DETERMINATION OF HEAVY METALS IN COCOA BEANS FROM SOME MAJOR COCOA GROWING REGIONS IN GHANA

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Abstract
In this study, the levels of six heavy metals namely: cadmium (Cd), lead (Pb), copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn) were determined in cocoa beans from four major cocoa-growing regions of Ghana, viz Ashanti, Western, Eastern and Central Regions. Samples were digested with aqua regia acid mixture and atomic absorption spectroscopy was used for the determination of the metals. The method of determination was validated by analyzing two standard reference materials and levels measured compared favourably with reported values. Analysis of the heavy metal concentrations generally showed the following pattern: Fe≈Mn≈Zn≈Cu>>Cd>Pb in beans from Western and Ashanti Regions while those from Central and Eastern Region was Zn>Fe>Cu≈Mn>>Cd>Pb. The concentrations expressed in $\mu g\ g^{-1}$ varied from 0.003 to 0.095 for Pd, 0.005 to 0.095 for Cd, 10.47 to 55.17 for Cu, 0.50 to 72.36 for Fe, 4.45 to 72.64 for Mn and 24.05 to 68.25 for Zn. Analysis of the data by variance showed that the difference in concentrations are statistically insignificant (P>0.05) suggesting that the metals might have reached the study areas through the same sources.The levels of the metals detected in this study are, however, below the Codex Alimentaruis'maximum levels in fruits and vegetables and maximum permissible levels for cocoa powder and cocoa mass suggesting that their presence may notpose serious health hazard in the cocoa beans.

Key words: Heavy metal, Atomic absorption spectroscopy, Cocoa beans, Digestion, validation, statistical analysis.

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1. INTRODUCTION

The heavy metal concentrations are so high in soils of many areas that they can poison the soil-plant system, degenerate the soil, and reduce the quality of products of crops (Alloway, 1992). Due to their cumulative effects and long-terminteractions, accumulation of heavy metals in soil can negativelyaffect regional eco-safety and pose a threat to relevant animals and plants (Chang *et al*., 2014). Moreover, they can threaten the health of animals and human beings upon bioaccumulation in food chain (Beevers et al.,1965 and Allow,ay 1994). Based on this, the studies of the heavy metal composition in food crops is increasingly becoming veryimportant and relevant not only to nutritionists and toxicologists but to the general public as well. Though some micro elements are very essential for the proper functioning of the body, the toxicity of others makes their presence in food a cause for concern. The sources of these metals in food may vary widely ranging from the soil on which the plants are grown to the conditions they are subjected to during and after crop production. Anthropogenic activities such as mining and smelting of metal ores, industrial emissions and application of insecticides and fertilizers have all contributed to the elevated levels of heavy metals in the environment (Alloway, 1994). The threat that heavy metals pose to human and animal health is exacerbated by their long-term persistence in the environment.Additionally, the consumption of heavy metal-contaminatedfood can seriously deplete some essential nutrients in the bodycausing a decrease in immunological defences, intrauterinegrowth retardation, impaired psycho-social behaviour,disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer (Agyekum*et al*., 2012).To substantiate this, toxicological effects of heavy metals such as lead on human beings include inhibition of haemoglobin formation, sterility, hypertension and mental retardation in children (Hermandez *et al*., 2003), while the major hazard to human health of cadmium is its chronic accumulation in the kidney where it causes dysfunction if the concentration in the kidney cortex exceeds 200mg/kg fresh weight (Gu et al. 2005). In addition, though copper is an essential element, it may be toxic to both humans and animals when its concentration exceeds the safe limits and its concentration in some human tissues can lead to cancerous or non-cancerous effects (Bakirdere *et al*., 2008 and Tama *et al*., 2005).Heavy metals from soil enter plants primarily through the roots system. In general plant root is the important site for uptake of chemicals from soil (Bell,1992).

Cocoa products are regarded to be among some of the most widely consumed foods worldwide. Cocoa beans are the raw materials from which the widely patronized products such as chocolate, candies, cocoa powder and beverages are produced. The possibility of finding heavy metals in these products is as a result of their accumulation in the raw cocoa beans. Unfortunately, body of evidence lends credence to the relatively high levels of heavy metals in cocoa products as compared to other food products (Bahiya et al., 2005 andRankin *et al.,* 2005). Several suggestions have been stipulated as to the origins of these metal contaminants but it is widely believed to be from the raw cocoa beans.

