HEAVY METALS CONTENT AND PHYTOCHEMICALS IN SOME SEASONINGS IN KUMASI METROPOLIS (GHANA)

BY

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DECLARATION

I hereby declare that this submission is my own work towards the MSc and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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DEDICATION

To my mum, Mrs. Mina Amponsah Darko.

ABSTRACT

Seasonings are flavour and taste enhancers that are used sparingly but frequently in Ghanaian meals. They contain some phytochemicals that are beneficial to man. Seasonings may be contaminated by heavy metals during cultivation, processing and handling. There is therefore the need to ascertain the safety of the seasonings consumed in Ghana. Thirty two (32) flavour enhancers were purchased from Asafo, railway and central markets in Kumasi in the Ashanti Region of Ghana. Iron (Fe), zinc (Zn), copper (Cu), cadmium (Cd) and lead (Pb) levels were measured using Flame Atomic Absorption Spectrometry and mercury (Hg) was determined by Cold Vapour Atomic Absorption Spectrometry. Phytochemical screening was performed using standard screening methods. In unmixed seasonings, Fe ranged from 19.4mg/kg to 971.40mg/kg, Zn was from 2.40mg/kg to 34.60mg/kg, Cu was from 0.9mg/kg to 10.10mg/kg, Cd was from ND to 0.9mg/kg (ND- not detectable) and Pb ranged from 0.6mg/kg to 1.8mg/kg. In mixed seasonings, concentration ranged from 83.36mg/kg to 480.82mg/kg for Fe, 1.72mg/kg to 26.78mg/kg for Zn, 1.73mg/kg to 7.70mg/kg for Cu, ND to 0.06mg/kg for Cd and 0.63mg/kg to 1.39mg/kg for Pb. Bouillon cubes had metal levels in the ranges of 9.66mg/kg to 52.45mg/kg for Fe, 0.83mg/kg to 8.93mg/kg for Zn, 0.66mg/kg to 3.59mg/kg for Cu, ND to 0.06mg/kg for Cd and 0.37mg/kg to 2.18mg/kg for Pb. Hg was not detectable in all seasonings. Positive and negative correlations were obtained between metals in some of the seasonings. The results indicated that Fe, Zn and Cu were below permissible levels whereas Pb and Cd were above permissible levels. However, it is unlikely for a person to consume amounts of seasoning in a day that will exceed the RDA for each metal. This suggests that intake of the seasonings will have negligible health effects on consumers. In the phytochemicals screening, 25% of all the seasonings contained saponins, 44% had coumarins, 9% had alkaloids and 25% were found to contain terpenoids. The seasonings may have health benefits in addition to their organoleptic properties due the presence of these phytochemicals.

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CHAPTER ONE

1. INTRODUCTION

1.1 BACKGROUND

Heavy metals have bio-importance as trace elements but, the biotoxic effects of many of them in human biochemistry are of great concern. They are natural components of the Earth's crust which cannot be degraded or destroyed. They enter our bodies via food, drinking water and air (Lenntech, 2008).

Amongst the known heavy metals, lead, mercury, arsenic, nickel and cadmium are linked to human poisoning at certain levels. Other heavy metals such as cobalt, zinc, manganese and chromium, are required by the body in small amounts, but can be toxic in larger doses (Schroeder *et al.*, 1973; WHO, 1997).

Sources of heavy metals in the environment include air emissions from automobile exhaust, pesticides leaching into water bodies, metal smelters, and processing of wastes from mining and industry (Duruibe *et al.,* 2007; Opuene and Agbozu, 2008). Heavy metals are generally present in agricultural soils at low levels. Due to their cumulative behaviour and toxicity; they however, have a potential hazardous effect not only on plants but also on human health (Ozkutlu *et al*., 2006).

The amount of trace metals in food is of interest because of their essential nature (Onianwa, *et al.*, 1999) and effects on human life (Dundar and Saglam, 2004; Oktem *et al.,* 2005). Heavy metals such as iron, zinc and copper have biological importance. Iron aids in transport of oxygen in red blood cells and in muscles. Zinc is required for the optimum functioning of many enzymes involved in catalytic functions, maintenance of structural stability, and regulatory functions (Vallee and Auld, 1990). Copper acts as an antioxidant by protecting the brain and the nervous system (Wardlaw, 2003). The kidneys and central nervous system can be damaged with chronic exposure to mercury (Pamphlett *et al*, 1997). Exposure to lead (Pb) may lead to higher risks of heart attacks and strokes in adults (Needleman *et al*., 1990).

Seasonings include spices, herbs and bouillon cubes. Bouillon cubes are taste enhancers (Elemo and Makinde, 1984), whose major active ingredients include salts and monosodium glutamate (Nnorom *et al*., 2007). Spices and colourants are also added to bouillon cubes.

Spices are dried parts of plants, which are used as diet components often to improve colour, aroma, palatability and acceptability of food. They consist of rhizomes, barks, leaves, fruits, seeds, and other parts of the plants (Satter *et al.*, 1989).

These plants can be contaminated with heavy metals easily through environmental pollution (Ansari *et al.*, 2004). Contamination could result from the type of soil for cultivation, source of water used for irrigation, where the spices are processed, stored or sold. There have been reports indicating wide variations in the concentrations of trace metals in bouillon cubes, mixed spices, natural plant spices and nuts (Satter *et al.*, 1989; Garcia *et a*l., 2000; Ansari *et al.*, 2004).

Ekpo and Jimmy (2005) reported that food spices are widely used especially on outdoor foods mainly for edibility enhancement. Colourants which are added to some of these flavour enhancers may contain some trace metals such as lead etc. In the United States, two families were reported to have been poisoned by consuming lead-contaminated spices used in the preparation of the family's food **(**Woolf and Woolf, 2005).

Spices generally improve taste of food. Additionally, spices combat food borne microorganisms and reduce food poisoning, involve in antioxidant function and antimicrobial activity as well (Gupta *et al.*, 2009). Spices are also known to possess a wide range of medicinal values

(Onianwa, *et al.,* 1999).These benefits from spices are due to the presence of phytochemicals (Lampe, 2003; Gupta *et al.*, 2009).

An observation made in the markets in Kumasi (Ghana) indicated that, most of the spices are imported from United States, South Africa, Malaysia, China and Dubai and majority from India. Interaction with sellers of powdered ginger and pepper (red and green) spices in the Kumasi market indicated that, these spices are processed by the individual seller. These spices may easily be contaminated by heavy metals from the soil or aerial depositions as these spices are dried on the ground or on roof tops. Moreover, commercial mills used may also introduce some amount of metals into the seasonings due to wear and tear of the machinery.

1.2 JUSTIFICATION

Heavy metals are dangerous because they tend to bio-accumulate and are taken up and stored faster than they are broken down or excreted (INL, 2008). The incidence of the lead poisoning from spices used in preparation of food by two families in the United States, suggest the need for assessing the safety of spices used in Ghana as being done in other countries. Aluminum and Chromium levels in different spices and aromatic herbs widely consumed in Spain were studied using electro-thermal atomic absorption spectrometry (Garcia *et al*., 2000; Lopez *et al*., 2000). Trace heavy metals such as Fe, Cd, Zn, Mn, Pb, Cu, and Ni have been determined in some spices, bouillon cubes and food condiments in Turkey and Nigeria using inductively coupled plasma atomic emission spectrometry (ICP-AES) and atomic absorption spectrometry (AAS) (Divrikli *et al*., 2006; Ozkutlu *et al*., 2006; Nnorom *et al.,* 2007).

Research publications to ascertain the safety of the seasonings in terms of concentration of heavy metals of these seasonings sold on the Ghanaian markets are scanty. In addition there is paucity of publication on the phytochemical screening of spices though research indicates that they are repository of phytochemicals which are beneficial to health (Halvorsen *et al.*, 2002; Okwu, 2004; Gupta *et al.*, 2009).

This project therefore seeks to ascertain the safety and health benefits of seasonings in terms of the concentration of heavy metals and the phytochemicals content of seasonings sold in markets in Kumasi in the Ashanti region of Ghana.

1.3 HYPOTHESIS

Spices and bouillon cubes contain heavy metals at levels dangerous to human health.

1.5 OBJECTIVES

1. To determine the concentration of iron, zinc, cadmium, copper, mercury and lead in some seasonings in Ghana, using atomic absorption spectrometry.
2. To determine correlations that may exist between the metals in the seasonings.
3. Qualitative analysis of phytochemicals in the seasonings.

CHAPTER TWO

2. LITRATURE REVIEW

2.1 WHAT ARE SEASONINGS?

Seasoning is the process of imparting flavor to or improving the flavour of food. Seasonings include spices, herbs and bouillon cubes which themselves are frequently referred to as *seasonings* (www.en.wikipedia.org/wiki/seasonings, 2011).

According to Oxford Food and Nutrition Dictionary, seasonings used to mean salt and pepper, but may include any herbs, spices, and condiments added to a savory dish (www.answers.com>...>Literature&Languages>Dictionary, 2011).

Figure 2.1: Examples of seasonings.

2.2 SPICES

Spices are dried parts of plants, which are used as diets components often to improve color, aroma, palatability and acceptability of food (Satter *et al.,* 1989). They can also be defined as aromatic vegetable substances, in the whole, broken, or ground form, whose significant function in food is seasoning rather than nutrition (Lampe, 2003). They consist of rhizomes, barks, leaves, fruits, seeds, and other parts of the plants (Satter *et al.,* 1989).

2.3 CHEMICAL NATURE

Spices are repository of many chemically active compounds that impart flavour, fragrance and piquancy. Flavoring properties of most spices are due to volatile oils and in some cases, to fixed oils and small amount of resin, which are known as oleoresins. A blend of different compounds such as alcohols, phenols, esters, terpenes, organic acids, resins, alkaloids, and sulphur containing compounds in various proportions produce the flavours. Besides these flavouring components, spices contain components such as proteins, carbohydrates, fibre, minerals, tannins and polyphenol (Jose and Joy 2004).

2.4 CLASSIFICATION OF SPICES

Spices could be classified or grouped according to different systems of classification based on:

(i) Botanical analogies or families

(ii) Economic and commercial importance

(iii) Climatic requirement (tropical, subtropical, temperate, etc.)

(iv)Number of seasons required to complete the life cycle (annuals, biennials, perennials, etc.)

(v) Morphology of the useful parts

Since spices come from various woody shrubs and vines, trees, aromatic roots, flowers, seeds, rhizomes and fruits of herbaceous plants. The most convenient system of classification may possibly be based on the morphological characters (Jose and Joy, 2004).

2.5 QUALITY EVALUATION OF SPICES

The quality of a spice is assessed by intrinsic as well as its extrinsic characters. The former consists of chemical quality, *i.e.* the retention of chemical properties like volatile oil, alkaloids and oleoresins while the latter emphasizes physical qualities such appearance, texture, shape, the presence or absence of unwanted materials or colour, etc.

In addition, certain health requirements are also implemented as export quality standard viz, pesticide residues, aflatoxin, heavy metals, sulphur dioxide, solvent residues and microbiological quality. However, physicochemical quality remains the ultimate attribute, while considering export requirement of spices because these properties delineate its grade in the market. The physico-chemical characteristics vary widely depending on the variety, agro-climatic conditions existing in the area of production, harvest and post-harvest operations (Jose and Joy, 2004).

2.6 USES OF SPICES

2.6.1 Organoleptic properties

Spices are used to enhance food flavor, color, and palatability (Sherman and Billing, 1999). Nutmeg and basil improve food flavour whereas garlic, pepper and rosemary increase pungency of food (Peter, 2004).

2.6.2 Nutritional properties

Most spices are rich source of proteins, vitamins and minerals such as sodium, iron, potassium and phosphorous. Parsley is the richest source of vitamin A whereas coriander is the richest source of vitamin C and A (Peter, 2004).

2.6.3 Antimicrobial properties

Spices preserve food by delaying spoilage in food (Ravindran *et al.,* 2002). Essential oils extracted from spices and herbs contain active antimicrobial compounds. Sulfur containing compound such as allicin, isolated from garlic oil, inhibits the growth of both gram-negative and gram-positive bacteria (Shelef, 1983). Phenolic compounds, carvacrol and thymol from oregano and thyme are effective fumigants against fungi on stored grain (Paster *et al.,* 1995). Black pepper contains the compound piperine which inhibits *Clostridium botulinum* (Nakatani, 1994). Amchur, an Indian spice made from dried pulp of unriped mango used as souring agent to provide desired acidity in food is reported to contain terpenoids. This was found to have antimicrobial effect on *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Gupta *et al*., 2009). Antimicrobial activity was evident in furostanol saponins, a compound found in seeds of *Capsicum sp (annum* L. var *acuminatum)* against yeast strains (De Marino *et al*, 2008).

2.6.4 Antioxidant properties

Antioxidants are added to foods to preserve the lipid components from quality deterioration (Peter, 2004). Spices produce phytochemicals that reduce free radicals in the body (Sherman and Billing, 1991). Reactive Oxygen Species which consist of the free radicals generate oxidative stress and generate pathophysiological disorders such as inflammations, cancer and arthritis (Gulsin and Boyukokuroglu, 2002). Dietary antioxidants oppose this and lower risk of disease (Atoui *et al.,* 2005). Essential oils, oleoresins and aqueous extracts of spices such as thyme, marjoram, sage, basil, fenugreek and fennel possess anti-oxidative properties (Peter, 2004). The oleoresin of rosemary inhibits oxidative rancidity and retard the development of off-flavor in some products such as meat (Giese, 1994).

Ginger (*Zingiber officinale*) has been identified in several studies as a plant with high antioxidant content (Shobana and Naidu, 2000; Halvorsen *et. al*., 2002). Extracts of several commonly used Indian spices also have been shown to inhibit lipid peroxidation. According to a study, relative antioxidant activities from highest to lowest were found in cloves, cinnamon, pepper, ginger, and garlic (Shobana and Naidu, 2000). Antioxidant activity was found to be high in Linalool, a terpene in coriander seed and Curcumin in tumeric (Zhao *et al.*, 1989; Krishnakantha and Lokesh, 1993). Flavonoids and tannins are phenolic compounds that act as primary antioxidants or free radical scavengers (Polterait, 1997).

2.6.5 Medicinal properties

All spices are medicinal and used extensively in indigenous system of medicine (Mahindru, 1982). Extracts from herbs and spices are used as infusions, decoctions, macerations, tinctures, fluid extracts, teas, juices, syrups, poultices, compresses, oils, ointments and powders (Peter, 2004). Garlic, ginger, cinnamon, and chilies are used in the treatment of dysentery, kidney stones, arthritis, and high blood pressure (Sherman and Billing, 1999). Some spices are used as brain stimulants, and aphrodisiacs (Hirasa and Takemasa, 1998). Alkaloids have analgesic, anti-spasmodic and bactericidal effects and this is the basis for their use as basic medicinal agents (Okwu, 2004). The essential oil of coriander is used as analgesic, dill and anise oils as antipyretic, coriander, celery, parsley and cumin oils as anti-inflammatory. Methanol extracts of allspice, marjoram, tarragon and thyme strongly inhibited platelet aggregation induced by collagen in humans(Peter, 2004). There is growing evidence that quercetin, a flavonol might inhibit the growth of tumour cells containing type II estrogen binging sites, including breast, colon, ovarian, leukaemia, gastrointestinal and meningioma cancer cells (Patil *et al.*, 1995). Quercetin and related compounds might also have protective effect against cardiovascular diseases and stroke by participating in the reduction of platelet aggregation and vasoconstriction (Boots *et al.*, 2008).

2.6.7 Colouring agents

Some spices are used as colouring agents due to the presence of colour components (Hirasa and Takemasa, 1998). The use of paprika and turmeric mostly is to impart colour than enhancing taste. Curcumin in turmeric produce orange yellow colour whereas zeaxanthin, a yellow hue in paprika and capsanthin a dark red tint in red pepper (Ravindran *et al.*, 2002).

The above characteristics exhibited in spices are due to secondary metabolites (Lampe, 2003).

2.7 Antioxidant and antimicrobial mechanisms of phytochemicals

Antioxidants are substances which when present in low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substance (Halliwell and Guteridge, 1989). In case of foods and beverages, antioxidants are related to the protection of specific oxidation substrates or the formation of specific oxidation.

Antioxidants can be considered as either preventive or chain breaking antioxidants. Preventive antioxidants inhibit oxidation by reducing the rate of chain initiation. They convert hydroperoxides to molecular products that are not potential sources of free radicals (Burton *et al.*, 1985). The hydroperoxide product causes the initiation process.

Chain breaking antioxidants are generally phenols or aromatic amines. Their antioxidant activity is due to their ability to trap peroxyl radicals as shown below (Krishnaiah *et al*., 2007).

$O_2^- + \text{antioxidant} \longrightarrow O_2 + \text{antioxidant} + \text{heat}$

The mechanism of polyphenol toxicity against microbes may be related to inhibition of hydrolytic enzymes (proteases and carbohydrolases) or other interactions to inactivate microbial adhesins, cell envelope transport proteins, non-specific interactions with carbohydrates, etc (Cowan, 1999).

2.8 BOUILLON CUBES

The bouillon cubes are taste enhancers, added to augment the taste properties of foods (Elemo and Makinde, 1984). The major active ingredients in these cubes are salts and monosodium glutamate (Nnorom *et al*., 2007).

In Ghana, manufacturers of bouillon cubes indicate the presence of added spices, vegetable fats, meat or poultry extracts, and sea foods (shrimps). In 1991, a study of heavy metals in shrimps from the Korle Lagoon reported that lead and mercury are present though at low concentrations but cadmium was not detected (Biney and Ameyibor, 1991). However, Nyarko and Evans (1998) reported that there are higher levels of lead, iron, copper cadmium and zinc in shrimps found in (Korle lagoon) the coastal waters of Ghana. This supports the need for periodic surveillance of food products for possible contamination by heavy meals.

2.9 HEAVY METALS

Heavy metals refer to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Duruibe *et al*., 2007).

Heavy metal poisoning could result, for instance, from drinking contaminated water (e.g. water flowing through lead pipes), high ambient air concentrations near emission sources, or intake via the food chain. They can enter a water supply system by industrial and consumer waste. Breaking down of soils from acidic rain also release heavy metals into streams, lakes, rivers, and groundwater (Lenntech, 2008).

Heavy metals are dangerous because they tend to bio-accumulate (i.e. their concentration increase in a biological organism over time, compared to their concentration in the environment). The metals accumulate in living things any time they are taken up and stored faster than they are broken down (INL, 2008).

2.9.1 Sources of emission

Heavy metals are emitted into the environment by both natural and anthropogenic causes. The major causes of emission are the anthropogenic sources specifically from mining operations (Hutton and Symon, 1986; Nriagu, 1989) and combustion of fuels, metal-working industries and phosphate fertilizers. It has been estimated that, for example, the anthropogenic emissions of cadmium are in the about 30 000 tons per year (Schützendübel and Polle, 2002).

