OCCURRENCE LEVELS OF HEAVY METALS IN FERMENTED COCOA BEANS AND COCOA DERIVED PRODUCTS PRODUCED IN GHANA

THIS THESIS IS SUBMITTED TO THE

UNIVERSITY OF GHANA

BY

JOHN OPOKU DANQUAH

(10395395)

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE

AWARD OF MPHIL FOOD SCIENCE DEGREE

JULY 2015

DECLARATION

This is to certify that this thesis is the result of research undertaken by John Opoku Danquah towards the award of MPhil Food Science degree in the Department of Nutrition and Food Science, University of Ghana, under the supervision of Professor Emmanuel Ohene Afoakwa and Professor Firibu K. Saalia. All references made to other people's work have been duly acknowledged.

……………………………… ……………………………..

John Opoku Danquah **Date**

(Student)

………………………………… ……………………………..

Professor Emmanuel Ohene Afoakwa **Date**

(Supervisor)

…………………………………….. ………………………………

Professor F. K. Saalia **Date**

(Co – Supervisor)

DEDICATION

I dedicate this thesis to the Lord Almighty for his goodness and mercies and to my wife, Anita Opoku – Danquah and my wonderful children, Jaidan and Jodene Opoku – Danquah.

ACKNOWLEDGMENT

I acknowledge the invaluable contribution of my supervisors; Prof. Emmanuel Ohene Afoakwa and Prof. F.K. Saalia towards the successful completion of this thesis. I appreciate your mentorship and friendship throughout the period we worked together. Frankly, you have contributed immensely in making me a better person than I first came to the department. Thank you. Again, my heartfelt gratitude and appreciation goes to all the Lecturers of the Nutrition and Food Science Department. I truly admire the competitiveness and rigor at the department. Your very lives have challenged me and I am proud to be a product of this great family.

Indeed, behind every successful man, there is a woman. To the woman behind my success; my wife and best friend, I say “ayekoo” and may God richly bless you for your prayers, support and understanding during this challenging period of my life. I am indeed a privilege husband. When David talked about the virtuous woman, I am convinced he was talking about you.

Now to all my friends and family, especially, Mr. Kofi Danquah, the Afful’s, Nana Ama Oforiwaa, Akwasi Akomeah Agyekum, John Opong-Otoo, Stephen Nyarko, Dr. Jerry Ampofo (Bobx), John Kongor, Abena Agyeibea Asamoa, Afia Asantewaa Wiafe and all my colleagues at Ghana Standards Authority. I say God richly bless you for whatever contribution you have made to my life or to the success of this work. I am most fortunate to have you as my family and friends. Thank you and God bless you all.

ABSTRACT

Cocoa is the mainstay of the economy of Ghana as it contributes about 28% of foreign exchange earnings. In recent times, cocoa has come under scrutiny because of high levels of heavy metals which are generally considered toxic. Due to the toxicity of these metals, there have been several agitations to reduce their levels as much as possible in foods; thus the setting up of stringent standards in several countries which in most cases are not supported by any scientific basis. The objective of this study was to assess the levels of heavy metals (Pb, Cd, Hg, As, Cu, Fe, Zn, Mn, Ni and Cr) in cocoa beans and cocoa derived products produced in Ghana and to ascertain their compliance or otherwise with EU No.488/2014 and FAO/WHO standards. Cocoa beans were sampled from Ashanti, Brong Ahafo, Eastern and Western regions while semi-finished and finished products were obtained from a major cocoa processing company in Ghana. The samples were digested using a microwave digester and the metals determined using atomic absorption spectrometry techniques.

The levels of Pb in cocoa beans across the regions ranged from 0.01 mg/kg in Western region to 0.084 mg/kg in Brong Ahafo region with a mean of 0.05 mg/kg. There was no significant difference ($P>0.05$) in the content of Pb across the regions and all samples analyzed were below the 1.0 mg/kg FAO/WHO recommended levels. Cadmium ranged from 0.081– 0.097 mg/kg with Ashanti and Brong Ahafo regions reporting the highest and lowest levels respectively. Cd across the four regions was below the 0.6 mg/kg EU recommended maximum limit for cocoa powder. The levels of mercury and arsenic were below the detection limits of 0.01 mg/kg and 0.03 mg/kg respectively. Copper ranged from 23.937 mg/kg in Western region to 29.139 mg/kg in Ashanti region with a mean of 27.295 mg/kg. The content of Cu in the Western region was significantly lower ($P<0.05$)

than the other regions. None of the samples exceeded the 50 mg/kg EU recommended limits. There was significant difference in Pb and Cd concentrations between the organic and conventional cocoa beans.

In assessing the impact of mining on levels of heavy metals in relation to cocoa, mining districts generally reported slightly higher levels than non-mining districts. Amongst the toxic metals, Cd reported the highest levels ranging from 0.057–0.113 mg/kg with levels of Pb between 0.05 mg/kg and 0.044 mg/kg for mining and non-mining districts respectively. There was significant difference ($P<0.05$) in the levels of Cd between the mining and non- mining districts. The results suggest that mining activities may have influenced the levels of Cd in cocoa beans. However, the levels of Cd were below the recommended maximum limits for specific cocoa products stated in (Eu) No. 488/2014 Mercury was not detected in all the samples except in Bogoso and Goaso which are both mining districts. For cocoa products, cocoa butter reported the least levels of all the metals while cocoa powder reported the highest concentrations of 0.220 mg/kg for Lead. Generally, the levels of heavy metals found in the cocoa beans and cocoa products were all below the recommended maximum limits and are thus considered safe.

TABLE OF CONTENT

Contents ....Page

DECLARATION...i

DEDICATION..ii

ACKNOWLEDGMENT iii

ABSTRACT..iv

TABLE OF CONTENT...vi

LIST OF TABLES x

LIST OF FIGURES xi

LIST OF ABBREVIATIONS xii

CHAPTER ONE 1

1.0 INTRODUCTION..1

1.1 Background 1

1.2 Statement of problem 3

1.3 Rationale for the study 3

1.4 Main objective...4

1.5 Specific objectives...5

CHAPTER TWO 6

2.0 LITERATURE REVIEW..6

2.1 Taxonomy and Botany of cocoa plant...6

2.2 Global cocoa production 7

2.3 Cocoa production in Ghana...8

2.4 Organic cocoa beans..11

2.5 Cocoa diseases and pests 12

2.6 Processing of cocoa 13

2.7 Benefits of cocoa 15

2.7.1 Economic importance of cocoa 15

2.7.2 Dietary and health benefits 15

2.8 Occurrence of heavy metals in cocoa 17

2.9 Sources of heavy metals 18

2.9.1 Natural factors 18

2.9.2 Industrial 19

2.9.3 Fertilizer applications 19

2.9.4 Pesticide application 20

2.10 Translocation of heavy metals into the food chain 21

2.11 Toxicity of heavy metals 23

2.11.1 Effect on human beings 23

2.11.2 Effect on soil ecosystem 26

2.12 Levels of heavy metals in cocoa beans and cocoa products 26

2.13 Methods of analysis for heavy metals 28

2.13.1 Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) 29

2.13.2 Atomic absorption spectroscopy (AAS) 30

CHAPTER THREE 31

MATERIALS AND METHODS 31

3.1 Study area 31

3.2 Sample collection 31

3.3 Chemicals and reagents 35

3.4 Sample preparation 36

3.4.1 Digestion procedure 36

3.4.2 Analysis of Pb, Cd and As 36

3.4.3 Analysis of Cu, Fe, Zn, Mn, Ni and Cr 37
3.4.4 Analysis of mercury 37
3.5 Detection limits 38
3.6 Quality control and assurance 38
3.7 Data analysis 39

CHAPTER FOUR 40

4.0 RESULTS AND DISCUSSION 40

4.1 Regional distribution of heavy metals in fermented cocoa beans 40
4.1.1 Distribution of Pb, Cd, Hg and As 40
4.2 Impact of mining on heavy metal contamination of cocoa beans 45
4.3 Comparing levels of heavy metals in organic and conventional cocoa beans 49
4.4 Effect of processing on concentration of heavy metal in different cocoa products 53

CHAPTER FIVE 57

5.0 CONCLUSIONS AND RECOMMENDATION 57

5.1 Conclusions 57
5.2 Recommendations 58

REFERENCES 59

APPENDICES 73

Appendix A: Microwave Program for Digesting Cocoa and Cocoa Products 73
Appendix B: Working Conditions for Atomic Absorption Spectrometer 74
Appendix C: Results of Certified Reference Materials 76

Appendix D: Results of Heavy metals in cocoa and cocoa products 77

Appendix E Graphical Representation of Results 80

Appendix F: Summary of ANOVA Results 82

LIST OF TABLES

Table 1: Distribution of heavy metals in mining and non-mining districts 47

Table 2: The levels of heavy metals in cocoa products along the production line 56

LIST OF FIGURES

Figure 1: Main cocoa growing Countries; Source: www.worldagroforestry.org 8

Figure 2: Schematic diagram of the chocolate manufacturing process; ………………..14

Figure 3: Exhibit of cocoa samples for analysis 32

Figure 4 Schematic diagram of the chocolate manufacturing process, Source: FAO/WHO (2015) 33

Figure 5: Map of Ghana showing the study sites 34

Figure 6 : Map of study sites for organic cocoa beans 35

Figure 7: Distribution of Lead and Cadmium in cocoa beans in the various regions 41

Figure 8: Distribution of Cu, Fe, Zn and Mn in cocoa beans across the regions 43

Figure 9: Distribution of Pb and Cd in organic and conventional cocoa 51

Figure 10: Distribution of Cu, Fe, Zn and Mn in organic and conventional cocoa 52

LIST OF ABBREVIATIONS

%	Percentage
AAS	Atomic Absorption Spectrophotometer
As	Arsenic
ASH	Ashanti Region
ATSDR	Agency for Toxic Substances and Disease Registry
BAF	Brong Ahafo Region
CAC	Codex Alimentarius Commission
Cd	Cadmium
Cr	Chromium
CRM	Certified Reference Material
EAS	Eastern Region
EPA	Environmental Protection Agency
EU	European Union
F-AAS	Flame Atomic Absorption Spectrometer
FAO	Food and Agriculture Organisation
FAPAS	Food Analysis Performance Assessment Scheme
Fe	Iron
g	gram
GAIN	Global Agricultural Information Network
GF-AAS	Graphite Furnace Atomic Absorption Spectrometer
GSA	Ghana Standards Authority
HCL	Hollow Cathode Lamp
HCl	Hydrochloric acid
Hg	Mercury

ICCO	International Organization for Cocoa
ICP- AES	Inductively Coupled Plama – Atomic Emission Spectroscopy
IITA	Institute of International Tropical Agriculture
IMO	Institute for Marketecology
mg/kg	milligram per kilogram
mg/L	milligram per Litre
Mn	Manganese
Ni	Nickel
^{o}C	Degree Celsius
Pb	Lead
ppb	Parts per billion
ppm	Parts per million
PTFE	Polytetrafluroethylene
STD	Standard deviation
WES	Western Region
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Heavy metal contamination of food is of important concern in many countries due to the health issues associated with them. Heavy metals such as lead, cadmium, mercury and arsenic are generally considered toxic to humans; but others such as iron, copper, zinc, manganese can be beneficial to the body for normal physiological and biochemical functions (Lee and Low, 1985). However, at elevated levels all heavy metals could be toxic and may cause adverse health effects (Bradl, 2005; Dyer, 2007). There is therefore the need to constantly monitor the occurrence of heavy metals in food to ensure that they are within safe and acceptable limits (McLaughlin *et al.*, 1999). Heavy metals can bio-accumulate in human tissues and cause health risk even when consumed at low levels. Cadmium (Cd) exerts toxic effects on the kidney, respiratory and skeletal systems and is classified as a human carcinogen (WHO, 2010); copper (Cu) has been implicated in liver cirrhosis (Graham and Cordano, 1976) while toxicity of Zinc (Zn) may lead to anaemia and lethargy (Fairweather-Tait, 1988). Lead (Pb) toxicity manifests itself in some cancers of the skin, lung and bladder (Gazza, 1990; Groten and VanBladeren, 1994).

In recent times, cocoa (*Theobroma cacao L*), a major raw material for the chocolate industry has come under strict scrutiny for high concentrations of heavy metals. Cocoa products contain varying amounts of cocoa solids ranging from 20– 90% and it has been shown that there is a significant correlation between cocoa solids and traces of metals in cocoa products (Yanus *et al.*, 2014). However, the consumption of chocolate and cocoa products continue to increase globally due to its nutritive and health benefits. Several research findings suggest that the consumption of cocoa products can delay certain age related sicknesses such as diabetes, hypertension, cardiovascular diseases, cancer and sexual weakness. Cocoa is also a known rich source of antioxidants and magnesium that help prevent cell damage and reduce the risk of strokes and heart attacks respectively (Aikpokpodion *et al*., 2013a).

Apart from the dietary benefits, cocoa is the mainstay of the economy of Ghana. It contributes about 28% of foreign exchange earnings and is the main source of income to many farmers in Ghana (Lowor and Shiloh, 2013). Currently, Ghana produces about 870 metric tonnes of cocoa making her the second world largest producer of cocoa beans after Cote d'Ivoire (Afoakwa, 2014). Ghana's contribution to world production for 2011/2012, 2012/2013 and 2013/2014 according to the International Organization for Cocoa (ICCO, 2014) was 879, 835 and 870 thousand metric tonnes respectively. To maintain and increase production to achieve a million metric tonnes target, the Government of Ghana has been pursuing programs and policies to boost cocoa production (Ahenkorah *et al.*, 1981; Dorman *et al*., 2004; Osei, 2007). One of such program is the mass cocoa spraying exercise where by government assists cocoa farmers with the application of agrochemicals and fertilizers. This initiative by government has been attributed to the recent high yield of cocoa beans (Vigneri, 2007).

Incidentally, the high application rates of fertilizers and pesticides have been implicated in the accumulation of heavy metals in soils and cocoa beans (He *et al.*, 2005). According to Aikpokpodion *et al. (*2013a), when pesticides are applied to destroy pest, only 15% of the pesticide applied is taken up by the target while the remaining 85 percent is distributed within the air and the soils. The uptake and translocation of heavy metals into the food chain is thus from the soil and the cuticles of the cocoa pod. The desire for safe and quality foods have increased the need for alternative forms of agricultural production capable of reducing the introduction of food safety hazards such as heavy metals in food. Given the extensive use of agrochemicals in conventional farming, there is a strong advocacy for organic farming. The objective of organic farming is to avoid the use of pesticides and other chemicals in crop production. This farming method has caught the attention of farmers worldwide as is it believed that organic farming may result in the reduction of heavy metal and other chemical contamination in cocoa.

1.2 Statement of problem

Cocoa beans and cocoa products worldwide have come under strict scrutiny from trading partners due to the possibility of high levels of heavy metals; and considering that almost all cocoa produced in Ghana are exported raises a lot of concern. Presently, there is no harmonized established regulation concerning the acceptable levels of heavy metals in cocoa and cocoa products apart from what may exist in individual countries. In most cases, the stringent limits set by individual countries are not supported by any scientific basis and could affect the international trade of the commodity as well as destroy the livelihood of smallholder farmers in producing countries. In view of this, the Codex Alimentarius Commission has initiated work to establish and harmonize acceptable levels for Cadmium in cocoa and cocoa products. This work may in the future be extended to include other heavy metals. Ghana, being a major cocoa producing country, is obligated to provide data on the occurrence levels of heavy metals in cocoa beans and cocoa derived products produced in Ghana and make appropriate proposals and recommendations for the establishment of maximum limits (ML’s) that would protect public health and promote trade.

1.3 Rationale for the study

Agrochemicals have since the past decades been applied on cocoa farms in Ghana with the aim of boosting crop yield and controlling pests and diseases. The increase in small scale mining activity is a source of heavy metal contamination particularly, mining activities within cocoa growing areas. The Eastern, Ashanti and some parts of the Western and Brong Ahafo regions have all experienced an increase in small scale mining activities. These mining activities have indirect impact on heavy metal contamination in cocoa beans due to the use of heavy metal-containing chemicals like mercury and Lead which are often used in the process of refining Gold (Lowor and Shiloh, 2013).With studies showing an increase in heavy metal contamination of soil ensuing the activities of small scale miners (Bonzongo *et al*., 2004; Akabzaa *et al*., 2005; Asante *et al*., 2005), there is the need to constantly monitor the environment and cocoa beans to ensure that the levels of heavy metals are within safe limits to ensure consumer safety. Other studies also suggest that the presence of trace amounts of metals in foods is influenced by processing conditions and added ingredients (Lowor and Shiloh, 2013). However, not much research has been conducted to investigate the levels of contamination contributed by the process of converting cocoa beans into semi- finished and finished products.

This study therefore seeks to evaluate the concentrations and distribution characteristics of heavy metals in cocoa beans and cocoa products produced in Ghana and to establish the heavy metal contents of organic cocoa beans as well as their conventional counterparts.

1.4 Main objective

The objective of this study was to evaluate the occurrence and levels of heavy metals in cocoa and cocoa products produced in Ghana.

1.5 Specific objectives

The specific objectives of the study were;

i. To determine the regional distribution of heavy metals: Pb, Cd, As, Hg, Cu, Fe, Zn, Cr, Ni and Mn in fermented cocoa beans.
ii. To establish the impact of mining on heavy metal contamination of cocoa beans.
iii. To compare the levels of heavy metal contamination in organic cocoa versus conventional cocoa beans.
iv. To investigate the levels of heavy metals (Pb, Cd, As, Hg, Cu, Fe, Zn) in intermediate chocolate products (cocoa shell, cocoa nibs, cocoa liquor, cocoa cake, cocoa butter) during chocolate processing.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomy and Botany of cocoa plant

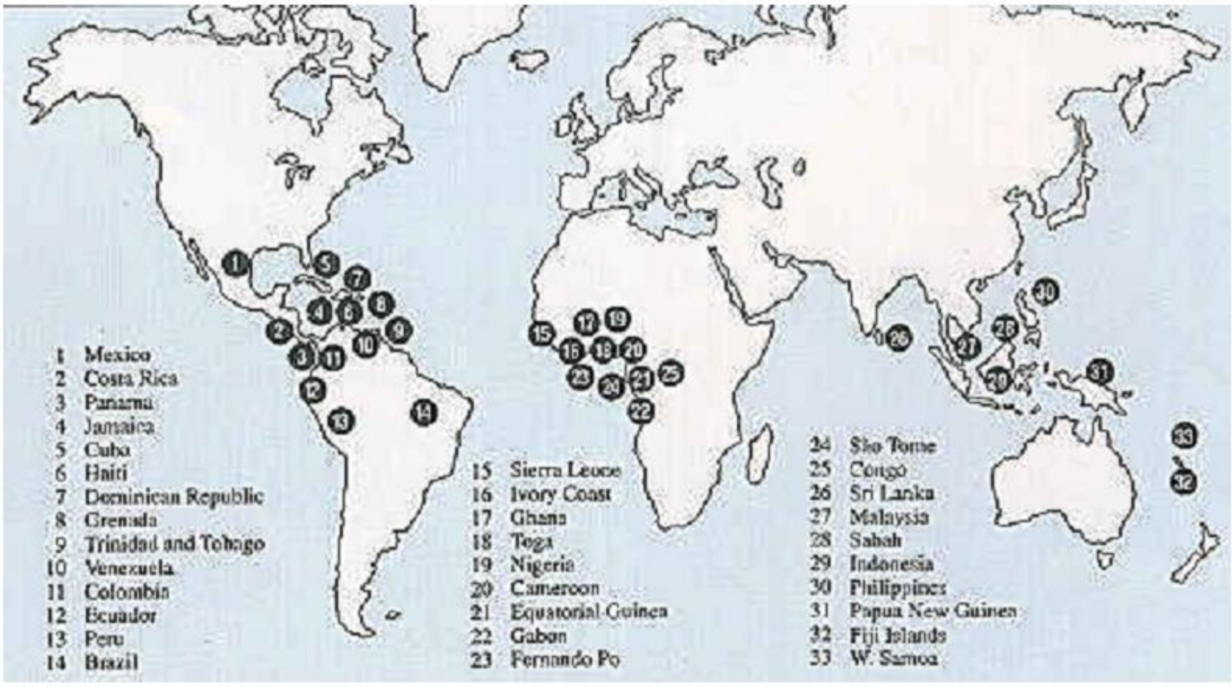
Theobroma cacao L. belongs to the family of Malvaceae (alternatively Sterculiaceae). The family Malvaceae, includes 22 species where the cocoa tree (*Theobroma cacao L*) is the dominant cropped species (Orwa *et al*., 2009). Other local species of importance are *T. bicolor* (pataste), *T. angustifolium* (cacao de mono) and *T. grandiflorum* (cupuassu). The cola nut (Cola acuminata) is a related species (Afoakwa, 2014). All cultivated cacao is classified into a single species *T. cacao* and subdivided into three well-defined groups of cacao: Forastero, Criollo and Trinitario which is a hybrid of Forastero and Criollo. The Forastero group now forms the greater part of all cocoa grown. It is a hardy and vigorous tree producing beans with a strong flavor (Wood, 1991). Amelonado with a smooth yellow pod and more pale to deep purple beans is the Forastero variety most widely grown in West Africa and Brazil. The largest producers of these cocoa beans are Ivory Coast and Ghana, where Forastero was established very early in the cocoa trade. The top producing countries primarily grow Forastero because it is disease resistant (Afoakwa, 2014).

