ISSN 2278-2540 | DOI: 10.51583/IJLTEMAS | Volume XII, Issue VII, July 2023

Determination of Heavy Metals in Cocoa Products Consumed in Nairobi County Kenya

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DOI: https://doi.org/10.51583/IJLTEMAS.2023.12709

Received: 03 July 2023; Accepted: 10 August 2023; Published: 24 August 2023

Abstract: - Cocoa is an important cash crop obtained from the beans of the Theobroma cacao tree belonging to the family Malvaceae. Its products are rich in several classes of phenolic compounds such as Flavanols, procyanidins, and anthocyanins. These compounds are attributed to copious health benefits. In addition to the bioactive compounds, cocoa contains heavy metals whose origin and levels depend on natural and anthropogenic activities. Beyond permissible limits, these metals are linked to deleterious damage to several systems in the human body. This study aims to determine the concentration of some heavy metals in Cocoa products and compare the levels with the permissible exposure limits stipulated by international health agencies. This was achieved by wet digestion of the strategically sampled cocoa products and their analysis using the atomic absorption spectrometry technique. The average concentration of Lead (Pb), Copper (Cu), Zinc (Zn), and Manganese (Mn) in cocoa powder was 0.154±0.021, 0.270±0.037, 0.794±0.063, and 0.334±0.015 respectively. The average concentration of Pb, Cu, Zn, and Mn in cocoa butter was 0.329±0.008, 0.028±0.038, 0.116±0.075, and 0.124±0.008 respectively. The average concentration of Pb, Cu, Zn, and Mn in chocolate milk was 0.463±0.003, 0.042±0.066, 0.296±0.015, and 0.162±0.010 respectively. Cadmium levels were below the detection limit in all the samples. The results obtained in all the samples were within the WHO and KEBS permissible levels in food products.

Keywords: Cocoa, Bioactive compounds, Heavy metals, Permissible limit, Atomic Absorption Spectrometry, Below detection limits, World Health Organization (WHO), and Kenya Bureau of Statistics (KEBS).

I. Introduction

Cocoa is an important cash crop obtained from the beans of the Theobroma cacao tree belonging to the family Malvaceae. It is an economic product with a massive boost to the gross domestic product (GDP) of cocoa-producing countries [1]. This is because cocoa is considered the main exporter of several cocoa-producing companies in West and Central Africa and offers an ocean of employment opportunities. According to Belwal, T. *et al.*, 2022, in the year 2022 the annual production of cocoa was about 4.72 million tons of Cocoa seeds and by the year 2025, the cocoa market is expected to grow at a compound annual growth rate of 7.33%.

Cocoa is among the main ingredients used in the production of confectionery, chocolate, and cosmetics. Archeological studies carried out recently on ceramic artifacts suggest that cocoa beans were harvested and consumed more than 5500 years ago [3]. This crop was first developed as a crop in many ancient South American cultures with the Aztecs and Mayans beings the most well-known of these indigenous populations.

Figure 1: Image of Cocoa pods.

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The four primary varieties of cocoa trees are Criollo, Nacional, Forastero, and Trinitario [4]. The most common variety is Forastero, this is attributed to its high resistance to diseases and higher productivity. The rarest among the four varieties is Nacional. However, it is of the greatest economic value due to its refined taste, more aromatic, and less bitter compared to other varieties [5]. From this crop, products such as cocoa powder, chocolate liquor, cacao nibs, dark chocolate, milk chocolate, baking chocolate, criollo, and cocoa butter can be made. These products are made from chocolate liquor (a paste prepared from the cocoa seed, the fruits of *Theobroma cacao.*)

Alexander Von Humboldt described the cocoa bean as a small space concentrated with a wealth of valuable nourishments by nature. They are rich in several classes of phenolic compounds, some of which are Flavanols, procyanidins, and anthocyanins. Procyanin B1, Procyanin B2, (-)-Epicatechin, and (+)-catechin are the most abundant flavanol in cocoa powder) [6], methylxanthines (e.g., caffeine and theobromine), anthocyanins, flavones, phenols (Clovamide and deoxyclovamide), hydroxylated stilbene derivatives, and phenolic acids [7 – 9]

The bountiful compounds in Cocoa bean products generate tastes, aroma, and a plethora of health benefits that have spellbound humans for eons. These health benefits include; anti-inflammation [10], cardio-protection [11], anti-neurodegeneration [12], blood sugar and weight control, and healthy teeth and skin. The quantity of the compounds in the cocoa beans depends on their genotype and origin, post-harvest treatment of the beans, industrial processes, the class of the soil, and the age of the cacao tree [1, 13].

