

Carcinogenic and Non-Carcinogenic Health Risk Assessment of Heavy Metals Consumption Through Spices on Human Health in Karachi, Pakistan

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ABSTRACT

Spices are used in daily cooking, it is crucial that people monitor their contents in order to preserve their health. This study compared local open markets in Karachi to supermarket-packaged spices and investigated the prevalence of heavy metal contamination in commonly used spices. We gathered five open markets spice samples and compared them with the similar kinds of packages. Atomic absorption spectrometry was used to measure the levels of heavy metals and the hazard index (HI) was used to evaluate potential health risks, which is based on non-carcinogenic effects. Two-way ANOVA was then used to analyze the data. The results showed that all the spice samples contained trace amounts of heavy metals. The total hazard quotient (THQ) and hazard index (HI) of these metals was however lower than the safety threshold (both less than 1), indicating that there was no threat of much

hazard due to normal consumption. Nickel (Ni), chromium (Cr), lead (Pb) and copper (Cu) in spices such as cinnamon, dried ginger and fenugreek were also significantly positively correlated. Notably, the values of estimated cancer risk were within acceptable limits. All this indicates that the ingestion of these spices either as loose or packaged spice will not be a health hazard within the region under investigation.

Keywords: Heavy metals, Spices, Health risks, ETAAS, FAAS

1. Introduction

Spices play a significant role in both food and medicine due to their excellent anti-inflammatory, antibacterial, anti-diabetic, anti-hypertensive, anti-inflammatory, and chemo-protecting qualities (Kaefer & Milner, 2008; Ninfali et al., 2005; Sung et al., 2012; Tajkarimi et al., 2010). They have been used to enhance food's flavor, aroma, and color (Oladeji et al., 2023; Tefera & Teklewold, 2021). In the pharmaceutical, textile, and other industries, they are also appreciated for their coloring, as preservatives, and as fumigants (Mekassa & Chandravanshi, 2015).

In Asian countries, spices are the primary food ingredients used in all homes regularly to enhance the food flavor as well as for medicinal purposes. Therefore, their frequent use makes these spices important. Ginger, cinnamon, and tulsi are currently used as a worldwide medication to treat rheumatism, headaches, respiratory issues, and digestive problems because of their strong anti-inflammatory, and anti-oxidation qualities (Kushwaha et al., 2022). Garlic is also a medicinal herb used to treat cardiovascular disorders, cancers, and atherosclerosis. Black seeds have the potential to address a wide range of common and unusual health conditions that individuals face (Tawab & Fatima, 2006). Spices and herbs are consumed for numerous purposes, especially as part of a healthy lifestyle (Winiarska-Mieczan et al., 2023). According to the World Health Organization, over 80% of people worldwide get their main healthcare from conventional medicine. Hence, many compounds found in plants utilized by practitioners of traditional medicine can be used to treat both viral and chronic illnesses (Fatima et al., 2011; Onyiba, 2022; Sahoo et al., 2021; Singh, 2024). Despite, many benefits and extensive use of raw materials and products of plant origin, they could potentially contain hazardous substances accumulated from the environment (Oladeji et al., 2023). Additionally, they could pick up heavy metal contamination from the growth, storage, and processing processes (Moussa et al., 2024; Thomas et al., 2012). Environmentally toxic chemicals are widely disseminated and enter the food chain, where they are present in various degrees in food and feed. Consumer health suffers because of the bioaccumulation and absorption of certain substances. Heavy metals, such as lead, arsenic, and mercury, are the most harmful elements, which can have various toxic and mutagenic effects. They disturb mineral homeostasis and ionic equilibrium, cause oxidative damage to cellular structures and DNA, and result in neoplastic alterations (Chan, 2003; Embuscado, 2015; Kowalska, 2021).

The primary goal of this study was to assess any potential carcinogenic and non-carcinogenic health risks associated with some heavy metals accumulated in spices; such as cinnamon, dried ginger, Turmeric, black seeds, and fenugreek, across various regions of Karachi. This investigation holds significant importance as these spices are consumed by the Pakistani population regularly and metals have a tendency to bioaccumulate in living organisms owing to the accumulation of these toxins even if taken in small amounts daily.

2. Material and methods

2.1. Sample Collection

Commercially available unpacked spice samples including cinnamon, dried ginger, Turmeric, black seeds, fenugreek, were collected from local markets of Karachi namely: Empress Saddar, Jona, Nazimabad, Gulshan-e-Iqbal, and Korangi market. Selection was based on their extensive used in Karachi, Pakistan. Packed spices of same type were also collected as unpacked spices but they are sold in a packed form that is normally processed. The samples were carried to the lab in polyethylene plastic bags that had been tagged and sealed. Spice samples were dried in an oven at 60 °C for 24 h. The spices were ground and blended using a Hanchen plant grinder (Fig. 1).