The Criollo trees are not as hardy as the Forastero variety and they produce softer pods containing 20-30 ivory or very pale purple beans. Criollo cacao typically has red or yellow pods with some being green or white (Asare, 2006). The pods have a bumpy or warty skin with pointed tips. The beans on the other hand, vary from light purple to white in color, and they are plump and full**.** In general, the beans from Criollo cacao are considered to have a finer flavour than other cocoa varieties and are therefore considered as "flavour beans". The Criollo trees are not very disease-resistant and hence, making it difficult for farmers to grow and maintain (Verheye, 2010). It is grown throughout the entire Central American region including Mexico. It is most notably present in the states of Tabasco and Oaxaca. Trinitario plants are not found in the wild as they are cultivated hybrids of Forastero and Criollo varieties. Trinitario cocoa trees are grown mainly in the Caribbean area but also in Cameroon and Papua New Guinea. The mostly hard pods are variable in colour and they contain 30 or more beans of variable colour but white beans are rare (Asare, 2006; Afoakwa, 2014).

2.2 Global cocoa production

Although Cocoa is native of the Amazon basin and other tropical areas of South and Central America where wild varieties still grow in the forests, the cocoa growing area has extended to the Caribbean and beyond. Most of the world's cocoa is grown in a narrow belt 10 degrees either side of the equator because the trees grow well in humid tropical climates with regular rains and a short dry season. Even temperatures between 21 and 23^{o}C with a fairly constant rainfall of 1,000 to 2,500 mm per year, are needed without hot dry winds and drought. Many countries now grow cocoa but the main producers are West Africa – (Ghana, Nigeria and Cote d'Ivoire), South America – (Brazil and Ecuador) and Asia – (Malaysia and Indonesia) (Afoakwa, 2014: ICCO, 2014).

Figure 1: Main cocoa growing Countries; Source: www.worldagroforestry.org



9s ss\q/5

2.3 Cocoa production in Ghana

Cocoa has historically been a key economic sector and a major source of export and fiscal earnings for Ghana (Bulir, 1998). In recent years, cocoa production has doubled from 395,000 tons in 2000 to 740,000 tons in 2005. The country recorded an all-time high of 1004 000 MT in 2012. This remarkable achievement earned Ghana the world's second largest exporter of cocoa beans after Cote d'Ivoire (GAIN, 2012). In terms of world cocoa exports, Ghana has maintained its position as the second largest exporter of cocoa beans for the period of 2005–2011. Within the same period, the country ranked 8th, 9th and 7th in cocoa butter export in 2005, 2006 and 2009 respectively. Cocoa paste exports however decreased such that Ghana was no longer ranked among the top 9 exporters of cocoa paste worldwide in 2008 and 2009 although between 2005 and 2007, Ghana ranked in the 3rd or 4th position (FAO/WHO, 2015).

Similar to other countries, cocoa production in Ghana is predominantly practiced by smallholder farmers. About 800,000 households are estimated to be involved, growing cocoa mostly on plots of 2–3 hectares with small plantations (Afoakwa, 2014). “Amazonica” and “Amelonado” cocoa varieties were previously cultivated in Ghana. The hybrid varieties were introduced in 1984 and performed better than the older varieties. By 2002, 57% of farmers from the three main areas of production were cultivating hybrid varieties (Vigneri, 2005). In addition to the hybrid varieties producing more fruit per pod, they also bear fruit earlier (3 instead of 5 years) (Kolavalli and Vigneri, 2011). The few setbacks with the hybrid varieties are that more care is needed (in terms of handling), their highest output occur in the presence of optimal weather conditions and additional farming practices such as fertilizer application, pruning and spraying of pesticides are required (Asante-Poku and Angelucci, 2013). With hybrid varieties, farmers are also required to make more harvest rounds at the beginning and at the end of the season (Bloomfield and Lass, 1992; Boahene, 1999).

Although production of cocoa in Ghana is increasing with estimated output of 1 004 000 MT in 2012, compared to 710 000 MT in 2009 and 904 000 MT in 2010, planted area has fallen slightly from 1.82 million hactares in 2008 to 1.63 million hactares in 2010. Yields have however been fairly stable since 2005, ranging from 0.38 to 0.42 tonnes /ha between 2005 and 2010. Out of the ten regions of Ghana, cocoa production takes place in only six with the Western region having the highest production value (over 50%), followed by Ashanti region (16 %), with Eastern and Brong Ahafo regions together accounting for about 19% of total production (COCOBOD, 2012).

The optimum annual rainfall level for cocoa production is about 1250 – 3 000 mm, although the preferred level is 1500 – 3 000 mm (IITA, 2009). A minimum and maximum temperature range of 18-21° C and 30 – 32° C respectively is required for optimum growth (IITA, 2009). In Ghana, cocoa production predominantly occurs in the rain forest, deciduous forest and transitional zones (Asante-Poku and Angelucci, 2013). Cocoa farmers in Ghana continue to rely on the traditional methods such as the hoe and cutlass method for farming (GAIN, 2012). Cocoa cultivation in Ghana is also predominantly rain fed and the best conditions for cocoa farming are those in which there is favorable rainfall during the night followed by sunny days as these results in healthy-looking trees with fully filled pods (Asante-Poku and Angelucci, 2013). The main cropping season in the country is October-February/March while there is also a smaller/light mid-crop cycle, which occurs from around April/May to mid-September (GAIN, 2012).

In order to maximize foreign currency earnings, the Ghana COCOBOD has introduced an extended duration for harvesting and marketing in the longer crop seasons for the main crop (October to May) and limited the duration for the light crop season (June-September).This is because the light crop season typically result in smaller production volume in comparison to the main crop variety, although the same type of bean quality is cultivated (GAIN, 2012). Ghana’s cocoa yield has been on average 25% less than the average yield of the ten largest cocoa producing nations and nearly 40% below the average yield of neighboring Côte d’Ivoire (Mohammed *et al.*, 2011). Reasons for Ghana’s low yields include the relatively old age of Ghana’s cocoa trees, pests and diseases such as black pod and mistletoes infestation, low investments into cocoa farming and the absence of widespread row planting (Mohammed *et al.*, 2011).

2.4 Organic cocoa beans

The term ‘‘organic’’ is widely used to describe and define both a chemical-free agricultural product as well as an environmentally sustainable method of farming. Codex defines organic agriculture as a holistic production management system which promotes and enhances agro ecosystem health, including biodiversity, biological cycles, and soil biological activity (CAC, 1999).It emphasizes the use of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. This is accomplished by using where possible, cultural, biological and mechanical methods as opposed to using synthetic materials to fulfill any specific function within the system (CAC, 1999). The very first cocoa certified to have been produced on an organic plantation during the 1980’s was introduced onto the international market from Bolivia (Augstburger *et al*., 2000). During the 1990’s, other countries such as the Dominican Republic, Brazil, Mexico, Ghana and the Ivory Coast have joined (Augstburger, *et al*., 2000).

Until recently, the production of cocoa by most farmers was devoid of agricultural inputs since farming was on subsistence basis and inputs were considered as too expensive (Afari-Sefa *et al*., 2010). For a farm to be considered as organic, it should involve growing of cocoa with minimal use of allowable chemicals such as fungicides and organic insecticides. Organic farming is a regulated way of farming that imitates or mimics nature. In 2009, the Cocoa Abrabopa Association became the first cocoa farmers’ association in Ghana to obtain certification from UTZ Certified for organic production. UTZ Certified stands for sustainable farming and better opportunities for farmers, their families and our planet. The program enables farmers to learn better methods, improve working conditions and take better care of their children and the environment (www.utzcertified.org). Over 500 Ghanaian cocoa producers, which is only a small fraction of total producers, successfully met the required standards (Nalley *et al*., 2012). Despite the proliferation of organic cocoa certifying agencies, the amount of organic cocoa available on the market is still minimal. These agencies includes: Fairtrade, the International Federation of Organic Agriculture Movements (IFOAM), the Rainforest Alliance, UTZ Certified and IMO Switzerland.

2.5 Cocoa diseases and pests

The agro-ecosystem of cocoa is saddled with diverse insect and pests infestation. Notable among them is the brown cocoa mired, *Sahlbergella singularis*, Hagi (Hemiptera, Miridae) which is responsible for 25–30% yield loss annually (Afoakwa, 2014). Other insect of economic importance are the cacao pod borer, *Characoma stictigrapta Hmps* (Lepidoptera: Noctuidae), the shoot feeders, *Anomis Leona Shaus*, *Earias biplaga Wlk* (Lepidoptera: Noctuidae) and *Sylepta retractalis Hmps* (Lepidoptera: Pyralidae) (Afoakwa, 2014). The mealy bug is another prominent insect of great economic importance. It is usually endemic in areas with the cocoa shoot virus disease. The mealy bug is a vector of a number of cocoa disease including Planococcoides njalensis Laing, Planococcus citri Risso and Ferrisiana virgata Ckll (Homoptera:Pseudococcidae)(Gerard, 1967; Youdewei, 1974, Ojo,1980; Ndubuaku *et al*. 2003; Afoakwa, 2014). Unlike the mealy bug, the shield bug, (heteroptera, Pentatomidae), the pod miner, Mamara species (Lepidoptera Lithocoletidae) and the root-feeding termites, (nigenensis Sjost) are significant minor pests of cocoa. These and other minor pests such as the sap-sucking psyllid, *Mesohomotoma* (Tyora) *tessmanni Aulman* (Homoptera : Psyllidae) and the cacao thrips, *Selenothrips rubrocinctus Giard* (Thysanoptera:Thripidae) occasionally attain the status of major pests when agro-ecological conditions in young cacao or ageing cacao plantations which are undergoing rehabilitation become more favourable to them

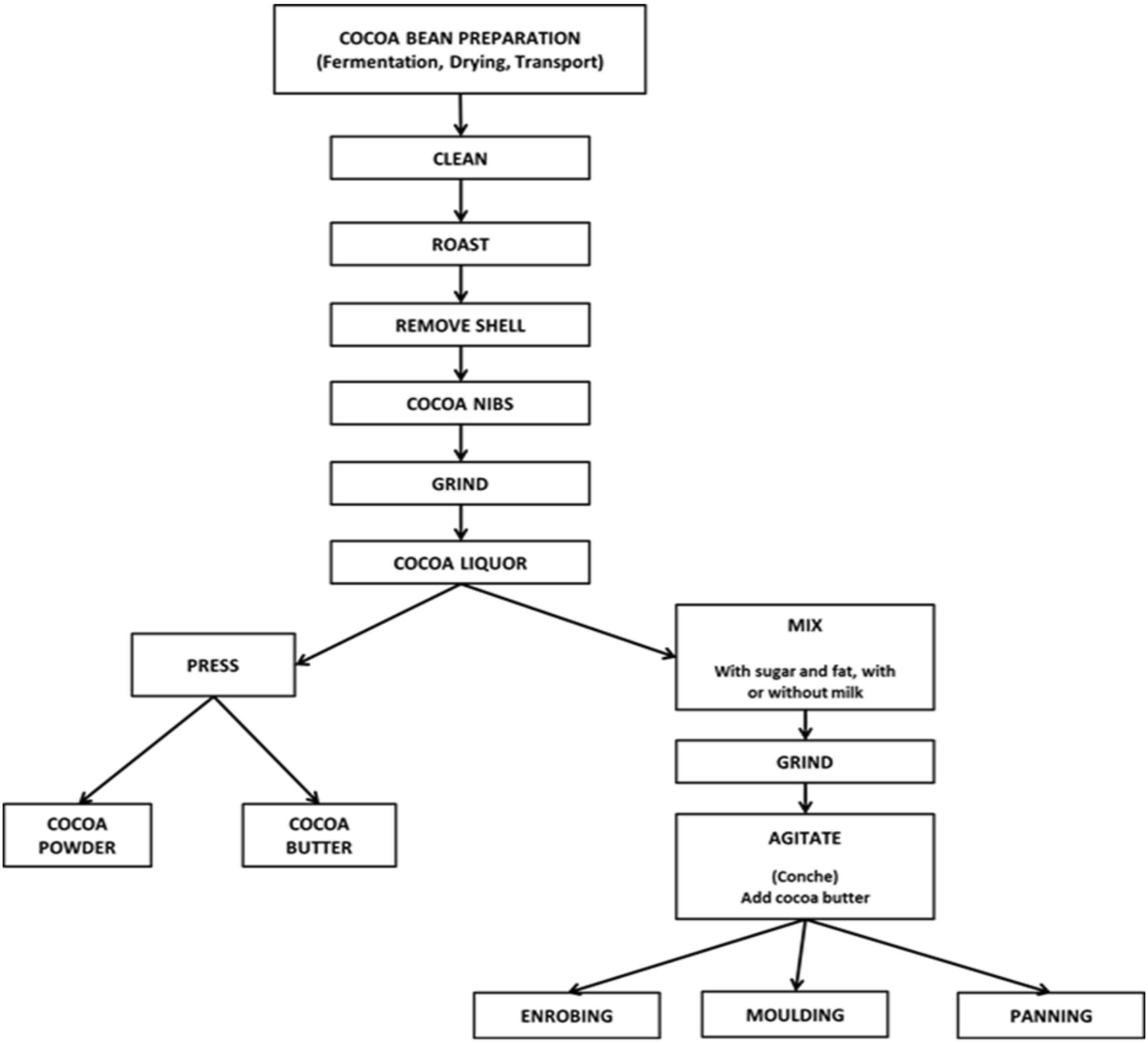
(Igboekwe, 1984). The Phytophtora pod rot disease caused by the fungus Phytophtora megakarya and the cocoa swollen shoot disease caused by Cacao Swollen Shoot Virus are the most important economic losses attributable to black pod disease of cacao. Economic loss from these diseases could be in the 30-90%. The secondary invasion of pathogenic fungi such as *Calonectria rigidiuscula* following attack by mind and shoot feeders causes dieback resulting in stagheadedness.

2.6 Processing of cocoa

The International Cocoa Standards require cocoa of tradable quality to be fermented, thoroughly dried, free from smoky beans, abnormal or foreign odor and free from any evidence of adulteration (Afoakwa, 2010; GAIN, 2012). The beans must be free from living insects, broken beans, and fragments. Pieces must also be seasonably uniform in size. In the world cocoa market, cocoa beans are most valued for their flavor (GAIN, 2012).

After fermentation, the beans are either exported or further processed through sorting, cleaning and roasting (at temperature of 120°C to 149°C) to develop the color and flavor (ICCO, 2012). The beans are then crushed to release the nib from the shell and then winnowed through a tunnel to separate the nib and shell. After this, the nibs are crushed into a mass, which is then heavily pressed until the mass is separated into butter (55–60%) and powder (FAO/WHO, 2015).

Figure 2: Schematic diagram of the chocolate manufacturing process; Source: FAO/WHO(2015).



2.7 Benefits of cocoa

2.7.1 Economic importance of cocoa

For decades, agriculture has been a major foreign exchange earner for most African economies. Amongst the different agricultural produce, cocoa is a highly exploited cash crop in the West and Central African sub-region (mainly Cote d'Ivoire, Ghana, and Cameroon). It has been reported that the West African sub-region produced 70.4% of the world output of 4.23 million metric tons in the 2013/14 year (ICCO, 2012). Ghana which is the world's second largest producer of cocoa benefit immensely from cocoa production in terms of foreign exchange generation, domestic income, and source of revenue for the provision of socio-economic infrastructure. Cocoa has consistently supported the economy of Ghana for the past sixty years. On foreign earnings, the cocoa crop generates about $2 billion annually (COCOBOD, 2012) and is a major contributor to Government Revenue and GDP.

In the southern forest belt, where cocoa is produced, aggregate figures suggest that through the 1990s, cocoa-farming households experienced improvements in their living conditions compared with food crop farmers (Kolavalli and Vigneri, 2011). Currently, the cocoa sector, offers livelihoods for over 800,000 farmers in the southern tropical belt of the country (Afoakwa, 2014). Household surveys indicate that poverty among cocoa-producing households dropped to 23.9% in 2005 down from 60.1% at the beginning of the 1990s (Kolavalli and Vigneri, 2011).

2.7.2 Dietary and health benefits

The seeds of the cocoa plant are mainly used to manufacture chocolate, cocoa based drinks and other confectioneries. The link between chocolate and health has been recorded as long as Cortés arrival in Mesoamerica (Dillinger *et al*., 2000; Afoakwa, 2010).

Codices document that chocolate was drank to maintain health in the 1500s. Although the early adopters did not have access to current technology to analyze subcellular biomedical benefits, they recognized health-promoting benefits during the era. Researchers continue to investigate the role cocoa plays in health and evidence suggests that there are many positive attributes that cocoa provides to the consumer. Epidemiological evidence about beneficial effects of chocolate came from the Kuna Indian population of the islands of Panama. This population is characterized by a low prevalence for atherosclerosis, type 2 diabetes and hypertension due to the daily inatake of homemade cocoa drinks by indigenous Kuna Indians (McCullough *et al*., 2006). These traits disappeared after migrating to urban areas on mainland Panama and subsequent changes in diet (i.e. consumption of much less cocoa which is commercially processed), hence negating the genetic nature of the traits (McCullough *et al*., 2006). Further, epidemiological evidence has come from a longitudinal study on the lifestyle and cardiovascular risk in a cohort of older men (Buijsse *et al*., 2006). This study found cocoa intake to be inversely related to blood pressure. Even after multivariate adjustment, the mean systolic blood pressure was 3.8 mmHg lower in the highest cocoa intake group compared with the lowest intake group (Wison and Hurst (2015).).

The nutritional qualities of chocolate have been acknowledged by several authors and some people have called it a complete food. It contains 40–60% fat, antioxidants, nitrogenous compounds, minerals and many beneficial biochemical compounds. The fat predominantly found in dark chocolate, cocoa butter contains approximately 33% oleic acid (monounsaturated), 25% palmitic acid (saturated), and 33% stearic acid (saturated) (Afoakwa, 2010). Cocoa contains large concentrations of flavonoids, epicatechin, catechin, and procyanidins (Natsume *et al*., 2000). Cocoa has the maximum levels of flavonoids greater than even tea and wine and is also rich in procyanidin flavonoids, comparable with levels in procyanidin-rich apples (Lowor and Shiloh, 2013).

The nitrogenous compounds of cacao include both proteins and the methylxanthines theobromine and caffeine which are central nervous system stimulants, diuretics, and smooth muscle relaxants (Manton, 2006). Minerals such as potassium, phosphorus, copper, iron, zinc, and magnesium also potentiate the health benefits of chocolate. Chocolate also contains valeric acid which acts as a stress reducer despite the presence of the stimulants caffeine and theobromine in the chocolate. Epidemiological evidence and numerous studies suggest the beneficial effects of cocoa consumption on cardiovascular diseases (atherosclerosis, hypertension), and diabetes (Manton, 2006).

2.8 Occurrence of heavy metals in cocoa

According to International Best Practices, cocoa beans must meet sanitary and phytosanitary standards to ensure consumer safety (Lowor and Shiloh, 2013). In view of this, stringent international standards are being set and likewise revised to regulate the levels of potential contaminants in cocoa and cocoa products. One group of contaminants that has attracted a lot of attention recently is heavy metals because of their toxicity. The term “heavy metals” refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004). “Heavy metal” is a general collective term, which applies to a group of metals and metalloids with atomic density greater than 4 g/cm^3, or 5 times greater than water (Huton and Symon, 1986; Nriagu and Pacyna, 1988; Hawkes, 1997).

However, being a heavy metal has little to do with density but concerns chemical properties. Heavy metals are totally non-degradable to non-toxic forms, although they may be ultimately transformed into insoluble and biologically unavailable forms. The route of exposure to heavy metals in humans is oral, dermal and inhalation. This occurs among multiple pathways such as food, drinking water, residential and occupational exposure. Heavy metals are found everywhere in the ecosystem; in soils, water, sediments and in plants in varying concentration. In recent times, there are about 35 metals of environmental concern of which twenty three are heavy metals; antimony, arsenic, bismuth, cadmium, cerium, chromium, cobalt, copper, gallium, gold, iron, lead, manganese, mercury, nickel, platinum, silver, tellurium, thallium, tin, uranium, vanadium and zinc.

2.9 Sources of heavy metals

2.9.1 Natural factors

Natural trace elements levels in soil are derived from the parent materials. This is typical of remote or mountain areas where impacts of human activity are relatively small. In rocks, heavy metals exist as ores in different chemical forms from which they are recovered as minerals (Khan *et al.*, 2011). Heavy metal ores include sulphides, such as iron, arsenic, lead, lead-zinc, cobalt, gold silver and nickel sulphides; oxides such as aluminium, manganese, gold, selenium and antimony. Some exist and can be recovered as both sulphide and oxide ores e.g. iron, copper and cobalt. Ore minerals tend to occur in families where metals that exist naturally as sulphides would mostly occur together, likewise for oxides. Therefore, sulphides of lead, cadmium, arsenic and mercury would naturally be found occurring together with sulphides of iron (pyrite, FeS_2) and copper (chalcopyrite, $CuFeS_2$) as minors, which are obtained as by-products of various hydrometallurgical processes or as part of exhaust fumes in pyrometallurgical and other processes that follow after mining to recover them (Khan *et al.*, 2011).

