ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt
International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

Concentrations, Dietary Intake, and Risk Assessment of Heavy Metals (Pb, Cr, and Ni) from Some Brands of Biscuits Sold in Kathmandu, Nepal

Jasana Maharjan[1], Pramod Kumar Yadav[2], Pawan Raj Shakya[1]*

[1]Department of Chemistry, Padmakanya Multiple Campus, Tribhuvan University, Kathmandu, Nepal
[2]Department of Chemistry, Thakur Ram Multiple Campus, Tribhuvan University, Birgunj, Nepal

Article Information

Received: 12 May 2024
Revised version received: 17 June 2024
Accepted: 20 June 2024
Published: 28 June 2024

Cite this article as:
J. Maharjan et al. (2024) Int. J. Appl. Sci. Biotechnol. Vol 12(2): 100-114. DOI: 10.3126/ijasbt.v12i2.66062

***Corresponding author**
Pawan Raj Shakya,
Department of Chemistry, Padmakanya Multiple Campus, Tribhuvan University, Kathmandu, Nepal
Email: pawansh2003@yahoo.com

Abstract

Biscuits are flour-based bakery food with high global recognition and acceptance. However, heavy metal contamination in these foodstuffs is of great concern these days since they are a potential threat to consumer health. In this study, the concentration of Pb, Cr, and Ni was determined using a Flame Atomic Absorption Spectrophotometer (FAAS) in a total of 10 popular biscuit brands belonging to four major classes *viz*., hard sweet, crackers, short dough, and cookies, sold in local grocery shops of Kathmandu, Nepal. Besides, potential health risks of selected metals through consumption of the foodstuffs were also assessed for children and adults as two receptor groups using USEPA deterministic approaches. Results revealed that the concentrations of Pb and Cr in tested samples were in a range of 0.47- 1.00 mg/kg (mean 0.77 mg/kg) and 1.02 - 1.25 mg/kg (mean 1.17 mg/kg) respectively. Nickel was, however below the detection limit. The statistical analysis using one-way ANOVA revealed a significant difference ($P < 0.05$) in the mean concentration of heavy metals in different samples. The estimated average daily dose (ADD_{ing}) of selected heavy metals through the ingestion pathway was below the Provisional Tolerable Daily Intake (PTDI) limits. The hazard index (HI) was within the safe limit (<1) indicating no non-carcinogenic risk to both the receptor groups. Similarly, the metals posed no carcinogenic risks to the groups since the cancer risk (CR) index was within the safe limit (10^{-6} - 10^{-4}). However, regular monitoring of the production chain and toxic contaminants in the foodstuffs is highly recommended.

Keywords: Biscuits; dietary intake; heavy metals; Kathmandu; risk assessment.

Introduction

Biscuits are the oldest and most loved cereal-based bakery food of British origin derived from the Latin word *biscoctus* which means twice baked (Chavan *et al*., 2016). It is a crispy, nutritious, convenient, and versatile snack with a longer shelf-life and good taste. Wheat flour, sugar, fat, leavening agents, milk solids, salts, and emulsifiers are the key ingredients used in biscuits (Mancebo *et al*., 2015). Besides, coloring agents, flavoring agents, fortifying agents, improvers, etc., may be added to increase the nutritive value, texture, taste, and flavor of the foodstuffs (Haider *et al*., 2022). Biscuits serve as an important source of carbohydrates, vegetable proteins, bioactive compounds, fat and dietary fiber, and some vitamins and minerals (Ayensu *et al*., 2019). The foodstuffs can be categorized into hard sweets, crackers, short dough (molded biscuits), and cookies based on their appearance, sensory attributes, fat or sugar content, or even glazes and cream (Smith, 1972; Davidson, 2019).

Heavy metals in foodstuffs like biscuits are a great challenge to food safety, quality, and security. Due to the high global demand for improved foods, WHO has enlisted nourishment security among its 11 needs (Gizaw, 2019). The contaminants get their way to the food through any chain of manufacturing processes starting from raw material (Arigbede, 2019), manufacturing and processing, baking conditions, storage conditions, environmental contamination (Ungureanu *et al*., 2022), and colorful packing materials containing non-food grade printing inks (Kim *et al*., 2008). Heavy metals such as Pb, Cr, Ti, Zn, and Cu can migrate from the printed surface to the food contact surface through four mechanisms such as blocking, rubbing, peeling, and diffusion (Bradley *et al*., 2005).

Heavy metals are persistent and non-biodegradable environmental contaminants with bio-accumulative and bio-absorptive capacity (Zhang *et al.*, 2016; Dehghani *et al.*, 2017). The toxicity of heavy metals is highly influenced by the nature and concentration of heavy metals as well as the resistance of the host body (Das *et al*., 2011). They are broadly classified into essential and toxic elements. Essential elements or trace elements include elements such as Fe, Zn, Cu, Mo, etc., which act as micro-nutrients and play important roles in different metabolic activities, physiological and biochemical reactions such as bone concentration, protein, lipid, and metabolism and act as biocatalyst and control the glucose level in the blood (Mollazadeh, 2014; Akinola *et al*., 2008). They cause different acute or chronic toxicity if present below or above their permissible limits. Metals like Cd, Pb, Hg, etc. are toxic elements that have no biological functions. They cause different health hazards and toxic effects on the human body even in low concentrations (Iwegbue, 2012).

Heavy metal contamination in frequently consumed foodstuffs like biscuits is of high concern and a serious issue challenging consumer health. Among the major pathways, ingestion of food is the predominant and obvious route of heavy metals to the human body (Zhuang *et al*., 2009; Rahman *et al*., 2014; Nawab *et al*., 2018). Excessive adsorption and accumulation of heavy metals in different vital body organs such as kidneys, liver, bones, brain, heart, lungs, etc., leads to disruption in the physiological processes (Biffa *et al.*, 2020), cumulative toxicity, and multiple organs damage such as neurological disorders, liver, kidney, reproductive organ damage, cancer, birth defects, respiratory tract, and bone diseases, anemia, gastrointestinal disorder, cardiovascular disorder, etc (Adefris, 2011; Balali-Mood, 2021; Rai *et al*., 2019; Jarup, 2003; Amjad *et al*., 2021).

Many studies have been conducted in the related areas in many parts of the world. Some of the literature includes pertinent works in countries like Iraq (Haider *et al*., 2022; Taher *et al.*, 2023), Romania (Ungureanu *et. al*., 2022), Iran (Sobhanardakani, 2019), Nigeria (Illupeju *et al*., 2019; Oyekunle *et al.*, 2021) and Serbia (Kovacervic *et al*., 2019). However, there are a limited number of studies on the contamination of heavy metals in foodstuffs like biscuits and assessing their potential health risks through consumption in a developing country like Nepal. In light of this event, the present study was carried out to estimate heavy metals (Pb, Cr, and Ni) in some popular brands of biscuits sold in local grocery shops in Kathmandu and assess their potential health risks in children and adults as two receptor groups through their consumption. The present study is worth conducting to provide baseline information on account of public health importance and maintaining food safety.

Materials and Methods

Sample collection

In the initial phase of the present work, a market survey was made extensively to observe the sales, demand, and consumption ratio of different brands of biscuits locally available in grocery shops in Kathmandu. Ten popular brand names of biscuits of the high consumer demand category were selected on a lottery basis from a name list of biscuits surveyed earlier. Three replicates of each selected brand product (n=30) with different batches and packing dates were purchased for analysis assuming possible variations in the elemental concentrations. The purchased samples belonged to four different classes *viz.*, hard sweet, cracker, short dough, and cookies based on their labeling and ingredients used. Biscuit names, coding, class, ingredients, and manufacturing companies are summarized in Table 1**.**

Table 1: Details of the selected biscuit samples under the present study.

