KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

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LEVELS OF SOME HEAVY METALS AND NUTRITIONAL COMPOSITIONS OF COCOA BEANS FROM SELECTED COCOA- GROWING AREAS IN ASHANTI AND WESTERN REGIONS OF GHANA

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BY

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CERTIFICATION

I hereby declare that this submission is my own work towards the award of Master of Philosophy in Environmental Chemistry 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 this university or elsewhere, except where due acknowledgement has been made in the text.

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ABSTRACT

In this study, the levels of six heavy metals namely: cadmium, lead, copper, manganese, iron and zinc were determined in cocoa beans from some cocoa-growing areas in the Western and Ashanti Regions of Ghana using atomic absorption spectroscopy. The metal levels in the cocoa beans from the Western Region, expressed in mg/ kg varied from 0.045 to 0.066 with mean value of 0.054 for cadmium, from 0.013 to 0.030 with mean value of 0.020 for lead, from 46.47 to 55.17 with mean value of 51.98 for copper, from 48.36 to 64.65 with mean value of 55.18 for manganese, from 43.80 to 53.11 with the mean value of 47.51 iron and from 43.04 to 52.06 with the mean value of 48.29 for zinc. Those of Ashanti Region ranged from 0.050 to 0.065 with the mean value of 0.056 for cadmium, from 0.014 to 0.020 with the mean value of 0.017 for lead, from 47.43 to 54.17 with the mean value of 49.10 for copper, from 47.15 to 57.34 with the mean value of 54.62 for manganese, from 50.23 to 63.87 with the mean value of 54.63 for iron and from 53.02 to 58.71 with the mean value of 56.49 for zinc.

Upon proximate analyses of four of the samples chosen at random (two samples from each region), it was found that all the samples were of high fat and carbohydrate content. The percentages of fat were 45.52%, 43.85%, 45.57% and 36.72% in samples from Kasapen, Asampaneye, Bekwai and Juaso respectively. Those of carbohydrates were 32.56%, 32.08%, 31.62% and 42.88% in cocoa samples from Kasapen, Asempaneye, Bekwai and Juaso respectively. Levels of proteins were 14.34%, 13.97%, 13.74% and 13.91% in the samples from the towns as listed in the order above. The samples from Kasapen in the Western Region gave 3.15%, 3.40% and 1.03% for moisture, ash and fibre contents respectively. The moisture, ash and fibre content in samples from Asempaneye also in the Western Region were 4.39%, 3.69% and1.02% respectively. Samples from Bekwai in the Ashanti Region gave 4.46%, 3.52% and1.09% for moisture, ash and fibre contents respectively. The moisture, ash and fibre contents were 2.32%, 3.19% and 1.07% respectively in the samples from Juaso in the Ashanti Region of Ghana.

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DEDICATION

I dedicate this work to my two beautiful daughters: Akua Dufie Asante-Nnuro and Afia Amoakoaa Asante-Nnuro.

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CHAPTER ONE

1.0 BACKGROUND.

With the development of industries and modernization of agriculture, soil pollution has become increasingly serious. The heavy metal concentrations are so high in soils of many areas that they can poison the soil-plant system, degenerate the soil, and reduce the quality of products of crops [1]. Moreover, they can threaten the health of animals and human beings upon bioaccumulation in food chain [1, 2]. Based on this, the studies of the elemental composition of food crops is increasingly becoming much relevant not only to nutritionists and toxicologists but to the general public as well. Though some micro elements are very essential for the proper functioning of the body, the toxicity of others makes their presence in food a cause for concern. The sources of these metals in food may vary widely ranging from the soil on which the plants are grown to the conditions they are subjected to during and after crop production. Conditions such as mining and smelting of metal ores, industrial emissions and application of insecticides and fertilizers (anthropogenic activities) have all contributed to the elevated levels of heavy metals in the environment [3]. The threat that heavy metals pose to human and animal health is exacerbated by their long-term persistence in the environment. To substantiate this, toxicological effects of heavy metals like lead on human beings include inhibition of hemoglobin formation, sterility, hypertension and mental retardation in children [4], while the major hazard to human health of cadmium is its chronic accumulation in the kidney where it causes dysfunction if the concentration in the kidney cortex exceeds 200mg/kg fresh weight [5]. In addition, though copper is an essential element, it may be toxic to both humans and animals when its concentration exceeds the safe limits and its concentration in some human tissues such as thyroid can be changed depending on the tissue state providing even cancerous or non-cancerous effects [6,7]. The interaction between these metals and solid phases of soil, water and air within and above soil depends on a variety

of chemical factors and determination of heavy metal transport and fate. Absorption of metals from soil water and soil particles is most important chemical determinant that limits mobility in soil [8]. Heavy metals from soil enter plants primarily through the root system. In general, plant roots are the most important site for uptake of chemicals from soil [9].

Cocoa products are regarded to be one of the most widely consumed foods worldwide. Cocoa beans are the raw material from which the widely patronized products like chocolate, candies, cocoa powder and beverages are produced. The possibility of finding heavy metals in these products is as a result of their accumulation in the raw cocoa beans.

Unfortunately, body of evidence lends credence to the relatively high levels of heavy metals in cocoa products as compared to other food products [10, 11]. Several suggestions have been stipulated as to the origins of these metal contaminants but it is widely believed to be from the raw material (cocoa beans).

For some decades now, cocoa has been and it continues to be the backbone of the Ghanaian economy in terms of foreign revenue and domestic incomes [12]. To corroborate this, Ghana currently produces about 1,000,000 metric tons of cocoa annually and the second largest producer in the world and it is estimated to cover 1.5 million hectors of land [13]. Undeniably, cocoa from Ghana is revered to be of the best quality with high demand in the world market. Due to these quality characteristics of Ghana's cocoa, they are mostly used as reference standard for cocoa produced from other parts of the world [14].

It is worth mentioning that though cocoa beans from Ghana have been reported to be of relatively low levels of heavy metals within the acceptable limits, most of these research works date back to the 1990s [15]. Owing to this, the safe levels of heavy metals in the Ghanaian cocoa beans can be questionable as there has been an alarming increase in mineral mining activities as well as other anthropogenic activities in cocoa-growing areas.

The patronage in cocoa products is likely to be on the ascendency following the recent discovery of high levels of polyphenols in cocoa and their great benefits to health [16, 17]. The polyphenols (catechins and epicatechins) have been detected to have good antioxidant and free radical scavenging properties leading to lower risk of cardiovascular diseases. Recent research has confirmed that cocoa has higher levels of essential polyphenols than in red wine and tea [18]. This may lead to high consumption of cocoa products and therefore calls for analytical interventions to determine the levels of heavy metals and nutritional contents in the raw material (cocoa beans).

1.1 STATEMENT OF PROBLEM

As plants constitute the foundation of the food chain, some concerns have been raised about the possibility of toxic concentrations of certain heavy metals being transported from plants to higher strata of the food chain. Therefore the high demand for quality cocoa beans from Ghana over the years has suffered rejection at the Japanese market due to the presence of high level of contaminants in the cocoa beans [19]. The situation is alarming by the fact that almost all Ghanaian cocoa farmers apply insecticides, herbicides, fertilizers and fungicides on their cocoa farms to reduce pest to enhance maximum yield. These activities are likely to introduce heavy metals like Cd, Fe, Pb, Cu, Mn and Zn etc. into the soil which eventually will end up in the edible parts of crops through phytoextraction by the crops and translocation throughout the plant system. Again, there is little literature on the nutritional values of the basic nutrients in cocoa beans. Due to this, nutritionists may find it difficult to have enough answers to why, how, when and the conditions under which cocoa beans and its products should be taken.

1.2 JUSTIFICATION

In Ghana, cocoa takes about 25% of the total export earnings and it is the second most important export commodity after gold [20]. This, coupled with the high demand of Ghana's

cocoa beans at the world market and worldwide patronage of cocoa products, makes it imperative to analyze the level of contaminants (heavy metals) so as to determine if the levels conform to the international standards. This will augment the work of the cocoa quality assurance services to consolidate the high demand for cocoa beans from Ghana "domestically" and internationally. Knowledge of the nutritional standing of cocoa beans will serve as grounds for nutritional and health advice.

1.3 OBJECTIVE OF STUDY

The objectives of this research are:

- To determine the levels of some heavy metals in cocoa beans from Ghana
- To compare the levels of heavy metals in Ghanaian cocoa beans with the permissible levels established by international food safety.
- To determine the proximate compositions in cocoa beans.

1.4 SCOPE OF STUDY

This work focuses on the determination of six heavy metals namely: Cd, Fe, Pb, Cu, Mn and Zn (These metals are toxic with high atomic number and specific gravity greater than 5.0. They include some metalloids, transition metals, lanthanides and actinides.) as well as the nutritional contents (carbohydrate, protein, fat, ash, fibre and moisture) in cocoa beans sampled from some ten selected towns in Ashanti Region and Western Region .Five towns were selected from each of the regions and in every town visited, cocoa samples were obtained from five different farmers giving a total sample size of fifty. Some of the samples were taken from the farmers in their raw state from their farms before drying while others were obtained in their dried states.

CHAPTER TWO

2.0 LITERATURE SURVEY

2.1 INTRODUCTION

The literature on the analysis of heavy metals in cocoa beans is sparse. There is much literature on the levels of heavy metals in cocoa products than in raw beans.

However, this chapter will seek to review the existing literature and work done on heavy metals in cocoa beans since the possible route of heavy metals into cocoa products is mainly through the raw material (cocoa beans).

2.2 HEAVY METALS CONCENTRATION IN FOOD.

With the development of industries and modernization of agriculture, soil pollution and its concomitant toxicological problems is increasingly alarming.

Efforts have been made in the recent years to improve the productivity of the low nutrient status of soils in tropical Africa in order to enhance food productivity and sustain the projected population growth. To ensure the success of these objectives, the use of inorganic fertilizers has more than doubled in recent past. Superphosphate fertilizers contain not only major elements necessary for plant nutrient and growth, but also trace metal impurities such as Cd and Pb [21, 22, 23, 24, and 25]. It is, therefore, an important anthropogenic source of soil contamination with heavy metals [26, 27]. The concentration of heavy metals added to soils through phosphate fertilizer depends on the origin of crude phosphate rock from which the fertilizer is manufactured [28]. The presence of trace metals in food consumed may, however, not be due to anthropogenic sources only. Most heavy metals are found to be naturally occurring forming part of the soil on which plants are grown.

These are taken up into the plant through absorption and translocation to other parts of the plant. While the absorption of very small amounts of trace metals is thus unavoidable, their presence which normally results in toxic amounts is chiefly due to human activities [29].

They are mainly released into the atmosphere through mining and smelting of ores that contain these metals. Through their release into the atmosphere, most of them ultimately end up in soils and sediments which are taken up by plants. If the levels are elevated, it may lead to increased uptake by plants [30] and hence increase their levels in food consumed from these plants [31]. Among an array of heavy metals, Cu, Co, Fe, Ni and Zn are essential micronutrient mineral elements whereas Cd, Hg, Pb and As have no known physiological functions in plants and are potential toxins. However, elevated levels of both essential and non-essential heavy metals in the plough layers of crop land pose serious threat for human health and agriculture. The excessive uptakes of these metals from the soil create dual problems. Metal concentrations in plants vary with plant species [32]. Plant uptake of heavy metals from soil occurs either passively with the mass flow of water into roots, or through active transport across the plasma membrane of root epidermal cells.

Under normal growing conditions, plants can potentially accumulate certain metal ions in order of magnitude greater than the surrounding medium [33]. The presence of some heavy metals in food may have some useful purposes but the potential toxicity of others; especially in high concentrations is the concern for many scientific studies as far as heavy metals are concerned [34]. Heavy metals like iron, zinc, copper which form an integral part of healthy life in man are mostly found naturally in foodstuffs [35]. Practically, they form essential components of some important enzymes and take part in most biological processes in man. Typical examples are the presence of iron as an essential component of hemoglobin and cytochrome [36]. Zn is required for the functioning of a number of metalloenzymes, including alcohol dehydrogenase and carbonic anhydrase. Its deficiency creates many

disorders such as growth retardation; poor wound healing and impaired immune function [37]. Again, copper aids in numerous biochemical processes in the body such as hemoglobin formation, antioxidant defense mechanism. However, its deficiency may result in hypochromic anemia but osteoporosis and kidney damage result for high levels of its exposure [38].

The heavy metals mentioned above, in smaller quantities, are important for biological activities. However, large quantities of these metals may cause chronic and acute toxicity. Ailments like lower energy level, damage to lungs, liver and cancer may result [35].

In spite of the **"necessary evil"** nature of the above-mentioned metals, some heavy metals like cadmium, arsenic, lead and mercury have no known biological function in the living system and as such their presence in human body causes major health threats at long-term exposure even at low doses. They are also implicated in causing carcinogenesis, mutagenesis and teratogenesis [39].

In the nutshell, heavy metals intake by human through ingestion arises from the uptake of these heavy metals by plants from fertilizers, sewage sludge manure and atmospheric deposition [40]. Anthropogenic sources such as phosphate fertilizer application, fossil fuel combustion and other industrial activities contribute the most importance source of heavy metal exposure.

2.2 COCOA PRODUCTION IN GHANA

In 1879, cocoa was introduced in Ghana from Fernando Po and it is attributed to a Ghanaian farmer called Tetteh Quashie [41]. Cocoa has since then become a major backbone of the Ghanaian economy. Ghana in the mid-1960s became the leading producer and exporter of cocoa with 570,000 tons per annum [42]. This is because cocoa production is characterized by its relative advantage of quick maturity and less labour intensive over other economically

important crops like oil palm, coconut, cola and sheer nut at that time. However, due to factors like poor administration, effect of pest, diseases and contamination, cocoa production suffered a decline from over 550,000 tons from the mid-sixties (1964/65) to less than 160,000 tons in 1983/84 in a period of a decade [43]. In 1990s cocoa production in Ghana rejuvenated both in quantity and quality as a result of effective fermentation and drying through the initiative by the Ghana Cocoa Board. As a result of this initiative, there was an improvement in the taste of cocoa from Ghana and to add to this, it was associated with low levels of contamination (from heavy metals, pesticides etc.) as compared to beans from other cocoa producing countries [44]. The intermittent reduction in cocoa production over the years was as a result of the fact that Ghanaian cocoa farmers reverted to traditional methods of controlling pest because they could not afford to buy pesticides. Recently, the greatest challenge confronting cocoa production in Ghana is the ability to increase cocoa yield through the use of these modern and sophisticated methods of averting pest without compromising the quality of cocoa beans for human consumption.

From the time of ripening of the cocoa pods to the marketing and processing of the beans, many processes are adopted by the Ghanaian cocoa farmer in order to ensure the quality of beans produced. This has culminated into the current production of 1,000,000 tons annually.

2.3 PROCESSES INVOLVED IN COCOA HARVESTING

The harvesting of cocoa involves three stages.

1. Pod plucking is the first process undertaken by farmers after being convinced by the conventions that the cocoa is ready to be harvested. This stage involves the physical and the actual harvesting of the cocoa pods from the tree using the hook, knife or cutlass. The precaution here is that much care is taken to make sure that no physical damage is caused to the cocoa tree. The cocoa pods should be plucked at the appropriate time as early harvesting may result in small-sized beans with low fat content over ripened pods will have their pulp

containing less sugar which will negatively affect fermentation processes and last but not the least, may result in salty beans.

On the other hand, late harvesting may expose the beans to rodent attack which will lead to reduction in yield or pods as well as the beans will get rotten.

The next stage is the pod opening . At this stage, cutlass is used or by hitting the pod against a stone. It is done in such a way that the beans in the pods are not damaged. It is believed that if opening is done after a day or two after plucking, it enhances the flavor of the beans. Again, delay for the above stipulated days increases fermentation. It is imperative to separate the placenta from the beans before fermentation to avoid clustering of the beans. Since cocoa beans used for the production of chocolates and other cocoa toffees rely on the flavor which is enhanced by fermentation, it is necessary to go through the fermentation processes well. The most popular fermentation procedure in Ghana is the heap method. In this method, the harvested beans are heaped on banana or plantain leaves and further covered with extra leaves. It usually takes about 6 days for the fermentation to be completed. To ensure uniform fermentation of the beans, the heap is turned and mixed on the 2^{nd} and 4^{th} days within the 6 days of fermentation. After fermentation, drying is employed in order to make the beans ready for export or industrial use.

2.3.1 Some benefits of drying cocoa beans

It removes both water and excess acidity from the beans; it also ensures longer storage of the cocoa beans. The commonest method of drying in Ghana is the use of the sun (sun drying) [45]. During drying, turning is done from time to time to ensure a uniform mixing and exposure of the beans to sunlight. There is a possibility of aerial deposition of some heavy metals onto the shells of the beans during drying.

Through the adoption and implementation of quality control practices which are in concord with the international standard by Cocoa Board, Ghana consistently produces cocoa beans which are of high quality for several years.

One of the cardinal interventions is the "mass spraying" exercise which is under taken four times each year in cocoa-growing Regions of the country. This ensures the prevention of pest and diseases but it also helps to curtail the indiscriminate use of unauthorized pesticides. All these have culminated into the achievement of the one million metric tons of cocoa currently.

2.4 HEALTH EFFECTS OF COCOA POLYPHENOLS

The discovery of polyphenols in cocoa and its products in the early 1909 has given the grounds for a lot of interest in the scientific world [46, 47]. These compounds are not only found in cocoa but also in fruits, red wine and tea [48].

However, apart from the discovery of these compounds in foods, the health effects of these compounds are of great importance to the chemists, food scientists and nutritionists.

Polyphenols have in recent times become the centre of research for many scientists due to their health benefits to man. Some of these benefits include; anti-carcinogenic, anti-microbial and anti-inflammatory effects. The type of polyphenol present in cocoa products has been found to be flavanols (flavan -3-ols).

They form part of flavonoid which is also a sub- class of polyphenols. The flavanols in cocoa may be monomeric, dimeric or polymeric. The two main monomeric flavanols present in cocoa are:

(-) – epicatechins and (+)-catechins. Their structures are presented below;

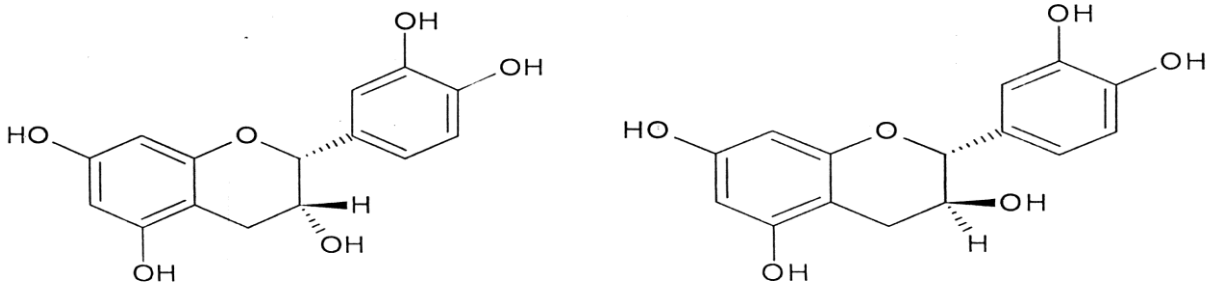


Figure 2.1 Molecular structures of monomeric flavanols in Cocoa

The two main types of dimeric flavanol which occur in cocoa are: Proanthocyanidin B_2 and B5 since the dimeric flavanols are also called proanthocyanidins. The polymeric combinations of these monomers called procoyanidins of chains up to 10 units have been found in cocoa [49]. The major components of the polyphenols in cocoa beans are the epicatechins.

These polyphenols content in the products of cocoa depend on several factors and conditions. These determinant factors range from biological to processing conditions. To develop a desired flavor in cocoa beans, optimum fermentation and drying of the cocoa beans are crucial.

Depending on the atmospheric conditions, fermentation takes between 5 to 6 days. Conversely, fermentation in spite of its importance in the production of quality cocoa beans, has been found to decrease the flavonoid content of cocoa beans [50]. Other processes like drying and roasting also contribute to the decrease in the flavonoid content in cocoa beans. However, as shown by several studies, the intake of cocoa may have cardiovascular benefits through anti-platelet function, higher density of lipoprotein, cholesterol oxidation and

lowering of blood pressure [51, 52]. It has recently been published in a publication on polyphenol content in cocoa bean from different countries that cocoa beans from Ghana are of very high antioxidant properties and so rated to be of high significance to health [53].

2. 5 MECHANISMS OF HEAVY METAL UPTAKE BY PLANT

Contaminant uptake by plants and its mechanisms have been explored by several researchers. It could be used to optimize the factors to improve the performance of plant uptake. According to Sinha et al. [54], the plants act both as "accumulators" and "excluders". Accumulators survive despite concentrating contaminants in their aerial tissues. The contaminants biodegrade or bio transform into inert forms in their tissues. The excluders restrict contaminant uptake into their biomass. Plants have evolved highly specific and very efficient mechanisms to obtain essential micronutrients from the environment, even when present at low ppm levels. Plant roots, aided by plant-produced chelating agents and plant-induced pH changes and redox reactions, are able to solubilize and take up micronutrients from very low levels in the soil, even from nearly insoluble precipitates. Plants have also evolved highly specific mechanisms to translocate and store micronutrients. These same mechanisms are also involved in the uptake, translocation, and storage of toxic elements, whose chemical properties simulate those of essential elements. Thus, micronutrient uptake mechanisms are of great interest to phytoremediation [55].

The range of known transport mechanisms or specialized proteins embedded in the plant cell plasma membrane involved in ion uptake and translocation include (1) proton pumps (″-ATPase's that consume energy and generate electrochemical gradients), (2) co- and anti-transporters (proteins that use the electrochemical gradients generated by ″-ATPase to drive the active uptake of ions), and (3) channels (proteins that facilitate the transport of ions into the cell). Each transport mechanism is likely to take up a range of ions. A basic problem is the interaction of ionic species during uptake of various heavy metal contaminants. After

uptake by roots, translocation into shoots is desirable because the harvest of root biomass is generally not feasible. Little is known regarding the forms in which metal ions are transported from the roots to the shoots [55].

Plant uptake-translocation mechanisms are likely to be closely regulated. Plants generally do not accumulate trace elements beyond near-term metabolic needs. These requirements are small ranging from 10 to 15 ppm of most trace elements that suffice for most needs [55]. The exceptions are "hyper accumulator" plants, which can take up toxic metal ions at levels in the thousands of ppm. Another issue is the form in which toxic metal ions are stored in plants, particularly in hyper accumulating plants, and how these plants avoid metal toxicity. Multiple mechanisms are involved. Storage in the vacuole appears to be a major one [55].

Water, evaporating from plant leaves, serves as a pump to absorb nutrients and other soil substances into plant roots. This process, termed evapotranspiration, is responsible for moving contamination into the plant shoots as well. Since contamination is from roots to the shoots, which are harvested, contamination is removed while leaving the original soil undisturbed. Some plants that are used in phytoextraction strategies are termed "hyper accumulators." They are plants that achieve a shoot-to-root metal-concentration ratio greater than one. Non-accumulating plants typically have a shoot-to-root ratio considerably less than one. Ideally, hyper accumulators should thrive in toxic environments, require little maintenance and produce high biomass, although few plants perfectly fulfill these requirements [56].

Metal accumulating plant species can concentrate heavy metals like Cd, Zn, Co, Mn, Ni, and Pb up to 100 or 1000 times those taken up by non-accumulator (excluder) plants. In most cases, microorganisms, bacteria and fungi, living in the rhizosphere closely associated with plants, may contribute to mobilize metal ions, increasing the bioavailable fraction. Their role

in eliminating organic contaminants is even more significant than that in case of inorganic compounds [57, 58]. Heavy metal uptake by plant through phytoremediation technologies is using these mechanisms of phytoextraction, phytostabilisation, rhizofiltration, and phytovolatilization as shown in figure 2.2

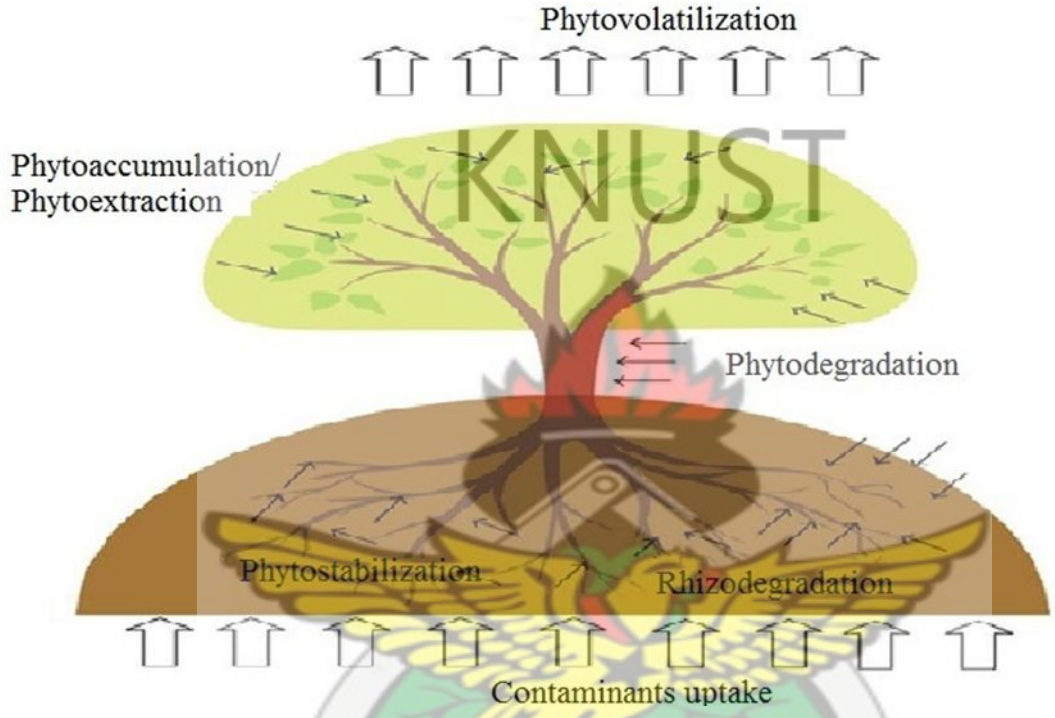


Figure 2:2: Mechanisms of heavy metals uptake by plant through phytoremediation process. (61)

Phytoextraction is the uptake/absorption and translocation of contaminants by plant roots into the above ground portions of the plants (shoots) that can be harvested and burned gaining energy and recycling the metal from the ash [63].

Phytostabilisation is the use of certain plant species to immobilize the contaminants in the soil and groundwater through absorption and accumulation in plant tissues, adsorption onto roots, or precipitation within the root zone preventing their migration in soil, as well as their movement by erosion and deflation [63].

Rhizofiltration is the adsorption or precipitation onto plant roots or absorption into and sequesterization in the roots of contaminants that are in solution surrounding the root zone by constructed wetland for cleaning up communal wastewater [62].

Phytovolatilization is the uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified form of the contaminant to the atmosphere from the plant.

Phytovolatilization occurs as growing trees and other plants take up water along with the contaminants. Some of these contaminants can pass through the plants to the leaves and volatilize into the atmosphere at comparatively low concentrations [62].

Plants also perform an important secondary role in physically stabilizing the soil with their root system, preventing erosion, protecting the soil surface, and reducing the impact of rain. At the same time, plant roots release nutrients that sustain a rich microbial community in the rhizosphere. Bacterial community composition in the rhizosphere is affected by complex interactions between soil type, plant species, and root zone location. Microbial populations are generally higher in the rhizosphere than in the root-free soil. This is due to a symbiotic relationship between soil microorganisms and plants. This symbiotic relationship can enhance some bioremediation processes. Plant roots also may provide surfaces for sorption or precipitation of metal contaminants [59].

In phytoremediation, the root zone is of special interest. The contaminants can be absorbed by the root to be subsequently stored or metabolized by the plant. Degradation of contaminants in the soil by plant enzymes exuded from the roots is another phytoremediation mechanism [60].

For many contaminants, passive uptake via micropores in the root cell walls may be a major route into the root, where degradation can take place [62].

2.6. SOURCES OF HEAVY METALS IN CONTAMINATED SOILS

Heavy metals occur naturally in the soil environment from the pedogenetic processes of weathering of parent materials at levels that are regarded as trace (<1000 mg kg−1) and rarely toxic [64, 65]. Due to the disturbance and acceleration of nature's slowly occurring geochemical cycle of metals by man, most soils of rural and urban environments may accumulate one or more of the heavy metals above defined background values high enough to cause risks to human health, plants, animals, ecosystems, or other media [66]. The heavy metals essentially become contaminants in the soil environments because (i) their rates of generation via man-made cycles are more rapid relative to natural ones, (ii) they become transferred from mines to random environmental locations where higher potentials of direct exposure occur, (iii) the concentrations of the metals in discarded products are relatively high compared to those in the receiving environment, and (iv) the chemical form (species) in which a metal is found in the receiving environmental system may render it more bioavailable [66]. A simple mass balance of the heavy metals in the soil can be expressed as follows [67, 68]

$$M\,\mathrm{t\,o\,t\,a\,l} = (M\,p + M\,a + M\,f + M\,\mathrm{a\,g} + M\,\mathrm{o\,w} + M\,\mathrm{i\,p}) - (M\,\mathrm{c\,r} + M\,l)$$

where " M " is the heavy metal, " p " is the parent material, " a " is the atmospheric deposition, " f " is the fertilizer sources, " a g " are the agrochemical sources, " o w " are the organic waste sources, " i p " are other inorganic pollutants, " c r " is crop removal, and " l " is the losses by leaching, volatilization, and so forth. It is projected that the anthropogenic emission into the atmosphere, for several heavy metals, is one-to-three orders of magnitude higher than natural fluxes [69]. Heavy metals in the soil from anthropogenic sources tend to be more mobile, hence more bioavailable than pedogenic, or lithogenic ones [60, 71]. Metal-bearing solids at contaminated sites can originate from a wide variety of anthropogenic

sources in the form of metal mine tailings, disposal of high metal wastes in improperly protected landfills, leaded gasoline and lead-based paints, land application of fertilizer, animal manures, biosolids (sewage sludge), compost, pesticides, coal combustion residues, petrochemicals, and atmospheric deposition [21, 73, 74] are discussed hereunder.