2.9.2 Industrial

Among the causes of emission of heavy metals today, industrial activities dominate the global flux of heavy metals especially Pb in the environment (Nriagu and Pacyna, 1988; Flegal and Smith, 1995) and has become the predominant source of contaminants in many food products including candies. The industrial sources of heavy metals include factories of batteries, soaps, paints, cosmetics, metal fabrication, plastics, corrugated iron sheets, pharmaceuticals, breweries, tanneries, former copper smelting plant as well as emissions from power stations.

Mining activities also contribute significantly to heavy metal contamination of the environment. During mining some residual metals remain as tailings scattered in open and partially covered pits which are transported through wind and floods, creating various environmental problems (Habashi, 1992). In some cases, even long after mining activities have ceased, the emitted metals continue to persist in the environment. A typical hard rock mine is believed to operate between 5–15 years, however, metal contamination that occurs as a consequence of hard rock mining persist for hundreds of years after the cessation of mining operations (Duruibe *et al*., 2007). Zinc (and occasionally lead) refineries are repositories of cadmium (Cd); lead (Pb) is emitted during its mining and smelting, generally, metals are emitted during their mining and processing activities (Lenntech, 2004).

2.9.3 Fertilizer applications

Some trace and heavy metals such as Cd and Pb may enter the soil as impurities of fertilizers (He *et al*., 2005). Mortvedt and Beaton (1995) reported that, phosphorus fertilizers are sources of heavy metals in the agricultural systems. On the average, phosphate rock contains 11, 25, 188, 32, 10, and 239 mg/kg- of As, Cd, Cr, Cu, Pb and Zn, respectively. He *et al*. (2005) observed that, Cu, Zn, Pb, and As can accumulate in soil if metal-containing chemicals are frequently used. For instance, repeated use of phosphate fertilizers such as triple superphosphate may result in accumulation of these elements and increase the contamination potential, especially of Cd in the soil (He *et al*., 2005).

Moore *et al*., (1998), reported that soil receiving repeated applications of inorganic manures and pesticides especially fungicides exhibited high concentrations of extractable metals and subsequently resulted in increased heavy metal concentrations in runoffs. In a typical cocoa farm, contamination may come from the application of inorganic manures and pesticides. Area deposition of these contaminants ends up in diverse places; from the soil, leaves, tree trunks and the fruits (Aikpokpodion *et al*., 2013a). Possible translocation of these metals from the soil to the cocoa beans is imminent. When crops are treated with pesticides, only about 15% of the applied pesticide is taken by the target while the remaining is distributed within the air and the soil (Aikpokpodion *et al*., 2013a). For example, due to the ability of copper to permeate the cuticle of cocoa pods after application, it was reported that 11% of the total copper residue in cocoa beans was absorbed from the applied copper fungicide via the pod (Aikpokpodion *et al*., 2013b).

2.9.4 Pesticide application

The possibility of detecting some heavy metals as residues in cocoa cannot be avoided due to the fact that heavy metal like copper is a major component of most fungicides used on cocoa globally. According to Aikpokpodion *et al*. (2013a), lead (Pb) in cocoa products have become a concern and the sources of lead conceivably include farming practices e.g. the application of fertilizers, lead-containing pesticides, composts and other soil additives. Two other anthropogenic activities by cocoa farmers that could be potential sources of Pb contamination in cocoa plantations are: the use of old Pb-acid batteries by cocoa farmers to control termites on their plantations and the presence of Pb as impurity in the raw materials for Cu based fungicide production (Aikpokpodion *et al.*, 2013a). According to African pegmatite, all copper fungicides are manufactured using scrap copper which contain Pb as impurity. The purity of the final product is measured by the amount of impurity removed from the raw material (Aikpokpodion et al., 2012).

2.10 Translocation of heavy metals into the food chain

Various chemical processes results in the translocation of heavy metals along the food chain. In a typical cocoa farm, contamination may come from the application of inorganic manures and pesticides. Ariel deposition of these contaminants ends up in diverse places; on the soil, leaves, tree trunks and the fruits. According to Aikpokpodion *et al*. (2013a), when crops are treated with pesticides, only 15% of the applied pesticide is taken by the target while the remaining is distributed within the air and the soil. Metals such as copper that have the ability to permeate the pods of cocoa get absorbed. It has been suggested that 11% of the total copper residue in cocoa beans was absorbed from the applied copper fungicide via the pod (Aikpokpodion *et al*. 2013b).

Insoluble metals are solubilized when heavy metal containing ores such as pyrite (FeS_2) and other sulphide minerals in aquifers are exposed to air and water in the presence of oxidizing bacteria, such as *Thiobacillus ferrooxidans,* are oxidised to produce metal ions, sulphate and acids (Ogwuegbu and Muhanga, 2005).

$$2FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4$$

$$2FeSO_4 + 2H_2SO_4 \rightarrow Fe_2(SO_4)_3 + SO_2 + 2H_2O$$

$$Fe_2(SO_4)_3 + 2FeAs + 9/2O_2 + 3H_2O \rightarrow 2H_3AsO_4 + 4FeSO_4 + S$$

(Source: Duruibe *et al.*, 2007)

These metal ions are leached and carried by acidic water downstream. They can be acted upon by bacterial and methyllated especially mercury to yield organic forms, such as monomethylmercury and dimethylcadmium.

$$M + \text{organic matter} \xrightarrow{\text{water bacteria}} CH_3M \text{ and } (CH_3)_2\,M$$

(Source: Duruibe *et al.*, 2007)

When agricultural soils are polluted, these metals are taken up by plants and consequently accumulate in their tissues. Animals that graze on such contaminated plants and drink from polluted waters, as well as marine lives that breed in heavy metal polluted waters also accumulate such metals in their tissues, and milk, if lactating (Habashi, 1992; Horsfall and Spiff, 1999). Humans are in turn exposed to heavy metals by consuming contaminated plants and animals, and this has been known to result in various biochemical disorders.

Furthermore, metal binding in soil is a common mechanism occurring through interaction of humic substances with oxides of Al, Mn, and Fe and is regarded as the active fraction (Dube *et al.*, 2001). Aikpokpodion *et al*. (2013a) citing Kashem *et al*. (2007) reported that water soluble and exchange forms of heavy metals are the most active and bioavailable. According to him, bioavailability is a term used to describe the release of a chemical from a medium of concern to living receptors such as plant roots. Misra *et al.*, (2009), on the other hand describes metal bioavailability as the fraction of heavy metals in the soil that is accessible to the food chain. Plants accumulate heavy metals in their edible portion when they are planted on contaminated fields. The accumulation of heavy metals according to Dudka and Chlopecka (1990), is influenced by the concentrations of the pollutants, chemical species of the pollutant in the soil, soil physicochemical properties as well as the plants growth characteristics. Heavy metals are mostly transported via the plant root through soluble movement, mass flow and diffusion (Aikpokpodion *et al.*, 2013b).

Since microorganisms are unable to decompose copper and other heavy metals, the long term use of fungicides can lead to accumulation of heavy metals in the soil which subsequently end up in the food chain.

When heavy metals are released into the soils, they occur as cations and strongly interact with the soil matrix and become mobile as a result of changing environmental conditions (Facchinelli *et al.*, 2001). Heavy metals such as Pb, Cd, Hg, and As do not only accumulate and circulate in the soil ecosystem but are picked up by crops grown on these contaminated soils (Ghrefat and Yusuf, 2006). The translocation of heavy metals from the soil to the plant is influenced by several factors that include but not limited to the following; type of soil and type of plant, soil pH, humus content, available organic matter, and soil treatment with agrochemicals, meteorology and the presence of other elements.

2.11 Toxicity of heavy metals

2.11.1 Effect on human beings

According to Mudgal *et al.*, (2010), there are about thirty heavy metal metals that play a pivotal role in the functioning of living organisms and recognized as essential elements for life. Substantial evidence supports the importance of these trace elements in human nutrition. These trace elements are involved in various biochemical functions and physiological mechanisms of the body as some of these forms are integral enzyme cofactors. Examples of such metals which play irreplaceable roles in the human physiology include zinc, copper, iron, selenium, magnesium, manganese etc. and thus termed essential trace elements. These essential trace metals become toxic when their levels in tissues exceed their limit of tolerance, i.e. when taken in excess or at high levels of exposure (Rehman and Syed, 2012). On the other hand, deficiency of these essential elements in human physiology may lead to disease conditions with visible clinical symptoms.

The transition between essentiality and toxicity varies from element to element. While some heavy metals are essential, there are others such as lead, mercury, arsenic, and cadmium that have no useful role in human physiology and may be toxic even at trace levels of exposure (Hu, 2002). The toxicity of these metals most commonly affect the brain, lungs and the kidney while others such as arsenic, are clearly capable of causing cancer (Dahiya *et al*. 2005).

Children are the most vulnerable age group to any kind of contamination in the food chain (Dahiya *et al*. 2005). Research has shown that the absorption rate in human beings for heavy metal is between 10-80% with children absorbing about 50% (Nartey *et al*. 2012). Lead, Cadmium, Mercury, Arsenic and Copper are regarded as environmental contaminants and their presence in foods can be harmful to human beings. At high levels, Pb in food may result in food poisoning in humans and cause damaging effect to the hematopoetical, hematic, renal, gastrointestinal systems (Aikpokpodion *et al.* 2013a)Ingested lead accumulates in different organs of the body and can cause under development of the central nervous system in foetus and newborn babies. It is dangerous because it can damage the brain and the peripheral nerves (Dahiya *et al*., 2005). Exposure to lead has also been associated with reduced IQ, learning disabilities, slow growth, hyperactivity, antisocial behaviour and impaired hearing (Dahiya *et al*., 2005). Lead is also known to damage the kidney, liver and reproductive system, basic cellular processes and brain function (Ackah, 2012).

Increased levels of Cu according to Aikpokpodion *et al*., (2013a) is associated with arthritis, fatigue, insomnia, scoliosis, osteoporosis, heart disease, cancer, migraine, heart seizures, gum diseases and memory loss. Nickel on the other hand, is the main known contaminant resulting from the manufacture of chocolate and is beneficial as an activator of many enzyme systems in trace amounts. However, at high levels it may accumulate in the lungs and cause bronchial hemorrhage (Selavpathy and Sarala Devi, 1995; Underwood, 1977). Other symptoms of copper include nausea, weakness, and dizziness.

Cadmium is a toxic metal and accumulates in several biological systems with a half-life of about thirty years (Ackah, 2012). It presence in foods has been implicated in several diseases such as the itai-itai sickness in Japan and others like gastrointestinal pains, nausea, respiratory distress, diarrhoea, impaired reproduction, kidney damage and hypertension (Underwood, 1977).

Chromium enhances the action of insulin; a hormone that is critical for the metabolism and storage of fats, carbohydrates and proteins in the body (Ackah, 2012). However, at elevated levels, Cr can cause respiratory problems, birth defects, infertility and tumor formations. Other symptoms include skin lesions, dermatitis and incidence of lung cancer (Ackah, 2012).

Iron is considered an essential element because it forms an integral part of many proteins and enzymes that maintain good health and is also involved in the circulation of oxygen in the body. Despite the essentiality of Fe, at high levels it may increase capillary permeability, reduce cardiac output and interfere with clotting mechanism, thereby augmenting hemorrhagic tendencies (Ackah, 2012).

Manganese is essential for growth and maintenance of good health. It is very vital for the development of the skeleton, activation of enzymes and may act as an antioxidant to prevent cell damage (Ackah, 2012). However, research has shown that at elevated levels, Mn may adversely affect the respiratory tract and the brain (Ackah, 2012).

2.11.2 Effect on soil ecosystem

Soils are the major sink or reservoir for heavy metals released into the environment by anthropogenic activities. From the environmental point of view, these heavy metals are largely immobile in the soil and thus tend to accumulate and persist in agricultural soils for a long time. The concentration and distribution of heavy metals in the soil often differ from metal to metal, probably as a result of their differential accumulation rates (Gyamfi, 2012). Unlike most organic contaminants which are oxidized to carbon dioxide (CO_2) by microbial action, heavy metals do not undergo microbial or chemical degradation (Andriano, 2003). Changes in their chemical forms (speciation) and bioavailability are however possible. Heavy metals are of high ecological significance since they are not removed from the soil through self-purification but rather accumulate in reservoirs and enter the food chain (Loska and Wiechula, 2003).

Heavy metal contamination of soil poses risks to animals, plants and the entire ecosystem through direct or indirect contact with contaminated soils. Apart from the hazards it pose to living organisms, heavy metal contamination deteriorates the soil ecosystem by severely inhibiting the bio-degradation of organic contaminants and reducing the usability of agricultural lands with a consequential effect on food security, quality and safety (Wuana and Okieimen, 2011).

2.12 Levels of heavy metals in cocoa beans and cocoa products

Even though there are a number of studies on the contribution of agrochemicals to the heavy metal levels in soils and cocoa beans elsewhere in the world (Alloway, 1995), there is scarcity of such studies in Ghana. COCOBOD in 2007, led by a team of scientists conducted research which alluded to the fact that agrochemicals contribute to the levels of heavy metals in soils and cocoa beans (Osei, 2012). Nartey *et al.*, (2012) corroborated their findings when they reported on the levels of heavy metals in fertilizers, cocoa nibs and cocoa shells on a particular cocoa farm in a year. They observed an appreciable level of heavy metals in the fertilizers applied on the farm than levels in the soils. They also observed that soils with lower pH had low levels of heavy metals. They attributed this phenomenon to the fact that acidic soils solubilize the heavy metals and leach them deep into the soil. They also found that the levels of heavy metals detected in cocoa beans were in agreement with levels set for fruits and vegetables by Codex Alimentarius Commission. Again, the levels of metals detected in the samples on fertilizer amended soils were higher than those obtained from natural soils. Agyen, 2011, conducted a research on heavy metals levels in cocoa beans from an inorganically farmed cocoa plantation and organic farm in Kwabibirem in the Eastern region of Ghana. The results showed a slight increase in the levels of heavy metals in the inorganically farmed cocoa bean than from the organic. Nevertheless, metals levels in the organic as well as the inorganic were within safe limits.

Work done on the content of cadmium in cocoa and cocoa products worldwide suggest varying levels of cadmium. A study in Canada reported 0.02-0.86mg/kg for chocolate with >50% cocoa solids and 0.02-1.25mg/kg with a mean of 0.34mg/kg for cocoa powder (FAO/WHO, 2015). Chocolate from Ecuador with cocoa solids >50% cocoa solids were reported to have concentrations ranging from 0.03–1.56 mg/kg with an average value of 0.378 mg/kg. On the contrary, chocolates with less cocoa solids (<50%) had Cd level of 0.062 mg/kg (FAO/WHO, 2015). The high level of cadmium in cocoa from Central America is believed to be related to the specific local constituents of the soil (Ackah, 2012).

The European Union (EU) is recommending that milk chocolate with ≥ 30% cocoa solids should contain a maximum of 0.3 mg Cd/kg (EFSA, 2011). In a study conducted by Aikpokpodion *et al*. (2013a) to assess the levels of some heavy metals in cocoa beans in

Nigeria, it was reported that concentration of cadmium in cocoa beans from Nigeria were below the recommended EU maximum limits of 0.3 mg/kg. This was attributed to low contamination of agricultural soils in Nigeria. On the contrary, Peru and Venezuela reported high levels for cadmium ranging between 0.03–2.51 mg/kg and 0.03–3.52 mg/kg respectively (FAO/WHO, 2015).

Dahiya *et al*. (2005) in studying the concentrations of lead, nickel and cadmium in chocolates and candies in Mumbai India, reported mean concentration of cadmium as 0.244mg/kg for cocoa-based chocolates; 0.071 mg/kg for milk based chocolates and a sugar based candies ranged from 0.001–0.027 mg/kg. According to Yanus *et al*. (2014), there is a linear correlation between the levels of trace element in chocolate and the cocoa solid contents; thus the concentration of Pb and Cd are expected to be higher in dark chocolate than in milk chocolate. They further suggested that since cadmium is associated with free fat cocoa solids, the content of cadmium in cocoa powder is about twice higher than the content in cocoa butter.

2.13 Methods of analysis for heavy metals

Elements in a vast array of substances can be determined in the laboratory using one or more of the following fixed laboratory assays: Atomic Absorption Spectroscopy (AAS), Atomic Fluorescence Spectroscopy (AFS), Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), Hydride Generation Atomic Absorption Spectroscopy (HGAAS), Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), X−ray fluorescence (XRF), Electron Microprobe (EM), Flame Photometer (FP) and Instrumental Neutron Activation Analysis (INAA). Depending on the matrix, these instruments accurately measure elements in environmental sample to parts per billion (ppb) concentrations i.e. µg/L and µg/Kg in solid samples respectively (Melamed, 2005).

The choice of a particular technique, however, depends on factors such as speed of analysis, availability of the instrument, technical expertise of the analyst or technician and the cost of analysis among others. Before any element is determined with any of these instruments, pre-treatment of sample with acidic extraction (acidic oxidation digestion) or with target reagents is required. The significance of pre-treatment is that all elemental species are converted into the inorganic form for easier detection and measurement. These laboratory assays measure elements accurately but they are expensive to operate and maintain. They are also bulky, requiring fully equipped and staffed laboratories to maintain and operate.

2.13.1 Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES)

ICP-AES is a hyphenated analytical technique which measures characteristic emission spectra by optical spectrometry. Samples are nebulized and the resulting aerosol is transported to the plasma torch. Element-specific emission spectra are produced by radio-frequency inductively coupled plasma. The spectra are dispersed by a grating spectrometer, and the intensities of the emission lines are monitored by photosensitive devices (Jeffery *et al.*, 1989). Background correction is required for trace element determination. Background is measured adjacent to analyte lines on samples during analysis. The position selected for the background-intensity measurement on either or both sides of the analytical line is determined by the complexity of the spectrum adjacent to the analyte line. Alternatively, users may choose multivariate calibration methods. In this case, point selections for background correction are superfluous since whole spectral regions are processed (Jeffery *et al.*, 1989).

2.13.2 Atomic absorption spectroscopy (AAS)

It is a technique in which the absorption of light by free gaseous atoms in a flame or furnace is used to measure the concentration of atoms. AAS is based on absorption of monochromatic light by a cloud of atoms of the analyte metal. In AAS, a liquid sample is aspirated into a nebulizer system. The sample then mixes with an oxidant gas which is drawn under pressure into a burner to form an aerosol. The flame which uses either air-acetylene or nitrous-oxide acetylene operates at a temperature of 2400^oC and 2800^oC respectively. Within the flame, the aerosol undergoes processes such as evaporation of the solvent and excitation of the gaseous metallic element. To determine the concentration of the analyte, a light beam from a lamp usually a Hollow Cathode Lamp (HCL) whose cathode is made of the element being determined is passed through the flame. A photomultiplier tube attached to the AAS can detect the amount of reduction of the light intensity due to absorption (absorbance) by the analyte. The absorption is proportional to the concentration of the metal ions following the Beer-Lambert Law (Skoog *et al*., 1998).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The four most productive regions for cocoa production (out of the 6 regions in Ghana) representing Brong Ahafo, Ashanti, Eastern and Western regions were selected for the study. From these regions, three districts each were randomly selected for sample collection. Konongo, Tepa, Juaso districts were selected for Ashanti Region while Kade, Suhum, Akim Oda were selected for the Eastern Region. In the Brong Ahafo Region, the districts selected were Kukuom, Sunyani and Goaso while Juaboso, Sefwi Bekwai and Bogoso were selected from the Western region.

3.2 Sample collection

About one (1) kg each of fermented cocoa beans from the various districts was obtained from the Quality Control Division (QCC) of the Ghana Cocoa Board (COCOBOD), Tema for the 2013/2014 main season. For reproducibility and representativeness, independent replicates samples were collected for the study.

With respect to the sampling of cocoa beans from organic farms, three (3) communities at Akwadum in the Suhum district which are recognized by COCOBOD as practicing organic farming were used for the study. The three (3) communities included Brong Densuso, Brong No. 1 and Obuotupan. Similar to the sampling from the conventional cocoa beans, 1 kg of fermented cocoa beans was sampled from the farms. Samples were collected in triplicates from each of the three communities.

To investigate the effect of processing on the levels of Lead, Cadmium, Arsenic, Mercury, Copper, Zinc and Iron; raw cocoa, semi-finished and finished cocoa products (cocoa nibs, cocoa liquor, cocoa powder, cocoa butter and chocolate) were sampled at various intermediate stages of the manufacturing process from a major cocoa manufacturing company in Ghana. About 600g of each product was sampled from three different batches for analysis. Below is a process flow diagram (Figure 2) showing the processing of cocoa beans into finished and semi -finished products.

