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RESEARCH ARTICLE

Evaluation of heavy metals in different brands of chocolates marketed in Kathmandu, Nepal, and their associated health risks

Jasana Maharjan and Pawan Raj Shakya*

Department of Chemistry, Padmakanya Multiple Campus, Tribhuvan University, Kathmandu, Nepal
**Corresponding E-mail: pawansh2003@yahoo.com*

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Abstract

Chocolates are among the sweet food items consumed by all age groups particularly children in Nepal. However, this foodstuff may be contaminated with heavy metals from the raw ingredients, production, and packaging methods, which could bring serious health issues. Therefore, this study aimed to determine cadmium (Cd), nickel (Ni), and lead (Pb) by Flame Atomic Absorption Spectrophotometer (FAAS) in a total of thirty-seven different brands of milk-based, cocoa-based, and sugar-based chocolates available in local grocery shops of Kathmandu city, Nepal and to evaluate associated health risks in children and adults using USEPA deterministic approaches. The results revealed concentrations of Cd, Ni, and Pb in the range of 0.021 – 0.585, 1.90 – 7.24, and 0.57 – 4.29 mg/kg respectively in studied chocolates and an overall mean concentration of 0.199, 4.22, and 1.94 mg/kg respectively. The observed concentrations exceeded the maximum permissible limits set by FAO/WHO (2001). A higher concentration of all studied metals was found in cocoa-based chocolate compared to milk-based and sugar-based chocolates. The positive and significant correlations ($p < 0.05$) among Cd, Ni, and Pb in studied chocolates indicate the possibility of contamination from common sources. Similarly, the estimated ADD_{ing} values were higher for all metals in cocoa-based chocolate for both children and adults. However, children were more prone to metals exposure than adults since their dietary intake was higher than adults. Cocoa-based chocolate in this study posed a non-carcinogenic risk to both children and adults since their hazard index (HI) values exceeded the acceptable limit (>1.0). In addition, Cd and Ni posed carcinogenic risks to both receptor groups through the consumption of all three categories of chocolate. Therefore, this study suggested the use of less contaminated raw materials in chocolates as well as regular monitoring of the production chain as an attempt to ensure the quality and safety of the food products.

Keywords: Chocolates, heavy metals, dietary intake, health risk, Kathmandu

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Introduction

Chocolates are one of the most important confectionery products in the human diet and can be eaten at any time of the day. They are the favorite food items for children and are also gifted as an expression of love and fondness from family and friends on several occasions [1]. In addition, they are nutritious, easy to digest, compact in size, affordable, and can be stored for a long time. A variety of chocolates and candies with different tastes and flavors are easily available everywhere in local grocery shops and departmental stores. The composition of several types of chocolates depends on the ingredients used and the manufacturing process employed. Some basic ingredients used in chocolates are cocoa solids, milk, sugar, liquor, cocoa butter, glucose, vegetable oil, emulsifiers, buffering agents, etc [2]. Chocolates are a good source of carbohydrates, saturated fatty acids, proteins, sugar, vitamins, and minerals such as zinc, magnesium, phosphorous, calcium, potassium, and iron [3]. Due to the presence of flavonoids and alkaloids such as theobromine and phenyl ethyl amine in chocolates, they have antioxidant, anti-inflammatory, anti-depressant, and stimulant properties that help to lower blood pressure [4].

Despite having nutritional and medicinal values, contamination of chocolate products by heavy metals is, however becoming an unavoidable problem and emerging as a serious global concern [5]. All the confectionery manufacturers are inclined to a highly competitive market so they intend to attract consumers by using different colorful packages, wrappers, flavors, and different attractive colors and shapes in chocolates. Heavy metals may reach the chocolate products through several pathways such as raw ingredients like cocoa powder used in sweets [6], manufacturing process, manufacturing vessels, unsafe storage [7], colorful printing inks [8], and colorful packaging and wrappers [9]. Children are the most targeted groups so they are at high potential risk due to heavy metal toxicity as the metals are easily and effectively absorbed in children causing different bio-toxic effects [10].