2.6.1 Fertilizers

Historically, agriculture was the first major human influence on the soil [75]. To grow and complete the lifecycle, plants must acquire not only macronutrients (N, P, K, S, Ca, and Mg), but also essential micronutrients. Some soils are deficient in the heavy metals (such as Co, Cu, Fe, Mn, Mo, Ni, and Zn) that are essential for healthy plant growth [76], and crops may be supplied with these as an addition to the soil or as a foliar spray. Cereal crops grown on Cu-deficient soils are occasionally treated with Cu as an addition to the soil, and Mn may similarly be supplied to cereal and root crops. Large quantities of fertilizers are regularly added to soils in intensive farming systems to provide adequate N, P, and K for crop growth. The compounds used to supply these elements contain trace amounts of heavy metals (e.g., Cd and Pb) as impurities, which, after continued fertilizer, application may significantly increase their content in the soil [77]. Metals, such as Cd and Pb, have no known physiological activity. Application of certain phosphatic fertilizers inadvertently adds Cd and other potentially toxic elements to the soil, including Hg, and Pb [78].

2.6.2 Pesticides

Several common pesticides used fairly extensively in agriculture and horticulture in the past contained substantial concentrations of metals. For instance in the recent past, about 10% of the chemicals that were approved for use as insecticides and fungicides in UK were based on compounds which contain Cu, Hg, Mn, Pb, or Zn. Examples of such pesticides are copper-containing fungicidal sprays such as Bordeaux mixture (copper sulphate) and copper oxychloride [77]. Lead arsenate was used in fruit orchards for many years to control some

parasitic insects. Arsenic-containing compounds were also used extensively to control cattle ticks and to control pests in banana in New Zealand and Australia, timbers have been preserved with formulations of Cu, Cr, and As (CCA), and there are now many derelict sites where soil concentrations of these elements greatly exceed background concentrations. Such contamination has the potential to cause problems, particularly if sites are redeveloped for other agricultural or nonagricultural purposes. Compared with fertilizers, the use of such materials has been more localized, being restricted to particular sites or crops [79].

2.6.3 Bio solids and Manures

The application of numerous biosolids (e.g., livestock manures, composts, and municipal sewage sludge) to land inadvertently leads to the accumulation of heavy metals such as As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Tl, Sb, and so forth, in the soil [74]. Certain animal wastes such as poultry, cattle, and pig manures produced in agriculture are commonly applied to crops and pastures either as solids or slurries [80]. Although most manure are seen as valuable fertilizers, in the pig and poultry industry, the Cu and Zn added to diets as growth promoters and As contained in poultry health products may also have the potential to cause metal contamination of the soil [80, 81]. The manures produced from animals on such diets contain high concentrations of As, Cu, and Zn and, if repeatedly applied to restricted areas of land, can cause considerable buildup of these metals in the soil in the long run.

Biosolids (sewage sludge) are primarily organic solid products, produced by wastewater treatment processes that can be beneficially recycled [59]. Land application of biosolids materials is a common practice in many countries that allow the reuse of biosolids produced by urban populations [63]. The term sewage sludge is used in many references because of its wide recognition and its regulatory definition. However, the term bio- solids are becoming more common as a replacement for sewage sludge because it is thought to reflect more accurately the beneficial characteristics inherent in sewage sludge [82]. It is estimated that in

the United States, more than half of approximately 5.6 million dry tonnes of sewage sludge used or disposed of annually is land applied, and agricultural utilization of biosolids occurs in every region of the country. In the European community, over 30% of the sewage sludge is used as fertilizer in agriculture [82]. In Australia over 175 000 tonnes of dry biosolids are produced each year by the major metropolitan authorities, and currently most biosolids applied to agricultural land are used in arable cropping situations where they can be incorporated into the soil [80].

There is also considerable interest in the potential for composting biosolids with other organic materials such as sawdust, straw, or garden waste. If this trend continues, there will be implications for metal contamination of soils. The potential of biosolids for contaminating soils with heavy metals has caused great concern about their application in agricultural practices [83]. Heavy metals most commonly found in biosolids are Pb, Ni, Cd, Cr, Cu, and Zn, and the metal concentrations are governed by the nature and the intensity of the industrial activity, as well as the type of process employed during the biosolids treatment [84]. Under certain conditions, metals added to soils in applications of biosolids can be leached downwards through the soil profile and can have the potential to contaminate groundwater [85]. Recent studies on some New Zealand soils treated with biosolids have shown increased concentrations of Cd, Ni, and Zn in drainage leachates [86, 87].

2.6.4 Wastewater

The application of municipal and industrial wastewater and related effluents to land dates back 400 years and now is a common practice in many parts of the world [88]. Worldwide, it is estimated that 20 million hectares of arable land are irrigated with waste water. In several Asian and African cities, studies suggest that agriculture based on wastewater irrigation accounts for 50 percent of the vegetable supply to urban areas [54]. Farmers generally are not bothered about environmental benefits or hazards and are primarily interested in maximizing

their yields and profits. Although the metal concentrations in wastewater effluents are usually relatively low, long-term irrigation of land with such can eventually result in heavy metal accumulation in the soil.

2.6.5 Metal Mining and Milling Processes and Industrial Wastes

Mining and milling of metal ores coupled with industries have bequeathed many countries, the legacy of wide distribution of metal contaminants in soil. During mining, tailings (heavier and larger particles settled at the bottom of the flotation cell during mining) are directly discharged into natural depressions, including onsite wetlands resulting in elevated concentrations [55]. Extensive Pb and Zn ore mining and smelting have resulted in contamination of soil that poses risk to human and ecological health. Many reclamation methods used for these sites are lengthy and expensive and may not restore soil productivity. Soil heavy metal environmental risk to humans is related to bioavailability. Assimilation pathways include the ingestion of plant material grown in (food chain), or the direct ingestion (oral bioavailability) of, contaminated soil [56].

Other materials are generated by a variety of industries such as textile, tanning, petrochemicals from accidental oil spills or utilization of petroleum-based products, pesticides, and pharmaceutical facilities and are highly variable in composition. Although some are disposed of on land, few have benefits to agriculture or forestry. In addition, many are potentially hazardous because of their contents of heavy metals (Cr, Pb, and Zn) or toxic organic compounds and are seldom, if ever, applied to land. Others are very low in plant nutrients or have no soil conditioning properties [80].

2.6.6 Air-Borne Sources

Airborne sources of metals include stack or duct emissions of air, gas, or vapor streams, and fugitive emissions such as dust from storage areas or waste piles. Metals from airborne

sources are generally released as particulates contained in the gas stream. Some metals such as As, Cd, and Pb can also volatilize during high-temperature processing. These metals will convert to oxides and condense as fine particulates unless a reducing atmosphere is maintained [57]. Stack emissions can be distributed over a wide area by natural air currents until dry and/or wet precipitation mechanisms remove them from the gas stream. Fugitive emissions are often distributed over a much smaller area because emissions are made near the ground. In general, contaminant concentrations are lower in fugitive emissions compared to stack emissions. The type and concentration of metals emitted from both types of sources will depend on site-specific conditions. All solid particles in smoke from fires and in other emissions from factory chimneys are eventually deposited on land or sea; most forms of fossil fuels contain some heavy metals and this is, therefore, a form of contamination which has been continuing on a large scale since the industrial revolution began. For example, very high concentration of Cd, Pb, and Zn has been found in plants and soils adjacent to smelting works. Another major source of soil contamination is the aerial emission of Pb from the combustion of petrol containing tetraethyl lead; this contributes substantially to the content of Pb in soils in urban areas and in those adjacent to major roads. Zn and Cd may also be added to soils adjacent to roads, the sources being tyres, and lubricant oils [58].

2.7 SOME FACTORS INFLUENCING PLANT UPTAKE OF METALS.

The soil represents the major repository of trace elements over geologic time. On a worldwide basis, soil shows an average composition close to the earth crust but the near – surface parent material from which soils are derived is not uniform and soil-forming processes differ markedly from one climate region to another, accounting for considerable overall variations in trace metal concentrations [89].

The major factor governing the availability of nutrients, metals and ions to plants in the soil is chiefly the uncomplexed ion [90]. This is due to the fact that in order for root uptake to occur,

a soluble species must exist adjacent to the root membrane for some finite period. The form of this solution species will have an enormous influence on its longevity in soil solution, mobility in soils and on the rate and extent of uptake, and perhaps mobility and toxicity in the plant [91]. Once deposited, metal- containing materials are subject to chemical and microbial modification with metal solubility ultimately approaching thermodynamic equilibrium with native soil minerals and organic matter. The rate and extent of solubility are governed by the physicochemical properties of the deposited material, soil processes and soil properties. Evidence indicates that soil microorganisms may play an important role by producing soluble ligands with high affinity for metals [92, 93]. Metals entering the soil as stable organo-complexes, such as those used in fertilization to correct micronutrient deficiencies or those possibly present in discharge from a nuclear fuel separation facility, may be initially highly stable [94, 95]. The duration of solubility and mobility in the soil will be a function of the stability of the complex to be substituted by major competing ions such as Ca and H [96, 97] and the stability of the organic ligand to microbial decomposition [58]. It may be concluded that the soil's physicochemical parameters which are most important in influencing the solubility of metals include: solution composition (organic and inorganic soluble), Eh and pH type, density of charge on soil colloids and reactive surface area [98]. These phenomena will be dependent upon soil properties including metal concentration and form, particle size distribution, quantity and reactivity of hydrous oxides, mineralogy, microbial activities and aeration [90]. These soil properties vary geographically and will be a function of the combined effects of parent material, topography, climate, time, man's activities and biological processes [99].

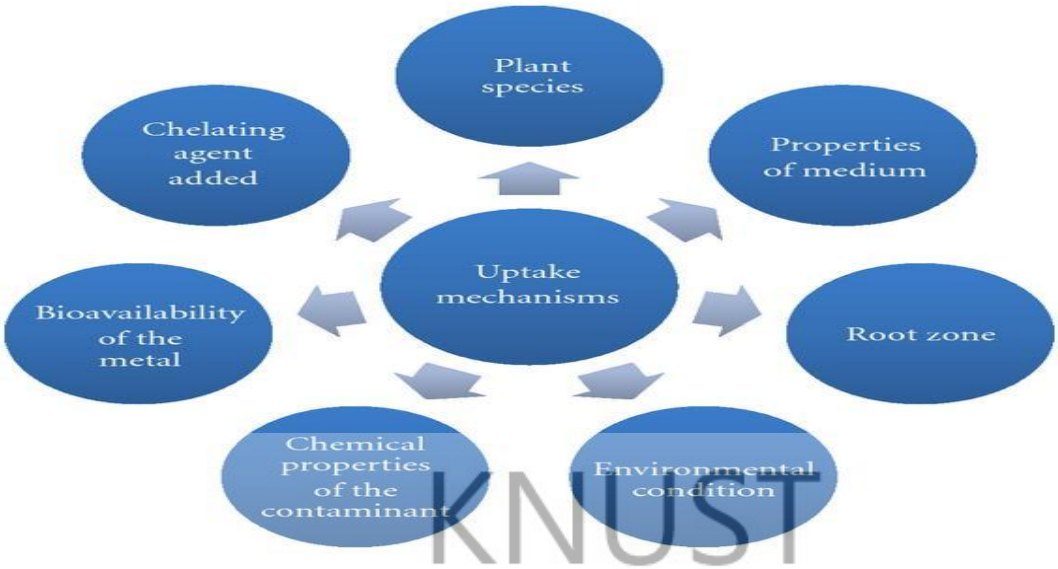


Figure 2.3: Factors affecting the uptake mechanisms of heavy metals. (61)

2.7.1 Properties of Medium

Agronomical practices are developed to enhance remediation (pH adjustment, addition of chelators, fertilizers) [84]. For example, the amount of lead absorbed by plants is affected by the pH, organic matter, and the phosphorus content of the soil. To reduce lead uptake by plants, the pH of the soil is adjusted with lime to a level of 6.5 to 7.0 [74].

2.7.2 The Root Zone

The Root Zone is of special interest in phytoremediation. It can absorb contaminants and store or metabolize it inside the plant tissue. Degradation of contaminants in the soil by plant enzymes exuded from the roots is another phytoremediation mechanism. A morphological adaptation to drought stress is an increase in root diameter and reduced root elongation as a response to less permeability of the dried soil [60].

2.7.3 Vegetative Uptake

Vegetative Uptake is affected by the environmental conditions [100]. The temperature affects growth of substances and consequently, root length. Root structure under field conditions differs from that under greenhouse condition [60]. The success of

phytoremediation, more specifically phytoextraction, depends on a contaminant-specific hyperaccumulator [101]. Understanding mass balance analyses and the metabolic fate of pollutants in plants are the keys to proving the applicability of phytoremediation [102].

Metal uptake by plants depends on the bioavailability of the metal in the water phase, which in turn depends on the retention time of the metal, as well as the interaction with other elements and substances in the water. Furthermore, when metals have been bound to the soil, the pH, redox potential, and organic matter content will all affect the tendency of the metal to exist in ionic and plant-available form. Plants will affect the soil through their ability to lower the pH and oxygenate the sediment, which affects the availability of the metals [103], increasing the bioavailability of heavy metals by the addition of biodegradable physicochemical factors, such as chelating agents and micronutrients [87].

2.7.4 Addition of Chelating Agent

The increase of the uptake of heavy metals by the energy crops can be influenced by increasing the bioavailability of heavy metals through addition of biodegradable physicochemical factors such as chelating agents, and micronutrients, and also by stimulating the heavy-metal-uptake capacity of the microbial community in and around the plant. This faster uptake of heavy metals will result in shorter and, therefore, less expensive remediation periods. However, with the use of synthetic chelating agents, the risk of increased leaching must be taken into account [87]. The use of chelating agents in heavy-metal-contaminated soils could promote leaching of the contaminants into the soil. Since the bioavailability of heavy metals in soils decreases above pH 5.5–6, the use of a chelating agent is warranted, and may be required, in alkaline soils. It was found that exposing plants to EDTA for a long period (2 weeks) could improve metal translocation in plant tissue as well as the overall phytoextraction performance. The application of a synthetic chelating agent (EDTA) at 5 mmol/kg yielded positive results [79]. Plant roots exude organic acids such as citrate and

oxalate, which affect the bioavailability of metals. In chelate-assisted phytoremediation, synthetic chelating agents such as NTA and EDTA are added to enhance the phytoextraction of soil-polluting heavy metals. The presence of a ligand affects the biouptake of heavy metals through the formation of metal-ligand complexes and changes the potential to leach metals below the root zone [104].

2.8 METHODS FOR ANALYSING HEAVY METALS.

The determination of heavy metals in organic matrices requires analytical techniques with high sensitivity and low detection limits. These methods with the above-mentioned specifications include inductively coupled plasma mass spectrometry (ICP –MS), inductively coupled plasma-optical emission spectrometry (ICP-OES), Electro-thermal atomic absorption spectrometry (ETAAS) which is the most widely used, flame atomic absorption spectrometry (F-AAS), graphite furnace spectrometry (GF-AAS) and X-ray fluorescence, neutron activation analysis (NAA).

2.8.1 Principles Of Atomic Absorption Spectroscopy

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert's Law. In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, that is wavelength, is specific to a particular electron transition in a particular element. In general, each wave length corresponds to only one element, and the width of an absorption line is only of the order of a few Pico meters (pm), which gives the technique its elemental selectivity.

The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer –Lambert's Law.

The instrumentation is designed in such a way that, in order to analyze a sample for its atomic content, it is to be atomized. The atomizers most commonly used nowadays are flames and electro thermal (graphite tube) atomizers. The atoms should then be irradiated by optical radiation and the radiation source could be an element-specific line radiation source or a continuum radiation source. The radiation then passes through a monochromator in order to separate the element-specific radiation from any other radiation emitted by the radiation source, which is finally measured by a detector. Figure 2.2 shows a block diagram of atomic absorption spectrometer.

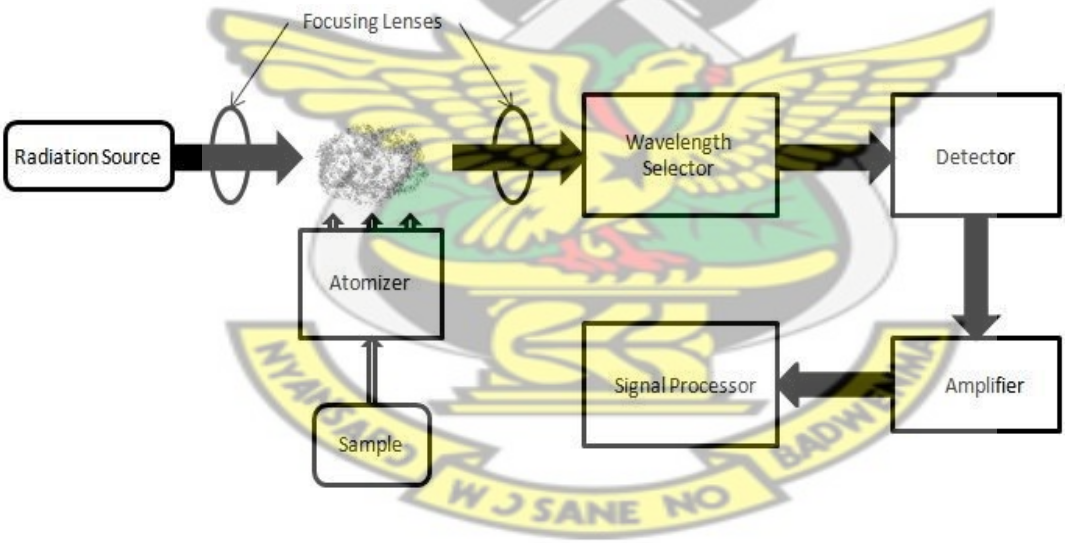


Figure 2.4: Schematic diagram of the operation of atomic absorption spectrometer [105]

2.9 TOXICITY OF THE ANALYSED HEAVY METAL

A heavy metal is a member of a loosely- defined subset of elements that exhibit metallic properties. It mainly includes the transition metals, some metalloids, lanthanides, and actinides. Many different definitions have been proposed –some based on density, some on

atomic number or atomic weight and some on chemical properties or toxicity [106]. Heavy metals constitute a very heterogeneous group of elements widely varied in their chemical properties and biological functions. The term heavy metals" defined as commonly held for those metals, which have specific weights more than $5gcm^{-3}$ [107]. Heavy metals are kept under environmental pollutant category due to their toxic effects in plants, humans and food.

The toxicity of these metals has two main aspects: (a) the fact that they have no known metabolic functions, but when present in the body they disrupt normal cellular processes, leading to toxicity in a number of organs; (b) the potential , particularly, of cadmium and lead and mercury to accumulate in the biological tissues by a process known as bioaccumulation. This occurs because the metals, once taken up into the body, are stored in particular organs like the kidney and liver which are excreted at a slow rate compared with their intake. The toxicities of the individual heavy metals are discussed below:

2.9.1 Lead (Pb)

The toxic effects of lead, like those of mercury, have been principally established in studies on people exposed to lead in the course of their work. Short-term exposure to high levels of lead may affect brain function by interfering with neurotransmitter release and synapse formation [108]. Exposure to lead has been associated with reduced IQ, Learning disabilities, slow growth, hyperactive, antisocial behavior and impaired hearing. Lead is known to damage the kidney, liver and reproductive system, basic cellular processes and brain function [109]. In addition to this, the most critical effect of low-level lead exposure is an intellectual development of young children and, like mercury, lead easily crosses the placental barrier and accumulates in the foetus.

2.9.2 Cadmium (Cd)

Cadmium can mainly be found in the earth's crust. It always occurs in combination with Zinc (Zn). Cadmium is also an inevitable by –product of industrial zinc, lead and copper extraction.

After being applied, it enters the environment mainly through the ground, because it is found in manures and pesticides. The principal toxic effect of cadmium is its toxicity to the kidney, although it has also been associated with lung damage (including induction of lung tumor) and skeletal changes in occupationally exposed populations. Cadmium is relatively poorly absorbed into the body, but once absorbed, it is rather slowly excreted. Like other metals, it accumulates in the kidney causing renal damage. An exposure to significantly higher cadmium levels occur when people smoke. Tobacco smoke is first transported to the liver through the blood. There, it is bond to protein to form complexes that are transported to the kidney. This accumulation damages the filtering mechanism of the kidney causing difficulty in the excretion of essential proteins and sugars from the body.

2.9.3 Copper (Cu)

Research shows that short period of exposure to high levels of copper can cause gastrointestinal disturbance, including diarrhoea, stomach cramps, nausea and vomiting. Using water with elevated levels of copper over many years may cause liver and kidney damage. The seriousness of these effects can be expected to increase with increased copper levels or length of exposure.

Children under one year of age are more sensitive to copper than adults. Other persons who are highly susceptible to copper toxicity include people with liver damage or Wilson's disease [110].

Long-term exposure to copper can cause irritation of the nose, mouth and eyes and can also cause headaches, stomach- aches, dizziness etc. High uptake of copper may lead to liver and Kidney damage and even death.

2.9.4 Zinc (Zn)

Although Zinc is an essential requirement for good health, excess zinc can be harmful. Excessive absorption of the metal suppresses copper and iron absorption [111]. The U.S. Food and Drugs Administration (FDA) has stated that zinc damages nerve receptors in the nose, which can cause anosmia. Reports of anosmia were also observed in the 1930s when Zn preparations were used in a failed attempt to prevent polio infections [112]. The problem with consuming too much zinc is that it actually prohibits one's metabolism from absorbing the other vitamins and minerals needed by the body. Again, Zn toxicity lowers the body's immunity and good cholesterol levels. In August 2008, the journal **"Neurology"** reported on four patients suffering from neuropathy and other neurological symptoms typical of zinc poisoning and copper deficiencies.

2.9.5 Iron (Fe)

The University of Maryland Medical Centre reports that there may be evidence that high iron levels may increase one's risk of certain cancers, including breast cancer. The " Journal of clinical Biochemistry and Nutrition" also notes that breast cancer is the most common type of cancer among women around the globe, and excessive iron intake may be one reason why so many women- develop this type of cancer [113] due to the storing of excess amount of iron in the body may also increase the risk of heart disease.

The office of Dietary Supplements of the National Institute of Health notes that too much iron accumulation in the body, if encouraged can lead to heart diseases. [114]

2.9.6 Manganese (Mn)

Manganese is a common element in the earth's crust, water and particulate matter in the atmosphere. This element is used in manufacturing steel, dry cell batteries, electrical coils, ceramics, fertilizers and fungicides. However, toxic effects from oral ingestion of manganese are rare and include lethargy, changes in muscle tone and posture, coma and involuntary movements.

2.10 REMEDIATION OF HEAVY METALS FROM CONTAMINATED SOILS

The overall objective of any soil remediation approach is to create a final solution that is protective of human health and the environment [115]. Remediation is generally subjecedt to an array of regulatory requirements and can also be based on assessments of human health and ecological risks where no legislated standards exist or where standards are advisory. The regulatory authorities will normally accept remediation strategies that centre on reducing metal bioavailability only if reduced bioavailability is equated with reduced risk, and if the bioavailability reductions are demonstrated to be long term [115]. For heavy metal-contaminated soils, the physical and chemical form of the heavy metal contaminant in soil strongly influences the selection of the appropriate remediation approach. Information about the physical characteristics of the site, type and level of contamination at the site must be obtained to enable accurate assessment of site contamination and remedial alternatives. The contaminant in the soil should be characterized in order to establish the type, amount, and distribution of heavy metals in the soil. Once the site has been characterized, the desired level of each metal in the soil must be determined. This is done by comparison of observed heavy metal concentrations with soil quality standards for a particular regulatory domain, or by performance of a site-specific risk assessment. Remediation goals for heavy metals may be set as total metal concentration or as leachable metals in soil, or as some combination of these. Several technologies exist for the remediation of metal-contaminated soils. Gupta et al.

[116] have classified remediation technologies of contaminated soils into three categories of hazard-alleviating measures: (i) gentle in situ remediation, (ii) in situ harsh soil restrictive measures, and (iii) in situ or ex situ harsh soil destructive measures. The goal of the last two harsh alleviating measures is to avert hazards either to man, plant, or animal while the main goal of gentle in situ remediation is to restore the malfunctionality of soil (soil fertility), which allows a safe use of the soil. At present, a variety of approaches have been suggested for remediating contaminated soils. USEPA [117] has broadly classified remediation technologies for contaminated soils into (i) source control and (ii) containment remedies. Source control involves in situ and ex situ treatment technologies for sources of contamination. In situ or in place means that the contaminated soil is treated in its original place; unmoved, unexcavated; remaining at the site or in the subsurface. In situ treatment technologies treat or remove the contaminant from soil without excavation or removal of the soil .Ex situ means that the contaminated soil is moved, excavated, or removed from the site or subsurface. Implementation of ex situ remedies requires excavation or removal of the contaminated soil. Containment remedies involve the construction of vertical engineered barriers (VEB), caps, and liners used to prevent the migration of contaminants. Another classification places remediation technologies for heavy metal-contaminated soils under five categories of general approaches to remediation: isolation, immobilization, toxicity reduction, physical separation, and extraction [118]. In practice, it may be more convenient to employ a hybrid of two or more of these approaches for more cost effectiveness. The key factors that may influence the applicability and selection of any of the available remediation technologies are: (i) cost, (ii) long-term effectiveness/permanence, (iii) commercial availability, (iv) general acceptance, (v) applicability to high metal concentrations, (vi) applicability to mixed wastes (heavy metals and organics), (vii) toxicity reduction, (viii) mobility reduction, and (ix)

volume reduction. Soil washing, phytoremediation, and immobilization techniques are among the best demonstrated available technologies (BDATs) for heavy metal-contaminated sites.

2.10.1 Immobilization technique

Ex- situ and in- situ immobilization techniques are practical approaches to remediation of metal-contaminated soils. The ex-situ technique is applied in areas where the highly contaminated soil must be removed from its place of origin, and its storage is connected with a high ecological risk (e.g., in the case of radio nuclides). The advantages of the method are: (i) fast and easy applicability and (ii) relatively low costs of investment and operation. The disadvantages include (i) high invasivity to the environment, (ii) generation of a significant amount of solid wastes (twice as large as volume after processing), (iii) the by-product must be stored on a special landfill site, (iv) in the case of changing of the physicochemical condition in the side product or its surroundings, there is serious danger of the release of additional contaminants to the environment, and (v) permanent control of the stored wastes is required. In the in-situ technique, the fixing agents amendments are applied on the unexcavated soil. The technique's advantages are (i) its low invasivity, (ii) simplicity and rapidity, (iii) relatively inexpensive, and (iv) small amount of wastes are produced, (v) high public acceptability, (vi) covers a broad spectrum of inorganic pollutants. The disadvantages of this immobilization are (i) it is only a temporary solution (contaminants are still in the environment), (ii) the activation of pollutants may occur when soil physicochemical properties change, (iii) the reclamation process is applied only to the surface layer of soil (30–50 cm), and (iv) permanent monitoring is necessary [115, 119]. The immobilization technology often uses organic and inorganic amendment to accelerate the attenuation of metal mobility and toxicity in soils. The primary role of immobilizing amendments is to alter the original soil metals to more geochemically stable phases via sorption, precipitation, and complexation processes [120]. The mostly applied amendments include clay, cement,

zeolites, minerals, phosphates, organic composts, and microbes [118]. Recent studies have indicated the potential of low-cost industrial residues such as red mud [121] and termitaria [122] in immobilization of heavy metals in contaminated soils. Due to the complexity of soil matrix and the limitations of current analytical techniques, the exact immobilization mechanisms have not been clarified, which could include precipitation, chemical adsorption and ion exchange, surface precipitation, formation of stable complexes with organic ligands, and redox reaction [123]. Most immobilization technologies can be performed ex -situ or in-situ. In situ processes are preferred due to the lower labour and energy requirements, but implementation will depend on specific site conditions.

2.10.2 Solidification/ stabilization (s/s)

Solidification involves the addition of binding agents to a contaminated material to impart physical/dimensional stability to contain contaminants in a solid product and reduce access by external agents through a combination of chemical reaction, encapsulation, and reduced permeability/surface area. Stabilization (also referred to as fixation) involves the addition of reagents to the contaminated soil to produce more chemically stable constituents. Conventional S/S is an established remediation technology for contaminated soils and treatment technology for hazardous wastes in many countries in the world [124].The general approach for solidification/stabilization treatment processes involve mixing or injecting treatment agents to the contaminated soils. Inorganic binders such as clay (bentonite and kaolinite), cement, fly ash, blast furnace slag, calcium carbonate, Fe/Mn oxides, charcoal, zeolite [125], and organic stabilizers such as bitumen, composts, and manures [126], or a combination of organic-inorganic amendments may be used. The dominant mechanism by which metals are immobilized is by precipitation of hydroxides within the solid matrix [127]. Solidification/stabilization technologies are not useful for some forms of metal contamination, such as species that exist as oxyanions (e.g., $Cr_2O_7^{2-}$, ASO_3^{-}) or metals that

do not have low-solubility hydroxides (e.g., Hg). Solidification/stabilization may not be applicable at sites containing wastes that include organic forms of contamination, especially if volatile organics are present. Mixing and heating associated with binder hydration may release organic vapors. Pretreatment, such as air stripping or incineration, may be used to remove the organics and prepare the waste for metal stabilization/solidification [128]. The application of S/S technologies will also be affected by the chemical composition of the contaminated matrix, the amount of water present, and the ambient temperature. These factors can interfere with the solidification/stabilization process by inhibiting bonding of the waste to the binding material, retarding the setting of the mixtures, decreasing the stability of the matrix, or reducing the strength of the solidified area [129]. Cement-based binders and stabilizers are common materials used for implementation of S/S technologies [130]. Portland cement, a mixture of calcium silicates, aluminates, aluminoferrites, and sulfates, is an important cement-based material. Pozzolanic materials, which consist of small spherical particles formed by coal combustion (such as fly ash) and in lime and cement kilns, are also commonly used for S/S. Pozzolans exhibit cement-like properties, especially if the silica content is high. Portland cement and pozzolans can be used alone or together to obtain optimal properties for a particular site [131]. Organic binders may also be used to treat metals through polymer microencapsulation. This process uses organic materials such as bitumen, polyethylene, paraffins, waxes, and other polyolefins as thermoplastic or thermosetting resins. For polymer encapsulation, the organic materials are heated and mixed with the contaminated matrix at elevated temperatures (120°C to 200°C). The organic materials polymerize and agglomerate the waste, and the waste matrix is encapsulated [84,133]. Organics are volatilized and collected, and the treated material is extruded for disposal or possible reuse (e.g., as paving material) [128]. The contaminated material may require pretreatment to separate rocks and debris and dry the feed material. Polymer encapsulation

requires more energy and more complex equipment than cement-based S/S operations. Bitumen (asphalt) is the cheapest and most common thermoplastic binder [131]. Solidification/stabilization is achieved by mixing the contaminated material with appropriate amounts of binder/stabilizer and water. The mixture sets and cures to form a solidified matrix and contain the waste. The cure time and pour characteristics of the mixture and the final properties of the hardened cement depend upon the composition (amount of cement, pozzolan, and water) of the binder/stabilizer.