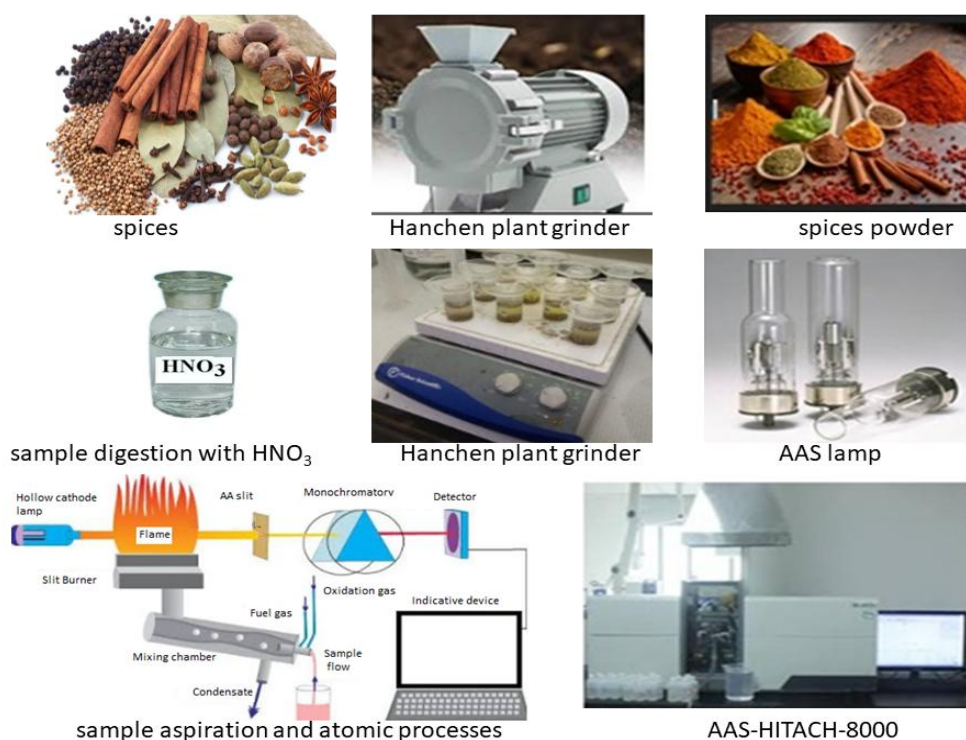


Fig. 1. Flow chart for experimental design

2.2. Chemicals and reagents

Nitric acid 69% from Analar NORMAPUR, VWR, BDH Chemicals (UK); hydrochloric acid 35% from Scharlau (Spain); hydrogen peroxide (35%), H₂SO₄ (50%) from Merck (Darmstadt, Germany); double deionized water used throughout the experiment. The standards for toxic metals (Pb, Hg, Cr, Ni, and Cu) with a concentration of 1000 mgL⁻¹ from Accu Trace (USA) were obtained. All the working standards were prepared from their stock standard solutions on daily basis.

2.3. Instrumentation

Pb, Cr, and Ni analysis were performed by electro-thermal atomization atomic absorption spectrometry (ETAAS), Cu by flame atomic absorption spectrometry (FAAS) Hitachi Model Z-5000 respectively. Similarly, cold-vapor hydride generation atomic absorption spectrometry (CVHG-AAS) was utilized for Hg. The recommended current was applied to the hollow cathode lamps according to the manufacturers'

user manual instructions, following the instrument conditions detailed elsewhere (Altunay, Elik, & Kaya, 2020). Operating instrumental parameters for the determination of metals are given in Table 1,

Table 1: *Measurement conditions for flame /electro-thermal atomization atomic absorption spectrometry*

Flame atomization atomic absorption					
Operating parameters	Pb	Cr	Ni	Hg	Cu
lamp current (mA)	7.5	7.5	10	6	7.5
Wavelength	283.3	359.3	232	253.7	324.8
slit width nm	1.3	1.3	0.2	1.3	1.3
Electro-thermal atomization atomic absorption					
Temperature programming	Temperature °C			Time (sec)	
Drying	80-120			30	
Ashing	300			30	
Atomization	1500			10	
Cleaning	2400			3	
Common parameters					
Sample volume				10 μL	
Background correction				D²	
Carrier gas Argon				200 mL/min	

2.4. Analysis of the health risks associated with using spices

2.4.1. Non-carcinogenic risk assessment

The hazardous evaluation related to human health was determined based on the rates of intake of spices and loads of metals. The methods of measuring human health risk involve estimating the carcinogenic and non-carcinogenic parameters such as total hazard quotient (THQ), hazard index (HI), and incremental lifetime cancer risk (ILCR) (Islam et al., 2023; Oladeji et al., 2023). The average daily intake (ADI) value is calculated by using equ.1 the amount consumed daily body weight and metal concentrations in various spices.

$$ADI = C_i \times IR \times ED \times EF / BW \times AT \quad (1)$$

Where C_i metal (mg/kg) is the heavy metal content in spices, IR is the ingestion rate, ED is the exposure duration (70 years), EF is the exposure frequency (365 days/year), BW is the body weight of adults that exposed to spices (67 kg) for this study, AT is the period over which the dose is averaged (365 days/year multiplied by number of exposure years (70 years)) (Dagneu & Tefera, 2023; Meseret et al., 2020).

The Total hazard quotient THQ is the ratio of the determined average dose of a pollutant to a reference dose level. The THQ assesses the non-carcinogenic risks of regularly consuming contaminants found in spices and herbs (Oladeji et al., 2023). The calculation of THQ in this study was using eq. 2.

$$THQ = ADI / R_f D \quad (2)$$

Where, $R_f D$ represents the reference dosage (mg/kg/day) of metals under consideration. $R_f D$ values for Pb, Hg, Cr, Ni, and Cu were found to be 0.004, 0.0003, 0.02, 0.003, and 0.04 mg/kg/day respectively (Gebeyehu & Bayissa, 2020; Hull et al.,

2015; Islam et al., 2023; Oladeji, 2024; Tefera & Teklewold, 2021).