For some decades now, cocoa has been and it continues to be the backbone of the Ghanaian economy in terms of foreign revenue and domestic incomes (COCOBOD, 1995). To corroborate this, Ghana currently produces about 1,000,000 metric tons of cocoa annually and is the second largest producer in the world. It is estimated that cocoa plants coverabout 1.5 million hectares of land (Franzan *et al*., 2007). Undeniably, cocoa from Ghana is revered to be of the best quality with high demand in the world market. Due to these quality characteristics of Ghana's cocoa, they are mostly used as reference standards for cocoa produced from other parts of the world (Fold 2008). It is worth mentioning that though cocoa beans from Ghana have been reported to contain relatively low levels of heavy metals and are within the acceptable limits, most of these research works date back to the 1990s (Redersen *et al*., 1994).Owing to this, the safe levels of heavy metals in the Ghanaian cocoa beans can be questionable as there has been an alarming increase in mineral mining activities as well as other anthropogenic activities in cocoa-growing areas. The patronage in cocoa products is likely to be on the ascendency following the recent discovery of high levels of polyphenols in cocoa and their great benefits to health (Engler *et al*., 2004 and Cooper *et al*., 2008). The polyphenols (catechins and epicatechins) have been detected to have good antioxidant and free radical scavenging properties leading to lower risk of cardiovascular diseases. Recent research has confirmed that cocoa has higher levels of essential polyphenols than in red wine and tea (Lee *et al.,* 2003).

As plants constitute the foundation of the food chain, some concerns have been raised about the possibility of toxic concentrations of certain heavy metals being transported from plants to higher strata of the food chain. The high demand for quality cocoa beans from Ghana over the years was affected by rejection in the Japanese market due to the detection of high levels of contaminants in the cocoa beans (Stateman, 2006). The situation is becoming more alarming due to the fact that almost all Ghanaian cocoa farmers now apply insecticides, herbicides, fungicides and fertilizers on their cocoa farms to control pestsand to enhancemaximum yield. These activities are likely to introduce heavy metals like Cd, Fe, Pb, Cu, Mn and Zn into the soil which eventually will end up in the edible parts of the crops through phytoextraction by the crops and translocation throughout the plant system.

In Ghana, cocoa takes about 25% of the total export earnings and it is the second most important export commodity after gold (Nartey *et al*., 2012). This, coupled with the high demand of Ghana's cocoa beans at the world market and worldwide patronage of cocoa products, makes it imperative for independent analyses of the levels of heavy metals so as to determine if the levels conform to the international standards. This will augment the work of the cocoa quality assurance services to consolidate the high demand for cocoa beans from Ghana domesticallyand internationally. The main objective of this study was, therefore to determine the levelsof the selected metals with the view of establishing the health status of cocoa beans in term of the heavy metals contamination as well as statistically deducing whether the differences in the levels are significant or insignificant.

2. MATERIALS AND METHODS

2.1 Geographical description of the sampling area

Ghana is situated on the west coast of Africa about 750km north of the equator between latitude 4^0 and 11.5^0 N and longitude 3.11^0 west. It shares boundaries with Burkina Faso to the north, Togo to the east, La Cote d' Ivoire to the west and Gulf of Guinea (part of Atlantic Ocean) to the south. Generally, the climate of Ghana is tropical and two main types of vegetation exist. These are the rain forest and savannah grassland. The forest vegetation is characterized by high temperatures and heavy rainfall almost throughout the year and is usually divided into rain forest and semi-deciduous forest. The forest vegetation promotes very rapid plant growth. Cocoa thrives well in the forest regions of Ghana which covers the south western part and comprises 6 out of the 10 political regions of the country.Samples of cocoa beans from Western, Ashanti, Eastern and Central Regions were used for this work. Fig 1 shows a map of major cocoa-growing towns in the four Regions where the cocoa beans used for the analyseswere taken.