Ex- situ S/S can be easily applied to excavated soils because methods are available to provide the vigorous mixing needed to combine the binder/stabilizer with the contaminated material. Pretreatment of the waste may be necessary to screen and crush large rocks and debris. Mixing can be performed via in-drum, in-plant, or area-mixing processes. In-drum mixing may be preferred for treatment of small volumes of waste or for toxic wastes. In-plant processes utilize rotary drum mixers for batch processes or pug mill mixers for continuous treatment. Larger volumes of waste may be excavated and moved to a contained area for area mixing. This process involves layering the contaminated material with the stabilizer/binder, and subsequent mixing with a backhoe or similar equipment. Mobile and fixed treatment plants are available for ex- situ S/S treatment. Smaller pilot-scale plants can treat up to 100 tons of contaminated soil per day while larger portable plants typically process 500 to over 1000 tons per day [128]. Solidification/stabilization techniques are available to provide mixing of the binder/stabilizer with the contaminated soil in situ. In situ S/S is less labor and energy intensive than ex situ process that requires excavation, transport, and disposal of the treated material. In situ S/S is also preferred if volatile or semivolatile organics are present because excavation would expose these contaminants to the air [132]. However, the presence of bedrock, large boulders cohesive soils, oily sands, and clays may preclude the application of in situ S/S at some sites. It is also more difficult to provide uniform and complete mixing

through in situ processes. Mixing of the binder and contaminated matrix may be achieved using in-place mixing, vertical auger mixing, or injection grouting. In-place mixing is similar to ex situ area mixing except that the soil is not excavated prior to treatment. The in situ process is useful for treating surface or shallow contamination and involves spreading and mixing the binders with the waste using conventional excavation equipment such as draglines, backhoes, or clamshell buckets. Vertical auger mixing uses a system of augers to inject and mix the binding reagents with the waste. Larger (6–12 ft diameter) augers are used for shallow (10–40 ft) drilling and can treat 500–1000 cubic yards per day [133]. Deep stabilization/solidification (up to 150 ft) can be achieved by using ganged augers (up to 3 ft in diameter each) that can treat 150–400 cubic yards per day. Finally injection grouting may be performed to inject the binder containing suspended or dissolved reagents into the treatment area under pressure. The binder permeates the surrounding soil and cures the place [128].

2.10.3 Vitrification

The mobility of metal contaminants can be decreased by high-temperature treatment of the contaminated area that results in the formation of vitreous material, usually an oxide solid. During this process, the increased temperature may also volatilize and/or destroy organic contaminants or volatile metal species (such as Hg) that must be collected for treatment or disposal. Most soils can be treated by vitrification, and a wide variety of inorganic and organic contaminants can be targeted. Vitrification may be performed ex situ or in situ although in situ processes are preferred due to the lower energy requirements and cost [134]. Typical stages in ex situ vitrification processes may include excavation, pretreatment, mixing, feeding, melting and vitrification, off-gas collection and treatment, and forming or casting of the melted product. The energy requirement for melting is the primary factor influencing the cost of ex situ vitrification. Different sources of energy can be used for this purpose, depending on local energy costs. Process heat losses and water content of the feed should be

controlled in order to minimize energy requirements. Vitrified material with certain characteristics may be obtained by using additives such as sand, clay, and/or native soil. The vitrified waste may be recycled and used as clean fill, aggregate, or other reusable materials [128]. In situ vitrification (ISV) involves passing electric current through the soil using an array of electrodes inserted vertically into the contaminated region. Each setting of four electrodes is referred to as a melt. If the soil is too dry, it may not provide sufficient conductance, and a trench containing flaked graphite and glass frit (ground glass particles) must be placed between the electrodes to provide an initial flow path for the current. Resistance heating in the starter path melts the soil. The melt grows outward and down as the molten soil usually provides additional conductance for the current. A single melt can treat up to 1000 tons of contaminated soil to depths of 20 feet, at a typical treatment rate of 3 to 6 tons per hour. Larger areas are treated by fusing together multiple individual vitrification zones. The main requirement for in situ vitrification is the ability of the soil melt to carry current and solidify as it cools. If the alkali content (as Na_2O and K_2O) of the soil is too high (1.4 wt. %), the molten soil may not provide enough conductance to carry the current [89,135]. Vitrification is not a classical immobilization technique. The advantages include (i) easily applied for reclamation of heavily contaminated soils (Pb, Cd, Cr, asbestos, and materials containing asbestos), (ii) in the course of applying this method, qualification of wastes (from hazardous to neutral) could be changed.

2.10.4 Assessment of Efficiency and Capacity Of Immobilization

The efficiency (E) and capacity (P) of different additives for immobilization and field applications can be evaluated using the expressions:

$$\boldsymbol{E}(\%) = \frac{\boldsymbol{M_0 - M_e}}{\boldsymbol{M_0}} \times \mathbf{100},$$

$$\boldsymbol{P} = \frac{(\boldsymbol{M_0 - M_e})\boldsymbol{V}}{\mathbf{m}},$$

Where E = efficiency of immobilization agent; P = capacity of immobilization agent; M_e = equilibrium extractable concentration of single metal in the immobilized soil (mg L−1); M_o = initial extractable concentration of single metal in preimmobilized soil (mg L−1); V = volume of metal salt solution (mg L−1); m = weight of immobilization agent (g) [136]. High values of E and P represent the perfect efficiency and capacity of an additive that can be used in field studies of metal immobilization. After screening out the best efficient additive, another experiment could be conducted to determine the best ratio (soil/additive) for the field-fixing treatment. After the fixing treatment of contaminated soils, a lot of methods including biological and physiochemical experiments could be used to assess the remediation efficiency. Environmental risk could also be estimated after confirming the immobilized efficiency and possible release [135].

2.10.5 Soil Washing

Soil washing is essentially a volume reduction/waste minimization treatment process. It is done on the excavated (physically removed) soil (ex situ) or on-site (in situ). Soil washing as discussed in this review refers to ex situ techniques that employ physical and/or chemical procedures to extract metal contaminants from soils. During soil washing, (i) those soil particles which host the majority of the contamination are separated from the bulk soil fractions (physical separation), (ii) contaminants are removed from the soil by aqueous chemicals and recovered from solution on a solid substrate (chemical extraction), or (iii) a combination of both [137]. In all cases, the separated contaminants then go to hazardous waste landfill (or occasionally are further treated by chemical, thermal, or biological processes). By removing the majority of the contaminants from the soil, the bulk fraction that remains can be (i) recycled on the site being remediated as relatively inert backfill, (ii) used on another site as fill, or (iii) disposed of relatively cheaply as nonhazardous material. Ex situ soil washing is particularly frequently used in soil remediation because it (i) completely

removes the contaminants and hence ensures the rapid cleanup of a contaminated site [138], (ii) meets specific criteria, (iii) reduces or eliminates long-term liability, (iv) may be the most cost-effective solution, and (v) may produce recyclable material or energy [139]. The disadvantages of the technique include the fact that the contaminants are simply moved to a different place, where they must be monitored, the risk of spreading contaminated soil and dust particles during removal and transport of contaminated soil, and the relatively high cost. Excavation can be the most expensive option when large amounts of soil must be removed, or disposal as hazardous or toxic waste is required .Acid and chelator soil washing are the two most prevalent removal methods [140]. Soil washing currently involves soil flushing an in situ process in which the washing solution is forced through the in-place soil matrix, ex situ extraction of heavy metals from the soil slurry in reactors, and soil heap leaching. Another heavy metal removal technology is electroremediation, which mostly involves electrokinetic movement of charged particles suspended in the soil solution, initiated by an electric gradient [141]. The metals can be removed by precipitation at the electrodes. Removal of the majority of the contaminants from the soil does not mean that the contaminant-depleted bulk is totally contaminant free. Thus, for soil washing to be successful, the level of contamination in the treated bulk must be below a site-specific action limit (e.g., based on risk assessment). Cost effectiveness with soil washing is achieved by offsetting processing costs against the ability to significantly reduce the amount of material requiring costly disposal at a hazardous waste landfill [142].Typically, the cleaned fractions from the soil washing process should be >70–80% of the original mass of the soil, but, where the contaminants have a very high associated disposal cost, and/or where transport distances to the nearest hazardous waste landfill are substantial, a 50% reduction might still be cost effective. There is also a generally held opinion that soil washing based on physical separation processes is only cost effective for sandy and granular soils where the clay and silt content (particles less than 0.063 mm) is less

than 30–35% of the soil. Soil washing by chemical dissolution of the contaminants is not constrained by the proportion of clay; as this fraction can also be leached by the chemical agent. However, clay-rich soils pose other problems such as difficulties with materials handling and solid-liquid separation [143]. Full-scale soil washing plants exist as fixed centralized treatment centres, or as mobile/transportable units. With fixed centralized facilities, contaminated soil is brought to the plant, whereas with mobile/transportable facilities, the plant is transported to a contaminated site, and soil is processed on the site. Where mobile/transportable plant is used, the cost of mobilization and demobilization can be significant. However, where large volumes of soil are to be treated, this cost can be more than offset by reusing clean material on the site (therefore avoiding the cost of transport to an off-site centralized treatment facility, and avoiding the cost of importing clean fill).

2.10.6 Principles of Soil Washing

Soil washing is a volume reduction/waste minimization treatment technology based on physical and/or chemical processes. With physical soil washing, differences between particle grain size, settling velocity, specific gravity, surface chemical behaviour, and rarely magnetic properties are used to separate those particles which host the majority of the contamination from the bulk which are contaminant-depleted. The equipment used is standard mineral processing equipment, which is more generally used in the mining industry [137]. Mineral processing techniques as applied to soil remediation have been reviewed in literature [144].With chemical soil washing, soil particles are cleaned by selectively transferring the contaminants on the soil into solution. Since heavy metals are sparingly soluble and occur predominantly in a sorbed state, washing the soils with water alone would be expected to remove too low an amount of cations in the leachates, chemical agents have to be added to the washing water [145]. This is achieved by mixing the soil with aqueous solutions of acids, alkalis, complexants, other solvents, and surfactants. The resulting cleaned particles are then

separated from the resulting aqueous solution. This solution is then treated to remove the contaminants (e.g., by sorption on activated carbon or ion exchange) [137].The effectiveness of washing is closely related to the ability of the extracting solution to dissolve the metal contaminants in soils. However, the strong bonds between the soil and metals make the cleaning process difficult [99,146]. Therefore, only extractants capable of dissolving large quantities of metals would be suitable for cleaning purposes. The realization that the goal of soil remediation is to remove the metal and preserve the natural soil properties limits the choice of extractants that can be used in the cleaning process [147].

2.10.7 Chemical Extratants for Soil Washing

Owing to the different nature of heavy metals, extracting solutions that can optimally remove them must be carefully sought during soil washing. Several classes of chemicals used for soil washing include surfactants, co-solvents, cyclodextrins, chelating agents, and organic acids [148]. All these soil washing extractants have been developed on a case-by-case basis depending on the contaminant type at a particular site. A few studies have indicated that the solubilization/exchange/extraction of heavy metals by washing solutions differ considerably for different soil types. Strong acids attack and degrade the soil crystalline structure at extended contact times. For less damaging washes, organic acids and chelating agents are often suggested as alternatives to straight mineral acid use [149]. Natural, low-molecular-weight organic acids (LMWOAs) including oxalic, citric, formic, acetic, malic, succinic, malonic, maleic, lactic, aconitic, and fumaric acids are natural products of root exudates, microbial secretions, and plant and animal residue decomposition in soils [150]. Thus metal dissolution by organic acids is likely to be more representative of a mobile metal fraction that is available to biota [151]. The chelating organic acids are able to dislodge the exchangeable, carbonate, and reducible fractions of heavy metals by washing procedures [142]. Although many chelating compounds including citric acid [150], tartaric acid [152], and EDTA [140]

for mobilizing heavy metals have been evaluated, there remain uncertainties as to the optimal choice for full-scale application. The identification and quantification of coexisting solid metal species in the soil before and after treatment are essential to design and assess the efficiency of soil-washing technology [153]. A recent study [154] showed that changes in Ni, Cu, Zn, Cd, and Pb speciation and uptake by maize in a sandy loam before and after washing with three chelating organic acids indicated that EDTA and citric acid appeared to offer greater potentials as chelating agents for remediating the permeable soil. Tartaric acid was however recommended in events of moderate contamination. The use of soil washing to remediate contaminated fine-grained soils that contained more than 30% fine fraction has been reported by several workers [155]. Khodadoust et al. [156] have also studied the removal of various metals (Pb, Ni, and Zn) from field and clay (kaolin) soil samples using a broad spectrum of extractants (chelating agents and organic acids). Chen and Hong [157] reported on the chelating extraction of Pb and Cu from an authentic contaminated soil using derivatives of iminodiacetic acid and L-cyestein. Wuana et al. [158] investigated the removal of Pb and Cu from kaolin and bulk clay soils using two mineral acids (HCl and H_2SO_4) and chelating agents (EDTA and oxalic acid). The use of chelating organic acids—citric acid, tartaric acid and EDTA in the simultaneous removal of Ni, Cu, Zn, Cd, and Pb from an experimentally contaminated sandy loam was carried out by Wuana et al. [154]. These studies furnished valuable information on the distribution of heavy metals in the soils and their removal using various extracting solutions.

2.10.8 Phytoremediation

Phytoremediation, also called green remediation, botanoremediation, agroremediation, or vegetative remediation, can be defined as an in situ remediation strategy that uses vegetation and associated microbiota, soil amendments, and agronomic techniques to remove, contain, or render environmental contaminants harmless [159]. The idea of using metal-accumulating

plants to remove heavy metals and other compounds was first introduced in 1983, but the concept has actually been implemented for the past 300 years on wastewater discharges [160]. Plants may break down or degrade organic pollutants or remove and stabilize metal contaminants. The methods used to phytoremediate metal contaminants are slightly different from those used to remediate sites polluted with organic contaminants. As it is a relatively new technology, phytoremediation is still mostly in its testing stages and as such has not been used in many places as a full-scale application. However, it has been tested successfully in many places around the world for many different contaminants. Phytoremediation is energy efficient, aesthetically pleasing method of remediating sites with low-to-moderate levels of contamination, and it can be used in conjunction with other more traditional remedial methods as a finishing step to the remedial process. The advantages of phytoremediation compared with classical remediation are that (i) it is more economically viable using the same tools and supplies as agriculture, (ii) it is less disruptive to the environment and does not involve waiting for new plant communities to recolonize the site, (iii) disposal sites are not needed, (iv) it is more likely to be accepted by the public as it is more aesthetically pleasing than traditional methods, (v) it avoids excavation and transport of polluted media thus reducing the risk of spreading the contamination, and (vi) it has the potential to treat sites polluted with more than one type of pollutant. The disadvantages are as follow (i) it is dependent on the growing conditions required by the plant (i.e., climate, geology, altitude, and temperature), (ii) large-scale operations require access to agricultural equipment and knowledge, (iii) success is dependent on the tolerance of the plant to the pollutant, (iv) contaminants collected in tissues may be released back into the environment in autumn, (v) contaminants may be collected in woody tissues used as fuel, (vi) time taken to remediate sites far exceeds that of other technologies, (vii) contaminant solubility may be increased leading to greater environmental damage and the possibility of leaching. Potentially useful

phytoremediation technologies for remediation of heavy metal-contaminated soils include phytoextraction (phytoaccumulation), phytostabilization, and phytofiltration [161].

2.10.9 Phytoextraction (Phytoaccumulation)

Phytoextraction is the name given to the process where plant roots uptake metal contaminants from the soil and translocate them to their above soil tissues. A plant used for phytoremediation needs to be heavy-metal tolerant, grow rapidly with a high biomass yield per hectare, have high metal-accumulating ability in the foliar parts, have a profuse root system, and a high bioaccumulation factor [162]. Two approaches have been proposed for phytoextraction of heavy metals, namely, continuous or natural phytoextraction and chemically enhanced phytoextraction [163]

2.10.10 Continuous or Natural Phytoextraction

Continuous phytoextraction is based on the use of natural hyperaccumulator plants with exceptional metal-accumulating capacity. Hyperaccumulators are species capable of accumulating metals at levels 100-fold greater than those typically measured in shoots of the common nonaccumulator plants. Thus, a hyperaccumulator plant will concentrate more than 10 mg kg−1 Hg, 100 mg kg−1 Cd, 1000 mg kg−1 Co, Cr, Cu, and Pb; 10 000 mg kg−1 Zn and Ni [164]. Hyperaccumulator plant species are used on metalliferous sites due to their tolerance of relatively high levels of pollution. Approximately 400 plant species from at least 45 plant families have been so far reported to hyperaccumulate metals [165]; some of the families are Brassicaceae, Fabaceae, Euphorbiaceae, Asterraceae, Lamiaceae, and Scrophulariaceae [166]. Crops like alpine pennycress (Thlaspi caerulescens), Ipomea alpine, Haumaniastrum robertii, Astragalus racemosus, Sebertia acuminate have very high bioaccumulation potential for Cd/Zn, Cu, Co, Se, and Ni, respectively [165]. Willow (Salix viminalis L.), Indian mustard (Brassica juncea L.), corn (Zea mays L.), and sunflower (Helianthus annuus L.) have reportedly shown high uptake and tolerance to heavy metals

[167]. A list of some plant hyperaccumulators are given in Table 6. A number of processes are involved during phytoextraction of metals from soil and these involve: (i) a metal fraction is sorbed at root surface, (ii) bioavailable metasl move across cellular membranes into root cells, (iii) a fraction of the metal absorbed into roots is immobilized in the vacuole, (iv) intracellular mobile metal crosses cellular membranes into root vascular tissue (xylem), and (v) metal is translocated from the root to aerial tissues (stems and leaves) [165]. Once inside the plant, most metals are too insoluble to move freely in the vascular system so they usually form carbonate, sulphate, or phosphate precipitate immobilizing them in apoplastic (extracellular) and symplastic (intracellular) compartments [168]. Hyperaccumulators have several beneficial characteristics but may tend to be slow growing and produce low biomass, and years or decades are needed to clean up contaminated sites when using them. To overcome these shortfalls, chemically enhanced phytoextraction has been developed. The approach makes use of high biomass crops that are induced to take up large amounts of metals when their mobility in soil is enhanced by chemical treatment with chelating organic acids [169].

2.10.11 Chelate-Assisted (Induced) Phytoextraction

For more than 10 years, chelant-enhanced phytoextraction of metals from contaminated soils have received much attention as a cost-effective alternative to conventional techniques of enhanced soil remediation [169]. When the chelating agent is applied to the soil, metal-chelant complexes are formed and taken up by the plant, mostly through a passive apoplastic pathway [169]. Unless the metal ion is transported as a noncationic chelate, apoplastic transport is further limited by the high cation exchange capacity of cell walls [168]. Chelators have been isolated from plants that are strongly involved in the uptake of heavy metals and their detoxification. The chelating agent EDTA has become one of the most tested mobilizing amendment for less mobile/available metals such as Pb [170]. Chelators have been isolated

from plants that are strongly involved in the uptake of heavy metals and their detoxification. The addition of EDTA to a Pb-contaminated soil (total soil Pb 2500 mg kg−1) increased shoot lead concentration of Zea mays L. (corn) and Pisun sativum (pea) from less than 500 mg kg−1 to more than 10,000 mg kg−1. Enhanced accumulation of metals by plant species with EDTA treatment is attributed to many factors working either singly or in combination. These factors include (i) an increase in the concentration of available metals, (ii) enhanced metal-EDTA complex movement to roots, (iii) less binding of metal-EDTA complexes with the negatively charged cell wall constituents, (iv) damage to physiological barriers in roots either due to greater concentration of metals or EDTA or metal-EDTA complexes, and (v) increased mobility of metals within the plant body when complexed with EDTA compared to free-metal ions facilitating the translocation of metals from roots to shoots [171]. For the chelates tested, the order of effectiveness in increasing Pb desorption from the soil was EDTA > hydroxyethylethylene-diaminetriacetic acid (HEDTA) > diethylenetriaminepentaacetic acid (DTPA) > ethylenediamine di(o-hyroxyphenylacetic acid) EDDHA [170]. Vassil et al [172] reported that Brassica juncea exposed to Pb and EDTA in hydroponic solution was able to accumulate up to 55 mg kg−1 Pb in dry shoot tissue (1.1% w/w). This represents a 75-fold concentration of lead in shoot over that in solution. A 0.25 M threshold concentration of EDTA was required to stimulate this dramatic accumulation of both lead and EDTA in shoots. Since EDTA has been associated with high toxicity and persistence in the environment, several other alternatives have been proposed. Of all those, EDDS ([S, S]-ethylenediamine disuccinate) has been introduced as a promising and environmentally friendlier mobilizing agent, especially for Cu and Zn [170]. Once the plants have grown and absorbed the metal pollutants, they are harvested and disposed of safely. This process is repeated several times to reduce contamination to acceptable levels. Interestingly, in the last few years, the possibility of planting metal hyperaccumulator crops over a low-

grade ore body or mineralized soil, and then harvesting and incinerating the biomass to produce a commercial bio-ore has been proposed [173] though this is usually reserved for use with precious metals. This process called phytomining offers the possibility of exploiting ore bodies that are otherwise uneconomic to mine, and its effect on the environment is minimal when compared with erosion caused by opencast mining [161].

2.10.12 Assessing the Efficiency of Phytoextraction

Depending on heavy metal concentration in the contaminated soil and the target values sought for in the remediated soil, phytoextraction may involve repeated cropping of the plant until the metal concentration drops to acceptable levels. The ability of the plant to account for the decrease in soil metal concentrations as a function of metal uptake and biomass production plays an important role in achieving regulatory acceptance. Theoretically, metal removal can be accounted for by determining metal concentration in the plant, multiplied by the reduction in soil metal concentrations [163]. It should, however, be borne in mind that this approach may be challenged by a number of factors working together during field applications. Practically, the bioaccumulation factor, f , amount of metal extracted, M (mg/kg plant) and phytoremediation time, t_p (year) [174] can be used to evaluate the plant's phytoextraction efficiency and calculated by assuming that the plant can be cropped n times each year and metal pollution occurs only in the active rooting zone, that is, top soil layer (0–20 cm) and still assuming a soil bulk density of 1.3 t/m^3, giving a total soil mass of 2600 t/ha.

$$f = \frac{\text{Metal concentration in plant shoot,}}{\text{Metal concentration in soil}}$$

$$\text{M (mg/kg plant)} = \text{Metal concentration in plant tissue} \times \text{Biomass,}$$

$$t_p \text{ (year)} = \frac{\text{Metal concentration in soil needed to decrease} \times \text{Soil mass}}{\text{Metal concentration in plant shoot} \times \text{plant shoots biomass} \times n}$$

2.10.13 Prospects of Phytoextraction

One of the key aspects of the acceptance of phytoextraction pertains to its performance, ultimate utilization of by-products, and its overall economic viability. Commercialization of phytoextraction has been challenged by the expectation that site remediation should be achieved in a time comparable to other clean-up technologies [161]. Genetic engineering has a great role to play in supplementing the list of plants available for phytoremediation by the use of engineering tools to insert into plants those genes that will enable the plant to metabolize a particular pollutant [175]. A major goal of plant genetic engineering is to enhance the ability of plants to metabolize many of the compounds that are of environmental concern. Currently, some laboratories are using traditional breeding techniques, others are creating protoplast-fusion hybrids, and still others are looking at the direct insertion of novel genes to enhance the metabolic capabilities of plants [175]. On the whole, phytoextraction appears a very promising technology for the removal of metal pollutants from the environment and is at present approaching commercialization.

2.10 .14 Possible Utilization of Biomass after Phytoextraction

A serious challenge for the commercialization of phytoextraction has been the disposal of contaminated plant biomass especially in the case of repeated cropping where large tonnages of biomass may be produced. The biomass has to be stored, disposed of or utilized in an appropriate manner so as not to pose any environmental risk. The major constituents of biomass material are lignin, hemicellulose, cellulose, minerals, and ash. It possesses high moisture and volatile matter, low bulk density, and calorific value [163]. Biomass is solar energy fixed in plants in form of carbon, hydrogen, and oxygen (oxygenated hydrocarbons) with a possible general chemical formula $CH_{1.44}O_{0.66.}$ Controlled combustion and gasification of biomass can yield a mixture of producer gas and/or pyro-gas which leads to the generation of thermal and electrical energy [176]. Composting and compacting can be employed as

volume reduction approaches to biomass reuse [177]. Ashing of biomass can produce bio-ores especially after the phytomining of precious metals. Heavy metals such as Co, Cu, Fe, Mn, Mo, Ni, and Zn are plant essential metals, and most plants have the ability to accumulate them [178]. The high concentrations of these metals in the harvested biomass can be "diluted" to acceptable concentrations by combining the biomass with clean biomass in formulations of fertilizer and fodder.

2.10. 15 Phytostabilization

Phytostabilization, also referred to as in-place inactivation, is primarily concerned with the use of certain plants to immobilize soil sediment and sludges [179]. Contaminants are absorbed and accumulated by roots, adsorbed onto the roots, or precipitated in the rhizosphere. This reduces or even prevents the mobility of the contaminants preventing migration into the groundwater or air and also reduces the bioavailability of the contaminant thus preventing spread through the food chain. Plants for use in phytostabilization should be able to (i) decrease the amount of water percolating through the soil matrix, which may result in the formation of a hazardous leachate, (ii) act as barrier to prevent direct contact with the contaminated soil, and (iii) prevent soil erosion and the distribution of the toxic metal to other areas [168]. Phytostabilization can occur through the process of sorption, precipitation, complexation, or metal valence reduction. This technique is useful for the cleanup of Pb, As, Cd, Cr, Cu, and Zn [178]. It can also be used to reestablish a plant community on sites that have been denuded due to the high levels of metal contamination. Once a community of tolerant species has been established, the potential for wind erosion (and thus spread of the pollutant) is reduced, and leaching of the soil contaminants is also reduced. Phytostabilization is advantageous because disposal of hazardous material/biomass is not required, and it is very effective when rapid immobilization is needed to preserve ground and surface waters [178].

2.10.16 Phytofiltration

Phytofiltration is the use of plant roots (rhizofiltration) or seedlings (blastofiltration) to absorb or adsorb pollutants, mainly metals, from groundwater and aqueous-waste streams rather than the remediation of polluted soils [161]. Rhizosphere is the soil area immediately surrounding the plant root surface, typically up to a few millimetres from the root surface. The contaminants are either adsorbed onto the root surface or are absorbed by the plant roots. Plants used for rhizofiltration are not planted directly in situ but are acclimated to the pollutant first. Plants are hydroponically grown in clean water rather than soil, until a large root system has developed. Once a large root system is in place, the water supply is substituted for a polluted water supply to acclimatize the plant. After the plants become acclimatized, they are planted in the polluted area where the roots uptake the polluted water and the contaminants along with it. As the roots become saturated, they are harvested and disposed of safely. Repeated treatments of the site can reduce pollution to suitable levels as was exemplified in Chernobyl where sunflowers were grown in radioactively contaminated pools [180].

2.11 PROXIMATE ANALYSIS

It is a method used for the quantitative analysis of different macronutrients in feed based on the Weende analysis that was developed in 1860 by Henneberg and Stohmann in Germany. It is also defined by the American society for Testing and Materials (ASTM) as the determination by prescribed methods of moisture, volatile matter, fixed carbon and ash. The chemical composition of the various materials was determined using the methods described in the AOAC [181]. The parameters under investigation were; moisture, ash, crude protein, crude fat, crude fiber and total carbohydrate contents.

2.11.1 Total Nitrogen

The proximate system, where "protein" is measured as total nitrogen multiplied by a specific factor, continues to dominate food composition studies. Most cited values for "protein" in food composition databases are in fact derived from total nitrogen or total organic nitrogen values. In the majority of cases, total nitrogen is measured using a version of the Kjeldahl method [182]. In this method, the organic matter is digested with hot concentrated sulphuric acid. A "catalyst mixture" is added to the acid to raise its boiling point, usually containing a true catalytic agent (mercury, copper or selenium) together with potassium sulphate. All organic nitrogen is converted to ammonia, which is usually measured by titration or, more rarely, calorimetrically. In the original method, a relatively large analytical portion (1–2 g) was used, but this requires large amounts of acid. Micro-Kjeldahl methods are much more commonly used as they produce a reduced amount of acid fumes and also require less acid and catalyst mixture. Environmental considerations exert considerable pressure to ensure the safe disposal of mercury and, especially, to minimize acid usage.

The micro methods can be automated at several levels [183]. Automation of the distillation and titration stages work well but automation of the digestion has proved quite difficult.

2.11.2 Crude Protein

Since the development of the proximate system of analysis, "crude protein" values have been calculated by multiplying the total nitrogen (N) content by a certain factor. This factor was originally 6.25, based on the assumption that proteins contain 16 percent of N. It has been known for a considerable time that proteins of plant origin (and gelatin) contain more N and therefore require a lower factor. Jones, Munsey and Walker [184] measured the nitrogen content of a wide range of isolated proteins and proposed a series of specific factors for different categories of food. These factors have been widely adopted and were used in the FAO/WHO [185] review of protein requirements. Several authors have criticized the use of

these traditional factors for individual foods. Heidelbaugh *et al [186*] evaluated three different methods of calculation (use of the 6.25 factor, use of traditional factors and summation of amino acid data) and found variations of up to 40 percent. Sosulski and Imafidon [187] produced a mean factor of 5.68 based on the study of the amino acid data and recommended the use of 5.70 as a factor for mixed foods.

