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ESTIMATION OF HEAVY METALS IN UNBRANDED CHOCOLATES SOLD IN KARACHI WITH COMPARISON TO OTHER PLACES AROUND THE WORLD

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ABSTRACT

Heavy metal contamination in food products is a major concern for human health, and chocolate, being a favourite sweet foodstuff of children and adults, possesses a greater threat of heavy metal exposure to humans. Twenty-five chocolate samples obtained from local markets in Karachi, Pakistan, are studied in this research to determine the heavy metal concentration. The studied metals include Pb, Ni, As, Cr, and Fe, and the results obtained in the present study are compared to the findings of the previous research conducted worldwide. According to the findings of the study, the mean concentrations of studied heavy metals were found in the order of As < Cr < Ni < Pb < Fe. The Fe level ranged from 0.31 to 7.12 ppm with a mean concentration of 2.901 ppm, which is less than that reported in the previous studies. The Pb level ranged from 0.54 to 4.76 ppm with a mean concentration of 2.707 ppm, which is the highest as compared to the previous studies. The Ni concentration ranged from 0.11 to 1.74 ppm with a mean concentration of 0.567 ppm, which is almost similar to the other studies. Maximum As and Cr concentrations were 0.098 and 0.87 ppm. Exposure to these metals can cause severe health issues in humans, particularly children, who are more susceptible to the negative health impacts of these heavy metals.

Keywords: Karachi, Chocolates, Heavy metals, unbranded

INTRODUCTION

Heavy metal contamination has expanded widely over the world, disrupting the ecosystem, and providing major health risks to humans (Rai *et al.,* 2019) through activities such as mining (Gyamfi *et al.*, 2019; Sun *et al.*, 2018) use of fertilizer and pesticide (Chen *et al.,* 2020; Qaswar *et al.,* 2020), atmospheric deposition (Chen *et al.*, 2019; Harmanescu *et al.,* 2011), volcanic eruptions, erosion, and industrial processes (Engwa *et al.,* 2019). The classification of trace metals into necessary and harmful is difficult since it is mostly determined by the dose of exposure (Islam *et al.,* 2015). Some metals, despite their vital function in coenzymes, can be harmful in large quantities (for example, copper (Cu), chromium (Cr), and nickel) (Ni) (Ullah *et al.*, 2017; Engwa *et al.*, 2019). Several toxic heavy metals and metalloids (for example, As, Pb, Cd, and Hg) are classed as non-essential to metabolism and other biological activities. These metals are harmful in a variety of ways (Gall *et al.*, 2015), as a result, the United States Environmental Protection Agency, and the Agency (ATSDR) for Toxic Compounds and Disease Registry have listed them on the top 20 list of harmful substances (Khalid *et al.,* 2017; Rai, 2018; Xiong *et al.,* 2016). Certain heavy metals, such as Cu, Fe, Zn and Cr (III) are essential components of metabolic processes such as cytochromes and enzymes, which are inextricably linked to biota metabolic functioning (Alloway, 2013; Broadley *et al.,* 2012; Elmorsi *et al.,* 2019) .

Food serves as the major cause of heavy metal exposure to human body (Bortey-Sam *et al.,* 2015; Ihedioha *et al.,* 2014). One of the most important problems for food security and safety is heavy metal contamination as it is harmful to human health (Guo *et al.*, 2010; Motesharrei *et al.*, 2016; Stavins, 2019). The research of heavy metal concentrations in foods is crucial, as there is rising concern about imported foods from all over the world (Maxwell and Neumann, 2009). The investigation of heavy metals in various foods is important since certain of these chemical components are either vital or hazardous to the human health (Åkesson *et a.l*, 2015; Salama and Radwan, 2017). Children are most vulnerable to the negative health impacts of heavy metal exposure through food due to their body weight (Samsuddin *et al.*, 2016) and chocolates, gums, candies, sweets and biscuits are children's favourite food (Iwegbue *et al.,* 2015) as sweets are preferences of children are stronger than those of adults (Vennerød *et al.*, 2018). Many studies have been conducted in different countries to assess the danger of human exposure to hazardous metals from dietary sources such as from soil and crops (Jia *et al.,* 2018) seasonings (Aigberua *et al.*, 2018), dietary supplements (Piekara *et al.,* 2020) and biscuits and sweets (Adegbol *et al*., 2015; Devi *et al.*, 2016; Salama and Radwan, 2017; Dawodu, 2019; Kovačević *et al.*, 2019; Adimula *et al.*, 2019; Salama, 2019; Sobhanardakani, 2019; Oyekunle *et al.,* 2021; Pasqualone *et al.*, 2021).