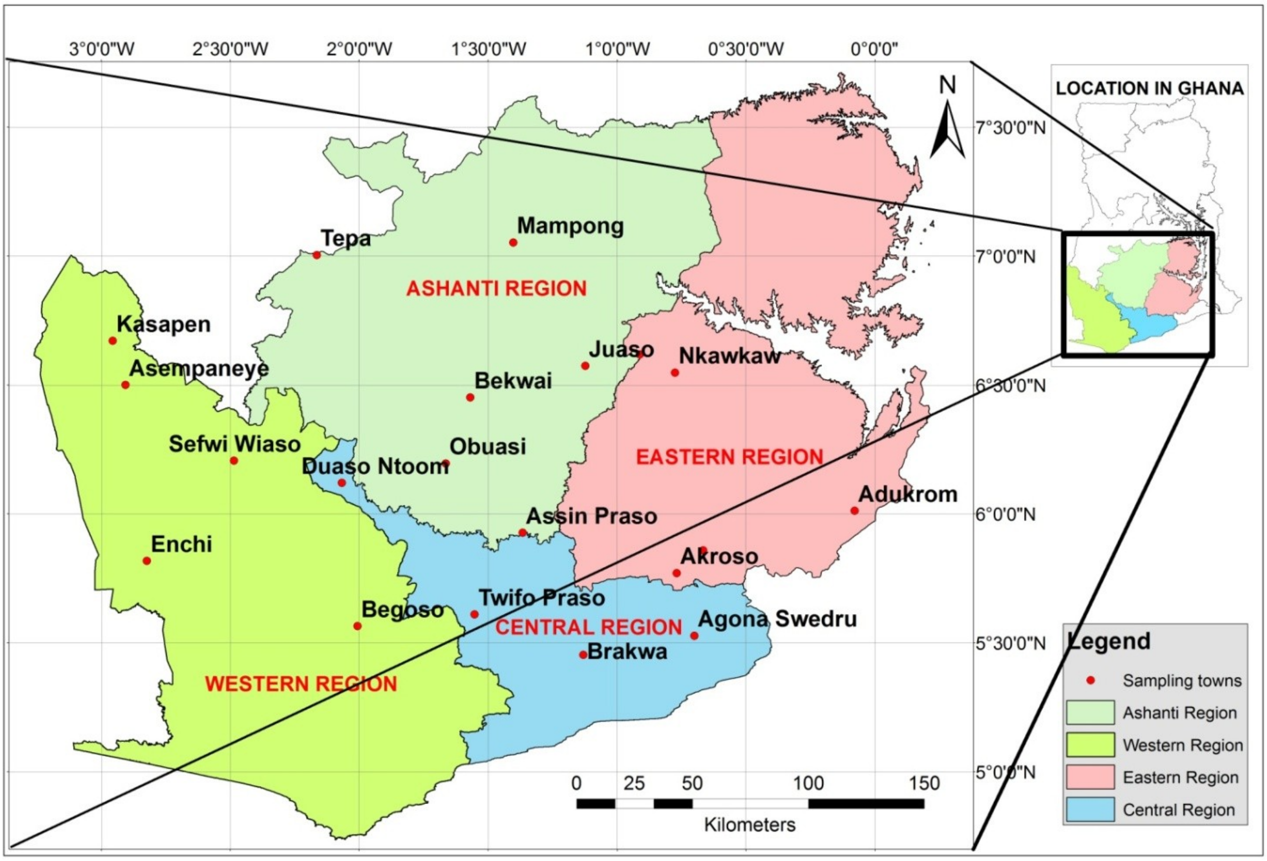

Fig1 Map showing the twenty major cocoa-growing towns where samples were taken.

2.2 Collection of samples

Samples of dried cocoa beans were obtained from cocoa farmers in some selected cocoa growing towns in Ashanti, Western, Eastern and Central Regions of Ghana. In each Region, five communities totaling twenty in all were selected for sampling. The communities were selected in such a way as to cover the geographical area of each of the Regions with regards to cocoa production. Some of the cocoa beans were obtained in the form of their fruits (pods). The beans from these fruits were subjected to fermentation and sun dried until they were fully dried. Sampling was done such that the towns from which cocoa beans were obtained were well spread throughout the Regions. In each community dried samples of cocoa beans were obtained from five different cocoa farmers and samples kept in clean and dried polyethylene bags.

2.3 Chemicals and reagents

All reagents used for this work were of analytical grade. Digestion of samples was performed using aqua regia (HNO_3 and HCl in ratio 3:1)were both obtained from Merck, Germany. De-ionized water was obtained from the Department of Chemistry, Kwame Nkrumah University of Science and Technologyand was used for all the analytical work.

2.4 Sample preparation and analysis

The dried beans were milled with Frltsch mortar grinder P-2 into fine cocoa powder and subsequently subjected to sample digestion.The aqua regia for the digestion was prepared by mixing 3:1 volumes of HCl and HNO_3 respectively in a fume hood. The prepared aqua regia was stored for 2 days to ensure a complete reaction and a uniform homogenous mixture between the acids before digestion of the samples commenced. One gram of the milled cocoa sample was digested with 30ml of the aqua regia in a pre-cleaned teflon cup by heating on a hot plate at 200^{o}C for about 20 minutes. The digest after cooling, was transferred into a 50 ml volumetric flask. De-ionized water was added to make it up to the 50 ml mark before being transferred and stored in pre-cleaned polypropylene tubes for analysis.

2.5 Atomic Absorption Spectrometry

Quantitative determination of the metallic elements was done using Atomic Absorption Spectrometer
(AAS) model PU9200X. Solutions of the cocoa prepared by acid digestion were aspirated directly by the aspiration tube with air-acetylene flame into the AAS. Standard metal solutions obtained from Chem Tech Analytical Limited, UK were used for calibration. Solutions of the SRM also prepared by acid digestion were as well analyzed by the AAS method.