In addition to the bioactive compounds, cocoa contains elements such as magnesium, iron, Copper, Phosphorous, Zinc etcetera. These elements have bioaccumulated in plants through absorption (from the soil) and surface deposition (from the air). The aforementioned elements are categorized into essential elements, major elements, and heavy metals [14]. Their availability in the ecosystem is attributed to natural sources such as weathering processes and anthropogenic activities.

The term heavy metals refer to any metallic chemical element that has a density greater than 5g/ cm^3 and is toxic or poisonous at low concentration [15]. Examples of heavy metals are mercury (Hg), cadmium (Cd), chromium (Cr), and lead (Pb). Over the past few decades, their levels have skyrocketed due to rapid urbanization and industrialization, constant application of agrochemicals, and unreasonable mining and waste management [16]. With this high concentration of heavy metals in the soil and consequently, around the root zone of the plants, the plants' system absorbs the heavy metals alongside water and nutrients [17].

Heavy metals contamination is a notable issue that has created immense attention in the scientific community due to unreasonable anthropogenic activities carried out everywhere in the globe which are amassing them into the ecosystem [18 – 20]. Beyond the permissible limit, the consumption of heavy metals is associated with deleterious damage to several systems in the human body such as the skeleton, immune, endocrine, circulatory, nervous etcetera [21, 22].

Therefore, this study aims to determine the concentration of some heavy metals in Cocoa products and compare the levels with the permissible exposure limits stipulated by international health agencies. The atomic absorption spectroscopy technique was used in the study since it is known to determine the concentration of approximately 70 elements. The concentration of a particular element can be computed in the technique by taking into account the amount of light of a specific wavelength the gaseous atoms of the sample absorb based on Beer-Lambert law. Some of the metals whose concentration can be determined using this technique are Cadmium, Zinc, Copper, Manganese, Lead, Chromium, Mercury etcetera [23].

II. Statement of the problem

Heavy metals find their way into cocoa products and consequently into the human body through ingestion (drinking and eating). In the body, these metals result in the aforementioned copious health complications. Therefore, since there are no studies conducted in the Nairobi markets or supermarkets concerning the determination of heavy metals in cocoa products, there is an urgency to determine the levels of heavy metals in cocoa products. This will aid relevant organizations to put forward measures to reduce the hazards associated with the presence of heavy metals in toxic levels in cocoa products as much as possible.

III. Main Objective

The main aim of this project is to determine toxic heavy metals in cocoa products consumed in Nairobi County supermarkets.

3.1: Specific Objectives

i. To determine the concentration of heavy metals (lead, copper, cadmium, zinc, and manganese) in Chocolate milk, Cocoa butter, and Cocoa powder consumed in Nairobi County.
ii. To compare the measured values with the permissible exposure limits stipulated by international health agencies.

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3.2: Justification of The Study

Due to the exponential growth in industrialization and urbanization, there has been a dramatic increase in the levels of heavy metals accumulating in the environment. Beyond permissible limits set down by international health agencies, these elements pollute the environment and severely damage living organisms when they accumulate in their bodies. When ingested by human beings in foods such as cocoa products, they accumulate in the body with time resulting in a grave health setback [16]. Therefore, this is an alarm to public health that require urgency to determine heavy metal concentrations in cocoa products.

IV. Materials and Methods

4.1: Area of Study

Nairobi is the capital city of Kenya and a hub of business in Eastern Africa. It is located in the south-central part of Kenya lying about 199km south of the Equator and between latitude 1.2921°S and longitude 36.8219°E. Statistics from the 2019 census show that 4.397 million people were living in Nairobi [24].

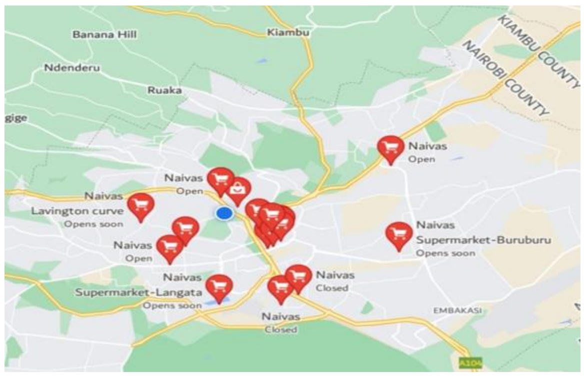

Figure 2: Description of the supermarkets from which samples were purchased.

