

The detection of Heavy Metal Concentrations by Inductively Coupled Plasma (ICP) in Libyan Cocoa Samples and Associated Health Risk Assessment

Hamad Hasan^{1*}, Amaal Yousuf², Marwa Khalifa², Monira Saleh¹

¹Chemistry Department, Faculty of Science, Omar Al-Mukhtar University, Libya

²Higher Institute of Medical Sciences and Technology, Al-Marj, Libya

Corresponding email. hamad.dr@omu.edu.ly

Abstract

Cocoa (*Theobroma cacao* L.) is one of the most widely consumed agricultural products worldwide, used in the production of chocolate and various beverages. Despite its nutritional and health-promoting properties, concerns have arisen due to contamination with heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn), and aluminum (Al), which may pose health risks. In this study, ten cocoa samples from local markets in Derna, Libya, were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to determine heavy metal concentrations. Results revealed elevated levels of Cd (7.06 µg/g), Cr (10.97 µg/g), and Cu (10.62 µg/g), all significantly above the permissible Maximum Residue Limits (MRLs), while Zn and Al also exceeded safety thresholds. Risk assessment showed a Target Hazard Quotient (THQ) of 1.17 for Cd, indicating potential health hazards, and a Hazard Index (HI) of 1.49, suggesting cumulative non-carcinogenic risks from chronic exposure. These findings highlight the importance of continuous monitoring and strict regulation of cocoa products to minimize public health risks associated with heavy metal contamination.

Keywords: Heavy metals, ICP-MS, Cocoa, Libya.

Introduction

Cocoa trees, are mostly grown in Asia, South America, Central America, and West Africa. Ghana, Indonesia, Nigeria, Brazil, Ecuador, Malaysia, and the Ivory Coast are the top eight cocoa-producing nations in the world. Around 8.0% of the world's 3.972 million tons of cocoa were produced in Indonesia [1]. A valuable luxury commodity, cocoa is essential to chocolate and other cocoa-based items that are eaten globally [2]. Cocoa-based products are among the most widely eaten foods in the world. Cocoa beans are used to make several popular items, including chocolate, cocoa powder, and beverages. However, there aren't enough studies on these beans' nutritional value, particularly when it comes to important components like proteins, carbohydrates, lipids, and other essential minerals.

Despite the established health advantages of cocoa, current concerns over food safety have been raised by the presence of heavy metals, including nickel (Ni), cadmium (Cd), chromium (Cr), and lead (Pb) in cocoa beans and in various cocoa products like cocoa butter, cocoa paste, cocoa powder, and chocolate. Research on the chemical makeup and characteristics of cocoa has revealed that its high quantity of polyphenolic components, especially flavonoids, which function as antioxidants, may enhance human health when consumed in moderation. These substances are important for supporting general health. According to certain studies, cocoa has a higher antioxidant capacity than red wine and green tea [3]. One of cocoa's health advantages is its capacity to scavenge free radicals [4-6].

The amounts of cadmium, nickel, and lead were found to vary from 0.001 to 2.730 grams/kg, 0.041 to 8.290 grams/kg, and 0.049 to 8.040 grams/kg, respectively. 42 out of 69 samples had lead levels over the allowable limit of 0.30 grams/kg, according to the Polish national standard for lead established by the Polish regulatory body. Nineteen samples had levels of cadmium above the allowable limit of 0.05grams/kg. A class of metallic and non-metallic elements having an atomic density higher than 4 g/cm³ is usually referred to as "heavy metals". Arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), lead (Pb), mercury (Hg), nickel (Ni), and vanadium (V) are among these metals, which are hazardous and non-essential elements. On the other hand, some heavy metals, like copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn), are necessary for life [7]. When exposed to heavy metals in large quantities or over extended periods of time, they can harm humans, animals, and plants. Their pervasive discharge from residential, industrial, agricultural, chemical, and technical sources, which pollutes the air, water, and land, makes them even more dangerous. Although human activities frequently speed up the release and transit rates of certain metals, increasing their concentrations in nature, natural occurrences like volcanic eruptions and ocean movements also help recycle these metals in the environment [8].