Hazard index is equivalent to the sum of THQ for Pb, Hg, Cr, etc as shown in eq 3. (Islam et al., 2023). The HI was used to calculate the total non-carcinogenic risk to human health posed by exposure to various contaminants. HI stands for the overall or total risk factor for all heavy metals in spices. On the other hand, if THQ/HI ratios are higher than 1, the population may have health problems. At the same time, the population will not present any potential health risks if THQ/HI ratios are less than 1 (Ghasemidehkordi et al., 2018; Khan et al., 2009; Mohammadi et al., 2019).

$$HI = THQ \text{ Pb} + THQ \text{ Hg} + THQ \text{ Cr} + THQ \text{ Ni} + THQ \text{ Cu} \quad (3)$$

2.4.2. Carcinogenic risk assessment

Cancer risk refers to an individual's increased probability of developing cancer due to contact with hazardous carcinogenic compounds such as Pb, Cd, and Cr (Islam et al., 2023). The carcinogenic risk index (CRI) was assessed by multiplying the estimated average daily intake by the oral cancer slope factor (CSF) for corresponding heavy metals. The carcinogenic risk assessment for selected heavy metals or their lifetime cancer risk was expressed by using eq. 4 (Barbes, 2023).

$$CRI = ADI \times CSF \quad (4)$$

The values of CRI were determined based on Pb, Hg, Cr, Ni, and Cu levels in spices. The CSF for Pb, Hg, Cr, Ni, and Cu were explained by USEPA (*united state environmental protection agencies*) (2012, 2017). The CSF levels (per mg/kg/day) for Pb, Hg, Cr, Ni, and Cu were 0.0085, N/A, 0.5, 0.84, and 1.70 respectively (Aendo et al., 2022; Samaila et al., 2021). The average CRI values ranging between 10^{-6} to 10^{-4} mg/kg are acceptable, however, CRI values less than 10^{-6} mg/kg are considered to pose no significant health risks for humans, while CRI levels greater than 10^{-4} indicate harmful effects for humans (Dagnew et al., 2023; Islam et al., 2023). The total carcinogenic risk index (TCRI) of carcinogen is the overall potential of carcinogenic risk of individual metal from the selected study areas. For the estimation of TCRI, the following eq. 5 was applied.

$$TCRI = CRI \text{ (Pb)} + CRI \text{ (Hg)} + CRI \text{ (Cr)} + CRI \text{ (Ni)} + CRI \text{ (Cu)} \quad (5)$$

2.5. Statistical analysis

Data were statistically analyzed using XL Stat, Excel 2013, and values were reported as mean $\pm$ standard deviation (SD). Two-way ANOVA was applied to test the difference among spices and between study regions. Differences are considered to be significant if $p < 0.05$. Pearson correlation coefficients were also applied to investigate the correlations among metal concentrations. All the statistical tests were conducted at a 95% confidence limit. Differences are considered to be significant if $p < 0.05$.

3. Results and Discussion

3.1. Method validation

3.1.1. Analytical figure of merit

Calibration graphs were established using the standard working solution of understudied metals. The calibration curve's linear ranges were 20–50 ppb for Pb, 1–5 ppb for Hg, 0.2–1.0 ppm for Cr, 20–70 ppb for Ni in ppb, 0.5–2 ppm for Cu, having coefficient of correlation (R^2) were 0.9987 for Pb, 0.9974 for Hg, 0.9975 for Cr, 0.9987 for Ni, 0.9981 for Cu. To ensure that the process is reliable, the limit of detection (LOD) is calculated from 3 s. Where "s" stands for the standard deviation (SD) derived from ten measurements of blank readings, the LODs for Pb, Hg, Cr, Ni,

and Cu, were 0.1, and 0.012, 0.42, 0.21, and 0.51, mgL⁻¹, respectively Table 2. Similarly, the LOQ is ten time of “s”.

3.1.2. Validation of methodology

The digestion method was optimized for analysis of Pb, Hg, Cr, Ni, and Cu in spice samples by the analysis of certified reference (CRMs), i.e., NIST-1570a Spinach Leaves for Hg, Cu and Ni and NIST-SRM 1515-Apple Leaves for Pb and Cr Table 3 show the good precision between the certified materials (CRMs) and the measured values in ppm mg/kg. Similarly, the standard addition and recovery test (97.3 to 98.6%) were performed on spice samples to authenticate the digestion method at a 95% confidence limit as depicted in Table 4. Both optimization and validation showed good accuracy and precision for understudied metals with the proposed digestion method.

Table 2: Validation parameters of heavy metals

Validation parameters	Pb (ppb)	Hg (ppb)	Cr (ppm)	N (ppb)	Cu (ppm)
LOD (mg/Kg)	0.1	0.012	0.42	0.21	0.51
LOQ (mg/Kg)	1.0	0.120	4.20	2.10	5.10
Correlation coefficient (R ²)	0.9987	0.9974	0.9975	0.9987	0.9981

Table 3: Comparison of certified materials and measured values in mg/kg with percent recovery

Toxic metals	Certified Value x ± s	measured value x ± s	% Recovery
NIST-1570a Spinach Leaves			
Hg	0.0297 ± 0.005	0.0286 ± 0.04	96.3
Cu	12.22 ± 0.75	12.08 ± 0.29	98.8
Ni	2.142 ± 0.046	2.102 ± 0.03	98.1
NIST-SRM 1515-Apple Leaves			
Pb	0.479 ± 0.06	0.468 ± 0.08	97.7
Cr	0.3 ± 0.02	0.296 ± 0.04	98.6

*Note: x = mean value

s = standard deviation at 95% confidence limit

Table 4: Standard addition and recovery of heavy metals in digested samples (mg/kg)

Metal	Initial Conc. (mg/kg)	Spiked Amount (mg/kg)	Measured Conc. (mg/kg)	Recovery (%)
Pb	0.490 ± 0.02	0.5	0.970 ± 0.03	96
Hg	0.028 ± 0.003	0.05	0.077 ± 0.004	98
Cr	0.390 ± 0.01	1	1.342 ± 0.02	95
Ni	2.200 ± 0.05	0.5	2.670 ± 0.06	94
Cu	12.18 ± 0.3	1	13.12 ± 0.40	94

*Note: mg/kg at 95% confidence limit,

%Recovery = (measured conc. – initial conc.) / spiked amount) × 100

3.2. Metal concentration

Cinnamon, dried ginger, Turmeric, black seeds, and fenugreek were tested for their contamination of toxic heavy metals i.e. Pb, Hg, Cr, Ni and Cu, respectively over the five regions of Karachi, Pakistan Fig. 2 represents the mean metal contamination

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and WHO limits.

