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Assessment of Contamination Levels and Estimation of Dietary Intake of Heavy Metals from Selected Imported Fruits and Vegetables in Ghana

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By

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Levels and dietary intake of heavy metals were assessed and evaluated in thirty two samples of fruit and vegetables collected from supermarkets in Accra, Ghana using atomic absorption spectroscopy. Differences ($p <= 0.05$) existed in Pb, Cd and Ni concentrations across the commodities collected. Levels of Pb, Cd and Cr exceeded safe limits established by FAO and EU. Washing reduced Cr in cabbage by 59% ($p <= 0.05$) but only reduced Pb in grapes by 12% ($p > 0.05$). Estimated daily and weekly intake through fruit and vegetable consumption were below provitional tolerable dietary intake established by the FAO. Although estimated dietary intake obtained in this study were below the maximum established limits, increased comsumption of these fruit and vegetables may bring about adverse health implications for the exposed population.

DEDICATION

This work is dedicated to members of my family: To the memory of my late parents - Kofi Dzimado and Mercy Akakpo for imparting in me the ability to cope with real-life challenges; my siblings - Komla Dzimado, Josephine Dzimado, Gray Dzimado, Joseph Dzimado, Alex Dzimado and Emmanuel Erasmus Dzimado for their prayers and encouragement.

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CHAPTER I

INTRODUCTION

Food safety is defined by the World Health Organization (WHO) as the assurance that the food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use (Rushing *et al*., 2010). There is a global tendency towards a greater consumption of fruits and vegetables due to a growing concern for a more balanced diet, with a lower proportion of carbohydrates, fats and oils and with a higher proportion of dietary fiber, vitamins, and minerals (Camelo, 2002). A recently published WHO/FAO report recommended a minimum of 400g of fruit and vegetables per day (excluding potatoes and other starchy tubers) for the prevention of chronic diseases such as heart disease, cancer, diabetes and obesity, as well as for the prevention and alleviation of several micronutrient deficiencies, especially in less developed countries (WHO, 2015). Epidemiological studies have shown that high intakes of fruit and vegetables are associated with a lower risk of chronic diseases; particularly, cardiovascular disease, type 2 diabetes and certain cancers i.e. of the mouth, pharynx, larynx, esophagus, stomach and lungs (EUFIC, 2012).

However, the safety of many fruits and vegetables is reportedly compromised as a result of the presence of chemicals and other contaminants. Large quantities of pollutants have continuously been introduced into ecosystems as a consequence of urbanization and industrial processes. During the last decades, the increasing demand of food safety has stimulated research regarding the risks associated with consumption of foodstuffs contaminated by pesticides, heavy metals and/or toxins (Ray *et al.*, 2010). Presently, some of the pollutants of most concern around the world are heavy metals (Antonious *et al*., 2010).

Elevated concentrations of heavy metals in harvested plant tissue could expose consumers to excessive levels of potentially hazardous chemicals (Antonious *et al*., 2010). Reportedly, dietary intake of heavy metals through contaminated fruits and vegetables may lead to various chronic diseases. Their toxicity can damage or reduce mental and central nervous function, lower energy levels, and damage blood composition, lungs, kidneys, liver and other vital organs (Kihampa *et al*., 2011). Long term effect of exposure to heavy metals may result in slowly progressing physical, muscular, and neurological degenerative processes that cause muscular dystrophy, and multiple sclerosis (Kihampa *et al.,* 2011). Subjecting humans to trace and heavy metals above the permissible limits will affect their health and may result in illness to human fetus, abortion and preterm labor, and mental retardation to children (Bempah *et al*., 2013). Heavy metals are given special attention throughout the globe due to their toxic and mutagenic effects even at very low concentrations (Ray *et al*., 2010).

Metals are persistent pollutants that can be biomagnified in the food chain, becoming increasingly dangerous to human and wildlife (Suruchi *et al*., 2011). Contamination of the environment by heavy metals is of major concern because of their toxicity (Agnieszka, 2014). The implication associated with heavy metal contamination is of great concern, particularly in agricultural production systems (Ray *et al*., 2010). The uptake and accumulation of heavy metals in fruits and vegetables are influenced by many factors such as climate, atmosphere deposition, the concentration of heavy metals in vegetables, the nature of soil, and the degree of maturity of plants (Suruchi *et al*, 2011). This has led researchers all over the world to study heavy metals contamination in the air, water, and foods to avoid their harmful effects and to determine their permissibility for human consumption (Bempah *et al.,*2013). Contamination of fruits and vegetables with heavy metals may be due to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, the harvesting process, storage and/or at the point of sale (Bukvic *et al*.). Heavy metals are non-biodegradable and persistent environmental contaminants, which may be deposited on the surface and then absorbed into the tissues of vegetables (Ray *et al*., 2010). The content of heavy metals in plants depends on the location of their growth and harvest, as well as on the capacity of the plants to accumulate various substances from soil, water and air.

Estimation of dietary intakes of contaminants such as heavy metals is crucial because it provides the platform for assessing possible health risks associated with exposure to given contaminants at any given time. Hazard assessments are important tools that can be utilized in estimating hazards to human health and safety and establishing related countermeasures to reinforce food safety (Kim *et al*., 2012). These tolerable intakes can be estimated daily, weekly and monthly for a particular type of foodstuff through food recalls or diaries. Provisional maximum tolerable daily intake values represent permissible human daily exposure as a result of the natural occurrence of the substance in food and in drinking-water; while the provisional tolerable weekly intake value represents permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods (FAO-Codex, 1995).

In a study, pesticide residues and heavy metals level in some selected fruit and vegetables grown and sold in Ghanaian markets were ascertain (Bempah *et al*., 2011). Their findings regarding heavy metal levels and intakes indicated that they were within the safe limits established by the FAO/WHO. It was also found that, heavy metal contamination was high in vegetables than in fruit.

There is lack of information regarding the safety of imported fresh fruit and vegetables with respect to heavy metals contamination and their estimated maximum tolerable intakes in Ghana. However, growing consumer interest in product variety, freshness, convenience and year-round availability have escalated the importation of assorted temperate fruits and vegetables to meet the growing needs of the Ghanaian consumer. However, little is known as to their safety with respect to chemical residue including heavy metals.

The objectives of this study were to: 1 Determine heavy metal (Cu, Pb, Cd, Ni, and Cr,) concentrations in selected imported fresh fruit and vegetables sampled from four supermarkets in Accra, Ghana; 2. Determine if washing (simulated restaurant/consumer level wash) would affect the levels of heavy metals in the produce before consumption; 3. Ascertain whether the levels of these heavy metals meet the international requirements, and 4. Estimate the tolerable heavy metal intake of the selected imported fresh fruit and vegetables.

CHAPTER II

LITERATURE REVIEW

Consumption of Fruits and Vegetables

Fruits and vegetables are chiefly made up of cellulose, hemi-cellulose and pectin substances that give them their texture and firmness (Bukvic *et al*.). They bring us vitamins, minerals and fibre, some energy (mainly in the form of sugar), as well as certain minor components - often referred to as phytochemicals or secondary plant products - which are potentially beneficial for our health and also have beneficial antioxidative effects (EUFIC, 2012 and Elbagermi *et al*., 2012). They are sources of many under-consumed nutrients and consuming fruits and vegetables is associated with a decreased risk of chronic disease (CDC, 2014). Fruits and vegetables provide a diversified, flavored, colorful, tasty, low caloric, and protective micro-nutrient rich diet (Sachdeva *et al*., 2013). They constitute important component of a healthy diet and, if consumed daily in sufficient amounts, could help prevent major diseases such as CVDs and certain cancers (WHO, 2004). The beneficial health effects of fruits and their products depends on the amount consumed in a daily diet, type of fruit and the content of biologically active compounds (Krejpcio *et al*., 2005).The health benefits associated with regular consumption of fresh fruits and vegetables have been clearly demonstrated and encouraged by national and international nutrition and health authorities (Rushing *et al*., 2010). The CDC (2013) reported that eating fruits and vegetables lowers the risk of developing many chronic diseases and can also help with weight management. A recently published WHO/FAO report recommended a minimum of 400g of fruit and vegetables per day (excluding potatoes and other starchy tubers) for the possible prevention of chronic diseases such as heart disease, cancer, diabetes and obesity, as well as for the prevention and alleviation of several micronutrient deficiencies, especially in less developed countries (WHO, 2015). Epidemiological studies have shown that high intakes of fruit and vegetables are associated with a lower risk of chronic diseases; particularly, cardiovascular disease also type 2 diabetes and certain cancers i.e. of the mouth, pharynx, larynx, oesophageal, stomach and lungs (EUFIC, 2012).

Despite the increased awareness and sensitization programs underpinning the protective effects of fruits and vegetables, their consumption still falls short of the WHO/FAO recommended daily intake of 400 g per day in both developed and developing countries; meanwhile, supply on the other hand, more than meets the need (WHO, 2003).

Insufficient intake of fruits and vegetables is estimated to cause around 14% of gastrointestinal cancer deaths, about 11% of ischaemic heart disease deaths and about 9% of stroke deaths globally (WHO, 2015). Overall it is estimated that low fruits and vegetables intake is attributable to approximately 2.7 million (4.9%) annual deaths and 26.7 million (1.8%) disability adjusted life years (DALYs) and causes about 31% of ischaemic heart diseases (IHD), 11% of stroke and 19% of gastro-intestinal cancers (Sachdeva *et al*., 2013). According to WHO (2003), the International Agency for Cancer Research (IARC) estimates that the preventable percentage of cancer due to low fruit and vegetable intake ranges from 5-12 % for all cancers, and up to 20-30% for upper gastrointestinal tract cancers worldwide. In addition to causing premature deaths, these diseases cause complications and disabilities, limit productivity, decreases quality of life, require costly treatments with implicit social burden and adverse health financing outcome for individual, family and country (Sachdeva *et al*., 2013).

However, the consumption of fruits and vegetables depends on many factors which may change throughout life. As for dietary habits in general, a wide range of factors influence fruit and vegetable consumption; factors in our physical, social and cultural environment as well as personal factors, such as taste preferences, level of independence, and health consciousness (EUFIC, 2012). Available data indicates that income and education, gender and age, accessibility, availability and preference have impact on consumption levels.

In 2011/12, almost half (48.5%) or 8 million Australians aged 18 years and over reported that they consumed two or more serves of fruit per day (the recommended daily intake), while 8.2% or 1.3 million met the guideline for daily vegetable intake (National Heart Foundation, 2012). Adults in the United States consume fruit about 1.1 times per day and vegetables about 1.6 times per day (CDC, 2013). These data reveal that the mean vegetable intake (including pulses and nuts) in Europe is 220 g per day. Mean fruit intake is 166 g per day, implying that the average consumption of fruit and vegetables is 386 g per day. From a national food consumption data based on dietary surveys in order to assess food intake in Europe, EUFIC (2012), revealed that the mean vegetable intake (including pulses and nuts) in Europe is 220 g per day; mean fruit intake is 166 g per day, implying that the average consumption of fruit and vegetables is 386 g per day. This suggests 14 g short of the WHO recommended daily intake. In a study from 52 low and middle-income countries 77.6% of men and 78.4% of women consumed less than the minimum recommended servings of fruits and vegetables; same study reported 74% low fruits and vegetables consumption amongst adults in India (Sachdeva *et al*., 2013). Key findings form a data brief indicated that more than three-quarters of youth aged 2–19 years (77.1%) consumed fruit on a given day, almost 92% of youth aged 2–19 years consumed vegetables on a given day, nine out of 10 children aged 2–5 years consumed fruit, while only 6 out of 10 adolescents consumed fruit on a given day (CDC, 2014). Various studies in Ghana have considered the development of the Ghanaian fresh fruits and vegetables industry, however a study conducted in 2009 in 52 countries (mainly low and middle income including Ghana) revealed that overall, 77.6% of men and 78.4% of women interviewed consumed less than the five daily servings of fruits and vegetables (Mohammed *et al.,* 2014). In the same study conducted in 2009, fruit and vegetable consumption among men in Ghana was close to three times lower than consumption in men from Pakistan (36.6% against 99.2%) with a similar trend showing 38.0% and 99.3% for women in Ghana and Pakistan respectively. In the 2008 Ghana Demographic and Health Survey, only 28% of women and 21% of men consumed fruit on a daily basis, and about a quarter (24%) of women and 30% of men consumed vegetables daily (Amo-Agyei et al 2014).

Fruits and Vegetables Imported into Ghana

The increased import of fruits and vegetables is driven by the ever-growing demand by the health conscious middle class of Ghana's population. Assorted fresh fruits and vegetables, predominantly apples, grapes, cabbages, broccoli, strawberry, kiwi and passion fruits are brought into the country to meet this need since Ghana's climate cannot support the production of some these commodities.

The major suppliers for fresh fruits and vegetables imported into Ghana are Netherlands, South Africa, Egypt, Israel, Spain and Italy with occasional influx from UK, Germany, Indonesia, China and Japan. The bulk of these commodities are displayed for sale in local supermarkets while the surplus, especially apples and grapes, are vended on the streets.