S. N.	Sample code	Biscuit name	Net wt.(g)	Class	Ingredients	Manufacturing Company
1.	B-1	Nebico Coconut Crunches	75	Cookies	Wheat flour, sugar, HVO, salt, SMP, invert syrup, coconut powder, emulsifier (E322), antioxidant (E319), dough raising agents (E 500, E503)	Nebico Pvt. Ltd.
2.	B-2	Parle Happy Happy	30	Cookies	Refined wheat flour, sugar, refined palm oil, Choco chips, sugar, hydrogenated oil, coca solids, emulsifier, soya lecithin, artificial flavoring substances, invert	Antartic Biscuit Pvt. Ltd.

Table 1: Details of the selected biscuit samples under the present study.

S. N.	Sample code	Biscuit name	Net wt.(g)	Class	Ingredients	Manufacturing Company
					sugar, raising agents (E 500, E503), iodized salt	
3.	B-3	Britannia Marie gold	40	Hard sweet	Wheat flour, sugar, refined palm oil, invert syrup, raising agents, iodized salt emulsifier, calcium salt, dough conditioner, vitamins, ferrous salt and potassium iodate	Britannia Nepal Pvt. Ltd.
4.	B-4	Nebico Thin Arrowroot	35	Hard sweet	Wheat flour, sugar, HVO, salt invert syrup emulsifier (E322), antioxidant (E320) dough raising agents (E 500, E503), essence	Nebico Pvt. Ltd.
5.	B-5	Khajurico vita milk	25	Short dough	Wheat flour, sugar, vegetable oil, butter, milk powder, raising agents, flavor, salt	Khajurico Nepal Pvt. Ltd.
6.	B-6	Britannia digestive original	150	Short dough	Refined wheat flour, whole wheat flour (20%), refined palm oil, sugar, wheat bran (4.7%), liquid glucose, milk solids, malt dextrin, raising agents (E500, E503), iodized salt, emulsifier (322i, 471 and 472e), malt extract and dough conditioner (223)	Britannia Nepal Pvt. Ltd.
7.	B-7	Cadbury Oreo	43.75	Short dough	Refined wheat flour, sugar, fractionated fat, palm olein. Coca solids (3%), invert sugar, leavening agents E500, E503), starch, iodized salt, emulsifier (E322), flavoring agents	Mondeleez India Food Pvt. Ltd
8.	B-8	Parle Monaco	34.8	Crackers	Refined wheat flour, sugar, refined oil (palm olein), invert sugar syrup, iodized salt (25%) acidity regulators (270, 296), dough raising agents (E500, E503), emulsifier (472e), flour treatments (223, 110ii,100i)	Antartic Biscuit Pvt. Ltd.
9.	B-9	Risk farm Top herbs	60	Crackers	Refined wheat flour, sugar, edible vegetable oil (Palm, palm olein), herbs (green tops of *Allium schoenoprasum L*) (0.38%), salt, acidity regulators (170, 330), dough raising agents (E 500, E503), emulsifier (450, 472), dough conditioner (223), antioxidant (319), food color (102), flavors (milk, onion, butter)	Saj Food Pvt. Ltd.
10	B-10	Nebico sugar free crackers	300	Crackers	Wheat flour, vegetable shortening, SMP, salt, emulsifier (E322), antioxidant (E319), dough raising agents (E500, E503), sweeteners (E 955, E 965) enzyme and essence	Nebico Pvt. Ltd.

Source: Market survey

Sample Processing

The biscuit samples were packed in clean polythene bags immediately after purchase, well-labeled, and transported to the lab. Then, samples were oven-dried at 70-80 ºC for 2 h. The dried samples were homogenized to powder with a porcelain mortar and pestle. All the processed samples were stored in well-labeled clean and dry airtight zip-lock plastic bags and stored in desiccators until further chemical treatment.

Analytical Reagents and Chemicals

All reagents and chemicals used in the analysis were of analytical grade. Standard stock solutions (1000 ppm) for Pb, Cr, and Ni 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%) was 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

For heavy metal analysis, a standard dry ashing method was followed for sample digestion and preparation of sample solution (Ungureanu *et al*., 2022; Tegegne, 2015). Accurately, 2 g of the homogenized and powdered sample was transferred into a well-cleaned porcelain crucible. The crucible was placed in a muffle furnace at a temperature of 550 °C for 6 h until the completion of the ashing process. Then, the crucible with ash was sufficiently cooled, wetted with water, and then treated with 2.5 mL of (1:1) HNO_3. The crucible was covered with a watch glass and placed on the hot plate at a temperature of 90 – 95 °C for 1 h for digestion of the sample. The sample was evaporated to dryness and was transferred to a 50 mL volumetric flask. Then, the final volume was made up to the mark using distilled water through homogeneous mixing. Solutions of all samples were prepared analogously. The blanks (without samples) were also prepared similarly to the samples.

Instrumentation and Metal Analysis

The concentrations of Cd, Ni, and Pb in the digested samples were determined by a Flame Atomic Absorption Spectrophotometer (nova AA 350 model manufactured by Analytik Jena Germany). Standard solutions were prepared in series using the reference metals and then plotted standard calibration curves following instructions and specifications of the manufacturing company (Table 2).

Recovery Test

To ensure the reliability of the analysis, a recovery test was performed by adding a known quantity of analyte to the samples. Accurately, 20 µg of Pb, Cr, and Ni (NIST Traceable CRM manufactured by Sigma Aldrich) was added to the sample selected for recovery test to prepare the fortified sample. Fortified samples were treated as per the sample and the concentration of heavy metals in the fortified samples was determined by FAAS (Table 3).

Health Risk Assessment

Health risk assessment (HRA) is a model to estimate the nature and magnitude of health impact on a given period of exposure to environmental contaminants such as heavy metals (USEPA, 2011). Both non-carcinogenic and carcinogenic health risks were assessed for children and adults as receptor groups using the protocol described by the US Environmental Protection Agency (USEPA, 1989). The equations and receptor parameters (Table 4**)** used in the present study were based on USEPA (1989).

Table 2: The operating conditions of FAAS for metal analysis.

Heavy metals	Current (mA)	Slit width (nm)	Wavelength (nm)	Optical mode	Flame
Lead (Pb)	2.0	1.4	217	Single Beam	Air acetylene
Chromium (Cr)	4.0	0.2	357	Single Beam	Air acetylene
Nickel (Ni)	3.0	0.2	232	Single Beam	Air acetylene

Table 3: Recovery test of heavy metals (Pb, Cr, and Ni) in biscuit samples.

Heavy metals	**Quantity added (µg)**	**Quantity recovered (µg)**	**Recovery (%)**
Lead (Pb)	20	17.6	88.0
Chromium (Cr)	20	21.8	109.0
Nickel (Ni)	20	18.5	92.5

Table 4: Receptor parameters based on the US Environmental Protection Agency (USEPA, 1989)

Parameters	Units	Children	Adult
Age	Year	6- 12	21- 70
Ingestion rate (IRing)	g/day	40	40
Exposure frequency (EF)	days/year	320	365
Exposure duration (ED)	Year	7	70
Avg. body weight (BW)	Kg	30	60
Average time (AT)	Days	(365 days/year × ED)	(365 days/year × ED)

Average Daily Dose (ADD_{ing})

The average daily dose (ADD_{ing}) of each heavy metal through the consumption of biscuits was calculated using Eqn. 1 given below:

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

where, ADD_{ing} is the average daily dose of each heavy metal ingested from the biscuits in mg/kg-day, C is the concentration of heavy metal in mg/kg, IRing is the ingestion rate of the biscuit sample in g/day, EF is the ingestion rate in g/day, EF is the exposure frequency in days/year, ED is the exposure duration in years, BW is the body weight of the exposed individual in kg and AT, the period over which the dose is averaged in days and 10^{-3} is the conversion factor.

Non-Carcinogenic Health Risk

The non-carcinogenic health risk assessment for the receptor groups was carried out by estimating Hazard Quotient (HQ) and Hazard Index (HI) as described by USEPA (1989).