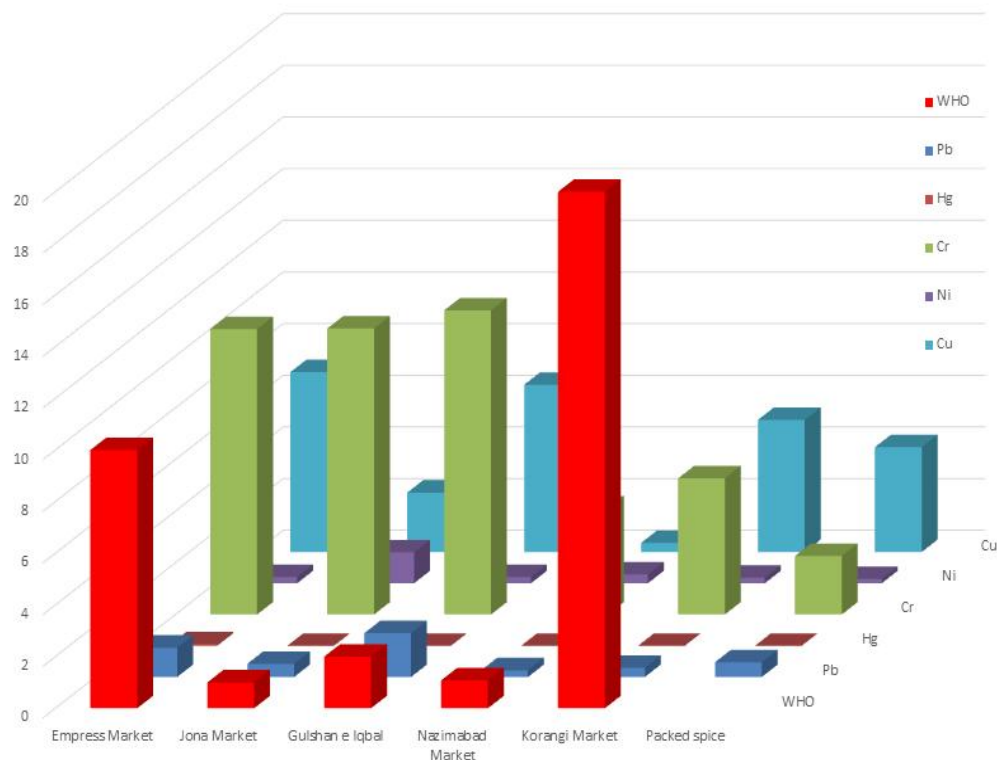


Fig. 2. Comparison of Heavy metals (Pb, Hg, Cr, Ni, and Cu) concentration across Empress Market, Jona Market, Gulshan e Iqbal Market, Nazimabad Market, Korangi and Packed spice with WHO limits

3.2.1. Lead

Lead is a heavy metal not necessary for any living being, and it can enter the human system through food, drink, and air. It creates a gradual toxicity even at lower concentrations and plays no positive role in human metabolism. There is no established safe exposure threshold for it. It has been found to build up mainly in humans and animals liver and kidneys (Belay et al., 2017; Ravisankar et al., 2019; Stojasavljević et al., 2019). **Table 3a-e** summarizes the mean concentrations of selected heavy metals in spices of different regions of Karachi. Significant differences in the amounts of heavy metals in different spices were observed that varied with their regions as well. The results of this study are compared with available literature and worldwide regulatory standards WHO/FAO. The Pb concentration range was between 0.260 and 1.696 mg/kg; all investigated spices had a Pb concentration within the WHO PML (10.0 mg/kg) (Organization, 2006). Some Pb levels in spices may be caused by deposition from air pollution, the distribution process, Pb-containing insecticides used during cultivation, metal smelting, or other factors.

Table 3a: Heavy metal concentration in spices and herbs of Empress market

S. No.	Spices and herbs	Lead (Pb) mg/Kg	Mercury (Hg) mg/Kg	Chromium(Cr) mg/Kg	Nickel(Ni) mg/Kg	Copper (Cu) mg/Kg
1	Cinnamon	1.904± 0.450	0.120± 0.045	9.380*± 0.749	0.285± 0.241	1.651± 1.451
2	Dried Ginger	2.721± 0.243	0.113± 0.051	14.72*± 0.551	0.471± 0.576	1.596± 1.997
3	Turmeric	0.382± 0.049	0.011± 0.051	7.530*± 0.047	0.142± 0.055	17.58± 9.642
4	Black pepper	0.354± 0.795	0.012± 0.041	10.98*± 0.142	0.166± 0.141	6.294±2.349
5	Fenugreek	0.321± 0.147	0.016± 0.051	12.67*± 0.058	0.166± 0.023	7.697± 4.717

*Note: Significantly above the limits of WHO

WHO maximum permissible limits for Lead (Pb): 10 mg/kg, Mercury (Hg): 1 mg/kg, Chromium (Cr): 2 mg/kg, Nickel (Ni): 1.08 mg/kg and Copper (cu): 20 mg/kg

Table 3b: Heavy metal concentration in spices and herbs of Jona market

S.No.	Spices and herbs	Lead (Pb) mg/Kg	Mercury (Hg) mg/Kg	Chromium(Cr) mg/Kg	Nickel(Ni) mg/Kg	Copper (Cu) mg/Kg
1	Cinnamon	1.214± 0.374	ND	14.82* ± 0.785	3.75*± 0.255	0.669± 0.544
2	Dried Ginger	1.750± 0.457	ND	10.14* ± 0.524	0.781± 0.820	2.470± 0.774
3	Turmeric	0.414± 0.914	ND	8.210* ± 0.711	0.882± 0.443	2.680± 0.687
4	Black pepper	0.354± 0.447	ND	9.214* ± 0.542	0.453± 0.471	6.445±0.23 4
5	Fenugreek	0.456± 0.368	ND	12.99* ± 0.812	0.115± 0.584	5.456± 0.932