Heavy metals are the most ubiquitous, non-biodegradable, and persistent environmental contaminants [11]. The levels of heavy metals are of great significance in foodstuffs because they are either essential or toxic to human health and cause different toxic effects upon exceeding the maximum permissible limits [12]. They make their path to the human body by different routes such as food, water, air, and dermal contact. Some heavy metals such as Pb, Cd, Cr, Ni, Fe, Cu, and Zn are commonly found in small concentrations in foods which may be bio-toxic to humans if present more than safe limits [13,14]. These contaminated food products contribute to human dietary intake and can accumulate gradually in the human body tissue over time which can damage different organs such as the kidney, liver, lungs, and central nervous system [15]. They also cause physical, muscular, and neurological degenerative diseases. Besides, the toxicity of metals could be neurotoxic, carcinogenic, mutagenic, or teratogenic [7].

Heavy metal contamination in chocolate products can arise at any stage from the chain of production till it reaches the consumers. The chain of foodstuff production includes the use of raw materials, processing method, packaging, transportation, delivery, storage, or marketing [16]. It is, therefore necessary to monitor regularly the concentration of metals in these food items in order to ensure that the metals do not cause any detrimental effect on human health. Children are the most susceptible age group to toxic metal intake from the consumption of such food items and hence, they are often at high risk than adults. The concentrations of heavy metals in selected brands of chocolates and candies have been carried out by many researchers around the world. Some of the literature includes studies in countries like India [2], Pakistan [17], Iran [18], Nigeria [19], Brazil [20], and Saudi Arabia [21]. However, the studies conducted on the concentration of heavy metals in chocolate products and their associated health risks to the human population in Nepal are still scanty in the literature. For this reason, this study aimed to determine the concentration of heavy metals (Cd, Ni, and Pb) in different brands of chocolates available in local grocery shops in Kathmandu and evaluate non-carcinogenic and carcinogenic health risks in children and adults. In light of this event, the findings of the present work would serve as a piece of baseline information for public awareness as well as helps support further investigation in the related area in the future.

Experimental

Sample collection

A total of thirty-seven different brands of chocolate samples that are most commonly favored by consumers were randomly selected and purchased from local grocery shops located in Kathmandu. The collected samples were grouped into three categories of chocolates *viz.*, milk-based, cocoa-based, and sugar-based based on their labeling and ingredients used. Among the analyzed samples were 15 different brands of milk-based, 10 cocoa-based, and 12 sugar-based. For the sake of convenience in analysis, the milk-based, cocoa-based, and sugar-based samples were coded as MBC, CBC, and SBC respectively. Three replicates of each brand product with different batches and packing dates were chosen randomly to observe possible variations in the elemental concentrations. The collected samples were dried at 70 ^{0}C for 2 hours. The dried samples were homogenized to powder in a stainless-steel blender and stored in plastic bottles at -4 ^{0}C till further analysis.

Analytical reagents and chemicals

All reagents used in the analysis were of analytical grade. Standard stock solutions (1000 ppm) for Cd, Ni, and Pb were certified and purchased from E. Merck, Germany. These solutions were diluted carefully to the required concentrations with doubly distilled water to prepare the calibration standards. Concentrated nitric acid (65%), and hydrogen peroxide (30%) were of analytical grade and used without further purification. All apparatus including the glassware and plastic vessels were treated with dilute (1:1) nitric acid for 24 h and then rinsed with distilled water before use. Doubly distilled water was used throughout the experiment.

Sample preparation

A standard protocol as described by Jalbani et al. [22] was followed for sample digestion and preparation of sample solution for metal analysis. Accordingly, 0.5 g of each brand of the homogenized samples was taken in a digestion tube. The content of the tube was treated with 10 mL of conc. HNO_3 (65 %, v/v) and 4 mL of H_2O_2 (30%, v/v) for the decomposition of organic matter. The mixture was placed in a chemical hood overnight so as to ensure complete dissolution and prevent foaming during the subsequent digestion process. The sample was digested at 80 ^{0}C, for 2-3 hours using a block digestor (Hanon SH420F Kjedahl) till the solution became completely clear. After complete digestion and sufficient cooling, the digested solution was filtered into a 25 mL volumetric flask using filter paper (Whatman 42). The digestion tube was rinsed with distilled water again and transferred the solution into the same volumetric flask. Then, the final volume was made with distilled water through homogeneous mixing. Solutions of all the samples were prepared in an analogous way and in triplicate. The blanks (without samples) were prepared in the same manner as the samples.