On average, Ghana imported 4197, 215 and 25.9 metric tons of apples (Fig.1), grapes (Fig. 2) and cabbage and other brassica vegetables (Fig. 3), respectively, between 2000 and 2012 (FAOSTAT, 2015). There were fluctuations in quantities of apples imported from 2000 to 2008 and increased steadily from 2009 to 2012. Quantities of grapes, however, kept increasing gradually from 2000 to 2007 with a much appreciable steady increase in quantities from 2008 to 2012.There were inconsistencies in the trend of quantities of cabbages and other brassica imported during the period. Quantities of apples, grapes, broccoli and cabbages imported for the year 2014 were 3005, 1286, 75 and 11.6 metric tons respectively

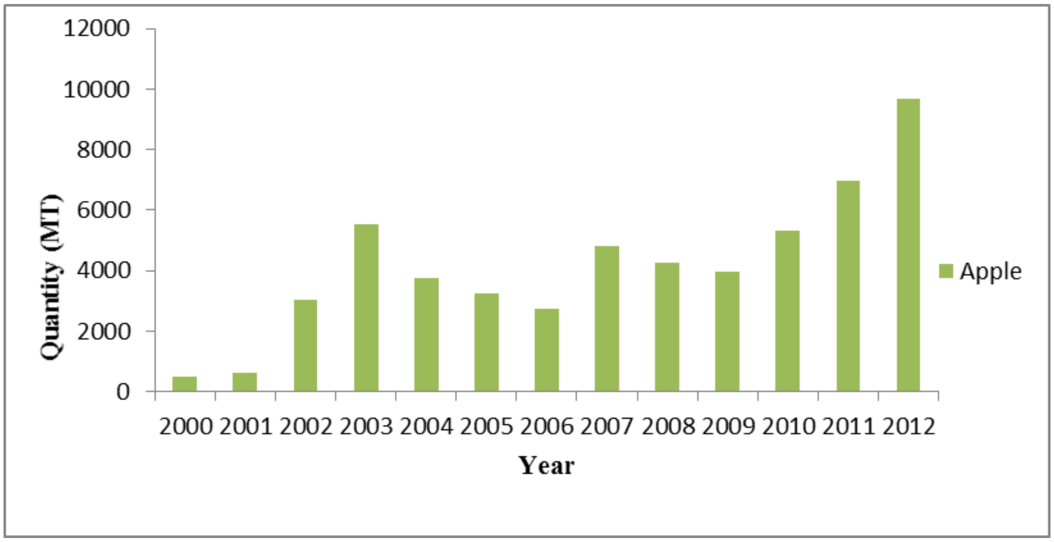

Figure 1 Amount of fresh apple fruits imported into Ghana for human consumption from the year 2000 to 2012

Source: Modified from FAOSTAT, 2015

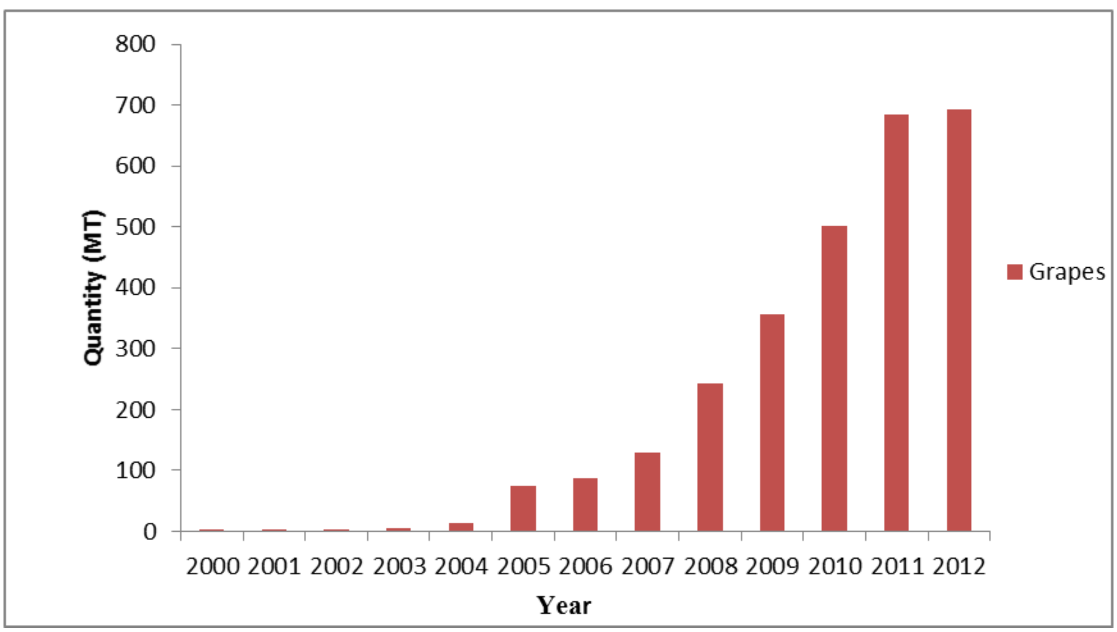

Figure 2 Amount of fresh table grapes imported into Ghana for human consumption from 2000 to 2012

Source: Modified from FAOSTAT, 2015

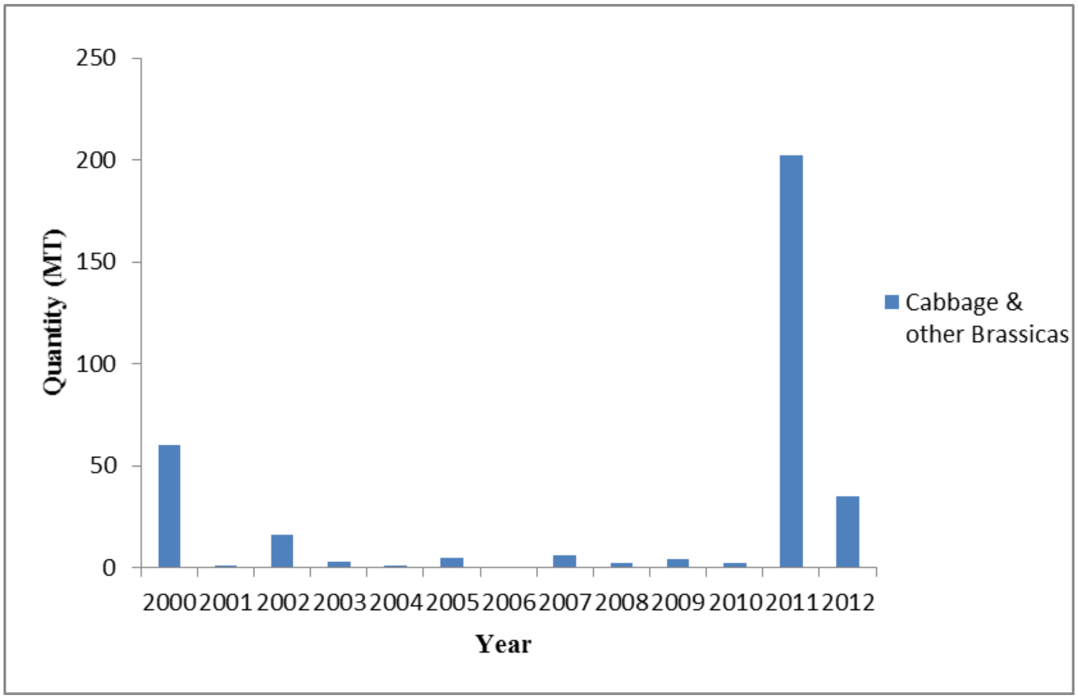

Figure 3 Amount of cabbage and other brassica vegetables imported into Ghana for human consumption from 2000 to 2012

Source: Modified from FAOSTAT, 2015

Sources of heavy metals and their pathways into plant tissue

The Codex Alimentarius defines contaminants as "Any substance not intentionally added to food, which is present in such food as a result of the production (including operations carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packing, packaging, transport or holding of such food or as a result of environmental contamination (FAO-Codex, 1995). "Reference to this definition, heavy metals can be classified as contaminants of great concern to food safety.

There are different sources of heavy metals in the environment such as (1) natural, (2) agricultural, (3) industrial, (4) domestic effluent, (5) atmospheric and (6) others

(Nagajyoti *et al.,* 2010). Metals and metalloids are present in the earth's crust, atmosphere and biosphere, and hence in foods; they are stable, environmentally persistent species of slow elimination that can easily accumulate in plants and tissues, and undergo transformations leading to even more toxic species (Bakkali *et al*., 2012). Heavy metals, such as cadmium, copper, lead, chromium and mercury are major environmental pollutants, particularly in areas with high anthropogenic pressure (Nagajyoti *et al.,* 2010). Land and water are precious natural resources on which the sustainability of agriculture and the civilization of mankind rely; but unfortunately, they have been subjected to maximum exploitation and severely degraded or polluted due to anthropogenic activities (Abdul *et al*., 2011). One of such pollutants is the heavy metals group. As a result, toxic metals may be absorbed by fruits and vegetables during production and finally enter the food chain at high concentrations capable of causing serious health risks to consumers. The term "heavy metals" refers to any metallic element that has a relative density greater than $4 g/cm^3$ (look for source). They are non-biodegradable and industrious natural contaminants which get saved on the surface and afterward retained into the tissues of vegetables (Sharma *et al.,* 2014). Plants take up heavy metals (HMs) by absorbing them from airborne deposits on the parts of the plants exposed to the air from the polluted environments as well as from contaminated soils through root systems (Elbagermi *et al*., 2012). Vegetables take up HMs and accumulate them in their edible and non-edible parts at quantities high enough to cause clinical problems to both animals and human beings (Guerra *et al* 2012). The uptake and accumulation of heavy metals in fruits and vegetables are influenced by many factors such as climate, atmospheric deposition, the nature of soil, and the degree of maturity of plants (Suruchi *et al*, 2011). Additionally, the uptake of metals from soil relies upon diverse elements, for example, their solvency, soil pH, plant development stages and content of manure in soil (Sharma *et al.,* 2014). The contamination of vegetables with heavy metals due to soil and atmospheric contamination poses a threat to quality and safety of the vegetables (Mohammed *et al*., 2012). Emissions of heavy metals from industries and vehicles may be deposited on the vegetable surfaces during their production, transport and marketing (Mohammed *et al*., 2012). Mohamed and Ahmed, (2006) confirmed this when they reported that heavy metal contamination may occur due to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, harvesting process, storage and/or sale The presence of heavy metals in fruits and vegetables have also been ascribed to anthropogenic sources/activities including the addition of manures, fertilizers, sewage sludge, and pesticides that uptake metals by modifying the physico-chemical properties of the soil such as pH, organic matter, and bioavailability of metals in the soil (Osma *et al*., 2013).

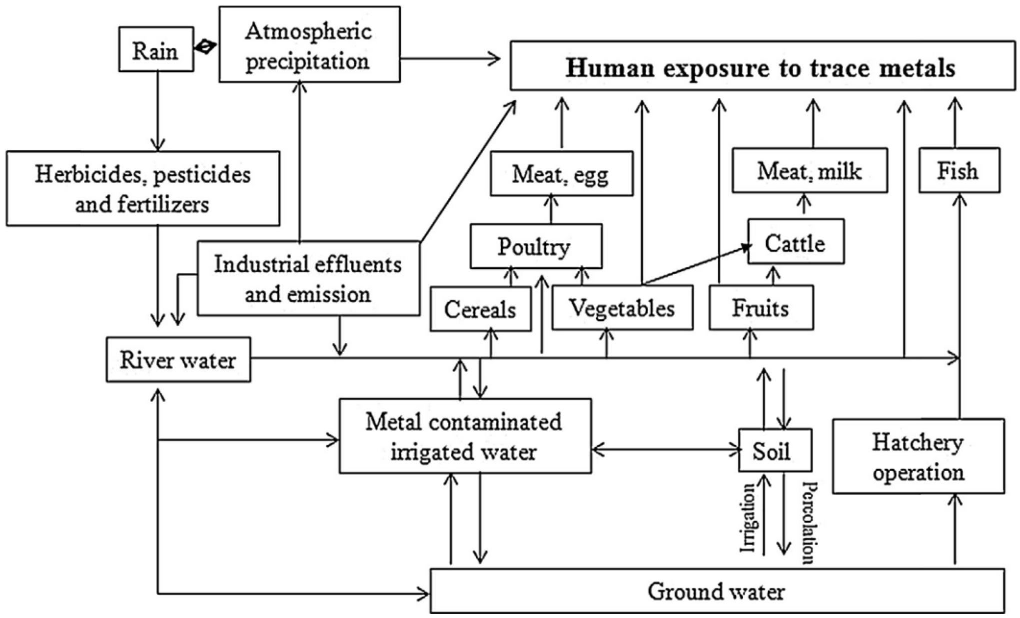

Figure 4 Possible pathways through which humans may be exposed to trace metals. Adapted from Islam and Others, 2014

Toxicity and health effects of heavy metals

Elevated concentrations of heavy metals in harvested plant tissue could expose consumers to excessive levels of potentially hazardous chemicals (Antonious *et al*., 2010). Dietary intake of heavy metals through contaminated vegetables may lead to various chronic diseases (Suruchi *et al*, 2011). Bioaccumulation of heavy metals in soft tissues interferes with normal physiological functions and generally exert their toxic effects by forming complexes with organic compounds (Aqiuno *et al.* 2012). Their toxicity can damage or reduce mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver and other vital organs; long term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that cause muscular dystrophy, and multiple sclerosis (Kihampa *et al*., 2011). Prolonged human consumption of unsafe concentrations of heavy metals in foodstuffs may lead to the disruption of numerous biological and biochemical processes in the human body (Bukvic *et al*.).

