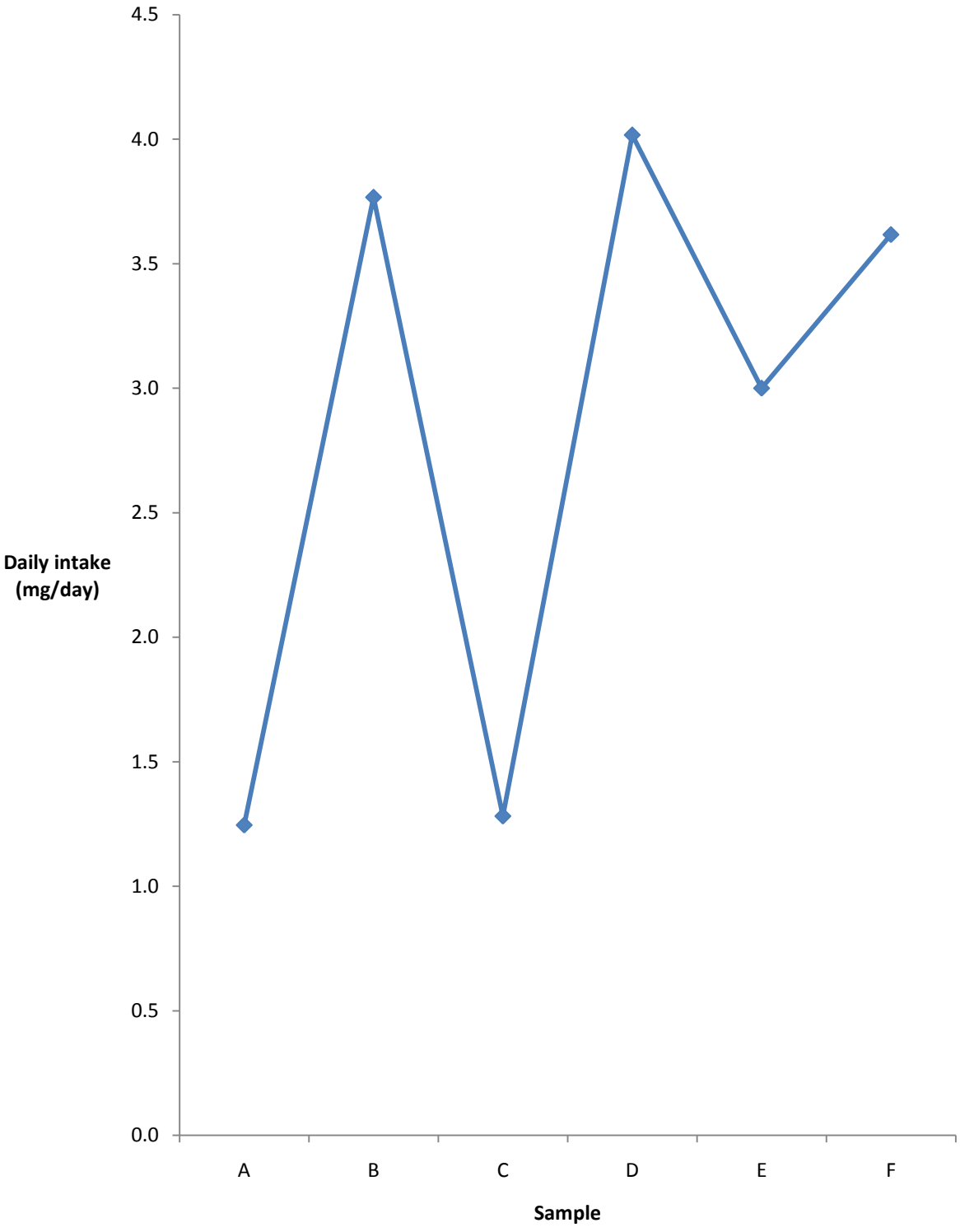


Fig. 4.7: Estimated daily intake of Cu in study samples

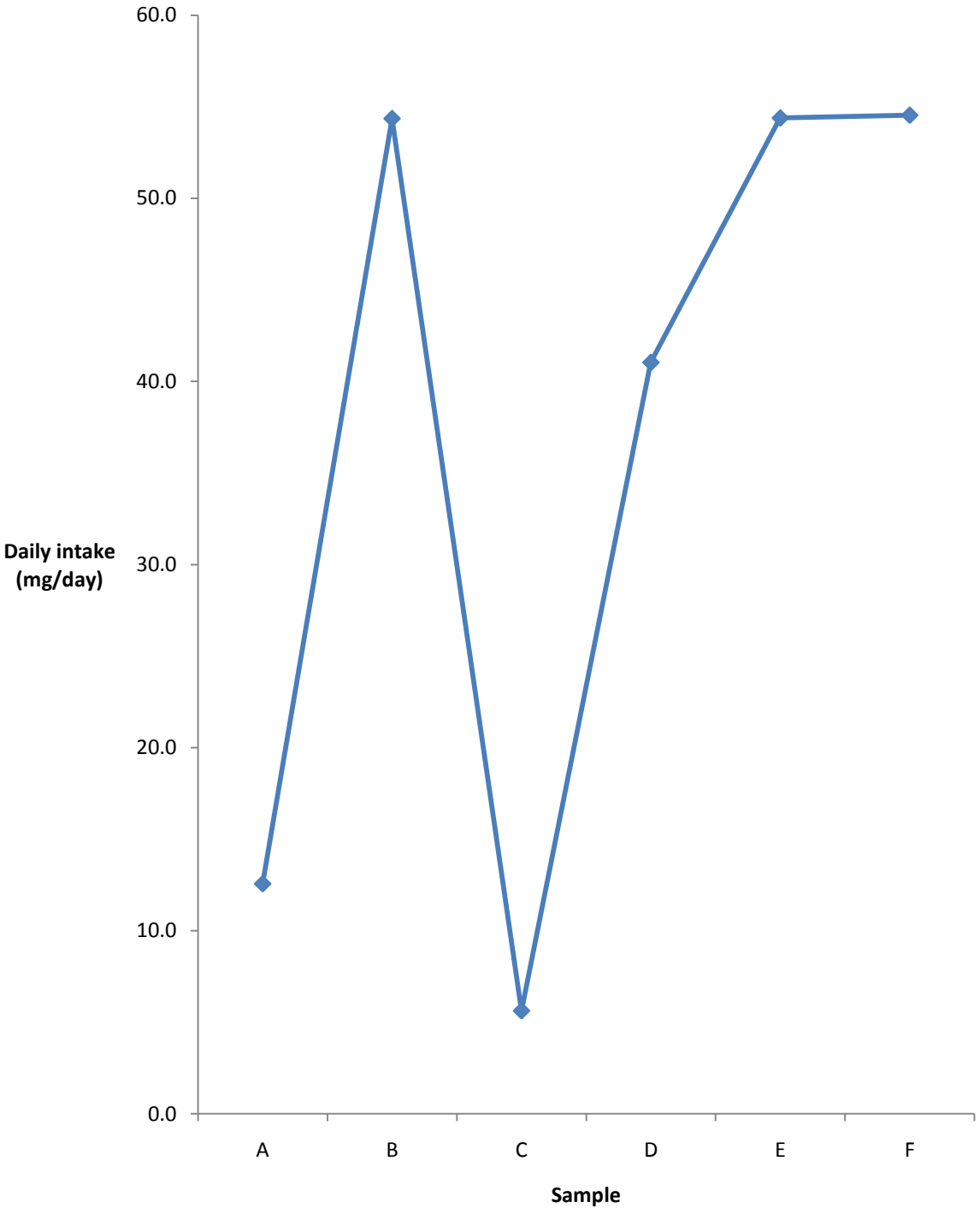


Fig. 4.8: Estimated daily intake of Mn in study samples

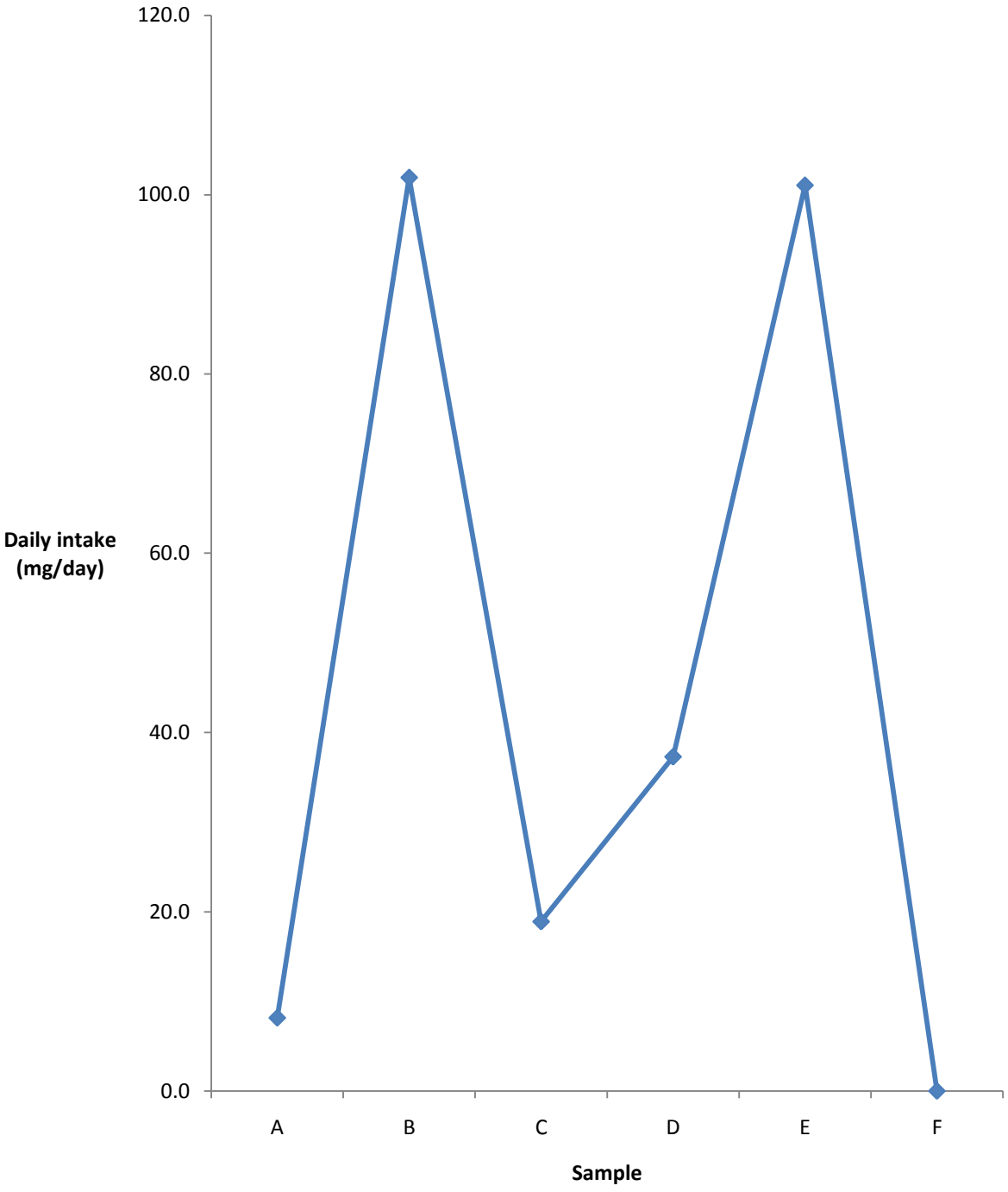


Fig. 4.9: Estimated daily intake of Fe in study samples

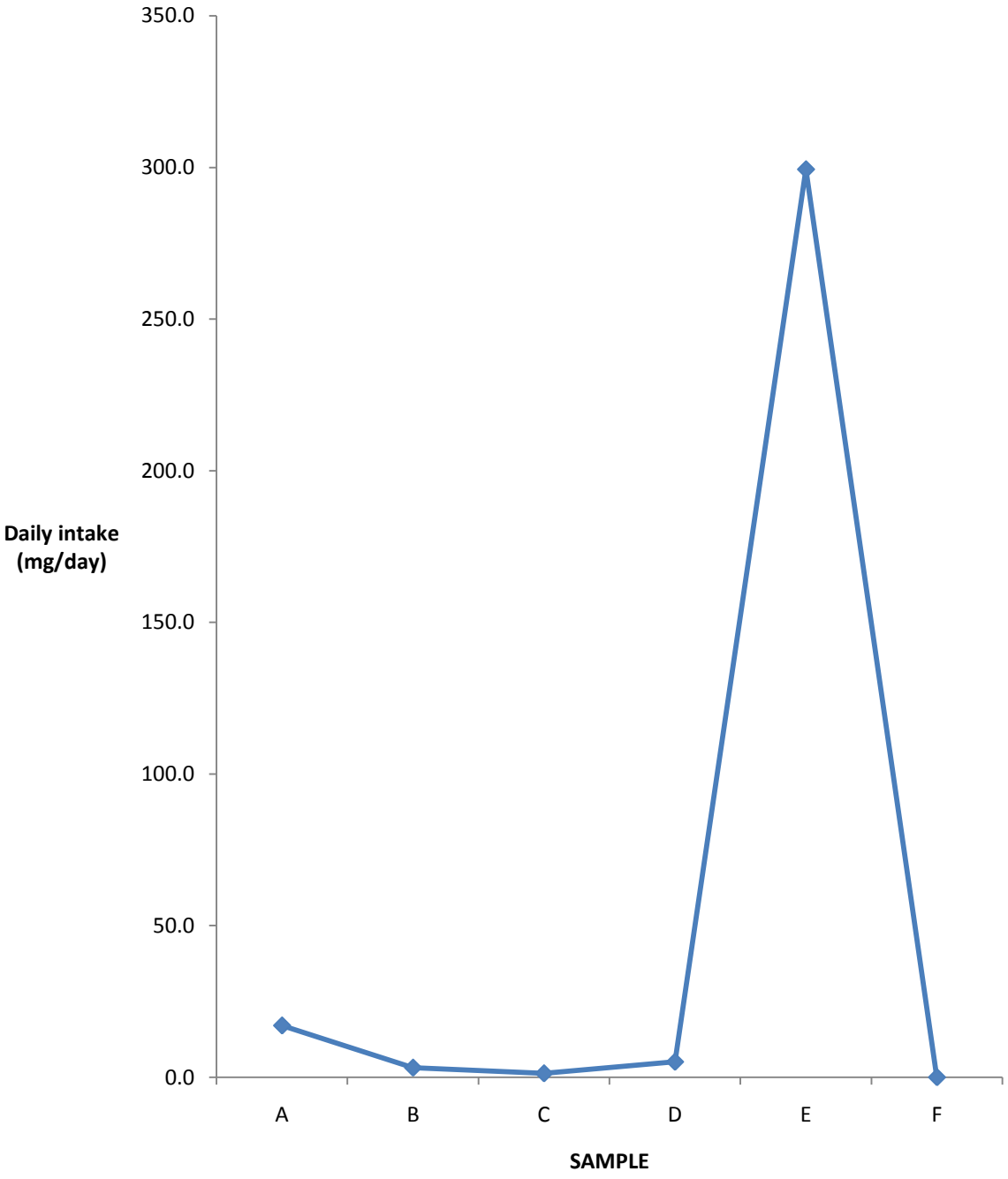


Fig. 4.10: Estimated daily intake of Ni in study samples

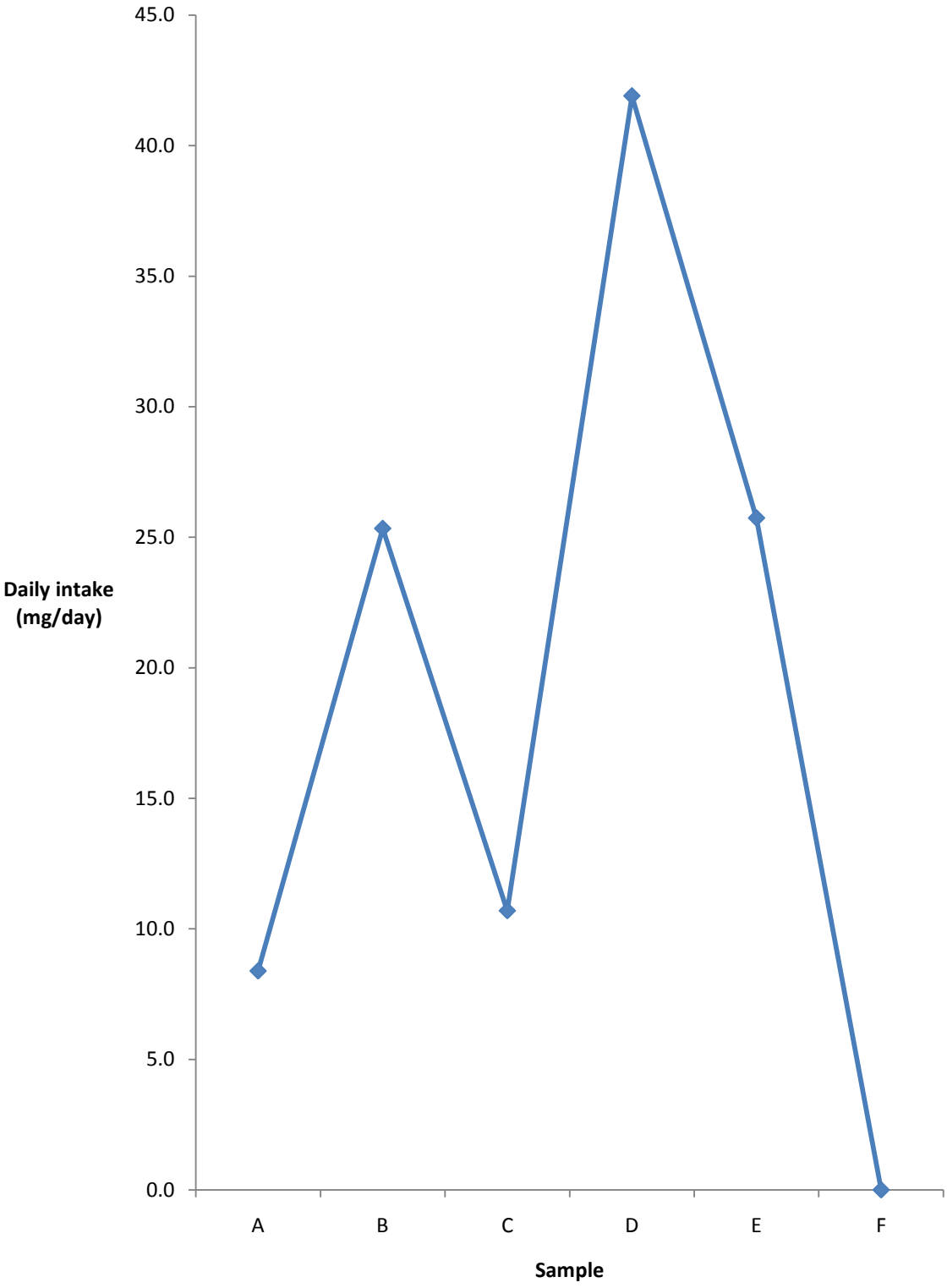


Fig. 4.11: Estimated daily intake of Zn in study samples

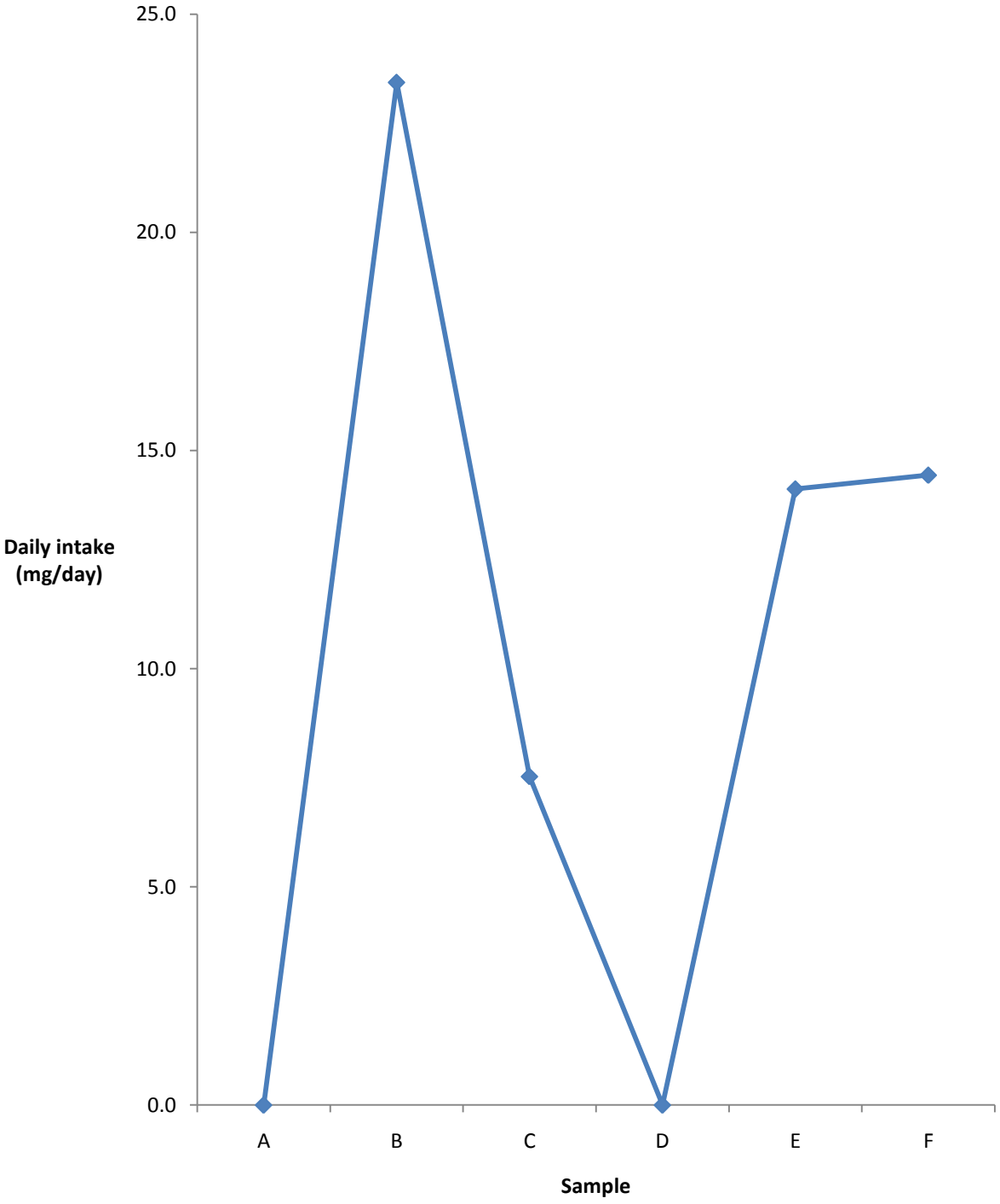


Fig. 4.12: Estimated daily intake of Ti in study samples

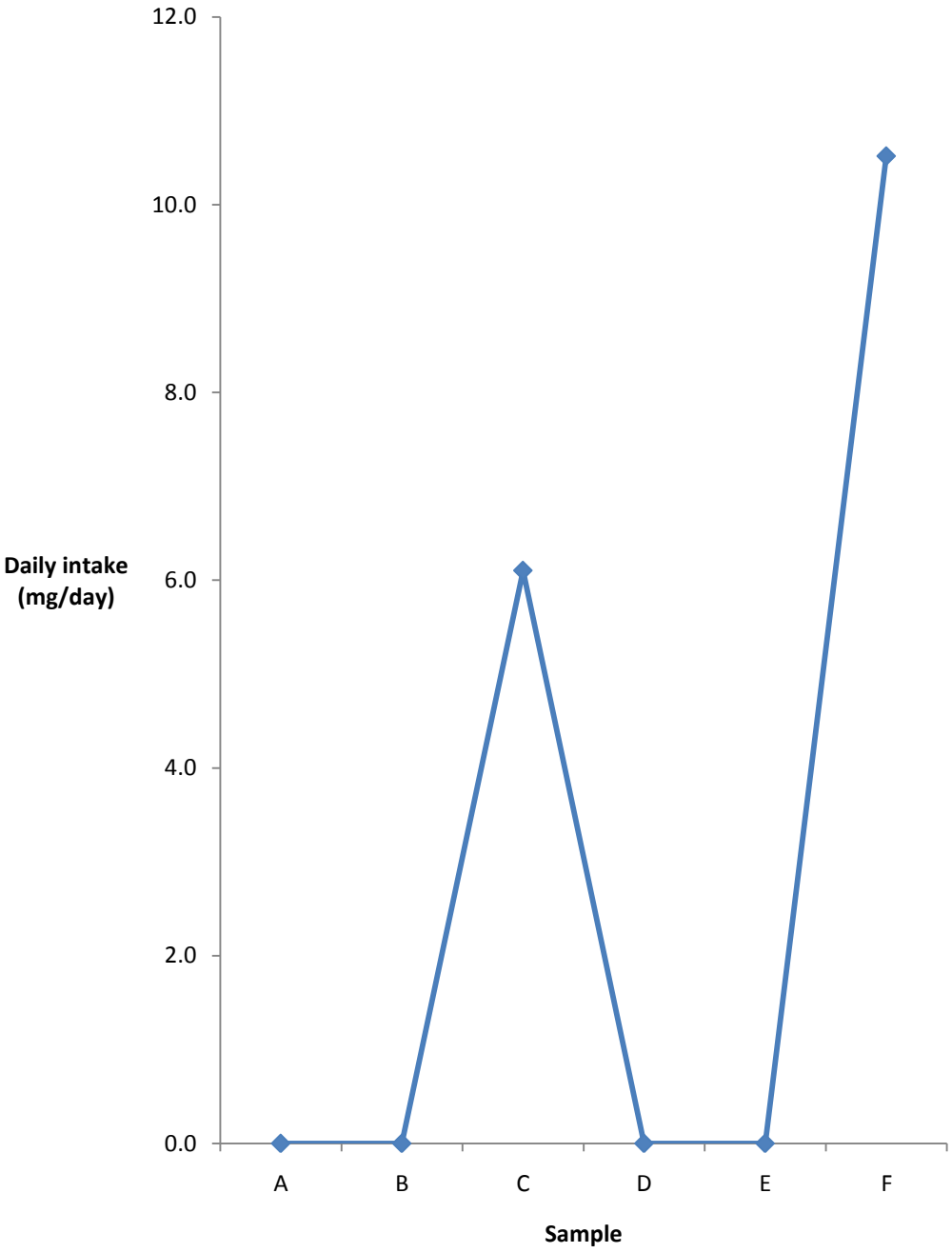


Fig. 4.13: Estimated daily intake of Cr in study samples

and E) – 10.5mg/day (Sample F) for Cr. The trends of daily intake of metals (DIMs) for the heavy metals in the samples were in the order:

Sample A: Ni > Mn > Zn > Fe > Cu > Ti = Cr

Sample B: Fe > Mn > Zn > Ti > Cu > Ni > Cr

Sample C: Fe > Zn > Ti > Cr > Mn > Cu = Ni

Sample D: Zn > Mn > Fe > Ni > Cu > Ti = Cr

Sample E: Ni > Fe > Mn > Zn > Ti > Cu > Cr

Sample F: Mn **>** Ti > Cr > Cu > Fe = Ni = Zn.

CHAPTER FIVE

5.0 DISCUSSION

5.1 METALS IN CANNED TOMATO PUREE

Although heavy metals such as Cu, Zn, Mn, Fe and Ni are essential in human nutrition, their determination in food products is important, since at high concentrations they pose health risk. In the present study, Cu was averagely high in Sample B compared to Sample A. Different sources of the raw tomatoes used in the processing could explain the difference in Cu levels in the two samples. However, due to unavailability of literature values and couple with the fact that there was no well defined limits for these metals in canned tomato puree, studies on vegetables were used for comparison. When the values of Cu were compared with values reported in literature, the values were found higher than 3.03 – 6.24µg/g reported by Fisseha (2002) in vegetable crops. Iyaka (2007) also reported 5.00µg/g which was lower than those reported in this study. Similarly, Mohammed *et al* (2003) in their assessment of essential and toxic elements in tomato reported higher levels of 4.3 – 57.1mg/g Cu. Generally, the results obtained in this study are higher than 5.00µg/g recommended limits for Cu (FAO/WHO, 2001). However, it should be noted that Cu is required as an essential dietary element. It is required for cellular metabolism in enzymatic and non-enzymatic systems. Copper acts as important metallic-activators of several enzymes. Deficiency of