Instrumentation and metal analysis

The concentrations of Cd, Ni, and Pb in the digested samples were determined by Flame Atomic Absorption Spectrophotometer (Perkin Elmer AAnalyst 800) using air-acetylene flame [23]. Standard solutions were prepared in series using the reference metals and then standard calibration curves were constructed at specific wavelengths for detecting the metals under study. The instrumental parameters were used as described by the manufacturer. The operating parameters of FAAS include wavelengths of 228.8, 232.0, and 228.3 nm for Cd, Ni, and Pb respectively, cathode lamp current as 7.5 mA for Cd, 10.0 mA for Ni and 7.5 mA for Pb and slit-width as 1.3 nm in Cd and Pb, and 0.2 nm in Ni.

At least three samples were selected from each category of the collected samples for the spike analysis of each analyte. A known quantity of analyte was added to 1.0 g of selected samples. Standard reference materials traceable to NIST manufactured by Merck, Germany were used to prepare fortified samples. Fortified samples were treated as per the sample and the heavy metal level in the fortified samples was identified by FAAS. The average percent recovery for Cd, Ni, and Pb was 99.6, 97.8, and 98.2 respectively. The standard deviation for the pretested samples was calculated to be 2.0, 2.5, and 2.8 % for Cd, Ni, and Pb respectively. The calculated detection limits were 9.0, 3.0, and 9.0 µg/L for Cd, Ni, and Pb respectively.

Health Risk Assessment

Health risk assessment estimates the total exposure to heavy metals (HMs) in humans through three different pathways *viz.*, ingestion, dermal absorption, and inhalation. However, ingestion is the main pathway of HMs exposure in the present study. For risk assessment of HMs in two receptor groups (children and adults) through the consumption of chocolates, equations (1-4) were used. The equation and receptor parameters (Table 1) used in the present study are based on US Environmental Protection Agency (USEPA) [24,25].

Table 1. Receptor parameters used for characterization of human exposure to HMs from chocolates.

Parameter	Units	Child	Adult
Age	year	6-12	21-70
Ingestion rate (IR_{ing})	g/day	30.0	50.0
Exposure frequency (EF)	days/year	320	365
Exposure duration (ED)	year	7.0	70
Avg. body weight (BW)	kg	30.0	60.0
Average time (AT)	days	(365 days/year × ED)	(365 days/year × ED)

Reference dose (R*f*D) and cancer slope factors (SF) for HMs

Parameter	Heavy metals (HMs)		
	Cd	Ni	Pb
R*f*D (mg/kg/day)	1.0×10^{-3}	2.00×10^{-2}	3.5×10^{-3}
SF $(mg/kg/day)^{-1}$	6.3	4.4	8.5×10^{-3}

Average Daily Dose (ADD_{ing}):

The average daily dose of each heavy metal through the consumption of chocolates was calculated using Eq. 1.

$$ADD_{ing} = C \times \frac{IR_{ing} \times EF \times ED}{BW \times AT} \times 10^{-3} \qquad \text{Eq. (1)}$$

Where, ADD_{ing} is the average daily dose of ingestion (mg/day/kg bw); C is the concentration of heavy metal (mg/kg) in a chocolate sample; IR_{ing} is the ingestion rate of the chocolate sample (g/day); EF is the exposure frequency (days/year); ED is the exposure duration (years); BW is the average bodyweight (kg) and AT is the averaging time for non-carcinogens (days) and 10^{-3} is the conversion factor. The receptor parameters are summarized in Table 1.

Non-carcinogenic Health Risk

The methodology for the estimation of non-carcinogenic risks in children and adults was applied in accordance with the provision of USEPA [25].

Hazard Quotient (HQ):

The non-carcinogenic risk for each heavy metal was assessed using the hazard quotient (HQ), which is the ratio of a single metal exposure level over a specified time period to a reference dose (R*f*D) for that metal derived from a similar exposure period. Eq. (2) was used for estimating HQ as follows:

$$HQ = \frac{ADD_{ing}}{RfD} \qquad \text{Eq. (2)}$$

Where ADD_{ing} is the average daily dose of ingestion (mg/day/kg) and R*f*D is the reference oral dose for heavy metals (mg/kg/day). The oral reference doses (RfD) for HMs are given in Table 1.