In principle, Southgate, 1974 [188] said it would be more appropriate to base estimates of protein on amino acid data and these were incorporated in the consensus document from the Second International Food Data Base Conference held in Lahti, Finland, in 1995, on the definition of nutrients in food composition databases [189]

If these recommendations are to be adopted, the amino acid data should include values for free amino acids in addition to those for protein amino acids because they are nutritionally equivalent. The calculations require very sound amino acid values (measured on the food) as discussed below, and involve certain assumptions concerning the proportions of aspartic and glutamic acids present as the amides and correction for the water gained during hydrolysis. Clearly, this approach would not be very cost-effective when compared with the current approach.

At the present time, it is probably reasonable to retain the current calculation method, recognizing that this gives conventional values for protein and that the values are not for true protein in the biochemical sense. However, it is important to recognize also that this method is not suitable for some foods that are rich in non-amino non-protein nitrogen, for example cartilaginous fish, many shellfish and crustaceans and, most notably, human breast milk, which contain a substantial concentration of urea.

2.11.3 Crude Fat

The values obtained for total fat or total material soluble in lipid solvents are very method-dependent. Carpenter, Ngeh-Ngwainbi and Lee (1993), [190], in their review for the AOAC of methods for nutritional labeling, set out the nature of the problems encountered. Gurr [191] discusses in detail the methods available for separating the different classes of lipids.

The classical method is based on continuous extraction performed on dried samples of food in a Soxhlet extractor, sometimes preceded by acid hydrolysis. This technique is time-consuming and subjects the extracted lipids to long periods of extraction at high temperatures. Its main drawback, however, is that it yields incomplete lipid extractions from many foods, especially baked products or those containing a considerable amount of structural fat. The extractant used is often petroleum spirit (which is less flammable than diethyl ether and less likely to form peroxides), which require completely dry analytical portions and the removal of mono-and disaccharides. Values obtained using this method requires close scrutiny before their inclusion in a database and their continued use is not recommended.

The use of mixed polar and non-polar solvents has been shown to extract virtually all the lipids from most foods. In the case of baked (cereal) products, however, incomplete extraction of fat may occur. Chloroform–methanol extraction is well known (Bligh and Dyer, 1959) [192]; this combines the tissue-penetrating capacity of alcohol with the fat-dissolving power of chloroform. The resultant extracts are complete but may also contain non-lipid materials and require re-extraction to eliminate these. The measurement of lipids after acid (Weibull and Schmid methods) or alkaline (Röse-Gottlieb method) treatment also provides good extraction from many foods. These techniques are recognized as regulated methods by the AOAC and the European Union. Alkaline methods are almost exclusively used for dairy foods and are the approved methods for such foods. The extracts from acid and alkaline

treatments are not suitable for fatty acid analysis because some oxidation and losses due to acid hydrolysis of fats may occur. The AOAC has adopted methods for determining total fat (also saturated, unsaturated and monounsaturated fats) in foods using acid hydrolysis and capillary gas chromatography (House, 1997) [193] to comply with the Nutrition Labeling and Education Act (NLEA) definition of fat.

2.11.4 Total Ash

Nutritionally, there is little value in recording ash values other than to provide an approximate estimate of the total inorganic material and to check for replication in the destruction of the matrix. A value for total ash is, of course, essential when it is necessary to calculate carbohydrate "by difference"

In dry ashing, the food is incinerated in a crucible, usually made of silica, although porcelain (can be used but less suitable) or platinum (very expensive but the least reactive) can be used. The food matrix must be destroyed by heating gently at first to char the sample and then at 500 °C in a muffle furnace (Wills, Balmer and Greenfield, 1980) [194] to prevent foaming of lipids (and sugars) until a white (or light grey) residue is produced. Heating above 500 °C can result in the loss of alkali metals.

In the case of "wet ashing" acid digestion, the food sample is heated with acid – usually a mixture of nitric and sulphuric acids. Perchloric acid is often included in the digesting acid mixture although this introduces the risk of explosion and the procedure must be carried out in a fume hood designed for the use of perchloric acid. Wet ashing offers the advantage that no reactions with the crucible can occur that can lead to the formation of insoluble silicates. Digestion can be carried out in a Kjeldahl flask but this requires a larger quantity of acid. Particularly for trace element analysis, digestion is best carried out in a sealed container. Tubes designed for this purpose are available from most laboratory suppliers. They are made

from resistant glass and have a cap with a plastic insert to provide an inert gas-tight seal. The analytical portion and the acid are placed in the tube, which is then capped and may be heated in a conventional or microwave oven. The tube is then allowed to cool completely before the gases are released with care.

2.11.5 Crude Fibre

Dietary fibre should be considered as part of the carbohydrates in foods. The major problem in the choice of method lies in the definition of dietary fibre and its interpretation in an analytical context. The term was first used in 1953, by Hipsley, [195] to describe the sum of the hemicelluloses, cellulose and lignin in food, in other words the components of plant cell walls in foods. Trowell, in 1972, [130,196] took up the term for "the indigestible components of the plant cell wall in foods". Both of these terms were too vague to use as a basis for an analytical method and it proposed that it be defined as "the sum of the plant polysaccharides and lignin that are not digested by the enzymes of the gastrointestinal tract" [197].

In this method the aim was to measure the carbohydrates specifically using colorimetric techniques. Englyst developed this approach using the more specific GLC methods, which gave values for the non-starch polysaccharides and incorporated a stage to convert resistant starch to non-enzymatically resistant starch.

In other parts of Europe, especially Sweden and Switzerland, and in the United States, the focus was directed at the "indigestibility of the polysaccharides and lignin". A gravimetric method was developed where the residue after starch removal is weighed to give a measure of total dietary fibre (TDF); this has evolved into the Official AOAC Method No. 982.29 (Prosky *et al.*, 1992) [198].

2.11.6 Water/Moisture

Values for water remain an essential constituent in food composition databases because water content is one of the most variable components, especially in plant foods. This variability affects the composition of the food as a whole. The range of methods for water analysis is summarized

The methods are based on the direct or indirect measurement of water removed from the food, changes in physical properties that change systematically with water content, or the measurement of the chemical reactivity of water (AOAC International, 2002) [199].

For the majority of foods in food composition databases, drying methods are adequate; although slight methodological differences can be observed, these differences are rarely significant. The AOAC Official Methods recommend a lower drying temperature (70 °C) for plant foods to minimize the destruction of carbohydrates. Where this occurs it is usually better to use vacuum drying or freeze-drying.

Vacuum drying is most efficient if a slow leak of dry air is passed through the oven. This approach has the advantage that the analytical portions can be left unattended for long periods. Vacuum drying at 60–70 °C is preferable to drying in an air oven, particularly for foods that are rich in sugars. However, for most foods drying in an air oven is satisfactory for food composition database purposes.

Drying in a microwave oven is very quick but requires continuous surveillance to avoid charring. Drying with infrared lamps has been very successfully automated (Bradley, 1998) [200]. Both of these methods, however, are more suitable for routine quality control.

2.11.7 Carbohydrates

The range of carbohydrates found in the human diet illustrates the nature of the task facing the analyst who wishes to follow the recommendations published by FAO/WHO (1998) [201] for measuring the carbohydrates in foods separately. Not all types of carbohydrates are, of course, present in all types of foods.

The distinctive metabolic and physiological properties of the different carbohydrates emphasize the fact that for nutritional purposes it is inadequate to consider the carbohydrates as a single component of foods.

The calculation of "carbohydrate by difference" using the Weende proximate system of analysis was a reflection of the state of knowledge of carbohydrate chemistry at the time. Moreover, the system was designed for animal feedstuffs, especially for ruminants, and most of the carbohydrates (except lignin-cellulose of which crude fibre was an approximate measure) would therefore be digested in the rumen.

For nutritional purposes carbohydrates can be considered as falling into three groups based on the degree of polymerization:

- sugars (mono- and disaccharides);
- oligosaccharides (polymers of three to nine monosaccharide or uronic acid units);
- Polysaccharides (polymers containing more than nine units), which fall into two broad categories: a-glucans (starches, starch hydrolysis products and glycogen) and a much more diverse group of non-a-glucans (non-starch polysaccharides [NSPs], which are the major constituents of dietary fibre).

These broad chemical groupings do not correspond precisely with physiological properties or with analytical fractions. The percentage of carbohydrate in food is therefore computed by summing all the percentages of the other parameters of analysis and subtract from 100%.

CHAPTER THREE

3.0 METHODOLOGY

This chapter deals with the steps in sampling, sample preparation and analyses of cocoa samples in order to obtain the concentrations of heavy metals present in them.

The equipment and chemical reagents as well as proximate test to determine the macro-nutritional values of the samples are also given. Quality assurance measures taken to ensure the reliability and reproducibility of the analytical data are also presented

3.1 GEOGRAPHICAL DESCRIPTION OF THE SAMPLING AREA

Ghana is situated on the west coast of Africa about 750km north of the equator between latitude 4^{o} and 11.5^{o} N and longitude 3.11^{o} west. It shares boundaries with Burkina Faso to the north, Togo to the east, La cote d'viore to the west and Gulf of Guinea (part of Atlantic Ocean) to the south. Generally, the climate of Ghana is tropical and two main types of vegetation exist. These are the rain forest and savanna grassland. The forest vegetation is characterized by high temperatures and heavy rainfall almost throughout the year and is usually divided into rain forest and semi-deciduous forest. The forest vegetation promotes very rapid plant growth [202, 203]. Cocoa thrives well in the forest regions of Ghana which covers the south western part and compresses 6 out of the 10 political regions of the country. Samples of cocoa beans from Western Region and Ashanti Region were used for this work. Figure 3.1 shows a map of major cocoa-growing towns from some of which cocoa beans used for the analysis in this work were taken.

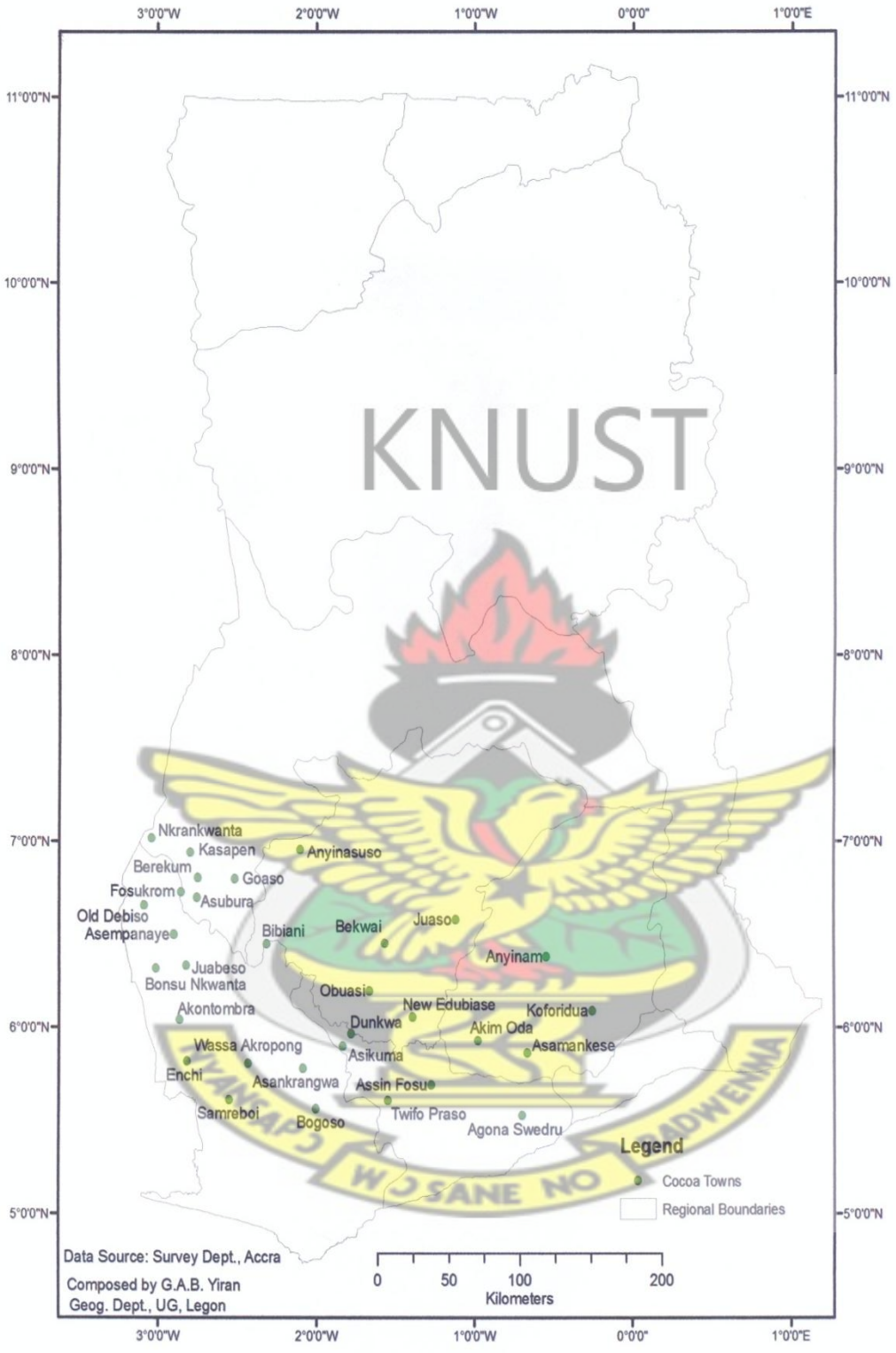


Figure 3.1: A map showing the major cocoa-growing towns from some of which samples were taken.

3.2 COLLECTION OF SAMPLES

Samples of fermented and dried cocoa beans were obtained from cocoa farmers in some selected cocoa growing towns in Ashanti and Western Regions of Ghana. However, few cocoa fruits were obtained from the farms of some farmers before the beans were dried.

Sampling was done such that the towns from which cocoa beans were obtained were well spread throughout the Regions with each of the towns having an equal opportunity of being chosen. Western Region was divided into five areas (Northern part, Eastern part, Southern part, Western part and Central part.). The names of the cocoa farming towns in one demarcated area were written on separate sheets of papers and thoroughly mixed up. One town was picked at random from that demarcated area. The same procedure was adopted in choosing one town from the other demarcated areas. In each town I visited dried samples of cocoa beans were obtained from five different cocoa farmers and kept in clean and dry polyethylene bags. The same procedure was employed during sampling in Ashanti Region. Figs 3.2-3.4 show some photographs during the sampling.

Figure 3.2: Cocoa fruit being collected from farmers at Mampong in the Ashanti Region of Ghana (AM005)

Figure 3.3: Some of the cocoa samples from Sefwi-Asempaneye in the Western Region of Ghana (SA005)

Figure 3.4: Cocoa beans from Juaso in the Ashanti Region of Ghana(AJ001)

Table 3.1: Sampling locations.

Region	Town	Number of samples
Western	1. Kasapen (SK)	5
	2. Enchi (SE)	5
	3. Sefwi – Juabeso(SJ)	5
	4. Asempaneye(SA)	5
	5. Bogoso(SB)	5
Ashanti	1.Juaso(AJ)	5
	2. Tepa(AT)	5
	3.Bekwai(AB)	5
	4. Obuasi (AO)	5
	5 Mampong(AM)	5
Total	**10**	**50**

3.3 SAMPLE PREPARATION AND PRE-TREATMENT

The dry cocoa beans samples obtained were kept in clean, dry glass bottles for further treatment. In handling the cocoa beans, gloves were worn to avert external contamination which would affect the analyses. Care was taken to also ensure that water and other reagents did not come into contact with the beans before subjecting them to milling, digestion and subsequently, analyses by the use of Flame Atomic Absorption Spectrometer. (F-AAS)

Figure 3.5: Cocoa beans before milling

Figure 3.6: Cocoa beans after milling

3. 4 CHEMICALS AND REAGENTS

All reagents used for this work were of analytical grade. Digestion of samples was performed using aqua regia of HNO_3 and HCl both obtained from Merck, Germany. Petroleum ether, Selenium catalysts were used for the proximate analyses for the determination of the fat protein and carbohydrate content in the cocoa beans.

De- ionized water (distilled water) obtained from the Chemistry Department, KNUST was used for all the analytical work.

3.5 EQUIPMENT AND GENERAL APPARATUS

All samples were collected, sealed and stored in pre-cleaned and dry low-density polyethylene bags. Samples were homogenized using a milling machine at the Pharmaceutical Chemistry Department at KNUST. Digested samples were stored in

polyethylene vials. The vials were pre-cleaned by soaking in ultrapure nitric acid for 24 hours, rinsing thoroughly with de-ionized water and dried at 30 ^{o}C in an oven. All glass wares used in the analysis were initially washed with detergent and water after which they were soaked in dilute nitric acid for 24 hours. The glass wares were then rinsed several times with de-ionized water and dried at 100^{o}C. Analysis of heavy metal concentration was performed using F-AAS of Buck Scientific VEP 210 model.

3.6 PREPARATION OF STANDARDS

Standards of heavy metals were prepared from multi- element standard stock solution (obtained from Inorganic Ventures Inc., USA) in 10% nitric acid and 2% HCl. Single-element standards of most of the elements having very low concentrations were also prepared for calibration. The working standard solutions were all prepared by serial dilution of the stock solution with de-ionized water in 100ml volumetric flask.

3.7 DIGESTION OF SAMPLES

This is a critical sample preparation step in quantitative analysis and steps had to be taken to ensure the reliability and reproducibility of the results by ensuring that samples were free from contamination and to avoid or minimize loss of analyte.

The aqua regia for the digestion was prepared by mixing 3:1 volumes of HCl and HNO_3 respectively in a hood. After storing the prepared aqua regia for 2 days to ensure a complete reaction and a uniform homogenous mixture between the acids, digestion of the samples commenced. One gram (1g) of the sample was weighed using a balance into 30ml of the aqua regia in a pre-cleaned Teflon cup.

The content of the sealed Teflon cup was heated on a hot plate at 200^{o}C in a hood and digestion continued for about 20 minutes. The digest after cooling, was transferred into a 50ml volumetric flask by filtering through a whatman no. 40 filter paper. De-ionized water

was added to make it up to the 50ml mark [204] before being transferred and stored in pre-cleaned polypropylene tubes for analysis.

All the samples were subjected to this procedure. Blank samples were digested and analyzed in the same way as described for the cocoa samples. Triplicate digestions were conducted for each sample.

3.8 QUALITY ASSURANCE

Sample containers and glassware used in the analysis were first cleaned with metal free nonionic detergent solution, rinsed thoroughly with de-ionized water and soaked in nitric acid for 24 hours. They were then washed several times with de-ionized water prior to the use. Blanks, consisting of de-ionized water, chemicals and reagents used for the digestion were subjected to similar sample preparation and analytical procedure in an effort to reduce the effect of contamination arising from chemical reagents, distilled water and glassware used in the analysis.

Accuracy of the method was evaluated through the analysis of two reference materials: NIST 1547 SRM certified Peach Leaves and IAEAV-10 SRM certified Hay Powder.

3.9 PROXIMATE ANALYSIS OF FOODS/FEEDS

The proximate compositions in cocoa beans determined in this work are moisture, ash, fat, protein, crude fiber and carbohydrates. These components are fundamental to the assessment of the nutritive quality of the samples being analyzed.

Six determinations in duplicate were made on each of samples. Blank determinations were also made.

3.9.1 Principles of moisture determination

Materials being analyzed were dried under standard conditions of temperature and pressure. The weight loss incurred is determined quantitatively as the moisture content.

3.9.1.1 Materials

- Analytical balance accurate to 4 decimal places
- Desiccator
- Thermostatically controlled oven
- Glass dishes

3.9.1.2 Method

Five grams (5.0g) of sample were transferred to previously dried and weighed dish. The dish was placed in an oven thermostatically controlled at 105^0C for 5 hours. The dish was removed and placed in a desiccator to cool. The cooled sample was weighed. Heating and cooling was repeated until constant weight was attained. Percent loss of weight was reported as moisture as:

Weight of dish = W_1

Weight of dish + Wet sample = W_2

Weight of dish + Dry Sample = W_3

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W2 - W1} \times 100$$

3.9.2 Crude Fat determination

The free lipid content (neutral fats – triglycerides) of samples and free fatty acids can be determined by extracting the dried material with a light petroleum fraction in a continuous extraction apparatus. The solvent is distilled off and the extract is dried and weighed. For continuous extraction method, the greater the presence of fresh solvent around the sample,

the more efficient of the extraction becomes. With this process, therefore, vapour of solvent moves up into the condenser, condensed as droplets, come into contact with the thimble, dissolves the sample and the sample is again vaporized and the processes is repeated.

3.9.2.1 Materials

- Analytical balance, accurate to 4 decimal places
- Laboratory oven
- Quick fit 250ml round – bottomed short flask with 24/29 socket
- Heating mantle
- Solvent distillation apparatus
- Cotton wool/ glass wool.
- Petroleum spirit (BP 60-80^{0}C)
- Anti-bumping granules
- Quick condenser

Quick fit 100ml soxhlet extractor EX 5/63

3.9.2.2 Method

Dried samples from moisture determination were transferred to 22 x 80 mm paper, placed a small ball of cotton wool or glass wool into the thimble to prevent loss of the sample. Anti-bumping granules were placed in previously dried (air oven at 100^{0}C) 250ml round bottom flask and weighed accurately. One hundred and fifty ml (150ml) of petroleum spirit (B. P 60-80^{0}C) was added to the flask and apparatus was assembled. Quick fit condenser was connected to the soxhlet extractor and refluxed for 4 hours on high or 16 hours on low heat on the heating mantle, the flask was removed the flask and evaporated on a steam bath. After heating the flak and fat/oil for 30 minutes in an oven at 103^{0}C, the weight of oil / fat collected was accurately determined as:

Weight of flask = W1

Weight of flask + fat = W2

Weight of fat = W2 - W1

Weight of sample W3

% crude fat = $\frac{\text{weight of fat (g)}}{\text{Weight of sample}} \times 100\%$

Symbolically, %crude fat = $\frac{W2 - W1}{W3} \times 100\%$

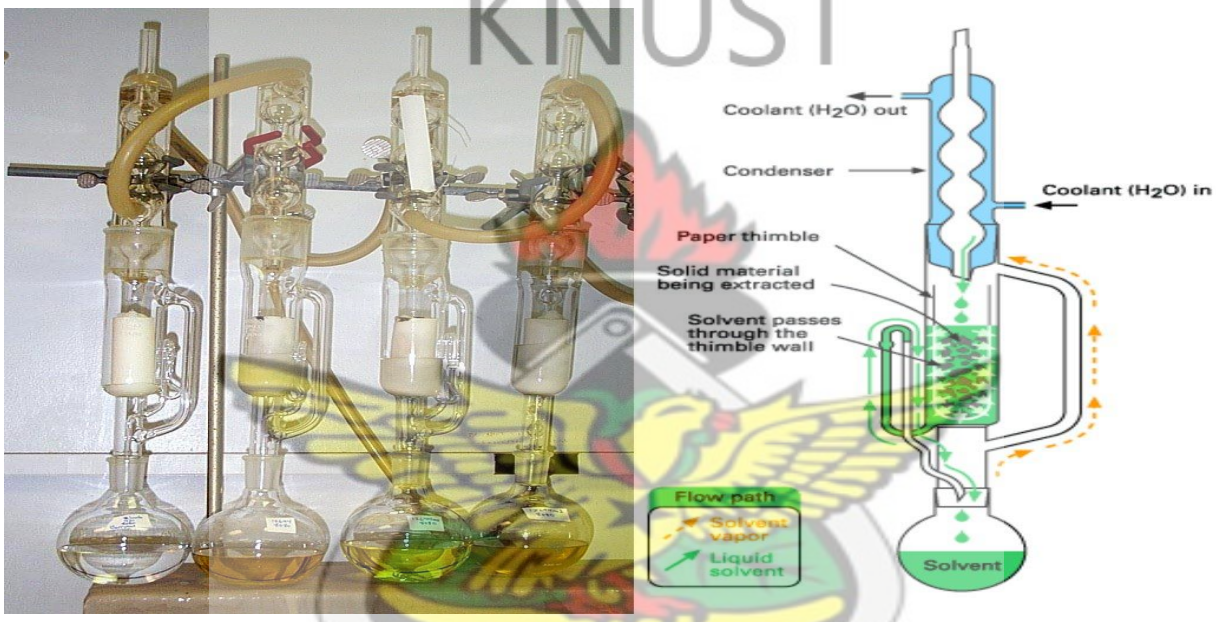


Figure 3.10: Set-up for the proximate analysis (soxhlet apparatus and diagram)

3.9.3 Crude Fibre determination

Crude fibre consists of cellulose, hemicelluloses and lignin. Lignin comprises of polymers of phenolic acids. Hemicellulose is made up of heteropolymers of polysaccharides.

Crude fibre is reported as the loss in weight on ignition of dry residue remaining after digestion of material with 1.25% H_2SO_4 and 1.25% NaOH under specified conditions. These two solvents remove protein and carbohydrate. The ignition step does away with the organic

matter/fibre . Crude fibre is always determined on defatted sample or only when the fat content is negligible.

3.9.3.1 Method

Samples from crude fat determination were transferred to a 750ml Erlenmeyer flask and approximately ½ gram of asbestos was added. After that 200ml of boiling 1.25% H_2SO_4 was added and the flask was immediately set on hot plate and connected to the condenser (cold finger type). The contents were boiled within 1 minute intervals and frequently until sample was thoroughly wetted. Care was taken to always keep material within the solution. At the end of 30 minutes, the flask was removed and immediately filtered through linen cloth in funnel and washed with boiling water until washings were no longer acidic. Charge and asbestos were washed back into flask with 200ml boiling 1.25% NaOH solution using wash bottle calibrated to deliver 200ml. The flask was connected to the condenser and boiled for exactly 30 minutes, filtered through fine cloth and washed thoroughly with boiling water.

Residue was transferred to Gooch crucible using a funnel with water from wash bottle, washed with approximately 15ml alcohol and then the crucible and its contents were dried for 1 hour at 100^0C. On cooling in a desiccator, the content was reweighed. The crucible was ignited in an electric furnace for 30 minutes, cooled and reweighed.

$$\% \text{ Crude fibre} = \frac{\text{loss in weight from incineration}}{\text{Weight of sample before defatting}} \times 100\%$$

3.9.4 Ash determination

The ash of foodstuff is the inorganic residue remaining after the organic matter has been burnt away. It should be noted however, that the ash obtained is not necessarily of the same composition as the mineral content as there may be some loss from volatilization. The ash

content can provide an estimate of the quality of the product, since high levels may indicate contamination

3.9.4.1 Materials

- Muffle
- Porcelain crucible/ silica dish
- Desiccator
- Analytical balance accurate to four decimal places

3.9.4.2 Method

2.0g of sample were transferred to a previously ignited and weighed crucible and placed in the muffle furnace (preheated to 600^0C) for 2 hours. Crucible was removed and allowed to cool in air somewhat but place in desiccator while still hot (transferring directly from furnace to desiccator), cooled and weighed. The heating, cooling and weighing were repeated till constant weight was obtained

3.9.4.3 Calculation

The % of ash content of samples was then calculated as: $\frac{X-Y}{W} \times 100\%$

Where X =weight of crucible + ash

Y = weight of crucible

W = weight of sample to be determined in (g) before ashing.

3.9.5 Protein determination

Although the Kjeldahl procedure has been modified many times, the basic procedure is still the most reliable technique for the determination of organic nitrogen. The method is based on the conversion of nitrogenous compounds in the analyzed substance of ammonium sulphate by digesting the material with concentrated sulphuric acid in the presence of a catalyst.

Copper sulphate, mercury and selenium are some of the catalysts used. Potassium sulphate is added in order to raise the boiling point. Ammonia is liberated from the digestion mixture by making the solution alkaline. It is then steam distilled to release ammonia. The ammonia is trapped in dilute acid (boric acid) and titrated.

The Kjeldahl method does not differentiate as to the type of nitrogen i.e. non- protein nitrogen. The usefulness of the method is further limited. Since the nitrogen in a great many organic compounds are not quantitatively converted into ammonia by this method. Pyridine, quinoline, quanandines, nitro- , nitroso-, azo-, diazo-, and hydrazo-, compounds are examples of compounds which cannot be directly digested. In many cases theoretical results may be obtained if the compounds are first reduced; nitrates require reduction with iron, or with phenol and zinc dust. Run a blank determination using the same amount of all reagents as used for the unknown sample. The blank will correct for traces of nitrogen in the reagents and should include digestion as well as distillation.

3.9.5.1 Materials

Boric acid

Hydrochloric acid

Sulphuric acid

Selenium- based catalyst (if not available then use 0.7g

Mercury oxide (HgO) and 15g of potassium sulphate

Sodium Hydroxide (40 %)

Mixed indicator (methyl red solution 0.1% and bromocresol green solution . 0.1% in 95% alcohol prepared separately, with the mixed indicator prepared from 20ml of the bromocresol green to each 4ml of methyl red.

3.9.5.2 Digestion

To the digestion flask, 2.00 gram of sample and a half of selenium based catalyst tablets and a few anti- bumping agents were added. A volume of 25ml concentrated H_2SO_4 was added to the flask and shaken for the entire sample to be thoroughly wet. The flask was placed on digestion burner and heated slowly until bubbling ceased resulting in the formation of a clear solution and cooled to room temperature. Digestion sample solution was transferred into a 100ml volumetric flask and made up to the mark.

3.9.5.3 Procedures Followed During Distillation

1. To flush out the apparatus before use, distilled water in a steam generator of the distillation apparatus was boiled with the connections arranged to circulate through the inner decomposition flask and out through the condenser, for at least 10 minutes. The receiving flask was lowered when the condenser was beneath the surface of the distillate. Heating continued for 30 seconds in order to carry over all liquid in the condenser. The burner was removed from the steam trap.
2. To the 250ml conical flask, 25ml of 2% boric acid and 2 drops of mixed indicator were added.
3. Liquid was drained form the steam trap leaving the stopcock, which drained the steam trap open.
4. The conical flask and its contents were placed under the condenser in such a position that the tip of the condenser was completely immersed in the solution.
5. 10ml of the digested sample solution was measured, the stopcock of the funnel on the steam jack was opened and the 10ml of the digested sample solution was poured in. Excess of 40% NaOH (about 15-20ml) was added to the decomposition flask and

the funnel stopcock was closed. To drive the liberated ammonia into the collection flask, steam was forced through the decomposition chamber by shutting the stopcock on the steam trap outlet.