Due to its long half-life in the body, chronic toxicity of lead is of most concern when considering the potential risk to human health (EFSA, 2010). Lead can be very harmful even at low concentrations when ingested over a long time and after absorption, lead is initially distributed to soft tissues throughout the body via blood, and then deposited in bone (Othman, 2010). FSAI (2009), indicated that, short-term exposure to high levels of lead can cause brain damage, paralysis (lead palsy), anaemia and gastrointestinal symptoms; whereas longer-term exposure can cause damage to the kidneys, reproductive and immune systems in addition to effects on the nervous system. In humans, the central nervous system is the main target organ for lead toxicity (EFSA, 2010). This can bring about varying levels of various mental dysfunctions in humans depending on the intensity of exposure, age and sex. In adults, exposure to lead may cause health problems including poor muscle coordination, nerve damage to the sense organs and nerves controlling the body, increased blood pressure, hearing and vision impairment, reproductive problems (e.g., decreased sperm count) and retarded fetal development even at relatively low exposure levels (U.S. EPA, 2013). For children, the U.S. EPA (2013) again reported that damage to the brain and nervous system, behavioral problems, anemia, liver and kidney damage, hearing loss, hyperactivity, developmental delays and in extreme cases, death can be caused by exposure to lead.

Cadmium is a relatively rare element, released to the air, land, and water by human activities. In general, the two major sources of contamination are the production an d utilization of cadmium an d the disposal of wastes containing cadmium. Increases in soil cadmium content will result in an increase in the uptake of cadmium by plants; the path way of human exposure from agricultural crops is thus susceptible to increases in soil cadmium (FAO-Codex, 1995) Cadmium (group IIB of the periodic table of elements) is a heavy metal posing severe risks to human health (Figure 5, Godt *et al.* 2005). Cadmium is released as a byproduct of various industrial activities, including the mining, galvanizing, and smelting of other metals like zinc, lead, and copper and it is used to produce batteries, fertilizers, and paint pigments (Aquino *et al*., 2012). The primary cadmium risk posed by the agricultural use of biosolids is the increased dietary cadmium intake of people consuming crops grown on these soils (Antonious *et al*., 2010). Crops grown on any growth or planting medium that is contaminated with cadmium has the potential of absorbing and retaining it in their biological systems. Cadmium poses a particular threat to human beings and animals because it is easily absorbed, relatively long retained in tissues and undergoes accumulation (Anna *et al*., 2012). Guerra and others, (2012) stated that, once in the human body, cadmium may remain in the metabolism from 16 to 33 years and is connected to several health problems, such as renal damages and abnormal urinary excretion of proteins. EFSA, (2009) reported that, cadmium absorption after dietary exposure in humans is relatively low (3–5 %) but it is efficiently retained in the kidney and liver in the human body, with a very long biological half-life ranging from 10 to 30 years Cadmium is a toxic and carcinogenic element and is a cumulative nephrotoxicant that is absorbed into the body from dietary sources and cigarette smoking (Krejpcio *et al*., 2005). Accumulated concentrations of cadmium in human body organs may cause different diseases in including but not limited to kidney problems, skeletal damage and reproductive disorders. Cadmium has been classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (group 1), with sufficient evidence for lung cancer and limited evidence for kidney, liver and prostate cancer (WHO, 2010).

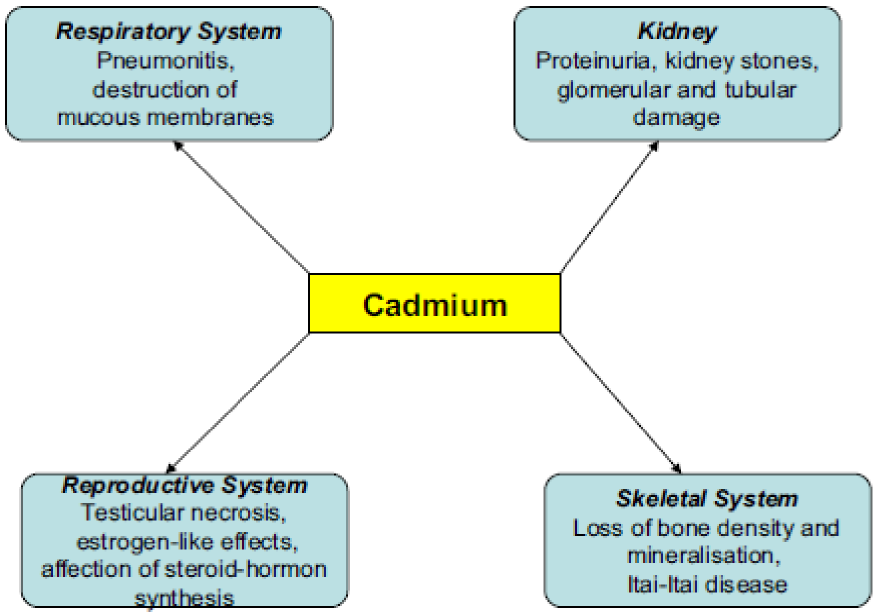

Figure 5 Effects of cadmium on several human organ systems

Adapted from Godt *et al.* 2005

Nickel (Ni) is the 24th most abundant element in the Earth's crust, comprising about 3% of the composition of the earth (Cempel *et al*., 2005). Based on this, its exposure can originate from a wide variety of sources. Food is the major source of nickel exposure, with an average intake for adults estimated to be approximately 100 to 300 micrograms per day (µg/d) (EPA, 2013). In adult humans, the body burden of nickel is estimated to average approximately 0.5 mg per 70kg, or approximately 7.3 µg per kg of body weight (Bastarache, 2008). Nickel has been demonstrated to disturb the mammalian reproductive functions at several levels of regulation (Cempel *et al*., 2005). Short-term (‘acute’) exposure to nickel causes allergic reactions in some individuals, both by touch and also from ingestion in food or water (EFSA, 2015).

Contamination of soil and ground water due to the use of Cr in various anthropomorphic activities have become a serious source of concern to plant and animal scientists over the past decade (Shanker et al., 2005). Chromium exists in the environment in several diverse forms such as trivalent (Cr(III)) and hexavalent, of which hexavalent chromium (Cr(VI)) is a carcinogen and a potential soil, surface water and ground water contaminant, while its reduced trivalent form ($Cr3+$) is much less toxic, insoluble and a vital nutrient for humans (Mandina et al., 2013). The toxicity of chromium depends on the oxidation state, chromium (VI) being more toxic than the trivalent form chromium (III) (Assem *et al*., 2014). For most people eating food that contains chromium (III) is the main route of chromium uptake, as chromium (III) occurs naturally in many vegetables, fruits, meats, yeasts and grains (Lenntech). The primary effects associated with exposure to chromium (VI) compounds are respiratory, gastrointestinal, immunological, hematological, reproductive, and developmental (ATSDR, 2012). WHO (2000), reported that bronchial tree is the major target organ for carcinogenic effects of chromium(VI) compounds, and cancer primarily occurs following inhalation exposure, uptake in the respiratory organs is of great significance in respect of the subsequent risk of cancer in humans. Acute oral exposure of humans to lethal or near-lethal doses of chromium (VI) has produced adverse gastrointestinal effects, including abdominal pain, vomiting, gastrointestinal ulceration, hemorrhage and necrosis, and bloody diarrhea (ATSDR, 2012). However, chromium (III) is present in the active centers of many enzymes, which is why it is classified as one of the essential elements

(Mandina *et al*., 2013) and is a nutrient required for normal energy metabolism (ATSDR, 2012).

At lower doses, copper ions can cause symptoms typical of food poisoning (headache, nausea, vomiting, diarrhea) (WHO, 2004). Copper is essential for good health; however, exposure to higher doses can be harmful and long-term exposure to copper dust can irritate your nose, mouth, and eyes, and cause headaches, dizziness, nausea, and diarrhea (ATSDR, 2004). High concentration of Copper may cause fever, hair and skin decoloration, irritation, respiratory tract, dermatitis, mouth and nausea (Rehman *et al.,* 2014). In humans, copper-induced hepatic damage is dependent on several factors including genetics, age and copper intake; with few reported cases of liver damage (centrilobular necrosis, jaundice, and increased aspartate aminotransferase activity) have been associated with intentional ingestion of a lethal dose of copper sulfate (ATSDR, 2011). The impairment of copper metabolism causes copper accumulation and toxic damage to cells primarily in the liver and the brain tissue, but also in the kidneys, eyes, joints and other organs (Angelova *et al*., 2011). In some cases, large quantities of copper can destruct many red blood cells which results in severe anemia (Angelova *et al*., 2011).

Maximum residue limits and intake of heavy metals

The Codex maximum level (ML) for a contaminant in a food or feed commodity is the maximum concentration of that substance recommended by the Codex Alimentarius Commission (CAC) to be legally permitted in that commodity (FAO-Codex, 1995). Whereas the term tolerable daily intake (TDI) is used by the International Program on Chemical Safety (IPCS) to describe exposure limits of toxic chemicals and the term acceptable daily intake (ADI) is used by the World Health Organization (WHO) and other national and international health authorities and institutes (European Medicines Agency, 2007).

Maximum and safe limits of heavy metal concentrations and intake levels have been established for various classes of food, water, soil, paints products etc. to safeguard consumers and environment against health, food safety and environmental hazards. Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food provides that maximum levels must be set for certain contaminants in foodstuffs in order to protect public health (EUR-Lex, 2007). Classically, maximum levels for mercury, lead, cadmium and tin in foodstuffs have been set by Commission Regulation No 1881/2006, the framework EU legislation which sets maximum levels for chemical contaminants in foodstuffs (FSAI, 2009). This Regulation establishes maximum levels (MLs) for these metals in a wide range of foodstuffs including milk, meat, fish, cereals, vegetables, fruit and fruit juices, and also sets a maximum level for mercury in fish and fish products (FSAI, 2009).

In a standard dealing with contaminants and toxins in food and feed, the Codex Alimentarius of the FAO (1995) designated the following: 0.05, 0.05 and 0.2 mg/kg maximum levels of cadmium for brassica fruits, fruiting vegetables and leafy vegetables respectively; and, 0.2, 0.3 and 0.3mg/kg maximum levels of lead for berries, brassica fruits and leafy vegetables respectively. The Commission of European Communities in setting maximum levels for certain contaminants in foodstuff designated 0.30mg/kg of lead in brassica vegetables, 0.20 mg/kg of lead in berries and small fruits and 0.10mg/kg of lead in fruits excluding berries and small fruits (EUR-Lex, 2006). In the same document, the commission set 0.050 mg/kg maximum level of cadmium in vegetables and fruit, excluding leaf vegetables, fresh herbs, fungi, stem vegetables, root vegetables and potatoes; and 0.20 mg/kg maximum level of cadmium in leaf vegetables, fresh herbs, celeriac and the following fungi: *Agaricus bisporus* (common mushroom), *Pleurotus ostreatus* (Oyster mushroom),*Lentinula edodes* (Shiitake mushroom).

In February 2015, EFSA published a scientific opinion on the risks to human health from nickel in food, particularly in vegetables, and also in drinking water. EFSA set a safe level, known as the tolerable daily intake (TDI), of 2.8 µg per kg of body weight (EFSA, 2015).

Incidence of Heavy Metals Residues in Food

Metals can also occur as residues in food because of their presence in the environment, as a result of human activities such as farming, industry or car exhausts or from contamination during food processing and storage (EFSA, 2015). Research and development regarding the occurrence of heavy metals in food items over the years have revealed both situations where heavy metal residues have exceeded or fallen below the maximum residues limits (MRL) established by regulatory bodies.

In some cases where the MRLs were exceeded, Rahman *et al.* (2013) reported 1.10-1.87 mg kg^{-1} of Pb in leaves and edible parts of vegetables irrigated with wastewater as compared to 0.014-0.08 mg kg^{-1} of Pb for those vegetables irrigated with fresh water whereas Cd concentrations in cauliflower irrigated with both fresh and wastewater were reported as mg kg^{-1} 0.039 and 0.08 mg kg^{-1} respectively. This indicated a substantial contribution of wastewater to Pb contamination in the vegetables. Values of cadmium obtained in unwashed cucumber and guava were 0.0981 and 0.1121ppm, respectively (Amin *et al.,* 2013) Brinjal showed more accumulation for Cr (0.06-0.97 mg kg–1) in most of the selected vegetables tested in a study by Rahman *et al.,* (2013). In onions, mean concentrations of Zn and Cd were reported as 61.97 mg/kg 0.20 mg/kg respectively (Bvenura and Afolayan, 2012). Akinola and Adenuga (2008), showed that the concentration of Pb (3.00±1.00µg/g) in pear fruits bought at Ikeja market were contaminated with Pb when compared with the maximum permissible concentration for Pb in food as established by FAO/WHO in 1984. Pb concentrations in spices collected from a local market in Mumbai ranged from a minimum of 1.51ppm in fennel seeds to a maximum of 2.92 ppm in cloves (Nikita *et. al*., 2014). Rehman *et al.* (2014) found and reported the concentration of Ni in fruits and vegetable in the order: *Lycopersicum esculentum* 15.95mg/kg, *Zizyphus jujube* 15.05mg/kg, *Psidium guajava* 14.37mg/kg, *Prunus armeniaca* 14.20mg/kg, *Allium cepa* 11.87mg/kg, *Allium sativum* 11.57mg/kg, *Citrus limon* 11.47mg/kg, *Habiscus esculentus* 11.03mg/kg, *Daucas carota* 10.53mg/kg and *Solanum tuberosum* 10.32 mg/kg. During an investigation of heavy metals vegetables, a maximum concentration of Pb (60.45mg/kg) was recorded in spinach whiles a minimum of (1.45 mg Kg-1) was obtained in coriander (Surichi *et al.,* 2011). Kihampa *et al*. (2011) stated that, among all heavy metals analyzed, the concentration of Zn (122.88 mg/kg dw) detected in *V. unguiculata* was the highest and that of Cd (0.28 mg/kg) detected in *A. blitum* was the lowest in all vegetables samples tested.