Hazard Index (HI):

In order to assess the overall potential for non-carcinogenic effects from more than one heavy metal, a hazard index (HI) is used. Since different heavy metals can cause similar adverse health effects, HI is calculated as the sum of hazard quotients (HQs). The index is based on the Guidelines for Health Risk Assessment of Chemical Mixtures of the US Environmental Protection Agency (USEPA) [24] and calculated using Eq. (3) as follows:

$$HI = \sum HQ = \sum \frac{ADD_{ing}}{RfD} \qquad \text{Eq. (3)}$$

In the event of $HI \leq 1$, adverse health effects would be unlikely to occur. However, potential non-carcinogenic effects would occur when $HI > 1$ as this indicates a significant non-carcinogenic risk posed to human health.

Lifetime Cancer Risk (CR)

Lifetime cancer risk (CR) is the lifetime probability of an individual developing any type of cancer due to carcinogenic daily exposure to a contaminant over a lifetime. Eq. (4) was used to estimate lifetime cancer risk [24].

$$CR = ADD_{ing} \times SF \qquad \text{Eq. (4)}$$

Where ADD_{ing} is the average daily dose of ingestion (mg/day/kg) and SF is the oral carcinogenic slope factor $(mg/kg/day)^{-1}$. The SF values for HMs are given in Table 1.

In general, the excess cancer risks with a CR value lower than 1.0×10^{-6} are considered to be negligible, a CR value above 1.0×10^{-4} is considered unacceptable, and a CR value lying between 10^{-6} and 10^{-4} is generally considered an acceptable range.

Statistical analysis

Data processing and statistical analysis were carried out on IBM-PC computer using EXCEL spreadsheets. Descriptive statistics (frequency, mean, range, standard deviation, etc.) were performed after the elemental analysis. Pearson's correlation coefficient was used to evaluate the correlation among the metals along with a significance test.

Results and discussion

Concentrations of heavy metals (HMs) in chocolates

The collected three different categories of chocolate samples were analyzed for Cd, Ni, and Pb, and their

Table 2. Concentration (mg/kg) of heavy metals in chocolates.

*Sample code	Statistical parameter	Heavy metals			
		Cd	Ni	Pb	$\sum_3$HMs
SBC	Mean ± SD	0.060 ± 0.012	2.51 ± 0.64	1.23 ± 0.53	3.80
(n = 12)	Range	0.021- 0.102	1.90 - 3.73	0.57- 1.96	-
CBC	Mean ± SD	0.375 ± 0.098	5.66 ± 1.09	3.09 ± 0.94	9.13
(n = 10)	Range	0.193 - 0.585	3.89 - 7.24	2.00 - 4.21	-
MBC	Mean ± SD	0.161 ± 0.047	4.49 ± 0.95	1.51 ± 0.46	6.16
(n = 15)	Range	0.076 - 0.289	3.72- 5.75	0.70- 2.54	-
Overall mean		0.199	4.22	1.94	6.36
**MAC (FAO/WHO, 2001)		0.05	0.1 - 0.5	0.1	-

*SBC: Sugar-based chocolate; CBC: Cocoa-based chocolate; MBC: Milk-based chocolate;
**MAC: Maximum allowable concentration

observed concentrations are depicted in Table 2.

The analytical results revealed variations in elemental concentrations among the three analyzed categories. It was found that concentrations of Cd, Ni, and Pb were in the range of 0.021 – 0.585, 1.90 – 7.24, and 0.57 – 4.29 mg/kg in studied chocolate samples respectively. In comparison to milk-based (MBC) or sugar-based (SBC), cocoa-based (CBC) chocolate had higher metal content. Accordingly, CBC measured comparatively the highest (9.13 mg/kg) and SBC the lowest concentration (3.80 mg/kg) of $\sum_3$HMs in the present study (Table 2). The findings of the present study are in agreement with Katiyar et al. [26] who also found relatively high metal levels in cocoa-based chocolates followed by milk-based and fruit-flavoured candies. Besides, all three categories of chocolate showed their overall mean concentrations in the descending order of Ni (4.22 mg/kg) > Pb (1.94 mg/kg) > Cd (0.199 mg/kg). However, all these mean values were found to exceed the maximum allowable concentration (MAC) recommended by FAO/WHO [27]. The sources of metal contamination in chocolates may be attributed to raw materials used, production processes, leaching from the vessels for storage or wrappers, packing, and also contamination from the environment [22]. Duran et al. [6] and Ochu et al. [7] also reported higher levels of metals in chocolate samples from unsafe storage conditions or during the production chain that involves the use of raw materials, processing, packaging, transportation, storage, or marketing. Besides, batch-batch discrepancies in production, differences in brand production methods as well as pollution from exogenous sources may equally contribute to metal contamination [27]. Thus, the chocolates provide a great contribution to the dietary intake of toxic metals, due to the high concentration observed. Moreover, dark chocolate reportedly contains a higher content of cocoa providing a reliable indicator of antioxidant activity along with an excellent nutritional source of Cu, Mn, Fe, and Mg [28].