Water, food, and the air are some of the ways that heavy metals can enter the human body. Even while some heavy metals are essential to the operation of living things, there is a serious risk when they are present in amounts above what is considered safe. These metals can pass through the placenta and build up in the growing baby's tissues. Furthermore, because heavy metals have a lengthy biological half-life, they might build up in different human organs and result in harmful side effects [9,10].

Induction-coupled plasma mass spectrometry (ICP-MS). It is a useful analytical technique for figuring out

the multiforming (qualitative) elements, their concentration (quantitative), and the abundance of isotopes of different matrices. One method for very sensitive metal and multielement metal analysis is induction-coupled plasma mass spectrometry (ICP-MS). It may be used for many different activities, such as environmental research, health care product analysis, the food and chemical industries, and, of course, clinical biology [11].

In Libya, the study of the contents of heavy metals was carried out in different samples [12-41], the evaluation of the effect of some hazardous compounds was estimated in many samples, Hydrocarbons [42-50], pesticides [51-52], plants [53-84], and others [86-95]. This study aims to measure the concentration of heavy metals in imported cocoa samples collected from some Libyan markets by using inductively coupled plasma [ICP].

Methods

Sampling

In this study, ten different commercial cocoa samples were collected from local markets in Derna city Libya, (2024). The samples were analyzed for the determination of heavy metals by Ionic Coupling Plasma (ICP) for metals: zinc, chromium, Iron, copper, cobalt, and magnesium. The types of samples were given in (Table 1):

Table 1: The studied cocoa samples

Sample. No	Name sample
1	Nesquik
2	Said
3	Ovaltine
4	Darlet
5	Corona
6	ALwdaq
7	CaCao Drink
8	Katsan Ovalite Cocoa powder
9	Raw cocoa
10	Dutch cocoa

Digestion of samples

For the determination of selected metals, about(0.5 g) powder sample was weighed and transferred into a (100 ML) beaker then dissolving it in (20 ml) of distilled water in a Beaker, then adding(5 ml)of concentrated nitric acid, heating and continuing to heat until the sample is almost dry, then leaving the solution to cool, then we filter the solution and take the leachate and add distilled water to a volume of (100 ml) then lifted aside to lose the heat, Leave the solution to cool [14-16]. Finally, stored in a polyethylene bottle for measuring metals. Measured by Inductively coupled plasma mass spectrometry (ICP-MS) at Cairo University, Egypt.

Risk assessment

The EDI of cocoa was computed by dividing the consumption rate by an average body weight of 60 kg to assess the possible health concerns for users. The acceptable daily intake (ADI) of cocoa was then compared to the EDI to calculate a risk index. The target hazard quotient (THQ), a technique created by the US Environmental Protection Agency, was used to evaluate the possible health concerns associated with prolonged exposure to chemical contaminants and was used to calculate the risk. Taking body weight, frequency, length of exposure, and amount consumed into consideration, the THQ compares the observed concentration to the oral reference dosage. A possible health concern is indicated by a THQ greater than 1.

$$THQ = ([Efr * ED_{tot} * IFR * C] / ([RfDO * BWa * ATn]) * 10^{-3}$$

Where:

EF_r: the exposure frequency (365 days/year); ED_{tot}: exposure duration (80 years for both Italian and Pakistani populations, based on survey results from ISTAT 2013). FIR: is the food ingestion rate (g day⁻¹); C: is the concentration (µg g⁻¹); RfD_o: is the oral reference dose (µg g⁻¹ day⁻¹). BWa: is the adult body weight (60 kg). ATn: is the average time for non-carcinogens (it is equal to EFr × ED_{tot}). The HRI assesses the potential danger associated with pesticide and metal residues detected in cocoa samples.

Results

Heavy metal contents

In this study some heavy metals were detected in cocoa samples by I.C.P.M.S The metals includes: Fe, Zn, Mn, Al, Cu, Ag, Pb, Cd, Cr and Sr on the other side these metals of Co, Ni, As, Ca and Sb were not detected in the studied samples, the contents were given in the Tables (2&3) and Figures (1&2). The contents of detected metals were fluctuated in the ranges of (0.34-12.31), (0.15-7.25), (ND-70.55), (ND-0.54), (0.11-1.04), (1.34-56.15), (0.14-3.39), (0.01-0.16), (0.12-1.19), (0.58-4.87 µg/g) For Ag, Al, Cd, Cr, Cu, Fe, Mn, Pb, Sr and Zn, respectively. Comparison of the arithmetic mean and standard deviation values of the metals in