2.6 Quality assurance/control

Sample containers and glassware used in the analysis were cleaned with metal free nonionic detergent solution, rinsed thoroughly with de-ionized water and soaked in nitric acid for 24 hours. They were then washed several times with de-ionized water prior to use. Blanks, consisting of de-ionized water, chemicals and reagents used for the digestion were subjected to similar sample preparation and analytical procedures in an effort to reduce the effect of contamination arising from chemical reagents, de-ionized water and glassware used in the analysis. In handling the cocoa beans, gloves were worn to avert external contamination which would affect the analyses.Accuracy of the method was evaluated through the analysis of two reference materials; NIST 1547 SRM certified Peach Leaves and IAEA V-10 SRM certified Hay Powder.

2.7 Statistical analyses

Statistical analysis used for the data analysis include application of descriptive statistics for deducing minimum, maximum and mean concentrations of detected metals and corresponding standard deviations. Data was subjected to one-way analysis of variance (ANOVA) to determine the statistical differences in concentrations of metals studied. All tests were regarded as statistically significant when $p < 0.05$. The calculations were performed using statistical software, SPSS version 16.

3. RESULTS AND DISCUSSION

3.1 Analysis of standard reference materials

Tables 1 and 2 present the results of analyses of the two certified reference materials. Values of heavy metals obtained for this work compared favourably with reported values. Within the limit of experimental errors, absolute errors for the analysis of IAEA V-10 SRM and NIST 1547 SRM as reported by this study ranged from 0.27 % to 5.32 % and 0.36 % to 10.19 % respectively.Percentage recoveriesof the metals from the SRMswere found to be between 96.88 % to 105.32% for the IAEA V-10 SRM and 93.70 % to 103.17 % for theNIST 1547 SRM. These results therefore confirm the reliability of the method for the determination of the metals.

Table 1 Analysis of IAEA V- 10 standard reference material for the heavy metals (µg/g)

Metal	This work	Reported values	% recovery	% absolute error
Pb	1.55±0.06	1.60±0.77	96.88	3.13
Fe	185.5±9.82	186±51.61	99.73	0.27
Zn	23.80±1.76	24±1.14	99.17	0.83
Mn	48.20±5.09	47±4.94	102.55	2.55
Cu	9.90±0.78	9.4±0.63	105.32	5.32

Table 2 Analysis of NIST 1547 standard reference material

Metal	This work	Reported values	% recovery	%absolute error
Pb	36.11±5.99	35.00±3.00	103.17	3.07
Fe	281.11±9.20	300.00±20.00	93.70	6.72
Zn	24.91±2.30	25.00±3.00	99.64	0.36
Mn	85.62±11.00	91.00±4.00	94.08	6.28
Cu	10.89±0.29	12.00±1.00	90.75	10.19

3.2 Statistical data for levels of the heavy metals in the cocoa beans

Statistical data showing the mean concentrations, the standard deviations and range indicating the minimum and maximum concentrations are presented in Tables 3, 4, 5 and 6. The results revealed significantly varying levels of heavy metals in the cocoa beans analyzed from the various areas. Analysis of the heavy metal concentrations generally showed the following pattern: Fe≈Mn≈Zn≈Cu>>Cd>Pb in beans from Western and Ashanti Regions while those from Central and Eastern Region was Zn>Fe>Cu≈Mn>>Cd>Pb. In all the samples the levels of the other four metals were far higher than those for lead and cadmium. Indeed, lead concentrations were the lowest in all the samples. The generally high concentrations of Fe, Zn, Cu and Mn in the samples were anticipated as they are regarded essential in living organisms. Analyses of the data by variance showed that the difference in concentrations are statistically insignificant ($P>0.05$) as presented in Tables 3, 4, 5 and 6. This suggests that the metals might have reached the study areas through the same sources.

The levels of lead measured from the cocoa beans ranged from 0.003 µg g^{-1} to 0.095 µg g^{-1} averaging 0.031 µg g^{-1} for all the samples. The highest level of 0.095 µg g^{-1} was recorded in beans sampled from Kwahu Praso and Assin Praso while the least value was detected in beans from Duaso Ntoom in the Central Region. However, on the town to town averages, Nkawkaw recorded the highest mean level of Pb followed by Kwahu Praso with Kasapen recording the lowest average. The levels detected in this study are, however, below the Codex Alimentaruis'Maximum level of 0.10 µg g^{-1} in fruits and vegetables and 1.00 µg/g maximum permissible levels for cocoa powder and cocoa mass. The levels reported here were meanwhile comparable to that of Rankin et al (2005). Rankin *et al* (2005) reported 0.005 µg g^{-1} in cocoa nibs from Nigeria using ICP–MS for measurement. Moreover, the levels were higher, but very close to the maximum level detected by Nartey *et al* (2012) who reported 0.07µg g^{-1} in cocoa nib from Wassa Akropong in the Western Region. Lead is one of the most toxic metals affecting almost all organs in the body (Goyer *et al.*, 2004; Smith 1984). Increase in lead concentrations in soils and subsequently in food may be due to the many application of the metal in paints, car batteries and in making antiknocking agent in fuel. This, many believe have a high possibility of ending up in the soil from deposits of exhaust fumes and other activities. Though the use of leaded fuel has been discontinued in Ghana, the metal is not biodegradable and may persist in the environment fora long time. Though cocoa beans as raw materials for the manufacture of cocoa products may contribute to the elevated levels of lead in cocoa products, the greatest percentage of lead contamination of cocoa products may occur during processing of beans due to various industrial processes that may require the use of metal parts some of which may contain trace amounts of the metal.

