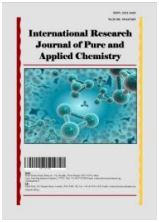

International Research Journal of Pure & Applied Chemistry

21(14): 36-46, 2020; Article no.IRJPAC.60362
ISSN: 2231-3443, NLM ID: 101647669

Assessment of Heavy Metals and Proximate Analysis of Cocoa Beans from Selected Cocoa Growing Areas in Ghana

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Authors' contributions

This work was carried out in collaboration among all authors. Field work and laboratory analysis of this study were carried out by authors WAN and DA. The project was designed and supervised by author JAMA while author SA prepared and submitted the manuscript for publication. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IRJPAC/2020/v21i1430245
Editor(s):
(1) Dr. Hao-Yang Wang, Shanghai Institute of Organic Chemistry, China.
Reviewers:
(1) Christopher Tyosue Kpega, Ahmadu Bello University Zari, Nigeria.
(2) Fayçel Ammari, Carthage University, Tunisia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/60362

Original Research Article

Received 15 June 2020
Accepted 20 August 2020
Published 03 September 2020

ABSTRACT

The levels of six selected heavy metals namely: lead (Pb), cadmium (Cd), copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn) have been investigated in cocoa beans sampled from Bekwai, Juaso, Kaspen, Asampaneye and Asamakese, five major cocoa-growing communities in Ghana. Aqua regia digestion procedure was applied for sample digestion and atomic absorption spectroscopy was used for the determination of the metals. The method for the determination of the metals was validated by analyzing standard reference material and levels of metals obtained compared favourably with reported values. Samples were also subjected to proximate analysis by determining moisture, ash, fibre, fat, protein and carbohydrate nutritional compositions of the beans. Method of proximate analysis as reported by Association of analytical chemists (AOAC) was used for estimation of nutritional compositions. Indeed, lead and cadmium concentrations were the lowest in all the samples. The generally high concentrations of Fe, Zn, Cu and Mn in the samples were

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anticipated as they are regarded essential in living organisms. The mean concentrations expressed in µg/g ranged from 0.013 to 0.042 for Pb, 25.36 to 54.24 for Cd, 36.94 to 58.71 for Zn, 7.12 to 64.65 for Mn and 10.14 to 54.17 for Cu. Proximate analysis of the cocoa beans showed carbohydrate and fat as the nutrients with the dominant composition. Correlation analysis of the metals and nutritional compositions indicated no clear relationship between the levels of the metals and the nutrients.

Keywords: Heavy metal; proximate analysis; atomic absorption spectroscopy; cocoa beans; nutrition.

1. INTRODUCTION

Cocoa has been the main stay of the economies of many West African countries including La Cote d' Ivoire, Ghana, Nigeria and Cameroon [1]. In Ghana it is believed that the cocoa sub-sector employs over eight hundred thousand (800,000) farmers and contributes about 70 – 100% of the incomes of these cocoa farmers [1]. The sub-sector again is considered as being the backbone of many agrochemical companies, input distributors, licensed cocoa buying companies [2,3]. Cocoa contributed 22.4% (463 million US$) of the total foreign exchange earning of Ghana and contributed 63% of the foreign export earnings from agricultural sector in 2002 [2]. A report published in the African bulletin magazine on Thursday 2nd September, 2010 quotes Dr. Frank Amoah as having said that cocoa engages over one million farmers, giving 17 to 20 percent of income to small scale farmers. He was also reported to have said that the sub-sector contributes about 5.5% of Ghana's Gross Domestic Product (GDP). At the same conference to adopt a common strategy to review the position of Ghana and La Cote d' Ivoire in the market with their 65% share in the world production of cocoa, Dr. Amoah was reported again to have said that since 2003, the sub-sector has grown over 30% and represented 37% of foreign currency earnings of the two country's national budgets in 2004 [4].