Chocolates are more likely to be contaminated from heavy metal pollution due to the variety of ingredients used in the preparation process (Devi *et al.*, 2016). Heavy metals such as Pb, Cr, Ti, Zn, Al, Cd, and Cu are also at danger of migrating from the printed surface to the contact surface. Most heavy metals, on the other hand, are introduced during the preparation and packing processes, when the sweets' surfaces are sticky, allowing the candy's surface to cling to the package's inner cover (Adegbola *et al.*, 2015), increasing the chance of contamination and heavy metal exposure. Different ingredients are involved in the preparation of chocolate with basic ingredients being cocoa powder, cocoa butter, sugar, and chocolate liquor *(Devi et al.*, 2016) and the ingredients can be a possible source of contamination in the chocolates. Heavy metal concentration has been reported in chocolates before (Dahiya *et al.*, 2005; Iwegbue, 2011) but no study has been conducted in Pakistan to estimate the heavy metal concentration in unbranded chocolates. In this study, the heavy metal concentration in unbranded chocolates is discussed for the first time in Pakistan and the concentrations found in the unbranded chocolates are compared with heavy metal concentrations reported in chocolates and candies in the previous studies.

METHODOLOGY

Study Area

Karachi has been selected as a study area which is Pakistan's largest financial centre, most populous, and industrialised city with a total area of 3,527 km^2, Karachi was estimated at 16.21 million in 2017 official census. However, unofficial numbers suggest that the population is closer to 25 million. Approximately 10,000 registered industrial units in the city produce food, pharmaceutical, chemical, fabric, paints, oil, steel, and paper products (with a substantial number in the informal part).

Sampling

A total of 25 samples of chocolate were collected from local markets/ vendor stalls of Hydri, Saddar, Gulshan-e-Hadeed, Liaqtabad, Orangi town and other street vendor stalls in populous areas of Karachi. While sampling several factors were taken into consideration comprising of ingredients, expiry date and packing conditions.

Sample Digestion

A 5 gm of chocolate sample was taken and wet digested using a 1:3 mixture of perchloric acid ($HClO_4$) and nitric acid (HNO_3). A colourless solution was formed after mild heating for 16 h, and it was evaporated to near dryness with 0.04 mol/l nitric acid. Solutions were made up to 10 ml after digestion and appropriate cooling of residues. In triplicate, all the samples were processed and digested (Kanmani and Gandhimathi, 2013).

Heavy Metals Analysis

Concentration of heavy metals (Pb, Ni, Cr, As, and Fe) were examined in all the digested samples by using appropriate Merck Super Nova60 kits. Examination of each sample was repeated 3 times.

RESULTS AND DISCUSSION

Twenty five chocolate samples were collected (C-1-C-25) and analysed for heavy metal concentration (Pb, Ni, As, Cr, and Fe) and all the samples were found to be contaminated with Pb, Ni, and Fe with significant concentrations, although As and Cr were not found in all the chocolate samples. Table 2 provides an overview of the health effects of heavy metals based on various research studies. The mean concentration of iron was found to be the highest among other heavy metals with a concentration of 2.901 ppm, followed by Lead, Nickel, Chromium, and Arsenic with concentrations of 2.707 ppm, 0.567 ppm, 0.190 ppm, and 0.010 ppm, respectively.