A mean cadmium concentration 0.052µg g^{-1} was obtained for all the samples with a range of 0.005 µg g^{-1} to 0.095 µg g^{-1}. The highest concentration 0.095 µg g^{-1} was detected in cocoa beans from Brakwa in the Central Region with Twifo Braso cocoa beans recording the least value. On the town to town averages, Brakwa recorded the highest level followed by Assin Foso with Twifo Praso recording the lowest average value. The values recorded in some of the communities were close to the maximum permissible levels of 0.100 µg g^{-1} in fruits, cocoa butter and chocolate but were far below the limit set by Codex in cocoa mass and cocoa powder of 1.0 µg/g (Rankin *et al*., 2005 and COPAL, 2004). Levels of cadmium obtained, however, compare favourably with the level set for plant parts at 0.l00 µg/g fresh weight. In 2006 the European Union on cocoa conference proposed for adoption, a level of 0.8 µg g^{-1}. It is believed that levels of cadmium in soils range from 0.010 µg g^{-1} to 7.000 µg g^{-1} (Sandstead, 1994). This metal is present in phosphate fertilizers in trace amounts and might be absorbed by plants grown with the use of these fertilizers (Clarkson, 1986; Flanders and Foulkes, 1996; IARC, 1993; Yost, 1979 and Cabrera, 1993). An important source of the element is the use of pesticides. In cocoa production pesticide spraying is done throughout the year to fight disease causing pests. This may lead to accumulation of the metal by the plant leading to toxicity. It is believed; however, that it accumulates more in the leaves than seeds (Solomons, 1998; Prasad, 1995).The seriousness of cadmium toxicity is that it accumulates in all levels of the food chain and concentrates in the liver and kidneys causing disorders. The pathway of cadmium toxicity is through consumption of contaminated food. However, the levels of cadmium in cocoa beans analyzed in this work may not pose any significant health hazard due to relatively low concentrations. The levels are in agreement with cadmium levels in cocoa reported by Mounicou *et al* (2003).

A mean copper level of 33.45 µg g^{-1} was detected in the samples with the least mean value of 10.47 µg g^{-1}detected in beans from Asamankese in the Eastern Region. The highest value of 55.17 µg g^{-1} was detected in the beans from Sefwi Wiawso in the Western Region. Generally, levels of copper detected in beans from Ashanti and Western Regions were higher than those from Eastern and Central Regions. Indeed, while the concentrations from Ashanti and Western Region beans were in a range of 46.47 µg $g^{-1}$to 55.17 µg g^{-1}, those from Eastern and Central Regions ranged from0.74 µg g^{-1} to 24.62 µg g^{-1}.The general higher concentrations of Cu in cocoa beans from Ashanti and Western Regions could possibly be linked to the abundance of vegetations in the two regions with the associated soils rich in minerals like Cu. Moreover, Ashanti and Western Regions have more cocoa farms per hectare of land than those in Eastern and Central Regions. These cocoa farms are sprayed with pesticides such as kocide 2000WP and fungikill 50WP to control pest infections. Meanwhile kocide 2000 WP and fungikill 50 WP contain copper hydroxide and 35 % copper respectively as active ingredients. Again the ever increasing mining activities in the two regions could also contribute to the higher concentrations of the metal. The United States Food and Nutrition Board (US FNB) has set the upper limit of copper at 10 mg day^{-1} whiles Linus Pauling Institute suggests 900.0 µg day^{-1} as the recommended dietary allowance (RDA) levels for adults. The values observed were far lower than the RDA value of 900.0 µg day^{-1} and the 10 mg day^{-1} set by US Food and

Nutrition Board (FNB, 2001). This shows that the level of copper in the samples cannot pose any health risk to humans. The metal is one of the essential minerals in foods.Irrespective of their concentration in the soil, the uptake of most heavy metals such as copper by plants is dependent on a number of factors such as pH, organic matter content and state of the metal. The uptake of copper for instance is improved under acidic conditions and the presence of high organic matter content of the soil (Tang *et al*., 1999). In spite of the fact that copper occurs naturally in all plants and essential fora number of biological processes in the body such as haemoglobin formation, drug and carbohydrate metabolism and antioxidant defense mechanisms, at excessive levels, however,toxicity may result. Mining activities and the application of phosphate fertilizers account for two of the main sources of release of copper into the soil and in the plant.