Undeniably, cocoa from Ghana is revered to be of the best quality with high demand in the world market. Due to these quality characteristics of Ghana's cocoa, they are mostly used as reference standard for cocoa produced from other parts of the world [5]. 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 [6]. 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. Anthropogenic activities such as mining and smelting of metal ores, industrial emissions and application of insecticides and fertilizers could contribute to elevated levels of heavy metals in the environment [7]. The threat that heavy metals pose to human and animal health is compounded by their long-term persistence in the environment. Additionally, the consumption of heavy metal-contaminated food can seriously deplete some essential nutrients in the body causing a decrease in immunological defences, intrauterine growth retardation, impaired psycho-social behaviour, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer [8]. Mechanism of metal toxicity include complexation of heavy metals with proteins to form complexes, in which carboxylic acid (–COOH), amine ($–NH_2$), and thiol (–SH) groups are involved. Such complexation modified biological molecules and lose their ability to function properly which result in the malfunction or death of the cells [9]. When metals bind to these groups, they inactivate important enzyme systems or affect protein structure, which is linked to the catalytic properties of enzymes. This type of toxin may also cause the formation of radicals which are dangerous chemicals that cause the oxidation of biological molecules [10].Toxicological effects of heavy metals such as lead on human beings include inhibition of haemoglobin formation, sterility, hypertension and mental retardation in children [11], 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 200 mg/kg fresh weight [12]. 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 [13, 14]. The high demand of Ghana's cocoa beans at the world market and worldwide patronage of cocoa products makes it imperative to analyze the level of contaminants (heavy metals) so as to determine if the levels conform to the international standards.

Cocoa products are considered to be one of the most widely consumed foods worldwide. Cocoa beans are the raw material from which the widely patronized products like chocolate, candies, cocoa powder and beverages are produced. However, there is little literature on the nutritional values of the basic nutrients such as proteins, carbohydrates, fats and other related nutrients in cocoa beans. Due to this, nutritionists may find it difficult to have enough answers to why, how, when and the conditions under which cocoa beans and its products should be taken. It is therefore, worthwhile to subject cocoa beans to proximate analysis to obtain nutritional knowledge on status of Ghana's cocoa beans. Analysis of foods and feeding stuffs for nitrogen (for protein), ether extract (for fat), crude fibre and ash (mineral salts), together with soluble carbohydrate calculated by difference is referred to as proximate analysis. Although proximates do not give the entire nutritional assay, they are an inexpensive way to track deviations from the quality of foods. Knowledge of the nutritional standing of cocoa beans will serve as grounds for nutritional and health advice.

2. MATERIALS AND METHODS

2.1 The Study Area

Ghana is situated on the west coast of Africa about 750km north of the equator between latitude 4° and 11.5° N and longitude 3.11° 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, and Eastern were used for this work. Fig. 1 is map of Ghana showing the towns in the three Regions where the cocoa beans used for the analyses were collected.