Cu causes low white blood cell count and poor growth. Excess intake of Cu can cause vomiting, nervous system disorder and Wilson's diseases (Lewis *et al*., 1995).

The mean concentration of Mn in Sample A was observed to be higher than Sample B, similar reason such as different in sources of tomato could be a major source of difference. The values obtained for both samples were higher than 5.56 – 1.973µg/g

reported by Awode (2008), and 0.006 – 0.108µg/g reported for imported mackerel (Abou-Arab *et al*., 1996). The levels of Mn in samples A and B were higher than 5.00µg/g FAO/WHO (2001) recommended limit.

The highest level of Fe was found in sample B. Also, in sample B relative to other metals, higher concentrations of Fe were obtained. This could be due to the fact that Fe is abundant in most soils, which could be readily available to the plants. In contrast to this study, Melaku (2009) reported the average concentration of Fe in canned tomato sold in Ethiopia markets as 6.95µg/g. In general, the concentrations of Fe in samples A and B were higher than 50.00µg/g recommended limit by FAO/WHO (2001) in foods. Iron plays many key roles in biological systems including oxygen transport (haemoglobin and myoglobin), respiration and energy metabolism (cytochromes and iron-sulfur proteins), destruction of hydrogen peroxides (hydrogen peroxidase and catalase), and DNA synthesis (ribonucleotide reductase) (Melaku, 2009). Deficiency of Fe is one of leading risk factors for disability and death worldwide. It results in anaemia which is recognized by its sysmptom such as low blood Fe level, small red blood cells and low blood haemoglobin values (Underwood, 1977).