2.9.2 Sources of human and plants exposure

Heavy metals may enter the human body through inhalation of dust, consumption of contaminated drinking water, direct ingestion of soil and consumption of food plants grown in metal-contaminated soil (Cambra *et al*., 1999; Dudka and Miller, 1999).

When agricultural soils are polluted, these metals are taken up by plants and consequently accumulate in their tissues (Trueby, 2003). Animals that graze on such contaminated plants and drink from polluted waters, as well as marine life that breed in heavy metal polluted waters also accumulate such metals in their tissues, and milk, if lactating (Horsfall and Spiff, 1999).

2.9.2.1 Vehicular traffic density

Certain trace elements are essential in plant nutrition, but plants growing in a polluted environment can accumulate these elements to high concentrations causing a serious risk to human health when they are consumed (Alloway *et al.,* 1990). Studies have found, for example, that the lead (Pb) burden found in the urban environment is strongly related to the vehicular traffic density (Daines *et al*., 1970) when leaded gasoline was in use. Wheeler and Rolfe (1979) found that Pb levels in vegetation increased linearly with traffic density. Other factors that influence the metal content in vegetation include distance from the road, the predominant wind direction, the local topography and season of the year. Buszewski *et al*., (2000), reported that high heavy metal concentrations were found in plants sampled from areas of high traffic density. Rodriguez-Flores and Rodriguez-Castellon, (1982) reported that the cadmium (Cd) and lead (Pb) levels in soil and vegetation decrease with increasing distance from the roadside. Nabulo, (2004) reported that the total heavy metal contents in soil decrease rapidly with increasing distance from the roads.

2.9.2.2 Water for irrigation

Water pollution by heavy metals is mainly caused by point source emissions from mining activities and a wide variety of industries. Chemicals usually present in wastewater are also an important concern for reuse application especially for irrigation of food crops. The mechanisms of food crop contamination by irrigation of reclaimed water may be either physical contamination, where evaporation and repeated irrigation may cause build up of contaminants on crops or uptake of the chemical constituents through roots from irrigation water or soil. Toxins include strong acid, strong base, cyanide, chrome, copper, mercury, and radiation materials. The fate and effects of pollutants discharged into a particular water body will depend not only on the amount of polluting substances emitted but also on the hydrological, physical, chemical and biological conditions characterizing the water body concerned (Wajahat *et al.,* 2006). A study by Awode *et al*., (2008) indicated that cadmium and nickel levels were high in pepper irrigated with water from the Challawa River in Nigeria.

2.9.2.3 Compost and sludge

Compost quality is determined in part by the amounts and types of heavy metals present (Genevini, *et al*, 1997). The presence of organic and inorganic contaminants in compost, pose a danger to the environment. Heavy metal is the main obstacle factor leading to restricted agricultural use of compost. Literature on the effect of compost use on heavy metal levels in the soil environment show that it varies according to soil types, plant species and compost quality. Increased zinc (Zn), copper (Cu) and lead (Pb) concentrations have often been observed, both in the soil and plants, while other heavy metals such as cadmium, nickel (Ni) and chromium(Cr) increase less consistently (Bevacqua and Mellano, 1993). In the long term, the use of sewage sludge can also cause a significant accumulation of Zn, Cu, Pb, Ni and Cd in soil and pants (Williams *et al.,* 1980; Mulchi *et al*., 1991).

2.9.2.4 Fertilizer application

Phosphorus fertilizers are essential to obtain high productivity. However, they contain heavy metals that can contaminate soil and threatens the health of animals and humans (Mortvedt, 1987). Soil contamination by heavy metals originating from phosphate fertilizers has become a concern in several countries. Thus, much research has been conducted to evaluate the presence of such metals in these amendments (McBride and Spiers, 2001; Prochnow *et al*., 2001; Loganathan *et al*., 2003). The heavy metal concentration in phosphate fertilizers is dependent on the type of rock phosphate used as raw material. The main Brazilian rock phosphates are low in heavy metals (Camargo *et al*., 2000).

Heavy metals such as arsenic (As), chromium (Cr), lead (Pb), mercury (Hg), nickel (Ni), and vanadium (V) occur in phosphate fertilizers. However, these metals are of less concern than Cd, because they are not as readily absorbed by plants from P-fertilized soils (Mortvedt, 1987). The application of fertilizers could increase the heavy metal bioavailability in soils due to the chemical alterations they provoke in the system (Tu *et al.*, 2002).

Cadmium concentration in maize amended with phosphate fertilizers was significantly affected not only by phosphate sources but also by their localization in the soil (Prochnow *et al*., 2001).

2.10 BIOCHEMISTRY OF TOXICITY

Poisoning by heavy metals is due to their interference with metabolic processes. When ingested, due to the acidity of the stomach, metals are converted to their stable oxidation states such as Zn^{2+}, Cd^{2+} and Hg^{2+}. They then combine with proteins and enzymes to form strong and stable chemical bonds (Ogwuegbu and Ijioma, 2003).

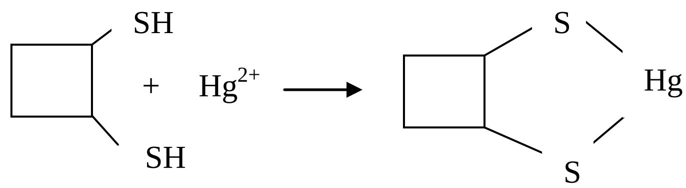

Figure 2.2: Binding of heavy metal to a protein or an enzyme active site (Mailman and Lawler, 2001)

An enzyme is inhibited from functioning when a poisoning metal replaces the hydrogen atoms or the metal groups (Holum, 1983). Substitution of heavy metals for the metals in metallo-enzymes is the mode of toxicity action by these metals. Due to the three dimensional structure of enzymes, binding is highly selective to the substrate specie upon which the substrate acts (Mailman and Lawler, 2001).

$$E + S \rightleftarrows E\text{-}S \longrightarrow E - S^* \longrightarrow E - P \longrightarrow E + P$$ **….** (Eq. 1)

(E = Enzyme; S = Substrate; P = Product; * = Activated Complex)

While at the E-S, E–S* and E-P states, an enzyme cannot accommodate any other substrate until it is freed. In a multi enzyme-complex, the product from one enzyme reacts with a second enzyme in a chain process, with the last enzyme yielding the final product as illustrated below:

$$A \xrightarrow{E1} B \xrightarrow{E2} C \xrightarrow{E3} D \xrightarrow{E4} F$$ (E1 to E4 are enzymes)….. (Eq. 2)

The final product (F) goes back to react with the first enzyme thereby inhibiting further reaction since it is not the starting material for the process.

Hence, the enzyme E1 becomes incapable of accommodating any other substrate until F leaves if utilized by the body. If the body cannot utilize the product formed from the heavy metal – protein substrate, there is a permanent blockage of the enzyme E1, which then cannot initiate any other bio-reaction of its function. Therefore, the metal remains embedded in the tissue and result in bio-dysfunctions (Holum, 1983).

A metal ion in the body's metallo-enzyme can be conveniently replaced by another metal ion of similar size (Ogwuegbu and Ijioma, 2003). Cadmium toxicity consequently, occurs when Cd^{2+} substitute Zn^{2+} which is present in many metallo-enzymes due to their chemical similarities (Mailman and Lawler, 2001).

2.11 Heavy metal content of spices and herbal plants in other countries

Heavy metals analysis of spices and herbal plants has been conducted in other parts of the world (Garcia et al., 2000; Divrikli *et al.,* 2003; Ozkutlu, *et al*., 2006; Krejpcio *et al*., 2007; Nnorom *et al.,* 2007). In Nigeria, the AAS was used to determine Fe, Cd Zn and Pb in some bouillon cubes, food condiments, food drinks and food beverages (Onianwa *et al*., 1999; Nnorom *et al.,* 2007).

Pb levels were below detection limit (0.02µg/g). Cd levels were between 0.84µg/g—4.80µg/g for bouillon cubes whiles mixed spices were between 0.85µg/g —4.90µg/g. Fe levels were between 3.65gµ/g – 419.05µg/g for bouillon cubes whiles mixed spices were between 92.35µg/g -- 419 µg/g. The levels of zinc ranged 1.60µg/g —90µg/g whereas mixed spices were between 3.4µg/g —22.55µg/g. The samples represented the most frequently and regularly consumed seasonings in Nigeria (Nnorom *et al.*, 2007).

Their results indicated that Pb and Cd were within safe limits specified by most food standards. The levels of Fe were attributed to the fact that plant foods and plants contain iron which complex to form structural components or storage compounds. Another reason was due to intentional fortification of the products or cumulative contribution of raw materials.

The elemental composition analysis by Akpanyung (2005) showed that the levels of iron (11.14mg/100g—17.1mg/g) and zinc (1.15mg/100g—2.73mg/100g) in bouillon cubes could not support a successful fortification of these cubes with iron and zinc.

Other studies carried out using the above spectrometric method, in different regions of Turkey showed that the levels of metals in the same samples differ. The levels of copper in basil, rosemary, laurel and lavender were 20.1, 9.2, 3.8 and 11.3µg/g respectively in a study in Anatolia whereas studies from Konya were 8.05, 6.66, 3.17 and 10.7µg/g (Divrikli *et al.*, 2003; Ozcan, 2004).

In the study carried out in Anatolia, the levels of other metals ranged between 0.2µg/g —2.7µg/g for Cd, 0.1—2.8µg/g for Pb, 30µg/g —945.3µg/g for Fe and 5.2µg/g —83.7µg/g for Zn.

In middle Anatolia, Ozcan (2004), reported the levels of Pb in basil, rosemary and lavender to be 2.10, 8.36 and 1.13µg/g. However in Western Anatolia, the levels of basil and rosemary were reported to be non detectable.

The level of iron in rosemary from Western Anatolia,Turkey, indicated 398.7µg/g which was higher than 375.2µg/g reported from Austria whereas that reported from Konya, Turkey were lower than 547µg/g (Chizzola *et al*.,2003; Ozcan, 2004; Divrikli *et al*., 2006).

A comparative study of zinc in rosemary, laurel and basil from Anatolia and Konya showed differences in concentration. The level of zinc was 22.62µg/g in rosemary, 5.22µg/g (root of laurel), 20.12µg/g (leaf of laurel) and 11.2µg/g in basil (Ozcan, 2004). The levels of zinc the afor mentioned spice from Konya were 15.6, 21.9 and 13.7µg/g respectively (Divrikli *et al*., 2006).

The levels of Pb and Cd were lower than those given by WHO and Food and Nutrition Board of the national Academy of Sciences, USA.

Using Inductive Coupled Plasma Atomic Emission Spectrocopy (ICP-AES) / ICAP-OES, the levels of metals were determined in commonly consumed spices in Turkey. Cd was not detected in any samples of nutmeg. The level of Cd in the rest of the samples ranged from13 mg kg^{-1}in clove to 206 mg kg^{-1}in black pepper. The content of Fe varied between 28 mg kg^{-1} in nutmeg to 374 mg kg^{-1} in black pepper. The zinc content of the plant samples ranged between 4 and 25 mg kg^{-1} with cardamom containing the highest (Ozkutlu *et al*., 2006).

Cadmium concentrations of medicinal plants studied in Italy, Egypt and Brazil were found to vary widely (De Pasquale, 1993; Abou-Arab *et al*, 1999; Caldas and Machado, 2004).

In another studies in Kampala, though not necessarily on spices and herbs, it was reported that traffic density and road side soils influence heavy metals levels in plants.

In this research, total cadmium (Cd) in soil was found at concentrations above the recommended maximum for agricultural soil at most of the sites whereas the total zinc (Zn) in soil was found within recommended concentrations at all sites except one.

However, elevated concentrations of Pb, Cd and Zn have been found in all crops particularly, leafy vegetables. Most of the heavy metal accumulations were in the leaves as compared to the roots, fruits and tuber. The order of heavy metal accumulation in leafy vegetables was; Leaf > Root > Peel > Fruit/Seed. Conversely, root crops accumulated most of these heavy metals in their roots and the order of accumulation was Root > Leaf > Peel > Tuber.

Accumulation of Pb in soils above background levels took place up to a distance of 30 m after which, it became constant at 28 mg/kg dry weight. Similarly, the Pb content in leafy vegetables decreased with increasing distance from the road edge. The study found background values of 50 mg/kg DW and 0.8 mg/kg DW for Zn and Cd in roadside soils respectively. A significant correlation was observed between Cd and Pb in roadside soils. Positive correlations were observed between Pb in soil, Pb in vegetation and Pb in air (window wipes).

Finally, the study found the dominant pathway for Pb contamination of leafy vegetables was from aerial deposition while Pb in root crops appeared to originate from the soil. Likewise, Zn vegetable contamination stemmed mainly from aerial deposition. On the other hand, the major pathway for contamination by Cd was found to be uptake by plants from the soil.

Recently, a study on the concentration of heavy metals in two brands of spices widely used in Pakistan was carried out using atomic absorption spectroscopy (Mubeen *et al.*, 2009).

From the study, concentration of Fe ranged from 144.5 - 1260 mgkg-^{1} while Cu was ranged from 9 - 44 mgkg^{-1} to 3.05 mg kg-^{1}on dry weight basis. The concentration of Cr was from 115 -368 mg kg-1 whereas Co and Cd ranged from 11.5 - 15 mgkg^{-1} and 0.5mgkg^{-1}. While variable levels of Pb were detected from 54 - 70 mgkg-^{1}. The results showed that concentrations of Cr and Pb of all spice samples under study were much larger than those of the minimum required limit values. From this study it was concluded that the spices are a sources of toxic heavy metal contamination (Mubeen *et al*., 2009).

The studies carried out in Turkey, indicated that seasoning contamination by heavy metals could depend on geological location from which the plant was picked for food though other factors may also play a role (Divrikli *et al.,* 2003; Ozcan, 2004; Divrikli *et al.,* 2006). It was also reported by Janitha *et al.*, (1988) that levels of iron in commercially ground spices increased to about 3-5 folds due to wear and tear of the machine.

The basic drive for all these researches was inadequate information on these plants and their products with respect to contamination by heavy metals. From the above studies, it can therefore be said that heavy metal contamination in food occurs from different sources and many factors greatly influence their levels in food.

2.12 THE ESSENTIAL HEAVY METALS (Copper, Zinc and Iron)

2.12.1 Copper in food

Dietary copper intake will vary considerably with the types of food consumed. The condition of the soils (e.g., copper content, pH, etc.) from which the foods are produced and drinking-water characteristics affect the levels of copper in food. Copper is distributed in foods such as liver, seafood (especially shellfish and crustaceans), grains, cereal products and potatoes.

It has been estimated (FDA, 1978) that these particular foods account for about 65% of the total dietary copper intake in adults.

In man the average daily requirement for adults is estimated at 2 mg, and for infants and children at 0.05 mg/kg bodyweight (Browning, 1969). The copper content of various foods ranges from 20 - 400 ppm (Underwood, 1962). The average daily dietary intake for adults is estimated at 2 - 5 mg, of which up to 0.7 mg are excreted in the urine (Browning, 1969).

2.12.1.1 The role of copper

Copper is an essential trace element and is a constituent of plants and of animal and human tissues. Copper as part of metalloproteins is vital for the proper functioning of cells in all biological systems. It also plays a role in iron metabolism by helping in oxygen transport as well as utilization and absorption of iron (Özçelik *et al.*, 2002).

Copper is necessary for the growth, development, and maintenance of bone, connective tis.sue, brain, heart and many other body organs. It is involved in the healing process, energy production, hair and skin coloring, and taste sensitivity. At sub-cellular level a number of enzymes, such as tyrosinase, contain Cu as part of their structure or require it for proper functioning (FAO/WHO, 1970). Copper acts as an antioxidant by defending the body against free radicals thereby protecting the brain and the nervous system (Wardlaw, 2003).

2.12.1.2 Copper toxicity

Copper is an essential trace element that is strongly bioaccumulated (Gangstad, 1986). Copper levels become critical in the presence of other metals such as zinc and molybdenum as they compete with each other for absorption (Underwood, 1962). An excess of molybdenum or zinc can induce or intensify a deficiency of copper. A similar but reverse pattern occurs when molybdenum or zinc is deficient and copper is in excess (Underwood, 1962; Gray and Daniel, 1964).

Continued intake of high levels of copper, leads to considerable accumulation in the liver (Bunch *et al*., 1965). A study by Buntain, (1961) indicated that long term administration of even low concentrations of copper results in some increased storage in the liver.

Effects of Copper deficiency

Copper deficiency can cause hypochronic anemia and loss of color from skin and hair due to the inability of body to manufacture collagen (Mason, 1979). It may also cause osteoporosis, poor bone formation and vascular abnormalities (Underwood, 1977). Copper deficiency also cause burning sensation in the throat and tonsils, malabsorption problems, iron-deficiency anemia baldness, heart disease, Menkes' Syndrome, nervous system impairment, low resistance to infection, poor tissue formation, impaired respiration and skin sores (Hoffman, 2009).

Effects of excess copper

Large doses of copper cause severe mucosal irritation and corrosion, widespread capillary damage, hepatic and renal damage, CNS irritation and depression.

Local skin corrosion with eczema and eye inflammation occurs. Rapid transfer of absorbed Cu to red cells causes haemolysis (Chuttani, 1965; Browning, 1969).

Contact of food or soft acid water with copper utensils may cause poisoning (Browning, 1969).

In mammals, injection or inhalation of copper and its compounds leads to haemochromatosis, liver injury or lung injury (Browning, 1969). Long-term effects are more likely in individuals with Wilson's disease, a condition which causes excessive absorption and storage of copper (NY SD, 1984). Chronic exposure to low levels of copper can lead to anemia (TOXNET, 1975-86).

2.12.2 Zinc in food

The richest food sources of zinc include oysters and meat products such as beef, veal, pork and lamb (Betts and Ma, 2000). To a lesser degree it is in most beans, nuts, whole grains and sunflower seeds. Phytates, which are found in whole grain breads, cereals, legumes and other products, have been found to decrease zinc absorption (Wise, 1995). The (US) recommended daily allowance from puberty in males is 15mg and 12mg for females. A higher amount is recommended for pregnant and lactating women (Reilly *et al.*, 1983). The recommended daily allowance for men is 11mg and 8mg for women (Wardlaw, 2003).

2.12.2.1 The role of zinc

Zinc plays important roles in growth and development, the immune response, neurological function, and reproduction. Many enzymes depend on zinc for their ability to catalyze vital chemical reactions. It serves as a co-factor for dehydrogenating enzymes and in carbonic anhydrase (Holum, 1983).