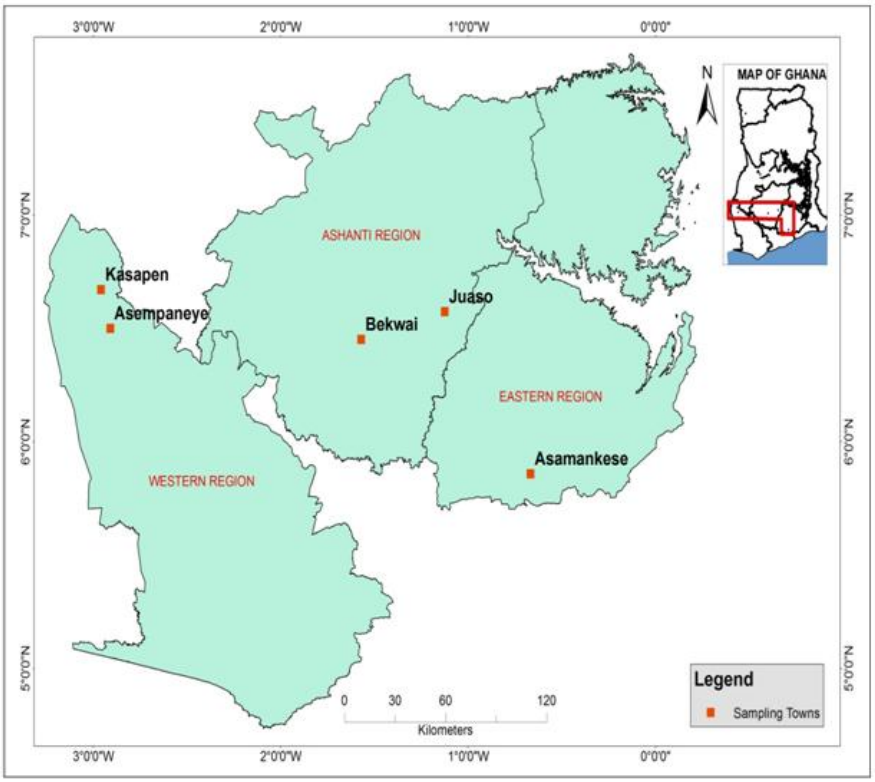

Fig. 1. Portions of map of Ghana showing the sampling communities

2.2 Collection of Samples

Samples of dried cocoa beans were obtained from cocoa farmers from Bekwai, Juaso, kasapen, Asempaneye and Asamankese. In each community samples were collected from six farmers. The farmers were selected in such a way as to cover the geographical area of the community 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. In each community dried samples of cocoa beans were obtained from the 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) both obtained from Merck, Germany. De-ionized water was obtained from the Department of Chemistry, KNUST and was used for all the analytical work.

2.4 Sample Preparation and Pre-Treatment

The dried cocoa beans samples obtained were subjected to further treatment. In handling the cocoa beans, gloves were worn to avert external contamination which would affect the analyses. Care was taken to also ensure that water and other reagents did not come into contact with the beans before subjecting them to milling, digestion and subsequently, analyses by the use of Flame Atomic Absorption Spectrometer.

2.5 Digestion of Samples

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 (1 g) of the milled cocoa sample was digested using 30 ml of the aqua regia in a pre-cleaned teflon cup by heating on a hot plate at 200°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 AAS analysis. All the samples were subjected to this procedure and blanks were prepared in a similar manner.

2.6 Atomic Absorption Spectrometer Analysis

The metallic elements were determined by Atomic Absorption Spectrometry (AAS) technique AAS model PU9200X. Solutions of the digested cocoa samples prepared by acid digestion were aspirated directly using the aspiration tube with air acetylene flame into the AAS. Standard metal solutions were used for calibration. Solutions of the standard reference material (SRM) prepared by the same acid digestion method were analyzed by the AAS for method validation.

2.7 Proximate Analysis of the Cocoa Beans

The proximate compositions in cocoa beans determined in this work are moisture, ash, fat, protein, crude fiber and carbohydrates. These components are fundamental to the assessment of the nutritive quality of food items. Samples were subjected to replicate determinations.

2.7.1 Determination of moisture

Five (5) grams of sample were transferred to previously dried and weighed dish. The dish was placed in an oven thermostatically controlled at 105°C for 5 hours. The dish was removed and placed in a desiccator to cool. The cooled sample was weighed. Heating and cooling was repeated until constant weight was attained. The weight loss incurred is determined quantitatively as the moisture content and percentage loss of weight was calculated as:

$$\% \text{ Moisture} = \frac{\text{loss of weight}}{\text{Weight of sample}} \text{x } 100$$

2.7.2 Determination of crude fat

About 2 g of sample was taken and placed in an extracted thimble and subjected to soxhlet extraction technique [15]. Two hundred millitres (200 ml) of petroleum ether as extracting solvent was placed in previously weighed 250 ml round bottom flask. Extraction was performed for about eight hours after which the thimbles and its content was removed and the solvent salvaged by distillation. The flask and its content were left

overnight in an oven at a low temperature of 30°C to completely evaporate the solvent leaving the oil. It was weighed and percentage crude fat calculated as:

$$\% \text{ Crude fat} = \frac{\text{weight of fat}}{\text{Weight of sample}} \text{x } 100$$