In some incidence where heavy metal residue were below the MRLs, Bvenura and Afolayan (2012) in their findings, stated that the mean concentrations of heavy metals in cabbage in a decreasing order of Mn (28.85 mg/kg) > Zn (27.38 mg/kg) > Cu (0.62 mg/kg) > Cd (0.24 mg/kg) > Pb (below limit of detection). Elemental assessment in fruits and vegetables revealed relatively low concentrations of Zn in *Habiscus esculentus*

1.35mg/kg followed by *Prunus armeniaca* 1.27mg/kg, *Psidium guajava* 0.68mg/kg, *Lycopersicum esculentum* 0.53mg/kg, *Citrus limon* 0.45mg/kg, *Daucascarota* 0.42mg/kg, *Solanum tuberosum* 0.38mg/kg, *Zizyphus jujuba* 0.36mg/kg, *Allium cepa* 0.33mg/kg, and *Allium sativum* 0.25 mg/kg (Rehman *et al*., 2014). From Borno State in Nigeria, negligible concentrations of Pb, (0.0132ppm) were reported in washed cucumber samples (Anim *et al*, 2013). Mean Cu concentrations (μg/g dry weight) in unwashed and washed samples of vegetables from different sites of Kolkata and South 24 Parganas of West Bengal, India were estimated as Brinjal (22.16, 11.75ug/g), Indian Spinach (971.16, 16.13 ug/g), Red Indian Spinach (63.23, 17.14 ug/g), Cauliflower (8.16, 6.11ug/g) and Cabbage (3.12, 0.45 ug/g) (Ray *et al.,* 2010). The evaluation of trace elements in grapes revealed 0.01, 0.017, 0.3, 0.11, 0.187 and 0.68mg/kg concentrations of nickel palladium cadmium zinc copper and iron respectively (Venugopal *et al*., 2011).

Incidents of Heavy Metals Intake in Food

In an assessment of heavy metals in vegetables and potential risk for human health, Fernando and others (2012), stated that the greatest contribution for Cd intake came from the 'monalisa' potato (0.00049 mg per day), followed by the 'prata' banana (0.00046 mg per day), 'pêra' orange (0.00041 mg per day) and carrot (0.00032 mg per day) among the test samples. Chandorkar and Deota (2013), reported 1.00, 0.69, and 9.08 mg/day of Cd, Pb and As respectively in a population of Vadodara in India. In south eastern Nigeria, Orisakwe (2012), reported true Pb and Cd intake for rice based meal as 3.53 and 0.034 g/kg respectively; whereas the true intake of Pb and Cd for cassava based meal were stated as19.42 and 0.049 g/kg respectively. Elbagermi *et al*. (2012), reported that if the mean levels of Pb (0.473 mg/kg), Cd (0.071 mg/kg), Cu (2.63 mg/kg), Zn

(3.7 mg/kg), Co (0.58 mg/kg), and Ni (1.49 7 mg/kg) are consumed daily, then contributions of heavy metals intake for an average human being from the fruits tested were 36.89 µg, 5.54 µg, 0.205 mg, 0.288 mg, 45.24 µg, and 0.116 mg, respectively. In case of vegetables, if the consumed daily mean levels of Pb, Cd, Zn, Cu, Co, and Ni were 0.25, 0.14, 8.15, 3.36, 0.51 and 0.24 mg/kg, respectively, the corresponding estimated daily intake were 24.8 µg, 13.3 µg, 0.8 mg, 0.33 mg, 49.7 µg, and 0.0231 mg, respectively. In Iran, a study involving the determination of heavy metals in selected edible vegetables, and estimation of their daily intake revealed 2.96, 2.50, 1.72 and 0.07 mg/day for Pb, Cu, Cr and Cd respectively (Maleki *et al.,* 2008).

CHAPTER III

MATERIALS AND METHODS

Study Area

Accra is the capital and largest city of Ghana with an estimated urban population of 2.3 million as of 2012. It lies within a geographic coordinate of latitude 5°33′21″ N and longitude 0°11′48″ W with an elevation of 108 ft above sea level located in southeastern Ghana, on the Gulf of Guinea. Accra is an important commercial, manufacturing, and communications center. It is the site of an international airport and a focus of the country's railroad system. Industries include vehicle and appliance assembly, petroleum refining, and the manufacture of foodstuffs, textiles, metal and wood products, plastics, and pharmaceuticals (Ghana Embassy, 2015).

The selected supermarkets; Palace, Marina, Shoprite and Melcom plus located in Accra are multinational business centers which provide a wide range of services and products to expatriates and the elite Ghanaian. They are therefore the hub and retail outlets for commonly imported fruits and vegetables in the country. Among these products are apples, table grapes, broccoli, cabbages, kiwi fruits, cauliflower, strawberry, passion fruits, pitches and blueberries with the later six rarely available. We limited the number of products sampled to four including apples, table grapes, broccoli, and cabbage. This was based on the most popular imported fruits and vegetables consumed in Ghana (FAOSTAT, 2015).

Sampling of Fruit and Vegetables

A preliminary visit was made to the sampling sites (supermarkets) in December 2014 to identify the varieties of the earmarked commodities that are in consistent supply and available to be purchased throughout the sampling period. As a result, golden yellow, red globe, and green cabbage varieties of apple fruits, table grapes and cabbage were respectively selected across all the supermarkets. However, broccoli displayed in the supermarkets were not labeled and attendants were unable to identify the varieties. In all, a total of sixteen (16) samples, comprising the four selected imported fruits and vegetables (apples, table grapes, cabbage and broccoli) were collected in January and February 2015 from the supermarkets for the experiment to test for their heavy metals concentrations and the effect of washing on the concentrations of these heavy metals (Pb, Cd, Cu, Ni and Cr).

For each of the four supermarkets (replications-blocks), samples of each commodity, averaging about 6kg, 5kg, 8kg and 5kg for apples, grapes, cabbage and broccoli, respectively, were randomly collected from the supermarkets during the early hours of the day. Samples were labeled and the lots for each product were separately kept in white polyethylene bags and transported to the Ghana seed testing laboratory for the necessary treatment prior to drying.

Sample Preparation

Samples of each commodity were taken from the composite samples and divided into two sub-samples. Each categorized as “washed” and “unwashed”. The washed categories were subjected to washing by agitating and rinsing in tap water which was not tested for its heavy metal concentration, for about 1min (a common washing procedure before products are consumed in Ghana). The washed samples were air dried for one hour to remove excess water. The other sub-group was not washed. The washed (W) and unwashed (U) samples were separately chopped up using a stainless steel knife for onward drying. As described by Elbagermi *et. al,*(2012), 1kg of each chopped sub-sample were weighed using ENTRIS 6202-1S balance (Sartorius, Germany) and oven-dried in Binder FD240 oven (Binder GmbH, Germany) at 105˚C for 24 hours. Dried samples were weighed in the ENTRIS 6202-1S balance and mechanically ground using a lab grinder model S102DS (Strand Manufacturing Co. Inc, USA). The mechanically ground samples were kept in zip-lock bags (brand) and immediately conveyed to the chemical laboratory of the Department of Nuclear and Environmental Chemistry of the Ghana Atomic Energy Commission (GAEC) for digestion and analysis.

Ashing and Digestion

Two grams (2g) of test portions of each powdered sample were weighed into 25 ml capacity ceramic crucibles acquired (MES Equipment, Ghana) for ashing. The weighing was done with ENTRIS 6202-1S balance at the Nuclear Chemistry lab of the GAEC. Using a plastic dropper, five drops of concentrated analytical grade of 6M HCL (1 + 1) (AOAC 999.11, 2002) purchased from MES Equipment were added to the weighed samples in the crucibles and covered. The crucibles with their contents were placed in a thermal programmable muffle furnace model 400321 (Lenton, UK) at an initial temperature of 100˚C (AOAC 999.11, 2002). The temperature was gradually increased at a rate of 50˚C/h to 450˚C and left to ash for eight (8) hours (AOAC 999.11, 2002). The ashed samples were left in the furnace to cool overnight. The ash was digested with 5ml 3N hydrochloric acid from MES Equipment and filtered through

Whatman filter paper into a 50ml volumetric flask (Kihampa *et al*., 2011 and Elbagermi *et. al.,*2012). The volume was made up to the mark with 3N hydrochloric acid and was transferred into a 50ml plastic bottle for instrument reading (Elbagermi *et. al* ,2012).

Determination of Heavy Metals with Atomic Absorption Spectrophotometer (AAS)

Heavy metals (Pb, Cd, Ni, Cr and Cu) were determined as absorbance, representing raw data as shown in Table 8, using atomic absorption spectrometer (Varian 240 FS, Germany). Working standard solutions of lead (Pb), chromium (Cr), cadmium (Cd), Nickel (Ni) and copper (Cu) purchased from MES Equipment were prepared from the stock standard solutions. After every five sample readings, standards were run to check the efficiency of the equipment. Calibration curves were prepared for each element and blank readings made before the commencement of every analyte reading. Detection limit for each metal was calculated as 3x the standard deviation of the mean of blank determinations (AOAC 999.11, 2002). The limits of detection (LOD) were 0.001, 0.002, 0.003, 0.001, and 0.002 mg/kg for Pb, Cd, Cu, Cr, and Ni, respectively. The standard operating conditions for the analysis of heavy metals using atomic absorption spectrometry used in this experiments are given in Table 1.

The actual concentrations of the metals in the samples were calculated using the following equation:

$$\text{Final Concentration (mg/kg)} = (C \times E) / S \qquad (1)$$

where C is the concentration of the test solution, E volume of the test solution (ml) which represents the final volume of digest used and S is sample weight (g) used during digestion (Akinola *et al*, 2008). Mean water-removable heavy metal concentrations (%reduction) was calculated as the percentage ratio of the difference between unwashed and washed mean fruits and vegetables concentration to unwashed mean fruits and vegetable concentrations (Suruchi *et al.,* 2011). The equation is as follows:

$$\% \text{ Reduction} = ((U - W)/U) \times 100 \qquad (2)$$

where U denotes concentration (mg/kg) of unwashed samples whiles W denotes concentration (mg/kg) of washed samples

Quality Assurance

To ensure the reliability of the results, appropriate quality assurance procedures and precautions were followed. Samples were carefully handled to avoid cross contamination. Glassware was properly cleaned, and reagents used were of analytical grades. Reagent blank determinations were used to apply corrections to the instrument readings. Repeated readings were made to ensure reproducibility of the method used.

Estimation of Dietary Intakes of Heavy Metals through the Fruits and Vegetables Consumption.

The estimated dietary intake of heavy metals through the consumption of fruit and vegetables tested were calculated in terms of tolerable daily intakes and tolerable weekly intakes (Elbagermi *et. al.,* 2012). These estimations were done according to the following equation:

$$\text{Daily intake of heavy metals (mg/day/person)} = [\text{Daily fruit or vegetable consumption}] \times [\text{mean concentration of heavy metals in fruit or vegetable}] \qquad (3)$$

$$\text{Weekly intake of heavy metals (mg/week/person)} = [\text{Weekly fruit or vegetable consumption}] \times [\text{mean concentration of heavy metals in fruit or vegetable}] \qquad (4)$$

The daily fruit and vegetable consumption (Table 2) was derived from average quantities of fruits and vegetables imported into Ghana during the 2013 and 2014 calendar year. The import figures were obtained from FAOSTAT 2015 and the 2013/2014 annual report of the Plant Protection and Regulatory Services Directorate of the Ministry of Food and Agriculture. This Directorate is mandated to inspect and certify plants and plant products entering and leaving the borders of Ghana, and as such takes records of all such materials. It is however important to note that postharvest losses were not adjusted for the calculation of the daily intake of fruits and vegetables.

Experimental Design and Statistical Analysis

Data was arranged in a two-way factorial arrangement with four (4) commodities and washing and vesus control as the factors. Fruits from four (4) supermarkets (same cultivars) were blocked and used as replications in a randomized complete block design. Data was analyzed using XLSTAT 2015.1.03.15485 to assess the levels of heavy metals and influence of washing on the concentrations of heavy metals in the fruits and vegetables tested. Analysis of Variance (ANOVA) was performed to separate means ($p \leq 0.05$), using Tukey's Honestly Significant Difference (HSD) test.

Table 1 Operational Conditions used in the AAS Varian 240 FS for determining the levels of heavy metals in the fruits and vegetables

Element	Wave Length (nm)	Slit Width (nm)	Lamp Current (MA)	Fuel	Support
Pb	232	0.2	4	Acetylene	Air
Ni	357.9	0.2	7	Acetylene	Air
Cr	228.8	0.5	4	Acetylene	Air
Cd	324.7	0.5	4	Acetylene	Air
Cu	217	1	5	Acetylene	Air

Obtained from the AAS Varian 240 FS Manual used in the National Nuclear Chemistry Research Laboratory of the Ghana Atomic Energy Commission.