6. The boric acid changed to bluish green as soon as it came into contact with ammonia and distillation continued for 1-5 minutes. The receiving flask was lowered so that the tip of the condenser was just above the liquid. The end of the condenser was washed with a little distilled water. Distillation continued for another 30 seconds and the burner was removed from the steam generator.
7. Before distilling another sample and on completion of all distillations, the apparatus was flushed as in step 1 above. Steam was made to pass until 5ml of distillate was obtained.

3.9.5.4 Titration

The distillate was titrated with 0.IM HCI solution. The acid was added until the solution became pink. The same procedure was followed for the blank (except the sample is omitted)

3.9.5.5 Calculation

$$\% \text{ Total nitrogen} = \frac{100 \times (V_A - V_B) \times M_A \times 0.01401}{W \times 10} \times 100$$

V_A- volume in ml of standard acid used in titration

V_B- Volume in ml of standard acid used in blank

M_A- Molarity of acid (HCL)

W- Weight in grams of the sample

The protein content is estimated by a process developed by a Danish Chemist/brewer, John Kjehdahl. He discovered that “all protein” contains about the same amount of nitrogen

(16%). He analyzed for nitrogen, which is relatively easy and calculated crude protein on the basis: 100/16 = 6.25, therefore; Nitrogen x 6.25 = crude protein.

∴ % Protein = 6.25 x % total nitrogen

Where 6.25 is the conversion factor (F)

3.9.5.6 Possible Errors and Disadvantages

- This procedure assumes that all nitrogen present in the samples is in protein form. This assumption is not necessarily true. Nitrogen could be in nucleic acid (RNA, DNA), urea.
- Different proteins need different correction factors because they have different amino acid sequences.
- The technique is time consuming to carry-out.
- The use of concentrated sulphuric acid at high temperature poses a considerable hazard, as does the use of some of the possible catalysts.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

The results obtained from the analyses of some heavy metals present in cocoa from some cocoa growing areas in Western Region and Ashanti Region are shown and discussed in this chapter.

4.1 QUALITY ASSURANCE OF ANALYSIS

In an effort to obtain results that are accurate and reproducible in these analyses , a number of quality control measures were ensured; from the initial sampling process to the final analyses of the samples using flame atomic absorption spectrometer (F-AAS) instrument.

Sampling of cocoa beans from Western and Ashanti Regions ensured that representative samples from the major cocoa –growing towns were taken. Sampling was done according to the codex general standard for contaminants and toxins in food [79]. Precautions were taken to reduce contamination during the handling and preparation of samples.

As mentioned earlier in this work, all reagents were of analytical grade and sample containers and apparatus were washed and rinsed thoroughly prior to their use. Since reagents could be reliable sources of contamination in analytical work, high purity reagents and distilled water were used in this work. Again, reagent blanks were analyzed. The concentrations reported in this work were thus actual concentrations of the samples relative to the reagent blanks. Moreover, the accuracy and reliability of the measurements were ascertained with the analysis of two reference materials; NIST 1574 SRM certified Peach Leaves and IAEA-V-10 SRM certified Hay Powder

These measured values for this work along with their corresponding certified or reported values for the reference materials are presented in Tables 4.1 and 4.2.

4.2 TABLE OF RESULTS

Table 4.1: Levels of heavy metals in IAEA – V-10 Standard reference materials (Hay Powder)

Metal	This work	Reported Values	Absolute Error	Percentage Error (%)
Pb	1.55(1.42-1.52)	1.6(0.8-1.9)	0.05	3.13
Cd	0.04(0.03-0.06)	0.03(0.02-0.05)	0.01	33.33
Fe	185.5(175-189)	186(117-190)	0.50	0.27
Zn	23.8(22.6-25.1)	24(23-25)	0.20	0.83
Mn	48.2(43.3-50.4)	47(44-51)	1.20	2.55
Cu	9.90(9.2-10.3)	9.4(8.8-9.7)	0.50	5.32

Table 4.2: Levels of heavy metals in NIST standard reference material 1547 (Peach Leaves)

Metal	This work	Reported Values	Absolute Error	Percentage Error (%)
Pb	43.48±2.1	45±3	1.52	3.38
Cd	0.12±0.03	0.11±0.02	0.01	9.09
Fe	289±8.8	300±20	11.00	3.67
Zn	27.56±2.1	25±3	2.56	10.24
Mn	89.76±3.1	91±4	1.24	1.36
Cu	11.74±2.0	12±1	0.26	2.17

$$\boldsymbol{Absolute\ Error} = |\text{Reported Values} - \text{Measured Values}|$$

$$\textbf{Percentage Error} = \frac{Absolute\ Error}{\text{Reported Values}} \times 100\%$$

Table 4.3: Levels of some heavy metals (mg/kg) in cocoa beans from Cocoa- growing towns in the Western Region of Ghana.

TOWN	SAMPLE	Cd	Pb	Cu	Mn	Fe	Zn
	SK005	0.045±0.002	0.013±0.00	48.46±0.05	57.85±0.03	44.58±0.04	39.38±0.03
	SK001	0.039±0.00	0.015±0.00	48.18±0.04	67.73±0.02	39.82±0.04	67.15±0.00
KASAPEN	**SK002**	0.025±0.001	0.011±0.001	56.00±0.11	49.15±0.07	48.35±0.04	46.65±0.04
	SK003	0.063±0.002	0.013±0.001	52.75±0.03	56.67±0.03	56.35±0.04	47.33±0.04
	SK004	0.055±0.000	0.014±0.00	54.17±0.02	41.83±0.02	41.78±0.04	52.84±0.02
MEAN		**0.045±0.001**	**0.013±0.0004**	**51.19±0.05**	**64.65±0.03**	**53.11±0.04**	**50.67±0.034**
ENCHI	**SE001**	0.045±0.003	0.013±0.001	45.91±0.06	45.66±0.02	41.81±0.06	41.65±0.02
	SE002	0.085±0.003	0.015±0.001	43.50±0.04	68.57±0.03	38.83±0.02	48.79±0.06
	SE003	0.061±0.003	0.014±0.002	43.65±0.04	62.84±0.03	55.32±0.03	44.83±0.01
	SE004	0.071±0.00	0.014±0.001	50.38±0.04	42.16±0.01	42.65±0.00	36.18±0.06
	SE005	0.062±0.002	0.019±0.002	48.92±0.05	61.74±0.02	42.80±0.05	48.25±0.02
MEAN		**0.065±0.002**	**0.015±0.003**	**46.47±0.05**	**56.19±0.02**	**44.28±0.03**	**43.94±0.03**
ASEMPANEYE	**SA001**	0.039±0.00	0.017±0.002	45.29±0.02	48.94±0.02	43.14±0.03	46.80±0.05
	SA002	0.025±0.001	0.021±0.002	65.25±0.04	57.92±0.05	46.72±0.03	43.26±0.02
	SA003	0.063±0.002	0.019±0.002	62.28±0.00	42.25±0.03	44.16±0.02	39.16±0.02
	SA004	0.055±0.00	0.018±0.001	47.41±0.06	58.26±0.02	49.42±0.00	43.77±0.05
	SA005	0.045±0.002	0.016±0.001	41.34±0.02	64.26±0.02	35.55±0.00	42.19±0.04
MEAN		**0.045±0.001**	**0.018±0.002**	**52..31±0.03**	**54.33±0.03**	**43.80±0.02**	**43.04±0.04**
SEFWI- JUABESO	**SJ001**	0.069±0.003	0.017±0.00	65.85±0.00	47.64±0.03	45.54±0.04	59.03±0.17
	SJ002	0.055±0.002	0.014±0.001	49.25±0.03	64.17±0.02	40.65±0.04	46.71±0.02
	SJ003	0.078±0.003	0.013±0.002	67.85±0.04	40.64±0.02	48.68±0.04	63.86±0.03
	SJ004	0.050±0.004	0.061±0.063	48.65±0.04	48.28±0.06	63.28±0.03	47.78±0.02
	SJ005	0.078±0.005	0.022±0.001	44.24±0.02	61.17±0.04	47.60±0.07	41.26±0.01
MEAN		**0.066±0.003**	**0.025±0.013**	**55.17±0.03**	**52.38±0.03**	**49.15±0.04**	**51.73±0.05**
BOGOSO	**SB001**	0.029±0.002	0.020±0.002	68.55±0.00	69.57±0.02	42.27±0.02	47.86±0.03
	SB002	0.080±0.002	0.017±0.002	69.85±0.04	46.18±0.06	49.91±0.05	53.18±0.05
	SB003	0.042±0.003	0.033±0.002	46.79±0.04	42.71±0.05	43.25±0.04	41.68±0.05
	SB004	0.038±0.003	0.044±0.002	41.28±0.03	36.16±0.02	45.25±0.04	49.35±0.03
	SB005	0.068±0.003	0.057±0.003	57.28±0.03	47.18±0.03	55.45±0.00	68.25±0.02
MEAN		**0.05±0.003**	**0.03±0.002**	**54.75±0.03**	**48.36±0.04**	**47.23±0.03**	**52.06±0.04**

Table 4.4: Levels of some heavy metals (mg/kg) in samples of cocoa beans from some Cocoa- growing towns in the Ashanti Region of Ghana.

TOWN	SAMPLE	Cd	Pb	Cu	Mn	Fe	Zn
JUASO	**AJ001**	0.066±0.005	0.014±0.001	61.29±0.15	61.17±0.03	66.45±0.14	48.86±0.02
	AJ002	0.052±0.00	0.021±0.001	49.87±0.10	46.26±0.02	48.43±0.06	49.78±0.04
	AJ003	0.078±0.005	0.021±0.001	43.49±0.15	41.72±0.04	68.16±0.01	60.64±0.09
	AJ004	0.051±0.002	0.032±0.001	55.65±0.09	49.39±0.04	43.81±0.03	57.27±0.02
	AJ005	0.042±0.003	0.012±0.001	60.56±0.12	37.20±0.05	44.34±0.14	62.15±0.02
MEAN		**0.058±0.003**	**0.02±0.001**	**54.17±0.1**	**47.15±0.04**	**54.24±0.08**	**55.74±0.04**
TEPA	**AT001**	0.085±0.00	0.012±0.001	43.75±0.10	67.71±0.02	63.67±0.12	48.94±0.03
	AT002	0.070±0.002	0.034±0.002	48.38±0.04	41.25±0.03	39.27±0.72	44.91±0.04
	AT003	0.076±0.00	0.015±0.001	44.56±0.09	71.15±0.03	48.45±0.05	42.93±0.02
	AT004	0.040±0.002	0.014±0.001	41.48±0.13	56.25±0.02	71.26±0.11	67.16±0.03
	AT005	0.024±0.00	0.013±0.001	63.36±0.03	49.68±0.03	44.39±0.04	61.15±0.02
MEAN		**0.059±0.0008**	**0.018±0.001**	**48.31±0.08**	**57.21±0.03**	**53.41±0.21**	**53.02±0.03**
BEKWAI	**AB001**	0.049±0.002	0.011±0.000	38.55±0.10	61.14±0.02	51.58±0.03	66.24±0.03
	AB002	0.041±0.003	0.012±0.001	63.65±0.10	48.35±0.03	47.81±0.0	671.14±0.02
	AB003	0.064±0.001	0.013±0.002	39.86±0.04	57.21±0.04	69.80±0.04	62.38±0.04
	AB004	0.045±0.005	0.023±0.001	47.81±0.05	63.82±0.02	42.17±0.07	49.35±0.03
	AB005	0.052±0.002	0.019±0.001	47.27±0.04	43.27±0.02	39.78±0.04	44.44±0.03
MEAN		**0.050±0.003**	**0.016±0.001**	**47.43±0.07**	**54.76±0.03**	**50.23±0.05**	**58.71±0.03**
OBUASI	**AO001**	0.062±0.001	0.016±0.001	41.71±0.04	48.87±0.05	66.59±0.06	49.18±0.03
	AO002	0.075±0.00	0.022±0.002	36.26±0.06	66.14±0.04	72.36±0.09	66.24±0.02
	AO003	0.057±0.003	0.012±0.001	56.75±0.10	66.73±0.02	48.86±0.07	60.78±0.03
	AO004	0.068±0.002	0.019±0.001	62.78±0.05	62.18±0.02	64.90±0.05	66.24±0.04
	AO005	0.061±0.004	0.014±0.001	41.81±0.05	39.22±0.03	66.63±0.09	44.93±0.02
MEAN		**0.065±0.002**	**0.017±0.001**	**47.86±0.06**	**56.63±0.03**	**63.87±0.07**	**57.47±0.03**
MAMPONG	**AM001**	0.060±0.002	0.013±0.001	38.94±0.02	44.36±0.02	63.48±0.14	49.28±0.03
	AM002	0.042±0.003	0.016±0.001	47.75±0.10	72.64±0.02	43.40±0.05	66.24±0.02
	AM003	0.056±0.004	0.012±0.001	61.35±0.06	58.75±0.03	53.25±0.00	60.78±0.03
	AM004	0.045±0.002	0.017±0.002	42.79±0.04	66.16±0.03	49.62±0.07	66.24±0.04
	AM005	0.050±0.002	0.013±0.001	47.70±0.05	44.79±0.04	47.36±0.09	44.93±0.02
MEAN		**0.051±0.003**	**0.014±0.001**	**47.71±0.05**	**57.34±0.03**	**51.42±0.07**	**57.49±0.03**

Table 4.5: The mean concentrations of heavy metals (mg/kg) in cocoa beans from the Western and Ashanti Regions of Ghana.

REGION	Mean Cd	Mean Pb	Mean Cu	Mean Mn	Mean Fe	Mean Zn
WESTERN	**0.054±0.002**	**0.02±0.0004**	**51.98±0.04**	**55.18±0.03**	**47.51±0.03**	**48.29±0.04**
ASHANTI	**0.056±0.0002**	**0.017±0.001**	**49.10±0.07**	**54.62±0.03**	**54.63±0.10**	**56.49±0.03**

Table 4.6: Moisture content

Sample Town		Wt of crucible	Wt of crucible + sample	Wt of crucible + Dry sample	Wt, of sample taken	Wt, of moisture	% moisture	Mean
Kasapen	1	38.7167	40.8382	40.7714	2.1216	0.0668	3.15	3.15
	2	43.6403	45.8273	45.7584	2.1870	0.0689	3.15	
Asempaneye	1	21.7893	23.8484	23.7593	2.0591	0.0891	4.44	4.39
	2	23.7281	25.7360	25.6469	2.0079	0.0891	4.44	
Bekwai	1	42.1867	44.2172	44.1255	2.030	0.0917	4.52	4.46
	2	48.9408	51.0137	50.9226	2.0729	0.0911	4.39	
Juaso	1	42.1129	44.1415	44.0957	2.0286	0.0458	2.26	2.23
	2	43.2416	45.2521	45.2078	2.0105	0.0443	2.20	

Table 4.7: Ash content

Sample		Wt of crucible	Wt of crucible + sample	Wt of crucible + Dry sample	Wt, of sample taken	Wt, of Ash	% ash	Mean
Kasapen	1	21.1226	23.1752	21.1958	2.0526	0.0732	3.57	3.40
	2	19.5487	21.5507	19.6132	2.0020	0.0645	3.22	
Asempaneye	1	19.7290	21.7595	19.7982	2.0305	0.0692	3.41	3.69
	2	18.7902	20.8305	18.8709	2.0403	0.0807	3.96	
Bekwai	1	18.1538	20.2742	18.2366	2.1204	0.0828	3.90	3.52
	2	18.8804	20.9438	18.9451	2.0634	0.0647	3.14	
Juaso	1	19.6858	21.7326	19.7494	2.0468	0.0636	3.11	3.19
	2	18.7208	20.7861	18.7883	2.0653	0.0675	3.27	

Table 4.8: Fat levels

Sample		Wt. of flask	Wt. of flask + fat	Wt. of sample taken	Wt. of Fat	% Fat	Mean
Kasapen	1	121.6800	122.6110	2.0373	0.9310	45.70	45.52
	2	122.9810	123.9050	2.0386	0.9240	45.33	
Sefwi Asempaneye	1	123.4610	124.3470	2.0256	0.8860	43.74	43.85
	2	122.8610	123.7530	2.0297	0.8920	43.94	
Bekwai	1	118.3380	119.2740	2.0417	0.9360	45.84	45.57
	2	119.4240	120.3450	2.0331	0.9210	45.30	
Juaso	1	183.9980	184.7420	2.0317	0.7440	36.62	36.72
	2	179.4240	180.2380	2.0421	0.7520	36.82	

Table 4.9: Fibre content

Sample		Wt. of crucible +Asbestos +fibre	Wt. of crucible + Asbestos	Wt. of Sample taken	Wt. of fibre	% fibre	Mean
Kasapen	1	25.7019	25.6808	2.0373	0.0211	1.04	1.03
	2	24.1040	24.0832	2.0386	0.0208	1.02	
Sefwi Asempaneye	1	29.6899	29.6695	2.0256	0.0204	0.01	1.02
	2	11.0339	11.0132	2.0297	0.0207	0.02	
Bekwai	1	11.5763	11.5543	2.0417	0.0220	1.08	1.09
	2	25.7076	25.6852	2.0331	0.0224	1.10	
Juaso	1	24.0715	24.0495	2.0317	0.0220	1.08	1.07
	2	11.6438	11.6222	2.0421	0.0216	1.06	

Table 4.10: Protein content

Sample		Initial Reading	Final Reading	Titric	Average Titre	Wt. of sample taken	% Protein
Kasapen	1	0.00	3.55	3.55		2.0456	14.34
	2	3.55	7.00	3.45	3.50		
Asempaneye	1	7.00	10.45	3.45		2.0561	13.97
	2	10.45	13.85	3.40	3.43		
Bekwai	1	13.85	17.20	3.35		2.0398	13.74
	2	17.20	20.55	3.35	3.35		
Juaso	1	20.55	24.00	3.45		2.0465	13.91
	2	24.00	27.35	3.35	3.40		

Table 4.11 Carbohydrate content

Town	Percentage of Carbohydrate
Kasapen	32.56
Sefwi- Asempaneye	32.08
Bekwai	31.62
Juaso	42.88

%ofcarbohydrate=100%-%Moisture+%Fat+%Protein+%Fibre+%Ash

4.3 GRAPHICAL REPRESENTATION OF RESULTS

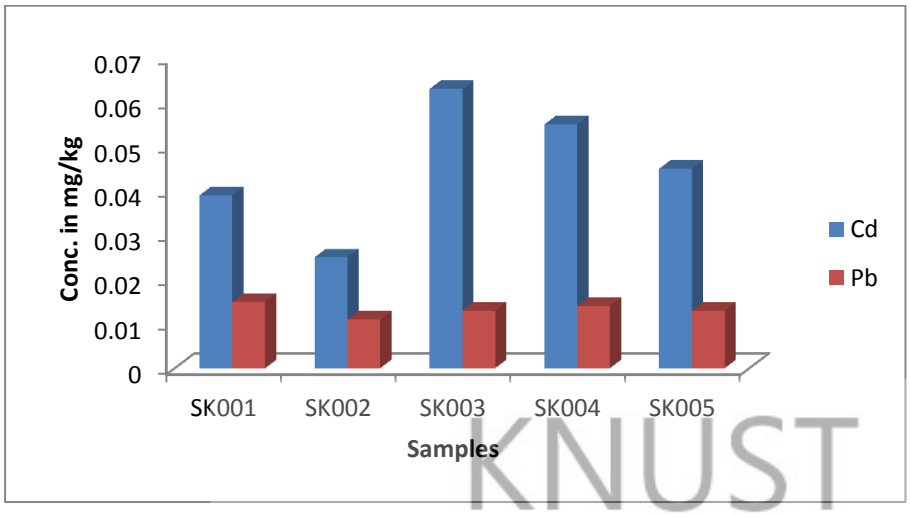


Figure 4.1: Levels of Cd and Pb in samples of cocoa beans from Kasapen in the Western Region of Ghana

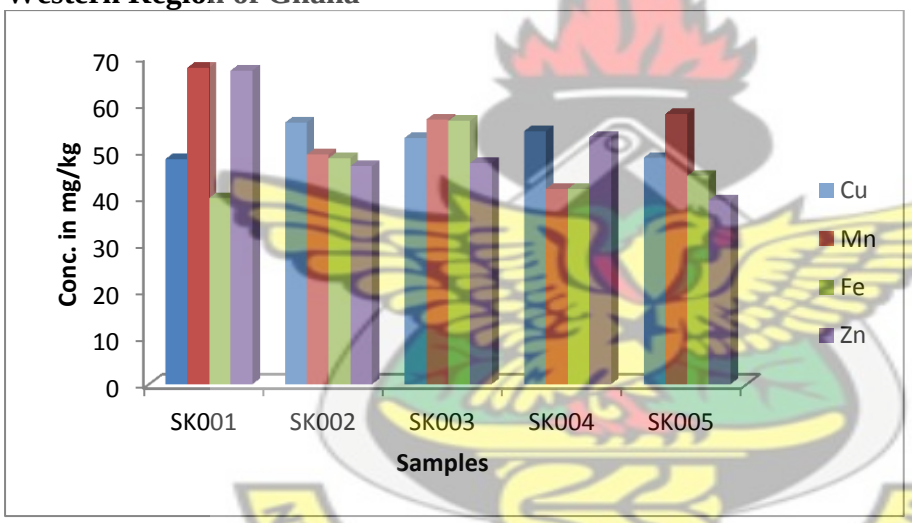


Figure 4.2: Levels of some heavy metals (Cu, Mn, Fe, and Zn) in samples of cocoa beans from Kasapen in the Western Region of Ghana

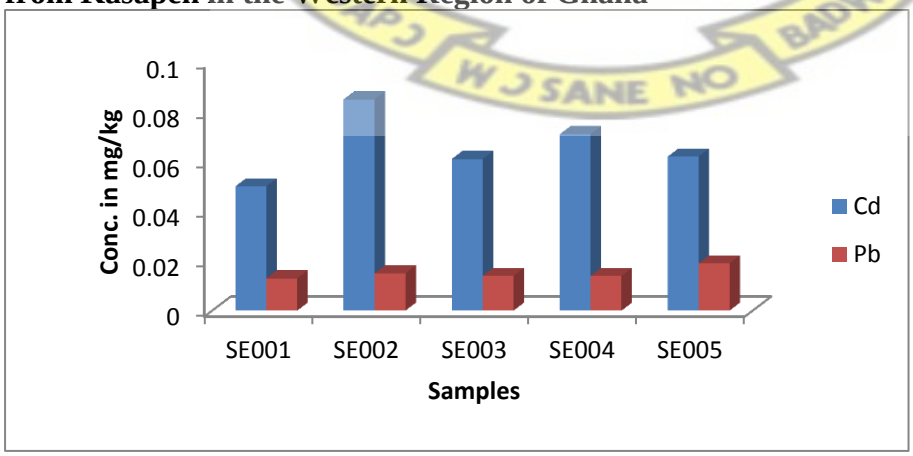


Figure 4.3: Levels of Cd and Pb in samples of cocoa beans from Enchi in the Western Region of Ghana

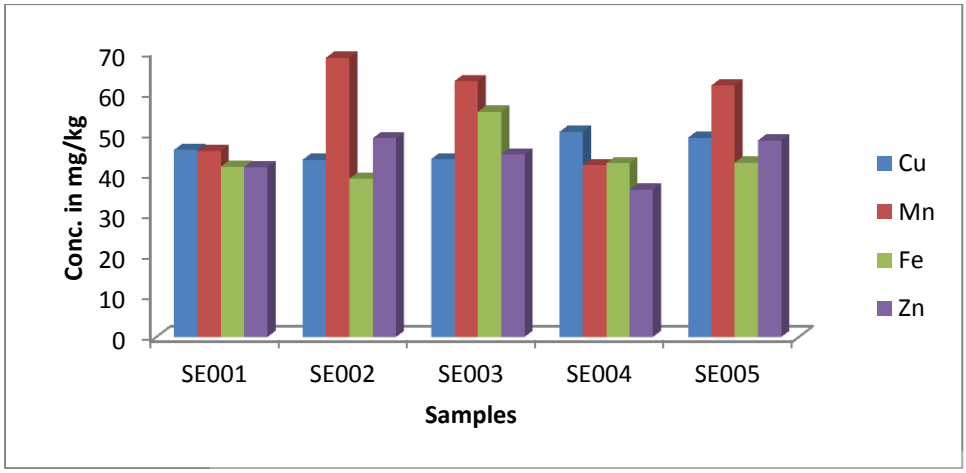


Figure 4.4: Concentrations of Cu, Mn Fe and Zn in samples of cocoa beans from Enchi in the Western Region of Ghana

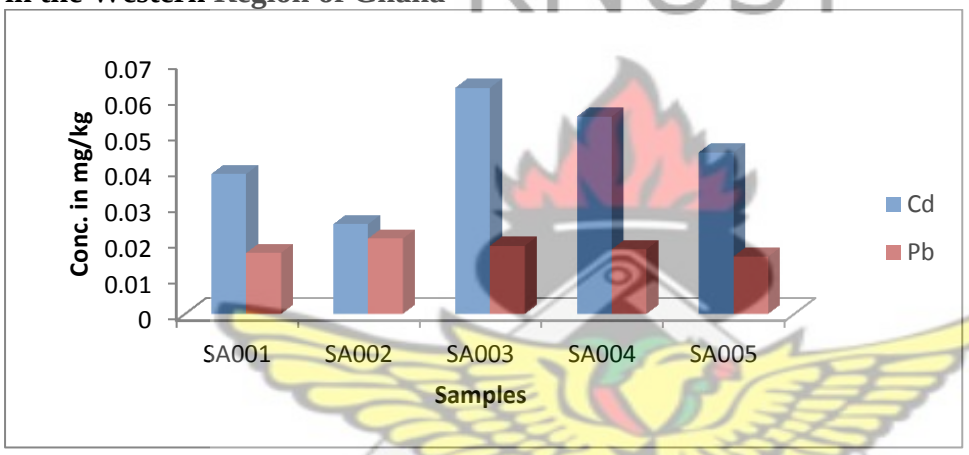


Figure 4.5: A graph showing the levels of Cd and Pb in samples of cocoa beans from Sefwi –Asempaneye in the Western Region of Ghana.