2.7.3 Determination of crude fibre

About 2 g of the sample was defatted using soxhlet extraction technique of AOAC (2002) [16]. The defatted sample was put in conical flask after washing and drying. About 0.5 g of asbestos was added to the sample in a 750 ml Erlenmeyer flask. About 200 ml of boiling 1.25 % (H_2SO_4) was added to the sample and heated on a hot plate to return the solution immediately to boiling. Care was taken to ensure that the sample did not stick to the walls of the flask. The flask was removed after 30 minutes and the content was immediately filtered on linen cloth and washed with distilled water until washings were no longer acidic with pH of about 6.8. The sample was returned to boiling as above but this time using 1.25% NaOH. The residue after treatment with NaOH was washed with approximately 15 ml alcohol and transferred into a dried crucible. It was then put in an oven at 105°C for one hour, cooled, weighed, and ashed for 30 minutes at 600°C. The sample was then cooled in a desiccator and reweighed. The loss in weight represents the content of fibre, computed as:

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

2.7.4 Determination of ash

A crucible was fire polished, cooled and then weighed. About 2 g of sample was weighed with the crucible and burnt in a muffle furnace at 600°C for two hours to ensure complete ashing. This was removed, cooled in a desicator and reweighed. This step was repeated until a constant weight was obtained. Percentage ash content was calculated as follows:

$$\% \text{ Ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \text{x } 100$$

2.7.5 Determination of protein

Protein determination was done using Kjedahl method [17]. About 2 g of the sample was taken and heated in 25 ml concentrated H_2SO_4 in the presence of a selenium catalyst. The sample solution was transferred into 100 ml volumetric flask and made to the mark. Five millilitres (5 ml) of the sample solution was pipetted into kjeldahl distillation flask, and 5 ml of 40 % sodium hydroxide added. This was distilled and the distillate collected into 20 ml of 2 % boric acid to which has been added 2 drops of mixed indicator solution. The distillate was then titrated with 0.01 M HCl from light green to a colourless. Duplicate analysis and blank determination were carried out. Percentage total nitrogen in the sample was calculated as follows:

$$\% \text{ total N} = \frac{100 \text{ x Va} - \text{Vb x Ma x } 0.01401 \text{ x} 100}{\text{W x } 100}$$

Va = volume of standard acid used in titration,
Vb = volume of standard acid used in blank titration,
Ma= Molarity of HCl, W = weight of sample.

$$\% \text{ Protein} = \% \text{ total nitrogen x F}$$

where F = 6.25

2.7.6 Determination of total carbohydrate

The total percentage carbohydrate content was determined using the method of difference as reported by the AOAC (2002) [16]. The percentage protein, crude fat, moisture and ash content of the sample were added and subtracted from hundred percent. The value obtained is the percentage carbohydrate content.

2.8 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. Accuracy of the AAS method was evaluated through the analysis of two reference materials; NIST 1547 SRM certified Peach Leaves and international atomic energy agency (IAEA), IAEA V-10 SRM certified Hay Powder. Proximate analyses were duplicated.

3. RESULTS AND DISCUSSION

3.1 Analysis of Standard Reference Materials

Table 1 present the results of analysis of the IAEA V-10 certified reference materials. Values of heavy metals measured for this work compared with reported values as shown in the Table. Within the limit of experimental errors, accuracy reported as percentage relative error and precision reported as percentage relative deviation ranged from -5.32% to 3.13% and 0.32% to 6.12% respectively.