Table 2 Annual and daily quantities of fruits and vegetables imported and corresponding daily quantities consumed in Ghana

Commodity	**Annual Import**		**Daily Import**		**Daily Consumption**
	MT	*Kg*	*MT*	*Kg*	*Kg*
Apple	3005.33	3005330	8.23	8233.78	0.00032
Grape	1286.23	1286230	3.52	3523.92	0.00014
Cabbage	11.6	11600	0.032	31.78	0.0000012
Broccoli	75.43	75430	0.21	209.53	0.0000081

Quantities consumed daily were computed from import figures obtained from the Plant Protection and Regulatory Services Directorate and FAOSTAT (PPRSD Annual Report, 2013-2014; and FAOSTAT 2015).

CHAPTER IV

RESULTS AND DISCUSSION

Levels of Heavy Metals in Fruits and Vegetables

Mean concentrations of Pb, Ni, Cd, Cr, and Cu in four imported fruits and vegetables sampled from supermarkets in Accra are summarized in Table 3. There were differences ($p \leq 0.05$) in the concentrations of Pb, Ni and Cr in the samples collected from all the supermarkets but not ($p > 0.05$) in Cd and Cu in any of the commodities sampled, n = 8. The concentrations of heavy metals varied considerably with Pb ranging from (1.46 – 2.01), Ni (0.27 – 0.53), Cd (0.36 – 0.54), Cr (0.19 – 0.32) and (Cu 3.66 – 4.58) mg/kg across the four commodities tested. This is probably, due to the fact that the uptake and accumulation of heavy metals in fruits and vegetables are influenced by many factors such as climate, atmosphere deposition, the nature of soil, and the degree of maturity of plants (Suruchi *et al*, 2011).

The highest and lowest concentrations of Pb were detected in grapes (2.01mg/kg) and broccoli (1.46 mg/kg), respectively. In a similar study, Elbagermi and others, (2012) reported that the concentration of Pb ranged between 0.02mg/kg in potatoes and 1.82 mg/kg in mangos. Ogunkule *et al*. (2014), also detected 1.62, 1.76, 1.84 and 1.93 mg/kg of Pb concentrations in mango, watermelon, cabbage and lettuce respectively. These findings are comparably consistent with the current outcomes, indicating that Pb can accumulate in plant tissues. The accumulation of Pb in plant tissues can occur through diverse mechanism in the environment such as cultivating plant on lead polluted soils and the use lead polluted water for irrigation. Lead is a natural environmental contaminant, but its ubiquitous occurrence results, to a great extent, from anthropogenic activities (EFSA, 2010).

The highest concentration of Ni (0.53mg/kg) was obtained in broccoli while the least (0.27 mg/kg) was detected in grapes (Table 3). This agrees with previous findings that Ni levels in foodstuffs generally range from less than 0.1 mg/kg to 0.5 mg/kg (Cempel and Nikel, 2006). This is also comparable to Ni concentrations observed in fruits and vegetables sampled from the markets in Musurata-India, with a maximum concentration of (5.143 mg/kg) and a minimum of 0.19 mg/kg detected in carrots (Elbagermi et al., 2012). Higher concentrations of Ni were reported in fruits and vegetables from: *Lycopersicum esculentum* - 15.95mg/kg, *Zizyphus jujube* - 15.05mg/kg, *Psidium guajava* - 14.37mg/kg, *Prunus armeniaca* - 14.20mg/kg, *Allium cepa* - 11.87mg/kg, *Allium sativum* - 11.57mg/kg, *Citrus limon* - 11.47mg/kg, *Habiscus esculentus* - 11.03mg/kg, *Daucas carota* - 10.53mg/kg and *Solanum tuberosum* - 10.32 mg/kg (Rehman et al., 2014). Nickel is one of many trace metals widely distributed in the environment, being released from both natural sources and anthropogenic activity, with input from both stationary and mobile sources; it is present in the air, water, soil and biological materials (Cempel *et al.,* 2005).

Concentrations of Cd ranged from 0.36 mg/kg to 0.54 mg/kg in broccoli and grapes respectively (Table 3). Concentration of Cd in vegetables were from 0.28 mg/kg in *A. blitum* to 1.48 mg/kg in *S. nigrum* (Kihampa *et al*., 2011). Mohamed *et al*. (2012), also reported 1.10 and 1.18 ug/kg of Cd in cabbage and tomato, respectively. Presence of

Cd in foods, especially fruits and vegetables, may be due to agronomic practices including pesticides and fertilizer applications. Highly contaminated areas may show higher Cd concentrations in locally produced food and the use of cadmium-containing fertilisers in agriculture increases cadmium concentrations in the crops and derived products (EFSA, 2009). Phosphate fertilizers also show a large Cd load; in Scandinavia, Cd concentration in agricultural soil increases by 0.2% per year (Godt *et al*., 2005).

Relative to the other heavy metals, the concentrations of Cr in the fruits and vegetables collected for this study were low with the highest quantity (0.32 mg/kg) detected in grapes and the lowest (0.19 mg/kg) in broccoli (Table 3). In Dar es Salaam, Tanzania, a work involving heavy metals in vegetables revealed comparably high Cr quantities ranging from a minimum of 1.15 mg/kg in *C. maxima* to a maximum of 29.39 mg/kg In *V. unguiculata* (Kihampa *et al.* 2011). Chromium in its naturally occurring state is in a highly insoluble form; however, most of the more common soluble forms found in soils are mainly the result of contamination by industrial emissions (FAO, 1999). These levels of Cr found in plant tissue, particularly fruits and vegetables, could be due possibly to uptake from soils polluted with the metal as high levels have been reported in soils. U.S. soil levels of total chromium range from 1 to 2,000 mg/kg, with a mean level of 37 mg/kg (ATSDR, 2004). Chromium from anthropogenic sources can be released to soils and sediments indirectly by atmospheric deposition, but releases are more commonly from dumping of Cr-bearing liquid or solid wastes such as chromate by-products ("muds"), ferrochromium slag, or chromium plating wastes (Mandina *et al.,* 2013).

Levels of Cu obtained in the samples tested were comparably high to the other heavy metals detected in this study. The levels detected were 3.66 mg/kg in grapes, 3.17

mg/kg in apples, 4.56 mg/kg in cabbage and 4.58 mg/kg in broccoli (Table 3). Rehman *et al.* (2014), stated maximum concentration of Copper (Cu) in *Prunus armeniaca* as 14.50mg/kg, followed by *Zizyphus jujube* - 12.30mg/kg, *Citrus limon* - 10.50mg/kg, *Habiscus esculentus* - 8.67mg/kg, *Lycopersicum esculentum* - 8.15mg/kg, *Allium sativum* - 7.45mg/kg, *Psidium guajava* - 6.43mg/kg, *Solanum tuberosum* - 5.45mg/kg, *Daucas carota* - 4.21mg/kg and *Allium cepa* - 3.77 mg/kg. Copper plays a crucial role in agricultural production as it is incorporated into many classes of plant protection products especially the fungicide group. In production systems where non-adherence to GAPs and best practices regarding pesticides applications exists, elevated levels of Copper contamination may occur. Krepjcio *et al*. (2005), attributed increased Cu content of strawberry and black currant to Cu-containing pesticides used in plant protection.

Effect of washing on Concentration of heavy Metals in Fruits and Vegetables

One objective of this study was to evaluate the effect of washing on the levels of heavy metals in the commodities tested. Amin *et al.* (2013), indicated that washing the samples reduced the level of metals from all the crops investigated. The use surfactants probably could have yielded much more reduction in the heavy metal contaminations in the sample. In some countries chemical disinfectant agents are used to decontaminate the surfaces of fruits and vegetables in addition to washing with water (WHO, 1998). In this work, washing had no effect ($p > 0.05$) on residual Pb in each of the produce tested, although there was tendency for washed fruits to contain less residual Pb (Table 4). Washing lowered ($p \leq 0.05$) residual Ni in all samples tested except apple (Table 4). However, Cd residue was reduced ($p < 0.05$) in all the samples whereas Cr and Cu were not ($p > 0.05$) in any of the commodities (Table 4). The highest reduction ($p \leq 0.05$)in concentration (mg/kg) of the trace metals in the fruits and vegetables was achieved for Cr in cabbage where unwashed and washed samples obtained 0.34 and 0.14 mg/kg respectively, 59% less after washing (Table 4). The percentage reduction of Cr in cabbage implies that the accumulation of Cr in the fruits and vegetables tested is not exclusively due to absorption from soils but also from the environment and other possible sources including industrial emissions. The industries with the largest contribution to chromium release include metal processing, tannery facilities, chromate production, stainless steel welding, and ferrochrome and chrome pigment production (ATSDR, 2012). The study area is, or close to the industrial hub of Ghana and more critically, this hub is the entry point of these imported commodities. The reduction in Cr concentration as a result of washing however, varied among the various products with 39%, 44% and 46% reductions in apple, grape and broccoli respectively.

The least reduction ($p > 0.05$) in concentration of the metals after the treatment occurred in grapes where 2.14 mg/kg Pb content was obtained in unwashed sample with a corresponding 1.88 mg/kg detected in the washed sample thus yielding 12% residue reduction. Generally, reduction in Pb concentration was not significant and relatively low across the other three (3) samples with 19%, 16% and 19% reductions in apple, cabbage and broccoli respectively (Table 5). Ray *et al*. (2010), observed that washing decreased the concentration of Pb by 77, 38, and 37 percentages in Indian spinach, cabbage and cauliflower respectively. Inferring from these outcomes, Pb contamination in the samples is not solely due to uptake from soil and agronomic practices during production but appreciable contribution emanate from environmental Pb. This is because, lead may reach and contaminate plants, vegetables, fruits and canned food through air, water and soil during cultivation (Al Othman, 2010). Heavy vehicular activities take place in and around the vicinities where the tested produce were purchased. On this premise, it could be remarked that the Pb contamination in the fruits and vegetables is partially owned to leaded gasoline used by these vehicles. The Industrial Revolution gave rise to an increase in the amount of lead in the environment and even a bigger increase occurred around 1920 when leaded gasoline was introduced; and in the 21st century, leaded gasoline is still not banned everywhere in the World (Al Othman, 2010).

Highest reduction in Ni content was achieved in grapes where 0.38 mg/kg was detected in unwashed sample and 0.16 mg/kg in washed sample resulting in 58% decrease in Ni content. This was followed by 57% Ni content reduction in cabbage, 36% in broccoli and 27% in apple. In a similar investigation, Ni saw a downward concentration after washing from 3.7 to 3 mg/kg in methi and from 1.6 to 0.8 mg/kg in coriander (Suruchi *et al*., 2011). It could, therefore, be agreed that, Ni abounds in the environment and can become surface contaminants on fruits and vegetables. Nickel is a naturally occurring metal that is sometimes present in food and water through environmental contamination, including as a result of human activity (EFSA, 2015). Again, Ni is said to be one of many trace metals widely distributed in the environment, being released from both natural sources and anthropogenic activity, with input from both stationary and mobile sources - it is present in the air, water, soil and biological material (Cempel and Nikel, 2006). Its emission into the air, hence on fruits and vegetables as surface contaminant could also be attributed to vehicular and some forms of industrial activities. Nickel finds its way into the ambient air as a result of the combustion of coal, diesel oil and fuel oil (Cempel and Nikel, 2006).

Appreciable contents of cadmium ($p \leq 0.05$) were also removed, Table 4. The highest percentage reduction of Cd (41%) was obtained in broccoli, Table 4. Cu content reduction was not significant ($p > 0.05$) after the washing treatment with 26% reduction in grapes. The difference between washed and unwashed fruits and vegetables with regard to these heavy metal concentrations suggests that heavy metal contamination of fruit and vegetables may be due many factors such as irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, the harvesting process, storage and/or at the point of sale (Bukvic *et al*.). Emissions of heavy metals from the industries and vehicles may be deposited on the vegetable surfaces during their production, transport and marketing (Mohammed *et al*., 2012).

Comparison of Observed Concentration of Heavy Metals with Established Safe Limits

The observed concentrations of Pb, Ni, Cd, Cr and Cu, in the fruit and vegetables were compared with the recommended limits established by the FAO/WHO and EU standards to assess their safe thresholds (Table 5). The FAO standard contains the main principles which are recommended by the Codex Alimentarius in dealing with contaminants and toxins in food and feed, and lists the maximum levels and associated sampling plans of contaminants and natural toxicants in food and feed which are recommended by the CAC to be applied to commodities moving in international trade (FAO-Codex, 1995). EU'S Commission Regulation (EC) No 466/2001 stated that, it is essential, in order to protect public health, to keep contaminants at levels which are toxicologically acceptable (Euro-Lex, 2006). Thirty (32) of the samples tested violated the safe limits of Pb, Cd and Cr set by the Codex and EU.