the studied cocoa samples with the minimum contaminant limits (MRLs) as determined by the Agency for Toxic Substances and Disease Registry and the minimum risk levels (MRLs). High concentrations of cadmium, chromium, and copper were obtained, followed by zinc and aluminum, except for strontium, which had a low concentration, compared to the minimum contaminant limits. The results of this study for the studied samples recorded contamination and toxicity. The values of the metal's cadmium, chromium, copper, zinc, and aluminum were (21.16 ± 7.06) , (10.87 ± 10.97) , (10.62 ± 10.09) , (1.25 ± 1.88) , and (2.05 ± 1.24) $\mu\text{g/g}$ for the metals, respectively, higher than the minimum contaminant limits. Tables (2 -4) show the contents of heavy metals in the studied cocoa samples:

Table 2. The contents of (Al, Fe, Mn, and Zn) in the studied cocoa samples.

Solution Label	Al 396.152 nm	Fe 238.204 nm	Mn 257.610 nm	Zn 213.857 nm
Sample 1	0.67	3.12	0.45	1.17
Sample 2	0.4	2.03	0.48	1.27
Sample 3	0.47	5.3	1.01	4.87
Sample 4	0.15	2.58	0.6	0.98
Sample 5	0.34	1.89	0.37	1.07
Sample 6	0.39	1.99	0.27	1.03
Sample 7	0.32	1.34	0.14	0.58
Sample 8	0.52	11.11	1.69	2.22
Sample 9	1.97	7.69	1.41	2.53
Sample 10	7.25	56.15	3.39	3.16
±SD	2.058	15.88	0.93	1.259
Average	1.248	9.32	0.981	1.888

Table 3: The contents of (Cu, Sr, and Ag) in the studied cocoa samples.

Solution Label	Cu	Sr	Ag
Sample 1	0.51	0.23	3.96
Sample 2	0.53	0.21	12.31
Sample 3	0.37	0.69	2
Sample 4	0.41	0.23	0.82
Sample 5	0.29	0.24	0.62
Sample 6	0.31	0.18	0.81
Sample 7	0.11	0.12	0.56
Sample 8	1.04	0.61	0.94
Sample 9	0.8	1.03	0.38
Sample 10	0.94	1.19	0.34
±SD	10.09	0.365	3.50
Average	10.62	0.473	2.274

Table 4. The contents of (Cr, Cd, and Pb) in the studied cocoa samples

Solution Label	Cr	Cd	Pb
Sample 1	0.05	0.01	0.1
Sample 2	0.03	0.02	0.11
Sample 3	0.05	70.55	0.13
Sample 4	0.06	0.01	0.12
Sample 5	0.03	0.01	0.13
Sample 6	0.02	0.01	0.14
Sample 7	0.03	0.01	0.01
Sample 8	0.13	0.01	0.12
Sample 9	0.03	0.01	0.11
Sample 10	0.54	0.03	0.16
±SD	10.87	21.16	0.037
Average	10.97	7.067	0.113

Comparison of the detected metal values with the Minimum Residual Limit (MRL) shown in (Table 5):

Table 5. Compared to the detected heavy metals with Minimum Residual Limit values.

Metal	Al	Cd	Cr	Cu	Pb	Sr	Zn
Concentration	1.24±2.05	7.06±21.16	10.97±10.87	10.09±10.62	0.11±0.03	0.47±0.36	1.88±1.25
MRLs	1	0.0005	0.0009	0.02	-	2	0.3

The results showed that all of the samples had high amounts of iron, while the other metals varied from sample to sample. The findings also revealed variations in the percentage distribution of the minerals under investigation. Iron (Fe) and manganese (Mn) are the primary minerals found in the cocoa samples, but there are also comparatively large concentrations of zinc and silver. The cocoa samples 1, 4, 5, 6, 6, 7, 8, 9, and 10 had the greatest percentage of aluminum values (32, 43, 38, 39, 42, 60, 48, and 78%) out of all the heavy metals found in the samples used in this investigation. Higher percentages of silver (41 and 71%) were found in samples 1 and 2. However, with values of 83%, Sample (3) had the highest cadmium proportion found. Other metals, including strontium, copper, lead, chromium, and cadmium, were also detected in trace amounts. The cocoa samples under investigation also showed a relative rise in manganese, zinc, silver, and aluminum levels.