Zinc stabilizes the structure of a number of proteins and cell membranes (King and Cousins 2006). Zinc helps to regulate gene expression by acting as transcription factors (binding to DNA and influencing the transcription of specific genes). Zinc also plays a role in cell signaling and has been found to influence hormone release and nerve impulse transmission (Troung-Tran *et al*., 2000). Zinc also play a role in apoptosis (gene-directed cell death), a critical cellular regulatory process with implications for growth and development, as well as a number of chronic diseases (Troung-Tran *et al*., 2000).

Zinc balances copper in the body and is essential for male reproductive activity (Nolan, 2003). Zinc is a component of retinol-binding protein, a protein necessary for transporting vitamin A in the blood. Zinc is also required for the enzyme that converts retinol (vitamin A) to retinal (Boron *et al.*, 1988; Christian and West, 1998).

2.12.2.3 Zinc toxicity

It has been reported that isolated outbreaks of acute zinc toxicity is as a result of the consumption of food or beverages contaminated with zinc released from galvanized containers. The major consequence of long-term consumption of excessive zinc is copper deficiency (FNB, 2001).

Zinc toxicity can occur in both acute and chronic forms. Acute and chronic adverse effects of high zinc intake include nausea; vomiting, loss of appetite, abdominal cramps, diarrhea, low copper status, altered iron function, reduced immune function, and reduced levels of high-density lipoproteins (FNB, 2001).

Effects of zinc deficiency

Zinc deficiency is associated with decreased release of vitamin A from the liver, which may contribute to symptoms of night blindness that are seen with zinc deficiency (Boron *et al.*, 1988; Christian and West, 1998). Chronic zinc deficient individuals show growth retardation. Studies in Children in Colorado and other developing countries have shown that zinc supplementation results in increased growth rates (Walravens *et al*., 1989; Hambridge and Krebs, 2000).

Delayed neurological and behavioral development in children also occurs as a result of zinc deficiency. It has been reported in China, India and Guatemala that zinc improved the neuro-psycologic and other functional activities in infants and children (Caufield *et al*., 1998; Sandstead *et al.*, 1998).

Zinc deficient individuals experience increase susceptibility to a variety of infections as the immune system is impaired (Shankar and Prasad, 1998).Research indicates that zinc deficiency may also potentiate the effects of toxins produced by diarrhea causing bacteria, E-coli (Wapnir, 2000). Recently, abnormal dark adaptation related to zinc deficiency in patients with cirrhosis of the liver and sickle cell disease has been reported. Zinc deficiency is known to affect testicular functions adversely in man and animals (Prasad, 1983).

The effects of excess zinc

At extremely high zinc intakes, toxicity symptoms such as nausea, vomiting, epigastric pain, lethargy, diarrhea and fatigue occur (Fischer *et al.*, 1990). Excess zinc causes induced copper deficiency with attendant symptoms of anemia and neutropenia, as well as impaired immune function and adverse effects on the ratio of low-density- lipoprotein to high-density-lipoprotein (Fosmire, 1990).

Milder gastrointestinal distress has been reported at doses of 50 to 150 mg/day of supplemental zinc. The major consequence of long-term consumption of excessive zinc is copper deficiency. Total zinc intakes of 60 mg/day (50 mg supplemental and 10 mg dietary zinc) have been found to result in signs of copper deficiency (King and Cousins, 2006).

2.12.3 Iron in food

Iron occurs in two forms in foods; heme and nonheme. Heme iron is found only in foods derived from animals, such as red meats, poultry and fish. Nonheme iron is found in both plant and animal foods. Heme iron is well absorbed by the body but most dietary iron is nonheme. It is absorbed at a relatively constant rate of about 23%. The rates of absorption of nonheme iron are lower, ranging from 2 to 20%, and are strongly influenced by dietary factors and body iron stores (Hoffman, 2009).

2.12.3.1 The role iron

Iron is a key element in the metabolism of almost all living organisms. In humans, iron is an essential component of hundreds of proteins and enzymes (Beard and Dawson, 1997; Fairbanks, 1999). Iron helps in the transport and storage of oxygen in higher animals. It helps in the transport of oxygen from the lungs to cells and assists in the return of carbon dioxide from cells to the lung for excretion (Wardlaw, 2003). Hemoglobin acquires oxygen rapidly during the short time it spends in contact with the lungs and to release oxygen as needed during its circulation through the tissues. Myoglobin functions in transport and short-term storage of oxygen in muscle cells, helping to match the supply of oxygen to the demand of working muscles (Yip and Dallman, 1996).

It also helps in transport of electrons during synthesis of ATP and critical to energy metabolism. Iron as heme-containing enzymes (Catalase and peroxidases) protect cells against the accumulation of hydrogen peroxide, a potentially damaging reactive oxygen species (ROS), by catalyzing a reaction that converts hydrogen peroxide to water and oxygen (Yip and Dallman, 1996). Ribonucleotide reductase is an iron-dependent enzyme that is required for DNA synthesis (Fairbanks, 1999). Thus, iron is required for a number of vital functions, including growth, reproduction, healing, and immune function (Suharno *et al.,* 1993; Lynch, 1997).

2.12.3.2 Iron toxicity

Potential iron toxicity occurs because very little iron is excreted from the body. This is due to the ability of iron to accumulate in body tissues and organs when normal storage sites are full (Corbett, 1995).

Effects of iron deficiency

The term iron deficiency refers to depleted body iron stores without regard to the degree of depletion or to the presence of anemia (Hoffman, 2009). Iron deficiency impairs red blood cells formation (Yip and Dallman, 1996).Insufficient iron for the synthesis of red blood cells may cause fatigue (Wardlaw, 2003). It impairs athletic performance and increase lactic acid production (Beard, 2001).

Severe iron deficiency causes brittle and concave nails, mouth sores, and taste atrophy. It also impairs wound healing (Lee, 1999; Hoffman, 2009). In general, iron deficiency causes reduced resistance to cold, inability to regulate body temperature and pica (Hoffman, 2009).

Effects of excess iron

Acute iron poisoning of excess iron causes; nausea, vomiting, abdominal pain, tarry stools, lethargy, weak and rapid pulse, low blood pressure, fever, difficulty breathing, and coma. Long-term damage to the central nervous system, liver and kidneys may occur (FNB, 2001; MDFC, 2000). Generally, an excess intake of iron may cause enlarged liver, skin pigmentation, lethargy, joint diseases, loss of body hair, amenorrhea, and impotence.

2.13 THE NON-ESSENTIAL HEAVY METALS (Lead, Cadmium and Mercury)

2.13.1 Lead in food

Lead (Pb) is a toxic trace element with no biological importance (Lenntech, 2008). It enters food through soil, air and packaging materials for the food material. The most convenient source of lead exposure in food or plants is aerial deposition (ATSDR, 1988; Wardlaw, 2003).

Lead enters into man through indigestion of contaminated food (Ferner, 2001). Contamination of foodstuff with lead has been reported. Corn flour contaminated with lead during grinding was reported to have poisoned a Greek family (Dona *et al.,* 1999).

Some pesticides used during growing of herbs and spices contaminate them (Galal-Gorchev, 1991). The use of herbs and spice for medicinal purposes has been found to contain significant amount of lead (CDCP, 1992; Bayly *et al.*, 1995).

The W.H.O has established potential tolerable weekly intake (PTWI) values for lead as well as other toxic metals. The PTWI value for lead for adult is 3.0mg or 430microgram per day (WHO, 1993). The total tolerable daily intake (TTDI) for lead in children is 6 μg per day. This may be exceeded especially in lead accumulated soils outdoor play environments (Calabrese *et al.*, 1997;

Viverette *et al.*, 1996). The PTWI established for all age groups is 25 μg per kg of body weight (WHO, 1993).

2.13.1.1 Effects of lead

One of the most prominent contaminants is lead. It is of particular concern especially for children because there is increasing evidence that relatively low concentrations of lead in the blood may greatly affect their mental development, an effect that persists into adulthood (Needleman *et al*., 1990). Studies have demonstrated that children are especially vulnerable to environmental Pb hazards (Mielke, 1999; Bearer, 2000; Dietert *et al*., 2000).

During developmental years thus 12-24 months, children are particularly prone to Pb in the environmental soil through hand to mouth behaviour (Calabresse *et al*., 1997; Manton *et al*., 2000). Women exposed to Pb during childhood subsequently pass the lead onto the foetus during pregnancy and to the neonate during nursing (Gulson *et al*., 1998). In this way, lead exposure is passed from a generation to another and the problems become intergenerational legacy of the community (Mielke *et al*., 2005).

Blood lead levels (PbB) as low as 10-100 μg/dl in adults have been associated with a wide range of adverse effects including nervous system disorders, anaemia and decreased haemoglobin synthesis, cardiovascular disease and disorders in bone metabolism, renal function and reproduction(Goyer, 1993). A relatively low exposure to Pb in children affects cognitive and behavioural development in children (Nriagu and Pacyna, 1988; Goyer, 1993).

Exposure to lead (Pb) may lead to higher risks of heart attacks and strokes in adults.

Widespread lead poisoning corresponds to intelligent quotient deficits and violent crime in children. These have implications to an array of social and psychological outcomes which may persist in adulthood (Nevin, 2000).

Other adverse health effects resulting from lead exposure include decreased heme biosynthesis, elevated hearing threshold and decreased serum levels of vitamin D. The neurotoxicity of lead is of particular concern, because neurobehavioral effects, such as impaired academic performance and deficits in motor skills, may persist even after blood lead (PbB) levels have returned to normal (Needleman, 1990).

Urban environments in general have received higher depositions of lead from vehicular emissions than rural areas (ATSDR, 1988).

Studies by Mahaffey, *et al*., (1982) indicated that nutritional deficiencies of iron or calcium, which are prevalent in children, may facilitate lead absorption and exacerbate its toxic effects.

2.13.2 Cadmium in food

Food is the main source of cadmium intake. Cadmium is more mobile in aquatic environments than most other metals and is bio-accumulative and persistent (USPHS, 1997). The continuing contamination of the environment from industrial and other sources is likely to increase the cadmium concentration in food.

Cadmium in water can influence its levels in food. Crustacea and shellfish from contaminated estuaries, and cereals irrigated with cadmium-containing water may exhibit elevated levels of this contaminant (WHO, 1972). Cereals and other leafy vegetables account for 50% of cadmium intake in which children intake range between 2-25ug/day and adults 10-50ug/day. High levels of cadmium may also be found in certain target organs, such as the liver and kidneys of mammals used for food (WHO, 1972).

Plants accumulate cadmium through some agricultural practices such as fertilizer application. The annual rate of cadmium input to arable land from phosphate fertilizers has been estimated at 5g/ha for the countries of European Economic Community (Hutton, 1982).The application of municipal sewage sludge to agricultural soils as fertilizers can also be a significant source of cadmium in food (Hutton and Symon, 1986).

More so, crop plants growing near to atmospheric sources of cadmium may contain elevated levels of cadmium (Carvalho *et al*., 1986). Smolders and McLaughlin (1996) observed that it is difficult to control cadmium concentrations in agricultural crops grown in saline soils due to the large effect of chloro-complexation on availability through plant uptake of free and complexed cadmium ions from soil solution.

2.13.2.1 Effects of cadmium

Cadmium is extremely toxic even at low levels. In humans, long term exposure results in renal dysfunction, characterized by tubular proteinuria (WHO, 1989). Cadmium is also associated with skeletal defects related to calcium loss which results in osteomalacia and osteoporosis (WHO, 1989).

Cadmium toxicity is characterized by chest pain, cough with foamy and bloody sputum, and death of the lining of the lung tissues because of excessive accumulation of watery fluids.

Depending on the severity of exposure, the symptoms of effects include nausea, vomiting, abdominal cramps, dyspnea and muscular weakness. Severe exposure may result in pulmonary odema and death. Pulmonary and renal effects may occur following sub-chronic inhalation exposure to cadmium and its compounds (Duruibe *et al.,* 2007).

2.13.3 Mercury in food

Mercury occurs naturally in the environment and is contributed to by industrial activity (FAO/WHO, 1972). There is much concern about the potential for human intoxication due to mercury in foodstuffs particularly, fish. The most serious toxic hazard arises from methylmercury residues in food (Lu *et al*., 1972). The levels found in food are higher than in treated water. The mercury in fish and foods from marine origin accounts for about 40% whereas fruits and vegetables accounts for about 20% (WHO, 1993).

The direct pollution of water by industrial sources is likely to affect fish more than other foods. Similarly, pollution caused by industrial wastes dumps will transfer mercury into the soil and water (FAO/WHO, 1972).

The discarding of electrical apparatus, paints, fluorescent lamps any other mercury containing substance on agricultural soil and near water bodies, through seepage into watercourses, may also cause contamination. The use of alkyl, alkoxyalkyl and inorganic mercurial fungicides may also be a source of contamination.

Low levels of total mercury can also occur in meat and dairy products and may include some methylmercury compounds, derived presumably from residues in feeds containing fish-meal or treated cereal grains (FAO/WHO, 1972).

A provisional tolerable weekly intake (PTWI) of 0.005 mg/kg (5 µg/kg) bodyweight (bw) have been decided for total mercury of which no more than 3.3 µg/kg body-weight should be methylmercury compounds. This is equal to 0.35 mg/week (350 µg/week) for a person weighing 70 kg (FAO/WHO, 1972). A new PTWI-value for methylmercury, corresponding to 1.6 µg/kg bw, which covers also pregnant women and their fetuses (the previous value was 3.3 μg/kg bw, and did not protect this risk group), has been developed (SCOOP, 2004). Typical dietary intakes were reported to be in the range 2-6 μg/day for children and 2-140 μg/day for adults (WHO, 1993).

2.13.3.1 Effects of mercury

Toxicological feature of mercury is reflected in the elemental, organic and inorganic forms. Methylmercury, an alkyl mercuric compound causes the greatest risk to human from dietary exposure.

Intoxication by elemental mercury or by methylmercury is revealed primarily by changes in behavior and by neurological signs. Specific sensory symptoms are also prominent in human methylmercury poisoning (Evans *et al.*, 1975).

The chemical form of mercury determines the biological effects which would occur (Kudsk, 1965). Methlymercury may induce alterations in the normal development of the brain of infants and may, at higher levels, induce neurological changes in adults. Children exposed to methyl mercury prior to birth may experience negative effects on their mental development (WHO 2000; WHO, 2003).

Major symptoms are acute pneumenitis, gastrointestinal disturbances, bloody diarrhoea and severe kidney injury with uraemia and anuria. Chronic exposure produces tremor and psychological disturbance and occasional proteinuria which may progress to nephrotic syndrome (Kazantzis *et al*., 1962; Joselow and Goldwater, 1967). Gingivitis, stomatitis, excessive salivation, occasional dermatitis, mercurialentis, non-specific fatigue, weight-loss and pallor have been reported (Bidstrup, 1964).

Chronic effects of inorganic mercury compounds include kidney damage and intestinal ulceration with haemorrhage (Bidstrup, 1964). However, chronic exposure increases urinary protein level (Joselow and Goldwater, 1967). Acute poisoning by mercury causes damage to kidneys which may be associated with intestinal symptoms, headache and weight loss (Berlin, 1986). Seizures, blindness, cerebral palsy and speech defects can occur in babies when the lactating mother is contaminated with mercury (Tsoumbrais and Tsoukali-Papadopoulou, 1994).

CHAPTER THREE

3. MATERIALS AND METHOD

3.1 Apparatus

All glassware and plastic containers used were washed with detergent solution followed by soaking in 10% (v/v) nitric acid overnight. They were rinsed with distilled water followed by 0.5 5% potassium permanganate, rinsed with distilled water and dried before use.

The atomic absorption Spectrometer (Spectr AA 220, Australia), was used for the determination of iron, copper, zinc, cadmium and lead. The Automatic Mercury Analyzer model HG-5000 (Sanso Seisakusho co., Ltd, Japan) was used for the determination of mercury.

3.2 Reagents

Analytical reagent (AnalaR) grade chemicals (BDH Chemicals Ltd., Poole. Emgland) were used throughout the study.

3.2.1 Preparation of Standard solutions

The iron, zinc, copper, cadmium and lead stock solutions of 1000ppm were prepared by dissolving 1g of pure metal in a minimum volume of 1% HNO_3 and diluted to 1L with 1% HNO_3. For Fe, 1g of Fe wire was dissolved in 50ml (1+1) HNO_3. It was diluted to 1 litre with distilled water in volumetric flasks.

Solution (100ppm) of iron, zinc, copper, cadmium and lead were prepared by pipetting 10ml each of the stock solution into 100ml volumetric flasks containing 10ml of 10% HNO_3 acid and made to the volumetric mark with distilled water.

3.2.2 Preparation of Mercury Standard Solution

Mercury stock standard solution (1000mg/L) was prepared by dissolving 0.0677g of $HgCl_2$ in 2ml HNO_3, 2ml $HCLO_4$ and 5ml H_2SO_4 in a 50ml digestion flask with heating on a hot plate at 200˚C for 30 minutes. The solution was then diluted to 50ml with distilled water.

3.2.3 Preparation of stannous chloride solution

Stannous solution (10%w/v) was prepared by dissolving 10g of hydrated salt ($SnCl_2.H_2O$) in 100ml 1M HCl. The solution was aerated with nitrogen gas at 50ml per minute for 30 minutes to expel any elemental mercury from it.

3.3 Sampling and Sample preparation

A total of thirty two (32) different powdered samples were randomly purchased from Asafo, Railway and Central markets in Kumasi, Ashanti Region of Ghana. Powdered ginger, red and green peppers were bought from farmers from the three markets. The samples were grouped into bouillon seasonings, mixed seasonings and unmixed seasonings as shown in Table 3.1, Table3.2 and Table 3.3. The samples were dried in an Astell Scientific dryer for two hours at 45˚C. They were cooled, stored in plastic bags in a dry place prior to analysis.