Cocoa products are widely used in Nairobi County where over 50% of the population uses cocoa products because they are affordable.

4.2: Project Design

The study involved three key steps to achieve the stated objectives i.e., choosing the study site, sample collection and transportation to the laboratory, sample preparation and digestion in the laboratory before analysis, and sample analysis.

4.3: Sample Collection and Transportation

Prior to the study, an in-depth field study was conducted to determine the best sampling sites. Three cocoa product samples; Cocoa powder, Chocolate milk, and Cocoa butter were purchased from Naivas supermarket in Nairobi County on 21st Feb, 2022. The locations of the supermarkets were consciously selected to encompass all the social classes. The samples were then transported to an analytical laboratory in the Department of Chemistry, University of Nairobi awaiting digestion and analysis. Figure 3 shows some cocoa products in the supermarket.

Figure 3: Cocoa products in Naivas supermarket.

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4.4: Sample Preparation and Digestion

Organic matter contains metals bonded to their organic matrix which makes it impossible to analyze them directly. According to Anna Turek *et al.*, 2019, the organic matrix can be effectively decomposed by a solution of nitric acid and hydrochloric acid (at a ratio of 1:3).

1g of each cocoa product sample was measured in triplets and put in different 100ml beakers which were well labeled. 7ml of 65% w/w (HNO_3) followed by 21ml of 37% w/w HCl were added in each sample and the mixtures were mixed vigorously and set on a hot plate for digestion for one and half hours at a temperature of 109 ºC. During the boiling process, 10ml of distilled water was then added into the beakers and heating continued until there was about 5ml of sample solution in the beakers, and the samples were allowed to cool.

Figure 4: Measured samples

The remaining content in the beakers was filtered into a 50ml volumetric flask and the sample solution was diluted with distilled water up to the marks then they were transferred to different sample bottles which had been washed and rinsed with concentrated HNO_3 to prevent ions from being absorbed by the walls of the bottles. Figure 7 shows the samples in sample bottles.

Figure 5: Samples being digested.

4.5: Apparatus and Reagents.

Apparatus used in this study were 100ml beakers, 50ml volumetric flasks, wash bottles, measuring cylinders, and watch glasses. Prior to the study, the apparatuses were soaked in analytical-grade nitric acid overnight and thoroughly cleaned with water and detergent the following day. They were then rinsed with deionized water and dried for 12 hours at 110 ºC.

Pieces of equipment employed in the study were a hot plate with a heat control and stirrer knob, Fischer scientific A-160 Analytical weighing balance, and Shimadzu Model AA-6300 (AAS).

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Figure 6: Photo of the Shimadzu Model AA-6300.

The reagents used were analytical grade Nitric acid, hydrochloric acid, deionized water, and stock solutions of lead, manganese, copper, cadmium, and zinc.

4.6: Development of the Calibration Curves

The dilution factor was used in this case to prepare different solutions of concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 ppm of Cadmium, Zinc, Lead, Copper, and Manganese from the respective stock solutions.

Analysis of each standard solution of every metal was carried out using the atomic absorption spectrometer to determine the absorbance of each one of them. The absorbance and the concentration of the solutions of a given metal were then used to generate the calibration curve in Microsoft Excel 2016. Distilled water was used at the beginning and when switching from one analyte to another to zero and flushing the AAS instrument.

4.7: Sample Analysis

After calibration, each sample was introduced into the instrument until a steady reading was obtained.

Figure 7: Sample solutions ready for analysis.

Deionized water was used as a blank solution as well as to wash the instrument after each analysis to return the reading of the instrument to its initial setting.

V. Results and Discussion

5.1: Introduction

According to Zhang, X. *et al.*, 2019 and Su, C *et al.*, 2014 cadmium, zinc, copper, and lead above permissible levels are associated with alarming environmental and health problems. The results from the triplet solutions from each sample were computed using Microsoft Excel 2016 to determine the average levels of cadmium, zinc, copper, manganese, and lead in the three samples. The averages are illustrated in Table 1.

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Table 1 average concentration of metal elements in the cocoa samples (in mg/kg).

SAMPLE	Cu	Pb	Cd	Zn	Mn
CO	0.270±0.037	0.154±0.021	BDL	0.794±0.063	0.334±0.015
B	0.028±0.038	0.329±0.008	BDL	0.116±0.075	0.124±0.008
M	0.042±0.066	0.463±0.003	BDL	0.296±0.015	0.162±0.010

Key:

Co – Cocoa powder

B – Cocoa butter

M – Chocolate milk

5.2: Concentration of Heavy Metals in Cocoa Products.