The concentration of Pb was found to be highest in sample C-13 and lowest in sample C-18 (milk-based chocolate), with Pb concentrations of 4.76 ppm and 0.54 ppm, respectively. Sample C-15 (caramel-based chocolate) had the highest concentration of Ni (1.74 ppm) and sample C-22 had the least Ni concentration (0.032) among the other samples. The Fe concentration was found to be highest in sample C-15 (caramel-based chocolate) and lowest in sample C-8, with Fe concentrations of 7.12 ppm and 0.31 ppm, respectively. Arsenic was only found in 6 samples with the highest concentration in sample C-12 (coconut-based chocolate) having an arsenic concentration of 0.098 ppm, whereas chromium was found in 15 samples, in which sample C-1 (coconut-based chocolate) had the highest concentration of 0.87 ppm.

Table 1. Average Heavy metals Concentration (Mean ± SE) in Unbranded Chocolates					
Sample Code	Mean Concentration ±SE (ppm)				
	Pb	Ni	As	Cr	Fe
C-1	4.21±0.305	0.67±0.056	BDL	0.87±0.136	1.44±0.113
C-2	2.23±0.161	0.23±0.021	BDL	0.45±0.107	2.12±0.156
C-3	2.65±0.162	0.76±0.041	BDL	0.33±0.113	2.76±0.196
C-4	4.11±0.173	0.097±0.063	BDL	0.56±0.101	0.61±0.058
C-5	3.29±0.150	0.51±0.0441	BDL	0.41±0.103	1.09±0.096
C-6	2.84±0.124	0.64±0.0335	BDL	0.098±0.056	0.71±0.0118
C-7	1.38±0.057	0.23±0.0285	BDL	0.65±0.114	2.41±0.174
C-8	3.17±0.133	0.19±0.093	BDL	0.088±0.0125	0.31±0.069
C-9	1.66±0.082	0.32±0.062	BDL	0.046±0.0105	0.91±0.121
C-10	1.09±0.095	1.22±0.0858	0.034±0.0102	BDL	1.69±0.118
C-11	2.41±0.105	0.45±0.0914	BDL	BDL	2.81±0.236
C-12	3.12±0.121	1.43±0.0428	0.098±0.0152	BDL	0.73±0.123
C-13	4.76±0.136	0.37±0.0912	BDL	BDL	1.78±0.214
C-14	4.12±0.129	1.41±0.0521	BDL	BDL	4.89±0.325
C-15	3.54±0.118	1.74±0.254	0.012±0.0096	0.098±0.0115	7.12±0.425
C-16	1.73±0.096	0.31±0.085	0.045±0.011	0.14±0.085	4.93±0.295
C-17	2.04±0.101	0.096±0.0109	0.067±0.026	0.23±0.055	5.91±0.415
C-18	0.54±0.075	0.052±0.0104	BDL	0.74±0.141	1.6±0.157
C-19	2.54±0.114	0.21±0.102	BDL	BDL	0.74±0.145
C-20	2.66±0.129	0.45±0.114	BDL	BDL	3.91±0.228
C-21	3.11±0.138	0.34±0.131	BDL	BDL	4.11±0.429
C-22	3.98±0.152	0.11±0.156	BDL	BDL	3.92±0.558
C-23	2.65±0.118	0.032±0.0141	BDL	BDL	5.71±0.414
C-24	1.75±0.097	0.78±0.194	BDL	BDL	6.44±0.508
C-25	2.09±0.104	1.54±0.215	BDL	0.044±0.025	3.88±0.237
Overall Mean	2.707	0.567	0.010	0.190	2.901