Hazard Quotient (HQ)

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

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

Where, ADD_{ing} is the average daily dose of ingestion (mg/kg/bw/day) and R*f*D is the reference oral dose for heavy metals (mg/kg/day). The oral reference doses (R*f*D) for heavy metals are 0.0036, 0.003, and 0.002 mg/kg/day for Pb, Cr, and Ni respectively (USEPA, 2010).

Hazard Index (HI)

For analyzing the cumulative non-carcinogenic effects of all metals (Pb, Cr, and Ni) under the present study, the risk parameter HI was used which is the sum of all HQ values (Eqn. 3).

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

∑HQ is not a quantitative estimation of the risk level of the population exposed to heavy metals (Chien *et al*. 2002; Wang *et al*. 2005) but indicates a level of concern due to exposure to the metals. If $HI \leq 1$, adverse health effects would be unlikely to occur but $HI > 1$ indicates a significant non-carcinogenic risk posed to human health on consumption of biscuits contaminated with studied heavy metals (Song *et al*., 2009).

Lifetime Cancer Risk (CR)

Lifetime cancer risk (CR) is the estimation of the probability of developing any type of cancer in the individual on daily exposure to a heavy metal over a lifetime. Eqn. 4 was used to estimate lifetime cancer risk.

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

where, ADD_{ing} is the average daily dose of ingestion (mg/day/kg) and SF is the oral carcinogenic slope factor in $(mg/kg/day)^{-1}$ where, SF values for Pb, Cr, and Ni are 0.0085, 0.5, and 4.4 $(mg/kg/day)^{-1}$ respectively. The cancer risk index with a CR value lower than 1.0×10^{-6} is considered to be negligible, a CR value above 1.0×10^{-4} is considered unacceptable, and a CR value lying between (10^{-6} - 10^{-4}) is generally considered an acceptable range.

Statistical Analysis

Data processing and statistical analysis were carried out on an IBM-PC computer using EXCEL spreadsheets and IBM SPSS 19. Descriptive statistics such as frequency, mean, range, standard deviation, and coefficient of variation were used as statistical parameters to present results of the study. Similarly, the Linear Correlation between the heavy metals in different biscuit brands was calculated using a t-test and Pearson Correlation Coefficient at a 5% significance level. The results were statistically analyzed by one-way analysis of variance (ANOVA).

Results and Discussion

Metal concentration in biscuit samples

The statistical summary of metal concentration in biscuit samples is presented in Table 5. The box and whisker plot of heavy metals representing minimum, maximum, median, 25th, and 75th percentile is depicted in Fig. 1.

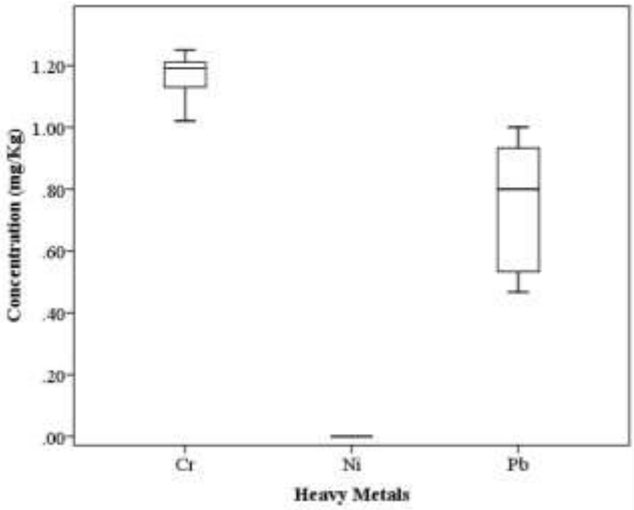

Fig 1: Box and whisker plot showing the concentration of Cr, Ni, and Pb with minimum and maximum values along with median, 25th, and 75th percentile (central box) in biscuit samples.

Table 5: Statistical summary of heavy metals (mg/kg) in tested biscuit samples.

Sample code	Heavy metals (mean ± SD; n=3)			$\sum_3$HMs
	Pb	Cr	Ni	
B-1	0.83 ± 0.17	1.02 ± 0.02	BDL	1.85
B-2	0.50 ± 0.22	1.24 ± 0.05	BDL	1.74
B-3	0.53 ± 0.13	1.25 ± 0.03	BDL	1.78
B-4	0.77 ± 0.13	1.18 ± 0.03	BDL	1.99
B-5	0.77 ± 0.09	1.20 ± 0.03	BDL	1.97
B-6	0.93 ± 0.20	1.13 ± 0.05	BDL	2.10
B-7	0.93 ± 0.09	1.17 ± 0.09	BDL	2.10
B-8	0.97 ± 0.13	1.12 ± 0.01	BDL	2.09
B-9	0.47 ± 0.17	1.21 ± 0.03	BDL	1.68
B-10	1.00 ± 0.08	1.21 ± 0.06	BDL	2.21
Mean	0.77 ± 0.06	1.17 ± 0.03	-	1.95±0.18
CV (%)	7.53	2.39	-	9.18
Minimum	0.47	1.02	-	1.68
Maximum	1.00	1.25	-	2.21
**MTC	0.3	2.3	67	-

**MTC = Maximum Tolerance Concentration (FAO/WHO, 2001)
BDL = Below Detection Level; CV = Coefficient of variation

Results revealed that the mean concentrations of heavy metals were in descending order of Cr > Pb > Ni, indicating a high level of Cr contamination in all tested biscuit samples. The sum of overall concentration of metals ($\sum_3$HMs) was found in the range of 1.68 – 2.21 mg/kg with a mean concentration of 1.95 mg/kg. Based on the overall metal content, the biscuit brands were found in ascending order of B-9 < B-2 < B-3 < B-1< B-5 < B-4 < B-8 < B-6 < B-7 < B-10, implying that the sample B-10 was highly contaminated with heavy metals.

Lead (Pb)

Lead is a toxic heavy metal that has no biological function in the body. Owing to its high level of toxicity, the International Agency for Research on Cancer (IAARC) has listed Pb as a human carcinogen (IAARC, 2012). Children are highly sensitive to lead toxicity due to high adsorption and faster metabolism of Pb than adults (Abt & Robin, 2020; Jarup, 2003). The toxic effects of the metal are renal dysfunction, nephropathies, anemia, abdominal pain, alterations of the gastrointestinal tract, Alzheimer's disease, and central nervous system damage including cancers of vital body organs (Paz *et al*, 2019; Fathabad *et al*., 2018). The concentration of Pb in tested biscuit samples was found in the range of 0.47 - 1.00 mg/kg with a mean concentration of 0.77 mg/kg. The maximum level of Pb was found in the B-10 sample with a concentration of 1.00 mg/kg. Similarly, B-9 confirmed the lowest Pb content among the samples with a mean concentration of 0.47 mg/kg. The mean concentration of Pb in the present study was found to be comparable with the studies conducted by Dada *et al.* (2017) and Iwegbue (2012) in which Pb content was reported in a range of 0.10 - 0.7 mg/kg and 0.001 - 1.1 mg/kg, respectively (Table 6). Similarly, in the previous studies conducted in biscuit samples (Table 6), the concentration of Pb was reported from BDL - 0.08 mg/kg (Adegbola *et al*., 2015), 0.066 mg/kg (Taher *et al*., 2023), 0.050 mg/kg (Illupeju *et al*., 2019) and 0.00009 - 0.029 mg/kg (Ungureanu *et al*., 2022). The level of Pb in the present study was higher than those values reported in the aforementioned studies (Table 6). However, Haider *et al*. (2022), Sobhanardakani (2019), and Arigbede *et al*. (2019) reported higher levels of Pb in their studied biscuit samples than in the present study. The level of Pb in all tested biscuit samples in the present study surpassed the threshold limit FAO/WHO of 0.3mg/kg, indicating a serious level of concern for consumer health.