The mean concentration of 38.08 μg g^{-1} and a range of 0.50 μg g^{-1} to 72.36 μg g^{-1} for iron were detected in the samples. The least value was recorded in Akim Akroso in the Birim South District of the Eastern Region while the highest value of 72.36 μg g^{-1} was recorded at Obuasi in the Ashanti Region. On town to town averages, Obuasi recorded the highest average concentration followed by Kasapen with Akim Akroso registering the least average iron concentration. The levels of the metal detected in the samples compare very well with those reported by Narteye*t al* (2012), particularly samples from Eastern and Central Regions.It is believed that acute toxicity of the metal can occur at 20-60 μg kg^{-1} body weight. Table 7 shows the recommended iron dietary allowance for humans at different age groups. Comparing the levels detected with these established levels, it can be said that values obtained are below the recommended dietary allowance(RDA) values (US FNB, 2001).

The levels of manganese obtained in this work ranged from 4.45 μg g^{-1} in samples obtained from Asamankese COCOBOD farms in the Eastern Region to a high value of 72.64 μg g^{-1} in a sample from Mampong in Ashanti Region. Considering average concentrations, Kasapen (64.65 μg g^{-1}) registered the highest, followed by Enchi (56.19 μg g^{-1}) with Asamankese registering the least value of 7.12 μg g^{-1}. On the whole, average concentrations recorded in Western and Ashanti Regions were higher than those from Eastern and Central Regions. Overdose of manganese is problematic though the mineral plays important roles in humans' health. Whiles the US EPA recommends 0.05 mg g^{-1} as the maximum allowable limit, US FNB sets the upper limits (ULs) for manganese at 10 mg day^{-1} for people at age 19 years and above whiles 9 mg day^{-1} is set for those between ages 14–18 years. The requirement for 4–8 years old has been set at 6 mg/day. Again the reference daily allowance (RDA) for manganese has been pegged at 1.8 -2.3 mg day^{-1} for adults at 19 and above years in foods, water and supplements. The good news is that the levels detected in the present study fall below standards set by international statutory bodies. This therefore points to the fact that cocoa beans produced by these communities do not pose any health risk and are safe for consumption with regard to manganese content.

A mean value 43.66 μg g^{-1}, was detected for zinc with a range of 24.05 μg g^{-1}recorded at Asamankese in the Eastern Region and high value of 68.25 μg $g^{-1}$was recorded at Begoro in Eastern Region. The results have revealed that the average concentrations of zinc in cocoa beans from Ashanti Region were the highest followed by those from Western Region. Indeed, cocoa beans from the Eastern and Central Regions recorded the least average concentration of zinc. The abundance in vegetations with associated soils rich in minerals in Ashanti and Western Regions could have accounted for the high Zn levels in beans from the two regions. The recommended dietary allowance for zinc has been pegged at 12 – 15 mg day^{-1} and the upper limit (UL) is set at 40 mg day^{-1} for all foods, water and supplements. However, the levels detected once again were far lower than the RDA values set for the metal zinc suggesting that there is no risk of contamination of the cocoa beans investigated with respect to zinc.

Table 3 Summary of statistical analysis of levels of heavy metals (µg g^{-1} dry weight) in cocoa bean from Western Region

M	Kasapen mean±SD range	Enchi mean±SD range	Asempaneye mean±SD range	Sefwi Wiaso mean±SD range	Begoso mean±SD range	Pvalue
Pb	0.013±0.004 0.011-0.015	0.015±0.003 0.013-0.019	0.018±0.002 0.012-0.021	0.025±0.013 0.013-0.061	0.030±0.002 0.017-0.057	0.63
Cd	0.045±0.001 0.025-0.063	0.065±0.002 0.045-0.085	0.045±0.001 0.025-0.063	0.066±0.003 0.050-0.078	0.050±0.003 0.029-0.080	0.87
Fe	53.11±0.04 39.82-56.35	44.28±0.03 38.83-55.32	43.80±0.023 5.55-49.42	49.15±0.044 0.65-48.68	47.23±0.030 42.27-55.45	0.96
Zn	50.67±0.034 39.38-67.15	43.94±0.03 36.18-48.79	43.04±0.04 39.16-46.80	51.73±0.05 41.26-63.86	52.06±0.040 41.68-68.25	0.85
Mn	64.65±0.03 41.83-67.73	56.19±0.02 42.16-68.57	54.33±0.03 42.25-58.25	52.38±0.034 0.64-64.17	48.36±0.040 38.16-69.57	0.99
Cu	51.19±0.05 48.18-56.00	46.47±0.05 43.50-50.38	52..31±0.03 41.34-65.25	55.17±0.034 4.24-67.85	54.75±0.030 41.28-69.85	0.97