The levels of all trace elements under study were analyzed using the AAS (Shimadzu Model AA-6300) for all the selected samples. The concentration of individual heavy metals in the different cocoa product samples is shown in Table 2 to Table 6 in mg/kg.

5.2.1: Copper

As detailed in Table 2, the levels of copper in all the samples were below the recommended permissible limits of 1.0 mg/kg of food stipulated by WHO [28]. The concentration of copper is highest in cocoa powder 0.270±0.021mg/kg and lowest in cocoa butter 0.027±0.008mg/kg.

Table 2 Average concentration of copper in the cocoa sample.

SAMPLES	AVERAGE (mg/kg)
CO	0.270±0.021
B	0.027±0.008
M	0.042±0.003

5.2.2: Lead

Lead is a hazardous waste emitted from industries and environments that negatively affects the function of the hepatic, endocrine, immune, reproductive, and gastrointestinal systems [29 – 31]. Its concentration in the different cocoa product samples is shown in Table 3.

Table 3 Average concentration of lead in the cocoa sample.

SAMPLES	AVERAGE (mg/kg)
CO	0.154±0.037
B	0.329±0.038
M	0.463±0.066

According to WHO and FAO, accepted a daily lead intake of 490 µm of lead for an adult [32]. Chocolate milk had the highest concentration of Pb (0.463±0.066) but within the permissible levels. The cocoa powder had the least concentration (0.154±0.037), as shown in Table 3 but still within the permissible levels.

5.2.3: Cadmium

Cadmium is one of the heavy metals that have no physiological functions but are associated with chronic ailments [33]. The concentration of cadmium in the different cocoa product samples is shown in Table 4. According to this study, cadmium was below the detection limit in all the cocoa product samples.

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Table 4 Average concentration of cadmium element in the cocoa sample.

SAMPLES	AVERAGE (mg/kg)
CO	BDL
B	BDL
M	BDL

5.2.4: Zinc

Zinc is one of the essential trace elements in the human body, particularly in immune responses [16]. However, in excess, it is deleterious to the body since it contributes to cardiovascular diseases, stomach damage, hypertension, and nausea [34]. Permissible levels of Zn in food as reported by WHO is 8mg/day for females and 11mg/day for males. In this study the concentration of Zn was in the range of 0.794±0.063mg/kg to 0.116± 0.075mg/kg, this is lower than the permissible levels. The cocoa powder had the highest concentration of Zn and cocoa butter had the lowest concentration. The concentration of zinc in the different cocoa product samples is shown in Table 5.

Table 5 Average concentration of zinc in the cocoa sample.

SAMPLES	AVERAGE (mg/kg)
CO (Cocoa powder)	0.794±0.063
B (Cocoa butter)	0.116±0.075
M (Chocolate milk)	0.296±0.015

5.2.5: Manganese

Manganese is categorized as an essential trace element whose deficiency leads to infertility and impaired skeletal growth. It is essential for intracellular activities. A human being weighing 70 kg contains Manganese in about 12 – 20 mg amount [35]. However, overexposure to the metal can result in ailments such as polycythemia, dystonia, and hepatic cirrhosis [36 – 38]. The concentration of manganese in the cocoa products samples is shown in Table 6.

Table 6 Average concentration of manganese in the cocoa sample.

SAMPLES	AVERAGE (mg/kg)
CO (Cocoa powder)	0.334±0.015
B (Cocoa butter)	0.124±0.008
M (Chocolate milk)	0.162±0.010

The WHO recommends permissible levels of Mn in foods to be in the range of, (1.00 to 12.00) mg/kg. The cocoa powder had the highest concentration of manganese 0.334±0.015mg/kg and cocoa butter had the lowest concentration of manganese 0.124±0.008mg/kg. According to the study, Mn concentrations were within the recommended daily dietary allowances of 2.3 mg for adult males and 1.8 mg for adult females [39].

5.3: Concentration of metal elements in the different samples

The concentration of heavy metals in the different individual cocoa product samples is shown in Figure 8.

5.3.1: Cocoa Powder

The end product is with a fine powdered consistency, having a mild and subtly bitter test. As shown in Figure 8 zinc has the highest concentration in cocoa powder of 0.794±0.063mg/kg. Manganese follows with a concentration of 0.334±0.015mg/kg, and manganese is followed closely by copper and lead. Cadmium was found to be below the detection limit. The concentration of these metals was within the permissible levels of WHO.