The most prominent sources of Pb contamination in biscuit samples may be attributed to contaminated soil, agrochemicals, and fertilizers containing lead (Aikpokpodian *et al*., 2013). Besides, automobile emissions, ingredients, and contaminated water used during the manufacturing process involving production, handling process, packing, and storage of biscuit products are potential sources of Pb content (Arigbede *et al*., 2019). Similarly, Kim *et al*. (2008) reported that Pb-based ink used to print the packaging of biscuits is an important source of Pb in biscuits.

Table 6: Comparison of heavy metal concentration in biscuit samples in the present study against different countries.

	Concentration of heavy metals (mg/kg)			
Countries	**Pb**	**Cr**	**Ni**	**References**
Nepal	(0.467 - 1.00)	(1.02 - 1.25)	ND	Present study
Iraq	(0.066 ± 0.008)	—	—	Taher *et al*. (2023)
Romania	(0.00009 - 0.029)	(0.004 - 0.065)	—	Ungureanu et. al. (2022)
Iraq	(2.07 - 29.97)	—	—	Haider *et al*. (2022)
Nigeria	(0.050 - 0.325)		—	Oyekunle *et al*. (2021)
Nigeria	(3.11 - 92.0)	(ND - 46.4)	—	Arigbede *et al*. (2019)
Nigeria	0.050	1.519	15.804	Illupeju *et al*. (2019)
Serbia	—	2.366	—	Kovacervic *et al*. (2019)
Iran	(1.00 - 4.00)	(0.010 - 2.200)	—	Sobhanardakani (2019)
Nigeria	(0.10 - 0.7)	—	—	Dada *et al*. (2017)
Nigeria	(ND - 0.08)	(0.12 - 0.25)	—	Adegbola *et al*. (2015)
Turkey	—	(0.26 - 0.66)	(0.27 - 0.67)	Harmankaya *et al*, (2012)
Nigeria	(0.001 - 1.1)	(0.1- 0.7)	(2.2 - 4.9)	Iwegbue (2012)

Chromium (Cr)

Chromium is an essential trace element that exists in two stable forms such as trivalent and hexavalent form. Trivalent chromium has a biological function in our body and its daily requirement is 1 µg/kg (Iwegbue, 2012). The significant roles of Cr(III) in the body are glucose and fat metabolism, synthesis of nucleic acids (DNA & RNA) and proper functioning of the immune system (Manore *et al*., 2009), reducing blood cholesterol level, control of diabetes (Mohan & Pittman, 2006). It also plays an important role as an electrolyte for different physiological processes in our body (Tegegne, 2015). The deficiency of Cr(III) results in the risk of coronary heart disease and diabetes (Mertz, 1969). Cr(VI) is highly toxic, carcinogenic, and mutagenic (Mclean & Beveridge, 2001) with cancer induction, especially bronchial carcinoma and lung cancer (IARC, 1990). The presence of Cr above the permissible limit leads to different health hazards such as liver and kidney damage, disorders of the nervous, respiratory, and circulatory systems, diabetes, heart attack, skin cancer and dermatitis, and ulcers of different organs (Kabata-Pendeans, 2016; Janbakhsh, 2018). The mean concentration of Cr in tested biscuit samples was found to be in the range of 1.02-1.25 mg/kg with a mean concentration of 1.17 mg/kg. A comparatively highest concentration of Cr was detected in the B-3 sample with a mean concentration of 1.25 mg/kg while B-1 detected the lowest of all with a mean value of 1.02 mg/kg. The levels of Cr in all samples were found below the maximum permissible level of FAO/WHO (2.3 mg/kg). The level of Cr in biscuit samples in previous studies (Table 6) was reported in the range of 0.1 - 0.7 mg/kg (Iwegbue, 2012), 0.004 - 0.065 mg/kg (Ungureanu *et al*., 2022), and 0.26 - 0.66 mg/kg (Harmankaya *et al*., 2012). The concentration of Cr in the present study was higher than in the reported studies. Similarly, a higher concentration of Cr was reportedly found to be 2.366 mg/kg (Kovacervic *et al*., 2019), BDL - 46.4 mg/kg (Arigbede *et al*., 2019), 0.010 - 2.200 mg/kg (Sobhanardakani, 2019), and 1.519 mg/kg (Illupeju *et al*., 2019) in different types of biscuits in different countries (Table 6). The concentration of Cr in these studies was appreciably higher than the Cr content in the present study. The contamination of Cr in biscuits may arise from the raw materials, production process, and leaching of Cr from containers (Arigbede *et al*., 2019; Ashraf, 2006). Similarly, Ochu *et al*. (2012) reported unsafe storage conditions, as well as the production chain, i.e., raw materials, processing, packaging, transportation, storage, or marketing. Moreover, the use of utensils made of alloys during the manufacture of foodstuffs may also induce Cr contamination.

Nickel (Ni)

Nickel is an important micro-nutrient having enzymatic function in our body. Excessive intake of Ni above the threshold limit results in bronchial hemorrhage, while other symptoms include nausea, weakness, and dizziness (Devi *et al*., 2016). Although Ni was found below the detection limit in the present study, Iwegbue (2012) reported Ni content in a range of 2.2 - 4.9 mg/kg in Nigerian biscuit samples while Harmankaya *et al*. (2012) reported Ni ranging from 0.27-0.67 mg/kg in the Turkish biscuits. Similarly, Illupeju *et al*. (2019) measured the level of Ni with 15.804 mg/kg in biscuit samples from Ibadan City, Nigeria.

The differences in the level of heavy metals in biscuit samples reported in the literature (Table 6) and the present study may be attributed to differences in heavy metal contamination in common ingredients used in biscuit manufacturing. One of the main ingredients includes wheat flour as the wheat plant could absorb heavy metals from the contaminated soil (Gavrilescu, 2022). Similarly, a chain of manufacturing processes such as baking, storage conditions, and packaging, as well as the extent of environmental pollution have a significant impact on the variability of metal contamination in biscuits (Pasqualone *et al*., 2021).

Statistical Analysis

The statistical analysis revealed that Pb and Cr were negatively correlated with each other with a correlation coefficient ($r = -0.5$ at $\alpha = 0.05$). The negative correlation between the metals indicates that Pb and Cr content in biscuit samples are not controlled by a single factor or source but is the combined effect of both natural and human activities (Suresh *et al*., 2012). The coefficient of variation (CV) is a simple measure of relative variability. It is the ratio of the standard deviation to the mean. The coefficient of variance is particularly useful for comparing variability between different measurements. The CV of heavy metals in the brands of biscuits ranged from BDL in Ni to 2.39% in Cr and 7.53% in Pb. Similarly, one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Differences (HSD) at a 95% confidence level test for post hoc multiple comparisons, was performed to test the significance of differences in total metal concentrations of heavy metals in biscuit samples. The analysis of variance for the concentrations of heavy metals studied in the various food groups indicates that there was a significant difference ($P < 0.05$) between the mean values of Pb, Cr, and Ni in biscuit samples (Table 7).

Health Risk Assessment

Average Daily Dose (ADD_{ing})

The average daily dose (ADD_{ing}) is highly influenced by a body weight, concentration of studied metals, and ingestion rate. In the present study, ADD_{ing} was calculated for two receptor groups *viz* children (6-12 yr) and adults (21-70 yr) using 40 g/day as ingestion rates for both age groups (Table 9). The other parameters involved in the estimation of ADD_{ing} are depicted in Table 9.