Health Risk Assessments

The health risks associated with consuming chemical pollutants (heavy metals) were evaluated using the Target Hazard Quotient (THQ) and the Hazard Index (HI) to ascertain the health hazards of heavy metals in cocoa samples. Regulatory agencies like the US Environmental Protection Agency (EPA) employ these fundamental ideas in risk assessment to explain the dangers of chemicals.

Target Hazard Quotient (THQ)

The connection between the oral reference dosage and the observed concentration is represented by the target hazard ratio, which is weighted by body weight, amount ingested, and frequency and duration of exposure to the chemical. Potential health risks are indicated by a THQ value of 1 or above, but no health risks are indicated by a value less than 1. The THQ findings for this investigation are displayed in (Table 6).

Table 6. The results of the Target Hazard Quotient (THQ).

Metal	THQ
Al	0.0002
Cd	1.17
Cr	0.203
Cu	0.044
Fe	0.002
Mn	0.001
Ag	0.075
Zn	0.001
Sr	0.0001

There is a chance of negative health impacts since the target hazard quotient (THQ) value for cadmium (1.17), which is greater than the acceptable limit for the target hazard quotient (1), is higher. There were no negative health impacts because the remaining values were within the safe limit of 1.

Hazard Index (HI)

The hazard index for each food type is the sum of the values of all the target hazard components. Chronic exposure to a group of toxic components from consuming a particular food type may lead to cumulative adverse health effects even if the hazard index is less than 1. However, if the target hazard ratio is greater than 1 ($HI \leq 1$), it may lead to adverse health effects, but not carcinogenic ones. The hazard index equation for the target

$$\text{is: } HI = THQ_1 + THQ_2 + THQ_3$$

In this study, the hazard index showed that the value of (1.49) was higher than the safe limit of one, meaning there is a potential non-carcinogenic health risk.

Discussion

In many nations, cocoa is a staple crop. It is utilized in the production of chocolate and several other culinary items, and it makes a substantial contribution to the world economy. Chocolate is a very nutrient-dense and high-energy dietary item, apart from the bioactive substances. Consuming cocoa in moderation can lower the incidence of cardiovascular disease and several risk factors. Despite all its advantages, eating a lot of cocoa might be bad for your health since it naturally includes a number of heavy metals [96]. Concerns over these metals' prevalence in a variety of food goods, including chocolate, have grown in recent years.

Both human and natural activity determine its source and amounts [3]. Because cocoa and its derivatives are consumed in large quantities, it is crucial to keep an eye on and assess the amounts of these toxins in different cocoa brands to guarantee their safety. Because of the World Health Organization's emphasis on the significance of studying medicinal plants, such as cocoa, food levels of heavy metals are often tracked to guarantee consumer safety. This study sought to evaluate and measure the levels of certain heavy metals in a range of meticulously and randomly chosen cocoa samples to address this requirement and investigate heavy metal contamination. Both branded and locally sourced cocoa goods were included in the tastings. By examining these samples.

To guarantee the safety and quality of this beverage, our study aimed to offer insightful information on possible exposure to heavy metals through cocoa intake. The amounts of heavy metal buildup in the examined cocoa samples varied, and these quantities exceeded the Agency for Toxic Substances and Disease Registry's allowable limits. It was found that, except for strontium and aluminum, the amounts of cadmium, chromium, copper, and zinc were all over the allowable limits. [4&5] This outcome suggests that pollutants and poisons are present. Cadmium is categorized as a carcinogenic chemical in humans by the International Agency for Research on Cancer (IARC).

This conclusion was supported by research [8], which found that the cocoa and chocolate samples from various parts of India had significant levels of lead and cadmium. These findings were different from those of the research, which examined six different kinds of heavy metals in Ghanaian cocoa beans. While the amounts of vital elements like iron and zinc were high, the findings revealed low levels of lead and cadmium. Heavy metal levels in cocoa products were evaluated by some studies [7&8], and the findings showed significant quantities that were over the permissible limits. The amounts of several heavy metals in cocoa products were compared to the exposure limits allowed by international health agencies, and revealed that all sample results fell below the acceptable range. Some of the samples under study had acceptable levels of lead and cadmium.