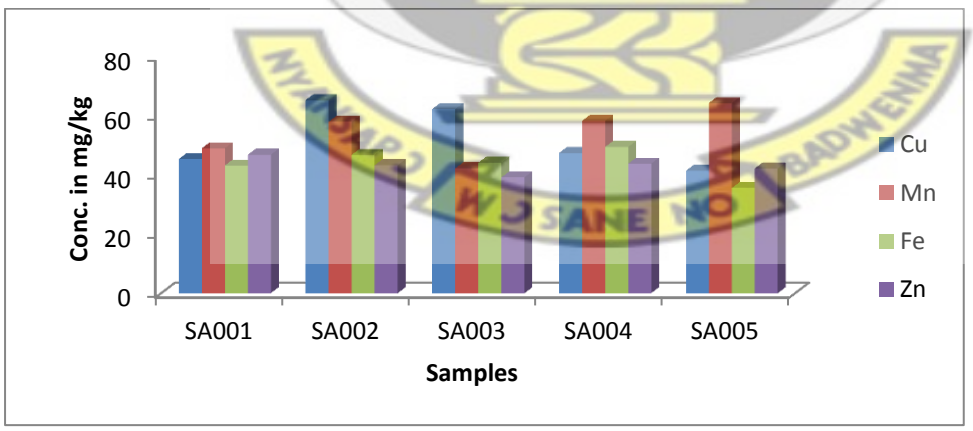


Figure 4.6: Levels of Cu, Mn, Fe and Zn in samples of cocoa beans from Sefwi-Asempaneye in the Western Region of Ghana

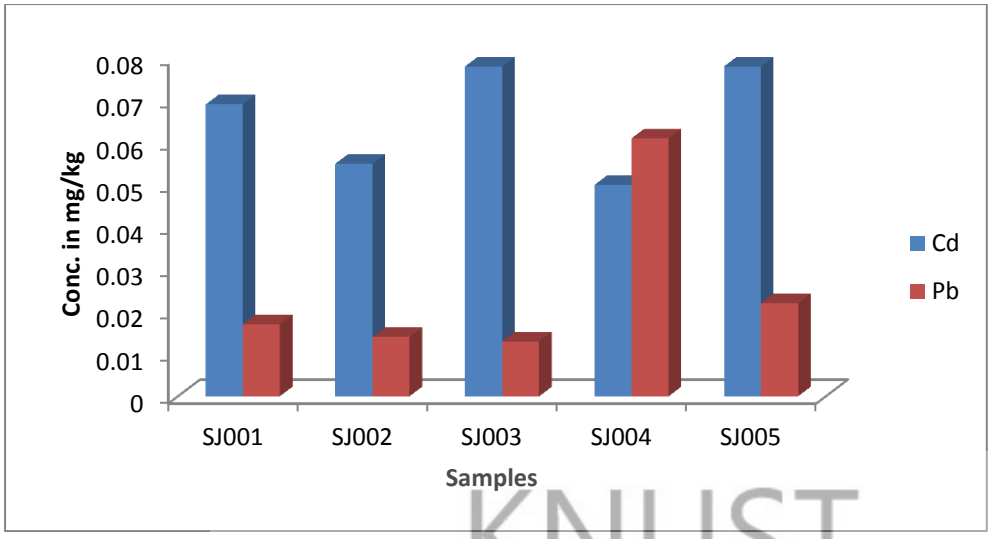


Figure 4.7: A graph showing the variations in the levels of Cd and Pb in samples of cocoa beans from Juabeso in the Western Region of Ghana

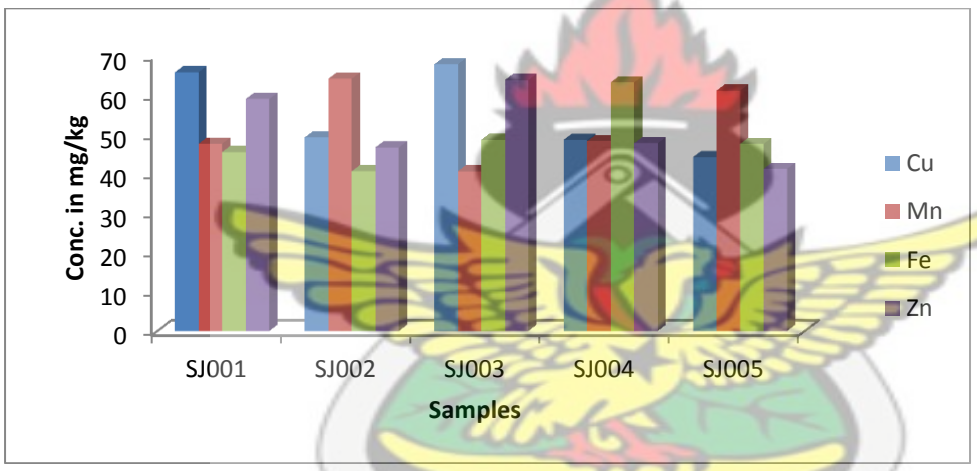


Figure 4.8: Levels of Cu, Mn, Fe and Zn in samples of cocoa beans from Juabeso in the Western Region of Ghana

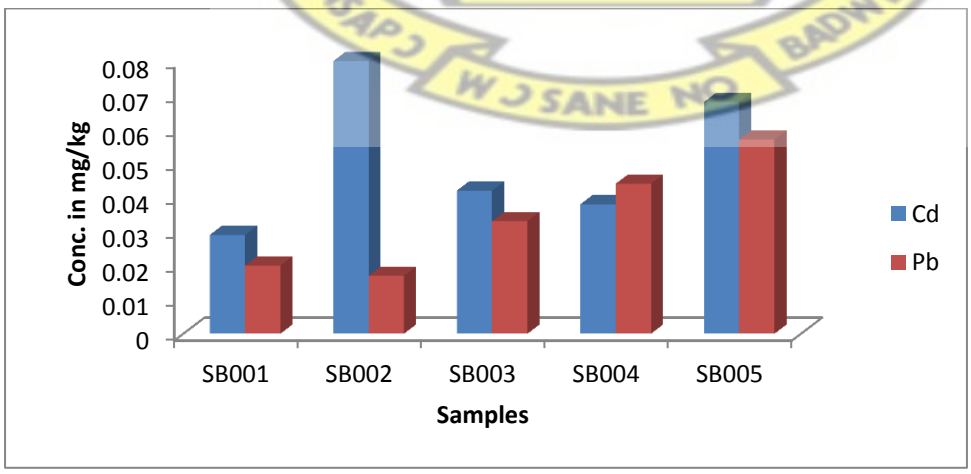


Figure 4.9: Levels of Cd and Pb in samples of cocoa beans from Bogoso in the Western Region of Ghana

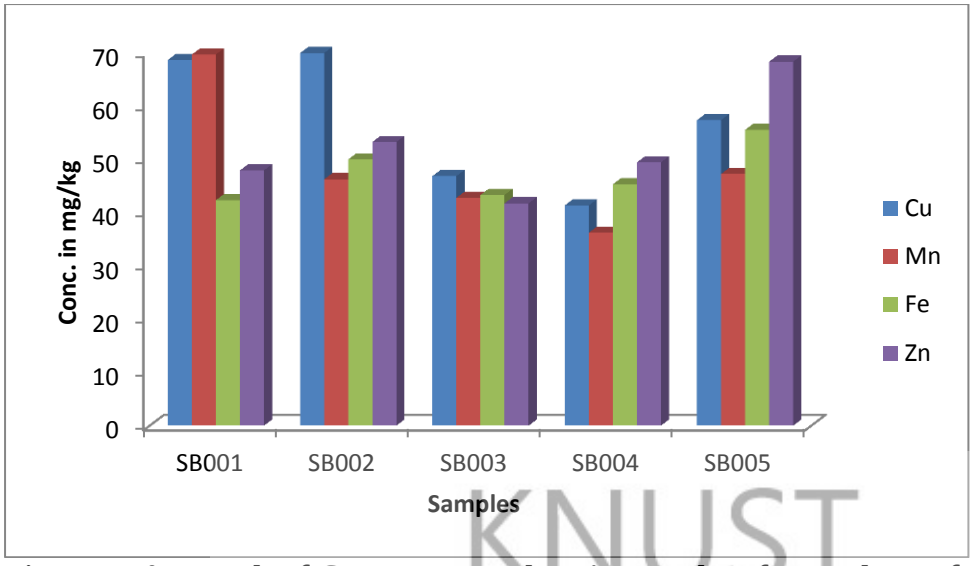


Figure 4.10: Levels of Cu, Mn, Fe and Zn in samples of cocoa beans from Bogoso in the Western Region of Ghana

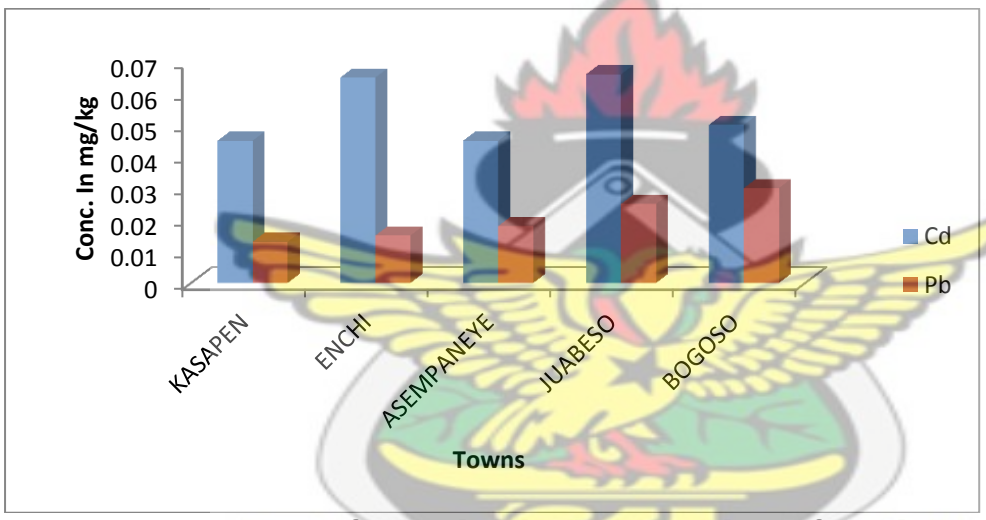


Figure 4.11: Variations of Cd and Pb levels in cocoa beans from the sample towns in the Western Region of Ghana

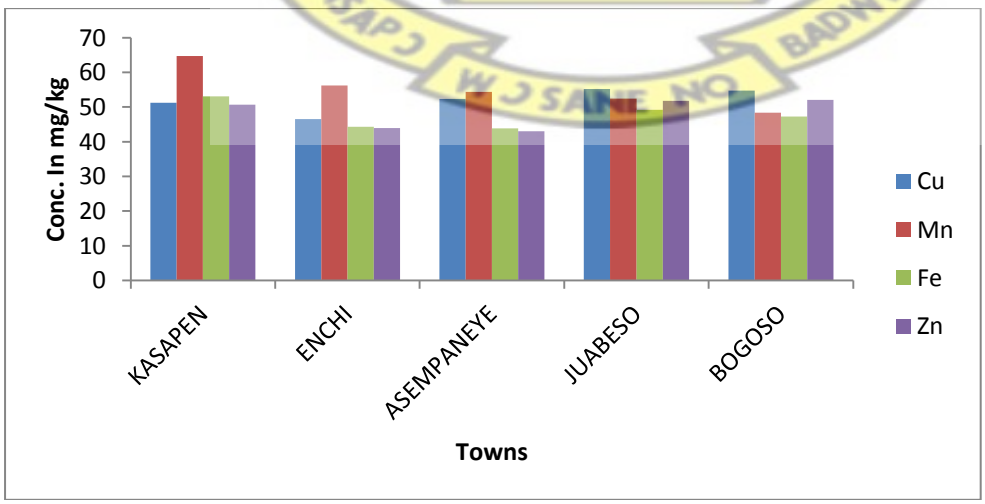


Figure 4.12: Variations of Cu, Mn, and Fe and Zn levels in cocoa beans from the sample towns in the Western Region of Ghana

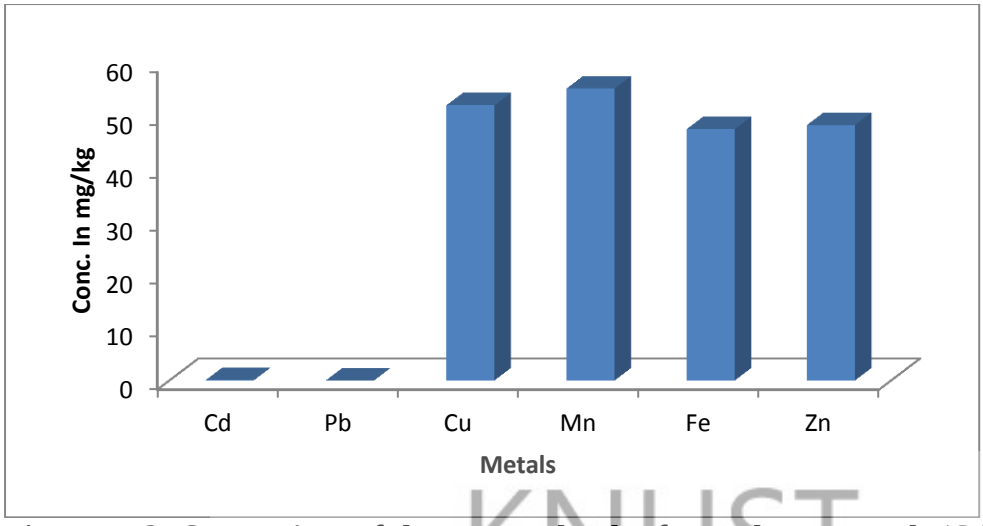


Figure 4.13: Comparison of the average levels of some heavy metals (Cd, Pb, Cu Mn, Fe and Zn) in samples of cocoa beans from the Western Region of Ghana.

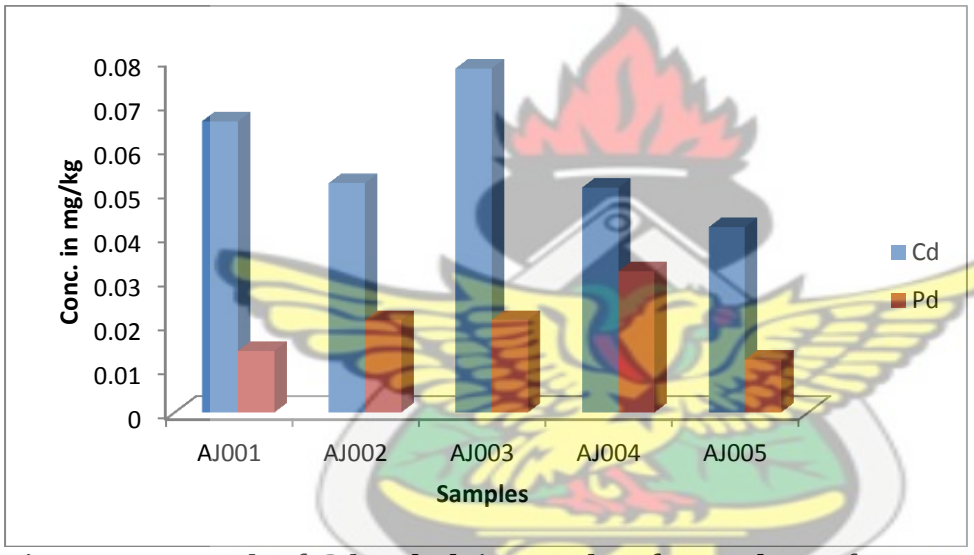


Figure 4.14: Levels of Cd and Pb in samples of cocoa beans from Juaso in the Ashanti Region of Ghana

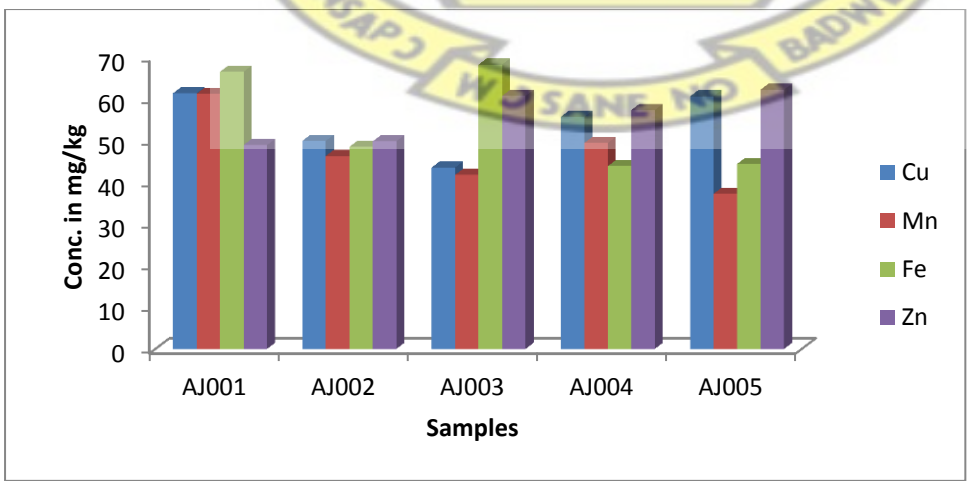


Figure 4.15: a graph showing the variations of the levels of Cu, Mn, Fe and Zn in samples of cocoa beans from Juaso in the Ashanti Region of Ghana

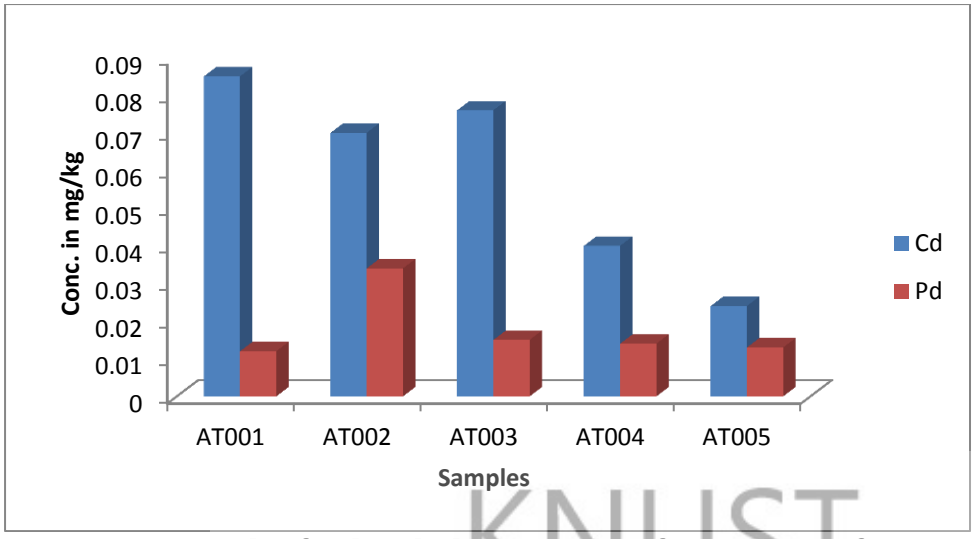


Figure 4.16: Levels of Cd and Pb in samples of cocoa beans from Tepa in the Ashanti Region of Ghana

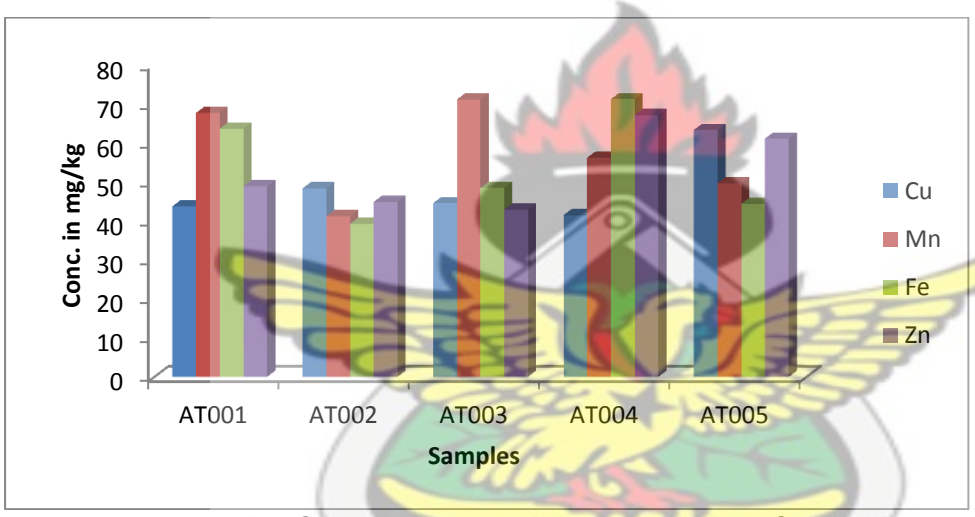


Figure 4.17: Levels of Cu, Mn, Fe and Zn in samples of cocoa beans from Tepa in the Ashanti Region of Ghana

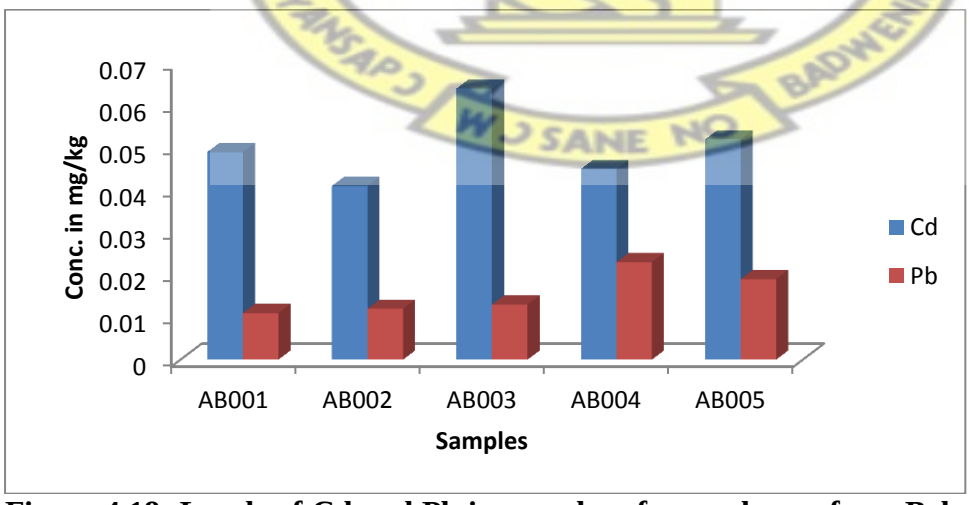


Figure 4.18: Levels of Cd and Pb in samples of cocoa beans from Bekwai in the Ashanti Region of Ghana

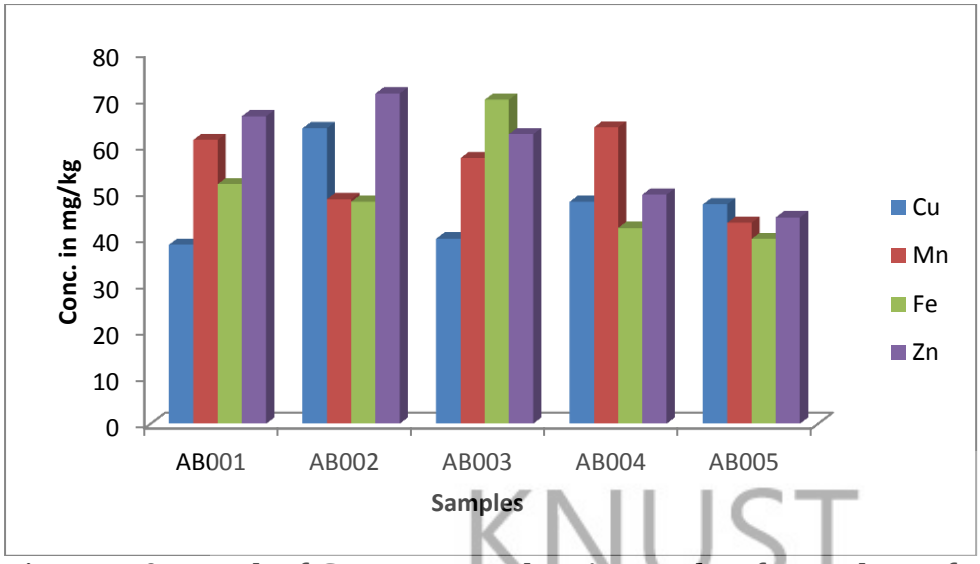


Figure 4.19: Levels of Cu, Mn, Fe and Zn in samples of cocoa beans from Bekwai in the Ashanti Region of Ghana

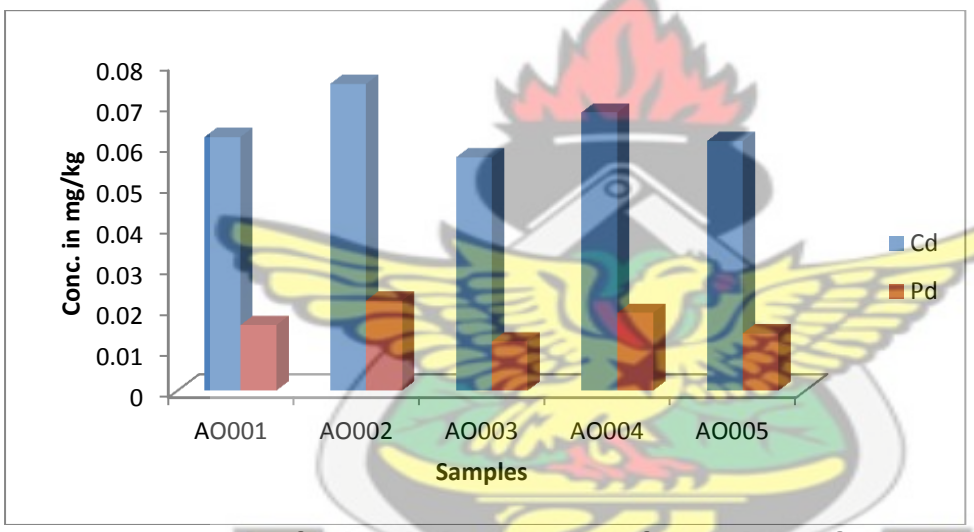


Figure 4.20: Levels of Cd and Pb in samples of cocoa beans from Obuasi in the Ashanti Region of Ghana

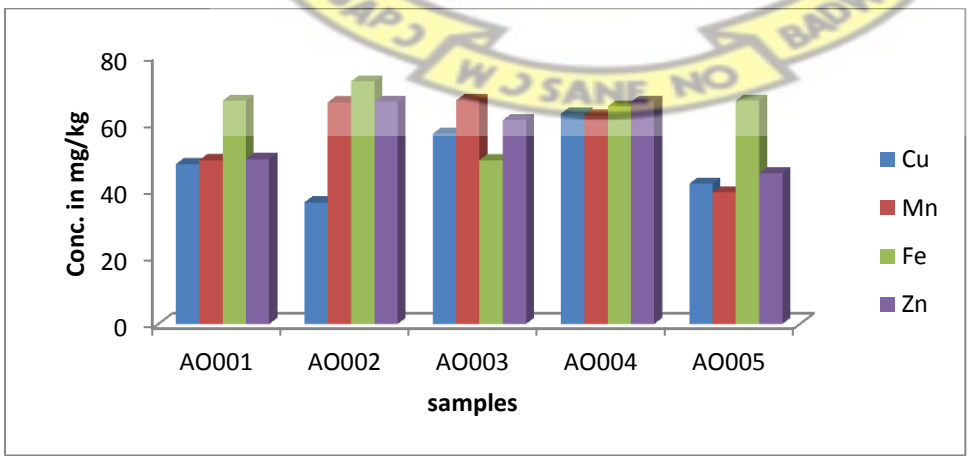


Figure 4.21: A graph showing the variations of the levels of Cu, Mn, Fe and Zn in samples of cocoa beans from Obuasi in the Ashanti Region of Ghana

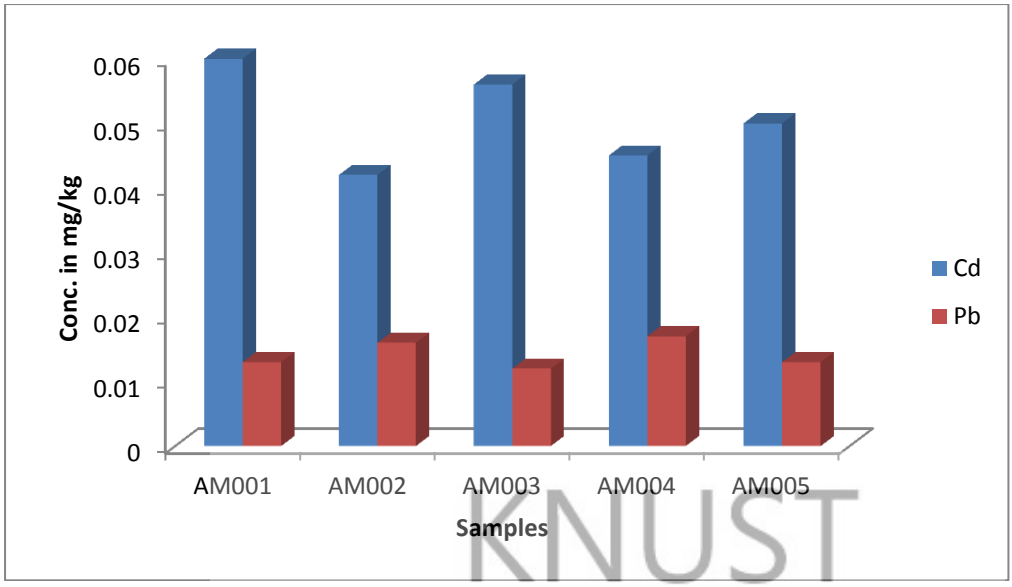


Figure 4.22: Levels of Cd and Pb in samples of cocoa beans from Mampong in the Ashanti Region of Ghana

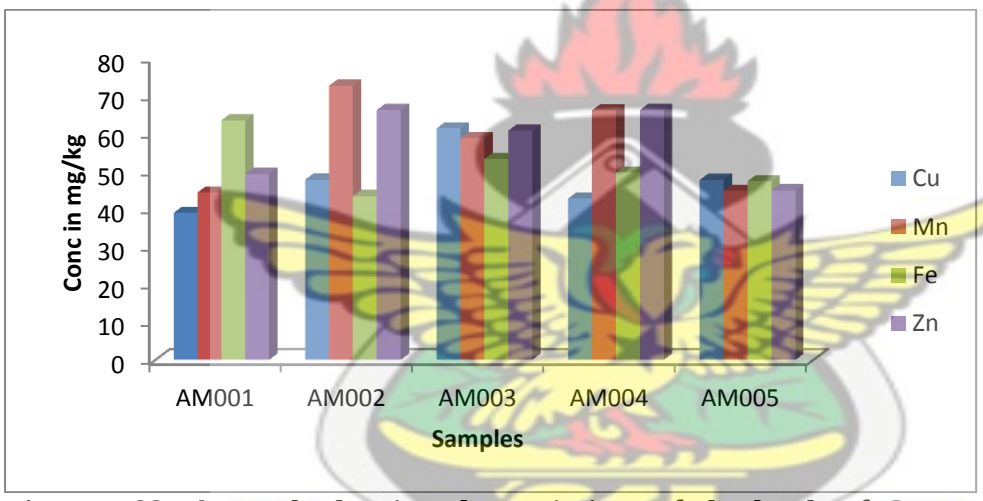


Figure 4.23: A graph showing the variations of the levels of Cu, Mn, Fe and Zn in samples of cocoa beans from Mampong in the Ashanti Region of Ghana

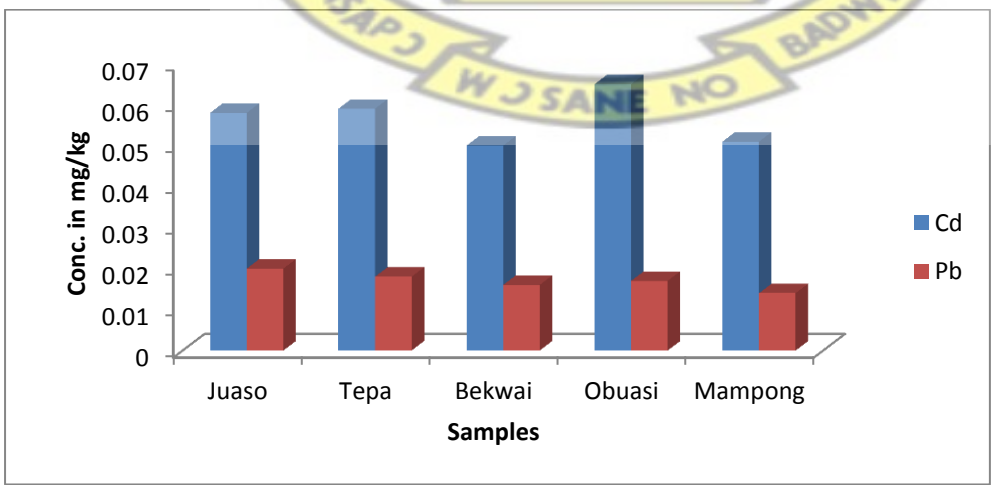


Figure 4.24: Comparison of Cd and Pb levels in cocoa beans from the sampled towns in the Ashanti Region of Ghana

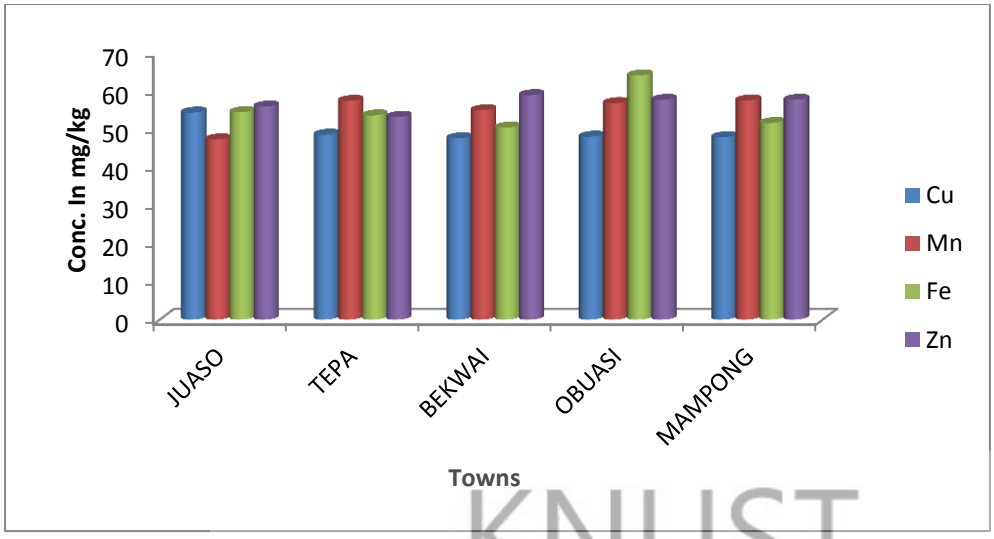


Figure 4.25: Comparison of Cu, Mn, Fe and Zn levels in cocoa beans from the sample towns in the Ashanti Region of Ghana

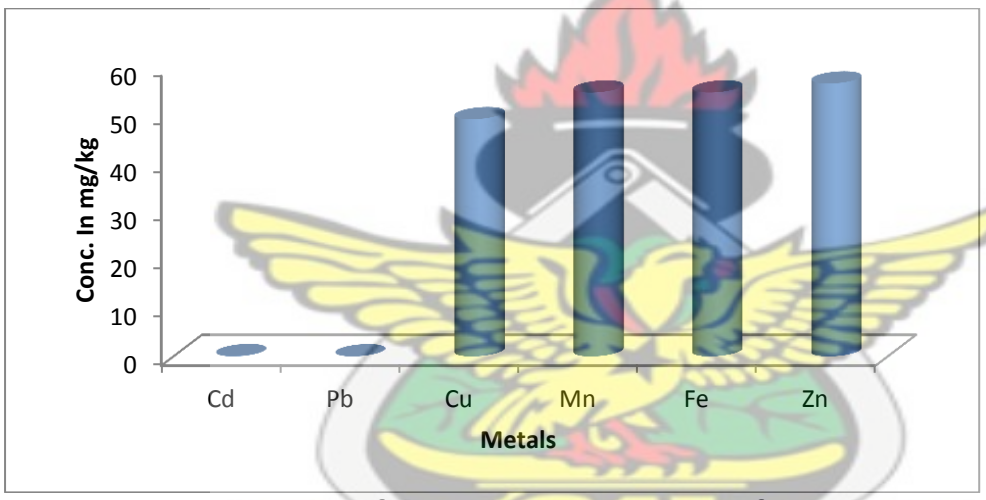


Figure 4.26: Comparison of the mean concentrations of some heavy metals (Cd, Pb, Cu, Mn, Fe and Zn) in samples of cocoa beans from the Western Region of Ghana.