The maximum acceptable limit of lead (Pb) levels established for apple is 0.1 mg/kg, grape is 0.2 mg/kg and the brassicas is 0.3 mg/kg (Table 6). Comparably, Pb levels detected in this study in all the commodities (apple, grapes, cabbage and broccoli) tested averaged eight (8) times higher than the safe limits established by both the FAO and the EU. Ray *et al*. (2010), investigated vegetables from different shopping malls in Kolkata, India and reported that Pb content of all the unwashed samples were above safe permissible levels recommended by WHO/FAO. Heavy metal contamination in a school vegetable garden in Johannesburg, South Africa which is the source of most imported fruits and vegetables in Ghana indicated that, 91% of vegetables samples exceeded the recommended limits for Pb, based on the Joint Codex Alimentarius Commission (Kootbodien *et al*., 2012). Also, in a study involving heavy metals in fruits and vegetables in Cape Eastern, South Africa, the concentrations of heavy metals in vegetables were in the range of 0.01 mg/kg – 1.12 mg/kg dry weight for Cd, 0.92 mg/kg – 9.29 mg/kg for Cu, 0.04 mg/kg – 373.38 mg/kg for Mn and 4.27 mg/kg – 89.88 mg/kg for Zn (Bvenura, 2012). In the same study, results of analysis of soils from the area revealed that Cd in soil was in the range of 0.01 mg/kg – 0.08 mg/kg, Cu levels were 4.95 mg/kg – 7.66 mg/kg and Pb levels were 5.15 mg/kg – 14.01 mg/kg. Levels above the safe limits indicate Pb contamination and have toxicological and health implications for consumption of such fruits and vegetables. Lead serves no useful purpose in the human body, but its presence in the body can lead to toxic effects, regardless of exposure pathway (ATSDR, 2007). Although Pb is not a nutritionally essential element, its monitoring is important because of its toxicity to human health (FAO, 1999). ATSDR (2007), remarked the nervous system as the most sensitive target of lead exposure and continued or repetitive exposures can cause a toxic stress on the kidney, if unrelieved, may develop into chronic and often irreversible lead nephropathy (*i.e.*, chronic interstitial nephritis)

The Food and Agriculture Organization designated 0.05 mg/kg maximum permissible limit Cd for fruits and brassica vegetables (FAO, 1999). The EU set safe limits for Cd at 0.05 mg/kg for most vegetables and fruits, and 0.20 for leafy vegetables, fresh herbs, celeriac and some fungi (Euro-Lex, 2006). The levels of Cd in this study (Table 6) exceeded the safe limits established by both regulatory bodies in all sixteen samples. Ray *et al*. (2010), confirmed these findings when their publication of a similar study highlighted that Cd content of all the unwashed samples found were above safe permissible levels recommended by WHO/FAO. Violation of these mandatory safe limits present food safety and health hazards to prospective consumers; and even implications for trade. Cadmium bioavailability, retention and consequently toxicity are affected by several factors such as nutritional status (low body iron stores) and multiple pregnancies, preexisting health conditions or diseases (EFSA, 2009). This leads to a variation in individual's vulnerability. Cadmium is primarily toxic to the kidney, especially to the proximal tubular cells where it accumulates over time and may cause renal dysfunction (EFSA, 2009).

The Commission Regulation (EC) No 466/2001 of the EU set 0.05 mg/kg as safe limit for Cr in fruits and vegetables (Euro-Lex, 2006). On the average, levels of Cr detected in the fruits and vegetables studied in this work are about four (4) times higher than the established acceptable permissible levels (Table 7). This asserts that such foods are unwholesome and could likely pose threat to human lives after continual and long-term consumption/exposures. However, Cr toxicity is dependent on the type of Cr compound present in the food item. Cr exists in the environment in several diverse forms such as Cr (0), Cr III and Cr VI species, and its toxicity depends on its valence state (Mandina and Tawanda, 2013). Cr III compounds are less harmful than Cr VI compounds (Assem and Zhu, 2007). It is therefore crucial to determine the type of Cr compound present in a given food item in order to prove its toxicity. This was however, beyond the scope of this work. Nonetheless, the regulation does not distinguish the type of Cr.

The level of safety or toxicity of Ni in the commodities examined in this study was not comprehensively ascertained because of lack of information on credible benchmarked safe limits for comparison. A requirement for nickel has not been conclusively demonstrated in humans (Cempel and Nikel, 2006). Numerous data credit Ni as an essential trace element required in the human body for proper metabolism. Ni is one of the essential element which is present in the environment in trace amount as well as it is considered as indispensable for number of metabolic reactions in living beings (Ismail *et al.,* 2011). However, the quantity one is exposed to could be detrimental or beneficial to the body. Cempel and Nikel (2006) recognized Ni as a nutritionally essential trace metal for at least several animal species, micro-organisms and plants, and therefore either deficiency or toxicity symptoms can occur when, respectively, too little or too much Ni is taken up.

Safe values for copper, lead, and cadmium in fruit and vegetables recommended by the WHO/FAO are 40, 0.3, and 0.2 mg/kg, respectively (Elbagermi *et al.,* 2012). Levels of Cu realized in the food products in this work are below the safe limits and can be categorized as safe for human consumption regarding Cu content (Table 7). Apart from its function as a biocatalyst, Cu is necessary for body pigmentation, for the maintenance of a healthy central nervous system, and for the prevention of anaemia, and it is interrelated with the function of Zn and Fe in the body (Elbagermi *et al.,* 2012).

Estimation of Dietary intake of heavy metal Through Fruit and Vegetables Consumption

Relevant to this study is to evaluate the tolerable intakes of these trace elements in the food products examined. These tolerable intakes can be estimated daily, weekly and monthly for a particular type of foodstuff through food recalls or diaries. The provisional tolerable daily intake (PTDI) value represents a permissible human daily exposure as a result of the natural occurrence of the substance in food and in drinking water; while the provisional tolerable weekly intake (PTWI) value represents a permissible human weekly exposure to those contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods (FAO-Codex, 1995). Estimated daily (Table 6) and weekly (Table 8) intakes of the heavy metals were estimated through the quantities of apple, grapes, cabbage and broccoli imported and consumed in Ghana. WHO (2010), documented a provisional tolerable weekly intake (PTWI) of 0.025 mg/kg bw for Pb, 0.007 mg/kg bw for Cd and a provisional maximum tolerable daily intake (PMTDI) of 0.5 mg/kg bw per day for Cu in foodstuff. The quantitative estimate of these contaminants is a crucial indicator/assessor of health risk for a given population. PTDI for a heavy metal refers to the intake of that particular metal from all the food groups and is an important indicator of health risk in the population exposed (Chandorkar and Deota, 2013).

Results from this work regarding Pb dietary intake in the fruits and vegetables examined were on the average seven (7) times lower than the reference safe limit of 0.025 mg/week, established by the World Health Organization (2010). Averagely, the estimated Cd intake was about six (6) times lower than the limit set by WHO (2010). Comparably, the highest estimated daily intake of Cu (0.0012 mg/day) was also several times lower than reference safe limit of 0.5 mg/day documented by WHO (2010). This confirmed results from Elbagermi *et al*. (2012), when they concluded that their estimated daily intakes for the heavy metals studied were below those reported by the FAO/WHO (2010). Contrarily, Chandorkar and Deota, (2013) estimated that PTDI for Cadmium was more than 15 times higher, Pb almost two times higher while for Arsenic it was ten times higher than the cutoff given by JECFA (2010).

Nickel and chromium dietary exposures were not comparable owing to unavailability of credible estimated safe dietary intakes. There are no maximum levels (MLs) for Ni in food, furthermore, no national survey data are available on Cr intakes and currently, there is no formal Recommended Dietary Allowance (RDA) for chromium (EFSA, 2015; USDA, 2001; and European Commission, 2003). Ni and Cr have been proven neither essential and associated with many beneficial activities, nor detrimental for the human body. Ni plays its role as a coenzyme in different beneficial biochemical reactions; and lower content of Ni in vegetables and fruits sources can lead to increased blood sugar level, hypertension and deficient growth in humans. On the other hand increased uptake of Ni in fruits and vegetables can reduce blood glucose level and lead to difficulty in breathing and nausea (Ismail *et al*., 2011). Assem and Zhu (2007) highlighted that chromium (III) is an essential trace element that is involved in the metabolism of glucose and fats; and depending on the type of chromium (III or VI) exposure may be harmful to health. Whether or not the estimated dietary intakes of these two trace metals are of health risk significance depends on how much and the type present in a given foodstuff.

Although the estimated daily and weekly intakes obtained in this study were below the maximum established limits, increased consumption of these fruits and vegetables may bring about adverse health implications for the exposed population.

Table 3 Mean concentrations (mg/kg) of heavy metals in fresh fruits and vegetables collected from supermarkets in Accra, Ghana

Product	Pb	Ni	Cd	Cr	Cu
Apple	1.52^{b}	0.51^{a}	0.47^{ab}	0.26^{a}	3.71^{a}
Grape	2.01^{a}	0.27^{b}	0.36^{b}	0.32^{a}	3.66^{a}
Cabbage	1.94^{a}	0.46^{a}	0.48^{ab}	0.24^{a}	4.56^{a}
Broccoli	1.46^{b}	0.53^{a}	0.54^{a}	0.19^{a}	4.58^{a}
SEM	0.026	0.007	0.012	0.040	0.98

Means within a column followed by the same letter are not different using Tukey's HSD ($\alpha = 0.05$).

Table 4 The effect of washing on the concentration(mg/kg) and % reduction of heavy metals in selected fruits and vegetables imported and sold in Accra, Ghana

Type	Product	Treatment	Pb	Ni	Cd	Cr	Cu
Fruit – Hard	Apple	U	1.68^{ns}	0.59^{a}	0.54^{a}	0.28^{ns}	4.05^{ns}
	Apple	W	1.36^{ns}	0.43^{a}	0.39^{b}	0.17^{ns}	3.38^{ns}
% reduction			19	27	28	39	17
Fruit – Soft	Grape	U	2.14^{ns}	0.38^{a}	0.44^{a}	0.41^{ns}	4.21^{ns}
	Grape	W	1.88^{ns}	0.16^{b}	0.28^{b}	0.23^{ns}	3.11^{ns}
% reduction			12	58	26	41	26
Leafy Green	Cabbage	U	2.11^{ns}	0.65^{a}	0.54^{a}	0.34^{ns}	5.03^{ns}
	Cabbage	W	1.78^{ns}	0.26^{b}	0.37^{b}	0.14^{ns}	4.08^{ns}
% reduction			16	57	37	59	19
Hard Vegetable	Broccoli	U	1.61^{ns}	0.64^{a}	0.64^{a}	0.24^{ns}	5^{ns}
	Broccoli	W	1.31^{ns}	0.41^{b}	0.44^{b}	0.13^{ns}	4.17^{ns}
% reduction			19	36	41	46	17
SEM			0.026	0.007	0.012	0.040	0.98

Means within a column within product followed by the same letter are not different using Tukey's HSD ($\alpha = 0.05$)Place all detailed caption, notes, reference, legend information, etc here

Table 5 Range and standard deviation of heavy metals residues (mg/Kg) in selected produce imported and sold in Accra, Ghana and maximum residue limits established by Codex and EU

						Maximum Residue Level	
Product	**Variable**	**Min.**	**Max.**	**Mean**	**Std. dev.**	**Codex**	**EU**
Apple	Pb	0.9	1.9	1.5	0.4	0.1	0.1
	Ni	0.4	0.7	0.5	0.1	-	-
	Cd	0.1	0.9	0.5	0.2	0.05	-
	Cr	0.1	0.5	0.2	0.1	-	0.05
	Cu	2	7.5	3.7	2	-	-
Grape	Pb	1.1	2.3	2	0.3	0.2	0.2
	Ni	0.1	0.4	0.3	0.1	-	-
	Cd	0.3	0.5	0.4	0.1	0.05	
	Cr	0.01	1.3	0.3	0.5	-	0.05
	Cu	0.9	6.6	3.7	2.2	-	-
Cabbage	Pb	1.6	2.2	1.9	0.2	0.3	0.3
	Ni	0.2	0.8	0.5	0.2	-	-
	Cd	0.3	0.9	0.5	0.2	0.05	0.2
	Cr	0.1	0.7	0.2	0.2	-	0.05
	Cu	1.9	9.6	4.6	2.9	-	-
Broccoli	Pb	1.1	1.8	1.5	0.2	0.3	0.3
	Ni	0.3	0.7	0.5	0.2	-	-
	Cd	0.4	0.7	0.5	0.1	0.05	0.2
	Cr	0.05	0.4	0.2	0.09	-	0.05
	Cu	1.2	7.5	4.6	2.5	-	-

Safe limits of the heavy metals were obtained from Codex Stan 193-1995 (WHO, 2010) of the United Nations and the Commission Regulation (EC) No 629/2008 of the European Union (EuroLex, 2010)

Table 6 Estimated daily intake (mg/day/person) and weekly intake (mg/week/person) through mean fruits and vegetables consumption (kg/person/day) for Pb, Ni and Cd in fruits and vegetables collected from supermarkets in Accra

Commodity	**Mean F&V Consumption**	**Pb**			**Ni**			**Cd**		
	Kg/person/day	*Mean Level. (mg/kg)*	*EDI (mg/day)*	*EWI (mg/week)*	*Mean Level (mg/kg)*	*EDI (mg/day)*	*EWI (mg/week)*	*Mean Level (mg/kg)*	*EDI (mg/day)*	*EWI (mg/week)*
Apple	0.00032	1.52	0.00049	0.0034	0.51	0.00016	0.0011	0.47	0.00015	0.0011
Grape	0.00014	2.01	0.00028	0.0019	0.27	0.000037	0.00026	0.36	0.00005	0.00035
Cabbage	0.0000012	1.94	0.0000023	0.000016	0.46	0.00000056	0.0000039	0.48	0.00000057	0.0000039
Broccoli	0.0000081	1.46	0.000012	0.000084	0.53	0.00000095	0.0000067	0.54	0.0000044	0.000031

The EDI and EWI were computed in Microsoft Excel using fruits and vegetables import figures obtained from the Plant Protection and Regulatory Services Directorate (PPRSD Annual Report, 2013 and 2014)

Table 7 Estimated daily intake (mg/day/person) and weekly intake (mg/week/person) through mean fruits and vegetables consumption (kg/person/day) for Cr and Cu in fruits and vegetables collected from supermarkets in Accra.