In the present study, the ADD_{ing} values of Pb for children and adult was found to be in the range of 0.546 - 1.169 µg/kg/day and 0.311 - 0.645 µg/kg/day, respectively (Table 9). The ADD_{ing} values of Pb in the present study for both receptor groups were below the Provisional Tolerable Daily Intake (PTDI) limit of 3.6 µg/kg/day (JECFA, 2011). This indicates the consumption of biscuits at the given ingestion rate of 40g/day is not likely to pose a health issue for both receptor groups. In the previous studies, ADD_{ing} values for Pb for children and adults were reported in the range of 0.00 - 0.0967 and 0.00 - 0.0414 µg/kg/day respectively (Ungureanu *et al*., 2022). Similarly, Sobhanardakani (2019) reported ADD_{ing} values in the range of 0.00793 - 0.0317 and 0.0017 - 0.0068 µg/kg/day for children and adults, respectively. Likewise, Arigbede *et al*. (2019) estimated ADD_{ing} values of Pb in the range of 3.55 - 105 and 2.07 - 61.3 µg/kg/day respectively for children and adults in which the reported ADD_{ing} values were comparatively higher than the present study.

Table 7. ANOVA test for heavy metals in biscuit samples.

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	7.104	2	3.552	232.938	.000
Within Groups	.412	27	.015		
Total	7.516	29			

Table 8. Post hoc test of heavy metals.

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Pb	Cr	-.40300*	.05522	.000	-.5399	-.2661
	Ni	.77000*	.05522	.000	.6331	.9069
Cr	Pb	.40300*	.05522	.000	.2661	.5399
	Ni	1.17300*	.05522	.000	1.0361	1.3099
Ni	Pb	-.77000*	.05522	.000	-.9069	-.6331
	Cr	-1.17300*	.05522	.000	-1.3099	-1.0361

* The mean difference is significant at the 0.05 level.

Table 9. Average daily dose (ADDing) of heavy metals (µg/kg/day) consumed by children and adults through tested biscuit samples.

Sample code	Pb		Cr		Ni	
	Children	Adult	Children	Adult	Children	Adult
B-1	0.974	0.556	1.192	0.680	-	-
B-2	0.584	0.333	1.450	0.827	-	-
B-3	0.623	0.355	1.461	0.834	-	-
B-4	0.897	0.512	1.379	0.787	-	-
B-5	0.897	0.512	1.403	0.800	-	-
B-6	1.081	0.622	1.321	0.754	-	-
B-7	1.091	0.622	1.368	0.780	-	-
B-8	1.130	0.645	1.309	0.747	-	-
B-9	0.546	0.311	1.414	0.807	-	-
B-10	1.169	0.677	1.414	0.807	-	-
Mean	0.899	0.514	1.371	0.782	-	-
Maximum	1.169	0.645	1.450	0.834	-	-
Minimum	0.546	0.311	1.192	0.680	-	-
*PTDI (JECFA, 2011)	3.6		200		5.0	

*PTDI: Provisional Tolerable Daily Intake,

JECFA: Joint FAO/WHO Expert Committee on Food Additives

In the present study, the range of ADD_{ing} values for Cr was 1.192 - 1.450 µg/kg/day and 0.680 - 0.834 µg/kg/day for children and adults, respectively (Table 9). The estimated values for Cr were below 200 µg/kg/day which is the PTDI limit (JECFA, 2011). This implies that the consumption of tested biscuit samples is safe for human health. The ADD_{ing} values of Cr for children and adults ranged between 0.013-0.22 and 0.0057 - 0.094 µg/kg/bw/day respectively in the study conducted by Ungureanu *et al*. (2022). Similarly, Sobhanardakani *et al*. (2019) reported ADD_{ing} values for both children and adults in the range of 0.0000793 - 0.0174 µg/kg/day. Arigbede *et al*. (2019) reported the ADD_{ing} values of Cr for children and adults in the range between 0.0 - 53 and 3.55 - 105 µg/kg/day, respectively, which are higher compared to the present study. The ADD_{ing} values for Pb and Cr in all biscuit samples for children were greater than in adults which was consistent with the previous studies. This indicates that children are at high risk due to their ability to absorb metals effectively which may cause retardation of physiological development (Kocak *et al*., 2005).

Non-Carcinogenic Risk Assessment

Hazard quotient (HQ) and hazard index (HI)

The estimated HQ values of heavy metals for children and adults based on the ADD_{ing} values are shown in Table 10 while the hazard index (HI) is depicted in Fig. 2.

The HQ values of Pb and Cr for children were estimated to be in the range of 0.156 - 0.334 and 0.397 - 0.487, respectively. Similarly, HQ values of Pb and Cr for adults were 0.089 - 0.193 and 0.227 - 0.278 respectively. The HQ values of both studied metals (Table 10) in all tested samples and receptor groups were within the safe limit (HQ < 1). The cumulative non-carcinogenic risk index, i.e. hazard index (HI) for children and adults ranged from 0.627 - 0.805 and 0.358 - 0.438 respectively (Figure 2). Both HQ and HI values in the present study exhibited the safe limit (< 1), implying that the consumption of biscuits in the given ingestion rate for both receptor groups is unlikely to cause non-carcinogenic health risks.

Table 10: Hazard quotient (HQ) of heavy metals for children and adults.

Sample code	HQ					
	Pb		Cr		Ni	
	Children	**Adult**	**Children**	**Adult**	**Children**	**Adult**
B-1	0.278	0.159	0.397	0.227	-	-
B-2	0.167	0.095	0.483	0.276	-	-
B-3	0.178	0.101	0.487	0.278	-	-
B-4	0.256	0.146	0.46	0.262	-	-
B-5	0.256	0.146	0.468	0.267	-	-
B-6	0.312	0.178	0.44	0.251	-	-
B-7	0.312	0.178	0.456	0.26	-	-
B-8	0.323	0.184	0.436	0.249	-	-
B-9	0.156	0.089	0.471	0.269	-	-
B-10	0.334	0.193	0.471	0.269	-	-
Mean	0.257	0.147	0.457	0.264	-	-
Min.	0.156	0.089	0.397	0.249	-	-
Max.	0.334	0.193	0.487	0.278	-	-

Min: Minimum; Max.: Maximum

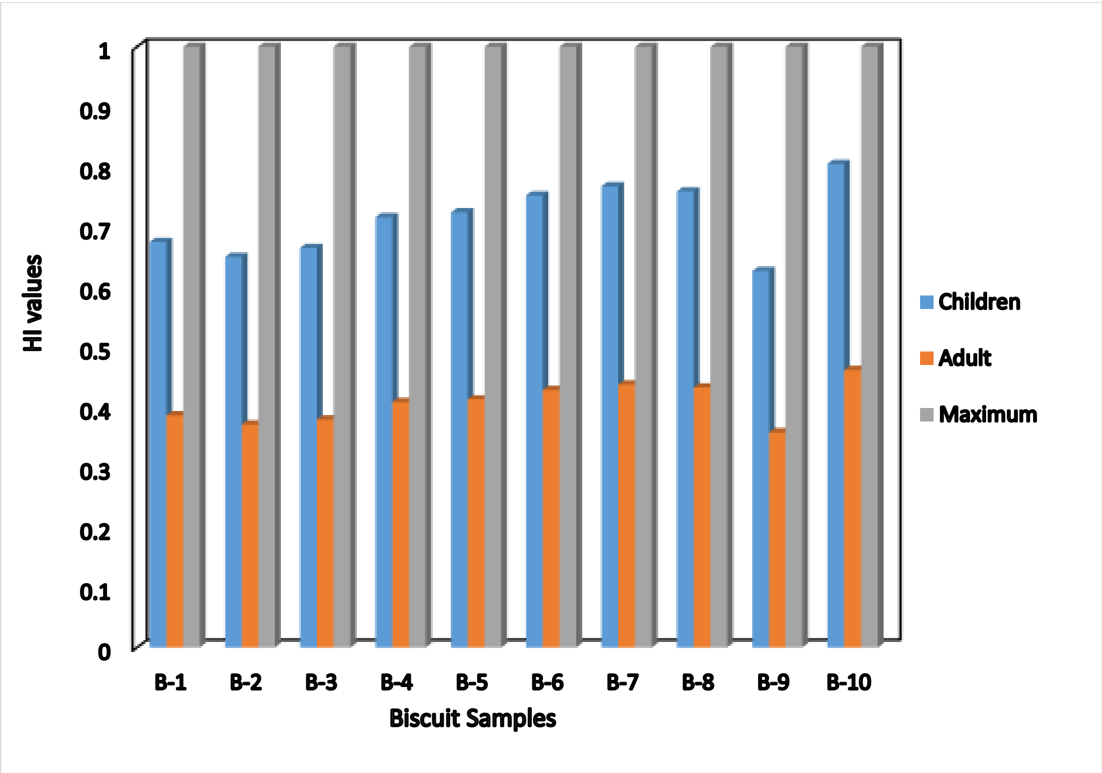

Fig. 2: Hazard index (HI) of heavy metals for children and adults.