The levels of Ni in sample A was observed to be higher than sample B, soil type where the crop was grown could be the main source of Ni and differences in Ni levels in the samples. However, when the levels of Ni was compared with values reported in literature, samples in this study were found higher than 0.80 – 0.89µg/g and 2.73 – 6.90µg/g reported by Fisseha (2002) and Awode *et al*. (2008), respectively. Zn is one of the important metals for normal growth and development in human beings. Deficiency of Zn can result from inadequate dietary intake and results in impaired absorption, excessive excreation or inherited defects in Zn metabolism (Colak *et al*., 2005; Narin *et al*., 2005). Zn

in canned tomato puree in this study was determined in the range of 26.5 – 35.2mg/g. While the highest level of Zn was determined in Sample B, the lowest level was in sample A. The levels of Zn in this study were higher than the 5.00µg/g recommended limit (FAO/WHO, 2001).

No Cr was detected in sample A. Detection of Cr in sample B might be taken up from soils through the roots of tomato plants or deposited on the surfaces of tomato plant leaves from airborne particulates or contaminated water might be used for irrigation and food processing or food processing operations might add the metal into tomato paste. However, the value obtained in this study was lower than 500 – 18900mg/g obtained in canned mackerel (Abou-Arab *et al*., 1996).

5.2 HEAVY METALS IN CHOCOLATES AND CANDIES

Heavy metal composition of food, candies and chocolates is of interest because of their essential or toxic nature. For example, Fe, Zn, Cu, Cr, Co, Ni and Mn are essential while Pb, Cd and Hg are toxic (Onianwa *et al*., 1999). Though, there are no well defined limits for these metals in chocolates and candies, rather daily intake limits of these metals in food are given by WHO, which will be discussed in a later section. Hence, comparison of the results obtained in this study with results reported in the literature on metals in candies and chocolates will be difficult, because limited studies are available, which are not easily accessible (Iwegbue, 2011). Hence, the results are compared with recommended standard limits.

Highest concentration of Cu was obtained in sample D (lollipop) with constituents mainly sugar and flour. Daily intake limit of Cu in the samples is higher than the 0.0733mg/g limits reported in vegetable. This could be related to high amount of copper in

the raw material and likely processing of these materials. There is greater risk of health effects from deficiency of copper intake than from excess copper intake. Acute toxicity due to ingestion of copper is infrequent in humans. However, when it occurs it is usually a consequence of consumption of copper in chocolates and candies or from accidental or deliberate ingestion of high quantities of copper salts. Symptoms include vomiting, lethargy, acute hemolytic anaemia, renal and liver damage, neurotoxicity, increased blood pressure and respiratory rates. In some cases, coma and death followed (Environmental Health Criteria for Copper, 1996). Chronic copper poisoning has not been described in general population (Aaseth and Norseth, 1986). Results of this study showed that chocolates and candies are significant sources of childhood copper exposure in Nigeria. Thus, long time consumption of products with high levels of Cu may pose a risk in reduction of total homocysteine and folate which are essential to body metabolism (Tamura and Turnland, 2004).

High level of Mn was observed in sample F, which is above 0.00004 – 0.0126mg/g reported in chocolates and candies (Iwegbue, 2011). This implies that impoundment of these products could be related to this reason. Metals are present in food either naturally or as a result of human activities such as agricultural practices, industrial emissions, car exhausts, or contamination during manufacturing. For chocolates and candies, contamination may also occur due to raw materials and water used. Manganese is present in most foodstuffs. The main contributors of manganese to the diet are cereals (10-30 mg/kg) as well as vegetables and fruits (0.5-5 mg/kg) (Beliles, 1994; Codex, 1995). Nuts may have a high content of manganese. In some countries, manganese has replaced organic lead as an additive in petrol. This might result in increasing concentrations of manganese in the environment and in foodstuffs in the future. Manganese is an essential trace element that

plays a role in bone mineralization, protein and energy metabolism, metabolic regulation, cellular protection from damaging free radical species, and the formation of glycosaminoglycans (Agency for Toxic Substances and Diseases Registry (ATSDR), 1997). Although manganese is an essential nutrient, exposure to high levels via inhalation or ingestion may cause some adverse health effects (ATSDR, 1997). Excess manganese affects the central nervous system and the neurological effects have been observed in case of occupational exposure. No problems are reported in connection with intake of manganese in foodstuff as manganese is considered one of the least toxic metals. Consistent with its role as an essential element, manganese and its inorganic compounds have a relatively low order of acute toxicity (Beliles, 1994). However, the absorption is increased in individuals with iron deficiency (Beliles, 1994). In humans, the degree of manganese absorption from the gastrointestinal system is generally low, of the order of 3 % (Beliles, 1994).

With the exception of samples A, D and F, Fe is the most abundant metal among all the metals studied. Fe ranged between ND – 102.5mg/g, higher than 0.0017 – 0.0123mg/g reported by Iwegbue (2011). High concentration of Fe in this study could be attributed to the fact that Fe is the fourth most abundant element (5%) in the earth's crust (Beliles, 1994) where soils are made and the raw materials for these products are grown. Iron is present in most foods and beverages. In general, liver, kidney, beef, ham, egg yolk, and soybeans have iron concentrations of the order of 30-150 mg/kg (Elinder, 1986). In several countries the cereal most commonly eaten, e.g., wheat flour, is fortified with iron in order to provide the necessary amount of iron in the diet (Nordic Council of Ministers, 1995). Food contamination with iron may originate from food processing equipment, and/or the containers used in packaging the products. The iron leaching into canned foodstuffs

depends on storage time of the food product. Rare cases of release of very high quantities of iron from food contact materials such as iron kitchen utensils have been observed (Codex, 1995). Iron is an essential trace metal (Joint Expert Committee on Food Additives (JECFA), 1983). Iron is mainly a deficiency problem and not a toxicological problem. Iron deficiency is generally the most common single nutritional deficiency in both developing and developed countries (Nordic Council of Ministers, 1995). Certain iron salts, mainly ferrous sulphate and ferrous succinate, are frequently used for the treatment and prevention of iron deficiency in humans (Beliles, 1994). Under normal conditions, about 5-15% of the iron is absorbed (Elinder, 1986). Ingestion of soluble iron salts by children in doses exceeding 0.5 g of iron can give rise to severe lesions in the gastrointestinal tract, followed by metabolic acidosis, shock and toxic hepatitis (Elinder, 1986).