Commodity	**Mean F&V Consumption**		**Cr**			**Cu**	
	Kg/person/day	*Mean level (mg/kg)*	*EDI (mg/day)*	*EWI mg/week*	*Mean level (mg/kg)*	*EDI (mg/day)*	*EWI mg/week*
Apple	0.00032	0.26	0.000082	0.00057	3.71	0.0012	0.0084
Grape	0.00014	0.32	0.000045	0.00032	3.66	0.00051	0.0036
Cabbage	0.0000012	0.24	0.00000029	0.000002	4.56	0.0000055	0.000039
Broccoli	0.0000081	0.19	0.0000015	0.000011	4.58	0.000037	0.00026

The EDI and EWI were computed in Microsoft Excel using fruits and vegetables import figures obtained from the Plant Protection and Regulatory Services Directorate (PPRSD Annual Report, 2013 and 2014)

CHAPTER V

SUMMARY AND CONCLUSION

Food safety and quality assurance in fresh produce should be ongoing processes that incorporate activities from the selection and preparation of the soil in agricultural operations through the final preparation and consumption of the food (UN, 2007). In general, varied levels of heavy metals (Pb, Ni, Cd, Cr and Cu) were detected in the fruits and vegetables (apple, grape, cabbage and broccoli) sampled from all the supermarkets. The presence of these contaminants in the fruits and vegetables could be attributed to two mechanisms: 'absorption' where there is uptake of the substances by plants during production and; 'adsorption' where these elements bind onto the surface of the food items during post-production activities. Washing reduced Cr in cabbage by 59% whereas Pb in grapes was reduced only by 12%. But levels of heavy metals in washed items were not reduced below the maximum residue limits. Though Pb, Cd and Cr content in the commodities tested exceeded the safe thresholds set by the regulatory bodies, their estimated dietary intakes were well below the reference dietary intakes established by the Joint Expert Committee on Food Additives of the WHO/FAO. Content of Cu were well below both the parametric reference estimated dietary intake and safe limits established by WHO/FAO. Ni content was not comparable because of safe limits and ceiling for dietary intakes have not been established. Estimated dietary intakes of the fruits and vegetables, indicated that negative health implication would not arise as a result of consuming such products in the short term but in the long run, heavy metals may bio-accumulate to pose health problems.

It is incumbent on governments to initiate and conduct monitoring programs, including total dietary studies to assure that safe or tolerable levels of contaminants are not exceeded. More sophisticated washing options can be explored to further reduce contaminants on the surface of produce before consumption. Further work can be carried out on these imported commodities to evaluate target hazard quotient (HQI) and hazard index (HI) of heavy metals for the exposed population.

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APPENDIX A

RAW DATA, STANDARD CURVES FOR THE VARIOUS ELEMENTS, MEAN CHART FOR WASHED AND UNWASHED SAMPLES

Table 8 Raw data (Absorbance) of heavy metals obtained by analyzing washed and unwashed fruits and vegetables samples through the use of Atomic Absorption Spectroscopy

	Lead		Nickel		Cadmium		Chromium		Copper	
Rep	W	U	W	U	W	U	W	U	W	U
APPLE										
1	1.8	1.95	0.4	0.65	0.33	0.475	0.13	0.23	2.03	2.2
2	1.7	1.875	0.45	0.6	0.28	0.425	0.1	0.18	2.23	2.5
3	1.08	1.425	0.475	0.58	0.25	0.4	0.13	0.2	3.25	4
4	0.88	1.475	0.375	0.55	0.7	0.875	0.33	0.53	6	7.5
	1.4	1.7	0.4	0.6	0.4	0.5	0.2	0.3	3.4	4.1
CABBAGE										
1	1.98	2.2	0.25	0.55	0.43	0.55	0.13	0.23	1.9	2.42
2	1.83	2.05	0.3	0.58	0.38	0.45	0.1	0.18	2.18	2.45
3	1.58	2.075	0.35	0.83	0.28	0.45	0.1	0.3	4.5	5.5
4	1.75	2.1	0.2	0.65	0.4	0.9	0.25	0.68	7.75	9.75
	1.8	2.1	0.3	0.7	0.4	0.6	0.1	0.3	4.1	5.0
GRAPE										
1	2.23	2.3	0.225	0.38	0.35	0.475	BDL	0.05	0.95	1.95
2	2.08	2.275	0.15	0.33	0.25	0.4	0.08	0.13	1.48	2.38
3	1.85	2.15	0.15	0.43	0.25	0.4	0.05	0.15	5.25	6.75
4	1.35	1.825	0.1	0.38	0.28	0.475	0.78	1.33	4.75	5.75
	1.9	2.1	0.2	0.4	0.3	0.4	0.3	0.4	3.1	4.2
BROCCOLI										
1	1.55	1.825	0.575	0.73	0.53	0.65	0.05	0.15	1.18	2
2	1.45	1.7	0.45	0.68	0.38	0.625	0.15	0.23	2.75	3.25
3	1.1	1.4	0.35	0.5	0.45	0.6	0.18	0.23	6.25	7.25
4	1.15	1.525	0.275	0.65	0.4	0.675	0.15	0.38	6.5	7.5
	1.3	1.6	0.4	0.6	0.4	0.6	0.1	0.2	4.2	5.0

W and U denote washed and unwashed samples respectively.

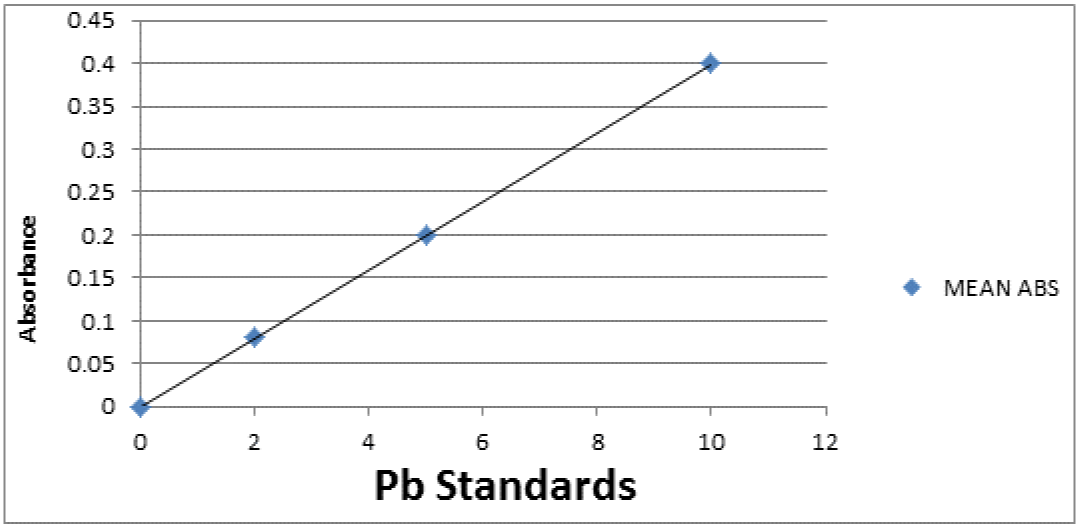

Figure 6 Pb standard curve generated during the calibration of the AAS instrument used to determine the heavy metal levels in fruits and vegetables tested

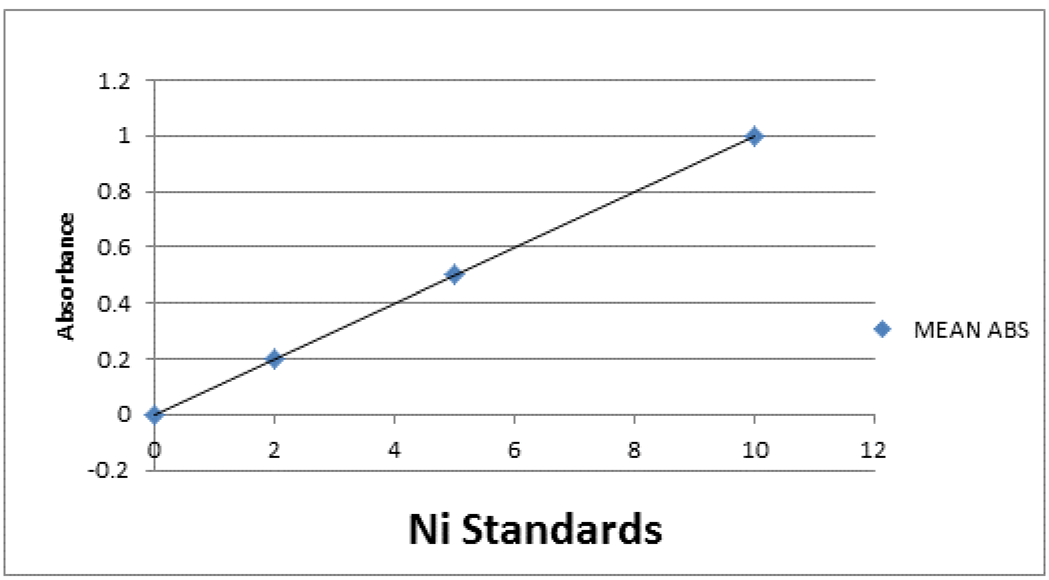

Figure 7 Ni standard curve generated during the calibration of the AAS instrument used to determine the heavy metal levels in fruits and vegetables tested

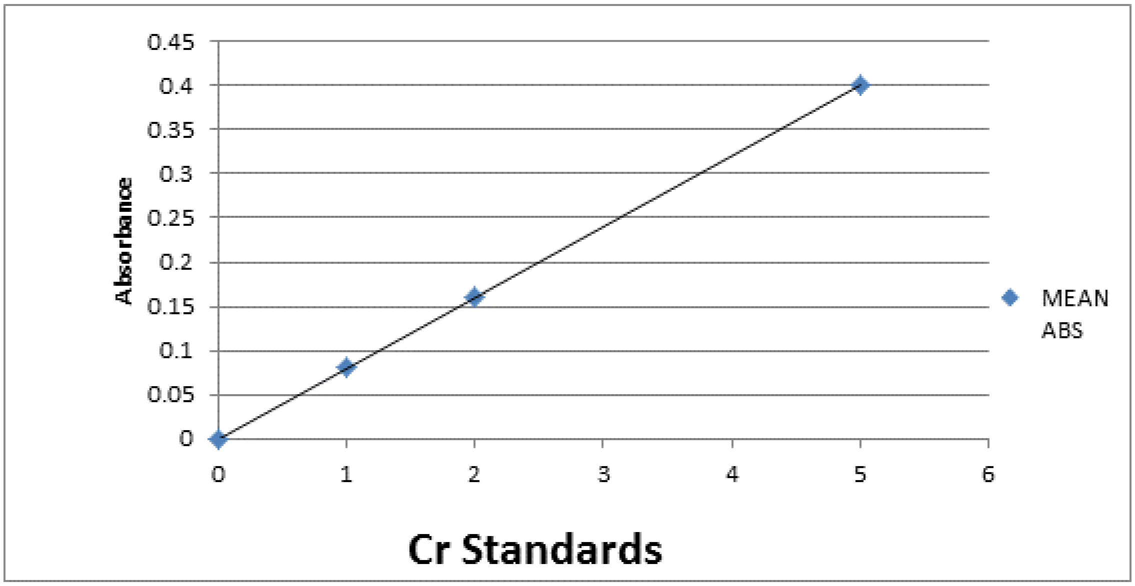

Figure 8 Cr standard curve generated during the calibration of the AAS instrument used to determine the heavy metal levels in fruits and vegetables tested

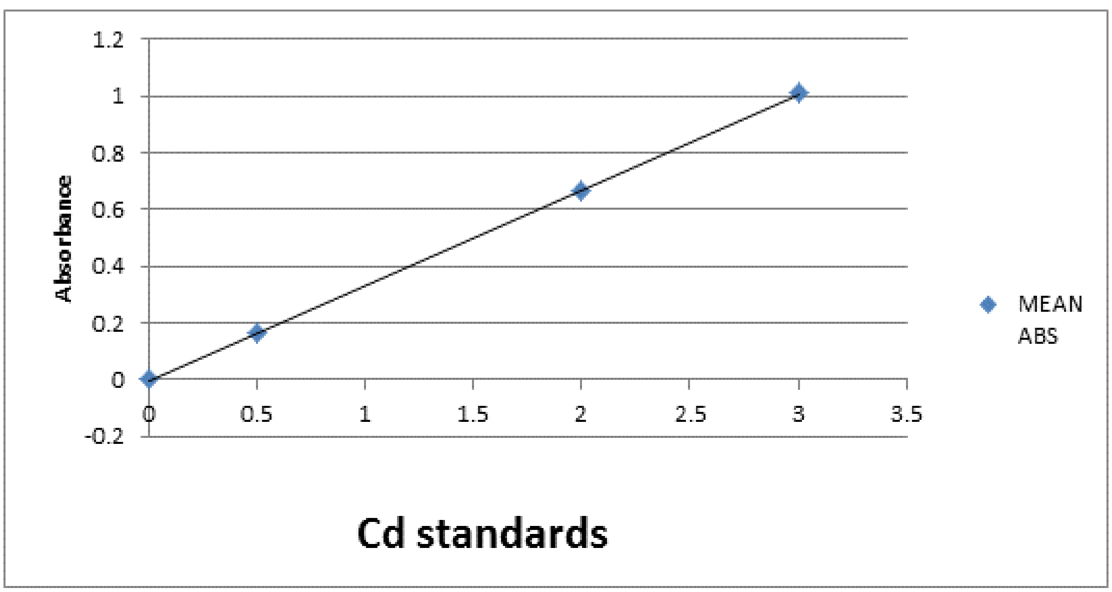

Figure 9 Cd standard curve generated during the calibration of the AAS instrument used to determine the heavy metal levels in fruits and vegetables t