Figure 3: Exhibit of cocoa samples for analysis

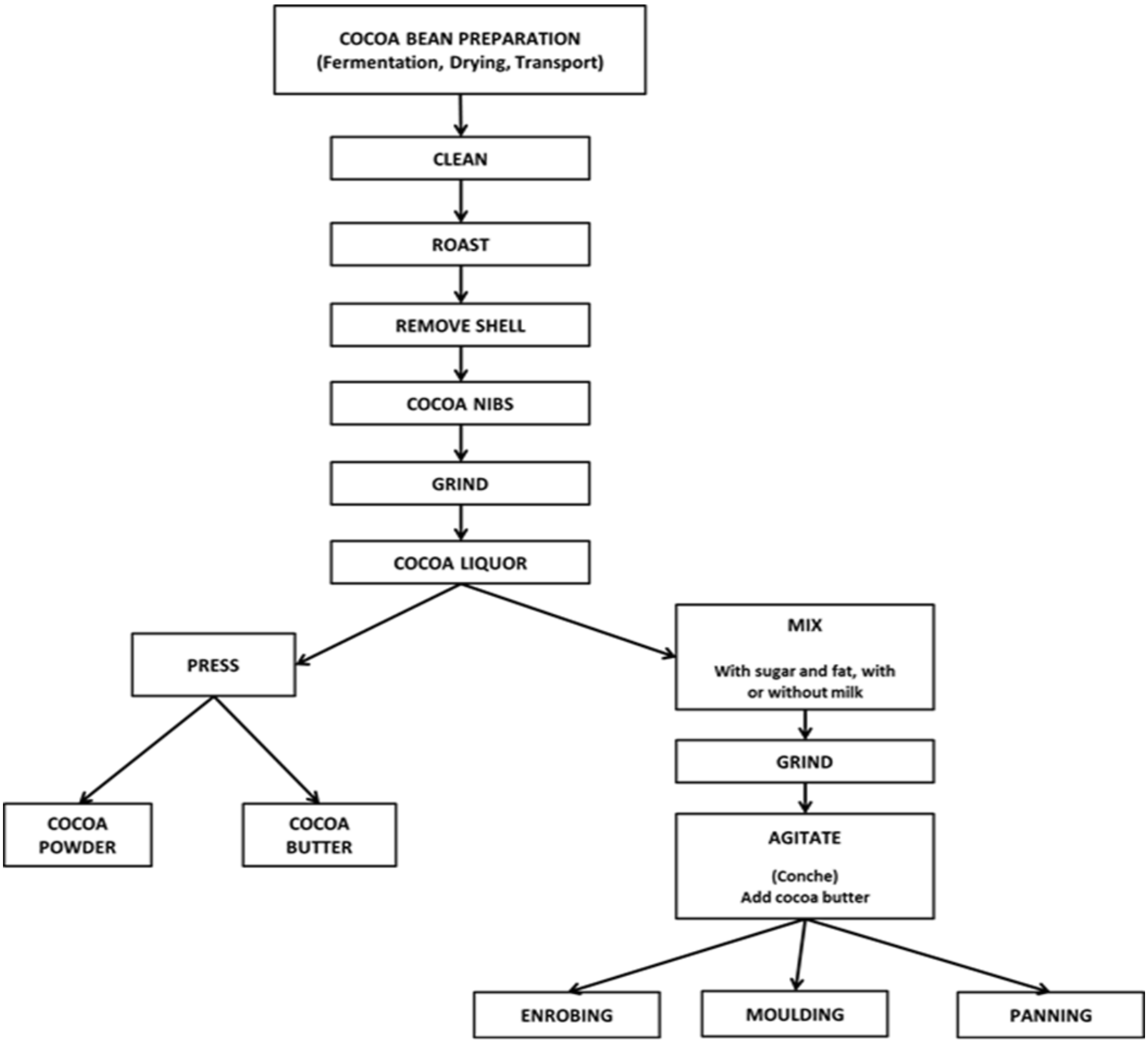
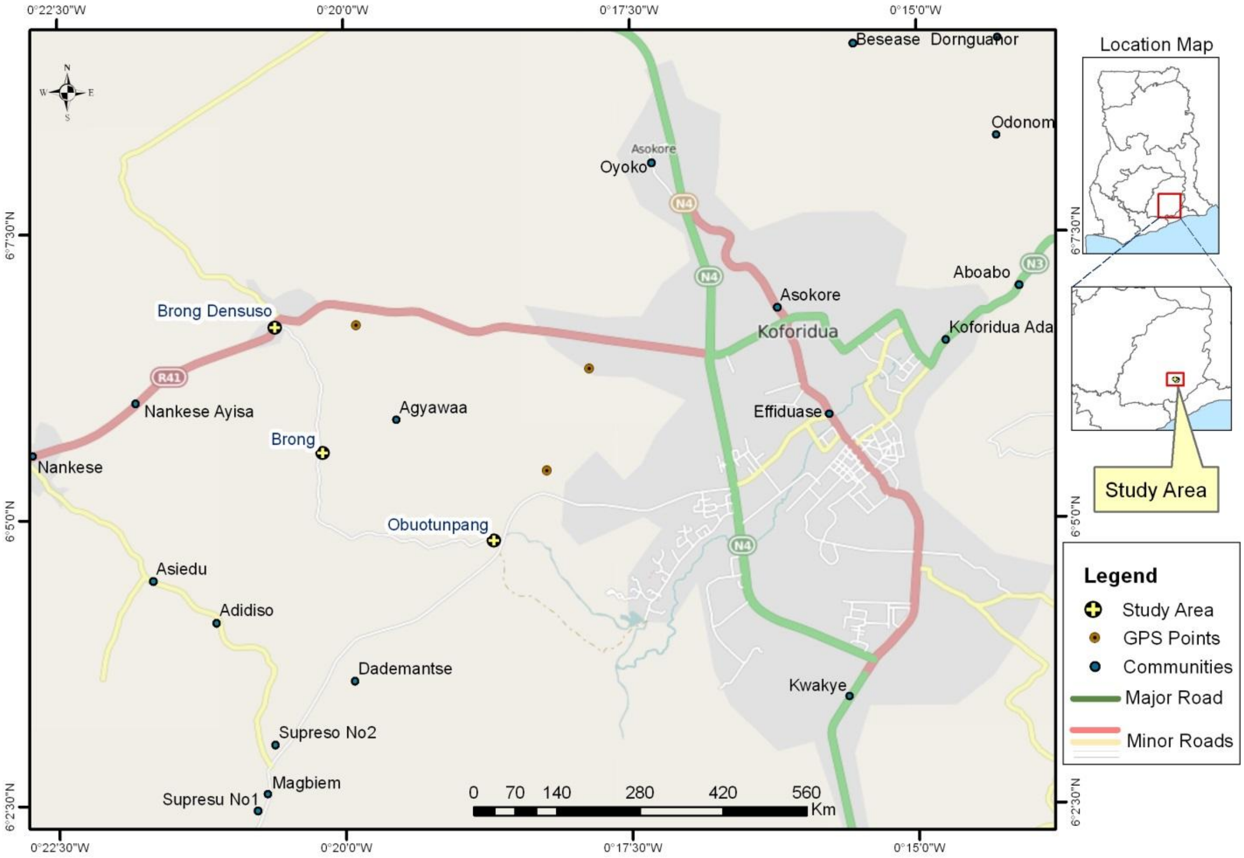


Figure 4 Schematic diagram of the chocolate manufacturing process, Source: FAO/WHO (2015)

Figure 5: Map of Ghana showing the study sites

Figure 6 : Map of study sites for organic cocoa beans



3.3 Chemicals and reagents

Water used for sample preparation and cleaning of glassware was deionized water (produced by Elga Purelab Prima). In order to avoid any trace metal contamination, glassware used for analyses were washed with water and detergent and soaked in an acid bath of 10% (v/v) HNO_3 overnight. The laboratory glassware were rinsed with deionized water and dried in an oven at 50^oC. All reagent used for the study were of analytical grade. Nitric acid (Merck, Germany) was added to samples using calibrated bottle dispenser (Boeco, Germany).

3.4 Sample preparation

Cocoa beans and cocoa products (nibs, liquor, cake and butter) were homogenized using a stainless steel mill (Christy and Norris, Chelmsford, Surrey, England, U.K.). Cocoa beans and cocoa products were microwave digested before further analysis.

3.4.1 Digestion procedure

Each sample was weighed in triplicate (1g) into a Teflon Polytetrafluroethylene (PTFE) vessel and digested with 5mL of HNO_3 (65 % v/v) and 3mL of H_2O_2 (30% v/v). The digestion was carried out in a microwave (Ethos 900, Tokyo, Japan) for 50 minutes using the digestion protocol in appendix A. Upon cooling to room temperature, the digested sample was transferred into 50 mL polypropylene tubes and made up to 25 mL with deionized-water. The digested sample was then used to determine the concentrations of heavy metals (Pb, Cd, As, Hg, Cu, Fe, Zn, Mn, Ni and Cr) using Atomic Absorption spectrometry (AOAC 999.10).

3.4.2 Analysis of Pb, Cd and As

The use of flame or graphite furnace technique is determined by the concentration of the metals to be determined. Lead, Cadmium and Arsenic were determined by graphite furnace atomic absorption spectrometer (Varian SpectrAA model 240FS, Tokyo, Japan) since they are present at very low concentrations that are usually not detected by F-AAS. In the GF-AAS (Varian GTA 120) technique, a pyrolytically coated tube with platform was used for the analysis and a programmed autosampler delivered a volume of 25uL that gave an absorbance within the linear range. The atomization temperatures of Pb, Cd and As were 2100^{o}C, 1800^{o}C and 2600^{0}C and were determined at wavelength of 283.3nm, 228.8nm and 193.7nm with lamp currents of 9.0mA, 4.0mA and 9 mA respectively.

3.4.3 Analysis of Cu, Fe, Zn, Mn, Ni and Cr

These trace metals were determined with Flame atomic absorption spectrometer (Thermo SolaarAA, Tokyo, Japan) because they are usually at level suitable for determination by FAAS. A working range of calibration standards (fluka analytical Sigma Aldrich Chemie GmbH) of Cu, Fe, Zn Mn, Ni and Cr were prepared daily and used to optimize the response of the atomic absorption spectrometer. The calibration standards allowed concentration of metals to be determined using linear regression. Cu, Fe Zn were determined at wavelength of 324.8 nm, 248.3 nm and 213.9 nm respectively using acetylene as the fuel and air as the support gas. The other elements Mn, Ni and Cr were determined at wavelength of 279.5 nm, 232.0 nm, and 357.9 nm respectively. The digest were aspirated into the spectrometer according to the manufacturer's guidelines for each element in the cookbook of the atomic absorption spectrometer (AOAC 999.10).

3.4.4 Analysis of mercury

Mercury was determined using the cold vapour technique with an AAS (Varian SpectrAA model 240FS, Tokyo, Japan) equipped with a hydride generation accessory (Varian VGA-77). $SnCl_2$ (25w/v) prepared in 20% (v/v) HCl was used as reductant. An uptake rate of 0.8–1.2mL/min of a mixture of 25% (w/v) $SnCl_2$ and H_2O were mixed with an uptake rate of 6–8mL/min of sample and aspirated into the reaction cell of the VGA. The resultant was separated by a liquid – gas separator. The mercury vapour then entered the quartz cell through fluorinated tubes. Mercury was determined at a wavelength of 253.7 nm with a lamp current of 4mA and a slit of 0.5 nm. Argon gas was used as the support gas. This procedure was first used to calibrate the equipment (Varian VGA-77) using calibration standards that ranged from 0.01–0.03mg/L. Calibration standards were prepared daily because mercury solutions are not stable for a long time even at fairly high concentration (CVAAS/BS EN 13806:2002).

3.5 Detection limits

The analytical detection limits (DL) for heavy metals in the digest were calculated as DL= x+3std; where x is the mean and std is the standard deviation of blank reading ($n \geq 20$). The calculated DL for the digest was approximately 0.001mgCd/kg, 0.03mgAs/kg, 0.01mgHg/kg and 0.01mgPb/kg. The rest were 0.01mg/kg for Cu, Fe, Zn, Mn, Ni and Cr. A DL is not static and will need to be reevaluated from time to time in accordance with changes in the blank levels (CAC, 2013).

3.6 Quality control and assurance

Quality assurance techniques were conducted during the analysis to validate the accuracy and reliability of the analytical results obtained. Reagent blanks were analysed with every batch of samples to correct sample readings for any contamination of heavy metal in reagents or water used. Certified reference materials (CRM Dorm 4, and Fapas proficiency material) with assigned concentrations were analyzed with every batch of analyses. The certified reference materials (Dorm 4) and PT (cocoa powder) were obtained from the National Research Council, Canada and Food Analysis Performance Assessment Scheme (FAPAS) respectively. Calibration check standard solutions were also analyzed at intervals (1 in every 10 samples) during analyses to monitor and control responses of the atomic absorption spectrometer. To ensure reproducibility of test results, samples were independently analyzed in triplicates.

3.7 Data analysis

Minitab 14 software was used for the statistical analysis of data. Differences between samples were determined using ANOVA and multiple range test (Fishers least significance difference, LSD) and significance was accepted at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Regional distribution of heavy metals in fermented cocoa beans

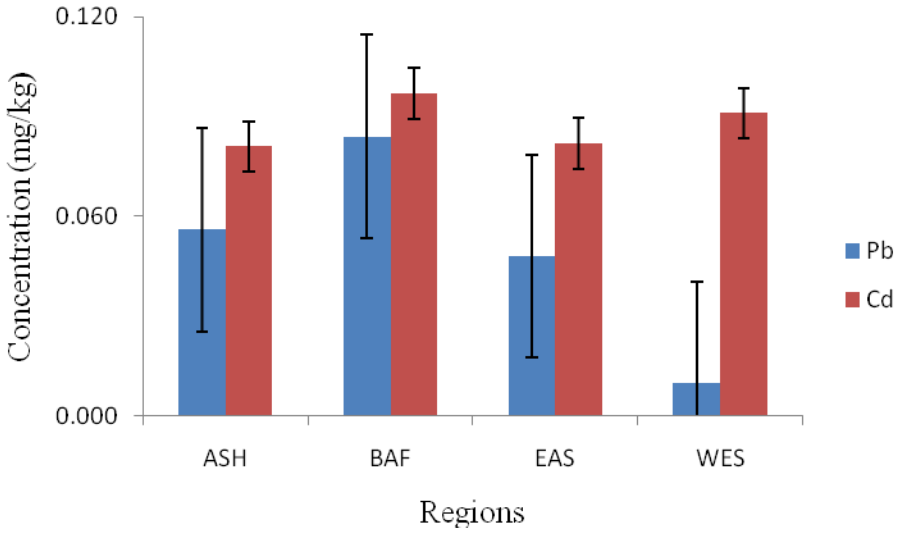
4.1.1 Distribution of Pb, Cd, Hg and As

The levels of Pb, Cd, As and Hg in foodstuffs are of interest as these are generally considered toxic to human beings (Lee and Low, 1985). The distribution of lead and cadmium varied across the regions as shown Figure 7 while Arsenic and mercury were not detected. The distribution of lead across the regions ranged from 0.01–0.084 mg/kg with an average value of 0.05 mg/kg. Brong Ahafo which recorded the highest concentration of Lead, ranged from 0.06–0.11 mg/kg with a mean of 0.084 mg/kg, while Western region had the least concentration of Pb ranged from below detection limit to 0.01 mg/kg. Ashanti and Eastern regions had mean concentrations of 0.056 mg/kg and 0.048 mg/kg respectively. Lead as mentioned earlier is toxic and may cause damage to the kidney, immune system, central nervous system and reproductive systems (Aikpokpodion *et al*., 2013a).

The coefficient of variation in lead varied widely with Ashanti region having 88%. The variation is mainly due to the heterogeneity in the samples as they were sampled at different periods from the selected districts within the region for analyses. There was no significant difference ($P > 0.05$) in the content of Pb among the regions studied. The results are lower than those reported by Aikpokpodion *et al*. (2013a) and agree with the results (0.05- 0.07mg/kg) by Nartey *et al*., (2012) when they both studied the distribution of Pb in cocoa beans in Nigeria and Ghana respectively. The results compare favorably with results obtained by Amankwah (2013), who also reported lower levels of Pb (0.002-0.095mg/kg). Aikpokpodion *et al*. (2013a) reported Pb levels ranging from 0.85-3.0 mg/kg with a mean of 1.97 mg/kg, while Nartey *et al*., (2012) reported 0.05–0.07 mg/kg.

Aikpokpodion *et al*. (2013a) suggested a possible food safety threat for consumers of cocoa products produced from the beans since it exceeded the EU proposed draft maximum limit for lead (1.0 mg/kg). Knezevic (1982) also reported Pb levels of 0.21-0.42 mg/kg in cocoa beans from Malaysia; Mounicou (2013) reported (0.001–0.769 mg/kg) while Lee and Low (1985) reported levels ranging from 3.54–4.25 mg/kg. The results obtained from the study therefore suggest that, Pb in cocoa beans produced in Ghana is very low compared to what has been reported in other countries and were below the Codex Alimentarius proposed draft maximum limit of 1.0 mg/kg for Pb (Amankwah, 2012).

Figure 7: Distribution of Lead and Cadmium in cocoa beans in the various regions



Cadmium is abundant in nature and can be released into the environment in different number of ways including natural activities such as volcanic activity and weathering of rocks (WHO, 2010). The content of Cd in cocoa beans across the regions ranged from 0.081 mg/kg in Ashanti region to 0.097 mg/kg in Brong Ahafo with a mean concentration of 0.088 mg/kg while Eastern and Western regions reported mean concentrations of 0.082±0.028 mg/kg and 0.091±0.003 mg/kg respectively. There was no significant difference in the content of Cd across the regions. The results agrees with research finding that reported average concentration of Cd as 0.17 mg/kg; and lower than (0.03–1.56mg/kg) with a mean value of 0.378 mg/kg in chocolates with cocoa solids content > 50% in Canada and Ecuador respectively (FAO/WHO, 2015). Amankwah, (2013), reported Cd range of 0.010mg/kg in Nkawkaw to 0.095mg/kg in Brakwa district in the central region with a mean of 0.046mg/kg.

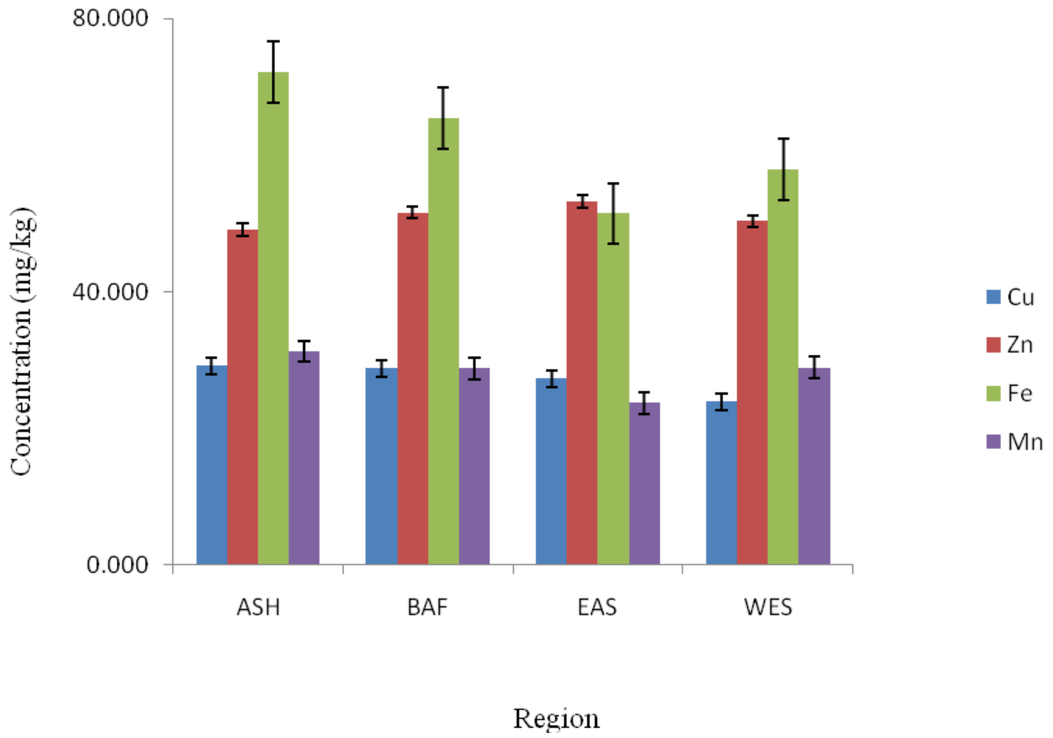
Studies carried out by Knezevic (1982) and Lee and Low (1985) reported Cd in the range of 0.48–1.83 mg/kg and 0.89–1.10 mg/kg respectively in cocoa beans from Malaysia while another study in Europe reported mean concentration of 0.183mg/kg Cd in cocoa beans analyzed (EFSA, 2012). Lowor and Shiloh, (2013) reported very low concentrations for Cd (< 0.01–0.48ug/kg) when they profiled Cd in cocoa beans using Neutron Analysis (NAA). Contrary to results of Pb that had higher levels in cocoa beans from Nigeria by Aikpokpodion *et al.* (2013), the results of Cd were similar to results from Ondo State which ranged from 0.04–0.08 mg/kg with an average of 1.97 mg/kg. Among the samples obtained from Ogun State, Cd was only detected in cocoa beans obtained from Sotiya at a concentration of 0.14 mg/kg. There was however, no detectable Cd among cocoa beans collected from Cross River State.