4.4 LEVELS OF HEAVY METALS IN COCOA BEANS

4.4.1 Lead

With reference to the determinations carried out in this study, a mean lead concentration ranging from 0.013mg/kg in samples from Kasapen to 0.03mg/kg in samples from Bogoso were obtained in cocoa beans from the individual towns of the Western Region of Ghana. This was obtained by computing the mean lead concentrations of the individual samples from

the various towns in the Western Region of Ghana (Kasapen, Asenpanaye, Juabeso, Enchi and Bogoso). However, the mean lead concentrations in cocoa beans from the aforementioned towns in the Western Region were found to be quite uniform. The levels of lead obtained in this work compare favourably with lead levesl in most fruits and vegetables Codex Almentarius has set a maximum of 0.1mg/kg for most of these fruits and vegetables (11) and the level of lead in cocoa beans from the western Region in comparison with the codex alimetarius maximum level is within the permissible limits. It is worth-mentioning that the level of lead in the individual five samples from each town before the mean for the towns were calculated were also found within the permissible limits from Table 4.3. This means that the unprocessed cocoa beans from the Western Region of Ghana are of quality in terms of the levels of lead in them. Figure 4.27 compares the mean levels of lead in cocoa beans from the sample towns in both Western Region and Ashanti Region with the Codex maximum limit.

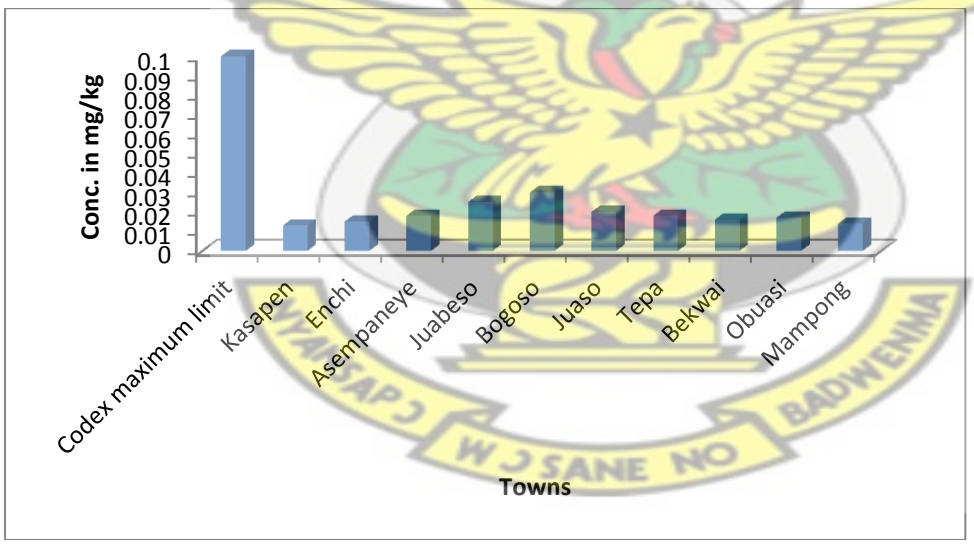


Figure 4.27: Comparison of the levels of lead (Pd) in cocoa beans from the sampling towns from both Western Region and Ashanti Region with codex maximum limit

Similar observations were made concerning the mean lead concentrations of individual samples towns in the Ashanti Region of Ghana. The mean lead concentrations, with reference to table 4.4 ranged from 0.014mg/kg in samples from Mampong to 0.02mg/kg in samples

from Juaso in the Ashanti Region of Ghana. The levels of lead in cocoa beans from the Ashanti region of Ghana also fall well below the maximum permissible limits.

Finally the mean lead concentrations in cocoa beans from the sample towns in the Western and Ashanti Regions were also compared with the codex maximum limits. Again, the levels of lead in all cocoa samples from Western and Ashanti Regions were found to fall below the codex maximum limits as:

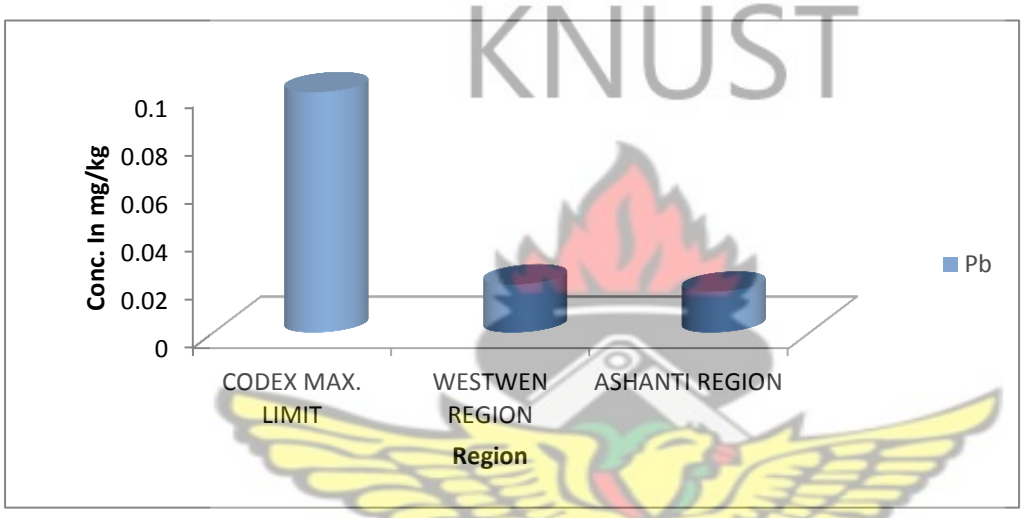


Figure 4.28: Comparison of the concentration of lead (Pb) in cocoa samples from Western and Ashanti Regions with the codex maximum limit

Comparatively, while the levels of lead obtained in this study fall well below the maximum permissible limits, Rankin et al [11] have reported even lower levels of lead (0.005kmg/kg) in cocoa beans from Nigeria using ICP-MS.

Lead is very detrimental to human health and it is regarded to be one of the most toxic metals with higher probability of incorporation into most foods. In most diseases, lead poisoning has been implicated and it is considered by the United States Agency for Toxic substances and Disease Registry (ATSDR) as one of the most toxic metals. What exacerbates the situation is that the targets of lead toxicity are the cardiovascular system (heart), kidneys and the developing nervous systems. Owing to this, children have been found to be more susceptible to the health effects of lead toxicity than adults.

Cocoa products have been reported to contain relatively high levels of lead as compared to other similar food products [10]. Though cocoa beans as a raw material for the manufacture of cocoa products contribute to the elevated levels of lead in cocoa products, the greatest percentage of lead contamination of cocoa products occur during processing of beans due to various industrial processes that may require the use of metal parts some of which may contain trace amounts of the metal [11]. Anthropogenic activities are the main sources of increase in the levels of lead in both soil and food. This is because lead has been used in a number of applications such as paints, car batteries and as components in anti-knocking agents in fuel.

4.4.2 Cadmium

Table 4.3 and Table 4.4 show the mean (±standard deviation) of the concentrations of cadmium in cocoa beans from the various towns in Western and Ashanti Regions of Ghana respectively. The concentration of cadmium (Cd) in cocoa beans from the Western Region ranged from 0.045mg/kg in samples from Kasapen and Asempaneye to 0.66mg/kg in samples from Juabeso. That of Ashanti Region ranged from 0.05mg/kg in samples from Bekwai to 0.065mg/kg in samples from Obuasi

The levels of cadmium in samples from the towns in the Western Region as well as Ashanti Region were found to be fairly uniform. Though the specific allowable limits for cocoa beans are not readily available, the levels of Cd obtained in this work compare favourably with the maximum limit of 0.1mg/kg fresh weight set for most plant parts (excluding the leaves). The mean levels of cadmium in cocoa beans from the sampling towns of Western and Ashanti Regions as compared to the Codex maximum permissible limit are represented in Figure 4.29. The mean levels of Cd in the entire cocoa samples from both Western and Ashanti Regions were found to be 0.054mg/kg and 0.056mg/kg respectively which are compared with the Codex maximum limit in Figure 4.30.

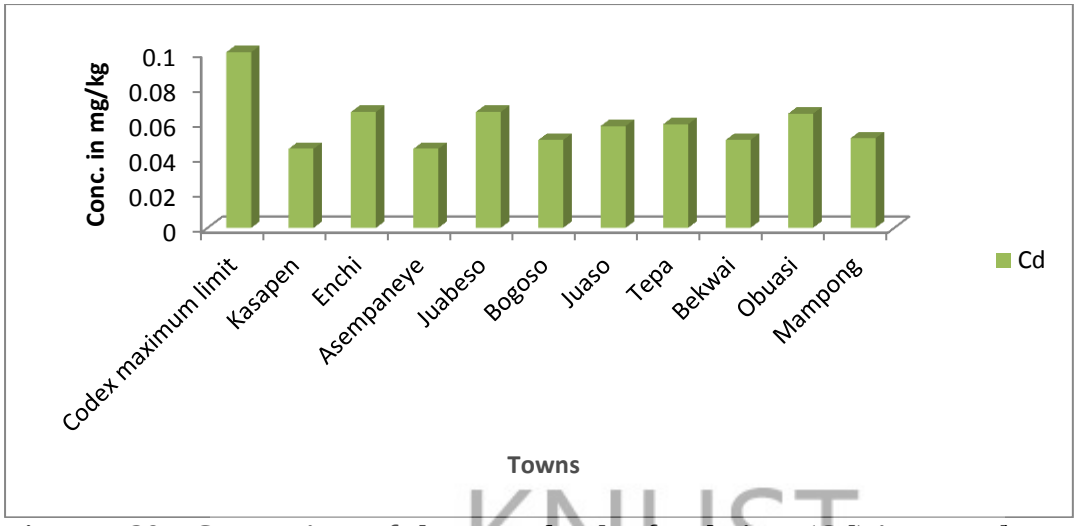


Figure 4.29: Comparison of the mean levels of cadmium (Cd) in cocoa beans from the sampling towns from both Western Region and Ashanti Region with the codex maximum limit

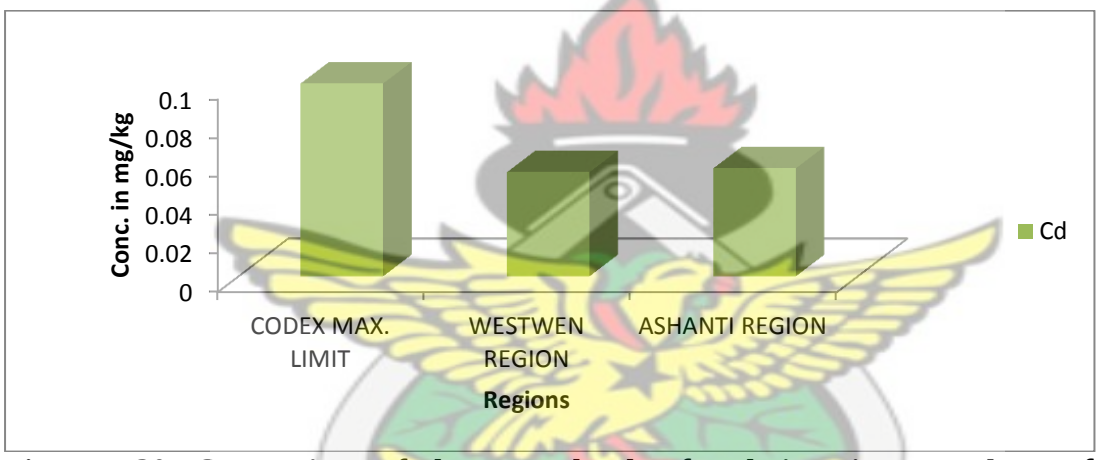


Figure 4.30: Comparison of the mean levels of cadmium in cocoa beans from the Western and Ashanti Regions with the codex maximum limit

The availability of cadmium in cocoa beans may be attributed to its natural occurrence in the soil and anthropogenic activities. Cadmium levels in the soil range from 0.01 – 7mg/kg [205]. Because of the high cation exchange capacity of most tropic soils the levels of heavy metals such as cadmium in the tropic soil are said to be very low [206].

Cadmium which is found in most fertilizers may get incorporated in plants grown with the use of these fertilizers. Once cadmium gets into the soil it is found to accumulate in the top soil where it binds strongly to organic matter and prevented from entering soil solution. The high sorption capacity of tropic soils may prevent its access to the plant and so reduce the risk

of contamination in food. The involvement and availability of cadmium in soil is low and best under low pH conditions [206]. Thus the use of pesticides containing trace amounts on cocoa plants to prevent and cure numerous diseases is a major contributory factor of the presence of heavy metals in soil and may consequently accumulate in plant and soil over time which may lead to toxicity. It is reported that cadmium tends to accumulate more in leaves of plants than in the seeds [207,208]. The seriousness of cadmium toxicity is that it accumulates in all levels of food chain and concentrate in the liver and kidneys causing disorders. The pathway of cadmium toxicity is through consumption of contaminated food. However, the levels of cadmium in cocoa beans analyzed in the work may not pose any significant health hazard due to relatively low concentrations. The levels are in agreement with cadmium levels in cocoa reported by Mounicou et al (2003) [209].

4.4.3 Other Elements (Zinc, Copper, Manganese and Iron)

The presence of these metals in food mostly is regarded relatively non- toxic or essential in living organisms. However, the levels of these metals analyzed in this work were much higher than the levels of cadmium and lead which are considered irrelevant in living organisms and are termed as toxic elements or metals. The mean levels of these metals in cocoa beans from the towns in the Western and Ashanti Regions are shown in Tables 4.5. Iron and zinc were the highest in all the samples in this work corroborating their normal composition in plants relative to other metals. The levels of these metals are mostly not of significant health interest especially at low concentrations because they are considered to be essential in plants, animals and man. Conversely, their presence at high concentrations beyond requirements may pose health problem to living organisms by interfering with the functions of other essential elements.

The mean concentration of zinc ranged from a low of 43.04mg/kg in samples from Asempaneye in the Western Region to 58.71mg/kg in samples from Bekwai in the Ashanti

Region. That of iron ranged from 43.80mg/kg in samples from Asempaneye in the Western Region to 63.874mg/kg in samples from Obuasi in the Ashanti Region. However, the concentrations of Zn and Fe in cocoa beans from the Western Region, upon the analysis, were found to be of lower levels as compared to those from Ashanti Region. The relative lower levels of these metals recorded from the Western Region may be attributed to factors such as heavy rainfall pattern in the region, soil characteristics and low levels in the soil. The Western Region is known to have the highest rainfall ievel in Ghana, a condition which may bring about leaching of most of these metals. The levels of Zn and Fe in the samples from both Ashanti and Western Regions are shown in figures 4.31 and 4.32.

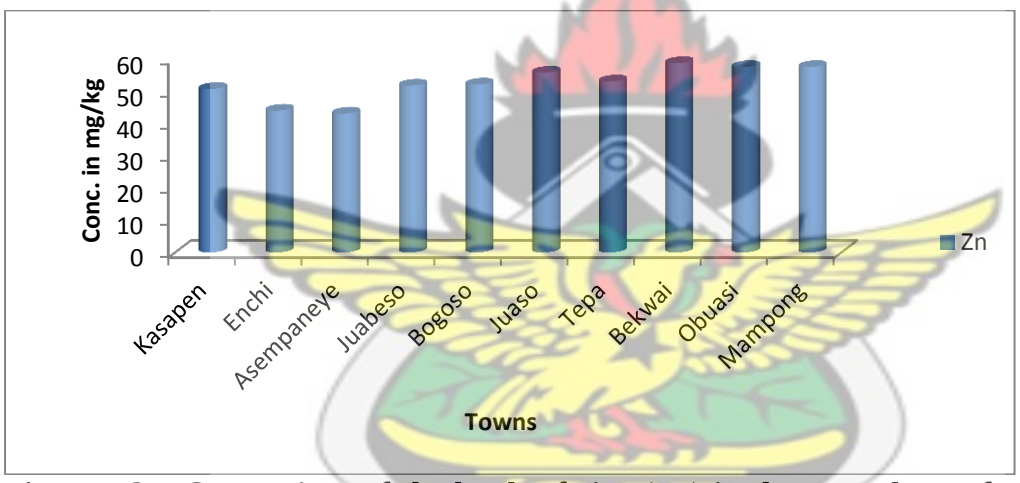


Figure 4.31: Comparison of the levels of zinc (Zn) in the cocoa beans from the sample towns in both Ashanti Region and Western Region.

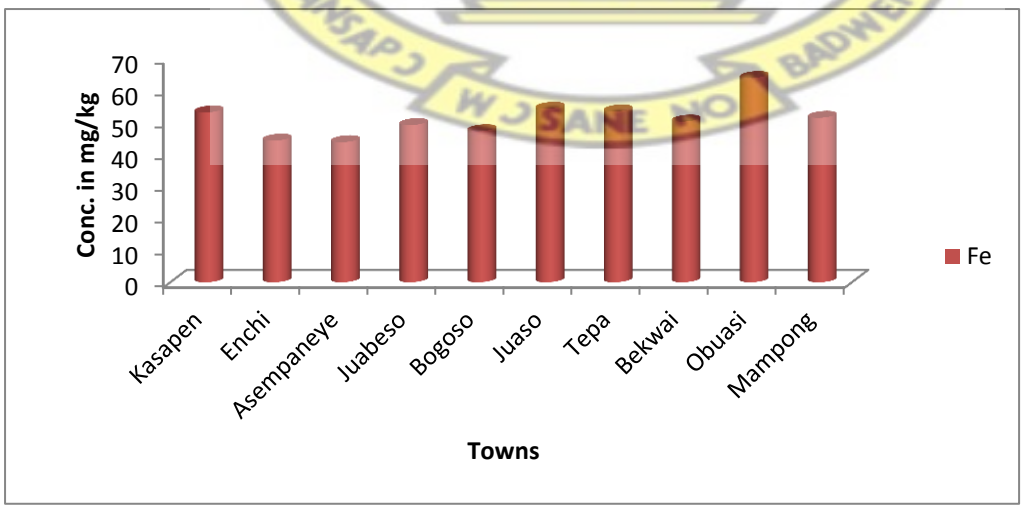


Figure 4.32: Comparison of the levels of iron (Fe) in cocoa beans from the sample towns in both Ashanti Region and Western Region.

The mean concentrations of manganese and copper were also high with that of manganese ranging from a low of 47.15.mg/kg in samples from Juaso in the Ashanti Region to 64.65mg/kg in samples from Kasapen in the Western Region.

That of copper also ranged from low of 46.47mg/kg in samples from Enchi to a high of 55.17mg/kg in samples from Juabeso all in the Western Region (Table4.5 and4.6). Cocoa is known to be a rich source of copper. Due to the continual use of copper –containing pesticides on cocoa farm to avert pests and diseases, these high levels of the metal in the beans are likely to increase over time. Copper intake in the body has been found to be primarily through diet, though this amount usually does not exceed the average dietary requirements of 10-12mg/day for adults [210].

The probable increase in the levels of copper in cocoa beans over the years ahead may be as a result of the fact that copper is probably the most widely used heavy metal on cocoa farms as fungicide. Oxides and hydroxides of copper are used as fungicides and sprayed on the cocoa plants about four times each year to prevent and control the occurrence of diseases and pests on the cocoa farms. Fungicides such as kocide 101 ($Cu(OH)_2$, 22%) and nordox 50 (Cu_2O, 50%) contain high levels of copper which upon application, get incorporated into the cocoa plant and the soil and may accumulate.

Irrespective of their concentration in the soil, the uptake of most heavy metals such as copper by plants is dependent on a number of factors such as pH, organic matter content, state of the metal etc. The uptake of copper for instance is improved under acidic conditions and the presence of high organic matter content of the soil [211].

In spite of the fact that copper occurs naturally in all plants (and animals) and essential in a number of biological processes in the body such as hemoglobin formation, drug and carbohydrate metabolism and antioxidant defense mechanisms, at excessive levels, however,

toxicity may result. Mining activities and the application of phosphate fertilizers account for two of the main sources of release of copper into the soil and in the plant [212].

Manganese is one of the commonest as well as most abundant metal in the environment. Manganese and its compounds exist naturally in the environment though its levels may be increased through human activities such as mining operations, burning of fossil fuels and the addition of pesticides and fertilizers on the farm. Once manganese settles on the soil, the soil type and its chemical state are some of the factors that determine its mobility.

Manganese is an essential trace element necessary for good health. In humans and animals, some of the remarkable roles played by Mn are: bone mineralization, protein and energy metabolism, metabolic regulation, cellular protection from damaging free radical species etc. [213]. Analysis in this work revealed that the levels of manganese in cocoa bean were found to be quite uniform in all the towns sampled across both Regions.

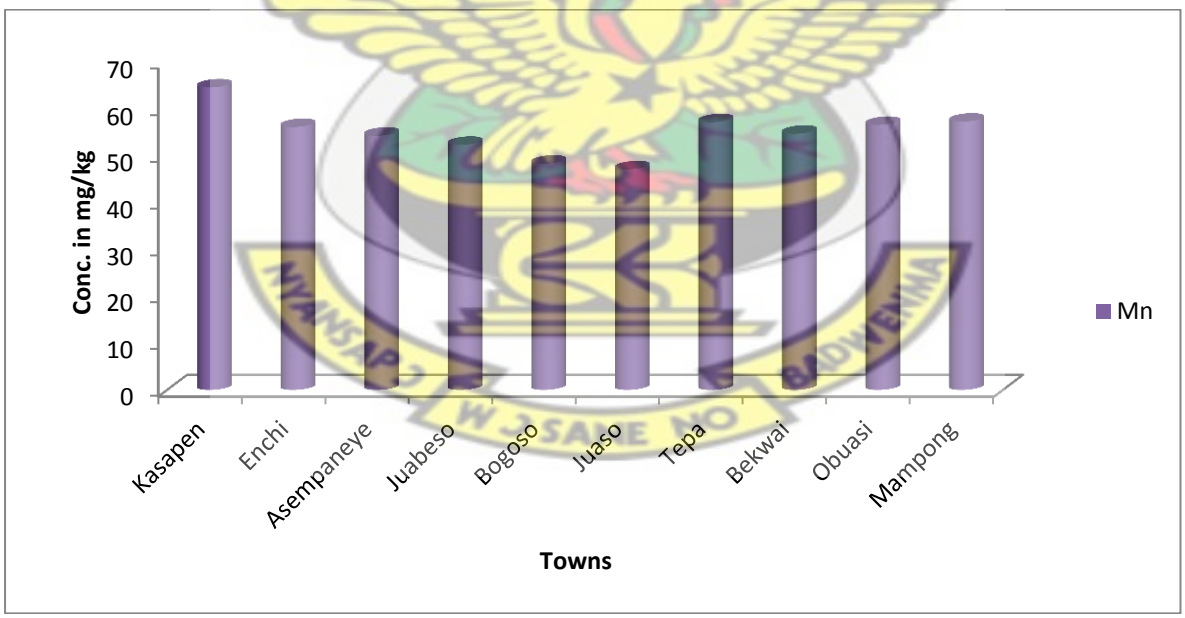


Figure 4.33: Comparison of the levels of manganese (Mn) in cocoa beans from the sample towns in both Ashanti Region and Western Region.

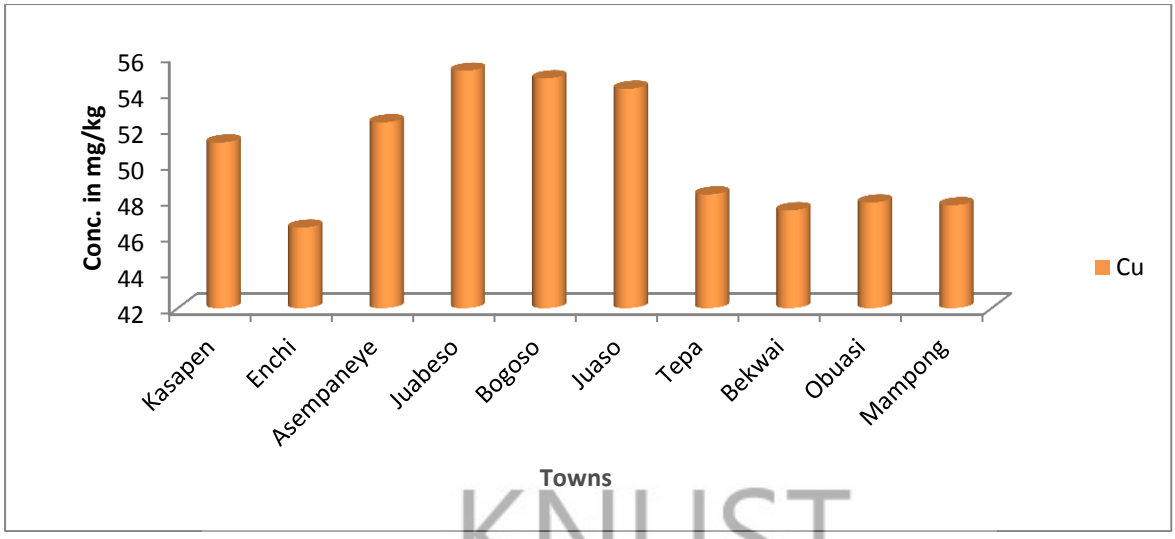
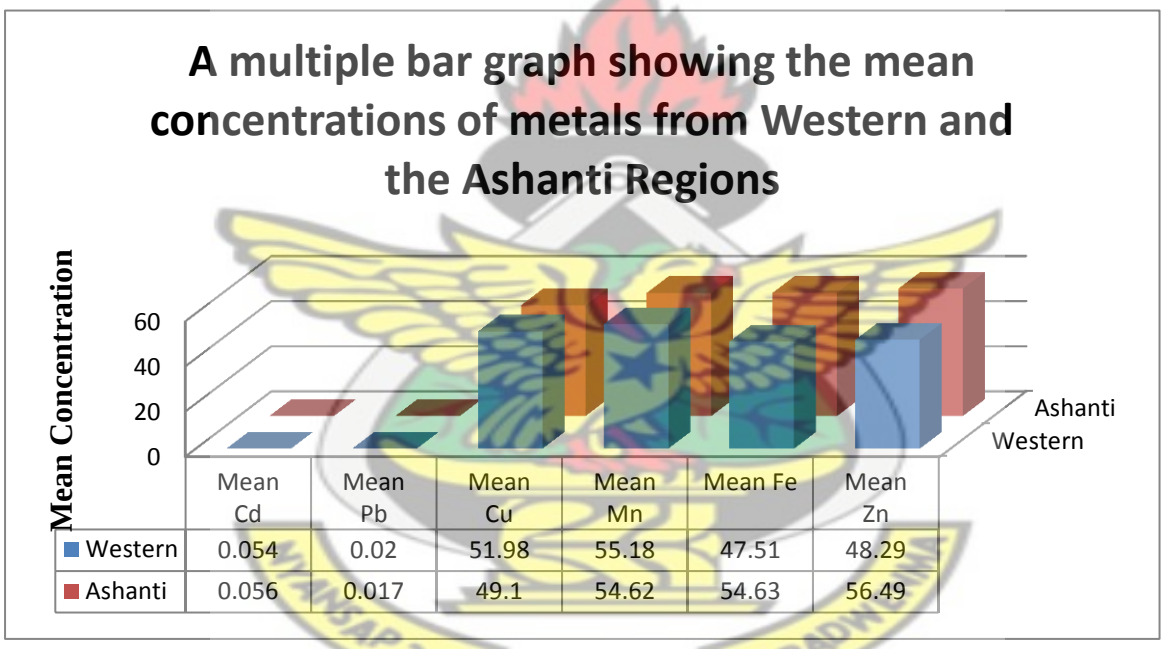


Figure 4.34: Comparison of the levels of copper in cocoa beans from the sample towns in both Ashanti and Western Regions.