Table 3.1 Seasonings code, names, country of origin and ingredients in unmixed seasonings

Sample group	Sample code	Sample	Source	Ingredients
Unmixed Seasonings (UM)	UM -1	Rosemary	China (CH)	Rosemary
	UM -2	Anise	Not Available	Anise
	UM -3	Garlic	India(IND)	Garlic
	UM -4	Nutmeg	France(FR)	Nutmeg
	UM -5	Prekese	Ghana(GH)	Prekese (*tertraplura tertraptera*)
	UM -6	Senegal Pepper	Not Available	Senegal Pepper (*xylopia aethiopia)*
	UM -7	Ashanti Pepper	Mali(ML)	Ashanti Pepper (*piper guineenese)*
	UM -8	Ginger	Ghana(GH-C)	Ginger
	UM -9	Ginger	Ghana(GH-A)	Ginger
	UM -10	Ginger	Ghana(GH-R)	Ginger
	UM -11	Green Pepper	Ghana(GH-C)	Green Pepper
	UM -12	Green Pepper	Ghana(GH-A)	Green Pepper
	UM -13	Green Pepper	Ghana(GH-R)	Green Pepper
	UM -14	Red Pepper	Ghana(GH-C)	Red Pepper
	UM -15	Red Pepper	Ghana(GH-A)	Red Pepper
	UM -16	Red Pepper	Ghana(GH-R)	Red Pepper

Table 3.2 Seasonings code, names, country of origin and ingredients in mixed seasonings

Sample group	Sample code	Sample	Source	Ingredients
Mixed Seasoning (M)	M- 1	Adobo(Red)	United States of America (USA)	Salt, garlic, onion, oregano, paprika, disodium guanylate, disodium inosinate, Coriander, cardamom
	M- 2	Adobo(Yellow)	United States of America (USA)	Salt, spices, dehydrated garlic, dehydrated onion, tricalcium phosphate, disodium guanylate, disodium inosinate.
	M- 3/M-5	Curry	France(FR)	Tumeric, mustard, cumin, salt, fennel, fenugreek, onion, coriander
	M- 4	Curry	Ghana(GH)/Nigeria(NIG)	Tumeric, ginger, fennel, coriander, salt, fenugreek, nutmeg, cumin
	M-6	Curry	South Africa(SA)	Spices, wheat cereal

Table 3.3 Seasonings code, names, country of origin and ingredients in bouillon seasonings

Sample	Sample code	Sample	Source	Ingredients
Bouillon Seasoning (BC)	BC-1	Chicken Seasoning	Senegal (SG)	Salt, hydrogenated palm oil, monosodium glutamate, disodium inosinate, wheat flour, chicken fat(BHA Propyl Gallate), sugar, autolysed yeast extract, dehydrated vegetables (parsley, onions), spices flavor, cotton seed, soy oil
	BC-2	Onion Seasoning	Cote D'ivoire (CTD)	Iodised salt, goat extract, glutamate, inosinate, amidon, disodium guanylate, sugar,spices,onion, colorant (E150 c, E124) spices extracts, matiere grasse vegetale, soy lecithin
	BC-3	Beef Seasoning	Cote D'ivoire (CTD)	Salt iodised, goat extract, iosinate, monosodium glutamate, sugar, vegetable oil, natural flavor, caramel colour, onion spices, disodium inosinate,bdisodium guanylate, beef meat, tumeric
	BC-4/BC-5	Beef/Chicken Seasoning	South Africa (S.A)	Iodised salt, monosodium glutamate, cornflour, sugar, disodium inosinate, colorant, flavouring, herbs and spices, hydrolysed vegetable protein
	BC-6	Shrimp Seasoning	Turkey (TUK)	Salt, monosodium glutamate, corn starch, sugar, disodium inosinate/ disodium guanylate , monosodium glutamate, vegetable oil,, yeast extract, shrimp powder, shrimp flavor,spices, colour (caramel, E150d),antioxidant synergist (citric acid)
	BC-7	Shrimp Seasoning	Ghana (GH)	Edible starch, monosodium glutamate, sugar, palm fat, shrimp, powder spices, tomatoe red (colourant), iodated salt
	BC-8	Soup/Stew Seasoning	Ghana (GH)	Iodated salt, flavor enhancer (E621, E627,E631), corn starch, sugar, spices and spices extract (celery,fenugreek, tumeric), vegetable fats, hydrolysed vegetable protein, beef flavour, colourants (E104, E110) citric acid, anticaking agents (E551)
	BC-9	Stew spice	Ghana (GH)	Iodated salt, flavor enhancer (E621, E627,E631), corn starch, sugar, spices and spices extract (celery, coriander), vegetable fats, hydrolysed vegetable protein flavours (tomatoes, onion, pepper), colourants (E104, E110, E124) citric acid, anticaking agents (E551)
	BC-10	Shrimp Seasoning	Ghana (GH)	Iodised salt, favour enhancers(E621, E627,E631), sugar, spices and spice extracts, shrimp powder, colourants(E110, E124), anti-caking agents (E551

3.3.1 Acid digestion

About 1g of each powdered sample was weighed into 50ml digestion tubes and 1ml H_2O added followed by 2ml HCl, 5ml HNO_3: $HCLO_4$ (1:1) and 2ml H_2SO_4.

Digestion tubes and contents were heated in digestion blocks at 200°C on Clifton hotplate (Model 67891, England) until clear solutions were obtained. The digests were cooled and filtered into 50ml volumetric flask. The digests were made to the mark by adding distilled water. Digestion was done in triplicate. Blanks were also prepared alongside.

The digests were subjected to Flame Atomic Absorption Spectrometer (Spectr AA 220, Australia), for the determination of Fe, Zn, Cu, Cd and Pb. The Automatic Mercury Analyzer model HG-5000 was used for Hg.

3.3.2 Determination of mercury

Determination of mercury in all the digests was carried out by Cold Vapour Atomic Absorption Spectrometry using an Automatic Mercury Analyzer model HG-5000 (Sanso Seisakusho co., Ltd, Japan) developed at National Institute for Minamata Disease (NIMD). The analyzer is an instrument designed specifically for the measurement of mercury using the cold vapour technique. During the determination, a known volume of the sample solution 5 ml was introduced into the reaction vessel using a micropipette (1-5 ml). The reaction vessel was immediately stoppered tightly and 0.5 ml of 10% (w/v) $SnCl_2.2H_2O$ in 1 M HCl was added from a dispenser for the reduction reaction. After 30 seconds, the four-way stopcock was rotated through 90^0 and the mercury vapour was swept into the absorption cell. Response was recorded on the strip chart recorder as very sharp peaks.

Concentration of mercury in samples was calculated by the formula below:

$$\text{Conc of Hg (mg/kg)} = \frac{\textit{Peak height of samples} - \textit{Peak height of blank}}{\textit{Peak height of standard} - \textit{Peak h eigtht of blank}} \times \frac{\textit{concentration of standard}}{\textit{weight of sample}}$$

3.4 Determination of Fe, Zn, Cu, Cd and Pb

The Flame Atomic Absorption Spectrometer (Spectr AA 220, Australia), was used for the determination of Fe, Zn, Cu, Cd and Pb. Standards and blank were aspirated into the flame for atomization using capillary tube. Lamp characteristic of the metal to be analysed was fixed. The response was measured after selecting the specific wavelength of light which was absorbed by the sample been analysed. The digests were also aspirated into the flame for atomization. The lamps were changed according to the metal being analysed. The concentrations of the analyte were determined from the calibration curve.

Concentration in mg/kg was calculated by the formula below:

$$\text{Concentration of metal (mg/kg)} = \frac{\textit{concentration of metal}\left(\frac{mg}{l}\right) \times \textit{volumeof digest}(L)}{\textit{weight of sample}(kg)}$$

3.5 Quality Assurance

The accuracy of the analytical methods was evaluated by analyzing a certified reference material, CRM (dogfish muscle, DORM-2). An amount of 0.5g and 1g of the CRM were weighed into digestion flasks and each sample was taken through the same digestion procedure as the seasoning samples. The digests were then analyzed for cadmium, lead, mercury, zinc, copper and iron.

3.6 Phytochemical screening of seasonings

3.6.1 Test for saponins

To 2g of each sample, 10ml of distilled water was added and boiled for 3-5 minutes. Samples were then filtered hot and the filtrates shaken vigorously. Formation of froth which persisted for a while was indicative of saponins (Egwaikhide and Gimba, 2007).

3.6.2 Test for flavonoids

Alcoholic solution of each sample was made and a small amount of magnesium ribbon followed by concentrated HCl drop wise, were added. A Brick red colouration was indicative of flavonoids (Patel1 et al., 2011).

3.6.3 Test for carotenoids

About 5g of each sample was extracted with ethanol. To 2ml of the extract, 3ml of antimony trichloride solution was added. A Dark blue colouration was indicative of carotenoids (Chem 369, KNUST Laboratory manual, 2009).

3.6.4 Test for coumarins

A small amount of each sample was moistened with water in a test tube. Each test tube was covered with a piece of filter paper moistened with dilute NaOH solution and placed in hot water bath. After 15minutes, the filter paper was removed and exposed to U.V light. Yellow green fluorescence indicated coumarins (Feigl, H. (1960).

3.6.5 Test for alkaloids

About 10ml of 1% HCl was added to 5g of each sample and left to stand for about 30 minutes with occasionally swirling. Solution was filtered and to 2ml of the filtrate, two drops of saturated aqueous solution of picric acid was added. The precipitates are indicative of the presence of alkaloids (Peach K and Tracey MV, 1995).

3.6.6 Test for terpenoids

A small amount of each sample was extracted with ethanol and evaporated to dryness in crucible. The extracts were re-dissolved in chloroform and few drops of 10% acetic anhydride followed by two drops of concentrated H_2SO_4 were added. A Reddish pink colouration is indicative of terpenoids (Patel1 et al., 2011).

3.6.7 Test for anthraquinones

A small amount of each sample was boiled with 25ml of 0.5M KOH and 4ml of concentrated H_2O_2. The mixture was cooled, filtered and acidified with a few drops of concentrated acetic acid. The acidified mixture was extracted with 15ml of benzene. The benzene extract were shaken with a small amount of concentrated NH_4OH. A Red colouration indicates the presence of anthraquinones (Chem 369, KNUST Laboratory manual, 2009).

3.7 Statistical analysis

Pearson's correlation coefficients were used to determine any correlation between the metals in the groups of seasoning, using the SPSS statistical package program version 16, 2007 and Microsoft Excel, 2007.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

The validity of the procedure was proved by the agreement between the certified and the determined values of the Certified Reference Materials as shown in Table 4.1 below.

Table 4.1 Reliability of results

Metal	Certified reference material (mg/kg)	Values obtained (mg/kg)
Fe	146	147.5
Zn	67.1	66.7
Cu	3.28	3.46
Cd	0.189	0.192
Pb	0.12	0.128

4.1 Heavy Metals in seasonings

Iron, zinc, copper, cadmium, mercury and lead levels are presented in three groups of seasonings namely unmixed (UM), mixed (M) and bouillon seasonings (BC). The majority of the seasonings were from Ghana. The mean (±standard deviation) and range of the concentration of metals in the various groups of seasonings are presented in Appendices 1, 2 and 3. The results are means of three replicates.

The levels of iron, zinc, copper and lead in unmixed seasonings are presented in Appendix 1. The iron, zinc, copper, cadmium and lead ranged from 19.4mg/kg to 971.40mg/kg, 2.40mg/kg to 34.60mg/kg, 0.9mg/kg to 10.10mg/kg, 0.6mg/kg to 1.8mg/kg respectively. Mercury was non-detectable for all samples analysed. The highest cadmium concentration in unmixed seasoning was 0.9mg/kg in Nutmeg (UM-4) while the rest of unmixed seasonings had no cadmium present.

Mixed seasonings had levels of iron ranging from 83.36mg/kg to 480.82mg/kg and zinc was from 1.72mg/kg to 26.78mg/kg (Appendix 2). That for copper was from 1.73mg/kg to 7.70mg/kg whereas lead, ranged from 0.63mg/kg to 1.39mg/kg. Cadmium concentration was highest in Adobo (red) M-1, (0.06mg/kg).

The levels of iron, zinc, copper and lead ranged from 9.66mg/kg to 52.45mg/kg, 0.83mg/kg to 8.93mg/kg, 0.66mg/kg to 3.59mg/kg and 0.37mg/kg to 2.18mg/kg respectively for bouillon cubes (Appendix.3). Onion, beef, shrimp and stew seasonings (BC-2, BC-4, BC-6, BC-9 and BC-10) had the highest concentration of cadmium (0.05mg/kg).

4.2 Iron (Fe) levels in unmixed, mixed and bouillon seasonings

The concentration of iron in unmixed seasonings in Figure 4.1 was between 19.4mg/kg and 971.40mg/kg. Red pepper purchased from the three markets showed varied concentrations. Red pepper concentration (UM- 14) from the Central market was almost three times that of Red pepper (UM-16) from Railway market whereas that from Asafo Red pepper (UM-15) was almost two times that of UM- 16. A wide variation was found in Fe concentration in Green pepper. Green pepper (UM-12) from Asafo had highest Fe level (226.2mg/kg) followed by Green pepper (UM-11) from Central market (94.3mg/kg) and Green pepper (UM-13) from the Railway market (10.59mg/kg). Ginger from Central market (UM-8) had the highest level of Fe (698.3mg/kg) whereas Ginger from Asafo market (UM-9) had the lowest Fe concentration of 408.4mg/kg. This suggests that Red pepper, Green pepper and Ginger samples from the three markets may come from different sources.

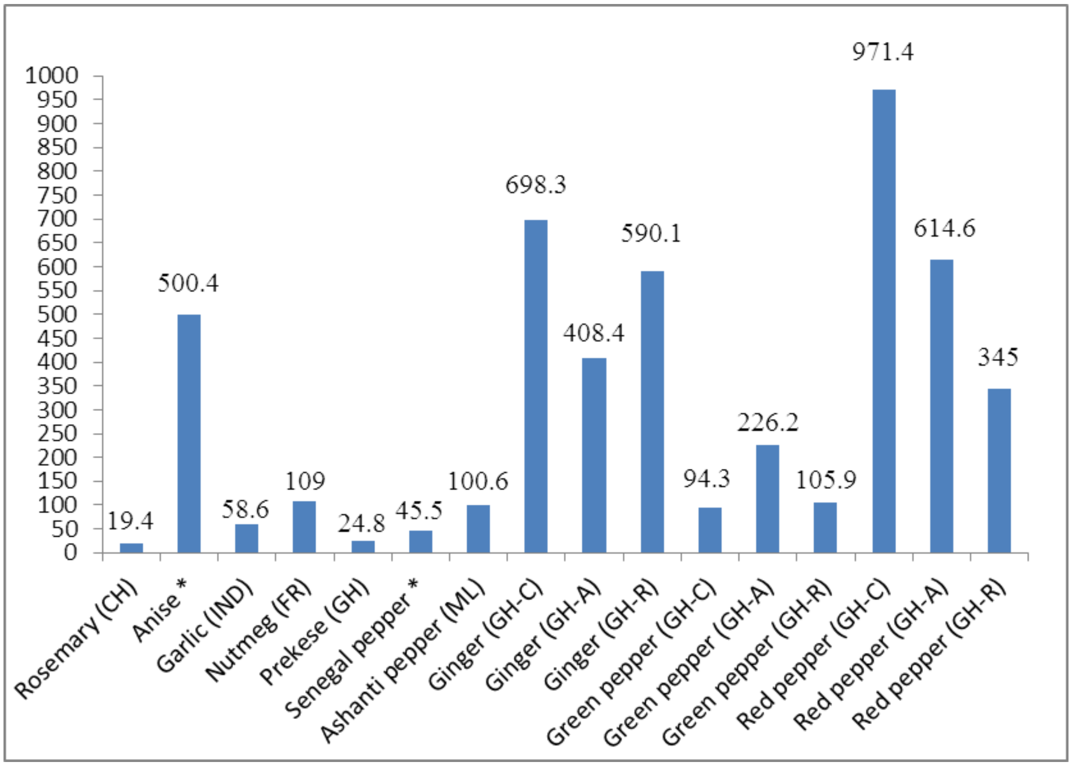

Figure 4.1: Concentration of Fe (mg/kg) in unmixed seasonings

The concentration of Fe in mixed spices ranged from 83.36mg/kg to 480.82.mg/kg with Adobo (yellow) (M-2) recording the least while Curry (M-4) had the highest (Figure 4.2). Four different brands of Curry were analysed. Curry (M-4) from Ghana had the highest value of Fe (480.82mg/kg) whiles Curry (M-5) from Nigeria had the lowest value of 149.13mg/kg. This suggests that Curry powder produced in Ghana maybe a rich source of Fe.

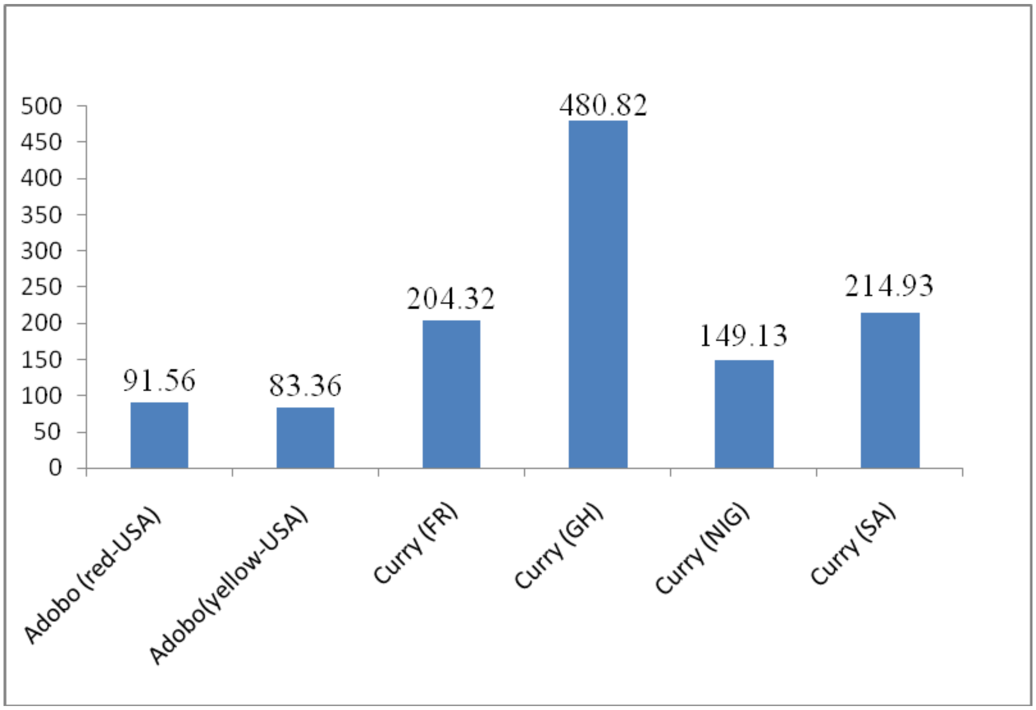

Figure 4.2: concentration of Fe (mg/kg) in mixed seasonings

The bouillon cubes had levels of iron between 9.66mg/kg in Stew spice (BC-9) Ghana and 52.45mg/kg in Chicken seasoning (BC-5) from South Africa (Figure 4.3). Shrimp bouillon from (TUK) Turkey (BC- 6) was higher in Fe concentration as compared to shrimp bouillons (BC- 10 and BC-7) from Ghana (GH). Beef bouillon (BC-3) from Cote d'Ivoire (CTD) was lower in Fe than beef bouillon from (S.A) South Africa (BC-4). Chicken and Beef seasoning from S.A and Shrimp seasoning from TUK may therefore be a good source of Fe.