BDL: Below detectable limit, SE: Standard Error

LEAD

Lead contamination in food can be through different routes, and the major route of lead exposure to non-smokers is through food and water (Othman, 2010). Unfortunately, despite being a major route of exposure, there are no limits defined by most countries for lead concentration in chocolates, except in Poland, where the acceptable limit for lead concentration in chocolates is 0.30 ppm (Jalbani et al., 2009). In this study, lead was found in all the chocolate samples in the range of 0.54–4.76 ppm. The values obtained in this study are compared with studies conducted worldwide over the past two decades to analyse the difference in heavy metal concentrations in different countries. The comparative data is shown in Table 3. According to previous research, the lead concentration in the current study was the highest. Most of the studies showed that the chocolate samples contained lead concentrations below 1 ppm, except in a few studies. A recent study found lead concentrations of 2.1 ppm in the United States and 1.8 ppm in Italy (Nnuro et al., 2020). Two previous studies in Pakistan, conducted in 2009 and 2012, found lead concentrations in chocolate and cocoa to be in the range of 2.480±19 ppm (Jalbani et al., 2009) and 0.061.4 (Rehman and Husnain, 2012). Health effects caused by lead are listed in Table 3.

NICKEL

Nickel is naturally present in the soil, and it is also emitted from volcanoes, but Ni pollution in the environment is mainly caused by fuel consumption or municipal and industrial waste generation (Genchi *et al.*, 2020). Ni is considered as an essential metal and is required by animals and plants in a very low concentration of about 0.001 to 2.64 micrograms per day (Ravishankar and Sridevi, 2020). In the present study, Ni is found in all the samples, and in this study, it is reported in the range of 0.034 to 1.74 ppm. In previous studies, significant levels of Ni were found in chocolate and cocoa samples, which were higher than the concentrations found in our study. The highest concentrations of Ni found in chocolate and cocoa samples are 4.23 ppm (Prakash *et al.*, 2014), 4.33 ppm (Jalbani *et al.*, 2009), 5.29 ppm (Prakash *et al*., 2014), 5.65 ppm (Devi *et al.*, 2016), 7.9 ppm (Iwegbue, 2011) and 8.29 ppm (Dahiya *et al.*, 2005), respectively, reported in different studies around the globe. The lowest concentration of Ni in chocolates has been reported in Pakistan in 2012, with a Ni concentration in the range of 0.0002–0.005 (Rehman and Husnain, 2012).

Chocolates are usually consumed by children more than adults, and the susceptibility of heavy metal poisoning is higher in children than in adults. Thus, heavy metal contamination in chocolates or raw materials used in chocolate production is a major public concern (Needleman, 2004). Even though Ni is a heavy metal and it can cause health effects in children and adults, there are no acceptable limits of Ni in candies and chocolates followed by any country. The health effects caused by Ni are discussed in Table 2.

Table 2. Health effects caused by heavy metals.

Heavy Metal	Health Effects	References
Lead	Encephalopathy, nausea and vomiting, CNS disease, circulatory and cardiovascular systems, learning and concentration problems in children, Accumulation of erythrocytes protoporphyrin, anaemia, abdominal pain, nephropathy, possibly human carcinogen.	(Ma, 2011; Järup, 2003; Soghoian and Sinert, 2009; El-Kady and Abdel-Wahhab, 2018)
Chromium	Kidney/renal dysfunction/failure, haemolysis and gastrointestinal haemorrhage, Collapse respiratory system through lung cancer and pulmonary fibrosis, shortness of breath wheezing, phlegm, nasal itching and soreness, pneumonia, impaired pulmonary functioning, and bronchitis	(Dong *et al.*, 2007) (Soghoian and Sinert, 2009) (Saha *et al.*, 2011)
Nickel	Renal dysfunction, affect body weight, allergy, cardiovascular and kidney diseases, lung fibrosis, lung, and nasal cancer.	(Buxton *et al.*, 2019)
Arsenic (metalloid)	Multi-organ dysfunction, tumors, encephalopathy, bone marrow, depression, hepatomegaly, melanosis, diarrhea, severe neuropathy, long QT, syndrome, peripheral vascular disease, black foot disease, lunger cancer, kidney cancer, bladder, and skin cancer, diabetes and cardiovascular diseases	(Järup, 2003; Soghoian and Sinert, 2009; Islam et al., 2015; El-Kady and Abdel-Wahhab, 2018; Palma-Lara e*t al.*, 2020)
Iron	malignant transformations, severe damage to cell organelles, tissue damage, mutation, Iron toxicosis, gastrointestinal bleeding, vomiting, diarrhoea, shocks, hypotension, lethargy, tachycardia, hepatic necrosis, metabolic acidosis, sometimes death, cancer, cell death	(Bhasin *et al.,* 2002; Jaishankar *et al.,* 2014)