The content of mercury and Arsenic were very low in all the samples analysed such that they could barely be detected by the AAS. Arsenic was not detected (n.d) in all the samples collected from the various locations within the regions but mercury was reported to have low concentrations ranging from n.d to 0.01 mg/kg. Brong Ahafo region was the only region that reported levels at the detection limit of 0.01 mg/kg. This low concentration of mercury in cocoa beans was confirmed by Lowor and Shiloh, (2013) who studying the impart of cocoa growing soils on the levels of heavy metals in cocoa noted that Hg was not detected in any of the samples tested using Neutron Activation Analysis (NAA).

4.1.2 Distribution of Cu, Fe, Zn and Mn

Contrary to some heavy metals such as Pb, Cd, Hg and As which have no known beneficial effects in human diet, Cu, Fe, Zn and Mn play significant role in health and are considered essential elements (EU, 2007). The distribution of these metals in cocoa beans across the regions is shown in Figure 8. Copper occurs naturally in the environment but its application in agriculture as the base for most fungicides and fertilizer has increased its levels in the environment (Lowor and Shiloh, 2013). The content of Cu in cocoa beans among the regions studied ranged from 23.937 mg/kg in Western region to 29.139 mg/kg in Ashanti region with an average concentration of 27.295 mg/kg. Eastern and Brong Ahafo had mean concentrations of 27.311–29.139 mg/kg respectively.

Figure 8: Distribution of Cu, Fe, Zn and Mn in cocoa beans across the regions



The content of Cu in the Western region which produces about 50% of the nation's cocoa (Anim-Kwapong, 2005), hd significantly lower levels ($p<0.05$) compared to Ashanti, Eastern and Brong Ahafo which were not significantly different ($P>0.05$).

Lowor and Shiloh (2013) reported 26.76mg/kg while Aikpokpodion, *et al*., (2013a), reported levels of 25 mg/kg and 26.1 mg/kg for Ogun and Ondo States respectively while 10–24 mg/kg with an average value of 18 mg/kg was reported for Cross River State. The results suggest the possibility of recording varying levels for Cu for different locations within the same country. Rehman (2012) reported (15.22–24.50 mg/kg); Knezevic (1982) reported (21.5–32.8 mg/kg), Amankwah (2013) reported (5.250-41.950 mg/kg), while Lee and Low (1985) reported Cu levels ranging from (15.22–24.50mg/kg) in cocoa beans. These results generally are comparable to the results obtained in this study. The results may be suggestive that the use of copper -based fungicide to prevent black pod disease over time may have accumulated levels of Cu in the beans. However, none of the regions or locations considered for the study exceeded the 50mg/kg recommended maximum limit set by the European Union.

The content of Zinc was the second highest metal after Iron in all the four regions (Figure 8). This is because Zn is widely abundant in nature and the source of zinc in most cocoa beans is mainly from the parent material from which the soils are formed (Oosterhuis, 2000). The concentration of zinc ranged from 49.113 mg/kg in Ashanti region to 53.263 mg/kg in the Western region with a mean value of 51.075 mg/kg. Brong Ahafo and Western region had average concentrations of 51.578 mg/kg and 50.347 mg/kg respectively. The content of Zinc in the Ashanti region and Western region were significantly different from Brong Ahafo and Eastern region. The results agreed with that of Nartey et al., 2012 (47.17mg/kg); Amankwah, 2013 (24.05 -50.785 mg/kg) but lower than that reported by Aikpokpodion *et al.*, (2013a). They reported 79–180 mg/kg with a mean value of 108 mg/kg and suggested that the high concentrations may be due to the inherent ability of the *Theobroma cacao* to absorb zinc from the soil. Zinc is widely known to interfere with Cu metabolism but knowledge about its toxicity in human is minimal (Barone, 1998).

The distribution of metals in the regions was in the order of Fe > Zn > Mn > Cu except in the Western region where the order was Zn > Fe> Cu> Mn (Figure 8). The content of Fe ranged from 51.450 mg/kg in the Eastern region to72.05 mg/kg in the Ashanti region with Brong Ahafo and Western region having 65.468 mg/kg and 57.907 mg/kg respectively. Amankwah (2013), reported a range of 0.5-160.60 mg/kg with a mean of 27.420 mg/kg while Nartey *et al*. (2012), reported Fe content of 38.730 mg/kg.

Manganese is naturally present in high concentrations in foods such as cereals, legumes, fruits, vegetables, grains, oysters and eggs. Mn was the third most abundant element detected in the cocoa beans with a range of 23.767–31.291 mg/kg and a mean concentration of 28.200 mg/kg. Ashanti region had the highest concentration of manganese while Eastern region reported the least levels. Brong Ahafo and Western region were 28.801 mg/kg and 28.939 mg/kg respectively. The content of Mn in cocoa from the Eastern region was significantly lower that cocoa beans from Ashanti, Brong Ahafo and Western regions. The results favorably compare with that of Nartey *et al.* (2012), who reported 33.60 mg/kg for cocoa beans from Wassa Akropong but lower than results of Amankwah (2013) who in a similar study reported 52.35mg/kg for cocoa beans from Kwaku Praso in the Eastern region.

4.2 Impact of mining on heavy metal contamination of cocoa beans

The activities of small scale miners have become very rampant in several of the cocoa growing areas in Ghana and mercury for example is often used in the process of refining gold (Lowor and Shiloh, 2013). The activities of these galamsey (small scale mining) operators are often not regulated and lead to indiscriminate pollution of agricultural lands and water bodies. To investigate the impact of mining activities on the levels of heavy metals in cocoa, samples were collected from mining and non- mining cocoa districts.

Table 1: Distribution of heavy metals in mining and non-mining districts

DISTRICT	Pb	Cd	Hg	As	Cu	Fe	Zn	Mn	Ni	Cr
Bogoso*	0.01^{a}	0.090±0.010^{bc}	0.01	N/D	25.037±2.782^{ab}	50.140±7.198^{a}	51.730±1.033^{bcd}	30.517±2.026^{bc}	0.01	N/D
Goaso*	0.083±0.025^{d}	0.103±0.021^{c}	0.01	N/D	28.027±3.196^{bc}	72.797±11.308^{b}	53..860±3.474^{d}	30.280±2.950^{bc}	0.01	N/D
Juaboso	N/D	0.090±0.020^{bc}	N/D	N/D	22.210±1.366^{a}	66.125±4.080^{ab}	50.283±2.079^{abc}	29.590±2.862^{bc}	0.01	N/D
Kade*	0.050±0.010^{bc}	0.113±0.015^{c}	N/D	N/D	29.107±0.690^{c}	52.710±2.121^{a}	52.060±0.272^{cd}	25.463±2.176^{ab}	0.01	N/D
Konongo*	0.030±0.010^{ab}	0.110±0.026^{c}	N/D	N/D	28.917±1.367^{c}	63.560±2.630^{ab}	47.630±1.830^{a}	34.360±3.830^{c}	0.01	N/D
Kukuom	0.065±0.007^{cd}	0.110±0.026^{c}	0.01	N/D	28.533±2.274^{bc}	56.660±2.022^{ab}	49.200±1.236^{abc}	30.057±4.975^{bc}	0.01	N/D
Suhum	0.043±0.012^{abc}	0.063±0.006^{ab}	N/D	N/D	24.277±1.589^{a}	61.173±13.587^{ab}	53.447±0.786^{d}	21.657±1.961^{a}	0.01	N/D
Tepa	0.030±0.010^{ab}	0.057±0.006^{a}	N/D	N/D	29.863±2.561^{c}	97.880±13.422	48.923±0.160^{ab}	30.387±4.600^{bc}	0.01	N/D

The values with * are mining districts, N/D – not detected; Same alphabet on the same column is not significant at $P < 0.05$

Table 1 shows the distribution of heavy metals in mining and non-mining areas. Mining districts as was expected recorded appreciably higher levels of heavy metals accumulation compared to the non-mining districts, however there were few incidences where non mining areas had higher levels. The mining districts were drawn from Bogoso (Western region), Goaso (Brong Ahafo), Kade (Eastern region) and Konongo (Ashanti region), while the non-mining areas included, Suhum (Eastern region), Juaboso (Western region), Kukuom (Brong-Ahafo) and Tepa (Ashanti region).

Of the toxic heavy metals studied, Cd levels were much more pronounced in both mining and non-mining areas. Cd values ranged from a low value of 0.057 mg/kg (Tepa) to a high value of 0.113 mg/kg (Kade). A similar study by Nartey *et al*. (2012) reported Cd levels in the range of 0.13 mg/kg to 0.57 mg/kg in cocoa nibs from Sefwi - Asawinso which is relatively greater than the highest concentration recorded in this work. Nevertheless, these levels were far below the permissible limits of 1.0 µg/g allowable for Cd in cocoa mass (Rankin *et al.* 2005).

Mercury (Hg) and Arsenic (As) residues in cocoa beans from this work were virtually below detectable limit. As levels were all below detectable limit in all the samples while only two mining areas, Bogoso and Goaso had levels of 0.01 mg/kg for Hg.

Pb residue in cocoa beans obtained, ranged from 0.01 mg/kg to 0.83 mg/kg with an average of 0.05 mg/kg and 0.044 mg/kg for mining and non-mining areas respectively. Cocoa beans obtained from cocoa plantations in Juaboso, a non-mining town had Pb residue below detection limit. Pb residues from Goaso was significantly higher ($P<0.05$) than any other sample within the mining and non -mining areas. This perhaps may be due to the increasing effect of mining activities on the levels of heavy metals in cocoa growing soils at Goaso which is a mining district. All the cocoa beans had Pb residue below the maximum residue limit (1.0 mg/kg) set by the European Union. The low concentration of lead (Pb) in the various beans does not dismiss the possibility of health threat on the consumers of cocoa products made from these beans. Lead can be very harmful even at low concentration when taken over a long time (Ellen *et al*., 1990 and Celik *et al*., 2004). After ingestion, the typical absorption rate of lead ranges from 3 to 80 %, whereas the typical absorption rates for dietary lead in adults and infants are 10 and 50% respectively. After absorption, lead is initially distributed to soft tissues throughout the body via blood and then deposited in bone. Lead is a chronic toxic chemical. It may cause damages to kidneys, the cardiovascular, immune, hematopoietic, central nervous and reproductive systems (Aikpokpodion *et al*. 2013a).

Although there are no known permissible limits for these essential metals in cocoa nibs, the levels obtained agreed with permissible levels reported in fruits and vegetables.. With the essential heavy metal, there were no distinctive patterns in their distribution in terms of mining and non-mining areas. Fe levels were relatively higher than the rest of the heavy metals. Cr on the other hand was below detection limit in all sampling areas including the mining areas. The results clearly suggest that though mining activities may have impacted on the levels of heavy metals in cocoa growing soils, it wasn't significant as all the samples collected from the mining districts were within the permissible limits specified in Codex Stan 193-1995 for fruits and vegetables.

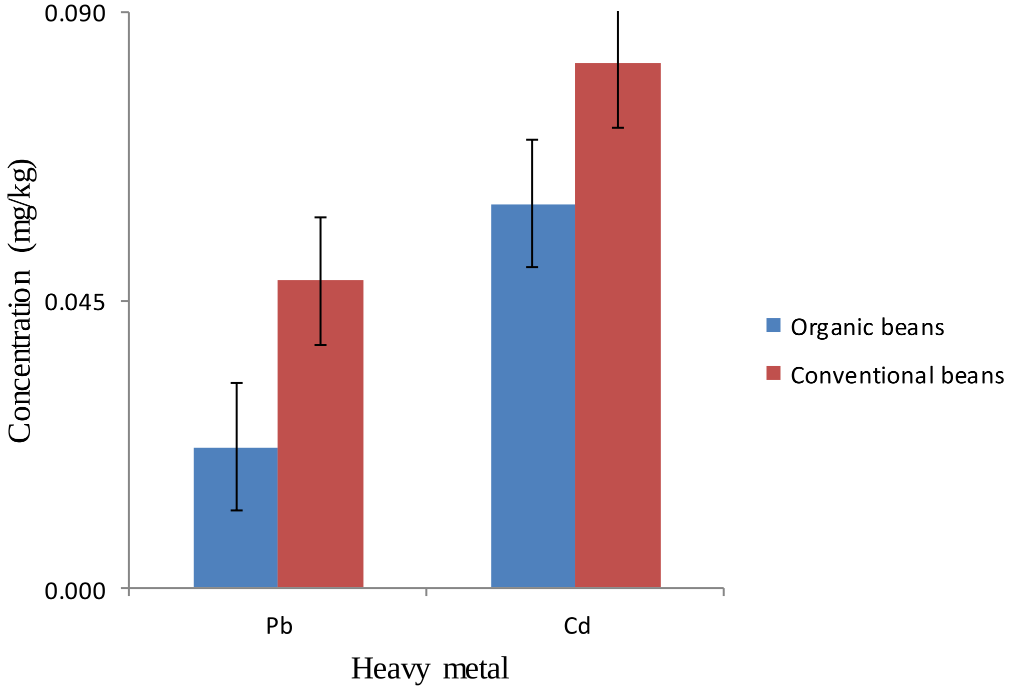
4.3 Comparing levels of heavy metals in organic and conventional cocoa beans

In establishing the impact of agrochemical application on the levels of heavy metals in cocoa beans, organic cocoa was collected from farms in the Eastern region and compared with their conventional counterparts from the same region as shown in Figure 9. Given the extensive use of agrochemical in the production of conventional cocoa, one expects to have higher levels of heavy metals accumulating in the beans. This is corroborated by Zarcinas *et al*. (2004) who reported that the application of phosphate fertilizers on cocoa farms in Malaysia is responsible for the high levels of Cd in cocoa (*Theobroma cacao*). Considering that cocoa in Ghana is predominantly grown with agrochemicals one does not expect otherwise.

The distribution of Pb in conventional and organic cocoa beans from the Eastern region is shown in Figure 9. The levels of Pb in the organic beans were slightly lower than the conventional beans ranging from 0.01– 0.03 mg/kg with a mean of 0.022 mg/kg while conventional cocoa ranged from 0.03–0.06 mg/kg with a mean of 0.048 mg/kg. Pb was significantly higher ($P<0.05$) in conventional cocoa than organic cocoa however all samples were well below the recommended maximum limit of 1.0 mg/kg specified for chocolates (GS 571, 2001).

The results suggest that even though Pb levels are below the proposed draft MLof 1.0 mg/kg, the application of agrochemicals may have had an impact on the levels of Pb in conventional cocoa. There is therefore the need to continuously monitor this environmental contaminant before it assume alarming levels in our soils and subsequently in cocoa beans produced in Ghana.

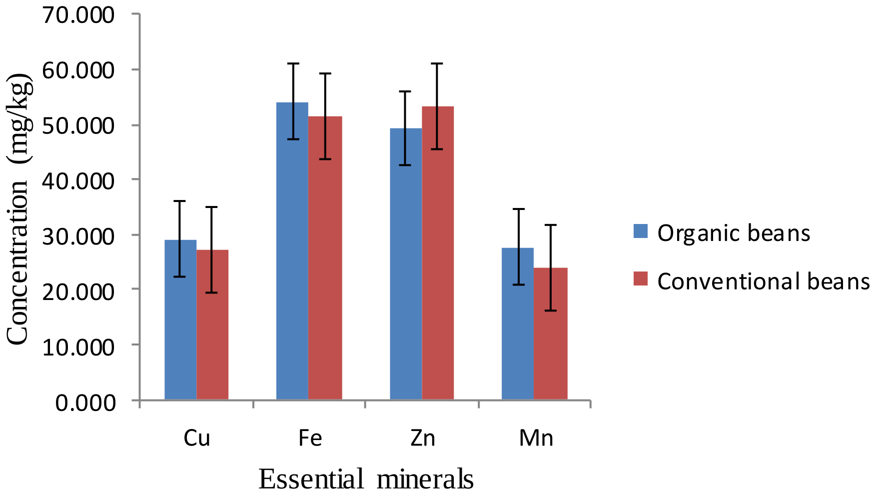
Figure 9: Distribution of Pb and Cd in organic and conventional cocoa



Cadmium which is easily transferred from the soil to plants varied widely between organic and conventional cocoa. Cd ranged between 0.06–0.13 mg/kg with a mean of 0.08 mg/kg in conventional cocoa, while organic ranged from 0.05–0.07 mg/kg with an average of 0.06 mg/kg. Cd levels in organic cocoa were significantly different ($P<0.05$) from conventional cocoa. The results, similar to that of lead, seem to suggest that the application of agrochemicals may have impacted or influenced the levels of Cadmium in conventional cocoa. However, the level of Cd in all the beans analysed were below the EU recommended limit of 0.8 mg/kg for chocolates with ≥ 50% total dry cocoa solids, and are therefore safe for consumption.

The levels of Fe, Zn, Cu and Mn in the organic cocoa was in the order of Fe > Zn > Cu > Mn whereas in conventional the order was Zn > Fe > Cu > Mn (Figure 10).

Figure 10: Distribution of Cu, Fe, Zn and Mn in organic and conventional cocoa



Contrary to Pb and Cd that were high in the conventional beans, all the essential elements (Cu, Fe, Zn, Mn) except Zinc were higher in organic cocoa than conventional. This is because copper inhibits the accumulation of Zinc. Copper which is the base for most fungicides for the prevention of black pod disease ranged from 23.19–31.50 mg/kg with a mean of 27.31 mg/kg in conventional cocoa and 27.46–30.94 mg/kg with a mean of 29.11 mg/kg in organic cocoa. There was however no significant difference ($P>0.05$) in the distribution of Copper between organic and conventional cocoa beans.

Comparing the conventional cocoa farm which uses lots of Cu based fungicides to organic farm which uses limited fungicides, the results suggest that the levels of Cu present may be emanating from the natural copper levels present in the soils and not due to any contamination. All the results obtained for the study were below the 50 mg/kg recommended maximum limit for Cu.

The content of Zinc in organic and conventional cocoa was 49.34 mg/kg and 53.26 mg/kg respectively. Plant mineral composition generally depends on the minerals present in the soils on which the plant is cultivated. The organic cocoa might have absorbed lot of Fe from the soil since it recorded the highest concentration among the essential elements with a mean of 54.09±6.85 mg/kg while 51.450±12.70 mg/kg was reported for conventional cocoa. There was no significant difference in the levels of the Fe between organic and conventional beans. Mn ranged from 19.73–27.27.61 mg/kg with a mean value of 23.77 mg/kg while Organic cocoa ranged from 23.29–30.61 mg/kg with an average value of 27.71 mg/kg. There was no significant difference in the levels of Mn between organic and conventional cocoa. The low levels of Pb, Cd, Hg and As and the relatively high levels of Cu, Fe, Zn and Mn which are regarded as essential elements in trace amounts may make cocoa a rich source of these minerals compared to seafood, grains nuts and green vegetables (Lowor and Shiloh, 2013).

4.4 Effect of processing on concentration of heavy metal in different cocoa products

The effect of processing on the concentration of heavy metals in semi-finished and finished cocoa products was assessed during the study. The results are presented in Table 2. Mercury (Hg) and Arsenic (As) were not detected in any of the five cocoa products analysed.

The content of Pb in cocoa butter, cocoa liquor and cocoa nibs were below the detection limit (<0.01mg/kg). Amongst the five samples analyzed for Pb, cocoa shell contained the highest level of Pb (0.283 mg/kg) whilst cocoa powder recorded 0.220 mg/kg. There was however no significant difference ($p>0.05$) between the content of Pb in cocoa shell and cocoa powder. The high level of Pb in cocoa shell is corroborated by Lee and Low (1985), who reported 6.15–8.28 mg/kg and suggested that cocoa shell contains more than twice the amount of metals as the nibs. However, since the shells are discarded during the manufacturing process, they may not contribute to the metal contents of the cocoa products. The level of Lead in this study is lower than that reported by Lee and Low (1985).

Cocoa powder as expected recorded the highest Cd content (0.127) followed by cocoa shell (0.077 mg/kg), cocoa liquor (0.063 mg/kg) and cocoa nibs (0.057 mg/kg). The content of Cd in cocoa nibs and cocoa liquor were significantly lower ($p< 0.05$) than cocoa powder and cocoa shell which were significantly different from each other.

Copper (Cu) on the other hand was not detected in cocoa butter. The cocoa product with the highest Cu content was cocoa powder with a mean Cu content of 52.267 mg/kg while cocoa nibs reported 31.987 mg/kg. Cocoa liquor and cocoa shell recorded 29.260 mg/kg and 28.543 mg/kg respectively. The content of Cu in cocoa liquor and cocoa shell was not significantly different ($p>0.05$), however, there was significant difference between the content of Cu in cocoa liquor, cocoa powder and cocoa nibs.