*Note: Significantly above the limits of WHO

WHO maximum permissible limits for Lead (Pb): 10 mg/kg, Mercury (Hg): 1 mg/kg, Chromium (Cr): 2 mg/kg, Nickel (Ni): 1.08 mg/kg and Copper (cu): 20 mg/kg

Table 3c: Heavy metal concentration in spices and herbs of Gulshan e Iqbal market

S.No.	Spices and herbs	Lead (Pb) mg/Kg	Mercury (Hg) mg/Kg	Chromium(Cr) mg/Kg	Nickel(Ni) mg/Kg	Copper (Cu) mg/Kg
1	Cinnamon	4.000± 0.422	ND	9.380* ± 0.749	0.285± 0.241	1.651± 1.451

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2	Dried	2.211±	ND	14.72*	±	0.471±	1.596±
	Ginger	0.561			0.551	0.576	1.997
3	Black seed	0.821±	ND	14.59*	±	0.164±	3.866±
		0.621			0.233	0.044	5.344
4	Turmeric	0.948±	ND	7.530*	±	0.142±	17.58±
		0.728			0.047	0.055	9.642
5	Fenugreek	0.502±	ND	12.67*	±	0.166±	7.697±
		0.303			0.058	0.023	4.717

*Note: Significantly above the limits of WHO

WHO maximum permissible limits for Lead (Pb): 10 mg/kg, Mercury (Hg): 1 mg/kg, Chromium (Cr): 2 mg/kg, Nickel (Ni): 1.08 mg/kg and Copper (cu): 20 mg/kg.

Table 3d: Heavy metal concentration in spices and herbs of Nazimabad market

S.N o.	Spices and herbs	Lead (Pb) mg/Kg	Mercury (Hg)mg/Kg	Chromium(Cr) mg/Kg	Nickel(Ni) mg/Kg	Copper (Cu) mg/Kg
1	Cinnamon	0.441±		4.560*	±	0.123±
		0.550	ND	0.019		0.375±
2	Dried	0.191±		1.631	±	0.541
	Ginger	0.451	ND	0.051		0.143±
3	Turmeric	0.021±		5.120*	±	0.441
		0.539	ND	0.017		0.475±
4	Black	0.354±		5.300*	±	0.283±
	pepper	0.795	ND	0.021		0.554
5	Fenugreek	0.056±		3.887	*±	0.026±
		0.557	ND	0.041		0.233±0.2
						31
						0.014±
						0.011

*Note: Significantly above the limits of WHO

WHO maximum permissible limits for Lead (Pb): 10 mg/kg, Chromium (Cr): 2 mg/kg, Mercury (Hg): 1 mg/kg, Nickel (Ni): 1.08 mg/kg and Copper (cu): 20 mg/kg

Table 3e: Heavy metal concentration in spices and herbs of Korangi market

S.No	Spices and herbs	Lead (Pb) mg/Kg	Mercury (Hg) mg/Kg	Chromium(Cr) mg/Kg	Nickel(Ni) mg/Kg	Copper (Cu) mg/Kg
1	Cinnamon	0.231	ND	6.180± 0.059	0.162±	2.721±
		±			0.240	0.551
		0.055				
2	Dried Ginger	0.018	ND	6.422± 0.051	0.255±	9.260±
		±			0.147	0.017
		0.060				

3	Turmeric	0.882 ± 0.159	ND	5.451 ± 0.455	0.211 ± 0.551	4.158 ± 0.042
4	Black pepper	0.354 ± 0.230	ND	2.854 ± 0.441	0.226 ± 0.552	5.294 ± 0.549
5	Fenugreek	0.276 ± 0.388	ND	5.451 ± 0.553	0.244 ± 0.443	4.157 ± 0.417

*Note: Significantly above the limits of WHO

WHO maximum permissible limits for Lead (Pb): 10 mg/kg, Chromium (Cr): 2 mg/kg, Mercury (Hg): 1 mg/kg, Nickel (Ni): 1.08 mg/kg and Copper (Cu): 20 mg/kg

Table 3f: Heavy metal concentration in packed spices and herbs

S. No.	Spices and herbs	Lead (Pb) mg/Kg	Mercury (Hg) mg/Kg	Chromium(Cr) mg/Kg	Nickel(Ni) mg/Kg	Copper (Cu) mg/Kg
1	Cinnamon	0.821 ± 0.011	ND	1.110 ± 0.020	0.110 ± 0.010	2.341 ± 0.051
2	Dried Ginger	0.618 ± 0.020	ND	2.432 ± 0.011	0.124 ± 0.011	5.250 ± 0.016
3	Turmeric	0.800 ± 0.159	ND	3.551* ± 0.022	0.151 ± 0.020	3.158 ± 0.022
4	Black pepper	0.300 ± 0.100	ND	1.814 ± 0.020	0.224 ± 0.021	5.494 ± 0.043
5	Fenugreek	0.331 ± 0.080	ND	2.411 ± 0.020	0.211 ± 0.012	4.057 ± 0.016

*Note: Significantly above the limits of WHO

WHO maximum permissible limits for Lead (Pb): 10 mg/kg, Chromium (Cr): 2 mg/kg, Mercury (Hg): 1 mg/kg, Nickel (Ni): 1.08 mg/kg and Copper (Cu): 20 mg/kg

Gulshan-e-Iqbal spices contained the highest mean level of Pb concentration 1.696 mg/kg, in contrast, Nazimabad market spices had the lowest Pb contamination 0.260 mg/kg while the Pb values in Empress, Jona and Korangi markets were 1.136 mg/kg, 0.512 mg/kg, and 0.3522 mg/kg, respectively (Fig. 3). The Pb content in all spice samples differs significantly ($p < 0.05$). The variety of Pb accumulations in different areas of Karachi may be caused by different levels of environmental pollution in those areas. Evaluating the findings of several studies, Pb content was detected to be similar or lower in Karachi than in earlier trials conducted in other regions. Pb contents in spices are comparable with the results reported from the market (Shim et al., 2019) and Italian market (Bua et al., 2016) while the concentration of Pb is higher in herbs from Poland (Kowalska, 2021), in areca nut, black cumin seed, carom seed, green cardamom, and pomegranate seed from Hyderabad, Pakistan (Baig et al., 2019), in Ethiopian spices (Adugna et al., 2024), Saudi Arab spices (Mir et al., 2024) with the permissible limits (WHO).