The higher HI values above the safe limit (.> 1) were reported in the study conducted by Arigbede *et al.* (2019) who found the HI range of 1.96- 38.3 and 1.14 - 22.3 for children and adults, respectively. Similarly, Ungureanu *et al.* (2022) also reported higher HI values surpassing the safe limit for children and adults. The HI values ranging from 0.005 - 0.00201 and 0.00235 - 0.0098 as reported by Sobhanardakani, (2019) for children and adults respectively, were within the safe limit and in good agreement with the present study.

Carcinogenic Risk Assessment

Carcinogenic risk (CR) and cumulative cancer risk (LCR)

The carcinogenic risk (CR) and cumulative cancer risk (LCR) of heavy metals on consumption of tested biscuit samples by both receptor groups are depicted in Table 11. In the present study, the CR values for Pb were found to be (4.64 - 9.94) $\times 10^{-6}$ and (2.64 - 5.75) $\times 10^{-6}$ for children and adults respectively. Similarly, CR values for Cr were found to be (5.96 - 7.25) $\times 10^{-4}$ and (3.40 - 4.20) $\times 10^{-4}$ for children and adults respectively. The cumulative cancer risk parameter (LCR) for children and adults was estimated as (6.04- 7.35) $\times 10^{-4}$ and (3.45- 4.23) $\times 10^{-4}$, respectively. In the present study, both CR and LCR values of Pb and Cr in all tested biscuit samples for both receptors were within the safe limit (10^{-6} - 10^{-4}). So, the consumption of the studied biscuit brands is unlikely to pose a cancer risk to both receptors.

The cumulative cancer risk parameter (LCR) for children and adults was estimated as (6.04- 7.35) $\times 10^{-4}$ and (3.45- 4.23) $\times 10^{-4}$, respectively (Table 11). In the present study, both CR and LCR values of Pb and Cr in all tested biscuit samples for both receptors were within the safe limit (10^{-6} - 10^{-4}). So, the consumption of all studied biscuit brands is unlikely to pose a cancer risk to consumers. Arigbede *et al.* (2019) also reported the CR values of Pb and Cr for children and adults as (2.1 - 208.5) $\times 10^{-5}$ and (4.0 - 6.2) $\times 10^{-2}$, respectively. However, the CR values for Cr exceeded the safe limit and were higher than in the present study.

Table 11. Carcinogenic risk (CR) and cumulative carcinogenic risk (LCR) indices of heavy metals for children and adults.

Sample code	Lifetime cancer risk (CR)						LCR =∑ CR	
	Pb		Cr		Ni			
	Children	Adult	Children	Adult	Children	Adult	Children	Adult
B-1	8.28×10^{-6}	4.73×10^{-6}	5.96×10^{-4}	3.4×10^{-4}	-	-	6.04×10^{-4}	3.45×10^{-4}
B-2	4.96×10^{-6}	2.83×10^{-6}	7.25×10^{-4}	4.1×10^{-4}	-	-	7.30×10^{-4}	4.13×10^{-4}
B-3	5.29×10^{-6}	3.02×10^{-6}	7.30×10^{-4}	4.2×10^{-4}	-	-	7.35×10^{-4}	4.23×10^{-4}
B-4	7.62×10^{-6}	4.35×10^{-6}	6.90×10^{-4}	3.9×10^{-4}	-	-	6.93×10^{-4}	3.94×10^{-4}
B-5	7.62×10^{-6}	4.35×10^{-6}	7.01×10^{-4}	4.0×10^{-4}	-	-	7.09×10^{-4}	4.04×10^{-4}
B-6	9.27×10^{-6}	5.29×10^{-6}	6.60×10^{-4}	3.8×10^{-4}	-	-	6.69×10^{-4}	3.85×10^{-4}
B-7	9.27×10^{-6}	5.29×10^{-6}	6.80×10^{-4}	3.9×10^{-4}	-	-	6.89×10^{-4}	3.95×10^{-4}
B-8	9.60×10^{-6}	5.48×10^{-6}	6.50×10^{-4}	3.7×10^{-4}	-	-	6.59×10^{-4}	3.75×10^{-4}
B-9	4.64×10^{-6}	2.64×10^{-6}	7.07×10^{-4}	4.0×10^{-4}	-	-	7.11×10^{-4}	4.02×10^{-4}
B-10	9.94×10^{-6}	5.75×10^{-6}	7.07×10^{-4}	4.0×10^{-4}	-	-	7.17×10^{-4}	4.05×10^{-4}
Mean	7.65×10^{-6}	4.37×10^{-6}	6.84×10^{-4}	3.9×10^{-4}	-	-	6.92×10^{-4}	3.94×10^{-4}
Min.	4.64×10^{-6}	2.64×10^{-6}	5.96×10^{-4}	3.4×10^{-4}	-	-	6.04×10^{-4}	3.45×10^{-4}
Max.	9.94×10^{-6}	5.75×10^{-6}	7.25×10^{-4}	4.2×10^{-4}	-	-	7.35×10^{-4}	4.23×10^{-4}

Min: Minimum; Max.: Maximum

Conclusions

In the present study, the concentration of heavy metals (Pb, Cr, and Ni) was determined in biscuit samples of some popular brands available in local grocery shops in Kathmandu and assessed their potential health risks in children and adults through dietary intake. Chromium content in all tested biscuit samples was within the safe limit while Pb exceeded the maximum permissible limit of FAO/WHO. However, Ni was found below the detection level in all tested samples under the present investigation. Assessment of health risks using different risk indices revealed children at higher risk compared to adults. The estimated average daily dose (ADD_{ing}) of Pb and Cr through dietary intake was below PTDI for both receptor groups. Both HQ and HI values for the receptors were within the safe limit (< 1) which implies that consumption of tested biscuit samples is unlikely to cause any non-carcinogenic health risks to them. Similarly, CR and LCR indices were also within the safe range ($10^{-6} – 10^{-4}$) for both receptors implying no potential carcinogenic risk on consumption of all tested biscuit samples under the present investigation. However, regular monitoring of the production chain and metal contaminants in such foodstuffs should be given high priority on account of consumer health issues and food safety.

Conflicts of Interest

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

Authors' Contribution

J. Maharjan: sample collection, investigation, data interpretation, and analysis, writing original draft, and reviewing; P. K. Yadav: resources, investigation, data interpretation, and analysis, and reviewing original draft; P. R. Shakya: supervision, resources, conceptualization, data interpretation, and analysis, writing-reviewing, and editing. All authors have read and made critical revisions of the manuscript for important intellectual content and approved the final version of the manuscript.

Acknowledgments

The authors are very much thankful to Aastha Scientific and Research Service Pvt. Ltd., Dillibazar, Kathmandu for providing the FAAS facilities. One of the authors, J. Maharjan is grateful to the Research Management Cell (RMC), Padmakanya Multiple Campus, Tribhuvan University for financial support through a mini research project.