Amongst the seven heavy metals analyzed, Iron recorded the highest levels in the order of cocoa powder (375.343 mg/kg) >cocoa shell (177.217 mg/kg) > cocoa liquor (167.720 mg/kg) > Cocoa nibs (25.223 mg/kg). The content of Fe in cocoa butter was below the detection limit.

The content of Zn in descending order was cocoa powder (83.683 mg/kg), cocoa liquor (51.253 mg/kg), cocoa shell (50.047 mg/kg); cocoa nibs (49.117 mg/kg); and cocoa butter (0.735 mg/kg). The content of Zn was significantly lower ($P<0.05$) in cocoa butter and significantly higher ($P<0.05$) in cocoa powder compared to the other products.

The residue levels of all the metals analysed except Cu are higher in cocoa shell than the nibs and generally agreed with the submission by Lee and Low (1985) that heavy metals accumulates more in the shell than the nibs. The levels of Pb, Cd and Zn in liquor and nibs are comparable as expected but Cu and Fe were significantly different ($P<0.05$). The content of Fe increased over a 100% when the cocoa nibs were processed into the

Liquor. This may be attributed to the metallic iron from machine parts (Ball mills) which are usually made of stainless steel rubbing against each other and causing wear and tear. This may be the reason why the ball mills over a long period of time decrease in size (Afoakwa, 2014).

Cocoa powder recorded the highest levels of metals among the products considered. This may be because the metals migrated/associated with the free fat cocoa solids when the liquor was pressed to obtain cocoa butter and cocoa cake. The removal of fats and moisture from the cocoa solids may be the reason why the metals content is higher than the cocoa liquor. Lee and Low (1985) reported similar finding when they reported an increase in metal contents from paste to cake and attributed it to loss of moisture during the heating process.

Cadmium and lead levels in chocolates as reported by Lee and Low (1985) were 0.32 mg/kg and 1.50 mg/kg respectively. Knevezic (1980) reported Cd (0.02–0.69 mg/kg) and Pb (0.02–0.69 mg/kg) in chocolates with varying content of cocoa solids. Their results are higher than Lead and cadmium levels obtained in locally manufactured chocolates analysed in this study.

Given the provisional tolerable weekly intake (PTWI) of 25 ug/kg and 7 ug/kg body weight (FAO/WHO, 1989) for lead and cadmium respectively, the very low levels of lead (BDL) and cadmium (0.009 ug/kg) in locally manufactured chocolates may not pose any adverse health effect to consumers.

Table 2: The levels of heavy metals in cocoa products along the production line

Cocoa products	Pb	Cd	Hg	As	Cu	Fe	Zn
Cocoa butter	N/D	N/D	N/D	N/D	N/D	N/D	0.735±0.106^{a}
Cocoa liquor	N/D	0.063±0.006^{ab}	N/D	N/D	29.260±0.193^{a}	167.720±4.723	51.253±1.300^{b}
Cocoa powder	0.220±0.082^{a}	0.127±0.006^{c}	N/D	N/D	52.567±2.645^{c}	375.343±18.886	83.683±4.220^{c}
Cocoa shell	0.283±0.012^{a}	0.077±0.012^{b}	N/D	N/D	28.543±0.605^{a}	177.217±25.986	50.047±1.113^{b}
Cocoa nibs	N/D	0.057±0.006^{a}	N/D	N/D	31.987±0.972^{b}	25.223±0.585	49.117±1.477^{b}
Chocolate	N/D	0.009±0.005	-	-	-	-	-

N/D – not detected; Same alphabet on the same column is not significant at $P < 0.05$; - means not tested

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

The conclusions drawn from this study are as follows:

i. The content of Pb, Cd, Hg, As, Cu, Fe, Zn and Mn in cocoa beans and cocoa products produced from Ghana are within the available recommended maximum limits and are thus, generally safe from heavy metal contamination.

ii. Cocoa beans from mining areas had similar heavy metals concentrations as compared to those from non -mining areas. The observed heavy metal concentrations were below the maximum limits and thus were safe. Hence mining activities have no negative impact on the heavy metals safety of cocoa beans in Ghana.

iii. Cocoa beans from conventional cocoa farming areas had relatively higher heavy metals concentrations as compared to those from organic farming areas. However, the observed heavy metals concentrations were all below the maximum limits and thus are safe. Hence the use of agrochemical application has no negative impacts on the heavy metals safety of cocoa beans in Ghana.

iv. Processing of cocoa into semi -finished and finished products has an increasing effect on the level of heavy metals contamination. Heavy metals concentration in cocoa powder was found to be higher than all the other products. However, the levels found with all the different heavy metals were all within the acceptable limits and thus are safe for human consumption.

5.2 Recommendations

Based on the findings from this study, the following recommendations are made;

i. Further studies should be carried out to conduct exposure assessment on the dietary levels of these elements in cocoa products..

ii. Further studies should be conducted to investigate the levels of heavy metals in the soils of cocoa growing regions to ascertain the correlation between soil and bean concentration.

iii. Environmental protection managers and other relevant stakeholders should use the findings obtained on evaluating the total concentrations and the distribution characteristics of heavy metals in organic and conventional cocoa beans to regulate the rate of agrochemical application and formulate policies to safeguard the safety of cocoa produced in Ghana as well as the environment.

REFERENCES

Adriano, D.C. (2003). Trace elements in terrestrial environment: Biogeochemistry, bioavailability and risk of metals. New York, NY: Springer. 45-47.

Abenyega, O., & Gockowski, J. (2003). Labour practices in the cocoa sector of Ghana with a special focus on the role of children. International Institute of Tropical Agriculture. 10–11.

Ackah, J. E. (2012). Distribution of heavy metals in cocoa farm soils in the western region of Ghana. (Mphil thesis). University of Ghana, Legon.

Afari-Sefa, V., Gockowski, J., Agyemnan, N., & Dziwornu, A. (2010). Economic cost–benefit of certified sustainable cocoa production in Ghana. Poster presented at the Joint 3rd African Association of Agricultural Economists (AAAE) and 48th Agricultural Economists Association of South Africa (AEASA) Conference, Cape Town, South Africa, 19–23 September.

Afoakwa, E.O. (2010). Chocolate Science and Technology. Oxford, UK: Wiley-Blackwell Publishers. 127-143.

Afoakwa, E. O. (2014). Cocoa Production and Processing Technology. Boca Raton, USA: Taylor and Francis Group, CRC Press. 48-50.

Agyen, K. E. (2011). Pesticide residues and levels of some metals in soils and cocoa beans in selected farms in the Kade area of the Eastern Region of Ghana. (MPhil thesis). Kwame Nkrumah University of Science and Technology, Kumasi.Agency for Toxic Substances and Disease Registry. (2000). Toxicological profile for manganese.(1–466). Atlanta, GA: US Department of Health and Human Services.

Ahenkorah, Y., Halm, B. J., Appiah, M. R., & Akrofi, G. S. (1981). Fertilizer use on cocoa rehabilitation projects in Ghana: 8th International Cocoa Research Conference, Cartagena, Colombia.

Aikpokpodion, P. E., Lajide, L., & Aiyesanmi, A. F. (2012). Metal fractionation in soils collected from selected cocoa plantations in Ogun State, Nigeria. *World Applied Sciences Journal*, *20*(5), 628–636.

Aikpokpodion, P.E., Atewolara-Odule, O.C., Osobamiro, T., Oduwole, O. O., & Ademola, S. M. (2013a). A survey of copper, lead, cadmium and zinc residues in cocoa beans obtained from selected plantations in Nigeria. *Journal of Chemical and Pharmaceutical Research*, *5*(6), 88–98.

Aikpokpodion, P. E., Lajide, L., & Aiyesanmi, A.F. (2013b). Impact of copper based fungicide on copper residue and mineral element distribution in cocoa beans and pods. *World Journal of Agricultural Science*, 9 (1), 10-16.

Akabzaa, T. M., Banoeng-Yakubu, B., & Seyire, J. S. (2005). Heavy metal contamination in some mining communities within Jimi river basin in Ashanti Region. *Journal of the Ghana Science Association*, *7*(1), 25-29.

Alloway, B.J. (1995). Heavy metals in soils. London: Blackie Academic and Professional press. 102-105.

Amankwah, D. (2013). Levels of some heavy metals and proximate analyses of cocoa beans from Eastern and Central regions of Ghana. (Mphil thesis). Kwame Nkrumah University of Science and Technology, Kumasi.

Anim-Kwapong, G. J., & Frimpong, E. B. (2005).Vulnerability of agriculture to climate Change: Impact on cocoa production. New Tafo Akim: Cocoa Research Institute of Ghana (CRIG).

AOAC (2005). Official Methods of Analysis.

Appiah, M. R., Sackey, S. T., Ofori-Frimpong, K., & Afrifa, A. A. (1997).The consequences of cocoa production on soil fertility in Ghana. *Ghana Journal of Agricultural Science*, *30*, 183–190.

Armah, F. A., Luginaah, I., & Obiri, S. (2012). Assessing environmental exposure and health impacts of gold mining in Ghana. *Toxicological and Environmental Chemistry*, 94(4), 786–798.

Asante, A. K., Agusa, T., Subramania, A., Ansa-Asare, O. D., Biney, C. A., & Tanbe, S. (2005). Contamination status of arsenic and other trace elements in drinking water in residents from Tarkwa, a historic mining township in Ghana. *Chemosphere*, *66*, 1513–1522.

Asante-Poku, A., & Angelucci, F. (2013). Analysis of incentives and disincentives for cocoa in Ghana. Technical notes series, MAFAP, FAO, Rome.

Asare, R. (2006). A review on cocoa agro-forestry as a means for biodiversity conservation. World Cocoa Foundation Partnership Conference, Brussels, 1-17.

Augstburger, F., Berger, J., Censkowsky, U., Heid, P., Milz, J., & Streit, C. (2000). Organic farming in the tropics and subtropics: Exemplary description of 20 crops Naturland bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australian and New Zealand. *Australian Journal of Soil Research*, *38*, 1037–1086.

Barone, A., Ebesh, O., Harper, R.G., & Wapnir, R. A. *(*1998). Placental copper transport in rats: Effect of elevated dietary zinc on foetal copper, iron and metallonthionein. *Journal of Nutrition, 128*(6), 1037-1041.

Bloomfield, E. M., & Lass, R. A. (1992). Impact of structural adjustment and adoption of technology on competitiveness of major cocoa producing countries. Working Paper 69. Paris: Organization for Economic Cooperation and Development.

Boahene, K., Snijders, T. A. B., & Folmer, H. (1999). An integrated socio economic analysis of innovation adoption: The case of hybrid cocoa in Ghana. *Journal of Policy Modeling,* 21(2), 167–84.

Bonzongo, J. C., Donkor, A. K., Nartey, V. K., & Lacerda, L. D. (2004). Mercury pollution in Ghana: A case study of environmental impacts of artisanal gold mining in sub-Saharan Africa. In L. T. D. Lacerda and R. E. Santelli (Eds.). Facets of environmental science and technology. Cambridge: Royal Society of Chemistry, 17–47.

Bradl, H. (2005). Heavy metals in the environment: Origin, interaction and remediation. *Interface Science and Technology*, 6, 1–27.

Buijsse, B., Feskens, E.J.M., Kok, F.J., & Kromhout, D. (2006). Cocoa intake, blood pressure and cardiovascular mortality: The Zutphen elderly study. *Archives of Internal Medicine*, 166,411-417.

Bulir, A. (1998). The price incentive to smuggle and the cocoa supply in Ghana. International monetary fund (IMF). Working Paper No. 98/88.

Codex Alimentarius Commission (1999). Guidelines for the production, processing, labelling and marketing of organically produced foods. Retrieved on June 12th 2015, at www.Fao.org.

Corguinha, A. P. B., Souza, G. A. de, Gonçalves, V. C., Carvalho, C. de A., Lima, W. E. A. de, Martins, F. A. D., & Guilherme, L. R. G. (2015). Assessing arsenic, cadmium and lead contents in major crops in Brazil for food safety purposes. *Journal of Food Composition and Analysis*, 37, 143–150.

Dahiya, S., Karpe, R., Hegde, A. G., & Sharma, R. M. (2005). Lead, cadmium and nickel in chocolates and candies from suburban areas of Mumbai, India .*Journal of Food Composition and Analysis*, *18*(6), 517–522.

Dillinger, T.L., Barriga, P., Escarcega, S., Jimenez, M., Salazar Lowe, D., & Grivetti, L.E. (2000). Food of the gods: Cure for humanity? A cultural history of the medicinal and ritual use of chocolate. *Journal of Nutrition*, *130*, 2057–2072.

Dorman, E. N. A., Van Huis, A., Leeuwis, C., Obeng-Ofori, D., & Dawson-Sakyi, O. (2004).Causes of low productivity of cocoa in Ghana: Farmer's perspectives and insights from research and the socio-political establishment. *NJAS Wageningen Journal of Life Sciences*, *11*(3/4), 237–259.

Dube, A., Zbytniewski, R., Kowalkowski, T., Cukrowska, E., & Buszewski, B. (2001). Adsorption and migration of heavy metals in soil. *Polish Journal of Environmental Studies*, *10*(1), 1–10.

Dudka, S., & Chlopecka, A. (1990). Effect of solid-phase speciation on metal mobility and phytoavailability in sludge-amended soil. *Journal of Water, Air and Soil Pollution,* 51, 153 – 160.

Duruibe, J.O., Ogwuegbu, M. O. C., & Egwurugwu, J. N. (2007a). Heavy metal pollution and Humanbiotoxic effects. *International Journal of Physical Sciences*, 2(5), 112–118.

Duruibe, J. O., Ogwuegbu, M. O. C., & Egwurugwu, J. N. (2007b). Pollution profiles of non- metallic inorganic and organic pollutants of drinking and potable waters due to mining activities in Ishiagu (Ebonyi State) of Nigeria. *International Journal of Physical Sciences*, *2*(8), 202-206.

Dyer, C. A. (2007). Heavy metals as endocrine disrupting chemicals. In A. C. Gore (Ed.), Endocrine-Disrupting Chemicals: From Basic Research to Clinical Practice. Totowa, NJ: Humana Press. 111–133.

European Food Safety Authority. (2011). Statement on tolerable weekly intake for cadmium. *European Food Safety Authority Journal*, 9(2), 1975.

European Food Safety Authority. (2012). Retrieved June 14, 2015, from http://efsa.europa.eu/

Facchinelli, A., Sacchi, E., & Mallen, L. (2001). Multivariate statistical and GIS-based approach to identify heavy metals sources in soils. *Environmental Pollution*, *114*, 13–324.

Fairweather-Tait, S. J. (1988). Zinc in human nutrition. *Nutrition Research Review*, *1*, 23–37.

FAO/WHO (2001). Draft Standards for Chocolates and Chocolate products. Joint FAO/WHO Standards Programme. CODEX Committee on Cocoa Products and Cocoa Chocolates, 19th Session, 3–5 October 2001, Fribourg, Switzerland, CX/CPC 01/3.

FAO/WHO (2015). Discussion paper on establishment of maximum levels of cadmium in cocoa. Joint FAO/WHO Standards Programme. CODEX Committee on Contaminants in Foods, 9th Session, 16-20th March, 2015, New Delhi, India, (CX/CF 15/9/1).

Flegal, A. R., & Smith, D. R. (1995). Measurements of environmental lead contamination human exposure. *Reviews of Environmental Contamination and Toxicology, 143*:1-45.

Gazza, F. (1990). Lead and cadmium: Sources, metabolism, dangers and presence in meat and meat Products. *Annalidella Facolta di Medicina Veterina,10,* 171–181.

Global Agricultural Information Network (2012). Cocoa Annual report. Retrieved on May2015.

Gerard, B.N. (1967). A review of 50 years of Applied Entomology in Nigeria Tree Crops. Proceedings of the Entomological Society of Nigeria. 22 – 23.

Ghrefat, H., & Yusuf, N. (2006). Assessing Mn, Fe, Cu, Zn, and Cd pollution in bottom sediments of Wadi Al-Arab Dam, Jordan. *Chemosphere*, *65*, 2114–2121.

Graham, G. G., & Cordano, A. (1976).Copper deficiency in human subjects. In S. A. Prasad, and D. Oberleas (Eds), The Nutrition Foundation. Trace elements in human health and diseases, Vol. 1: Zinc and copper. New York, NY: Academic Press. 75-79.

Groten, J. P., & VanBladeren, P. J. (1994). Cadmium bioavailability and health risk in food. *Trends in Food Science and Technology*, *5*, 50–55.

Gyamfi, H.G. (2012). Impact of copper-based fungicide application on copper contamination in cocoa soils and plants in the Ahafo Ano North District, Ashanti Region. MSc thesis. Kwame Nkrumah University of Science and Technology, Kumasi.

Habashi, F. (1992). Environmental issues in the metallurgical industry – progress and problems, environmental issues and waste management in energy and mineral production. Balkama, Rotherdam, 1143 -1153.

Hawkes, J.S. (1997). Heavy Metals. *Journal of Chemical Education*, *74*(11),1374.

He, Z. L., Yang, X. E., & Stoffella, P. J. (2005). Trace elements in agroecosystems and impacts on human biotoxic effects. *International Journal of Physical Sciences*, *2*(5), 112–118.

Horsfall, M. N. Jr., & Spiff, A.I. (1999). Speciation of heavy metals in intertidal sediments of the Okirika River System (Nigeria), Bull. *Chemistry Society of Ethiopian*, 13(1): 1–9.

Hu, H. (2002). Human health and heavy metals exposure. In McCally M. (Ed), Life Support: The Environment and Human Health. MIT press.

Hutton, M., Symon, C. (1986). The quantities of cadmium, lead, mercury and arsenic entering the U.K. environment from human activities. *Science Total Environment*. *57*: 129-150.

International Organization for Cocoa. (2012). The world cocoa economy: past and present. One hundred and forty – second meeting. Retrieved on June 15, 2015, from http://www.icco.org/

Ieggli, C. V. S., Bohrer, D., de Nascimento, P. C., de Carvalho, L. M., & Gobo, L. A. (2011). Determination of aluminum, copper and manganese content in chocolate samples by graphite furnace atomic absorption spectrometry using a microemulsion technique. *Journal of Food Composition and Analysis*, *24*(3), 465–468.

Igboekwe, A.D. (1984). Occurrence and distribution of the cacao psyllid, Mesohomotoma (Tyora) tessmanni (Homoptera:Psyllidae). Proceedings 9th International Cocoa Research Conference Lome, Togo, 597 – 601.

Institute of International Tropical Agriculture ((IITA) (2009). *Climate Change and Cocoa.* Retrieved on July 7, 2015, from www.iita.org.

Kashem, M.A.,Singh, B. R., & Kawai, S. (2007). Mobility and distribution of Cd, Ni and Zn in contaminated soil profiles from Bangladesh. Nutrient Cycling in Agroecosystems, *77*:187-198.

Keen, C. L, Holt, R.R, Oteiza, P. I, Fraga, C.G., & Schmitz, H. H. (2005). Cocoa antioxidants and cardiovascular health. *American Journal of Clinical Nutrition*, *81,* 298S -303S.

Khan, M. S, Zaidi, A., Goel, R., & Musarrat, J. (2011).Biomanagement of metals-contaminated soils. Springer Dordrecht Heidelberg London New York, 3.

Kolavalli, S., & Vigneri, M. (2011). Cocoa in Ghana: Shaping the Success of an Economy: The World Bank. Retrieved on June 15, 2015, from www.worldbank.org.

Lee, C. K., & Low, K. S. (1985). Determination of cadmium, lead, copper and arsenic in raw cocoa, semi -finished and finished chocolate products. *Pertanika*, *8*(2), 243–248.

Lenntech (2004). Water Treatment. Netherlands: Lenntech, Rotterdamseweg. Retrieved on March 4, 2015 from www. lenntech.com.

Leung, H. M., Leung, A. O. W., Wang, H. S., Ma, K. K., Liang, Y., Ho, K. C., & Yung, K. K. L. (2014). Assessment of heavy metals/metalloid (As, Pb, Cd, Ni, Zn, Cr, Cu, Mn) concentrations in edible fish species tissue in the Pearl River Delta (PRD), China. *Marine Pollution Bulletin*, *78*, 235–245.

Lowor, S. T., & Shiloh, O. (2013). Estimation of some metal and quality profile of Cocoa beans in Ghana. *Elixir Food Science*, *59*, 15415–15420.

Loska, K., & Wiechuła D. (2003). Application of principal component analysis for the estimation of source of heavy metal contamination in surface sediments from the Rybnik Reservoir. *Chemosphere*, *51:* 723-733.

Manach, C., Scalbert A., Morand C., Rémésy C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition*, *79* (95): 727 - 747.

Mahrizal, L., Nalley, L., Dixon, B. L., & Popp, J. (2012). Necessary price premiums to incentivize Ghanaian organic cocoa production: A phased, orchard management approach. *Horticultural Science*, *47*(11), 1617–1624.

Manton, W. I. (2006). Sources of lead in cocoa and chocolate. *Environmental Health Perspectives*, *114*(5), 274–275.