3.2.2. Mercury

Over the five regions of Karachi, Pakistan, Mercury contamination was found only in the spices gathered from Karachi's Empress Market (Fig. 3). Mercury concentrations in spices ranged from 0.011 mg/kg to 0.120 mg/kg, with a mean of 0.544 mg/kg. The mercury levels in frequently used spices were within WHO guidelines (Organization, 2006). It was observed from the present research that spices and herbs from Karachi were not contaminated with mercury, as mercury exhibits dangerous health effects on the body when regularly consumed via food. Human kidneys, brains, and developing fetuses can all suffer damage from high levels of mercury exposure (Kowalska, 2021; Moussa et al., 2024). When compared with the previous works, the Hg concentration was slightly lower than in other countries. Similarly, amounts of Hg in herbs and spices were in agreement with the results of other studies (Bua et al., 2016; Nordin & Selamat, 2013; Shim et al., 2019).

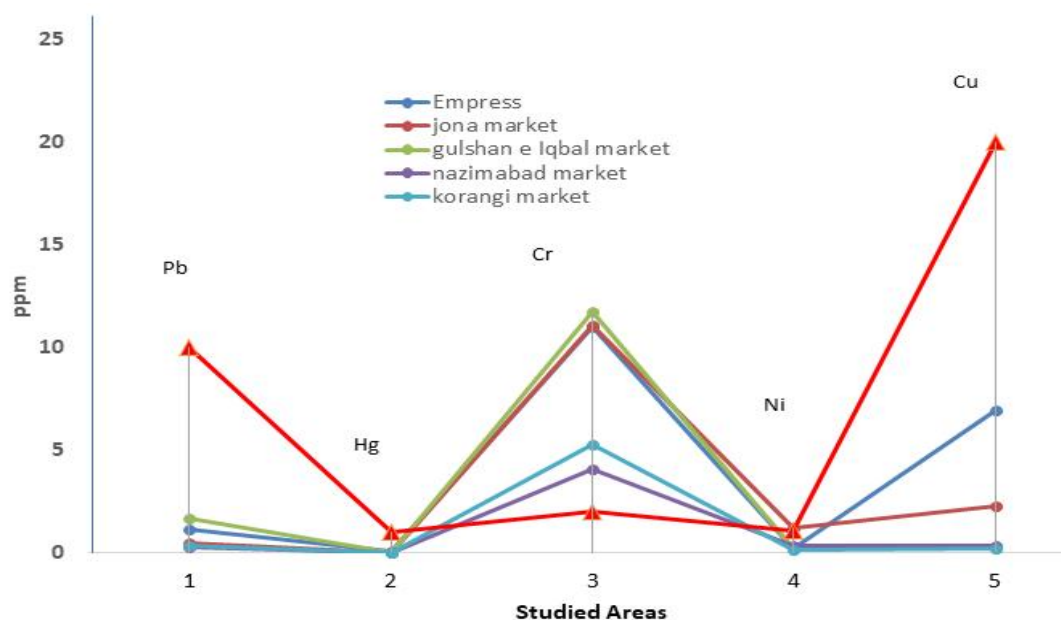


Fig. 3. Mean concentration of Heavy metals across studied areas and WHO limits

3.2.3. Chromium

Chromium is a toxic heavy metal, it contributes significantly to the body's physiological and biological processes when levels are within acceptable ranges (Lakshmanasenthil et al., 2013). However, its absence results in hyperglycemia. With increased body fat, as well as kidney injury from high Cr concentrations, *via* oxidation processes, the liver and blood cells (Bahiru et al., 2019).

The chromium content in all spice samples differed significantly $p < 0.05$ and ranged from 4.099 to 11.78 mg/kg. Cinnamon of Jona market contains the highest Cr concentration 14.82 mg/kg. According to this study, the majority of spices and herbs were found to be chromium-contaminated. Spices and herbs from Jona Market had the greatest mean Cr concentration 11.78 mg/kg. In contrast, samples taken from the Nazimabad Market had the lowest mean Cr concentration, which was 4.099 mg/kg (Fig. 3), which may be attributed to different environmental contamination over the different areas. The Cr concentration of the five regions of Karachi differs

significantly by $p < 0.05$. Cr is one of the known dangerous pollutants in the world. In large quantities, it poses a threat to both plants and animals. Compared with findings of previously published articles, the concentration of Cr was higher in black pepper reported from the Latvia market (Reinholds et al., 2017), ajwain from East Dembia contained significantly high Cr level (Tefera & Teklewold, 2021) and cumin from Saudi Arabian market also contained Cr level high (Seddigi et al., 2016). However, the concentrations of Cr in cinnamon in this study and thyme from India (Das & Das, 2018) were in agreement with the results reported in Karachi, Pakistan.

3.2.4. Nickel

The Nickel was found in the mean level of 0.219 - 1.196 mg/kg. Jona Market spices have the highest mean level while the lowest mean level was observed in Korangi market (Fig. 3). The nickel content was within the permissible limits (WHO 1.087 mg/kg) except cinnamon 3.75 mg/kg exceeded the Nickel concentration. It differs significantly over the region and within the same region also with $p < 0.05$. Compared with findings of previously published articles, the concentration of Ni in spices of Saudi Arabian markets (Seddigi et al., 2016), and Nigerian markets (Gaya & Ikechukwu, 2016) were within the acceptable limits while the Ni contamination was significantly higher in spices and herb collected from Hyderabad, Pakistan (Baig et al., 2019), cumin from the market of Asmara contained Ni with higher level as prescribed by WHO (Russom et al., 2019).