Cadmium (Cd)

The mean concentration of Cd in SBC, CBC, and MBC was found 0.060, 0.375, and 0.161 mg/kg respectively (Table 2). Accordingly, CBC measured the highest concentration of Cd followed by MBC and SBC. The observed concentration of Cd in each category of chocolate samples was also higher than the FAO/WHO [27] recommended safe limit of 0.05 mg/kg. Elevated Cd content in cacao and cocoa-based chocolates brings a serious health risk in humans although a recent threshold of 0.8 mg/kg has reportedly been established in some regulations [29]. Cocoa beans can accumulate Cd through cocoa plants naturally from soil, and hence, its concentration could vary significantly in chocolates [30]. The source of Cd in soils is from rock phosphate fertilizer, weathering of rocks, mining, and smelting. Besides milk and sugar, cocoa is a main ingredient in chocolate and hence the ingredient used could be a potential source of metal contamination in food products [22]. Cadmium is a non-essential metal and highly toxic. Therefore, the metal could be detrimental to living organisms even at low concentrations [31]. It could lead to the death of cells or increase their proliferation [16]. Cadmium is classified as a group IA human carcinogen by the International Agency for Research on Cancer (IARC) [32] due to its toxic nature. Chronic exposure to Cd results in lung, liver, kidney, and prostate cancer, skeletal damage, inhibits DNA repair enzymes, causes an inflammatory response and disturbance in calcium metabolism, and fragile bones [33].

Nickel (Ni)

Among HMs in the present study, Ni content was measured as highest in all analyzed categories of chocolate samples (Table 2) in contradiction with the findings of Alamgir et al. [34] who reported higher Pb content than Ni in studied chocolate samples. Accordingly, the mean concentration of Ni in SBC, CBC, and MBC was found to be 2.51, 5.66, and 4.49 mg/kg respectively. These mean values were found to cross the maximum allowable concentration (0.1 – 0.5 mg/kg) for Ni in the food product set by FAO/WHO [27]. The variations in the metal concentration among the samples could be due to the variation in raw ingredients [2]. Dahiya et al. [35] in their study reported the manufacturing process as a source of Ni contamination in chocolates when the metal is used as a catalyst. Similarly, Duran et al. [6] also reported processing methods and cocoa beans accumulated with Ni as the possible sources of Ni contamination in chocolates. Nickel is an essential trace element that plays an important role in enzyme activation for biological function in our body. But its presence above the permissible limit causes different toxic effects in humans such as contact dermatitis, headache, gastrointestinal disorder, lung fibrosis, nasal cancer, and respiratory problem [36]. The International Agency for Research on Cancer (IARC) [37] has determined metallic nickel and some nickel compounds as carcinogenic to humans.

Lead (Pb)

Lead is a serious cumulative body poison that enters into the body system through the air, water, and food [12]. Like Cd and Ni in the present study, Pb was found to contaminate all analyzed chocolate samples in descending order of CBC > MBC > SBC. The mean concentration of Pb ranged from 1.23 mg/kg (SBC) to 3.09 mg/kg (CBC). It was observed that Pb levels in these samples crossed the safe limit of 0.1 mg/kg recommended by FAO/WHO [27] suggesting these foodstuffs as Pb contaminated that might pose a risk to humans. Reportedly high Pb level in CBC is most likely due to soil texture contaminated with Pb from the injudicious use of agrochemicals and fertilizers during cocoa farming [38]. Rankin et al. [39] reported atmospheric emissions of leaded gasoline as a potential source of Pb contamination in chocolates as cocoa bean shells have a higher capacity to absorb Pb during the fermentation and sun-drying of unshelled beans at cocoa farms. Similarly, Kim et al. [9] in their report attributed the source of Pb contamination to chocolate packaging as the packaging materials contained significantly higher levels of Cr and Pb. The higher Pb content in the case of green and yellow-coloured packaging materials or wrappers might have originated from the Pb-based coloured ink used to print the packaging. Besides, heavy metal migration from packaging or wrappers to foodstuffs by direct or indirect contact has also been reported in some studies [40, 41]. Lead, like that of Cd, is a non-essential and highly toxic element that is associated with neurotoxicity, nephrotoxicity, and a variety of other health disorders [42]. Lead has been classified as a group IIA human carcinogen by IARC [37] due to its toxic nature. High bioaccumulation of Pb in the human body results in respiratory, neurological, urinary, and cardiovascular disorders and causes inflammatory responses in different body organs [43].