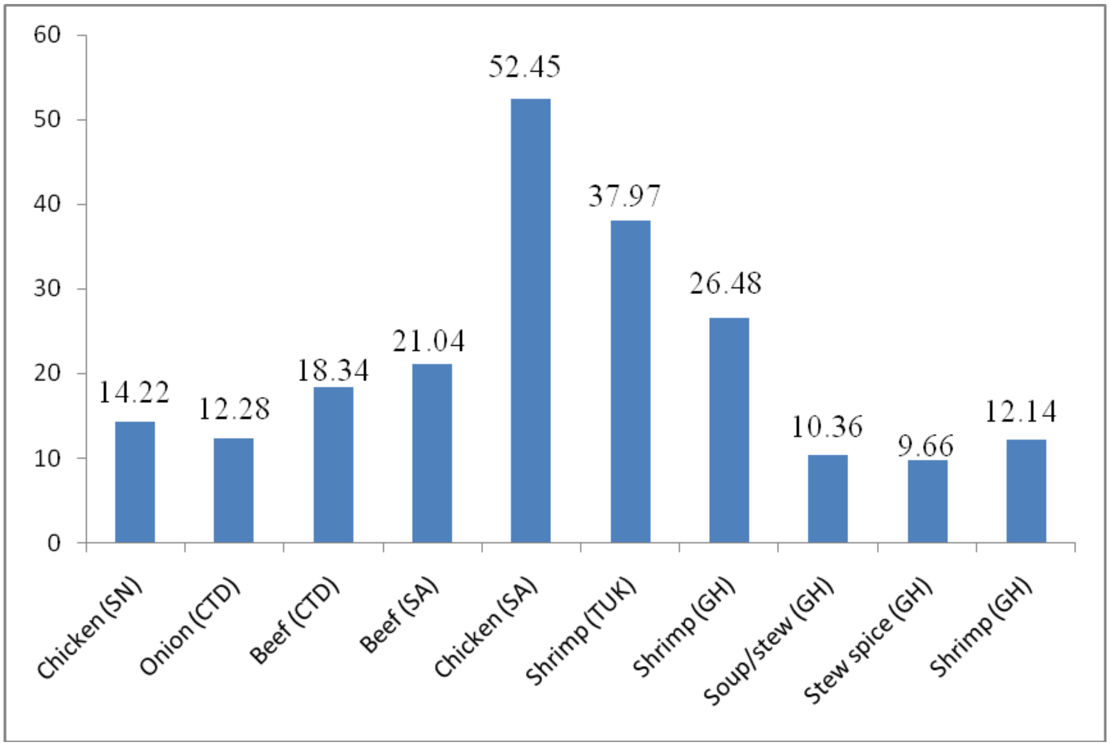

Figure 4.3: concentration of Fe (mg/kg) in bouillons

Rosemary (UM-1) had lower level of iron whereas in Ginger, levels were higher than those reported by Divrikli *et al*., 2006, Ozkutlu *et al*., 2006 as well as Koc and Sar, 2009. Levels of iron in Ginger (UM-8, UM- 9 and UM-10) and Garlic (UM-3) were also higher than levels reported by Hashmi *et. al*.,(2007). Generally, the levels of iron in unmixed seasonings were higher than mixed seasonings. The unmixed seasonings are mostly made up of combination of the same plants parts. The higher Fe content in unmixed seasonings could therefore be due to combination of the same plants parts (Nnorom *et al.*, 2007) produced from different places under different conditions. This is because sellers purchase the same plants parts such as fruits or leaves from different sources, mixed them and mill them into a single homogeneous product. The high levels of iron in seasonings could also be due to contamination during milling. Research indicates that commercial grinding of spices contaminates them to about between 3 and 5 folds, due to wear and tear of the machine parts (Janitha *et al*, 1988).

The levels of iron in the bouillon cubes were higher than reported by Akpanyung (2005). Fortification of food with iron is a means of combating iron deficiency (Blum, 1997). Bouillon cubes have been suggested as a fortification vehicle to combat micronutrients deficiency such as iron. Data has shown that bouillon cubes are widely used in many Ghanaian homes (LRC, 2006). The presence of Fe in the bouillon seasonings could be due to the added spices and herbs which are plant derived products.

The high levels of iron in all the seasonings could be due to the presence of iron which occurs naturally in plants in the form of metalloproteins, plant ferritins and the fact that iron forms part of the structural component of plants(Lynch, 1997).

Iron helps in the transport of oxygen from the lungs to cells and also is required for a number of vital functions, including growth, reproduction, healing, and immune function in the body (Lynch, 1997; Suharno *et al.,* 1993; Wardlaw, 2003).

Even though Fe is an essential element needed by the body, consumption of an excessive amount can lead to health effects. To safeguard the health of the public organization such as Nutrigold Technical (2007) and FAO/WHO have establish tolerable levels known as the Recommended Daily Allowance (RDA) and Provisional Tolerable Weekly Intake (PTWI) for most elements.

In order not to exceed the RDA of 14.8mg/day of Fe for a 60kg body weight, a person must not consume 762.89g of rosemary daily. The maximum amount of seasonings in grams (g) which when consumed will not exceed the RDA are reported in Table 4.2. However, it is unlikely for a person to consume such amounts in a day therefore Fe toxicity is insignificant.

Table 4.2 Maximum amount of seasoning intake in order to be below daily intakes of Fe, Zn, Cu, Cd and Pb

SAMPLE	SAMPLE CODE	SOURCE	Maximum amount of seasoning in grams(g) which when consumed will not exceed the RDA of Fe, Zn, Cu, Cd and Pb for a 60 kg body weight (bw)				
			Fe (RDA:14.8mg/day)	Zn (RDA:15mg/day)	Cu (RDA:2mg/day)	Cd (PTWI:7.5µg/kg bw)	Pb (PTWI: 25µg/kg bw)
Rosemary	UM-1	CH	762.89	5769.23	2,222.22	-	145.71
Anise	UM-2	*	29.58	433.53	215.05	-	205.71
Garlic	UM-3	IND	252.56	1006.71	625	-	214.29
Nutmeg	UM-4	FR	135.78	1388.89	198.02	68.57	197.14
Prekese	UM-5	GH	596.77	6250	689.66	-	214.29
Senegal pepper	UM-6	*	325.27	2941.18	363.63	-	308.57
Ashanti pepper	UM-7	ML	147.12	842.7	444.44	-	360.00
Ginger	UM-8	GH	21.19	852.27	425.53	-	120.00
Ginger	UM-9	GH	36.24	1063.83	540.54	-	240.00
Ginger	UM-10	GH	25.08	1271.2	1,333.33	-	133.93
Green pepper	UM-11	GH	156.94	773.2	1,538.46	-	153.06
Green pepper	UM-12	GH	65.42	887.57	1,428.57	-	197.14
Green pepper	UM-13	GH	139.75	974.03	298.51	-	240.00
Red pepper	UM-14	GH	15.23	1006.71	277.78	-	153.06
Red pepper	UM-15	GH	24.08	1578.94	259.74	-	214.29
Red pepper	UM-16	GH	42.78	2027.07	1,104.97	-	153.06
Adobo(red)	M-1	U.S.A	16.16	8720	1,156.07	1071.43	154.29
Adobo(yellow)	M-2	U.S.A	177.54	5434.78	346.62	1285.71	198.29
Curry	M-3	FR	72.43	848.4	312.99	6428.57	231.43
Curry	M-4	GH	30.78	1097.29	259.74	3214.29	197.14
Curry	M-5	NIG	99.24	1081.47	312.70	-	267.86
Curry	M-6	S.A	68.86	560.12	312.99	-	342.86

Table 4.2 Cont'd Maximum amount of seasoning intake in order to be below daily intakes of Fe, Zn, Cu, Cd and Pb

SAMPLE	Sample CODE	SOURCE	Maximum amount of seasoning in grams(g) which when consumed will not exceed the RDA of Fe, Zn, Cu, Cd and Pb for a 60 kg body weight (bw)				
			Fe (RDA:14.8mg/day)	Zn (RDA:15mg/day)	Cu (RDA:2mg/day)	Cd (PTWI:7.5µg/kgbw)	Pb (PTWI:25µg/kgbw)
Chicken seasoning	BC-1	SG	960.81	11,111.11	3,030.30	2,142.86	264.57
Onion seasoning	BC-2	CTD	829.13	1,807.2	2,564.10	1,285.71	98.30
Beef seasoning	BC-3	CTD	808.93	6,666.67	2,631.58	1,607.14	181.21
Beef seasoning	BC-4	S.A	703.42	6,224.07	1,709.40	1,285.71	113.98
Chicken seasoning	BC-5	S.A	282.17	1,679.73	557.10	-	170.07
Shrimp seasoning	BC-6	TUK	389.78	8,333.33	1,769.91	1,285.71	200.27
Shrimp seasoning	BC-7	GH	558.9	9,259.26	1,709.40	2,142.86	100.60
Soup/stew seasoning	BC-8	GH	700	7,425.74	1,600.00	1,607.14	148.80
Stew spice	BC-9	GH	652.7	11,627.91	2,127.66	1,285.71	579.14
Shrimp seasoning	BC-10	GH	820.27	16,666.67	1,162.79	1,285.71	180.07

4.3 Zinc (Zn) levels in unmixed, mixed and bouillon seasonings

Unmixed seasonings had zinc content between 2.40mg/kg and 34.60mg/kg (Figure 4.4). Anise (UM-2) recorded the highest amount of Zn whereas *Prekese* (UM-5) recorded the lowest. Ginger (UM-8) from Central market had the highest level of Zn (17.6mg/kg) whereas Ginger (UM-10) from Railway market had the lowest Zn concentration of 11.8mg/kg. The Green pepper from the various markets, showed a wide variation in Zn concentration. Green pepper (UM-11) from Central market had highest Zn content whereas Green pepper (UM-13) from Railway market had the lowest amount of Zn (Figure 4.4). Zinc level in Red pepper (UM-14), purchased from Central market was two times the concentration of Red pepper (UM-16) from Railway market. The differences in the concentration of Zn in the Green pepper, Red pepper and Ginger from the various markets suggest that the seasonings may come from different sources.

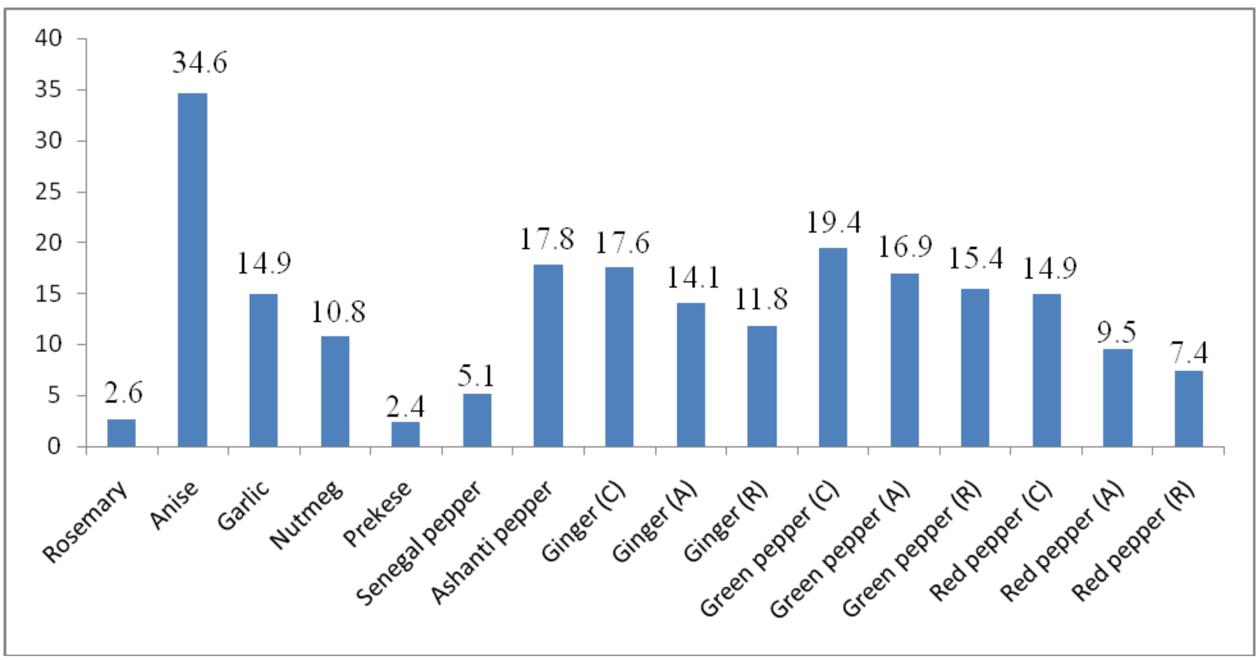

Figure 4.4: Concentration of Zn (mg/kg) in unmixed seasoning

In Figure 4.5, Zn levels in mixed seasonings was between 1.72mg/kg and 26.78mg/kg with Adobo(red) from U.S.A recording the least while Curry seasonings from S.A the highest. The four different brands of Curry analysed showed the Curry (M-3) from France showing the highest Zn content whereas Curry (M4) from Ghana had the least. Zn content in Curry seasonings from Ghana and Nigeria were very close. The levels of Zn in Curry seasonings was in the order Ghana < Nigeria < France < South Africa. This suggests that S.A Curry may be a rich source of Zn.

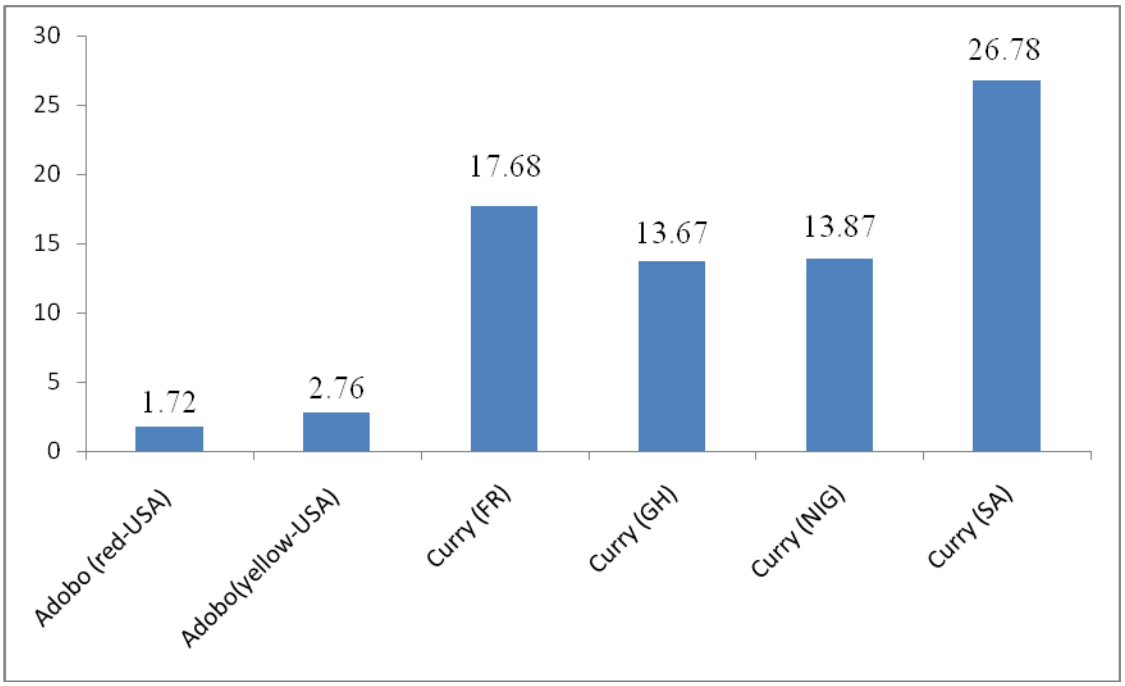

Figure 4.5: Concentration of Zn (mg/kg) in mixed seasonings

The zinc content in bouillon cubes was between 0.83mg/kg in Onion seasoning (BC-2) and 8.93mg/kg in Chicken seasoning (BC-5) as shown in Figure 4.6. The levels of Zn were relatively high as compared to other studies e.g. Zinc concentrations in bouillon cubes were lower than values reported in Nigeria (Akpanyung, 2005). With the exception of Chicken seasoning (BC-5), Zn concentrations were in a lower range; 0.83mg/kg to 2.41mg/kg as compared to 1.6mg/kg to

4.40mg/kg reported by Nnorom *et al.,* (2007). Chicken seasoning from S.A may be considered a good source of Zn.

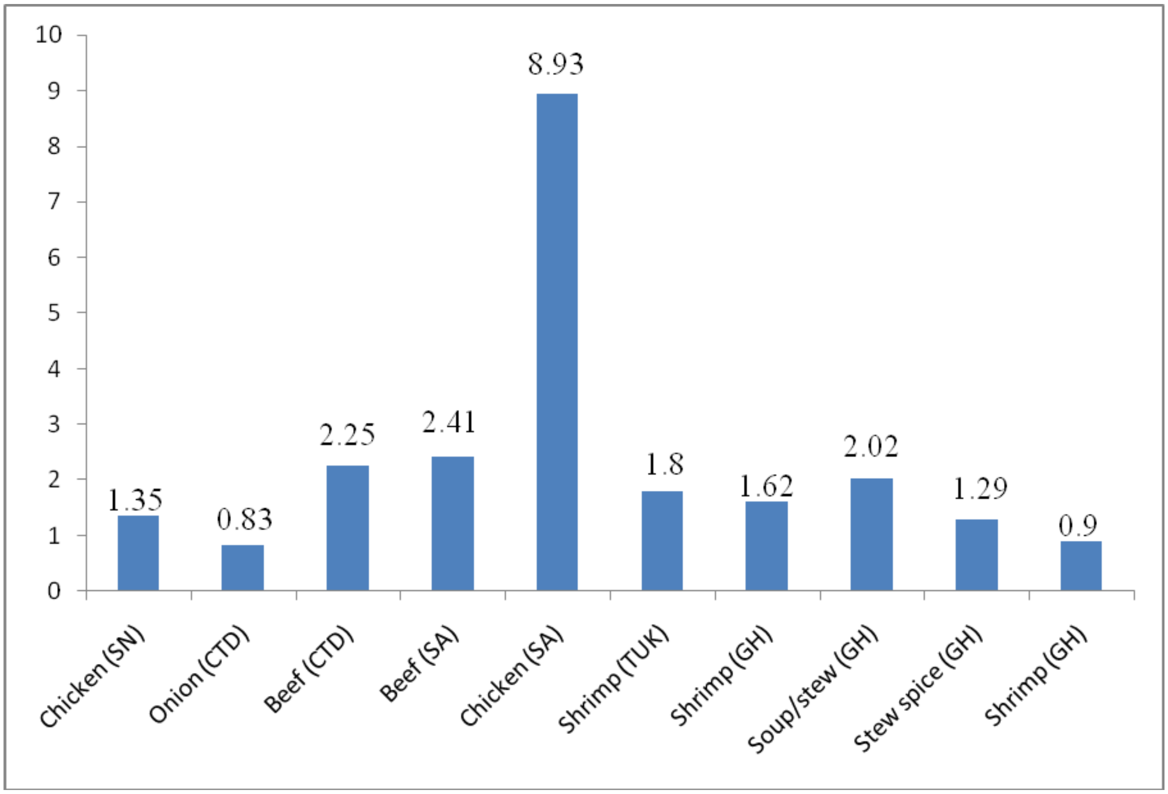

Figure 4.6: Concentration of Zn (mg/kg) in Bouillon

Ginger (UM-8, UM-9 and UM-10) had concentrations of Zn higher than that reported by Ozkutlu *et al*., (2006) and Hashmi *et. al.,* (2007). Rosemary (UM-1) had lower Zn level compared to other studies (Divrikli *et al*., 2006; Koc and Sar, 2009). Garlic (UM-5) had higher Zn content compared to reported levels by Hashmi *et. al.*,(2007). Green pepper (UM-11) from Central market recorded the highest Zn content while Green pepper from Railway market (UM-13) the least. Red pepper recorded a range of 7.4mg/kg to 19.4mg/kg as compared to 10.4mg/kg to 35mg/kg in Nigeria (Awode *et al.,* 2008). The levels of Zn in Curry seasonings in this study ranged from 13.87mg/kg to 26.78mg/kg as compared to 13.65mg/kg - 29.90mg/kg by Nnorom *et al.,* (2007).