SD = standard deviation, P = significance, M= Metals

Table 4 Summary of analysis of levels of heavy metals (µg g^{-1} dry weight) in cocoa beans from Ashanti Region

M	Juaso mean±SD range	Tepa mean±SD range	Bekwai mean±SD range	Obuasi mean±SD range	Mampong mean±SD range	Pvalue
Pb	0.020±0.001 0.012-0.032	0.018±0.001 0.012-0.034	0.016±0.001. 0.011-0.023	0.017±0.001 0.012-0.022	0.014±0.001 0.012-0.017	0.75
Cd	0.058±0.003 0.042-0.078	0.059±0.001 0.024-0.085	0.050±0.003 0.041-0.064	0.065±0.002 0.057-0.075	0.051±0.003 0.042-0.060	0.84
Fe	54.24±0.08 43.81-68.16	53.41±0.210 39.27-71.26	50.23±0.050 39.78-69.80	63.87±0.070 48.86-72.36	51.42±0.070 43.40-63.48	0.92
Zn	55.74±0.04 48.86-62.15	53.02±0.030 44.91-61.16	58.71±0.030 44.44-67.14	57.47±0.030 44.93-66.24	57.49±0.030 44.93-66.24	0.76
Mn	47.15±0.04 37.20-61.17	57.21±0.030 41.25-71.15	54.76±0.030 43.27-63.82	56.63±0.030 39.22-66.73	57.34±0.034 4.36-72.64	0.99
Cu	54.17±0.10 43.49-61.29	48.31±0.08 41.48-48.38	47.43±0.070 38.55-63.65	47.86±0.060 36.26-62.78	47.71±0.050 38.94-61.35	0.92

SD = standard deviation, P = significance, M= Metals

Table 5 Summary of analysis of heavy metals (µg g^{-1}dry weight) in cocoa bean from Eastern Region

M	Asamankese mean±SD range	Akim Akroso mean±SD range	Nkawkaw mean±SD range	Kwahu Praso mean±SD range	Adukrom mean±SD range	Pvalue
Pb	0.042±0.001 0.007-0.091	0.026±0.002 0.009-0.064	0.070±0.01 0.06-0.085	0.056±0.001 0.019-0.095	0.041±0.09 0.036-.048	0.96
Cd	0.047±0.009 0.015-0.085	0.041±0.010 0.020-0.090	0.049±0.004 0.01-0.09	0.033±0.003 0.015-0.085	0.047±0.001 0.020-0.070	1.00
Fe	25.86±0.069 13.70-48.70	10.91±0.092 0.50-16.06	35.07±0.15 4.85-57.30	33.70±0.11 13.25-57.30	27.32±0.12 5.85-41.90	0.86
Zn	36.94±0.163 24.05-49.60	33.19±0.22 26.90-37.15	37.74±0.14 36.20-39.50	39.99±0.13 34.15-50.75	32.93±0.12 25.70-36.65	0.83
Mn	7.12±0.097 4.45-9.20	11.45±0.066 9.10-13.30	9.80±0.086 6.80-16.80	18.24±0.12 6.80-52.35	8.93±0.13 6.65-14.50	0.63
Cu	10.74±0.079 5.25-22.70	11.64±.0.06 6.45-16.80	13.64±0.55 7.50-8.30	15.42±0.06 9.20-21.10	17.78±0.27 12.25-21.95	0.81

SD = standard deviation, P = significance, M= Metals

Table 6 Summary of analysis of heavy metals (μg g^{-1} dry weight) in cocoa beans from Central Region

M	Duaso Ntoom mean±SD range	Assin Praso mean±SD range	Twifo Praso mean±SD range	Brakwa mean±SD range	Agona Swedru mean±SD range	Pvalue
Pb	0.041±0.00 0.003-0.07	0.05±0.00 8.55-18.30	0.037±0.00 0.002-0.068	0.021±0.00 0.002-0.045	0.045±0.00 0.004-0.093	0.96
Cd	0.035±0.03 0.02-0.05	0.074±0.02 0.065-0.090	0.032±0.013 0.005-0.065	0.067±0.008 0.025-0.095	0.053±0.09 0.005-0.06	1.00
Fe	19.11±0.28 10.58-29.70	14.35±0.26 3.30-23.55	22.89±0.12 3.00-41.55	25.06±0.18 17.75-38.30	30.70±0.33 22.00-43.70	0.86
Zn	30.00±0.15 26.05-34.00	29.62±0.24 27.55-32.28	37.79±0.16 29.90-34.30	34.21±0.27 30.70-38.15	37.39±0.16 29.40-57.90	0.81
Mn	13.03±0.26 8.55-18.30	11.96±0.17 11.15-13.60	9.36±0.20 6.95-13.95	8.77±0.25 7.50-9.50	8.67±0.22 5.40-13.55	0.83
Cu	14.74±0.13 11.50-18.35	20.11±0.29 14.25-30.10	16.27±0.13 13.85-21.75	18.69±0.032 15.25-21.90	24.62±0.07 17.35-41.95	0.63