Ni is exceptionally high in sample E compared to others. This value is much higher than trace amount (0.0014 – 0.0079mg/g) obtained in chocolates and candies sold in southern Nigeria markets (Iwegbue, 2011). Nickel is found in small quantities in many foodstuffs (0.001-0.01 mg/kg) and in higher concentrations in foodstuffs such as grains, nuts, cocoa products and seeds (up to 0.8 mg/kg) (National Food Agency of Denmark, 1995). It is found as complex bound Ni^{2+} in the diets (Codex, 1995). The absorption and retention of nickel in the gastrointestinal tract is influenced by fasting and food intake. Food intake and gastric emptying are of substantial significance for the bioavailability of nickel from aqueous solutions. The absorption of free nickel ions released in the gastrointestinal tract may be 40 times higher than that of complex-bound nickel from foodstuff (Sunderman *et al*., 1989). The absorption of nickel from drinking water is increased by fasting (Nielsen *et al*., 1999). Inorganic nickel compounds are absorbed to 10% or less from the gastrointestinal tract (Norseth, 1986). Nickel intake via foodstuff does

not cause hazards for the majority of consumers (Codex, 1995). A subgroup of the population of Demark (app. 10%, mainly women) have contact allergy to nickel. Nickel allergy is only caused by absorption of nickel through the skin (Codex, 1995).

The highest value of Zn in chocolates and candies (42.5mg/g) in this study is higher than the reported value of 0.0006 – 0.008mg/g in chocolates and candies sold in southern Nigeria markets (Iwegbue, 2011). Zn is an essential trace metal (Elinder, 1986), the 25th most abundant element and is widely found in nature (Beliles, 1994). Zinc is present in the form of zinc ions or zinc salts (Codex, 1995). It is one of the most ubiquitous of the essential trace metals (Florence and Batley, 1980). The absorption of ingested zinc is highly variable (10-90%) (Elinder, 1986). Zinc is an essential element necessary for the function of a large number of metalloenzymes (ATSDR, 1992; Beliles, 1994). Zn acts to diminish the toxicity of cadmium and copper (Florence and Batley, 1980). Zn may be a modifier of the carcinogenic response; zinc deficiency or excessively high levels of zinc may enhance susceptibility to carcinogenesis (Beliles, 1994).

With the exception of sample D, Ti is present in all the samples analysed. Related reports of Ti in chocolates and candies are scarce. However, Ti is the ninth most common element in the earth's crust and occurs in a number of minerals (Beliles, 1994). There is no evidence indicating that titanium is an essential element for man (Nordman and Berlin, 1986). Titanium is used in the form of the dioxide as a colour additive in confectionary, dairy products, soft drinks, etc. (Directive 93/43/EEC). Also, titanium is used as additives to edible inks, toothpaste, and pharmaceuticals (Whitehead, 1991). Titanium compounds are generally considered to be poorly absorbed upon ingestion (Nordman and Berlin, 1986). Studies on titanium alloys used in inplants and titanium compounds used in cosmetics and pharmaceuticals do not indicate any local effects on tissues (Nordman and Berlin, 1986). A

distinct toxicological dichotomy exists between TiO, the insoluble, unreactive non-metabolized form devoid of toxicity, and the soluble, inorganic salts that metabolize normally with absorption, distribution, and excretion (Beliles, 1994). However, little information exists on how titanium acts as a toxic agent, and what does exist is of little or no value in understanding the toxic actions of titanium (Beliles, 1994).

Similar to other metals, concentrations of Cr in the samples are higher than 0.00004 – 0.003mg/g reported by Iwegbue (2011). The main sources of chromium are cereals, meat, vegetables and unrefined sugar, while fish, vegetable oil and fruits contain smaller amounts (Codex, 1995). Most foodstuffs contain less than 0.1 mg chromium per kg (Nordic Council of Ministers, 1995). Chromium is present in the diet mainly as Cr(III) (Codex, 1995). As with all metals, food contamination may be caused by atmospheric fall-out (Codex, 1995). Chromium is found in the environment mainly in the trivalent form. Hexavalent chromium, or chromate, may also be found in very small amounts, arising usually from anthropogenic sources (Beliles, 1994). Cr(III) has the ability to form strong, inert complexes with a wide range of naturally occurring organic and inorganic ligands (Florence and Batley, 1980). In most soils and bedrocks, chromium is immobilised in the trivalent state (Florence and Batley, 1980). Chromium is an essential element to man. Chromium is found at low levels in most biological materials. Cr(III), the most stable oxidation state in biological materials, is important for normal glucose metabolism, while Cr(VI) is highly toxic (Beliles, 1994; Costa, 1997; Nordic Council of Ministers, 1995). Cr(III) has a low toxicity due to low absorption (about 0.5%) (Nordic Council of Ministers, 1995). Toxic aspects of chromium are related to Cr(VI) (Nordic Council of Ministers, 1995), due to its high absorption, easy penetration of the cell membranes and its genotoxicity and oxidising properties (Codex, 1995).

5.3 ESTIMATED DAILY INTAKE OF METALS

As revealed from the results, Ni, Fe, Zn and Mn have the highest intake among the samples. It should be noted that the DIM estimation is a risk assessment designed to avoid underestimation of the risk. Thus, it incorporated several assumptions such as ingested quantities of metal ions correspond to the quantities that are absorbed (United States Environmental Protection Agency (USEPA), 1989). Hence, the DIMs values of Cu as revealed in the results showed that Sample A and D are below than 1.4mg/day recommended limit for Cu in UK (Ysart *et al*., 2009). However, the DIM for other samples were found to be 2 folds higher than the stipulated limits, with the largest contribution to the intake of Cu from Sample D.