Masoni, A., Ercoli, L., & Mariotti, M. (1996). Special properties of leaves deficient in iron, sulfur, magnesium, and manganese. *Agronomy Journal*, *88*: 937–43.

McCullough, M.L., Chevaux, K., & Jackson, L. (2006). Hypertension, the Kuna and the epidemiology of flavanols. *Journal of Cardiovascular Pharmacology*, *47*, S103-9.

McLaughlin, M. J., Hammon, R. E., Mclaren, R., Speir, G., & Rogers, T. W. (1999). A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australian and New Zealand. *Australian Journal of Soil Research*, 38, 1037–1086.

McKay, A., & Coulombe, H. (2003). Selective Poverty Reduction in a Slow Growth Environment: Ghana in the 1990s. Human Development Network, World Bank, Washington, DC.

Melamed, D. (2005). Monitoring arsenic in the environment: a review of science and technologies with the potential for field measurements. Analytica Chimica Acta, 532,1–13.

Misra, V., Tiwari, A., Shukla, B., & Seth, C.S. (2009). Effects of soil amendments on the bioavailability of heavy metals from zinc mine tailings. *Environmental Monitoring and Assessment, 155*:567-474.

Mohammed, D., Asamoah, D., & Asiedu-Appiah, F. (2011). Cocoa value chain - Implication for the Smallholder Farmer in Ghana. Department of Information Systems and Decision.

Moore, Jr. P. A., Daniel, T. C., Gilmour, J. T., Shreve, B. R., Edwards, D. R., & Wood, B. H. (1998). Decreasing metal runoff from Poultry litter with aluminum sulfate. *Journal Environment Quality*, *27,*92–95.

Mortvedt, J. J., & Beaton, J. D. (1995). Heavy metal and radionuclide contaminants in phosphate fertilizers. In Tiessen, H., (ed), Phosphorus in the global environment: transfer, cycles and management (pp.93–106). NewYork, NY: Wiley.

Mudgal, V., Madaan, N., Mudgal, A., Singh, R.B., & Mishra, S., (2010). Effect of toxic metals on human health. *The Open Nutraceuticals Journal*, 3; 94-99.

Nriagu, J.O., & Pacyna J.M. (1988). Quantitative assessment of world- wide contamination of air, water and soils by trace metals. *Nature 333*:134–139.

Nalley, L. L., Dixon, B. L., & Popp, J. (2012). Necessary Price Premiums to Incentivize Ghanaian Organic Cocoa Production: A Phased, *Orchard Management Approach*, *47*(11), 1617–1624.

Nartey, V. K., Haizel, M., Doamekpor, L. K., & Dankyi, E. (2012). Studies on the contribution of fertilizers to heavy metal levels in soils and cocoa from some cocoa farms in the Western Region of Ghana. *Journal of Natural Sciences Research, 2*(8), 111–121.

Natsume, M., Osakabe, N., & Yamagishi, M. (2000). Analyses of polyphenols in cacao liquor, cocoa, and chocolate by normal-phase and reversed phase HPLC. Bioscience, Biotechnology and Biochemistry, *64*, 2581-7.

Ndubuaku, T.C.N., Ojelade, K.T.M., Asogwa, E.U., & Anikwe, J.C. (2003). Reports and recommendations on the evaluation of Pulmic System PM 120 Knapsack sprayer for protection of cocoa farms in Nigeria. Tech. Rep. CRIN, Ibadan, Nigeria, 5pp.

Ntiamoah, A., & Afrane, G. (2008). Environmental impacts of cocoa production and processing in Ghana: Life cycle assessment approach. *Journal of Cleaner Production*, *16*, 1735–1740.

Ogwuegbu, M.O.C., & Muhanga, W. (2005). Investigation of lead concentration in the blood of people in the copperbelt province of Zambia. *Journal of Environment*, (1): 66 – 75.

Ojo, A.A. (1980). Insect pests and cocoa production in Nigeria. Proceedings of the Nigerian Cocoa Board Symposium. University of Ibadan, 42 – 47.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., & Anthony, S.(2009). Agroforestree Database: A tree reference and selection guide version 4.0.

Osei, I. (2007). Sustainable practices in the global cocoa economy: A producer's perspective. The 4th Indonesia International Cocoa Conference and Cocoa Dinner;

the Westin Resort, Nusa Dua - Bali, Indonesia. Retrieved from http://www.cocoafederation.com/events/

Peprah, K. (2014). Mitigating land degradation with chemical fertilizer application in the Asunafo Forest, Ghana. *International Journal of Science and Research, 3*(4), 476–482.

Quarmine, W., Haagsma, R., Sakyi-Dawson, O., Asante, F., Van Huis, A., & Obeng-Ofori, D. (2012). Incentives for cocoa bean production in Ghana: Does quality matter? *NJAS - Wageningen Journal of Life Sciences*, 60-63, 7–14.

Ramtahal, G., Chang Yen, I., Bekele, I., Bekele, F., Wilson, L., Sukha, B., & Maharaj, K. (2014). Cost-effective method of analysis for the determination of cadmium, copper, nickel and zinc in cocoa beans and chocolates. *Journal of Food Research*, *4*(1), 193–199.

Rankin, C. W., Nriagu, J. O., Aggarwal, J. K., Arowolo, T. A., Adebayo, K., & Flegal, A. R. (2005). Lead contamination in cocoa and cocoa products: Isotopic evidence of global contamination. *Environmental Health Perspectives*, *113*(10), 1344–1348.

Rehman, S., & Syed, M. H. (2012). Assessment of trace metal contents in chocolate samples by Atomic Absorption Spectrometry. *Journal of Trace Element Analysis, 1*, 1-11.

Selavpathy, P., & Sarala Devi, G. (1995). Nickel in Indian chocolates (toffees). *Indian Journal of Environmental Health, 37* (2), 123–125.

Skoog, D. A., Holler, F. J., & Nieman, T. A. (1998). Principles of instrumental analysis (5thed.). Philadelphia, PA: Harcourt Brace and Company.

Underwood, E.J. (1977). Trace elements in human and animal nutrition, (4th ed.). New York, NY: Academic Press. 55-59.

Verheye, W.H, (2010). Soils, plant growth and crop production, (Vol III), UK, Eolss

Publishers/UNESCO

Vigneri, M. (2007). Drivers of cocoa production growth in Ghana. Overseas Development Institute, project briefing No. 4. Retrieved on December 14, 2014 from http://www.odi.org.uk/resources/

Wang, Q. R., Cui, Y.S., Liu, X.M., Dong, Y.T., & Christie, P. (2003). Soil contamination and plant uptake of heavy metals at polluted sites in China. *Journal of Environmental Science and Health*, *38*(5), 823–838.

Wison, P.K., & Hurst, W. J. (Eds) (2015). Chocolate and health chemistry, nutrition and therapy. Cambridge, UK: Royal Society of Chemistry, 124.

Wittsiepe, J., Schnell, K., Hilbig, A., Schrey, P., Kersting, M., & Wilhelm, M. (2009). Dietary intake of nickel and zinc by young children - Results from food duplicate portion measurements in comparison to data calculated from dietary records and available data on levels in food groups. *Journal of Trace Elements in Medicine and Biology*, *23*, 183–194.

Wood, G.A.R. (1991). A history of early cocoa introductions. *Cocoa Growers Bulletin*, 44:6-11

WHO (2010). Exposure to cadmium: a major public health concern. *World Health Organization*, 3–6. Retrieved from http://www.who.int/ipcs/features/cadmium.pdf

Wuana, R.A., & Okieimen, F. E (2011). Heavy metals in contaminated soils: A review of sources, chemistry, risks and best available strategies for remediation. Ecology 20.

Yanus, R. L., Sela, H., Borojovich, E. J. C., Zakon, Y., Saphier, M., Nikolski, A., & Karpas, Z. (2014).Trace elements in cocoa solids and chocolate: An ICPMS study.*Talanta*, 119, 1-4.

Youdeowei, A. (1974). The cocoa mired problem in Nigeria. Proceedings at 4th Conference of

West Africa. Cocoa Ent., Legon, Ghana, 29 – 34.

Zarcinas, B.A, Ishak, C.F., Mclaughlin, M.J., & Cozens, G. (2004). Heavy metals in soils and crops in Southeast Asia. *Environmental Geochemistry and Health*, 26, 343–357.

APPENDICES

Appendix A: Microwave Program for Digesting Cocoa and Cocoa Products

Step	Time (mins)	Power (watts)	Pressure (Bar)	Temp °C
1	00:20:00	1000	50	200
2	00:20:00	1000	50	200
Vent: 00:10:00		Rotorctrl on		Twist on

Ref: Milestone Operator Manual MA127 (2009)

Appendix B: Working Conditions for Atomic Absorption Spectrometer

Optimization parameters for Flame Atomic Absorption Spectrometer (F-AAS)

ELEMENT	**WAVE LENGTH nm**	**LAMP CURRENT %**	**SLIT WIDTH nm**	**FUEL**	**SUPPORT**
Zn	213.9	75	0.5	ACETYLENE	AIR
Cu	324.7	75	0.5	ACETYLENE	AIR
Fe	248.3	75	0.5	ACETYLENE	AIR
Mn	279.5	75	0.5	ACETYLENE	AIR
Cr	357.9	100	0.5	ACETYLENE	AIR
Ni	2314	75	0.5	ACETYLENE	AIR

Optimization parameters for Graphite furnace Atomic Absorption spectrometer (GTA-AAS)

Elements	Pb	Cd	As
Background correction	Deuterium	Deuterium	Deuterium
Conc. Unit	ug/L	ug/L	ug/L
Instrument mode	Absorbance	absorbance	Absorbance
Sampling mode	Automix	Automix	Automix
Calibration mode	Concentration	Concentration	Concentration
Measurement mode	Peak height	Peak height	Peak height
Replicate standards	3	3	3
Replicate samples	3	3	3
Wavelength	283.3nm	228.8nm	193.5nm
Slit width	0.5	0.5nm	0.5%
Gain	42%	50%	80

Lamp current	9.0mA	4.0mA	4.0mA
Atomization temperature	2100^{o}C	1800^{o}C	2600^{o}C
Total volume	25uL	25uL	25Ul
Calibration Algorithm	Linear	Linear	Linear

Appendix C: Results of Certified Reference Materials

Concentration of Trace metals in (Dorm 4) from NRI (Canada)

Trace metal	Certified values(mg/kg)	Observed value (mg/kg)
Pb	0.416±0.053	0.36±0.08
Cd	0.306±0.015	0.314±0.06
Hg	0.410±0.055	0.354±0.03
As	6.80±0.64	5.848±1.30
Cu	15.9±0.9	15.11±3.022
Fe	341±27	296.67±89.00
Zn	52.2±3.2	49.59±10.99

Concentration of Lead and Cadmium in FAPAS Material (Cocoa powder)

Trace metal	**Certified Values (mg/kg)**	**Observed values (mg/kg)**
Pb,	0.037±0.017	0.034±0.01
Cd	0.141±0.061	0.129±0.070

Appendix D: Results of Heavy metals in cocoa and cocoa products

The Levels of Heavy metals in Cocoa beans from the various Districts

DISTRICT	Pb	Cd	Hg	As	Cu	Fe	Zn	Mn	Ni	Cr
Akim Oda	0.050±0.014^{bc}	0.070±0.010^{ab}	N/D	N/D	28.550±2.742^{bcd}	40.887±8.161^{a}	54.283±4.001^{e}	24.180±2.496^{ab}	0.01	N/D
Bogoso	0.01^{a}	0.090±0.010^{bcd}	N/D	N/D	25.037±2.782^{abc}	50.140±7.198^{ab}	51.730±1.033^{bcde}	30.517±2.026^{cd}	0.01	N/D
Goaso	0.083±0.025^{de}	0.103±0.021^{cd}	0.01	N/D	28.027±3.196^{bcd}	72.797±11.308^{c}	53.860±3.474^{de}	30.280±2.950^{cd}	0.01	N/D
Juaboso	N/D	0.090±0.020^{bcd}	N/D	N/D	22.210±1.366^{a}	66.125±4.080^{bc}	50.283±2.079^{abc}	29.590±2.862^{bcd}	0.01	N/D
Juaso	0.135±0.007^{f}	0.077±0.021^{abc}	N/D	N/D	28.637±3.655^{bcd}	51.973±4.284^{ab}	50.787±0.752^{abcd}	29.127±4.2519^{bcd}	0.01	N/D
Kade	0.05±0.01	0.113±0.015^{d}	N/D	N/D	29.107±0.690^{cd}	52.710±2.121^{ab}	52.060±0.272^{bcde}	25.463±2.176^{abc}	0.01	N/D
Konongo	0.03±0.01^{ab}	0.110±0.026^{d}	N/D	N/D	28.917±1.367^{cd}	63.560±2.630^{bc}	47.630±1.830^{a}	34.360±3.830^{d}	0.01	N/D
Kukuom	0.065±0.007^{cd}	0.110±0.026^{d}	0.01	N/D	28.533±2.274^{bcd}	56.660±2.022^{abc}	49.200±1.236^{ab}	30.057±4.975^{cd}	0.01	N/D
Sefwi Bekwai	N/D	0.093±0.015^{bcd}	N/D	N/D	24.563±3.600^{abc}	57.455±9.397^{abc}	49.027±0.327^{ab}	26.710±2.325^{abc}	0.01	N/D
Suhum	0.043±0.012^{abc}	0.063±0.005^{ab}	N/D	N/D	24.277±1.589^{ab}	61.173±13.587^{bc}	53.447±0.786^{cde}	21.657±1.961^{a}	0.01	N/D
Sunyani	0.097±0.015^{e}	0.077±0.021^{abc}	0.01	N/D	29.823±4.219^{d}	64.010±3.467^{bc}	51.673±3.294^{bcde}	24.700±1.315^{abc}	0.01	N/D
Tepa	0.03±0.01^{ab}	0.057±0.006^{a}	N/D	N/D	29.863±2.561^{d}	97.880±13.422^{d}	48.923±0.160^{ab}	30.387±4.535^{cd}	0.01	N/D

Key: N/D – not detected; Same alphabet on the same column is not significant at $P < 0.05$

Regional Distribution of Heavy metals across the Regions

REGION	**Pb**	**Cd**	**Hg**	**As**	**Cu**	**Fe**	**Zn**	**Mn**	**Ni**	**Cr**
Ashanti	0.056±0.049^{ab}	0.081±0.029^{a}	N/D	N/D	29.139±2.400^{b}	72.085±23.173^{b}	49.113±1.695^{a}	31.291±4.350^{b}	0.01	N/D
Brong Ahafo	0.084±0.021^{b}	0.097±0.025^{a}	0.01	N/D	28.794±2.990^{b}	65.468±9.309^{ab}	51.578±3.192^{bc}	28.801±4.028^{b}	0.01	N/D
Eastern	0.048±0.010^{a}	0.082±0.025^{a}	N/D	N/D	27.311±2.805^{b}	51.450±12.697^{a}	53.263±2.266^{c}	23.767±2553^{a}	0.01	N/D
Western	0.01^{a}	0.091±0.014^{a}	N/D	N/D	23.937±2.713^{a}	57.907±9.087^{ab}	50.347±1.657^{ab}	28.939±9.388^{b}	0.01	N/D

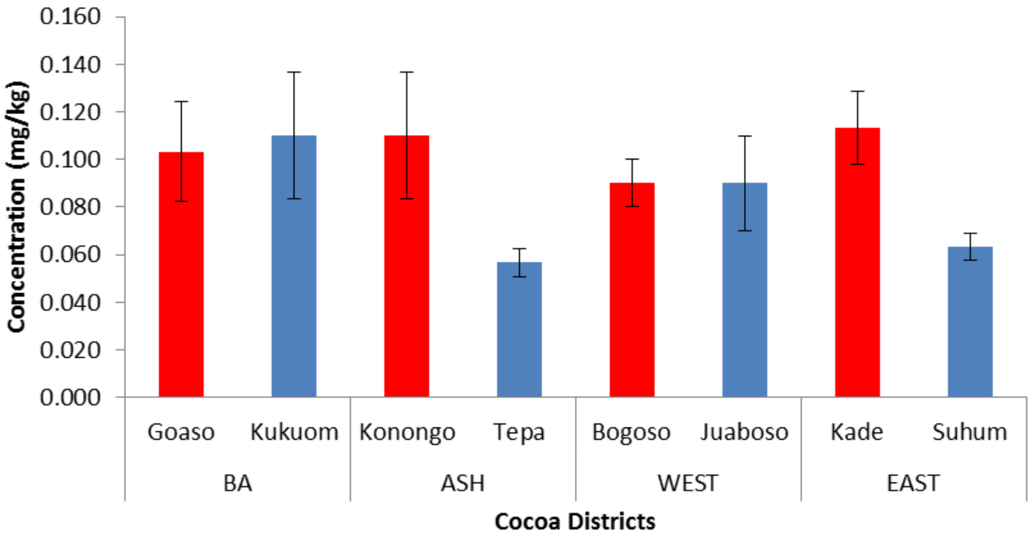
The Content of Heavy metals in conventional and Organic beans

TYPE	Pb	Cd	Hg	As	Cu	Fe	Zn	Mn	Ni	Cr
Organic cocoa beans	0.022±0.008^{a}	0.060±0.009^{a}	N/D	N/D	29.110±1.188^{a}	86.936±52.6809^{a}	49.336±3.804^{a}	27.709±2.575^{a}	N/D	N/D
Conventional cocoa beans	0.048±0.010^{b}	0.082±0.025^{b}	N/D	N/D	27.311±2.805^{a}	51.450±12.697^{a}	53.263±2.266^{b}	23.767±2.2553^{b}	N/D	N/D

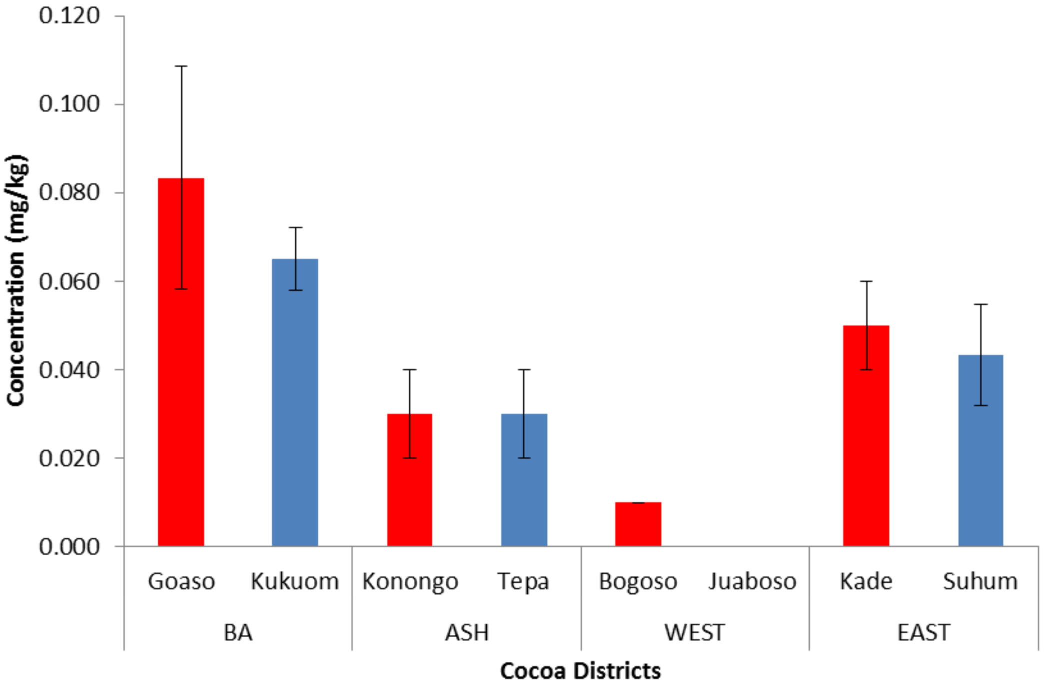
Distribution of heavy metals in mining and non -mining districts

Category	**Pb**	**Cd**	**Hg**	**As**	**Cu**	**Fe**	**Zn**	**Mn**	**Ni**	**Cr**
Mining	0.050±0.030[a]	0.104±0.019[b]	0.01	N/D	27.771±2.567[a]	61.246±11.845[a]	51.320±2.946[a]	30.155±4.089[a]	N/D	N/D
Non-mining	0.044±0.017[a]	0.080±0.027[a]	N/D	N/D	26.221±3.664[a]	72.273±20.139[a]	50.463±2.167[a]	27.923±4.979[a]	N/D	N/D