Correlation of HMs in chocolate samples

Correlation analysis is a valuable tool utilized in HMs data analyses for studying the inter-relationship of paired data [44]. The inter-relationship of metals can provide significant information about their pathways and sources of origin [45]. In this study, Pearson's correlation coefficient analysis among the studied HMs in all three categories of chocolate samples was applied and is depicted in Table 3. In SBC, Cd was strongly and positively related with Ni (r = 0.819, $p < 0.05$), and Pb (r = 0.948, $p < 0.05$), indicating a similar source of origin. Likewise, Ni was significantly and positively correlated with Pb (r = 0.943, $p < 0.05$). For CBC, Cd was positively correlated with Ni (r = 0.736, $p < 0.05$) and Pb (r = 0.635, $p < 0.05$) while Ni was strongly and significantly correlated with Pb (r = 0.949, $p < 0.05$). Similar to SBC, MBC also showed Cd positively and strongly correlated with Ni (r = 0.918, $p < 0.05$), and Pb (r = 0.825, $p < 0.05$) indicating a common source of origin. Also, Ni was strongly and significantly linked with Pb (r = 0.979, $p < 0.05$) in the same sample.

Table 3. Correlation of heavy metals in chocolate samples

Sugar-based chocolate (SBC)				Cocoa-based chocolate (CBC)				Milk-based chocolate (MBC)			
	Cd	Ni	Pb		Cd	Ni	Pb		Cd	Ni	Pb
Cd	1.000	*0.819	*0.948	Cd	1.000	0.736	0.635	Cd	1.000	*0.918	*0.825
Ni	*0.819	1.000	*0.943	Ni	0.736	1.000	*0.949	Ni	*0.918	1.000	*0.979
Pb	*0.948	*0.943	1.000	Pb	0.635	*0.949	1.000	Pb	*0.825	*0.979	1.000

*Correlation is significant at $p < 0.05$

The positive and significant correlations among Cd, Ni, and Pb indicate the possibility of contamination from common sources such as raw materials, processing methods, packaging, wrappers, storage, transportation, and also from the environment [6,7,22].

Comparison of HMs in chocolate samples with previous literature

A comparative overview of heavy metal levels in chocolate samples reported from different countries across the globe is displayed in Table 4.

Table 4. Comparison of heavy metal concentrations (mg/kg) in chocolate samples reported from different countries.

Country	Cd	Ni	Pb	References
Nepal	0.199	4.22	1.94	Present study
India	0.170	0.84	2.00	[2]
Pakistan	1.438	0.22	1.42	[17]
Iran	0.012	-	2.61	[18]
Nigeria	-	15.78	7.39	[19]
Brazil	0.029	-	0.05	[20]
Saudi Arabia	0.014	0.98	0.07	[21]
Malaysia	0.070	-	-	[46]

The mean concentrations of Cd, Ni, and Pb observed in this study are compared against those reported data (Table 4). Accordingly, the level of Cd (0.199 mg/kg) in this work was comparable with the chocolate samples available in India but higher than those available in Malaysia, Iran, Nigeria, Brazil, and Saudi Arabia (Table 4). Among the countries under the present comparison, Pakistan reported the highest concentration of Cd (1.438 mg/kg) in the marketed chocolate samples. For Ni, Nigeria reported the highest concentration (15.78 mg/kg) among the countries. The level of Ni (4.22 mg/kg) in this work was, however higher in comparison to those reported for chocolate samples available in Pakistan, India, and Saudi Arabia. Likewise, the level of Pb (1.94 mg/kg) in this study was found to be higher than those reported for Pakistan, India, Brazil, and Saudi Arabia but less than for Iran and Nigeria. The variations among the concentration reported by various researchers (Table 4), could be due to the variation in raw ingredients. Moreover, water and milk are basic raw ingredients, and their level also varies naturally. Hence, it may be assumed that the variation in the metal concentration in chocolates could be due to their geographical origin [13].