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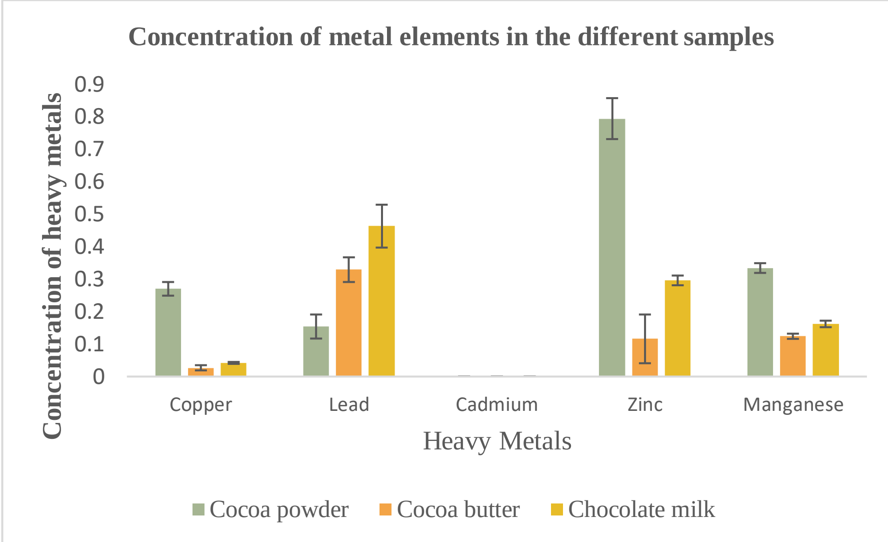

Figure 8: Concentration of heavy metals in the different samples.

5.3.2: Cocoa Butter

It is valued for its melting characteristic; it remains brittle at room temperature or low but melts just below body temperature. Figure 8 shows metal concentrations in cocoa butter. Cadmium was found to be below the detection limit. Lead had the highest concentration of 0.329±0.038mg/kg, and manganese followed with a concentration of 0.124±0.008mg/kg which is followed closely by zinc. Copper recorded the lowest concentration of 0.027±0.008mg/kg.

5.3.3: Chocolate Milk

Chocolate milk provides important nutrients such as calcium, protein, and vitamin D which may benefit health. Figure 8 shows metal concentrations in chocolate milk. Cadmium was found to be below the detection limit. Lead had the highest concentration of 0.463±0.066mg/kg, zinc followed with a concentration of 0.296±0.015mg/kg which is followed closely by manganese. Copper recorded the lowest concentration of 0.042±0.003mg/kg.

5.4: Average Concentration of Heavy Metals in Different Samples.

From Table 7, From study and data Table 4.10, Zn had the highest total concentration of 0.402±0.051mg/kg, followed by Pb, Mn, and Cu which had a concentration of 0.315±0.047mg/kg, 0.207±0.011mg/kg and 0.113±0.011mg/kg respectively. Cadmium (Cd) concentration was below the detection limit.

Table 7: Average concentration of heavy metals in cocoa.

METAL	TOTAL (mg/kg)
Cu	0.113±0.011
Pb	0.315±0.047
Cd	BDL
Zn	0.402±0.051
Mn	0.207±0.011

VI. Conclusion and Recommendation

6.1: Conclusion

All the concentrations of metals in all the samples were within the WHO and KEBS permissible levels in food products. Although most samples in the test for Lead metal were within permissible levels, chocolate milk had the highest concentration of

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(0.463mg/kg). The results obtained in this study show that all the heavy metals were within the right concentration, but care should be taken to ensure that people take the correct dosage to avoid complications.

In conclusion, the assessment and correlation of the environmental exposure levels of the cocoa product analyzed elements with human biological biomarkers or adverse effects were not done in this study. Therefore, conclusions about whether these levels were toxic at the biological levels should be drawn with caution as only assessed some exposure concentrations were assessed which might be different when reaching the human body.

6.2: Recommendations

The recommendations derived from the current study include;

1. The current study suggests more investigation studies to determine the contamination of heavy metals in other different cocoa products.
2. Monitoring of heavy metal concentration in cocoa products should be done regularly to make sure that they remain within the recommended permissible levels.
3. A better selection of fresh raw materials, including an analysis of toxic elements before processing cocoa products, could improve the situation.
4. The concentration of lead and cadmium should always be monitored in all processed products because they are toxic and can bring chronic conditions in people.

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