According to research, the quantities of harmful metals were over the allowable limit, with lead and cadmium having the highest levels and chromium having the lowest. Lead, cadmium, and nickel are just a few of the dangerous substances that can contaminate raw cocoa resources and products. [96] The conditions under which the cocoa trees are cultivated or the conditions under which the cocoa beans undergo different procedures like fermentation, drying, and grinding might be the cause of this. The metal tools used for mixing, grinding, and storing materials may potentially be the source of contamination. The cocoa plant's geographic origin and the production environment also affect the amounts of these components. According to certain research, these outcomes have been shown (Nickel and McMillan). Cadmium is taken up by cocoa trees from the soil, and as the tree grows, it builds up in the cocoa beans. Toxic amounts of pollution are quickly attained.

According to previous studies [1], cocoa goods stated to originate from Latin America had greater cadmium content than those coming from Africa, confirming that regional diversity affects cadmium levels. Samples were taken from eight cocoa-producing farms on the cadmium content of cocoa plants and soil properties in the city of Almirante, Bocas del Toro province. In addition to samples of cocoa tree leaves and fruits, soil samples were collected at a depth of 30 cm. The findings indicated that the soil's pH was 5.1, which is acidic, and that its organic matter content was over 3%, both of which are appropriate for the soil's ability to fix cadmium. The mean levels of bioavailable and total cadmium were 0.02 and 0.10 mg/kg, respectively [10]. Cocoa leaves had cadmium concentrations above the suggested threshold of 0.5 mg/kg. Cocoa beans have a low quantity of cadmium (0.25 mg/kg). The Hazard Index (HI) and the Target Hazard Quotient (THQ) were also used to calculate health hazards. Apart from cadmium, which had a value of 1.17, over the safe level, suggesting the presence of toxins in the examined samples, the Target Hazard Quotient readings were within the safe range. The Hazard Index score of 1.49 exceeded the internationally advised safe threshold. Suggesting that ingesting these goods in large quantities may pose a health risk. These findings align with the research conducted by some studies [9], which demonstrated that consistent cocoa eating, Chocolate that has been contaminated, can have major negative health impacts, such as an elevated risk of cancer and renal disease. It is advised to use aqueous extracts sparingly to get vital nutrients and safeguard consumers. To guarantee that daily consumption restrictions are not exceeded and to safeguard the public's health from potential hazards related to heavy metals, it is crucial to monitor and examine cocoa samples from Libya and other areas.

Different cocoa brands and geographical areas have been found to contain varying levels of heavy metals, underscoring the necessity of routine testing and food product quality management [1-3]. Differences in the fundamental physical and chemical properties of various agricultural regions can account for the notable diversity in metal levels throughout these locations. Soil type, water quality, mineral content, climate, pesticides, and the processes utilized to change the product into its consumable form are a few examples of these variables. The accumulation of metal in plant components is significantly influenced by each of these factors [5]. Thus, the levels of metals in different chocolate brands were significantly influenced by their place of origin. It is crucial to remember that the water solubility of various metals varies. Previous studies have reported similar variations in metal levels across different chocolate brands [7-9]. To ensure the safety of food products and consumers, further research and quality control in this area are suggested.

Conclusion

The analysis using ICP-MS revealed elevated concentrations of metals in cocoa samples collected from Derna, Libya. Metals exceeding the Maximum Residue Limits (MRLs) include: Cadmium (Cd): Average concentration of 7.06 µg/g, far above the permissible limit of 0.0005 µg/g. Chromium (Cr): 10.97 µg/g vs. the limit of 0.0009 µg/g. Copper (Cu): 10.62 µg/g, exceeding the limit of 0.02 µg/g. Zinc (Zn) and Aluminum (Al) also surpassed safe thresholds. The Target Hazard Quotient (THQ) for cadmium was 1.17, indicating potential health risks. The Hazard Index (HI) for all metals combined was 1.49, suggesting chronic exposure could be harmful.

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Conflict

The authors provided that no conflict of the results given in this study with those reported in any other study.

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