Zinc levels in all samples were lower than the standard level (100mg/kg) set by FAO/WHO (2003). The differences in Zn content from same plants parts purchased from the various markets could be due to the type of soil for cultivation and the drying environment. The high levels of zinc in the seasonings reflect the normal composition expected in plant derived products (Onianwa *et. al.,* 1999). Research indicates that micronutrients are generally higher in leaves than in other above ground parts such as fruits in plants (Basgel and Erdenmoglu, 2005). However, Zn in Rosemary, a leafy spice, was quite low. This may be due to low Zinc content in the soil used for cultivation.

Zinc plays important roles in growth and development in humans (Colak *et al.,* 2005). Zinc deficiency is of growing concern in the developing world because consumption of plants food has inhibitory effect on zinc absorption (Divrikli *et al.,* 2006). It is reported by Hotz and Brown (2004) that an estimated 20% of the world population was at risk of inadequate zinc intake. Delayed neurological and behavioral development in children occurs as a result of zinc deficiency (Caufield *et al.,* 1998). Zinc deficiency impairs the immune system of a person making him to be susceptible to infections (Shankar and Prasad, 1998).

In order not to exceed the RDA of 15mg/day of Fe for a 60kg body weight, a person must not consume more than 8720g of Adobo (red) daily. An amount in excess of this may have harmful effects in person. The maximum amount of seasonings in grams (g) which when consumed will not exceed the RDA are reported in Table 4.2. However, it is unlikely for a person to consume such amounts in a day therefore an exposure to toxic levels of Zn from consumption of the seasonings is negligible.

4.4 Copper (Cu) levels in unmixed, mixed and bouillon seasonings

The concentration of copper in unmixed seasonings ranged from 0.9mg/kg to 10.10mg/kg (Figure 4.7). Rosemary (UM-1) recorded the lowest Cu concentration whiles Nutmeg (UM-4) recorded the highest. The next highest Cu level was found in Anise (UM-2) followed by Red pepper from Railway market, Asafo market and Central in that order.

Ginger (UM-9 and UM-10) from Asafo and Railway markets recorded the same values for Cu. The concentrations of Cu in Green pepper from all three markets were very close. This suggests that though samples differ in localities, their Copper content maybe influenced by the same factors such as soil type.

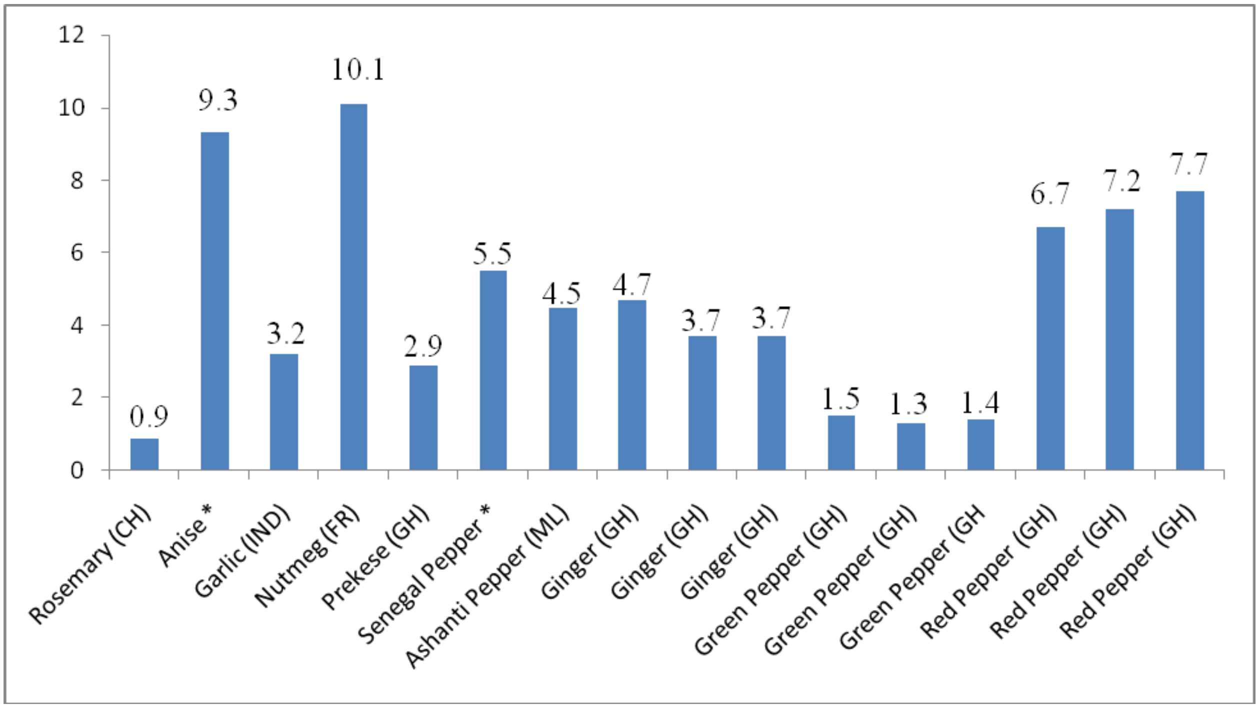

Figure 4.7: Concentration of Cu (mg/kg) in unmixed seasonings

The copper level in mixed seasonings was between 1.73mg/kg and 7.70mg/kg (Figure 4.8). The lowest level was found in Adobo (yellow) (M-1) from U.S.A and the highest in Curry (M-4) from Ghana. Copper in the four brands of Curry was highest in Curry from Ghana followed by Nigeria, South Africa and France in that order. The level of Cu in Adobo (red) (M-1) and Adobo (yellow) (M-2) both from U.S.A, was similar. This suggests that they may come from the same source. Though the highest content of copper was found in unmixed spice, copper content in mixed spices were generally higher than the unmixed spices. This could be attributed to the combination of the different spices evident in mixed seasonings.

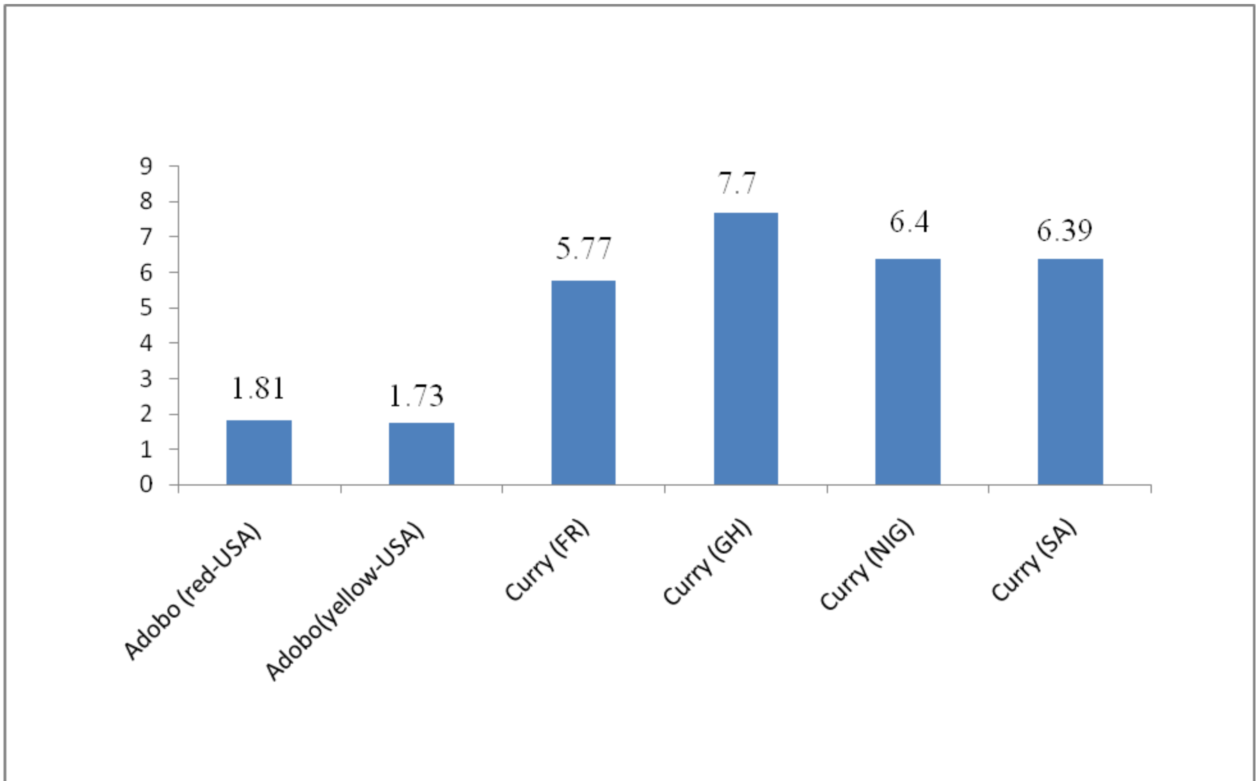

Figure 4.8: Concentration of Cu (mg/kg) in mixed seasonings

In the bouillon seasonings, Chicken seasoning (BC-1) recorded the lowest Cu concentration of 0.66mg/kg while Chicken seasoning (BC-5) recorded the highest concentration of 3.59mg/kg (Figure 4.9). This discrepancy could be due to differences in processing methods and also their different origins. Shrimp seasonings (BC-7 and BC-10) from Ghana differed in Cu concentrations. This may be attributed to the fact that they are produced by different companies and hence different processing methods. The Cu content in Beef seasoning (BC-4) from S.A was about two times that from Cote d'Ivoire. Chicken seasoning (BC-5) from S.A could be said to be rich in Cu as compared to Chicken seasoning (BC-1) from Senegal. It was about five times higher than the concentration of Cu in Chicken seasoning (BC-1).

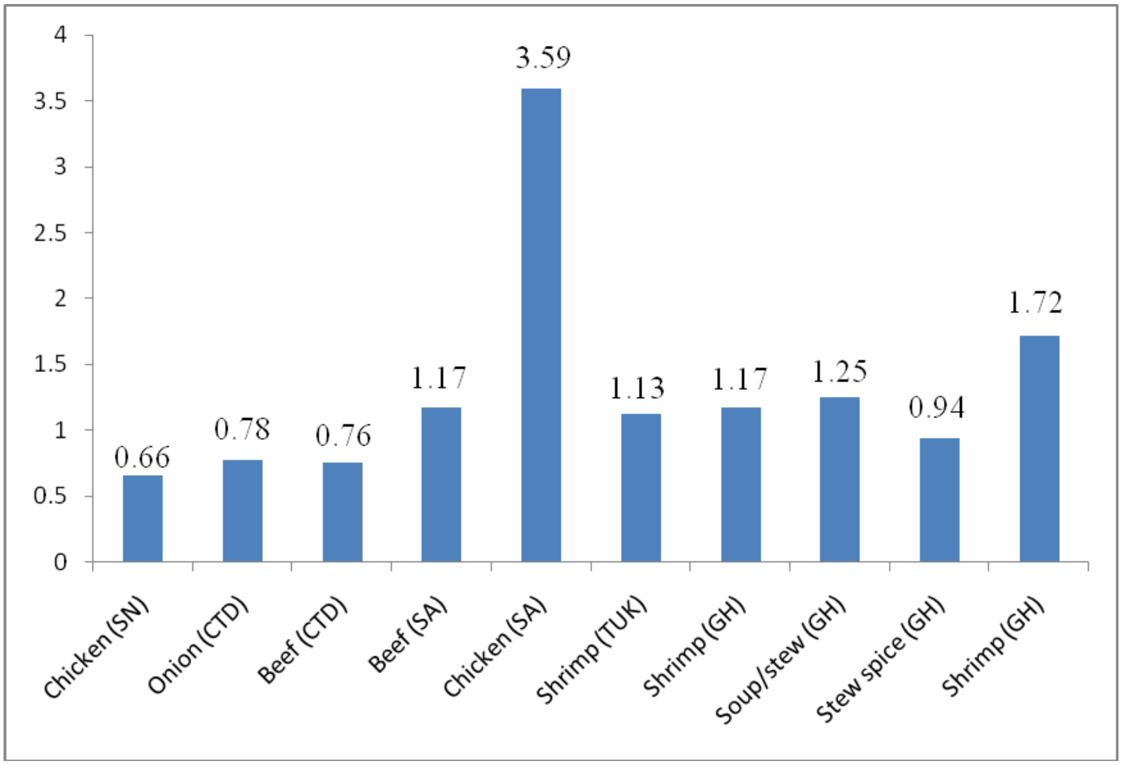

Figure 4.9: Concentration of Cu (mg/kg) in bouillons

Rosemary (UM-1) had a lower level of copper as compared to other studies (Ozcan, 2004; Divrikli *et al*., 2006; Koc and Sar, 2009). Ginger (UM-8, UM-9 and UM-10) had higher levels of copper compared to values reported in other literature (Ozkutlu *et al*., 2006; Hashmi *et. al.*, 2007; Krejpcio *et al.*, 2007). The level of copper in Garlic (UM-3) was also higher than that reported by Hashmi *et. al.,* (2007). Level of Cu in Nutmeg (UM-4), was 10.10mg/kg in this study which was higher than 7.28mg/kg reported by Krejpcio *et al.,* (2007). The level of Cu in all groups of seasonings was below the permissible level of 10mg/kg (FAO/WHO, 2003) in plants.

Copper helps in iron metabolism by helping in oxygen transport as well as utilization and absorption of iron (Özçelik *et al.,* 2002). It is also necessary for the growth, development, and maintenance of bone, connective tissue, brain, heart and many other body organs (FAO/WHO, 1970). However, in excess it becomes toxic and result in anemia with severe mucosal irritation and corrosion, capillary damage, hepatic and renal damage, CNS irritation and depression (Browning, 1969; TOXNET, 1975-86).

Considering the maximum amount of daily intakes in Table 4.2 the amount obtained for the various seasonings are unlikely to be consumed. This indicates that Cu toxicity is unlikely to occur from the consumption of the seasonings.

4.5 Cadmium (Cd) levels in unmixed, mixed and bouillon seasonings

Cadmium levels in unmixed seasonings ranged from ND to 0.9mg/kg (Figure 4.10). Nutmeg (UM-4) was found to contain the highest Cd content whereas the rest of the seasonings were non- detectable.

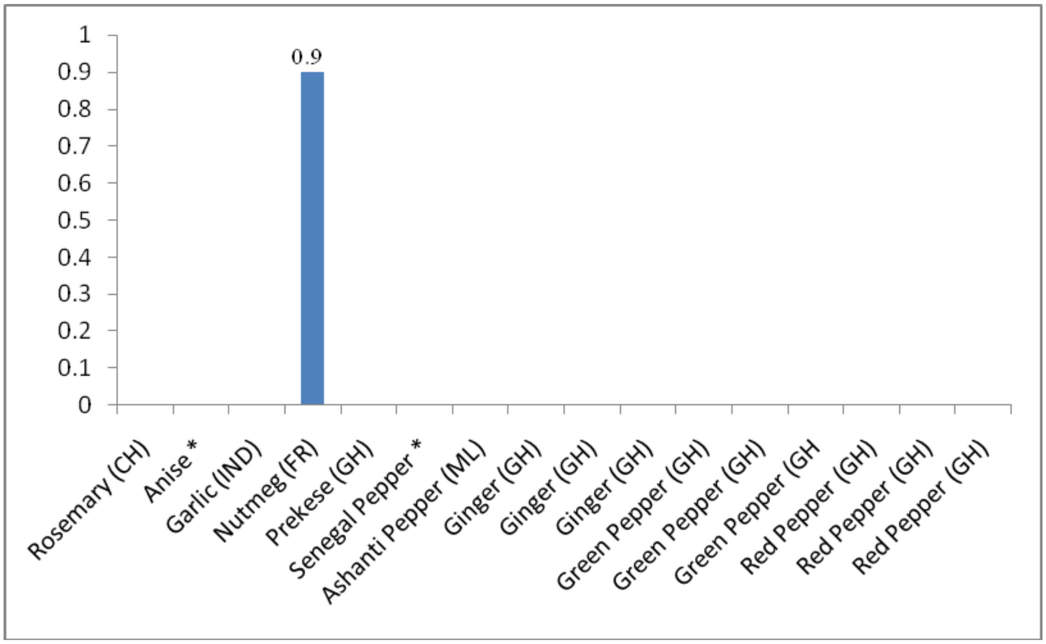

Figure 4.10: Concentration of Cd (mg/kg) in unmixed seasonings

In Figure 4.11, the cadmium levels in mixed seasonings ranged from ND to 0.06mg/kg. Adobo (red) (M-1) recorded the highest Cd content. Two of the four brands of Curry (M-5 and M-6) from Nigeria and South Africa were found to contain no Cd. However, the highest Cd level in the Curry samples was found in M-4 from Ghana though below the permissible limit (0.20mg/kg).