3.2 Levels of the Heavy Metals in the Cocoa Beans

Table 2 presents the mean concentrations, the standard deviations and range showing the minimum and maximum concentrations of the metals in the cocoa beans. The data showed significantly varying levels of heavy metals in the cocoa beans analyzed from the five communities. In all the samples the levels of the four metals, Fe, Mn, Zn and Cu were far higher than those for lead and cadmium. Indeed, lead and cadmium concentrations were the lowest in all the samples. The generally high concentrations of Fe, Zn, Cu and Mn in the samples is not surprise at all as these elements are important in living systems. Variance investigation of the results indicated that differences in concentrations are statistically insignificant ($P>0.05$) as shown in Table 2. Thus, the metals may have entered the sampling sites through the same sources. Some potential sources of the metal could be linked to the use of pesticide products such as kocide 2000 WP and fungikill 50WP to control pest infections by cocoa farmers. The pesticides, kocide 2000 WP and fungikill 50 WP contain copper hydroxide and 35 % copper as active ingredients respectively. .Application of these pesticide products is therefore, potential sources of copper to the environment. Cadmium and other trace metals are present in phosphate fertilizers in trace amounts and might be absorbed by plants grown with the use of such fertilizers [18,19]. Again, the ever increasing mining activities in the cocoa growing areas in Ghana could also contribute to the presence of the metals in the beans.

3.3 Distribution of the Metals in the Cocoa Beans

Fig. 2 shows the percentage distribution of the Pb and Cd in the beans while Fig. 3 also presents distribution for Fe, Zn, Mn and Cu. Pb and Cd were considered separately from the others four because of their toxicity to humans and wildlife. The other four metals were also put in one group as they are regarded as essential to living organisms.

3.3.1 Lead and Cadmium

With regard to the toxic metals studied, Cd concentrations were higher compared to those for Pb as shown in Fig 2. Indeed, the concentrations of cadmium in the beans were in some cases more than three times those of lead except in beans from Asamankese where the two metals have almost the same concentrations. The concentrations of Cd ranged from 0.045 µg/g to 0.058 µg/g while those for Pb were from 0.013 µg/g to 0.042 µg/g. The highest concentrations of Pb, 0.042 µg/g was recorded in beans obtained from Asamankese while the least value was detected in beans from Kasapen in the Western region. Meanwhile, the highest Cd level of 0.058 µg/g was registered in beans from Juaso while the lowest concentration level was obtained in beans sampled from kasapen and Asampaneye, both in the Western region and Asamankese in the Eastern region. The levels of lead and cadmium detected in this study are, however, below the Codex Alimentaruis'maximum level of 0.10 of µg/g in fruits and vegetables and 1.00 µg/g maximum permissible levels for cocoa powder and cocoa mass [20,21].

3.3.2 Zinc, Manganese, Iron and Copper.

The distribution of the four essential metals presented in Fig. 3 generally did not follow any particular pattern. However, Zn and Mn; Fe and Zn; Mn; Mn and Cu; Zn and Fe were the dominant metals in beans from Bekwai, Juaso, Kasapen, Asempaneye and Asamankese respectively. The concentrations of the metals were 47.45 µg/g to 58.71 µg/g, 47.15 µg/g to 55.74 µg/g, 50.67 µg/g to 64.65 µg/g, 43.04 µg/g to 54.33 µg/g and 7.12 µg/g to 36.94 µg/g in beans from Bekwai, Juaso, Kasapen, Asempaneye and Asaamankese respectively. Clearly, the concentrations of the four essential metals were comparatively lower in beans from Asamankese. Indeed, the lowest metal concentration (7.12µg/g) for the four metals was recorded for manganese in beans from Asamankese. The highest recorded metal concentration (64.65µg/g) was registered in beans from Kasapen in the Western region. The

results also showed that the concentrations of the four metals in the beans from the study areas were comparable except those from Asamankese, particularly, concentrations in beans from Bekwai, Juaso in Ashanti region and Kaspen in Western region. The abundance in vegetation with associated soils rich in minerals in Ashanti and Western regions could have accounted for the high levels of the four metals in beans from the two regions. Again, high mining activities in the two regions could also account for elevated metal concentrations.