References

Abt E and Robin LP (2020) Perspective on cadmium and lead in cocoa and chocolate. *Journal of Agricultural and Food Chemistry*, **68**(46): 13008-13015. DOI: 10.1021/acs.jafc.9b08295

Adefris A (2011) Heavy metal toxicity. Medscape Drugs, Diseases, and Procedures. Article 814960, 7 pp.

Adegbola RA, Adekanmbi AI, Abiona DL and Atere AA (2015) Evaluation of some heavy metal contaminants in biscuits, fruit drinks, concentrates, candy, milk products and carbonated drinks sold in Ibadan, Nigeria. *International Journal of Biological and Chemical Sciences*, **9**(3): 1691-1696. DOI: 10.4314/ijbcs.v9i3.47

Aikpokpodion PE, Atewolara-Odule OC, Osobamiro T, Oduwole OO and Ademola SM (2013) A survey of copper, lead, cadmium and zinc residues in cocoa beans obtained from selected plantations in Nigeria. *Journal of Chemical and Pharmaceutical Research*, **5**(6): 88-98. https://www.jocpr.com

Akinola MO, Njoku KL and Ekeifo BE (2008) Determination of lead, cadmium, and chromium in the tissue of an economically important plant grown around a textile industry at Ibeshe, Ikorodu area of Lagos State, Nigeria. *Advances in Environmental Biology*, 25-31.

Amjad M, Hussain S, Baloch ZUR and Raza R (2021) Determination of heavy metals in locally available chocolates in Lahore region. *Turkish Journal of Agriculture-Food Science and Technology*, **9**(6): 1144-1153. DOI: 10.24925/turjaf.v9i6.1144-1153.4262

Arigbede O, Olutona G and Dawodu M (2019) Dietary intake and risk assessment of heavy metals from selected biscuit brands in Nigeria. *Journal of Heavy Metal Toxicity and Diseases*, **4**: 1-15. DOI: 10.21767/2473-6457.10027

Ashraf W (2006) Levels of selected heavy metals in tuna fish. *Arabian Journal of Science and Engineering*, **31**: 89-92.

Ayensu J, Lutterodt H, Annan RA, Edusei A and Loh SP (2019) Nutritional composition and acceptability of biscuits fortified with palm weevil larvae (Rhynchophorus phoenicis Fabricius) and orange-fleshed sweet potato among pregnant women. *Food Science and Nutrition*, **7**(5): 1807-1815. DOI: 10.1002/fsn3.1024

Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR and Sadeghi M (2021) Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. *Frontiers in Pharmacology*, 227. DOI: 10.3389/fphar.2021.643972

Bradley EL, Castle L, Dines TJ, Fitzgerald AG, Gonzalez Tunon P, Jickells SM and Ramsay IA (2005) Test method for measuring non-visible set-off from inks and lacquers on the food-contact surface of printed packaging materials. *Food Additives and Contaminants*, **22**(5): 490-502. DOI: 10.1080/02652030500129253

Briffa J, Sinagra E and Blundell R (2020) Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon*, **6**(9), e04691. DOI: 10.1016/j.heliyon.2020.e04691

Canalis MSB, Leon AE and Ribotta PD (2017) Effect of insulin on dough and biscuit quality produced from different flours. *International Journal of Food Studies*, **6**(1): 13-23. DOI: 10.7455/ijfs/6.1.2017.a2

Chavan RS, Sandeep K, Basu, S and Bhatt S (2016) Biscuits, cookies and crackers: chemistry and manufacture. *Encyclopedia of Food and Health*, Academic press 437-444, ISBN 9780123849533. DOI: 10.1016/B978-0-12-384947-2.00076-3

Chien LC, Hung TC, Choang KY, Yeh CY, Meng PJ, Shieh MJ and Han BC (2002) Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan. *Science of the Total Environment*, **285**: 177-185. DOI: 10.1016/S0048-9697(01)00916-0

Dada EO, Ojo ON, Njoku KL and Akinola MO (2017) Assessing the levels of Pb, Cd, Zn, and Cu in biscuits and home-made snacks obtained from vendors in two tertiary institutions in Lagos, Nigeria. *Journal of Applied Sciences and Environmental Management* **21**(3): 521-524. DOI: 10.4314/jasem.v21i3.13

Das SK, Grewal A and Banerjee M (2011) A brief review: Heavy metal and their analysis. *International Journal of Pharmaceutical Sciences Review and Research*, **11**(1): 13-18.

Davidson I (2019) Biscuit, cookie and cracker production: Process, production and packaging equipment, (2nd edition). London: Elsevier Inc., Academic Press 1-12.

Dehghani MH, Zarei A, Mesdaghinia A, Nabizadeh R, Alimohammadi M and Afsharnia M (2017) Response surface modeling, isotherm, thermodynamic and optimization study of arsenic (V) removal from aqueous solutions using modified bentonite-chitosan (MBC). *Korean Journal of Chemical Engineering* **34**: 757–767.

Devi P, Bajala V, Garg VK, Mor S and Ravindra K (2016) Heavy metal content in various types of candies and their daily dietary intake by children. *Environmental Monitoring and Assessment* **188**: 86. DOI: 10.1007/s11814-016-0330-0

FAO/WHO, Codex Alimentarius Commission (2001) Food additives and contaminants. Joint FAO/WHO Food Standards Programme, & World Health Organization. Codex Alimentarius: General requirements (food hygiene). ALINORM 01/12A: 1-289.

Fathabad AE, Shariatifar N, Moazzen M, Nazmara S, Fakhri Y, Alimohammadi M, Azari A and Khaneghah AM (2018) Determination of heavy metal content of processed fruit products from Tehran's market using ICP- OES: A risk assessment study. *Food and Chemical Toxicology* **115**: 436-446. DOI: 10.1016/j.fct.2018.03.044.

Gavrilescu M (2022) Enhancing phytoremediation of soils polluted with heavy metals. *Current Opinion in Biotechnology* **74**: 21-31. DOI: 10.1016/j.copbio.2021.10.024

Gizaw Z (2019) Public health risks related to food safety issues in the food market: a systematic literature review. *Environmental health and preventive medicine* **24**(1): 1-21. DOI: 10.1186/s12199-019-0825-5

Haider NN, Altemimi AB, George SS, Baioumy AA, El-Maksoud AAA, Pasqualone A and Abedelmaksoud TG (2022) Nutritional Quality and Safety Characteristics of Imported Biscuits Marketed in Basrah, Iraq. *Applied Sciences* **12**(18): 9065. DOI: 10.3390/app12189065

Harmankaya M, Ozcan MM, Duman E and Dursun N (2012) Mineral and heavy metal contents of ice-cream wafer, biscuit and gofret wafers. *Journal of Agroalimentary processes and Technologies* **18**: 259-265.

IARC (1990) Chromium, nickel and welding. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, **49**: 1- 648.

IARC (2012) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans; World Health Organization International Agency for Research on Cancer: Lyon, France, 100C: 121-145.

Ilupeju O, Adimula VO, Onianwa PC, Ayom E and Baba AA (2019) Assessment of heavy metals in foods and adult dietary intake estimates. *African Journal of Science, Technology, Innovation and Development* **11**(2): 261-268. DOI: 10520/EJC-15735857c2

Iwegbue CMA (2012) Metal contents in some brands of biscuits consumed in southern Nigeria, *American Journal of Food Technology* **7**: 160-167. DOI: 10.3923/ajft.2012.160.167

Janbakhsh S, Hosseini Shekavabi SP and Shamsaie Mergan M (2018) Nutritional value and heavy metal content of fishmeal from the southwest Caspian Sea. *Caspian Journal of Environmental Sciences*, **16**(4): 307-317.

Jarup L (2003) Hazards of heavy metal contamination. *British Medical Bulletin* **68**(1): 167-182. DOI: 10.1093/bmb/ldg032

JECFA (Joint FAO/WHO Expert Committee on Food Additives). Evaluation of certain contaminants in food: seventy-second report of the joint FAO/WHO expert committee on food additives. WHO Technical Report Series; Rome: JECFA, 2011, No. 959-960.