Health risk assessment

Average daily dose (ADD_{ing}) of heavy metals

The ADD_{ing} of heavy metals depends on both the metal concentration in the food products and the associated ingestion rate. Our preliminary survey revealed 30 and 50 g/day of chocolates as ingestion rates for children and adults respectively. The ingestion rates for the target receptors were assumed on the basis of the weight and frequency of chocolate consumption. Additionally, body weight can also influence tolerance to contaminants. Therefore, an average body weight of 30 and 60 kg was considered for children (6-12 years) and adults (21-70 years) respectively in this study. Details on receptor parameters for the estimation of ADD_{ing} are given in Table 1.

Table 5. Average daily dose (ADD_{ing}) of heavy metals (mg/day/kg bw) from consumption of chocolates by children and adults.

Sample code	Average daily dose (ADD_{ing}) ($\times 10^{-3}$)					
	Cd		Ni		Pb	
	Children	Adult	Children	Adult	Children	Adult
SBC	0.05	0.05	2.20	2.09	1.08	1.02
CBC	0.33	0.31	4.96	4.71	2.71	2.57
MBC	0.14	0.13	3.94	3.74	1.32	1.26
Overall	0.17	0.16	3.70	3.51	1.70	1.61

The estimated ADD_{ing} of metal value through the products studied is shown in Table 5. Results revealed that the ADD_{ing} values for the samples ranged from 0.05 – 0.33 (Cd), 2.20 – 4.96 (Ni), and 1.08 – 2.71 mg/day/kg (Pb) for children while for adults 0.05 – 0.31 (Cd), 2.09 – 4.71 (Ni), and 1.02 – 2.57 mg/day/kg (Pb). The trend of estimated ADD_{ing} in all three chocolate categories was observed in the descending order of Ni >Pb> Cd for both children and adults. This indicates comparatively higher ingestion of Ni compared to Pb and Cd. Although the ADD_{ing} values between the two receptor groups were found to be almost comparable; children however showed slightly higher ADD_{ing} values compared to adults. Besides, the foodstuff studied showed their ADD_{ing} values in the descending order of CBC > MBC > SBC for both receptor groups. The overall mean ADD_{ing} values of Cd, Ni, and Pb for children were 0.17×10^{-3}, 3.70×10^{-3}, and 1.70×10^{-3} mg/day/kg respectively, and for adults 0.16×10^{-3}, 3.51×10^{-3}, and 1.61×10^{-3} mg/day/kg respectively. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) [47] has established Provisional Tolerable Daily Intake (PTDI) of 1.0, 5.0, and 3.6 µg/day/kg bw for Cd, Ni, and Pb respectively. Accordingly, all chocolate products in this study showed their respective ADD_{ing} values below the tolerable intake limits issued by JECFA [47]. In general, children can absorb metals more easily than adults. For instance, adults can absorb 10% of Pb through the gastrointestinal tract whereas 40-50% of the metal has been reported for children [48]. The susceptibility of young children to the adverse effects of toxic metals is reported to be higher than adults [49]. Therefore, the level of toxic contaminants in these food products and the raw materials used for their production are a matter of great concern for public health as well as food safety.

Non-carcinogenic and carcinogenic health risks

All three HMs (Cd, Ni, and Pb) were included for estimating the health risk because of their potential toxicity to humans [50]. The non-carcinogenic and carcinogenic health risks of these HMs were estimated for children (6-12 years) and adults (21-70 years). The human health risk of HMs through consumption of all three categories of studied chocolates was estimated separately and is presented in Tables 6 and 7. The estimated hazard quotient (HQ) and hazard index (HI) for non-carcinogenic risk representing both receptor groups are presented in Table 6.

Table 6. Non-carcinogenic risk of heavy metals in children and adults from consumption of chocolates.