3.2.5. Copper

Copper is an essential trace element of the body which is important in a variety of biochemical processes. However, at higher levels, Cu is toxic and affects mainly the kidneys and blood. Copper was found in the mean levels of 0.354–6.963 mg/kg with the lowest concentration of Cu in Fenugreek from Nazimabad market while highest concentration in Turmeric from Empress Market (Fig. 3). All the samples that have been recorded were within the levels of WHO limits.

Compared with the previously reported findings observed that the Cu concentration in spices and herbs from the Saudi Arabian market (Seddigi et al., 2016), Nigerian market (Gaya & Ikechukwu, 2016), Korean market (Shim et al., 2019) and spices from Ethiopian market (Tefera & Teklewold, 2021) were slightly same as our results while spice collected from Panjab, Pakistan reported the lower level of Cu contamination in spices (Batool & Khan, 2014).

3.3. Non-carcinogenic and carcinogenic risk assessment

According to the results in Table 4a-e, the average daily intake (ADI) of toxic trace metals was determined as related to the average spices consumption by the adults in these studied regions. Health evaluation based on the average daily intake of (ADI) of heavy metal pollutants is one of the basic health risk assessment techniques. In general, health hazard due to trace metal contamination depends on the average ADI (Meseret et al., 2020). The ADI values of Pb, Hg, Cr, Ni, and Cu from the consumption of the studied samples were within the tolerable daily intake reference dose limits recommended by USEPA. The values of the total hazard quotient (THQ, Table 4a-e) for heavy metals pollution demonstrated that the level of Pb, Hg, Cr, Ni, and Cu through spice consumption was below the acceptable safety value of 1 ($THQ < 1$), indicating that the local inhabitants in the studied regions will not be exposed to the serious health risks and safe for human consumption. The hazard index values were less than 1 over the five regions of Karachi. For this study, the oral exposure

route from the consumption of various spices was considered for the health risk assessment of carcinogens. The decreasing order of carcinogenic risk values of examined heavy metals were as follows $\text{Cr} > \text{Cu} > \text{Pb} > \text{Ni} > \text{Hg}$. The CRI values of Pb, Hg, Cr, Ni, and Cu for an adult population from the studied locations were in the acceptable range. Moreover, all of the TCRI amounts of all carcinogenic risk heavy metals (Pb, Hg, Cr, Ni, and Cu) were between 1×10^{-6} - 1×10^{-4} , demonstrating the acceptable range for adults from the studied metals in spices.

Table 4a: ADI, THQ and HI values of heavy metals in spices of Empress market

S. No	Pb		Hg		Cr		Ni		Cu		HI
	ADI	THQ	ADI	THQ	ADI	THQ	ADI	THQ	ADI	THQ	
Cinnamon	2.8×10^{-04}	7.1×10^{-02}	1.7×10^{-05}	6.0×10^{-02}	1.4×10^{-3}	4.7×10^{-1}	4.3×10^{-05}	2.1×10^{-03}	2.5×10^{-04}	6.2×10^{-03}	6.1×10^{-01}
Dried Ginger	4.1×10^{-04}	1.0×10^{-01}	1.6×10^{-05}	5.6×10^{-02}	2.2×10^{-3}	7.3×10^{-1}	7.0×10^{-05}	3.5×10^{-03}	2.4×10^{-04}	6.0×10^{-03}	9.0×10^{-01}
Turmeric	5.7×10^{-05}	1.4×10^{-02}	1.6×10^{-06}	5.5×10^{-03}	1.1×10^{-3}	3.7×10^{-1}	2.1×10^{-05}	1.1×10^{-03}	2.6×10^{-03}	6.6×10^{-02}	4.6×10^{-01}
Black pepper	5.3×10^{-05}	1.3×10^{-02}	1.7×10^{-06}	6.0×10^{-03}	1.6×10^{-3}	5.5×10^{-1}	2.5×10^{-05}	1.2×10^{-03}	9.4×10^{-04}	2.3×10^{-02}	5.9×10^{-01}
Fenugreek	4.8×10^{-04}	1.2×10^{-02}	2.3×10^{-06}	8.0×10^{-03}	1.9×10^{-3}	6.3×10^{-1}	2.5×10^{-05}	1.2×10^{-03}	1.1×10^{-03}	2.9×10^{-02}	6.8×10^{-01}

Table 4b: ADI, THQ and HI values of heavy metals in spices of Jona market

S. No	Pb		Hg		Cr		Ni		Cu		HI
	ADI	THQ	ADI	THQ	ADI	THQ	ADI	THQ	ADI	THQ	
Cinnamon	1.8×10^{-4}	4.5×10^{-2}	1.7×10^{-5}	6.0×10^{-2}	1.4×10^{-3}	4.7×10^{-1}	4.3×10^{-5}	2.1×10^{-3}	2.5×10^{-4}	6.2×10^{-3}	6.1×10^{-01}
Dried Ginger	2.6×10^{-4}	6.5×10^{-2}	1.6×10^{-5}	5.6×10^{-2}	2.2×10^{-3}	7.3×10^{-1}	7.0×10^{-5}	3.5×10^{-3}	2.4×10^{-4}	6.0×10^{-3}	9.0×10^{-01}
Turmeric	6.1×10^{-5}	1.5×10^{-2}	1.6×10^{-6}	5.5×10^{-3}	1.1×10^{-3}	3.7×10^{-1}	2.1×10^{-5}	1.1×10^{-3}	2.6×10^{-3}	6.6×10^{-2}	4.6×10^{-01}
Black pepper	5.2×10^{-5}	1.3×10^{-2}	1.7×10^{-6}	6.0×10^{-3}	1.6×10^{-3}	5.5×10^{-1}	2.5×10^{-5}	1.2×10^{-3}	9.4×10^{-4}	2.3×10^{-2}	5.9×10^{-01}
Fenugreek	6.8×10^{-5}	1.7×10^{-2}	2.3×10^{-6}	8.0×10^{-3}	1.9×10^{-3}	6.3×10^{-1}	2.5×10^{-5}	1.2×10^{-3}	1.1×10^{-3}	2.9×10^{-2}	6.8×10^{-01}