	Mean Cd	Mean Pb	Mean Cu	Mean Mn	Mean Fe	Mean Zn
Western	0.054	0.02	51.98	55.18	47.51	48.29
Ashanti	0.056	0.017	49.1	54.62	54.63	56.49

Figure 4.35: Comparison of the mean concentrations (mg/kg) of heavy metals (Cd, Pb, Cu, Mn, Fe and Zn) in cocoa beans from Western and Ashanti Regions of Ghana.

The above graphical representations of the levels of Pb, Cd, Cu, Mn, Fe and Zn in cocoa beans from the Ashanti and Western Regions show that the values are comparable values. Figures 4.1 to 4.10 compare the levels of these toxic metals in the individual samples from the towns in the Western Region, figures 4.11 and 4.12 compare their mean levels in the samples from the individual towns; figure 4.13 shows the levels of the heavy metals in the

entire samples of cocoa beans from the Western Region. Figures 4.14 to 4.23 compare the levels of the metals in the individual samples from the towns in the Ashanti Region while figures 4.24 and 4. 25 compare the mean levels of the heavy metals in the samples from the towns in the Ashanti Region. Figure 4.26 represents the levels of these metals in the entire samples from the Ashanti Region. Figure 4.35 compares the mean levels of the metals in both Western and Ashanti Regions.

Tables at appendix 3 show the work done by V. k Nartey et al (2012) at the Department of Chemistry, Legon (216). They determined the effects of various fertilizer types on the pH of soil, the levels of some heavy metals in soil samples and cocoa nibs from the Western Region of Ghana.

In most of the determinations made, the levels of heavy metals in fertilizer amended soils (FS) were higher as compared to natural soils (NS).This is due to the fact that the soils could be retaining those heavy metals sourced from the applied fertilizers. According to their work, the pH values of the fertilizer amended soils were lower than those of the natural soil (216). However, metals easily enter soil solutions at low pH level and become mobile; as such their intake by plants may increase (214).This phenomenon may contribute to the elevated levels of heavy metals in cocoa beans from fertilizer amended soils. This lends credence to the fact that the contributions of anthropogenic activities to the presence and subsequent uptake of heavy metals by plants are very enormous.

4.4.4 Proximate analyses

Based on the results obtained from the proximate analysis, the moisture content in cocoa beans from Kasapen and Asempaneye in the Western Region were 3.15% and 4.39% respectively while 3.40% and 3.69% were their ash contents, 45.52% and 43.85% were their fat contents, 1.03% and 1.02% were their fibre contents, 14.34% and 13.97% were their protein content and 32.56% and 32.08% were their carbohydrate contents respectively. Cocoa

beans from Bekwai and Juaso in the Ashanti Region also recorded 4.46% and2.23% for their moisture contents, 3.52% and 3.19% were their ash contents, 45.57% and 36.72% were their fat contents, 1.09% and 1.07% for their fibre contents, 13.74% and 13.91% for protein content while 31.62% and 42.88% for their carbohydrate content respectively. (Table 4.6 to 4.11).

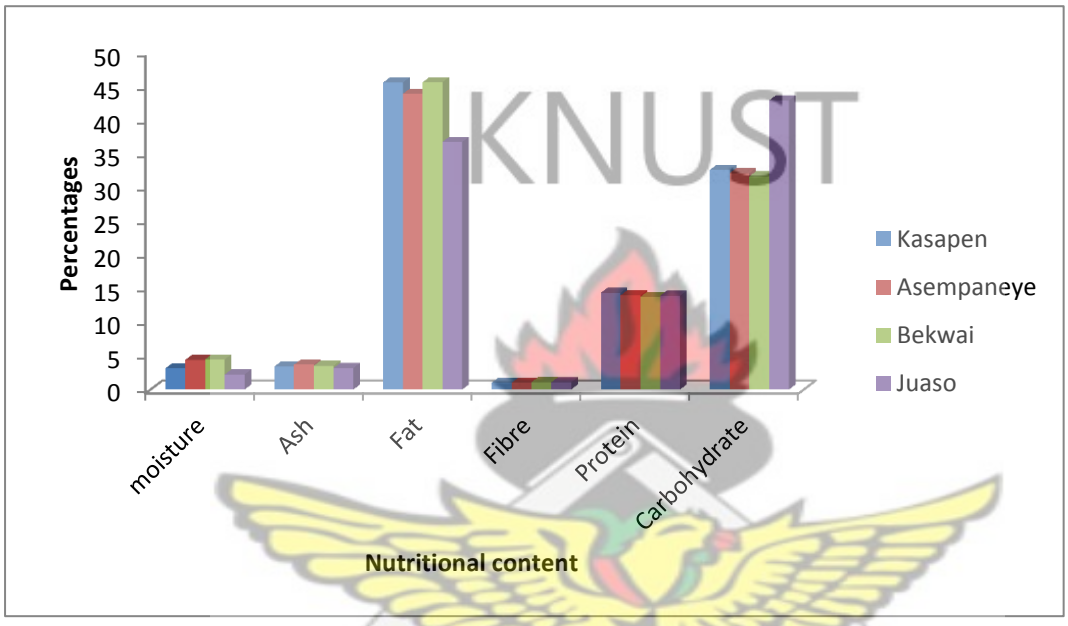


Figure 4.36: Percentages of nutritional contents in cocoa samples from Ashanti and Western Regions of Ghana.

The high percentages of carbohydrate and fat contents in the samples from both Western and Ashanti Regions confirm that cocoa and for that matter, those from Ghana are a rich source of energy. The results also show that cocoa is also a rich source of protein and therefore regarded as good sources of basic nutrient which can meet the recommended dietary demand of the consumers. The significant values of fat, energy and protein present in the cocoa samples studied is in conformity with what WHO (1985) reported which is that a male adult requires 2944KCal of energy/day and 49g of protein/day [215].

4.5 RELATIONSHIP BETWEEN NUTRITIONAL CONTENTS AND LEVELS OF HEAVY METALS IN COCOA SAMPLES

This section focuses on the relationship between the percentages of selected nutritional contents (carbohydrate and fat) and the levels of some heavy metals (lead and cadmium). Carbohydrate and fat contents were chosen because they were relatively high in all the samples from both Regions. Lead and cadmium were also selected because they are the most toxic among the heavy metals analyzed.

Juaso in the Ashanti Region recorded 42.88% as the highest percentage of carbohydrate among the four samples chosen for proximate analysis. It however also recorded the highest levels of lead (0.02mg/kg) and cadmium (0.058mg/kg). The inference which could be drawn from these results may be that higher carbohydrate content in cocoa beans may enhance lead and cadmium accumulation. Sample from Bekwai in the Ashanti Region recorded the highest percentage of fat (45.57%) but did not record the highest levels of lead and cadmium. It could be deduced that fat content may not enhance lead and cadmium accumulation.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

The analysis of the metals,Cd, Pb, Cu, Mn, Fe, and Zn in cocoa beans were performed by the use of F-AAS technique. The reliability of the procedures employed in the analysis was ascertained by the use of two standard reference materials results of which were in good agreement with their reported values

The concentrations of the metals obtained in this work were below the recommended maximum limits set by WHO/FAO and hence pose less or no risk upon consumption. From this work done, it can be concluded that cocoa beans produced in the country, mainly from the Western and Ashanti Regions provide generally safe levels of heavy metals and of good and quality nutritional values.

From the results of the analysis, metals concentrations did not differ significantly among the samples of the Western Region as well as the Ashanti Region of the country. The generally uniform levels of the metals in the various regions may be due to similar soil types and characteristics as well as general agricultural practices occurring within the various cocoa growing regions of the country. The concentrations of Cu, Mn, Zn, and Fe were observed to be comparatively quite high. Though, these metals are considered as essential elements in plants, higher concentrations than needed may pose some level of risk upon consumption. The relatively high levels of Cu are most likely due to its wide application on the cocoa farms as fungicides. Use of alternative sources of fungicides may go a long way in curbing the relatively high levels of this metal.

The proximate analysis carried out on the cocoa beans samples from the Western and Ashanti Regions of Ghana revealed that they are of high carbohydrate, fat and protein contents.

5.2 RECOMMENDATION

Since the processing of cocoa beans usually involves the pressing of the pulverized cocoa nib (cocoa mass/liquor) to separate the solids (powder/cake) from the cocoa butter (fats), it is recommended that a research is conducted to find which of the separated parts contain the highest levels of heavy metals and vice versa for scientific advancements.

Also, the processing of cocoa beans involves the separation of the shells from the nib and so it is recommended that the determination of the levels of heavy metals in both the shells and the nibs be made separately for comparison.

Analysis of soils from the various cocoa -growing regions is highly recommended to determine the levels of these metals in the soils and the extent of uptake into the cocoa plant.

Research to determine the levels of heavy metals especially, Hg in cocoa beans from the mining areas is recommended to ascertain the real effect of mining activities on the levels of Hg in cocoa beans.

A research into the effects of nutritional contents on the levels of heavy metals in cocoa beans is highly recommended

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APPENDICES
APPENDIX 1

Actual concentrations of heavy metals (mg/kg) in cocoa beans from some cocoa-growing towns in Western Region of Ghana- West Africa.

LABEL	Fe (mg/kg)	Fe (mg/kg)	Fe (mg/kg)	Mean Fe (mg/kg)	Deviation	Cu (mg/kg)	Cu (mg/kg)	Cu (mg/kg)	Cu (mg/kg)	Deviation
SK001	39.82	39.79	39.86	39.82	39.82±0.04	48.15	48.18	48.22	48.18	48.18±0.04
SK002	48.31	48.35	48.38	48.35	48.35±0.04	55.96	56.12	55.92	56.00	56.00±0.11
SK003	56.38	56.31	56.35	56.35	56.35±0.04	52.75	52.72	52.78	52.75	52.75±0.03
SK004	41.75	41.78	41.82	41.78	41.78±0.04	54.19	54.15	54.17	54.17	54.17±0.02
SK005	44.57	44.55	44.62	44.58	44.58±0.04	48.42	48.45	48.51	48.46	48.46±0.05
SE 001	41.82	41.75	41.86	41.81	41.81±0.06	45.85	45.92	45.97	45.91	45.91±0.06
SE 002	38.85	38.83	38.81	38.83	38.83±0.02	43.45	43.52	43.53	43.50	43.50±0.04
SE003	55.35	55.29	55.32	55.32	55.32±0.03	43.61	43.66	43.68	43.65	43.65±0.04
SE004	42.65	42.65	42.65	42.65	42.65±0.00	50.42	50.34	50.38	50.38	50.38±0.04
SE 005	42.81	42.75	42.84	42.80	42.80±0.05	48.95	48.94	48.86	48.92	48.92±0.05
SA 001	43.11	43.15	43.16	43.14	43.14±0.03	45.26	45.28	45.32	45.29	45.29±0.03
SA 002	46.75	46.71	46.69	46.72	46.72±0.03	65.25	65.21	65.29	65.25	65.25±0.04
SA 003	44.16	44.18	44.15	44.16	44.16±0.02	62.28	62.28	62.28	62.28	62.28±0.00
SA 004	49.42	49.42	49.42	49.42	49.42±0.00	47.44	47.35	47.45	47.41	47.41±0.06
SA 005	35.55	35.55	35.55	35.55	35.55±0.00	41.32	41.35	41.36	41.34	41.34±0.02
SJ 001	45.53	45.51	45.58	45.54	45.54±0.04	65.85	65.85	65.85	65.85	65.85±0.00
SJ 002	40.65	40.61	40.69	40.65	40.65±0.04	49.27	49.25	49.22	49.25	49.25±0.03
SJ 003	48.65	48.72	48.67	48.68	48.68±0.04	67.81	67.85	67.88	67.85	67.85±0.04
SJ 004	63.28	63.25	63.31	63.28	63.28±0.03	48.65	48.61	48.68	48.65	48.65±0.04
SJ 005	47.54	47.61	47.67	47.60	47.60±0.07	44.26	44.22	44.25	44.24	44.24±0.02
SB001	42.25	42.28	42.29	42.27	42.27±0.02	68.55	68.55	68.55	68.55	68.55±0.00
SB 002	49.85	49.95	49.92	49.91	49.91±0.05	69.82	69.85	69.89	69.85	69.85±0.04
SB 003	43.25	43.28	43.21	43.25	43.25±0.04	46.75	46.82	46.79	46.79	46.79±0.04
SB 004	45.29	45.24	45.22	45.25	45.25±0.04	41.25	41.28	41.31	41.28	41.28±0.03
SB 005	55.45	55.45	55.45	55.45	55.45±0.00	57.25	57.28	57.31	57.28	57.28±0.03
Blank	0.00	0.00	0.00	0.00	0.00±0.00	0.00	0.00	0.00	0.00	0.00±0.00

Concentration of Cd and Pb

Labels	Cd (mg/kg)	Cd (mg/kg)	Cd (mg/kg)	Mean Cd (mg/kg) *	Deviation	Pb (mg/kg)	Pb (mg/kg)	Pb (mg/kg)	Mean Pb (mg/kg)	Deviation
SK001	0.039	0.039	0.039	0.039	0.039±0.00	0.015	0.015	0.015	0.015	0.015±0.00
SK 002	0.025	0.026	0.25	0.025	0.025±0.001	0.011	0.011	0.012	0.011	0.011±0.001
SK 003	0.061	0.065	0.060	0.063	0.063±0.002	0.013	0.014	0.013	0.013	0.013±0.001
SK 004	0.055	0.055	0.055	0.055	0.055±0.000	0014	0.014	0.014	0.014	0.0140.00
SK 005	0.045	0.047	0.042	0.045	0.045±0.002	0.012	0.012	0.014	0.013	0.013±0.001
SE001	0.045	0.041	0.048	0.045	0.045±0.003	0.013	0.012	0.015	0.013	0.013±0.001
SE 002	0.085	0.089	0.082	0.085	0.085±0.003	0.014	0.016	0.014	0.015	0.015±0.001
SE 003	0.061	0.065	0.057	0.061	0.061±0.003	0.016	0.013	0.012	0.014	0.014±0.002
SE 004	0.071	0.071	0.071	0.071	0.071±0.00	0.013	0.014	0.015	0.014	0.014±0.001
SE 005	0.061	0.065	0.059	0.062	0.062±0.002	0.017	0.021	0.019	0.019	0.019±0.002
SA 001	0.038	0.042	0.045	0.042	0.042±0.003	0.017	0.018	0.016	0.017	0.017±0.001
SA 002	0.043	0.045	0.048	0.045	0.045±0.002	0.021	0.019	0.023	0.021	0.021±0.002
SA 003	0.061	0.065	0.072	0.066	0.066±0.005	0.018	0.021	0.017	0.019	0.019±0.002
SA 004	0.064	0.061	0.068	0.064	0.064±0.003	0.016	0.018	0.019	0.018	0.018±0.001
SA 005	0.037	0.042	0.045	0.041	0.041±0.003	0.015	0.017	0.016	0.016	0.016±0.001
SJ 001	0.072	0.065	0.069	0.069	0.069±0.003	0.017	0.017	0.017	0.017	0.017±0.000
SJ 002	0.053	0.055	0.057	0.055	0.055±0.002	0.012	0.015	0.014	0.014	0.014±0.001
SJ 003	0.082	0.078	0.075	0.078	0.078±0.003	0.011	0.015	0.013	0.013	0.013±0.002
SJ 004	0.045	0.051	0.055	0.050	0.050±0.004	0.15	0.016	0.016	0.061	0.061±0.063
SJ 005	0.072	0.079	0.084	0.078	0.078±0.005	0.021	0.022	0.024	0.022	0.022±0.001
SB001	0.031	0.029	0.026	0.029	0.029±0.002	0.018	0.019	0.022	0.020	0.020±0.002
SB 002	0.078	0.082	0.081	0.080	0.080±0.002	0.017	0.019	0.015	0.017	0.017±0.002
SB 003	0.042	0.045	0.038	0.042	0.042±0.003	0.032	0.035	0.031	0.033	0.033±0.002
SB 004	0.035	0.042	0.037	0.038	0.038±0.003	0.047	0.041	0.044	0.044	0.044±0.002
SB005	0.064	0.069	0.072	0.068	0.068±0.003	0.055	0.061	0.055	0.057	0.057±0.003
Blank	0.00	0.00	0.00	0.00	0.00±0.00	0.00	0.00	0.00	0.00	0.00±0.00

Concentrations of Mn and Zn

Labels	*Mn* (mg/kg)	*Mn* (mg/kg)	*Mn* (mg/kg)	*Mean* Mn (mg/kg)	*SD*	*Zn* (mg/kg)	*Zn* (mg/kg)	*Zn* (mg/kg)	*Mean* Zn (mg/kg)	*SD*
SK001	67.71	67.75	67.72	67.73	67.73±0.02	67.15	67.15	67.15	63.86	67.15±0.00
SK002	49.11	49.23	49.12	49.15	49.15±0.07	46.64	46.61	46.69	46.65	46.65±0.04
SK003	56.65	56.71	56.65	56.67	56.67±0.03	47.38	47.31	47.31	47.33	47.33±0.04
SK004	41.86	41.82	41.82	41.83	41.83±0.02	52.82	52.86	52.85	52.84	52.84±0.02
SK005	57.87	57.85	57.82	57.85	57.85±0.03	39.42	39.36	39.36	39.38	39.38±0.03
SE001	45.65	45.68	45.65	45.66	45.66±0.02	41.67	41.63	41.65	41.65	41.65±0.02
SE002	68.55	68.61	68.55	68.57	68.57±0.03	48.75	48.86	48.75	48.79	48.79±0.06
SE003	62.82	62.88	62.82	62.84	62.84±0.03	44.82	44.84	44.84	44.83	44.83±0.01
SE004	42.15	42.17	42.15	42.16	42.16±0.01	36.25	36.15	36.15	36.18	36.18±0.06
SE005	61.72	61.75	61.75	61.74	61.74±0.02	48.26	48.22	48.26	48.25	48.25±0.02
SA001	48.95	48.92	48.95	48.94	48.94±0.02	46.79	46.85	46.75	46.80	46.80±0.05
SA002	57.94	57.87	57.96	57.92	57.92±0.05	43.26	43.25	43.28	43.26	43.26±0.02
SA003	42.25	42.22	42.27	42.25	42.25±0.03	39.15	39.18	39.16	39.16	39.16±0.02
SA004	58.25	58.29	58.25	58.26	58.26±0.02	43.73	43.82	43.76	43.77	43.77±0.05
SA005	64.25	64.25	64.28	64.26	64.26±0.02	42.23	42.19	42.16	42.19	42.19±0.04
SJ001	47.62	47.68	47.63	47.64	47.64±0.03	58.92	58.94	59.23	59.03	59.03±0.17
SJ002	64.18	64.19	64.15	64.17	64.17±0.02	46.74	46.7	46.7	46.71	46.71±0.02
SJ003	40.61	40.65	40.65	40.64	40.64±0.02	63.84	63.89	63.85	63.86	63.86±0.03
SJ004	48.25	48.35	48.25	48.28	48.28±0.06	47.75	47.79	47.79	47.78	47.78±0.02
SJ005	61.22	61.15	61.15	61.17	61.17±0.04	41.25	41.26	41.26	41.26	41.26±0.01
SB001	69.55	69.59	69.57	69.57	69.57±0.02	47.82	47.88	47.87	47.86	47.86±0.03
SB002	46.13	46.25	46.16	46.18	46.18±0.06	53.23	53.15	53.15	53.18	53.18±0.05
SB003	42.65	42.75	42.72	42.71	42.71±0.05	41.65	41.65	41.74	41.68	41.68±0.05
SB004	36.16	36.18	36.15	36.16	36.16±0.02	49.36	49.32	49.37	49.35	49.35±0.03
SB005	47.15	47.21	47.17	47.18	47.18±0.03	68.24	68.27	68.24	68.25	68.25±0.02
Blank	0.00	0.00	0.00	0.00	0.00±0.00	0.00	0.00	0.00	0.00	0.00±0.00

APPENDIX 2

Concentrations of some heavy metals in cocoa beans (mg/kg) from some cocoa- growing towns in Ashanti Region of Ghana- West Africa

LABELS	Fe (mg/kg)	Fe (mg/kg)	Fe (mg/kg)	Mean Fe (mg/kg)	Deviation	Cu (mg/kg)	Cu (mg/kg)	Cu (mg/kg)	Mean Cu (mg/kg)	Deviation
AJ001	66.73	66.64	66.45	66.61	**66.45±0.14**	61.26	61.45	61.15	61.29	61.29±0.15
AJ002	48.4	48.5	48.41	48.43	**48.43±0.06**	49.90	49.75	49.95	49.87	49.87±0.10
AJ003	68.16	68.15	68.17	68.16	**68.16±0.01**	43.48	43.65	43.35	43.49	43.49±0.15
AJ004	43.80	43.85	43.79	43.81	**43.81±0.03**	55.74	55.65	55.57	55.65	55.65±0.09
AJ005	44.30	44.5	44.22	44.34	**44.34±0.14**	60.55	60.68	60.45	60.56	60.56±0.12
AT001	63.80	63.60	63.60	63.67	**63.67±0.12**	43.65	43.85	43.75	43.75	43.75±0.10
AT002	38.90	40.10	38.80	39.27	**39.27±0.72**	48.35	48.38	48.42	48.38	48.38±0.04
AT003	48.51	48.42	48.43	48.45	**48.45±0.05**	44.65	44.55	44.48	44.56	44.56±0.09
AT004	71.37	71.25	71.16	71.26	**71.26±0.11**	41.45	41.62	41.36	41.48	41.48±0.13
AT005	44.41	44.35	44.42	44.39	**44.39±0.04**	63.38	63.32	63.37	63.36	63.36±0.03
AB001	51.61	51.55	51.58	51.58	**51.58±0.03**	38.65	38.55	38.45	38.55	38.55±0.10
AB002	47.85	47.84	47.75	47.81	**47.81±0.06**	63.65	63.55	63.75	63.65	63.65±0.10
AB003	69.8	69.76	69.83	69.80	**69.80±0.04**	39.83	39.85	39.9	39.86	39.86±0.04
AB004	42.12	42.25	42.13	42.17	**42.17±0.07**	47.85	47.75	47.83	47.81	47.81±0.05
AB005	39.78	39.82	39.75	39.78	**39.78±0.04**	47.24	47.31	47.25	47.27	47.27±0.04
AO001	66.56	66.65	66.55	66.59	**66.59±0.06**	41.67	41.72	41.75	41.71	41.71±0.04
AO002	72.35	72.28	72.45	72.36	**72.36±0.09**	36.21	36.25	36.32	36.26	36.26±0.06
AO003	48.93	48.85	48.79	48.86	**48.86±0.07**	56.85	56.75	56.65	56.75	56.75±0.10
AO004	64.85	64.95	64.91	64.90	**64.90±0.05**	62.75	62.84	62.75	62.78	62.78±0.05
AO005	66.72	66.63	66.55	66.63	**66.63±0.09**	41.85	41.75	41.83	41.81	41.81±0.05
AM001	63.35	63.45	63.63	63.48	**63.48±0.14**	38.96	38.92	38.95	38.94	38.94±0.02
AM002	43.45	43.36	43.38	43.40	**43.40±0.05**	47.75	47.85	47.65	47.75	47.75±0.10
AM003	53.25	53.25	53.25	53.25	**53.25±0.00**	61.28	61.36	61.4	61.35	61.35±0.06
AM004	49.55	49.68	49.62	49.62	**49.62±0.07**	42.75	42.82	42.79	42.79	42.79±0.04
AM005	47.28	47.35	47.46	47.36	**47.36±0.09**	47.65	47.75	47.69	47.70	47.70±0.05
Blank	0.00	0.00	0.00	0.00	0.00±0.00	0.00	0.00	0.00	0.00	0.00±0.00

Concentrations of some lead and cadmium in cocoa beans (mg/kg) from some cocoa- growing towns in Ashanti Region of Ghana- West Africa

Labels	Cd (mg/kg)	Cd (mg/kg)	Cd (mg/kg)	Mean Cd mg/kg	Deviation	Pb (mg/kg)	Pb (mg/kg)	Pb (mg/kg)	Mean (mg/kg)	Deviation
AJ001	0.068	0.072	0.059	0.066	0.066±0.005	0.013	0.015	0.014	0.014	0.014±0.001
AJ002	0.052	0.054	0.049	0.052	0.052±0.002	0.023	0.021	0.020	0.021	0.021±0.001
AJ003	0.085	0.075	0.073	0.078	0.078±0.005	0.014	0.012	0.011	0.012	0.012±0.001
AJ004	0.048	0.054	0.052	0.051	0.051±0.002	0.033	0.031	0.033	0.032	0.032±0.001
AJ005	0.039	0.045	0.0.48	0.042	0.042±0.003	0.011	0.013	0.011	0.012	0.012±0.001
AT001	00.85	0.085	0.085	0.085	0.085±0.00	0.013	0.011	0.013	0.012	0.012±0.001
AT002	0.069	0.073	0.068	0.070	0.070±0.002	0.032	0.036	0.034	0.034	0.034±0.002
AT003	0.076	0.0.76	0.0.76	0.076	0.076±0.000	0.015	0.013	0.016	0.015	0.015±0.001
AT004	0.038	0.0.42	0.041	0.040	0.040±0.002	0.016	0.014	0.013	0.014	0.014±0.001
AT005	0.026	0.025	0.022	0.024	0.024±0.002	0.014	0.012	0.013	0.013	0.013±0.001
AB001	0.047	0.048	0.051	0.049	0.049±0.002	0.011	0.012	0.011	0.011	0.011±0.000
AB002	0.0.41	0.038	0.043	0.041	0.041±0.003	0.012	0.014	0.011	0.012	0.012±0.001
AB003	0.064	0.063	0.066	0.064	0.064±0.001	0.013	0.011	0.015	0.013	0.013±0.002
AB004	0.043	0.052	0.041	0.045	0.045±0.005	0.024	0.021	0.023	0.023	0.023±0.001
AB005	0.049	0.053	0.055	0.052	0.052±0.002	0.021	0.018	0.019	0.019	0.019±0.001
AO001	0.061	0.063	0.062	0.062	0.062±0.001	0.016	0.017	0.014	0.016	0.016±0.001
AO002	0.075	0.075	0.075	0.075	0.075±0.00	0.025	0.019	0.021	0.022	0.022±0.002
AO003	0.054	0.061	0.057	0.057	0.057±0.003	0.012	0.011	0.014	0.012	0.012±0.001
AO004	0.071	0.068	0.065	0.068	0.068±0.002	0.021	0.018	0.019	0.019	0.019±0.001
AO005	0.063	0.055	0.065	0.061	0.061±0.004	0.014	0.016	0.013	0.014	0.014±0.001
AM001	0.062	0.059	0.058	0.060	0.060±0.002	0.013	0.011	0.014	0.013	0.013±0.001
AM002	0.042	0.046	0.039	0.042	0.042±0.003	0.017	0.015	0.015	0.016	0.016±0.001
AM003	0.056	0.051	0.062	0.056	0.056±0.004	0.012	0.014	0.011	0.012	0.012±0.001
AM004	0.042	0.048	0.045	0.045	0.045±0.002	0.015	0.017	0.019	0.017	0.017±0.002
AM005	0.048	0.053	0.049	0.050	0.050±0.002	0.011	0.013	0.014	0.013	0.013±0.001

APPENIX 3

(216) Mean values for heavy metal levels(µg/g) in Natural soils (NS) and fertilizer amended soils (FS)

Sampling town	Cocoa type	Cu	Mn	Ni	Cd	Cr	Pb	Zn	Fe
Sefwi A/N	NS	8.14±0.01	233.40±9.20	20.60±1.30	ND	5.25±4.85	2.38±0.01	14.50±5.50	8600.00±1000
	FS	11.30±3.60	287.00±61.90	29.70±4.40	ND	8.00±7.00	2.60±0.30	14.40±0.60	7890.00±1880
Wassa Akr.	NS	2.01±0.47	28.80±2.03	5.71±0.06	ND	12.80±2.63	1.12±0.16	2.01±0.25	1659.80±440
	FS	2.82±0.22	14.10±2.90	7.03±1.62	ND	19.60±0.40	1.52±0.55	1.99±0.05	2500.00±230
Bogoso	NS	2.77±0.35	46.80±14.40	5.99±0.44	ND	13.60±0.57	1.32±0.41	2.43±0.83	2410.00±180
	FS	3.25±0.53	57.40±8.24	6.27±1.05	ND	13.00±1.20	1.76±0.36	2.76±0.17	2052.00±18

Asawinso/Nkatieso (A/N), Akropong (Akr).

(216) Mean values for heavy metal levels in cocoa nibs of cocoa from natural soil (NS) and fertilizer amended soil (FS) (µg/g)

Sampling locations	Cocoa type	Cu	Mn	Ni	Cd	Cr	Pb	Zn	Fe
Sefwi A/N	NS	19.15±5.59	25.30±9.76	0.25±0.13	0.13±0.01	ND	0.05±0.07	33.60±1.27	28.75±7.28
	FS	17.80±3.11	13.05±2.05	0.18±0.01	0.41±0.31	ND	0.07±0.04	38.96±7.14	36.75±2.76
Wassa Akropong	NS	11.70±2.59	26.33±3.71	0.38±0.05	0.43±0.26	ND	0.07±0.05	43.97±1.39	38.50±10.41
	FS	15.73±5.43	33.60±7.89	0.27±0.07	0.71±0.13	ND	0.16±0.02	47.17±9.67	38.73±10.8
Bogoso	NS	16.25±3.24	25.60±5.52	0.32±0.04	0.58±0.25	ND	0.07±0.14	44.35±1.06	29.33±5.67
	FS	16.95±2.05	24.75±6.57	0.32±0.12	0.54±0.23	ND	0.07±0.04	43.65±1.91	28.20±5.87

(216) Table 2: Mean values for Soil pH

Sampling location	Soil type	pH values
Sefwi Asawinso/Nkatieso	NS	6.45±1.86
	FS	5.90±0.95
Wassa Akropong	NS	4.21±0.65
	FS	3.87±0.12
Bogoso	NS	5.43±1.20
	FS	5.01±0.71

NS - Natural soil; FS – Fertilizer amended soil