The recommended dietary allowance for Zn has been pegged at 12 – 15 mg/day for all foods, water and supplements [22]. For Mn the US EPA recommends 0.05 mg/g as maximum allowable limit while, US FNB set the upper limit at 10 mg/day for people age at 19 years and above. It is believed that acute toxicity of Fe can occur at 20 – 60 µg/kg body weight [23]. In the case of Cu the United States of Food and Nutrition Board has set the upper limit of copper at 10 mg/day while Linus Pauling Institute suggests 900.00 µg/day as the recommended dietary allowance level for adults. Compared values observed or detected for the metals in the cocoa beans to international recommended values discussed above it can be adduced that the presence of the metals may not pose health hazard to humans.

3.4 Proximate Analysis

Results obtained for the proximate analysis of the beans are presented in Table 3. Margins of errors are standard deviations. Range of measurements showing the maximum and minimum values are also presented. The moisture content of the beans from the five study areas varied from 2.23 % to 4.46 % with an average value of 3.46 %. The highest value of 4.46 % was recorded for beans from Bekwai. Values obtained for the crude fat were from 45.57 % to 36.72 % averaging 41.72 % with cocoa beans from Bekwai registering the highest fat content. The average composition of crude fibre was found to be 1.37 % in a range of 1.02 % to 2.63 %. Beans from Asamankese recorded the maximum fibre composition, while those from Asampaneye had the lowest fibre composition. The results showed that fibre content in beans from the four other communities were almost same. Ash which is known to contain dietary minerals (sodium, potassium, iron, calcium), and vitamins (β-carotene, retinol, vitamin D_3, vitamin D_2, B Vitamins) was recorded with an average composition of 3.50 % in a range of 3.19 % to 3.72 %. The highest ash composition of 3.72 % was also registered in beans from Asamankese. An average protein content of 13.98 % was determined for the samples. Samples from Kasapen had the highest protein composition of 14.34 %. In proximate studies, carbohydrate calculated as percentage difference has ingredients such as dietary fibre, sugars and sugars alcohols. An average carbohydrate content of in the cocoa beans was 35.57 % with beans from Juaso recording the highest carbohydrate composition.

3.5 Relationship between Nutritional Contents and Levels of Heavy Metals in Cocoa Samples

Equations for linear regression plots between selected nutritional contents (carbohydrate and fat) and the levels of some heavy metals (lead, cadmium and iron) are presented in Table 4. Carbohydrate and fat compositions were chosen because they were relatively high in all the samples from all the regions. Lead and cadmium were also selected because they are the most toxic among the heavy metals studied and iron was considered for this analysis because of its nutritional importance to humans.

The results of the linear regression plot showed weak correlation between the selected metals and the nutrients with coefficient of correlation (R^2) ranging from 0.0351 to 0.5231. However, about 50 % correlation (R^2= 0.523) was obtained for fat compositions and levels for lead. Thus:

$$F = -282.08Pb + 47.869,$$

where F is % fat composition and Pb is level of lead in the samples.

Thus, from the regression equation, there is negative correlation between fat composition and levels of lead, suggesting that as the composition of fat increases, level of Pb to some extent decreases. Clearly, there is no obvious correlation between the nutrients and the metals.

Table 1. Analysis of IAEA V- 10 standard reference material for the heavy metals

Metal	Measured (µg/g)	Reported values (µg/g)	Accuracy (%)	Precision (%)
Pb	1.55±0.06	1.60±0.77	3.13	6.12
Fe	185.5±9.82	186.00±51.61	0.27	2.15
Zn	23.80±1.76	24.00±1.14	0.83	0.63
Mn	48.20±5.09	47.00±4.94	-2.55	0.32
Cu	9.90±0.78	9.40±0.63	-5.32	3.72