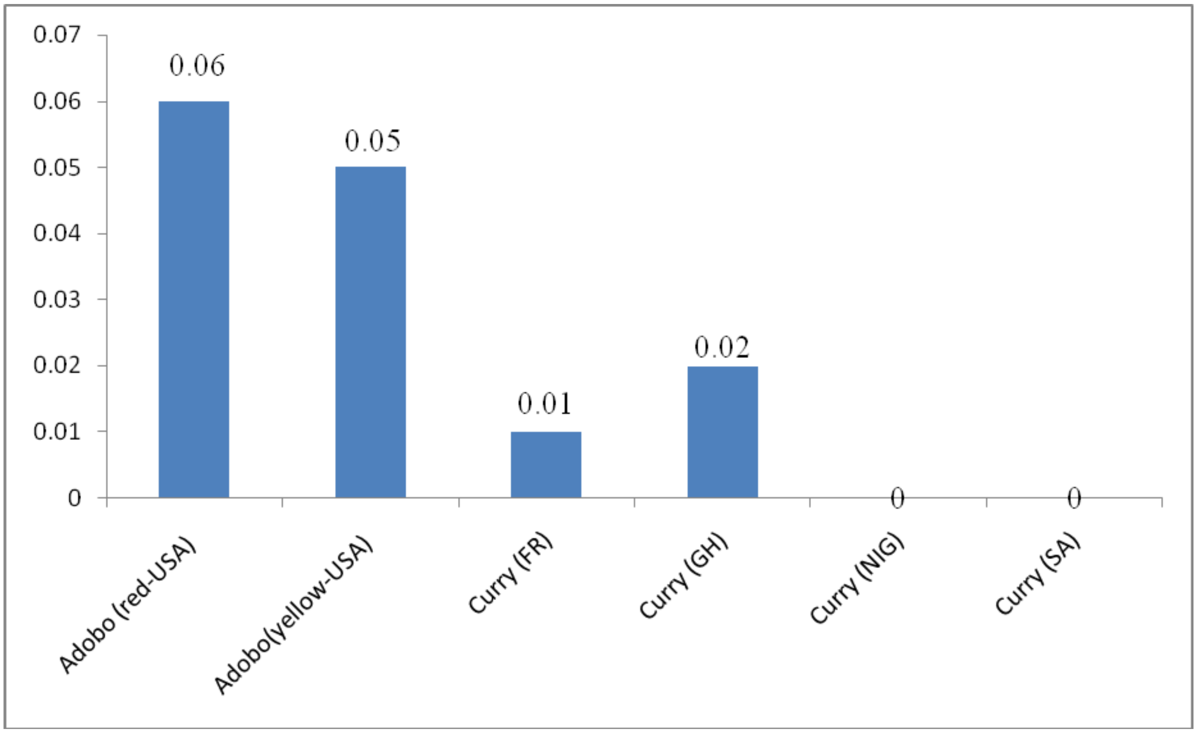

Figure 4.11: Concentration of Cd (mg/kg) in mixed seasonings

Cadmium levels in bouillon cubes reported in Figure 4.12, ranged from ND in Chicken seasoning (BC-5) to 0.05mg/kg in Beef seasoning (BC-4), Onion seasoning (BC-2), Stew spice (BC-9) and shrimp seasonings (BC-6 and BC-10). The cadmium content in the bouillon seasonings from GH, TUK, S.A and CTD were the same. This implies that all these bouillon cubes may have the same health effects when consumed.

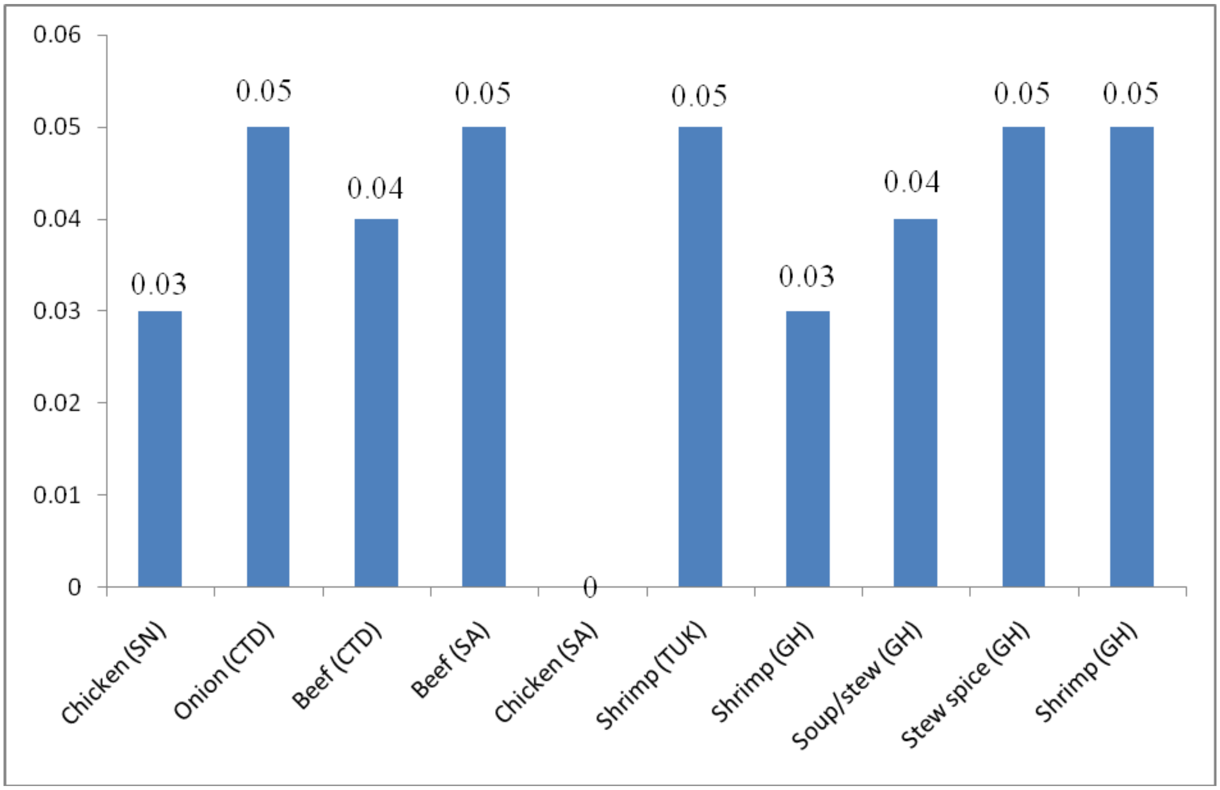

Figure 4.12: Concentration of Cd (mg/kg) in bouillon seasonings

Cadmium was not detectable in Rosemary as reported by Divrikli *et al*., (2006). Koc and Sar, (2009) reported 3.1μg/g of Cd in Rosemary. The concentration of cadmium in pepper was reported to range from 0.25mg/kg to 1.07mg/kg by Awode *et al.*, (2008) but was not detected in any type of pepper in this study. Also Krejpcio *et al.,* (2007), reported that cadmium levels in Ginger ranged from 0.02mg/kg to 0.04mg/kg but was not detected in this study. Concentration of Nutmeg (UM-4) was higher than reported levels (0.05mg/kg) by Krejpcio *et al.,* (2007). The level of cadmium in Curry seasonings were lower than levels reported in literature (Nnorom *et al.,* 2007).

According to Chizzola *et al*., 2003, cadmium levels could be considered normally low in plants. Generally, all the seasoning with the exception of Nutmeg were below the permissible level

(0.20mg/kg) required in spices set by the FAO/WHO (2001). Cadmium is said to be easily transported from the soil to the edible parts of plants (Mengel and Kirkby, 1982). The low Cd levels in the seasonings could be due to poor availability of the element from the soil to the plants parts and low amounts of cadmium in the soil.

It is reported that presence of cadmium in food is mostly derived from various sources of environmental contamination and has no biological importance (Adriano, 1984). Though cadmium content in the seasonings were low, its presence could be due to contamination of raw materials, technological processes or coloring agents used as they contain cadmium salts (Cabrera *et al.*, 1995). The use of phosphate fertilizers may increase the naturally occurring cadmium in the soil which may in turn increase the cadmium content in plants. Soil amended with sewage sludge also increase cadmium content in soil (Wajahat *et al.*, 2006) which may have a direct effect in plants.

Cadmium toxicity causes renal dysfunction, skeletal defects related to calcium loss which results in osteomalacia and osteoporosis (WHO, 1989).
Cadmium toxicity is characterized by chest pain, cough with foamy and bloody sputum, and death of the lining of the lung tissues due to excessive accumulation of watery fluids (Duruibe *et al.*, 2007).

In order not to exceed the daily intake of Cd calculated from the weekly intake of 7.5µg/kg bw, the maximum amount of seasonings that must be consumed are reported in Table 4.2. An excess amounts will cause toxicity. Toxicity is unlikely though, as it impossible to consume such a large amount in a day. This makes the seasonings safe for human consumption.

4.6 Lead (Pb) levels in unmixed, mixed and bouillon seasonings

In unmixed seasonings, the levels of Pb ranged from 0.6mg/kg to 1.8mg/kg. Ashanti pepper (UM-7) from Mali recorded the least Pb level while Ginger (UM-8) from GH (Central), recorded the highest (Figure 4.13). The second highest level of Pb was found in Ginger from Railway followed by Rosemary (UM-1) from China.

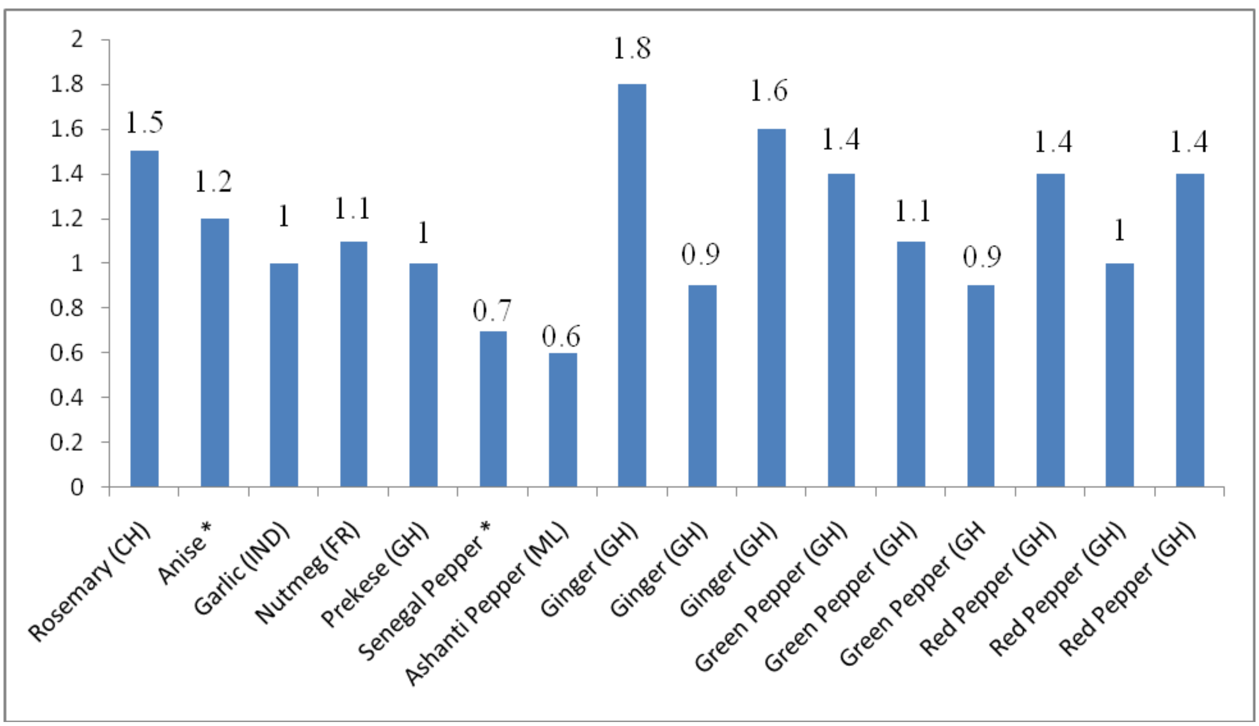

Figure 4.13: Concentration of Pb (mg/kg) in unmixed seasoning

Ginger, red pepper and green pepper from the various markets showed differences in the concentrations of Pb (Table 4.1). Ginger (UM-8) from Central market was found to contain the highest level of Pb, followed by Ginger (UM-10) from Railway and Ginger (UM-9) from Central market in that order. Red pepper (UM-14) from Central market had the same Pb content as Red pepper (UM-16) from the Railway market. The levels of Pb in Green pepper ranged from 0.9mg/kg and 1.4mg/kg. The Pb levels in Green pepper was in the order Central > Asafo > Railway. It can be observed that of the three markets, seasonings from Central had the highest Pb content. This could be attributed to the high traffic density near this market. This is also evident in seasonings from Railway market which is sandwiched between two busy roads. Kylander *et al.*, (2003) reported that Ghana still uses leaded petrol. Wheeler and Rolfe (1979) found that Pb levels in vegetation increased linearly with traffic density.

The Pb concentration in mixed seasonings in Figure 4.14 was between 0.63mg/kg and1.39mg/kg. The highest Pb level was found in Adobo (red) (M-1) from the U.S.A whilst the Curry seasonings (M-6) from S.A had the lowest. The second highest Pb level was found in Adobo (yellow) (M-2) also from U.S.A. Adobo (red) had lower Pb concentration than Adobo (yellow), both being products of the United States of America. Within the different brands of Curry, Pb levels ranged from 0.63mg/kg to1.05mg/kg. Curry (M-4) from Ghana had the highest Pb level whereas Curry (M-6) from S.A had the lowest. The presence of Pb in the seasonings could be due to combination of different plant parts that make up the ingredients of the mixed seasonings.

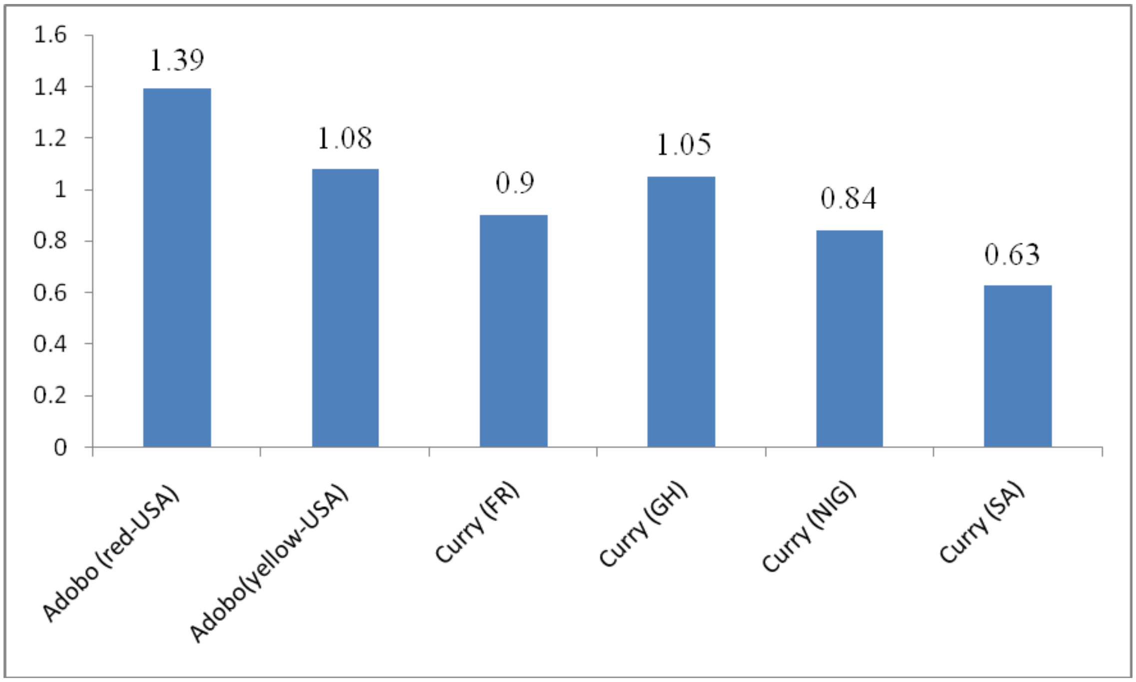

Figure 4.14: Concentration of Pb (mg/kg) in mixed seasonings

The level of Pb in bouillon seasonings ranged between 0.37mg/kg and 2.18mg/kg (Figure 4.15). Levels of Pb in Onion seasoning (BC-2) from CTD was found to be the highest whereas Soup/stew (BC-8) from GH, the lowest. The second highest Pb level was found in Shrimp bouillon (BC-7) from GH.

Chicken bouillon (BC-5) from S.A obtained Pb level two times that of Senegal (SG). Shrimp seasonings from GH (BC-7 and BC-10) were higher in Pb concentration than that from TUK (BC-6). The level of Pb in Beef seasonings from Cote d'Ivoire was lower than that from South Africa.

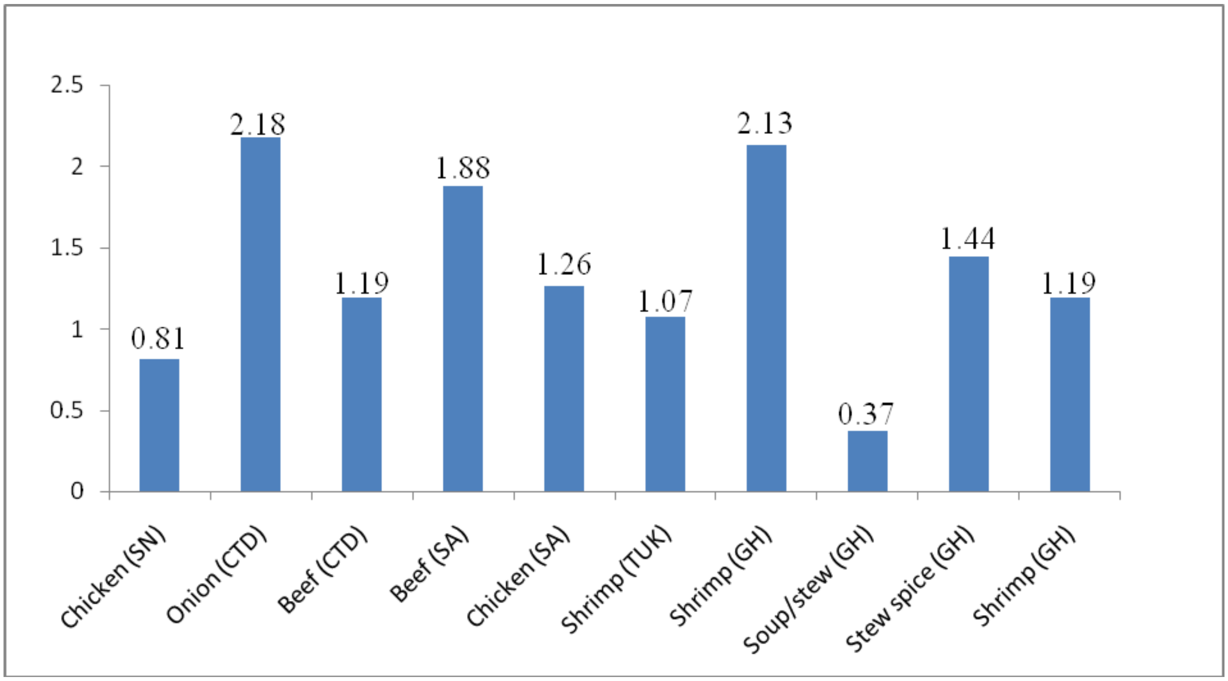

Figure 4.15: Concentration of Pb (mg/kg) in bouillon seasoning

Pb has been reported as non detectable in some spices and herbs (Divrikli *et al*., 2006; Nnorom *et al.*, 2007) and not investigated at all in other studies (Ozkutlu *et al*., 2006). However, in other studies (Chizzola *et al.*, 2003; Gupta *et al.*, 2003; Koc and Sar, 2009) the highest Pb level ranged from 2mg/kg to 200mg/kg as compared to 0.37mg/kg and 2.8mg/kg in this present study. The concentration of Pb in Nutmeg (UM-4) was higher than that reported (0.36mg/kg) by Krejpcio *et al*., (2007). The levels of Pb in mixed seasonings were higher than levels reported in literature (Nnorom *et al.*, 2007).