ARSENIC

Arsenic poisoning affects millions of people in the world each year, and ground water contamination by arsenic is the major cause of arsenic poisoning in humans. The other sources of arsenic poisoning in humans are through food or skin contact. Arsenic is abundant in the environment and even terrestrial animals and marine organisms contain significant concentrations of arsenic, i.e., 0.06–0.4 ppm and 0.78–25 ppm, respectively (Mochizuki, 2019). In this study, arsenic has been found in chocolate samples with a mean value of 0.010 ppm, which is less than that of lead and nickel, but arsenic is known to cause high toxicity even in low concentrations (Järup, 2003). Acceptable daily intake of As for adults is 11.8-13.9 ppm body weight per day (Roychowdhury *et al.*, 2003). Similar studies have been conducted in Pakistan and arsenic was not found in any of the samples. The comparative data of arsenic concentration found in different studies has been listed in Table 3. The highest concentration of arsenic was found in a chocolate sample from Libya with a concentration of 32.0 ppm (Alkherraz *et al.*, 2009), whereas the lowest concentration has been reported in Saudi Arabia with a concentration of 0.009 ppm (Salama and Radwan, 2017). Arsenic has been associated with numerous health issues, and health problems caused by arsenic are listed in Table 2.

Table 3. Comparative data of heavy metal concentration in Chocolates.

S #	Studied sample	Country	Heavy metal concentration (ppm) Range in chocolates and candies					Reference
			Pb	Ni	As	Cr	Fe	
1.	Unbranded chocolates	Pakistan	0.54-4.76	0.11-1.74	BDL-0.098	0.00-0.87	0.31-7.12	This Study
2.	Chocolates	India	n.d.–4.00	n.d.–5.65	N/A	N/A	N/A	(Devi *et al.*, 2016)
3.	Cocoa powder	Israel	0.103-0.009	N/A	0.006-0.041	1.31-0.121	N/A	(Yanus *et al.*, 2014)
4.	Cocoa butter	Israel	0.6785 - 0.6755	N/A	0.01027 - 0.00827	0.29-0.204	N/A	(Yanus *et al.*, 2014)
5.	Candies and chocolates	Brazil	<0.08–2.30	1.4–7.9	N/A	N/A	N/A	(Iwegbue, 2011)
6.	Candies and chocolates	Philippine	0.05–1.10	N/A	N/A	N/A	N/A	(Ieggli *et al.*, 2011)
7.	Chcolate cherry	Pakistan	2.48 ±0.19	4.33 ±0.25	N/A	N/A	N/A	(Jalbani *et al.*, 2009)
8.	Chocolates	India	0.24–8.04	0.05–8.29	N/A	N/A	N/A	Dahiya *et al.*, 2005
9.	Cocoa based chocolate	Nigeria	0.0119 - 0.0698	N/A	N/A	N/A	N/A	(Rankin *et al.*, 2005)
10.	Cocoa based chocolate	Turkey	1.24±0.05	0.12±0.04	N/A	3.96±0.06	9.86±0.23	(Duran *et al.*, 2009)
			1.33±0.16	0.14±0.03	N/A	3.87±0.10	9.98±0.36	
11.	Chocolates	Saudi Arabia	0.017-0.239	N/A	N/A	N/A	N/A	(Othman, 2010)
12.	Cocoa	Libya	00-2.1	N/A	1.0-32.0	2.0-16.4	N/A	(Alkherraz *et al.*, 2009)
13.	Cocoa	US	2.1	N/A	N/A	2.9	9.0	Nnuro *et al.*, 2020
		ITALY	1.8	N/A	N/A	2.0	1.0	
		FRACE	N. D	N/A	N/A	16.4	32.0	
14.	Cocoa chocolate	Indonesia	< 0.10	N/A	< 0.01	N/A	N/A	(Assa *et al.*, 2018)
15.	Chocolate	India	N/A	0.77 - 5.29	0.03-0.90	N/A	N/A	(Prakash *et al.*, 2014)
	Candies			1.02 - 4.23	0.10-4.50			
16.	Cocoa products	Saudi Arabia	0.132-0.049	3.153-0.170	0.0230-0.009	0.444-0.307	N/A	(Salama, 2019)
17.	Cocoa powder	Italy	1.228 ± 0.146	N/A	0.094 ± 0.013	N/A	N/A	(Lo Dico *et al.*, 2018)
18.	Dark chocolate	US	0.002-0.11	N/A	N/A	N/A	N/A	(Abt *et al.*, 2018)
19.	Milk chocolate	US	0.002-0.07	N/A	N/A	N/A	N/A	
20.	Chocolate	Pakistan	0.06-1.4	0.0002-0.005	N/A	N/A	0.015-0.051	(Rehman and Husnain, 2012)