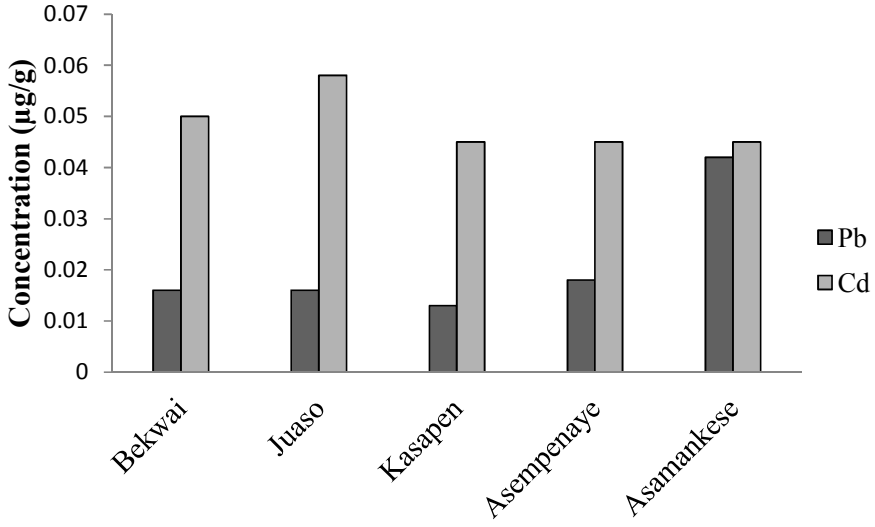

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Fig. 2. Distribution of lead and cadmium in the cocoa beans

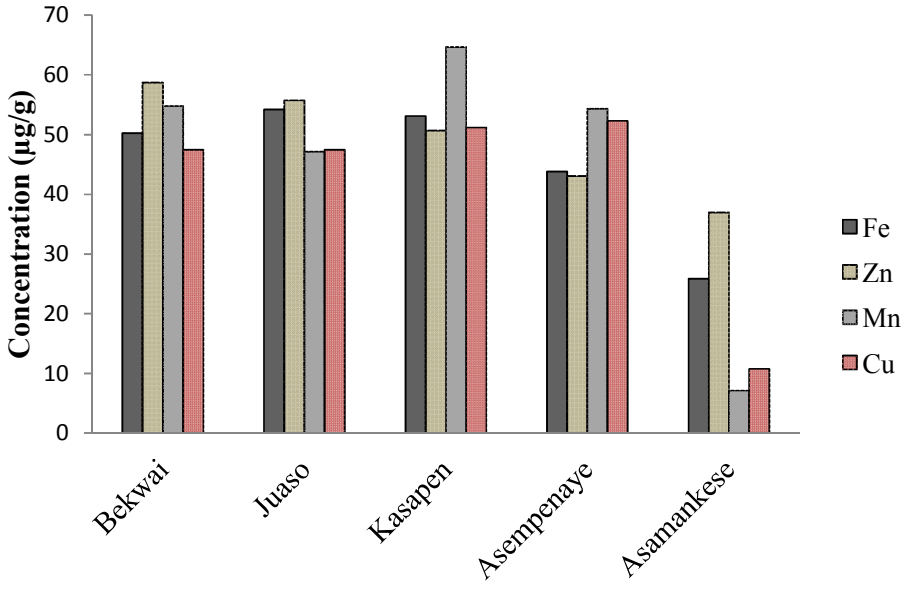

Fig. 3. Distribution of iron, zinc, manganese and copper in the cocoa beans

Table 2. Statistical analysis of levels of heavy metals (µg g^{-1}) in cocoa beans from selected cocoa growing communities

	Bekwai		Kasapen		Asempaneye		Juaso		Asamankese		
Metal	**mean±SD**	**range**	**mean±SD**	**range**	**mean±SD**	**range**	**mean±SD**	**range**	**mean±SD**	**range**	**Pvalue**
Pb	0.013±0.004	0.011-.015	0.016±0.001	0.011-0.023	0.018±0.002	0.012-0.021	0.020±0.001	0.012-0.032	0.042±0.001	0.007-0.091	>0.05
Cd	0.045±0.001	0.025-.063	0.050±0.003	0.041-0.064	0.045±0.001	0.025-0.063	0.058±0.003	0.042-0.078	0.045±0.009	0.015-0.085	>0.05
Fe	53.11±0.04	39.82-6.35	50.23±0.050	39.78-69.80	43.80±0.02	35.55-49.42	54.24±0.08	43.81-68.16	25.86±0.069	13.70-48.70	>0.05
Zn	50.67±0.034	39.38-7.15	58.71±0.030	44.44-67.14	43.04±0.04	39.16-46.80	55.74±0.04	48.86-62.15	36.94±0.163	24.05-49.60	>0.05
Mn	64.65±0.03	41.83-7.73	54.76±0.030	43.27-63.82	54.33±0.03	42.25-58.25	47.15±0.04	37.20-61.17	7.12±0.097	4.45-9.20	>0.05
Cu	51.19±0.05	48.18-6.00	47.43±0.070	38.55-63.65	52.31±0.03	41.34-65.25	54.17±0.10	43.49-61.29	10.74±0.079	5.25-22.70	>0.05

