



Research Article

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

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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.

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 sample coding, Net weight, class, and ingredients, are summarized in Table 1.

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

| S. N. | Sample code | Net wt.(g) | Class | Ingredients |
|-------|-------------|------------|---------|---|
| 1. | B-1 | 75 | Cookies | Wheat flour, sugar, HVO, salt, SMP, invert syrup, coconut powder, emulsifier (E322), antioxidant (E319), dough raising agents (E 500, E503) |
| 2. | B-2 | 30 | Cookies | Refined wheat flour, sugar, refined palm oil, Choco chips, sugar, hydrogenated oil, coca solids, emulsifier, |

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

| S. N. | Sample code | Net wt.(g) | Class | Ingredients |
|-------|-------------|------------|----------------|--|
| | | | | soya lecithin, artificial flavoring substances, invert sugar, raising agents (E 500, E503), iodized salt |
| 3. | B-3 | 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 |
| 4. | B-4 | 35 | Hard sweet | Wheat flour, sugar, HVO, salt invert syrup emulsifier (E322), antioxidant (E320) dough raising agents (E 500, E503), essence |
| 5. | B-5 | 25 | Short dough | Wheat flour, sugar, vegetable oil, butter, milk powder, raising agents, flavor, salt |
| 6. | B-6 | 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) |
| 7. | B-7 | 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 |
| 8. | B-8 | 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) |
| 9. | B-9 | 60 | Crackers | Refined wheat flour, sugar, edible vegetable oil (Palm, palm olein), herbs (green tops of <i>Allium schoenoprasum L</i>) (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) |
| 10 | B-10 | 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 |

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 °C 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) HNO₃. 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:

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

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, IR_{ing} is the ingestion rate of the biscuit sample in g/day, EF is the ingestion rate in g/day, ED 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 (RfD) for that metal derived from a similar exposure period. Eqn. 2 was used for estimating HQ as follows:

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

Where, ADD_{ing} is the average daily dose of ingestion (mg/kg/bw/day) and RfD is the reference oral dose for heavy metals (mg/kg/day). The oral reference doses (RfD) 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).

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

∑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 ≤ 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.

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

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.

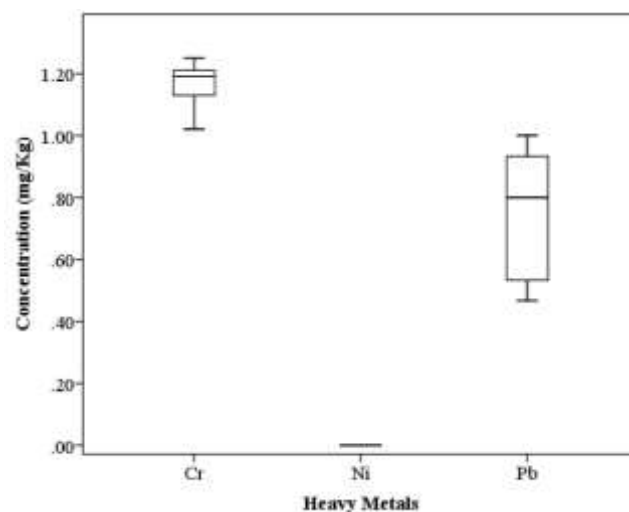


Fig 1: Box and whisker plot showing the concentration of Cr, Ni, and Pb with minimum and maximum values along with median, 25th, and 75th percentile (central box) in biscuit samples.

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

| Sample code | Heavy metals (mean ± SD; n=3) | | | Σ 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 (Σ₃HMs) 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.

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

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 $\mu\text{g}/\text{kg}/\text{day}$ and 0.311 - 0.645 $\mu\text{g}/\text{kg}/\text{day}$, 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 $\mu\text{g}/\text{kg}/\text{day}$ (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 $\mu\text{g}/\text{kg}/\text{day}$ 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 $\mu\text{g}/\text{kg}/\text{day}$ 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 $\mu\text{g}/\text{kg}/\text{day}$ 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 (ADD_{ing}) 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

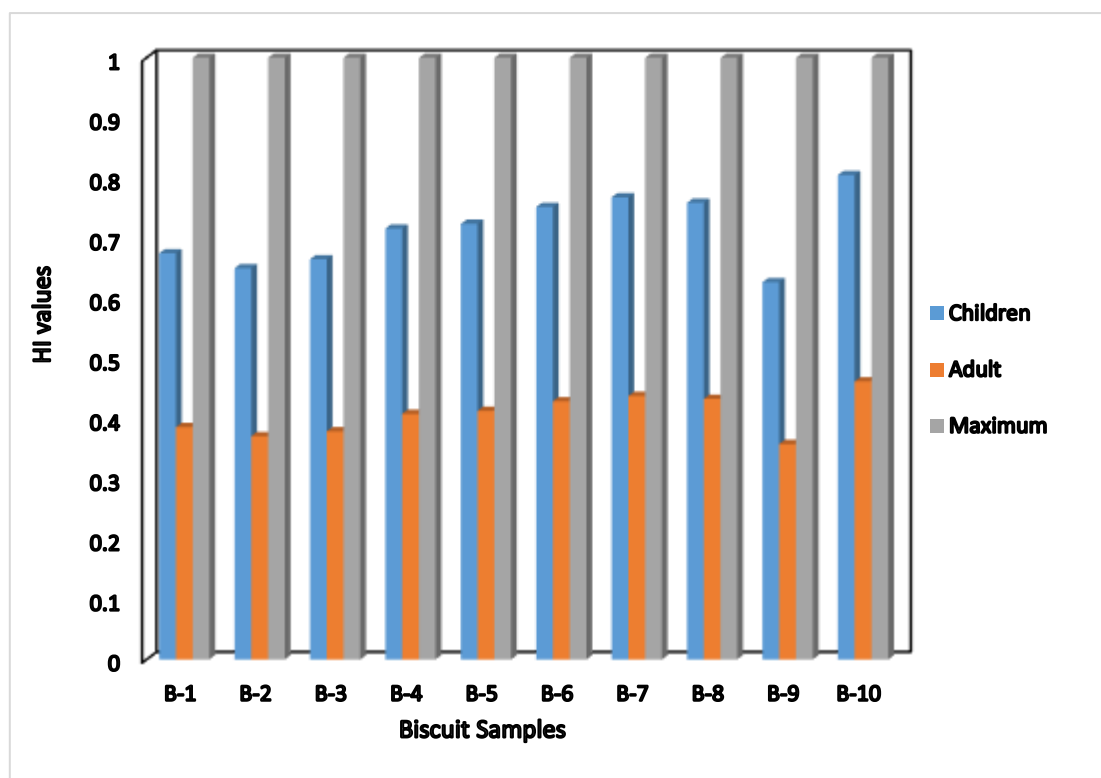


Fig. 2: Hazard index (HI) of heavy metals for children and adults.

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 = \sum 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.

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