SD = standard deviation, P = significance, M= Metals

Table 7Age groups and recommended dietary allowance (RDA) of iron (mg day^{-1}) for humans

Humans	Age group	RDA[1]
Infant (male/female)	0-6 months	0.27
Infant (male/female)	7-12 months	1.0
Children (male/female)	9-13 years	7.0 -8.0
Adult (female)	14-18 years	15.0
Adult (female)	19-50 years	18.0
Expectant mother	18years	27.0
Nursing mother	≤ 18 years	10.0
Nursing mother	> 18 years	9.0

[1] US FNB, 2001. Levels of Fe in cocoa beans ranged from 0.50 μg g^{-1} to 72.36 μg g^{-1}.

3.3 Comparison of mean concentrations of the metals in the cocoa beans from the four Regions

Fig. 2 compares the mean concentrations of the studied metals in the cocoa beans from the four Regions. Obviously, concentrations of Pb and Cd in the beans were insignificant compared to those of Fe, Cu, Zn and Mn. Cadmium concentrations in the samples were quite the same particularly, those from Western, Ashanti and Central Regions. Lead was the less significant metal in the cocoa beans with Ashanti and Western Regions recording the lowest concentrations. It is clear from the Fig 2 that the metals, Fe, Cu, Zn and Mn were the dominant metals. The four metals were, however, more prominent in samples from Ashanti and Western Regions than in samples from Eastern and Central. Indeed, cocoa beans from Ashanti registered the highest mean concentrations for Fe and Zn, while the beans from Western Region recorded the highest mean concentration for copper. However, concentrations of Mn in beans from Ashanti and Western Regions were almost the same. Meanwhile, mean concentrations for the four metals in the beans from Eastern and Central Regions were comparable but relatively lower as shown in Fig2.

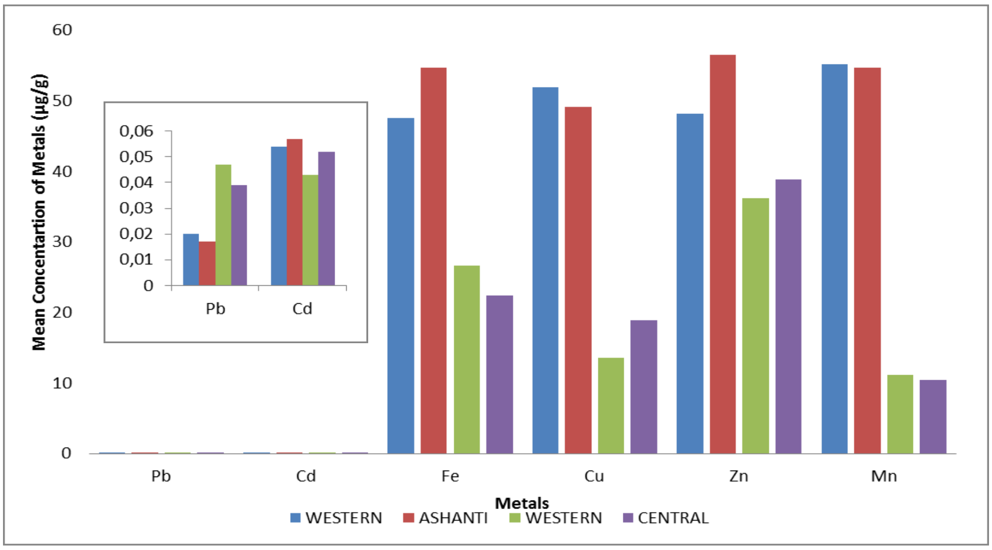

Fig.2 Distribution pattern of the metals in the cocoa beans.

4. CONCLUSION

The study revealed the presence of the heavy metals atvarying levels with lead and cadmium generally detected at lower concentrations in the cocoa beans. In all the samples the levels of the other four metals Cu, Fe, Zn and Mn were far higher than those for lead and cadmium. The general high concentrations of Fe, Zn, Cu and Mn in the samples were anticipated as they are regarded essential in living organisms. Analyses of the data by variance showed that the difference in concentrations are statistically insignificant ($P>0.05$). This suggests that the metals might have reached the study areas through the same sources.The levels of the metals detected in this study are, however, below the Codex Alimentaruis Maximum levels and tolerable upper limits set by reputable institutions such as US EPA, US FNB and suggested RDA by Linus Pauling Institute suggesting that their presence may not pose health hazard.

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