SD = standard deviation, P = significance

Table 3. Statistical data on percentage nutritional compositions of the cocoa beans

Nutrient	**Bekwai**		**Juaso**		**Kasapen**		**Asempaneye**		**Asamankese**	
	Mean±SD	**Range**	**Mean±SD**	**Range**	**Mean±SD**	**Range**	**Mean±SD**	**Range**	**Mean±SD**	**Range**
Moisture	4.46±0.09	4.39 - 4.52	2.23± 0.04	2.20-2.26	3.15± 0.16	3.10 - 3.32	4.39± 0.33	3.96 -4.44	3.06±0.82	2.58-5.14
Crude fat	45.57±0.38	45.30 -5.84	36.72±0.14	36.62-36.82	45.52±0.26	45.33-45.70	43.85±0.14	43.74-43.94	36.94±0.74	33.22-39.93
Crude fibre	1.09±0.01	1.08 -1.10	1.07±0.01	1.06 -1.08	1.03±0.01	1.02 - 1.04	1.02±0.01	1.01-1.02	2.63± 0.12	2.52-2.69
Ash	3.52±0.54	3.14 - 3.90	3.19±0.11	3.11 -3.27	3.40± 0.22	3.22 - 3.57	3.69± 0.38	3.41- 3.96	3.72±0.65	3.19-4.11
Protein	13.74±0.72	13.33 14.04	13.91±0.73	13.50-14.01	14.34±0.84	14.25-14.59	13.97±0.81	14.15 -14.47	13.95±0.87	13.19-14.42
Carbohydrate	31.62±0.35	30.60-32.76	42.88±0.21	2.56 -43.51	32.56±0.30	31.78 -33.08	32.08±0.33	32.17-38.73	39.30±0.64	33.71-45.30

% carbohydrate = 100% - (% moisture + % fat + % protein + % fibre + % ash)

Table 4. Relationship between nutrients composition and selected metals

Regression equation	Coefficient of correlation (R^2)
C=232.58Pb + 30.618	0.279
C=506.22Cd + 8.169	0.398
C= -0.0825Fe + 39.438	0.036
F = -282.08Pb + 47.869	0.523
F = -354.10Cd + 58.929	0.198
F = 0.172Fe + 33.911	0.197

C = % carbohydrate in the cocoa beans, F = % fat in the cocoa beans,
Pb = lead, Cd = Cadmium, Fe = iron

4. CONCLUSION

The investigation showed the presence of the six studied heavy metals in the cocoa beans with lead and cadmium generally detected at lower concentrations. The concentrations of the other four metals namely, Cu, Fe, Zn and Mn were far higher than those for lead and cadmium. This trend is not surprise at all as Fe, Zn, Cu and Mn are among the metals regarded essential in living organisms. 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 suggesting that their presence may not pose adverse health implications. Analysis of the metal concentrations by one way Anova showed that the difference in concentrations are statistically insignificant (P>0.05). Proximate analysis of the samples revealed that carbohydrates and fats constituting the dominant nutrition compositions of the beans. Correlation analysis showed that there is no clear relationship between the metal levels and nutrition compositions.

ACKNOWLEDGEMENTS

The authors would like to express appreciation to the Department of Chemistry, Kwame Nkrumah University of Science and Technology for supporting the work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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