Kabata-Pendias A (2010) Trace elements in soils and plants, Fourth edition, 231- 333, CRC Press, Florida, USA, 112-113.

Kim KC, Park YB, Lee MJ, Kim JB, Huh JW, Kim DH and Kim JC (2008) Levels of heavy metals in candy packages and candies likely to be consumed by small children. *Food Research International* **41**(4): 411-418. DOI: 10.1016/j.foodres.2008.01.004

Kocak S, Tokusoglu O and Aygan S (2005) Some heavy metals and trace essential detection in canned vegetable foodstuff by differential pulse polarography. *Electronic Journal of Environmental, Agricultural and Food Chemistry* **4**: 871-878.

Kovacervic S, Loncarevic I, Pajin B, Fistes A, Vasiljevic I, Lazovic M, Mrkajic D, Karadzic M and Podunavac-kuzmanovic, S (2019) Toward identification of the risk group of food products: Chemometric assessment of heavy metals content in confectionery products. *Food Additives* *and Contaminants,* Part A **36**(7): 1068-1078. DOI: 10.1080/19440049.2019.1606455

Mancebo CM, Picon, J and Gomez M (2015) Effect of flour properties on the quality characteristics of gluten-free sugar-snap cookies. *LWT-Food Science and Technology* **64**(1): 264-269. DOI: 10.1016/j.lwt.2015.05.057

Manore M, Meyer LN and Thompson J (2009) Mineral and Exercise. In: Sport Nutrition for Health and Performance. Thames and Hudson, New York, USA, 608.

McLean J and Beveridge TJ (2001) Chromate reduction by a pseudomonad isolated from a site contaminated with chromated copper arsenate. *Applied and Environmental Microbiology* **67**(3): 1076-84. DOI: 10.1128/AEM.67.3.1076-1084.2001

Mertz W (1969) Chromium Occurrence and Function in Biological Systems. *Physiological Reviews*, **49**: 163-239. DOI: 10.1152/physrev.1969.49.2.163

Ming-Ho Y (2005) Environmental toxicology: biological and health effects of pollutants, Chap. 12, CRC Press LLC, ISBN 1-56670-670-2, 2nd Edition, Boca Raton, USA.

Mohan D and Pittman CU (2006) Activated carbons and low-cost adsorbents for remediation of tri- and hexavalent chromium from water. *Journal of Hazardous Material* **137**(2): 762-811. DOI: 10.1016/j.jhazmat.2006.06.060

Mollazadeh N (2014) Metals health risk assessment via consumption of vegetables. *International Journal of Agriculture and Crop Sciences (IJACS)* **7**(8): 433-436. http://ijagcs.com/wp-content/uploads/2014/06/433-436.pdf

Nawab J, Farooqi, S, Xiaoping W, Khan S and Khan A (2018) Levels, dietary intake, and health risk of potentially toxic metals in vegetables, fruits, and cereal crops in Pakistan. *Environment Science and Pollution Researc* **25**(6): 5558–5571. DOI: 10.1007/s11356-017-0764-x

Ochu JO, Uzairu A, Kagbu JA, Gimba, CE and Okunola, OJ (2012) Evaluation of Some Heavy Metals in Imported Chocolate and Candies Sold in Nigeria. *Journal of Food Researc* **1**(3): 169–177. DOI:10.5539/jfr.v1n3p169

Oyekunle JAO, Durodola SS, Adekunle AS, Afolabi FP, Ore OT, Lawal MO, and Ojo OS (2021) Potentially toxic metals and polycyclic aromatic hydrocarbons composition of some popular biscuits in Nigeria. *Chemistry Africa* **4**(2): 399-410. DOI: 10.1007/s42250-020-00215-7

Pasqualone A., Haider NN., Summo C., Coldea TE, George SS and Altemimi AB (2021) Biscuit Contaminants, Their Sources and Mitigation Strategies: A Review. *Foods* **10**: 2751. DOI: 10.3390/foods10112751

Paz S, Rubio C, Frias I, Gutierrez AJ, Gonzalez-Weller D, Martin V and Hardisson A (2019) Toxic metals (Al, Cd, Pb, and Hg) in the most consumed edible seaweeds in Europe, *Chemosphere* **218**: 879–884. DOI: 10.1016/j.chemosphere.2018.11.165

Rahman MA, Rahman MM, Reichman SM, Lim RP and Naidu R (2014) Heavy metals in Australian grown and imported rice and vegetables on sale in Australia. Health hazard. *Ecotoxicology and Environmental Safety* **100**: 53–60. DOI: 10.1016/j.ecoenv.2013.11.024

Rai PK, Lee SS, Zhang M, Tsang YF, and Kim KH (2019) Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environment International* **125**: 365-385. DOI: 10.1016/j.envint.2019.01.067

Shobhanardakini S (2019) Heavy metals health risk assessment through consumption of some foodstuffs marketed in city of Hamedan Iran. *Caspian Journal of Environmental Sciences* **17**(2): 175-183. DOI: 10.22124/CJES.2019.3414

Smith WH (1972) *Biscuits, Crackers and Cookies*, (1st edition). Applied Science Publishers, London.

Song B, Lei M, Chien T, ZhengY, Xie Y, Li X and Gao D (2009) Assessing the health risk of heavy metals in vegetables to the general population in Beijing, China. *Journal of Environmental Science* **21**: 1702-1709. DOI: 10.1016/S1001-0742(08)62476-6

Suresh G, Sutharsan P, Ramasamy V and Venkatachalapathy R (2012) Ecotoxicology and Environmental Safety Assessment of spatial distribution and potential ecological risk of the heavy metals in relation to granulometric contents of Veeranam lake sediments, India. *Ecotoxicology and Environmental Safet,* **84**: 117–124. DOI: 10.1016/j.ecoenv.2012.06.027

Taher FA and Abojassim AA (2023) Assessment of Heavy Metals in Biscuit Samples Available in Iraqi Markets. *Biological Trace Element Research*. DOI: 10.1007/s12011-023-04013-3

Tegegne WA (2015) Assessment of some heavy metals concentration in selected cereals collected from local markets of Ambo City, Ethiopia. *Journal of Cereals and Oilseeds* **6**: 8-13. DOI: 10.5897/JCO15. 0138

Ungureanu EL, Mocanu AL, Soare, AD and Mustatea G (2022) Health risk assessment of some heavy metals and trace elements in semi-sweet biscuits: A case study of romanian market. *UPB Scientific Bulletin Series B* **84**(1): 123-136.

USEPA (1989) Risk Assessment Guidance for Superfund Volume 1: Human Health Evaluation Manual (Part A); Office of Emergency and Remedial Response: Washington, DC, USA, 1989.

USEPA (2011) Integrated risk information system. Washington, DC: US EPA. Available at: http://www.epa.gov/IRIS/

USEPA (US Environmental Protection Agency) (2010) Risk-based concentration Table. Washington DC.

Wang XL, Sato T, Xing BS and Tao S (2005) Health risks of heavy metals to the general public in Tianjin China via consumption of vegetables and fish. Science of the Total Environment **350**: 28- 37. DOI: 10.1016/j.scitotenv.2004.09.044

Zhang J, Yang J, Liu C and Zeng G (2016) Site-specific risk assessment and integrated management decision-making: a case study of a typical heavy metal contaminated site, Middle China. *Human and Ecological. Risk Assessment* **22**: 1224–1241. DOI: 10.1080/10807039.2016.1151348

Zhuang P, Mc Bride MB, Xia H, Li N and Li Z (2009) Health risk from heavy metals via consumption of food crops in the vicinity of Dobaoshan mine, South China. *Science of Total Environment* **407**: 1551- 1561. DOI: 10.1016/j.scitotenv.2008.10.061