Sample code	Hazard quotient (HQ)						Hazard index (HI)	
	Cd		Ni		Pb			
	Children	Adult	Children	Adult	Children	Adult	Children	Adult
SBC	0.050	0.050	0.110	0.104	0.308	0.291	0.468	0.445
CBC	0.330	0.310	0.248	0.235	0.774	0.734	1.352	1.279
MBC	0.140	0.130	0.197	0.187	0.377	0.360	0.714	0.677

The results showed HQ values for both children and adults close to each other. The estimated HQ values for both receptor groups are less than 1 in all chocolate samples. Jia et al. [51] also reported a THQ value of less than 1 for Cd in different brands of chocolate samples in China. The HI value represents the cumulative effect of all the concerned metals on the consumption of foodstuff. In this study, only CBC showed a higher HI value (>1) for both children and adults (1.352 and 1.279, respectively), while SBC and MBC had comparatively lower HI values for both receptor groups. This suggests a significant non-carcinogenic risk of cocoa-based chocolate (CBC) for both children and adults in this study area in agreement with the findings of Salama [21] who also obtained non-carcinogenic risk from cocoa products. However, the higher HI value for children than adults suggested that children were more vulnerable to non-carcinogenic risk than adults due to CBC consumption.

Table 7. Carcinogenic risk of heavy metals in children and adults from consumption of chocolates.

Sample code	Lifetime cancer risk (CR)					
	Cd		Ni		Pb	
	Children	Adult	Children	Adult	Children	Adult
SBC	3.15×10^{-4}	3.15×10^{-4}	9.68×10^{-3}	9.20×10^{-3}	9.18×10^{-6}	8.67×10^{-6}
CBC	2.08×10^{-3}	1.95×10^{-3}	2.18×10^{-2}	2.07×10^{-2}	2.30×10^{-5}	2.18×10^{-5}
MBC	8.82×10^{-4}	8.19×10^{-4}	1.73×10^{-2}	1.65×10^{-2}	1.12×10^{-5}	1.07×10^{-5}

The carcinogenic risk (CR) estimated for both children and adults is depicted in Table 7. It was found that the CR values for both receptor groups were close to each other in all studied chocolates. The CR values of Cd for both children and adults (3.15×10^{-4} - 2.08×10^{-3} and 3.15×10^{-4} - 1.95×10^{-3}, respectively) in all studied samples of chocolate exceeded the acceptable level of carcinogenic risk for human (1.0×10^{-6} – 1.0×10^{-4}). Similarly, the CR values of Ni for children and adults (9.68×10^{-3} - 2.18×10^{-2} and 9.20×10^{-3} - 2.07×10^{-2}, respectively) also exceeded the acceptable level. Therefore, it may be suggested that both Cd and Ni can pose a lifetime cancer risk on the consumption of all studied categories of chocolate. The CR values for Pb in all samples were, however within the acceptable range indicating no carcinogenic risk for both receptor groups. Hence, it is required to check raw materials including milk and milk products used in chocolates for residual metal levels and assess their possible health impacts for maintaining consumer safety [52].

Conclusions

In this study, concentrations of Cd, Ni, and Pb were investigated in three different categories of chocolate *viz.*, sugar-based, cocoa-based, and milk-based chocolate; and evaluated their associated health risks in children and adults. The analytical results confirmed that cocoa-based chocolate is a notable source of these metals as compared to milk-based and sugar-based chocolates. The higher concentration of studied metals in cocoa-based chocolate may be attributed to the metal-contaminated raw materials such as cocoa beans, cocoa solids, and cocoa butter used for their production. Among the metals, the highest concentration of Ni was observed in all chocolate types followed by Pb and Cd; all exceeding the maximum allowable concentrations set by FAO/WHO for the foodstuff. The positive and significant correlations ($p < 0.05$) among Cd, Ni, and Pb in studied chocolates indicate the possibility of contamination from common sources. Similarly, the ADD_{ing} values were estimated to be higher for Ni in cocoa-based chocolate for both children and adults; however, all values estimated for metals were below the maximum permissible limits issued by JECFA. Besides, children were also observed more prone to HMs exposure than adults since their ADD_{ing} values were found slightly higher than adults. This study also confirmed that cocoa-based chocolate posed a non-carcinogenic risk to both children and adults since their HI values exceeded the safe limit of 1. Likewise, Cd and Ni posed carcinogenic risks to both receptor groups through the consumption of all three categories of chocolate. Hence, to reduce dietary exposure to the human population, raw materials contaminated with a lower content of these metals should be used in chocolates, in addition to regular monitoring of the production chain to ensure the quality and safety of food products.

Conflict of interest

The authors declare that there is no conflict of interest pertinent to this work.

Author contributions

J. Maharjan: sample collection, investigation, data interpretation and analysis, writing original draft, and reviewing; **P. R. Shakya:** supervision, resources, conceptualization, data interpretation, and analysis, writing-reviewing, and editing. Both authors have read and agreed to the final version of the manuscript.

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