The level of Lead in all seasoning samples were above the permissible limit required in spices (0.3mg/kg) by the FAO/WHO (2003). Spices are reputed to acquire lead during growth in lead contaminated soils or in the course of milling or other processing procedures.

The use of pesticides contaminated with heavy metals during the growing of herbs and spices may also be a source of lead contamination in the final product (Galal-Gorchev, 1991).

The lead content in seasonings could also be attributed to the addition of lead during processing to impart colour, sweetness to taste or to increase the weight of these products (Kakosy *et al.*, 1996). This could also be due to combination of contaminated raw materials which reflects in all the groups of seasonings investigated. Research indicates that traffic density also increase the lead burden in the environment thereby increasing the lead content in vegetation (Rodriguez-Flores and Rodriguez-Castellon, 1982; Buszewski *et al*., 2000; Nabulo, 2004).

Lead is a non-essential toxic heavy metal (Schroeder et al., 1973; WHO, 1973). Increased levels of lead in man result in poor mental development in children, cardiovascular diseases, renal dysfunction and encephalopathy seizures (Schumann, 1990; Goyer, 1993).

The various amounts of seasonings in grams (g) which when consumed will not exceed the RDA as are reported in Table 4.2.

4.7 Correlation between metals in seasonings

A correlation analysis was employed to find out any relationship that may exist between metals in a particular group of seasoning. Correlation coefficients are shown in Tables 4.3, 4.4 and 4.5. Significant correlations were observed at 0.05 or 0.01.

A correlation coefficient is a number between -1 and +1 that measures the degree of association between two variables. A positive value for the correlation implies a positive association and a negative value for the correlation implies a negative or inverse association (Stevens, 2008).

That is, positive correlation indicates that both variables increase or decrease together, whereas negative correlation indicates that as one variable increases, the other decreases or vice versa.

Moderately negative correlation occurred between Cd and Fe whereas moderately positive correlation occurred between Pb and Fe in unmixed seasonings (Table 4.3).

Table 4.3 Correlation between metals in unmixed seasonings

	Iron	Zinc	Copper	Cadmium	Lead
Iron	1				
Zinc	0.364(*)	1			
Copper	0.363(*)	0.128	1		
Cadmium	-0.512(**)	-0.067	0.183	1	
Lead	0.516(**)	-0.02	0	0.223	1

* Correlation is significant at 0.05 level

** Correlation is significant at 0.01 level

Table 4.4 shows a moderate negative correlation between Cu and Fe and a moderately positive correlation between Pb and Cd in mixed seasonings. Strong positive correlations were also shown between Cu and Zn whereas a strong negative correlation was shown between Cd and Zn in mixed seasonings.

Table 4.4 Correlation between metals in mixed seasonings

	Iron	Zinc	Copper	Cadmium	Lead
Iron	1				
Zinc	0.472	1			
Copper	0.684(**)	0.812(**)	1		
Cadmium	-0.495	-0.871(**)	-0.798(**)	1	
Lead	-0.038	-0.628(**)	-0.453	0.626(**)	1

** Correlation is significant at 0.01 level

Bouillon seasonings showed strong positive correlations between Fe and Zn; Fe and Cu; Cu and Zn. A moderately negative correlation was shown between Cd and Fe; Cd and Zn and also between Cd and Cu (Table 4.5).

Table 4.5 Correlation between metals in bouillon cubes seasonings

	Iron	Zinc	Copper	Cadmium	Lead
Iron	1				
Zinc	0.954(**)	1			
Copper	0.912(**)	0.892(**)	1		
Cadmium	-0.721(**)	-0.586(**)	-0.549(**)	1	
Lead	-0.044	-0.078	-0.045	0.168	1

** Correlation is significant at 0.01 level

The moderately negative correlations in unmixed and mixed seasonings suggest that the probability that an increase in the level of one metal causing the other to decrease is not much. Moderately positive correlation in mixed seasonings implies that the probability that an increase in the level of one metal causing another to increase is not much.

The strong positive correlations between metals shown in mixed and bouillon seasonings indicate that the presence of the metals are possibly from the same source (Gökhan, 2009). Thus, metals present in the seasoning could be from a single source that is, the soil, water for irrigation, the processing method or any other source of heavy metal that may be present.

The strong negative correlation in mixed seasonings indicates that, the presence of the metals is possibly from multiple sources (Awode *et al.*, 2008).

4.8 Phytochemical screening of seasonings

In the phytochemical screening, positive sign (+) indicated presence of phytochemical, whereas negative sign (-) indicated the absence of phytochemical.

From the results of the phytochemical screening of the seasonings (Table 4.8) out of 32 seasonings, 8 contained saponins, 14 showed the presence of coumarins, 3 contained alkaloids and 8 had terpenoids. However, none contained anthraquinones, flavonoids and carotenoids.

Table 4.7 Phytochemical constituents of 32 seasonings from markets in Kumasi

Seasonings	samples	Sap	Cmr	Flv	Car	Alk	Ter	Anq
Rosemary	UM-1	-	+	-	-	-	+	-
Anise	UM-2	+	-	-	-	-	-	-
Garlic	UM-3	-	-	-	-	-	-	-
Nutmeg	UM-4	-	-	-	-	-	+	-
Prekese	UM-5	+	-	-	-	-	+	-
Senegal Pepper	UM-6	-	+	-	-	-	-	-
Ashanti Pepper	UM-7	-	-	-	-	-	-	-
Ginger	UM-8	+	+	-	-	-	-	-
Ginger	UM-9	+	+	-	-	+	-	-
Ginger	UM-10	+	+	-	-	+	-	-
Green Pepper	UM-11	-	+	-	-	+	-	-
Green Pepper	UM-12	-	+	-	-	-	-	-
Green Pepper	UM-13	-	+	-	-	-	-	-
Red Pepper	UM-14	+	+	-	-	-	-	-
Red Pepper	UM-15	+	+	-	-	-	-	-
Red Pepper	UM-16	+	+	-	-	-	-	-
Adobo(red)	M-1	-	-	-	-	-	-	-
Adobo(yellow)	M-2	-	+	-	-	-	+	-
Curry	M-3	-	-	-	-	-	+	-
Curry	M-4	-	-	-	-	-	+	-
Curry	M-5	-	-	-	-	-	+	-
Curry	M-6	-	-	-	-	-	+	-
Chicken seasoning	BC-1	-	-	-	-	-	-	-
Onion seasoning	BC-2	-	+	-	-	-	-	-
Beef seasoning	BC-3	-	-	-	-	-	-	-
Beef seasoning	BC-4	-	-	-	-	-	-	-
Chicken seasoning	BC-5	-	-	-	-	-	-	-
Shrimp seasoning	BC-6	-	-	-	-	-	-	-
Shrimp seasoning	BC-7	-	+	-	-	-	-	-
Soup/stew seasoning	BC-8	-	-	-	-	-	-	-
Stew spice	BC-9	-	-	-	-	-	-	-
Shrimp seasoning	BC-10	-	-	-	-	-	-	-

Sap—Saponins, Cmr —Coumarins, Flv —Flavonoid, Car —Carotinoids, Alk —Alkaloids

Anq—Anthraquinones, Ter –Terpenoids.

Most plants contain some phytochemicals and they mostly cluster in similar classes of species (Katzer, 2002). This may suggest why only few were present in a particular seasoning. In unmixed seasonings, Ginger (UM-8, UM-9 and UM-10) from Central, Asafo and Railway markets in Kumasi was found to contain the highest number of phytochemicals namely saponins, coumarins and alkaloids whereas Anise, Nutmeg, Senegal pepper, *Prekese* and Green pepper (UM-2, UM-4, UM-5, UM-6, UM-12 and UM-13) contained the least (Table 4.7). However in Ashanti pepper (UM-7), no phytochemical was present.

Coumarins and terpenoids were the only phytochemicals found in mixed seasonings. Terpenoids were present in all the mixed seasonings except Adobo (red) M-1. Coumarins were present in only Adobo (yellow), (M-2). The highest number of phytochemicals (coumarins and terpenoids) was in Adobo (yellow), (M-2). However no phytochemical was present in Adobo (red) (M-1) (Table 4.7).

Coumarins were found in Onion and Shrimp seasonings (BC-2 and BC-7) in the bouillon cubes whereas the rest of the cubes had no phytochemicals present (Table 4.7).

Phytochemicals have shown to exhibit antioxidant and antimicrobial effects (Shobana and Naidu 2000; Halvorsen *et al.,* 2002; Gupta *et al.,* 2009). Oxidative damage due to free radicals plays a role in the development of chronic, age-related degenerative diseases and dietary antioxidants oppose this and lower risk of diseases (Atoui *et al.,* 2005). Ginger (UM-9 and UM-10) had the highest number of phytochemicals, confirming its high antioxidant activity (Shobana and Naidu 2000; Halvorsen *et al.,* 2002). The presence of terpenoids, coumarins, alkaloids and saponins in some of the seasonings may imply that, these seasonings can be involved in antioxidant activities destroying free radicals in the body (Gonzalez and Marketon, 2003).

Okwu (2004) reported that saponins have antibiotic properties which help the body to fight infections. *Capsicum sp (annum* L. var *acuminatum)* a pepper specie which, was reported to contain saponins showed high antimicrobial activity against *Saccaromycoids, Kloeckera* and *Hanseniaspora* (De Marino *et al*, 2008). The seasonings, Ginger (UM-8, UM-9 and UM-10) and Red pepper (UM-14, UM-15 and UM-16) contain saponins which may therefore help fight infections. The alkaloids in Ginger (UM-8, UM-9 and UM-10) may suggest the seasonings having an analgesic effects and bactericidal effects thus being used as medicinal agents (Okwu, 2004). Amchur, an Indian spice was reported to contain terpenoids which had an antimicrobial effect on *Staphylococcus aureus* and *Psuedomonas aeruginosa* (Gupta *et al.*, 2009). This implies that the seasonings containing terpenoids may inhibit microbial function.

The presence of phytochemicals in some of the seasonings in the present study suggests that, the seasonings may not only impart flavor and taste to food, they may further function as food preservatives and improve health of consumers.

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The study showed that Iron, Zinc, Copper and Lead were present in all seasonings whereas 56.3% of the seasonings were found to contain Cadmium. Mercury was not detected in any of seasonings analysed. The levels of toxic metals, Cadmium and lead were mostly above the FAO/WHO permissible level. Positive and negative correlations were obtained in all groups of seasonings suggest that contamination in the seasonings may come from a single source or multiple sources.

Even though the data on the rate of consumption of seasoning in Ghana is unavailable, it is unlikely that a person will consume so high amounts of seasoning in a day. This suggest that intake of the seasonings will have negligible effects on consumers health. However, the high levels of toxic metals (Cd and Pb) suggest the need for regular monitoring of the seasonings for contamination.

Saponins, Coumarins, Alkaloids and Terpenoids were found to be present in some of the seasonings. This implies that the seasonings may not only impart flavor and taste to food but also provide some health benefits to consumers.

5.2 RECOMMENDATIONS

1. Further studies should be done to consider specific sources (e.g. soil, water of irrigation etc.) of contamination by heavy metals in the local seasonings.

2. Due to the varying sources of contamination by heavy metals in food and the large number of seasonings in our markets, regular monitoring of heavy metals should be done.

3. A further study to investigate the antimicrobial effects of the seasonings to know the potency of the phytochemicals should be done.

4. Food industries such as restaurants and food vendors and consumers should be educated in the dangers of using contaminated seasonings in daily meals.

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www.en.wikipedia.org/wiki/seasonings

www.answers.com>...>Literature&Languages>Dictionary

APPENDICES

Appendix 1: Mean levels (± SD) (mg/kg) of iron, zinc, copper, cadmium and lead in unmixed (UM) seasonings

SAMPLE	SOURCE	SAMPLE CODE	IRON	ZINC	COPPER	CADMIUM	LEAD
Rosemary	China (CH)	UM-1	19.4±1.6	2.6±0.4	0.9±0.2	ND	1.5±0.2
Anise	*	UM-2	500.4±5.9	34.6±1.9	9.3±0.1	ND	1.2±0.6
Garlic	India (IND)	UM-3	58.6±8.6	14.9±1.5	3.2±0.2	ND	1.0±0.1
Nutmeg	France (FR)	UM-4	109.0±1.6	10.8±2.0	10.1±2.6	0.9±1.6	1.1±0.6
Prekese	Ghana (GH)	UM-5	24.8±0.8	2.4±0.1	2.9±0.1	ND	1.0±0.8
Senegal pepper	*	UM-6	45.5±1.0	5.1±0.9	5.5±3.9	ND	0.7±0.1
Ashanti pepper	MALI (ML)	UM-7	100.6±0.8	17.8±0.4	4.5±0.2	ND	0.6±0.1
Ginger	Ghana (GH-C)	UM-8	698.3±1.4	17.6±1.8	4.7±1.3	ND	1.8±0.6
Ginger	Ghana (GH-A)	UM-9	408.4±10.5	14.1±0.7	3.7±0.2	ND	0.9±0.3
Ginger	Ghana (GH-R)	UM-10	590.1±4.0	11.8±1.8	3.7±0.7	ND	1.6±0.5
Green pepper	Ghana (GH-C)	UM-11	94.3±8.0	19.4±0.8	1.5±0.2	ND	1.4±0.1
Green pepper	Ghana (GH-A)	UM-12	226.2±0.0	16.9±1.1	1.3±0.0	ND	1.1±0.1
Green pepper	Ghana (GH-R)	UM-13	105.9±1.1	15.4±1.1	1.4±0.0	ND	0.9±0.2
Red pepper	Ghana (GH-C)	UM-14	971.4±1.8	14.9±1.6	6.7±0.2	ND	1.4±0.6
Red pepper	Ghana (GH-A)	UM-15	614.6±1.0	9.5±0.4	7.2±0.2	ND	1.0±0.0
Red pepper	Ghana (GH-R)	UM-16	345.9±12.7	7.4±0.7	7.7±0.3	ND	1.4±0.5

***** Source not known (C)- Central market (A)- Asafo market (R)- Railway market

Appendix 2: Mean levels (± SD) (mg/kg) of iron, zinc, copper, cadmium and lead in mixed (M) seasonings

SAMPLE	SOURCE	SAMPLE CODE	IRON	ZINC	COPPER	CADIUM	LEAD
Adobo (red)	United States of America (U.S.A)	M-1	91.56±5.15	1.72±0.32	1.81±0.04	0.06±0.00	1.39±0.64
Adobo(yellow)	United States of America (U.S.A)	M-2	83.36±3.49	2.76±0.93	1.73±0.03	0.05±0.00	1.08±0.03
Curry	France (FR)	M-3	204.32±4.37	17.68±0.46	5.77±0.14	0.01±0.01	0.91±0.10
Curry	Ghana (GH)	M-4	480.82±0.95	13.67±0.28	7.70±1.72	0.02±0.02	1.05±0.16
Curry	Nigeria (NIG)	M-5	149.13±4.15	13.87±0.80	6.40±0.13	ND	0.84±0.15
Curry	South Africa (S.A)	M-6	214.93±5.40	26.78±0.61	6.39±0.11	ND	0.63±0.10

ND – non detectable

Appendix 3: Mean levels (± SD) (mg/kg) of iron, zinc, copper, cadmium and lead in bouillon (BC) seasonings

SAMPLE	SOURCE	SAMPLE CODE	IRON	ZINC	COPPER	CADIUM	LEAD
Chicken seasoning	Senegal (SG)	BC-1	14.22±2.96	1.35±0.38	0.66±0.07	0.03±0.01	0.81±0.04
Onion seasoning	Cote d'Ivoire (CTD)	BC-2	12.28±3.35	0.83±0.72	0.78±0.07	0.05±0.02	2.18±0.03
Beef seasoning	Cote d'Ivoire (CTD)	BC-3	18.34±1.80	2.25±1.25	0.76±0.08	0.04±0.02	1.19±0.80
Beef seasoning	South Africa (S.A)	BC-4	21.04±1.24	2.41±0.52	1.17±0.06	0.05±0.00	1.88±0.04
Chicken seasoning	South Africa (S.A)	BC-5	52.45±1.56	8.93±0.82	3.59±0.36	ND	1.26±0.12
Shrimp seasoning	Turkey (TUK)	BC-6	37.97±0.83	1.80±0.13	1.13±0.27	0.05±0.02	1.07±0.31
Shrimp seasoning	Ghana (GH)	BC-7	26.48±0.06	1.62±0.50	1.17±0.1	0.03±0.03	2.13±0.26
Soup/stew seasoning	Ghana (GH)	BC-8	10.36±1.20	2.02±0.11	1.25±0.26	0.04±0.01	0.37±0.04
Stew spice	Ghana (GH)	BC-9	9.66±0.62	1.29±0.97	0.94±0.05	0.05±0.00	1.44±0.56
Shrimp seasoning	Ghana (GH)	BC-10	12.14±1.51	0.90±0.01	1.72±0.12	0.05±0.00	1.19±0.58

Appendix 4: Operating parameters for Flame atomic absorption Spectrometer (Spectr AA 220, Australia)

Element	**Wavelength (nm)**	**Slit width(nm)**
Fe	248.3	0.2
Cu	324.8	0.7
Zn	213.9	0.7
Cd	228.8	0.7
Pb	283.3	0.7