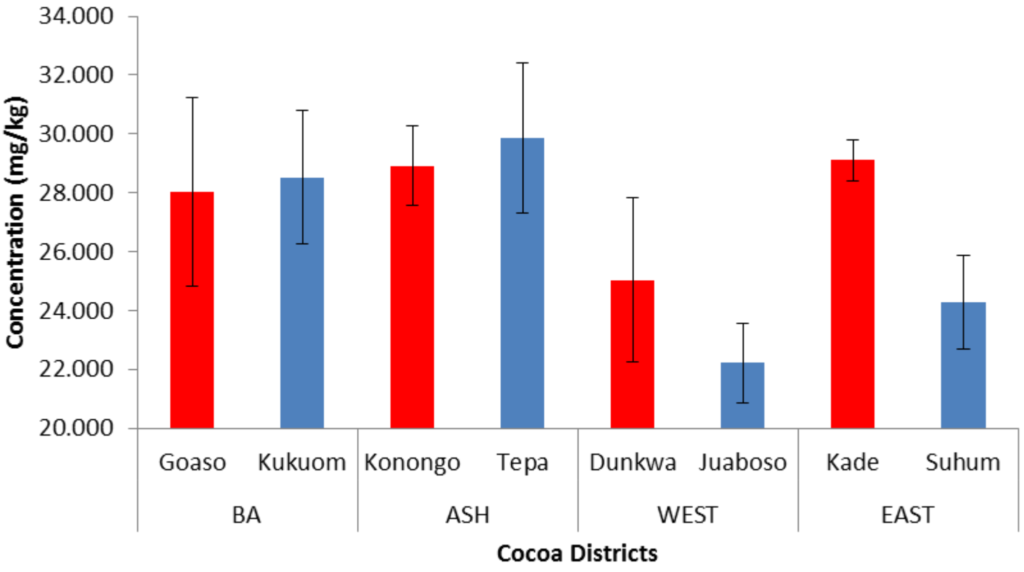
Appendix E Graphical Representation of Results



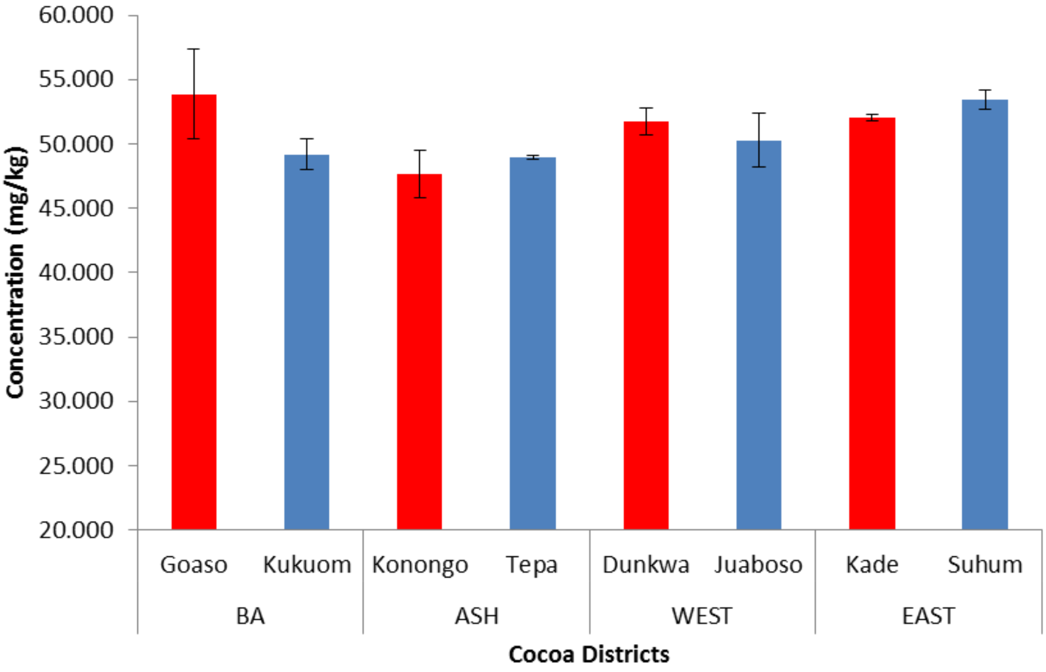
Distribution of Cd in mining and non-mining Districts



Distribution of Pb in mining and non-mining Districts



Distribution of Copper and non-mining Districts



Distribution of Zinc in mining and non –mining Districts

Appendix F: Summary of ANOVA Results

6/12/2015 2:17:53 PM

```
Welcome to Minitab, press F1 for help.
```

Summary of ANOVA results for Lead

Descriptive Statistics: Pb

```
Variable  Region        Mean     StDev  CoefVar   Minimum   Maximum
Pb        Ashanti      0.0563   0.0493    87.58    0.0200    0.1400
          Brong Ahafo  0.08375  0.02066   24.67    0.06000   0.11000
          Eastern      0.04750  0.01035   21.79    0.03000   0.06000
          Western      0.010000       *       *  0.010000  0.010000
```

One-way ANOVA: Pb versus Region

```
Source  DF        SS        MS     F      P
Region   3  0.008371  0.002790  2.83  0.063
Error   21  0.020725  0.000987
Total   24  0.029096

S = 0.03142   R-Sq = 28.77%   R-Sq(adj) = 18.59%

                               Individual 95% CIs For Mean Based on
                               Pooled StDev
Level        N     Mean    StDev  -+---------+---------+---------+--------
Ashanti      8  0.05625  0.04926                 (---*----)
Brong Ahafo  8  0.08375  0.02066                        (----*---)
Eastern      8  0.04750  0.01035               (----*---)
Western      1  0.01000        *  (------------*------------)
                                  -+---------+---------+---------+--------
                               -0.050     0.000     0.050     0.100

Pooled StDev = 0.03142
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Region

Individual confidence level = 98.89%

Region = Ashanti subtracted from:
Region          Lower    Center    Upper
Brong Ahafo  -0.01626   0.02750  0.07126
Eastern      -0.05251  -0.00875  0.03501
```

```
Western     -0.13908  -0.04625  0.04658

Region      -------+---------+---------+---------+--
Brong Ahafo             (----*---)
Eastern            (---*----)
Western       (--------*---------)
         -------+---------+---------+---------+--
             -0.10      0.00      0.10      0.20

Region = Brong Ahafo subtracted from:

Region     Lower    Center    Upper  -------+---------+---------+---------+--
Eastern  -0.08001  -0.03625  0.00751          (---*----)
Western  -0.16658  -0.07375  0.01908  (---------*--------)
                                     -------+---------+---------+---------+--
                                         -0.10      0.00      0.10      0.20

Region = Eastern subtracted from:

Region     Lower    Center    Upper  -------+---------+---------+---------+--
Western  -0.13033  -0.03750  0.05533      (--------*---------)
                                     -------+---------+---------+---------+--
                                         -0.10      0.00      0.10      0.20
```

Summary of ANOVA results for Cadmium

Descriptive Statistics: Cd

```
Variable  Region        Mean    StDev  CoefVar  Minimum  Maximum
Cd       Ashanti     0.08111  0.02892    35.65  0.05000  0.14000
         Brong Ahafo  0.09667  0.02500    25.86  0.06000  0.13000
         Eastern     0.08222  0.02539    30.87  0.06000  0.13000
         Western     0.09111  0.01364    14.97  0.07000  0.11000
```

One-way ANOVA: Cd versus Region

```
Source  DF        SS        MS     F      P
Region   3  0.001489  0.000496  0.87  0.469
Error   32  0.018333  0.000573
Total   35  0.019822

S = 0.02394   R-Sq = 7.51%   R-Sq(adj) = 0.00%
```

```
                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level        N    Mean    StDev  -------+---------+---------+---------+--
Ashanti      9  0.08111  0.02892  (----------*----------)
Brong Ahafo  9  0.09667  0.02500           (---------*----------)
Eastern      9  0.08222  0.02539  (----------*----------)
Western      9  0.09111  0.01364        (----------*----------)
                                 -------+---------+---------+---------+--
                                    0.075     0.090     0.105     0.120

Pooled StDev = 0.02394

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Region

Individual confidence level = 98.92%

Region = Ashanti subtracted from:

Region          Lower   Center    Upper
Brong Ahafo  -0.01500  0.01556  0.04611
Eastern      -0.02945  0.00111  0.03167
Western      -0.02056  0.01000  0.04056

Region       --------+---------+---------+---------+-
Brong Ahafo             (-----------*-----------)
Eastern             (-----------*------------)
Western               (-----------*-----------)
             --------+---------+---------+---------+-
                  -0.025     0.000     0.025     0.050

Region = Brong Ahafo subtracted from:

Region     Lower    Center    Upper  --------+---------+---------+---------+-
Eastern  -0.04500  -0.01444  0.01611  (-----------*-----------)
Western  -0.03611  -0.00556  0.02500     (-----------*-----------)
                                     --------+---------+---------+---------+-
                                          -0.025     0.000     0.025     0.050

Region = Eastern subtracted from:

Region     Lower   Center    Upper  --------+---------+---------+---------+-
Western  -0.02167  0.00889  0.03945        (------------*-----------)
                                    --------+---------+---------+---------+-
```

```
                        -0.025    0.000    0.025    0.050
```

Summary of ANOVA results for Copper

Descriptive Statistics: Cu

```
Variable  Region        Mean  StDev  CoefVar  Minimum  Maximum
Cu        Ashanti      29.139  2.399    8.23   25.310   32.550
          Brong Ahafo  28.794  2.990   10.38   25.230   34.280
          Eastern      27.311  2.805   10.27   23.190   31.500
          Western      23.937  2.713   11.33   20.660   28.170
```

One-way ANOVA: Cu versus Region

```
Source  DF      SS     MS     F      P
Region   3  152.34  50.78  6.79  0.001
Error   32  239.39   7.48
Total   35  391.74

S = 2.735   R-Sq = 38.89%   R-Sq(adj) = 33.16%

                         Individual 95% CIs For Mean Based on
                         Pooled StDev
Level        N    Mean  StDev   --+---------+---------+---------+-------
Ashanti      9  29.139  2.399                      (-------*------)
Brong Ahafo  9  28.794  2.990                        (------*-------)
Eastern      9  27.311  2.805               (------*-------)
Western      9  23.937  2.713   (-------*------)
                                --+---------+---------+---------+-------
                                22.5      25.0      27.5      30.0

Pooled StDev = 2.735

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Region

Individual confidence level = 98.92%

Region = Ashanti subtracted from:

Region        Lower  Center   Upper  -------+---------+---------+---------+--
Brong Ahafo  -3.836  -0.344   3.147             (------*------)
Eastern      -5.320  -1.828   1.664        (------*------)
Western      -8.694  -5.202  -1.710  (------*------)
                                     -------+---------+---------+---------+--
```

```
                              -5.0     0.0     5.0    10.0

Region = Brong Ahafo subtracted from:

Region    Lower  Center   Upper  -------+---------+---------+---------+--
Eastern  -4.975  -1.483   2.009       (------*------)
Western  -8.350  -4.858  -1.366  (------*------)
                                 -------+---------+---------+---------+--
                                      -5.0     0.0     5.0    10.0

Region = Eastern subtracted from:

Region    Lower  Center  Upper  -------+---------+---------+---------+--
Western  -6.866  -3.374  0.117     (------*------)
                                -------+---------+---------+---------+--
                                     -5.0     0.0     5.0    10.0
```

Descriptive Statistics: Zn

```
Variable  Region        Mean  StDev  CoefVar  Minimum  Maximum
Zn        Ashanti     49.113  1.695     3.45   45.880   51.450
          Brong Ahafo  51.58   3.19     6.19    48.04    57.68
          Eastern     53.263  2.266     4.25   50.470   58.460
          Western     50.347  1.657     3.29   48.280   52.430
```

One-way ANOVA: Zn versus Region

```
Source  DF      SS     MS     F      P
Region   3   84.78  28.26  5.40  0.004
Error   32  167.53   5.24
Total   35  252.31

S = 2.288   R-Sq = 33.60%   R-Sq(adj) = 27.38%

                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level         N    Mean  StDev  --+---------+---------+---------+-------
Ashanti       9  49.113  1.695  (-------*------)
Brong Ahafo   9  51.578  3.192            (-------*-------)
Eastern       9  53.263  2.266                   (------*-------)
Western       9  50.347  1.657       (-------*-------)
                                --+---------+---------+---------+-------
                                48.0      50.0      52.0      54.0
Pooled StDev = 2.288
```

```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Region

Individual confidence level = 98.92%


Region = Ashanti subtracted from:

Region        Lower  Center  Upper  -------+---------+---------+---------+--
Brong Ahafo  -0.457   2.464  5.386                (-------*-------)
Eastern       1.229   4.150  7.071                (-------*-------)
Western      -1.688   1.233  4.154          (--------*-------)
                                    -------+---------+---------+---------+--
                                         -3.5      0.0       3.5       7.0

Region = Brong Ahafo subtracted from:

Region    Lower  Center  Upper  -------+---------+---------+---------+--
Eastern  -1.236   1.686  4.607            (--------*-------)
Western  -4.152  -1.231  1.690      (-------*--------)
                                -------+---------+---------+---------+--
                                     -3.5      0.0       3.5       7.0

Region = Eastern subtracted from:

Region    Lower  Center  Upper  -------+---------+---------+---------+--
Western  -5.838  -2.917  0.004  (--------*-------)
                                -------+---------+---------+---------+--
                                     -3.5      0.0       3.5       7.0
```

Descriptive Statistics: Fe

```
Variable  Region        Mean  StDev  CoefVar  Minimum  Maximum
Fe        Ashanti      72.09  23.17    32.15    48.92   110.53
          Brong Ahafo  65.47   9.31    14.22    55.23    79.78
          Eastern      51.45  12.70    24.68    36.16    75.67
          Western      57.91   9.09    15.69    45.05    69.01
```

One-way ANOVA: Fe versus Region

```
Source  DF    SS   MS     F      P
Region   3  1901  634  2.79  0.061
Error   26  5907  227
Total   29  7808
```

```
S = 15.07   R-Sq = 24.35%   R-Sq(adj) = 15.62%


Individual 95% CIs For Mean Based on
                         Pooled StDev
Level        N   Mean  StDev  ------+---------+---------+---------+---
Ashanti      8  72.09  23.17                 (--------*--------)
Brong Ahafo  8  65.47   9.31               (---------*--------)
Eastern      8  51.45  12.70  (--------*--------)
Western      6  57.91   9.09      (---------*----------)
                         ------+---------+---------+---------+---
                             48        60        72        84

Pooled StDev = 15.07

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Region

Individual confidence level = 98.91%

Region = Ashanti subtracted from:

Region        Lower  Center  Upper   -+---------+---------+---------+--------
Brong Ahafo  -27.29   -6.62  14.06         (----------*---------)
Eastern      -41.31  -20.64   0.04   (----------*---------)
Western      -36.51  -14.18   8.15      (----------*----------)
                                     -+---------+---------+---------+--------
                                    -40       -20         0        20
Region = Brong Ahafo subtracted from:

Region    Lower  Center  Upper   -+---------+---------+---------+--------
Eastern  -34.69  -14.02   6.66     (---------*---------)
Western  -29.89   -7.56  14.77       (----------*----------)
                                 -+---------+---------+---------+--------
                                -40       -20         0        20

Region = Eastern subtracted from:

Region    Lower  Center  Upper   -+---------+---------+---------+--------
Western  -15.88    6.46  28.79            (----------*----------)
                                 -+---------+---------+---------+--------
                                -40       -20         0        20
```

Descriptive Statistics: Mn

```
Variable  Region        Mean  StDev  CoefVar  Minimum  Maximum
Mn        Ashanti      31.29   4.35    13.90    25.21    37.66
          Brong Ahafo  28.80   4.03    13.98    23.77    35.80
          Eastern     23.767  2.553    10.74   19.730   27.610
          Western     28.939  2.717     9.39   24.580   32.650
```

One-way ANOVA: Mn versus Region

```
Source  DF     SS    MS     F      P
Region   3  270.7  90.2  7.44  0.001
Error   31  376.1  12.1
Total   34  646.8

S = 3.483   R-Sq = 41.85%   R-Sq(adj) = 36.22%

                             Individual 95% CIs For Mean Based on
                             Pooled StDev
Level         N    Mean  StDev  ---------+---------+---------+---------+
Ashanti       9  31.291  4.350                      (-----*------)
Brong Ahafo   8  28.801  4.028                (------*------)
Eastern       9  23.767  2.553  (------*------)
Western       9  28.939  2.717                 (------*-----)
                                ---------+---------+---------+---------+
                                      24.5      28.0      31.5      35.0

Pooled StDev = 3.483
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Region

Individual confidence level = 98.93%
Region = Ashanti subtracted from:

Region         Lower  Center   Upper
Brong Ahafo   -7.086  -2.490   2.106
Eastern      -11.983  -7.524  -3.066
Western       -6.811  -2.352   2.106

Region       +---------+---------+---------+---------
Brong Ahafo          (-------*-------)
Eastern      (------*-------)
Western              (------*-------)
             +---------+---------+---------+---------
```

```
        -12.0      -6.0       0.0       6.0

Region = Brong Ahafo subtracted from:

Region    Lower  Center   Upper      +---------+---------+---------+---------
Eastern  -9.630  -5.035  -0.439        (-------*------)
Western  -4.458   0.138   4.733                (------*-------)
                                     +---------+---------+---------+---------
                                  -12.0      -6.0       0.0       6.0

Region = Eastern subtracted from:

Region   Lower  Center  Upper      +---------+---------+---------+---------
Western  0.714   5.172  9.631                          (-------*------)
                                   +---------+---------+---------+---------
                                -12.0      -6.0       0.0       6.0
```

Conversional and organic

6/12/2015 4:18:38 PM

```
Welcome to Minitab, press F1 for help.

Descriptive Statistics: Pb
Variable  Type            Mean     StDev  CoefVar  Minimum  Maximum
Pb        conver. Cocoa  0.04750  0.01035    21.79  0.03000  0.06000
          Organic cocoa  0.02200  0.00837    38.03  0.01000  0.03000


One-way ANOVA: Pb versus Type
Source  DF         SS         MS      F      P
Type     1  0.0020008  0.0020008  21.37  0.001
Error   11  0.0010300  0.0000936
Total   12  0.0030308

S = 0.009677   R-Sq = 66.02%   R-Sq(adj) = 62.93%

Level          N      Mean     StDev
conver. Cocoa  8  0.047500  0.010351
Organic cocoa  5  0.022000  0.008367

               Individual 95% CIs For Mean Based on
               Pooled StDev
Level          +---------+---------+---------+---------
conver. Cocoa                    (------*-----)
```

```
Organic cocoa    (-------*-------)
                 +---------+---------+---------+---------
               0.012     0.024     0.036     0.048

Pooled StDev = 0.009677
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 95.00%

Type = conver. Cocoa subtracted from:

Type              Lower     Center      Upper
Organic cocoa  -0.037642  -0.025500  -0.013358

Type          -----+---------+---------+---------+----
Organic cocoa  (-------*-------)
               -----+---------+---------+---------+----
                 -0.030    -0.015     0.000     0.015
```

Descriptive Statistics: Cd

```
Variable  Type            Mean    StDev  CoefVar  Minimum  Maximum
Cd        conver. Cocoa  0.08222  0.02539    30.87  0.06000  0.13000
          Organic cocoa  0.06000  0.00866    14.43  0.05000  0.07000
```

One-way ANOVA: Cd versus Type

```
Source  DF        SS        MS     F      P
Type     1  0.002222  0.002222  6.18  0.024
Error   16  0.005756  0.000360
Total   17  0.007978

S = 0.01897   R-Sq = 27.86%   R-Sq(adj) = 23.35%

                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level          N     Mean    StDev  ---------+---------+---------+---------+
conver. Cocoa  9  0.08222  0.02539              (--------*--------)
Organic cocoa  9  0.06000  0.00866  (--------*--------)
                                    ---------+---------+---------+---------+
                                         0.060     0.075     0.090     0.105

Pooled StDev = 0.01897
```

```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type
Individual confidence level = 95.00%

Type = conver. Cocoa subtracted from:

Type            Lower    Center    Upper
Organic cocoa  -0.04118  -0.02222  -0.00327

Type          ------+---------+---------+---------+---
Organic cocoa  (-----------*-----------)
            ------+---------+---------+---------+---
              -0.032    -0.016     0.000     0.016
```

Descriptive Statistics: Cu

```
Variable  Type           Mean  StDev  CoefVar  Minimum  Maximum
Cu        conver. Cocoa  27.311  2.805    10.27   23.190   31.500
          Organic cocoa  29.110  1.188     4.08   27.460   30.940
```

One-way ANOVA: Cu versus Type

```
Source  DF     SS     MS     F      P
Type     1  14.56  14.56  3.14  0.095
Error   16  74.23   4.64
Total   17  88.79

S = 2.154   R-Sq = 16.40%   R-Sq(adj) = 11.18%

                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level          N    Mean  StDev  --------+---------+---------+---------+-
conver. Cocoa  9  27.311  2.805  (---------*---------)
Organic cocoa  9  29.110  1.188           (---------*---------)
                                 --------+---------+---------+---------+-
                                      27.0      28.5      30.0      31.5
Pooled StDev = 2.154
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Type

Individual confidence level = 95.00%
Type = conver. Cocoa subtracted from:
Type           Lower  Center  Upper  ---+---------+---------+---------+------
Organic cocoa  -0.354   1.799  3.951           (------------*-------------)
                                     ---+---------+---------+---------+------
                                       -1.6       0.0       1.6       3.2
```