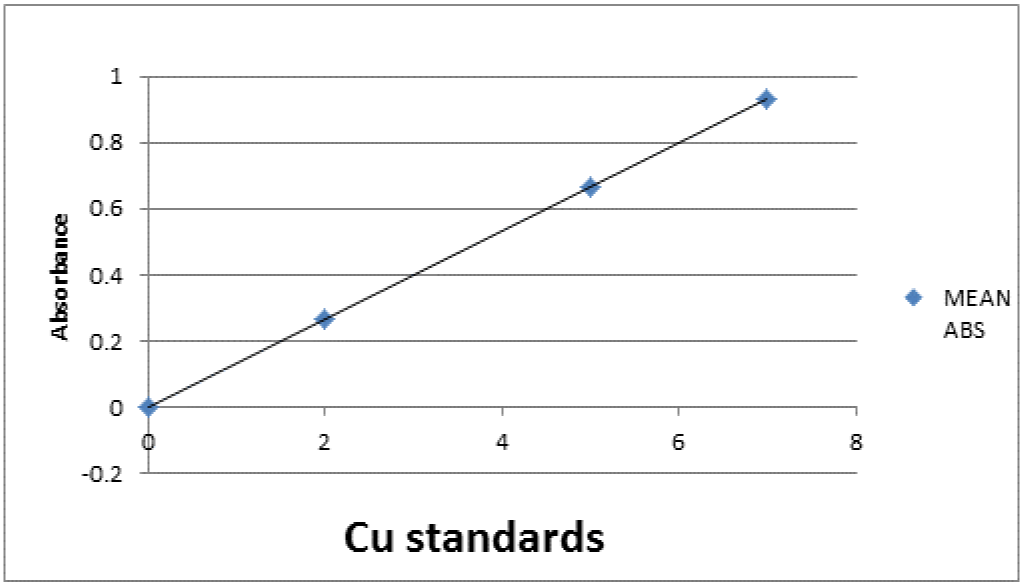

Figure 10 Cu standard curve generated during the calibration of the AAS instrument used to determine the heavy metal levels in fruits and vegetables tested

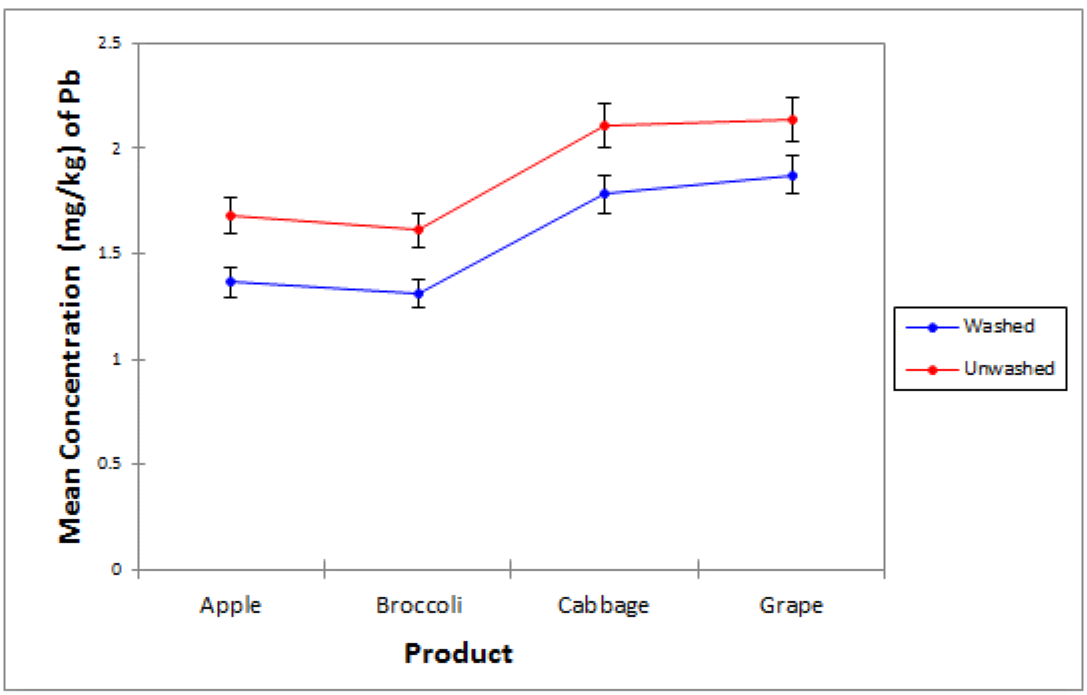

Figure 11 The relationship between the concentration (mg/kg) of Pb in washed and unwashed fruits and vegetable sampled from supermarkets in Accra, Ghana.

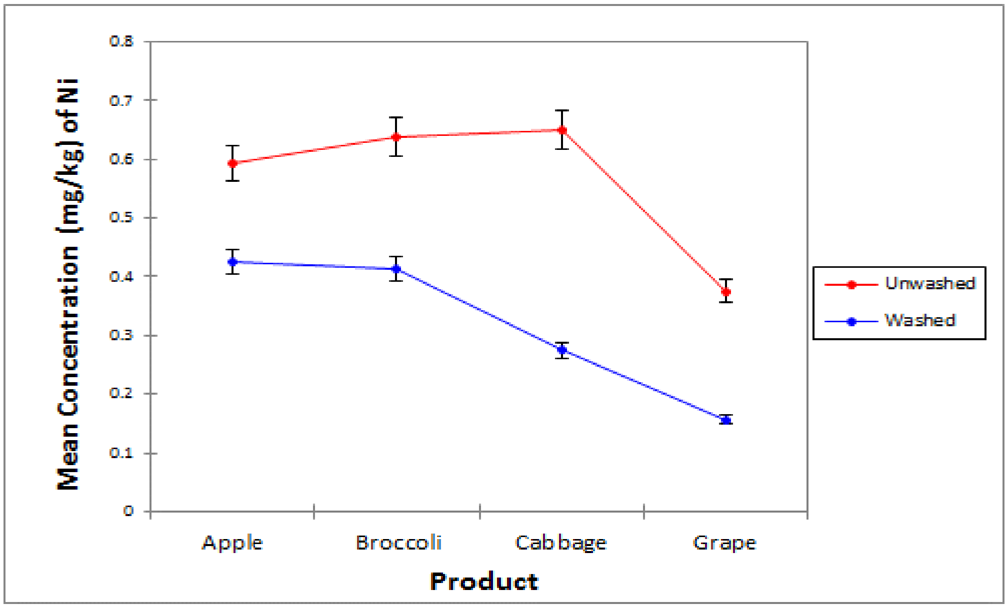

Figure 12 The relationship between the concentration (mg/kg) of Ni in washed and unwashed fruits and vegetable sampled from supermarkets in Accra, Ghana

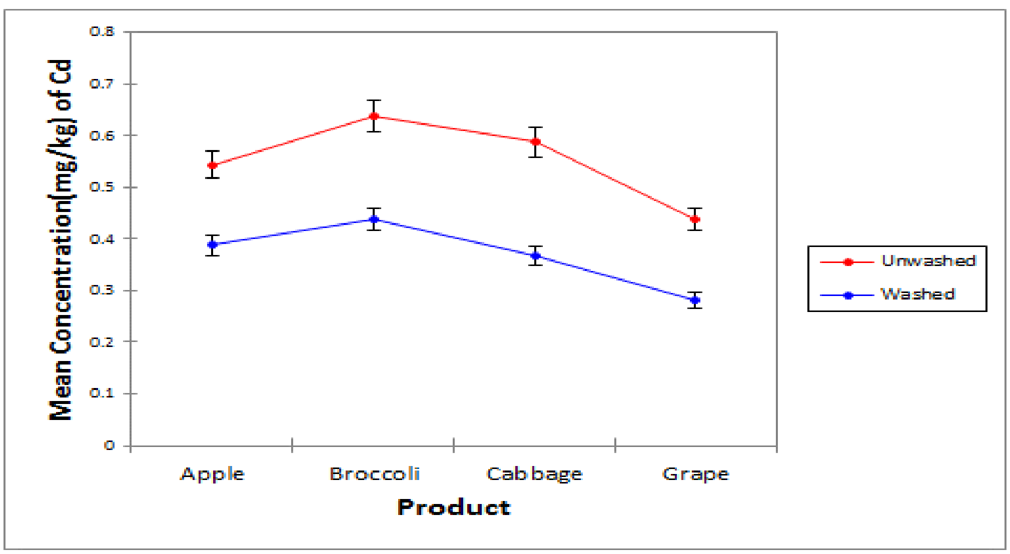

Figure 13 The relationship between the concentration (mg/kg) of Cd in washed and unwashed fruits and vegetable sampled from supermarkets in Accra, Ghana

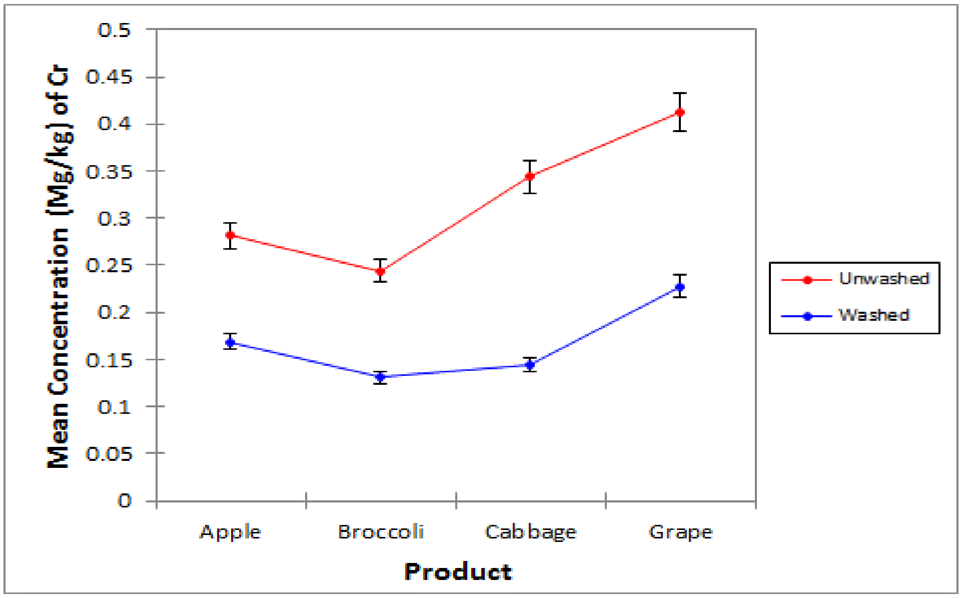

Figure 14 The relationship between the concentration (mg/kg) of Cr in washed and unwashed fruits and vegetable sampled from supermarkets in Accra, Ghana

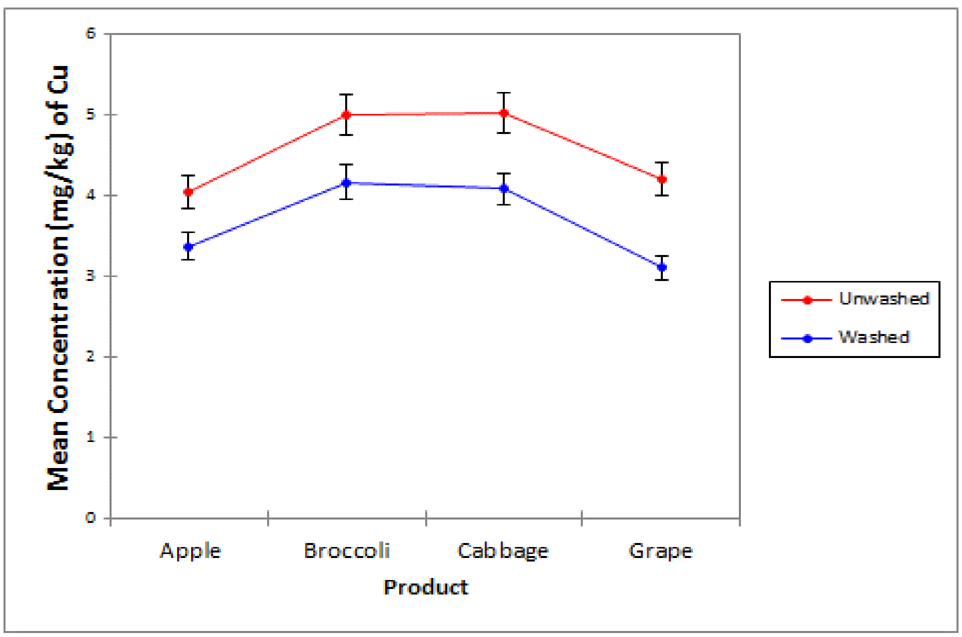

Figure 15 The relationship between the concentration (mg/kg) of Cu in washed and unwashed fruits and vegetable sampled from supermarkets in Accra, Ghana