CHROMIUM

Chromium is an essential component for human physical and biochemical processes, and a lack of it can result in a variety of health effects or diseases, such as infertility and cancer (Sawut *et al.*, 2018). Chromium exposure occurs through skin contact, inhalation, or via food, and the major route of exposure is through the ingestion of contaminated food. In this study, chromium has been found in most of the samples in the range of BDL to 0.87 ppm, which is far less than that reported in the previous studies. Several other studies reported chromium in chocolates, sweets, and cocoa, and the findings of the studies are listed in Table 3. The highest value of chromium has been reported in France with a chromium concentration of 16.4 ppm in the cocoa sample (Nnuro *et al.*, 2020), whereas the lowest concentration has been reported in Israel with a concentration of 0.29 ppm (Yanus *et al.*, 2014). Chromium can cause several health effects on humans, which have been listed in Table 3, including respiratory and lung diseases (Yang and Massey, 2019).

IRON

Iron is among the most abundant metals in the earth's crust, and it takes 26th place among the elements in the periodic table (Abbaspour *et al.*, 2014). Iron is a cofactor for several proteins and enzymes and is vital for human growth and survival (Silva *et al.*, 2019). Despite being an important factor for human growth, iron can cause several diseases in humans, more significantly in children. Children are especially vulnerable due to their high consumption of iron-containing products (Albretsen, 2006). being the favourite sweets of children, have been studied to find the heavy metal content in them and, according to the findings of this study, iron concentration in chocolate products has been in the range of 0.31-7.12 ppm, which is consistent with most of the studies listed in Table 3. However, a study conducted in France in 2020 shows high concentrations of Fe (32.0 ppm) in chocolate products (Nnuro *et al.*, 2020), and it does not consist of the present study. The health effects caused by iron exposure are discussed in Table 2.

CONCLUSION

The chocolate samples analysed in this research were found to be contaminated with all the studied heavy metals, including Fe, Pb, Ni, As, and Fe with significant concentrations. As with Fe, it was found to be at the highest concentration among the other heavy metals, with as having the lowest mean concentration. But heavy metals can cause severe health effects to humans, even in small quantities, so it is important to act against the market availability of contaminated chocolates and sweets. None of the studied samples were found to be safe for human consumption, and it is proven by previous studies that chocolate contamination with heavy metals is a serious public health concern. Data from several studies has been discussed in this report to highlight the significance of controlling heavy metal contamination in chocolates and raw ingredients used in making chocolate to save humans from heavy metal exposure through ingestion.

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