The contribution of overall DIM values resulting from levels of Mn as shown in the results showed that all the samples had their DIM values above the 4.5mg/day threshold limit recommended (Ysart *et al*., 2009). Similarly, for Fe and Zn, all the samples except Samples A and F were above the 15.0 and 11.0mg/day recommended for Fe and Zn daily dietary intake (Ysart *et al*., 2009). Also, for Ni, all the samples except Sample F were above the 0.1mg/day recommended limit while for Cr, Sample C and F were above 0.1mg/day the recommended limit.

Generally, the results revealed that Sample A (except for Cu and Fe), B, C (except for Cu), D and E have levels of the metals above the daily dietary recommended limit for all the studied metals in the samples. Thus, frequent intake of these contaminated products is likely to induce health effects arising largely from Cu, Mn, Fe, Ni and Zn.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The present study showed that not all the studied metals were found in the samples; Ti and Cr in samples A and D, Cr in sample C and E, and Fe, Ni and Zn in sample F were not detected. However, all the metals found in a sample were generally higher than reported values for vegetables and confectionery for tomato puree, chocolates and candies. The reasons for the high levels of the metals in the samples could arise from unsafe storage conditions or at any point in the production chain.

Also, evaluation of dietary intake of these products daily revealed that sample A (except for Cu and Fe), B, C (except for Cu), D and E were above the daily dietary recommended limit for all the studied metals. Thus a frequent intake of these contaminated products is likely to induce health effects arising largely from Cu, Mn, Fe, Ni and Zn.

The assessment provides some preliminary evidence that these imported products from China contained unacceptable levels of heavy metals with potential serious consequences for children and adults who consume the products. The results also agree with the argument of NAFDAC and SON of the questionable quality of products imported from China (This Day, 2007) which poses a potentially significant public health threat.

6.2 RECOMMENDATIONS

Despite the efficiency of the food regulatory agencies in Nigeria, the importation and distribution of contaminated foods still continues. However, the agencies should employ the use of portable X-ray fluorescence instruments readily available, as demonstrated by the investigation here, for effective screening of products or confirming

contamination by these heavy metals.

Also, more regulatory actions should be taken to restrict consumption of these contaminated foods as the future of any nation depends on the health, prosperity and progress of the forthcoming generation.

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Appendix I

Levels of heavy metals in sample A (mgg^{-1})

Sampling Zone	Cu	Mn	Fe	Ni	Zn	Ti	Cr
North-West	4	41	26.5	55	27	ND	ND
North-East	4	40	27	55.5	27.5	ND	ND
North-Central	4.2	40.5	26.4	55.7	27.2	ND	ND
South-West	4.1	42.5	26	55	27.8	ND	ND
South-East	4	41	26.2	56	26.5	ND	ND
South-South	4	40	27	55.5	27.5	ND	ND

ND: Not Detected

Appendix II

Levels of heavy metals in sample B (mgg^{-1})

Sampling Zone	Cu	Mn	Fe	Ni	Zn	Ti	Cr
North-West	3.5	54	102	3	25	23	ND
North-East	3.2	54	101	3.2	25	23.5	ND
North-Central	3.7	54.5	102.5	3.5	25	23	ND
South-West	4	54.8	102.2	3.1	25.5	23.8	ND
South-East	4.2	54	101.5	3	26	23.5	ND
South-South	4	54.8	102.2	3.1	25.5	23.8	ND

ND: Not Detected

Appendix III

Levels of heavy metals in sample C (mgg^{-1})

Sampling Zone	Cu	Mn	Fe	Ni	Zn	Ti	Cr
North-West	4	18	60	4	34.5	24	20
North-East	4.1	18.2	61	4.1	34.5	24.2	20
North-Central	4	18.5	61	4	34.8	24.5	20
South-West	4.5	18.4	62	4.5	35	25	19
South-East	4.2	18.6	62.5	4.2	35.2	24.6	20
South-South	4.2	18	62	4.2	34.5	24.5	20

ND: Not Detected

Appendix IV

Levels of heavy metals in sample D (mgg^{-1})

Sampling Zone	Cu	Mn	Fe	Ni	Zn	Ti	Cr
North-West	4	41	37.5	5	42	ND	ND
North-East	3.7	41	37	5	42.2	ND	ND
North-Central	4.2	40.5	37.2	5	42.1	ND	ND
South-West	4	41.2	37.5	5.2	42.5	ND	ND
South-East	4.2	41.3	37	5.1	40.1	ND	ND
South-South	4	41.2	37.5	5.2	42.5	ND	ND

ND: Not Detected

Appendix V

Levels of heavy metals in sample E (mgg^{-1})

Sampling Zone	Cu	Mn	Fe	Ni	Zn	Ti	Cr
North-West	3	54	100	300	26.5	14	ND
North-East	2.6	54.1	102	302	25	14.5	ND
North-Central	3.1	54	101	295	25.2	13.5	ND
South-West	2.8	54.5	100.7	292	25	13	ND
South-East	3.5	54.7	101.5	305	26.7	14.7	ND
South-South	3	55	101	302	26	15	ND

ND: Not Detected

Appendix VI

Levels of heavy metals in sample F (mgg^{-1})

Sampling Zone	Cu	Mn	Fe	Ni	Zn	Ti	Cr
North-West	3.5	54	ND	ND	ND	14	10
North-East	3.2	55	ND	ND	ND	14	10.5
North-Central	3.6	54.5	ND	ND	ND	13.6	10.3
South-West	3.7	54	ND	ND	ND	14.5	10.8
South-East	3.5	54	ND	ND	ND	15	11
South-South	4.2	55.7	ND	ND	ND	15.5	10.5

ND: Not Detected

Appendix VII

Estimated daily intake of metals (mg/day)

Sample	Cu	Mn	Fe	Ni	Zn	Ti	Cr
A	1.2	12.6	8.2	17.1	8.4	0.0	0.0
B	3.8	54.4	101.9	3.2	25.3	23.4	0.0
C	1.3	5.6	18.9	1.3	10.7	7.5	6.1
D	4.0	41.0	37.3	5.1	41.9	0.0	0.0
E	3.0	54.4	101.0	299.3	25.7	14.1	0.0
F	3.6	54.5	0.0	0.0	0.0	14.4	10.5
UK Limit	1.4	4.5	15.0	0.1	11.0		0.1