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Table 4c: ADI, THQ and HI values of heavy metals in spices of Gulshan e Iqbal market

S. No	Pb		Hg		Cr		Ni		Cu		HI
	ADI	THQ	ADI	THQ	ADI	THQ	ADI	THQ	ADI	THQ	
Cinnamon	6.0 x10 ⁻⁴	1.5 x10 ⁻¹	BDL	BDL	5.5 x10 ⁻⁴	1.8 x10 ⁻¹	5.6 x10 ⁻⁵	2.8 x10 ⁻³	1.8x10 ⁻⁵	4.6x10 ⁻⁴	3.4x10 ⁻¹
Dried Ginger	3.3 x10 ⁻⁴	8.2 x10 ⁻²	BDL	BDL	7.2 x10 ⁻⁴	2.4 x10 ⁻¹	5.7 x10 ⁻⁵	2.9 x10 ⁻³	3.2x10 ⁻⁵	8.1 x10 ⁻⁴	3.3x10 ⁻¹
Turmeric	1.4 x10 ⁻⁴	3.5 x10 ⁻²	BDL	BDL	9.0 x10 ⁻⁴	3.0 x10 ⁻¹	7.5 x10 ⁻⁵	3.8 x10 ⁻³	8.7 x10 ⁻⁵	2.2 x10 ⁻³	3.4x10 ⁻¹
Black pepper	6.3 x10 ⁻⁵	1.6 x10 ⁻²	BDL	BDL	1.3 x10 ⁻³	4.4 x10 ⁻¹	3.1 x10 ⁻⁵	1.5 x10 ⁻³	1.9 x10 ⁻⁵	4.6 x10 ⁻⁴	4.5x10 ⁻¹
Fenugreek	7.5 x10 ⁻⁵	1.9 x10 ⁻²	BDL	BDL	1.1 x10 ⁻³	3.8 x10 ⁻¹	2.2 x10 ⁻⁵	1.1 x10 ⁻³	9.7 x10 ⁻⁵	2.4 x10 ⁻³	4.0x10 ⁻¹

Table 4d: ADI, THQ and HI values of heavy metals in spices of Nazimabad market

S. No	Pb		Hg		Cr		Ni		Cu		HI
	ADI	THQ	ADI	THQ	ADI	THQ	ADI	THQ	ADI	THQ	
Cinnamon	6.6 x10 ⁻⁵	1.6x10 ⁻²	BDL	BDL	6.8 x10 ⁻⁴	2.3 x10 ⁻¹	1.8 x10 ⁻⁵	9.2 x10 ⁻⁴	5.6 x10 ⁻⁵	1.4 x10 ⁻³	2.5 x10 ⁻¹
Dried Ginger	2.9 x10 ⁻⁵	7.1 x10 ⁻³	BDL	BDL	2.4 x10 ⁻⁴	8.1 x10 ⁻²	2.1 x10 ⁻⁵	1.1 x10 ⁻³	1.3 x10 ⁻⁴	3.2 x10 ⁻³	9.3 x10 ⁻²
Turmeric	3.1 x10 ⁻⁶	7.8 x10 ⁻⁴	BDL	BDL	7.6 x10 ⁻⁴	2.5 x10 ⁻¹	7.1 x10 ⁻⁵	3.5 x10 ⁻³	4.2 x10 ⁻⁵	1.1 x10 ⁻³	2.6 x10 ⁻¹
Black pepper	5.3 x10 ⁻⁵	1.3 x10 ⁻²	BDL	BDL	7.9 x10 ⁻⁴	2.6 x10 ⁻¹	3.9 x10 ⁻⁶	1.9 x10 ⁻⁴	3.5 x10 ⁻⁵	8.7 x10 ⁻⁴	2.8 x10 ⁻¹
Fenugreek	8.4 x10 ⁻⁶	2.1 x10 ⁻³	BDL	BDL	5.8 x10 ⁻⁴	1.9 x10 ⁻¹	1.4 x10 ⁻⁴	6.9 x10 ⁻³	2.1 x10 ⁻⁶	5.2 x10 ⁻⁵	2.0 x10 ⁻¹

Table 4e: ADI, THQ and HI values of heavy metals in spices of Korangi market

S. No	Pb		Hg		Cr		Ni		Cu		HI
	ADI	THQ	ADI	THQ	ADI	THQ	ADI	THQ	ADI	THQ	
Cinnamon	3.4 x10 ⁻⁵	8.6 x10 ⁻³	B D L	B D L	9.2 x10 ⁻⁴	3.1 x10 ⁻¹	2.4 x10 ⁻⁵	1.2 x10 ⁻³	4.1 x10 ⁻⁴	1.0 x10 ⁻²	3.3 x10 ⁻¹
Dried Ginger	2.7 x10 ⁻⁶	6.7 x10 ⁻⁴	B D L	B D L	9.6 x10 ⁻⁴	3.2 x10 ⁻¹	3.8 x10 ⁻⁵	1.9 x10 ⁻³	1.4 x10 ⁻³	3.5 x10 ⁻²	3.6 x10 ⁻¹
Turmeric	1.3 x10 ⁻⁴	3.3 x10 ⁻²	B D L	B D L	8.1 x10 ⁻⁴	2.7 x10 ⁻¹	3.1 x10 ⁻⁵	1.6 x10 ⁻³	6.2 x10 ⁻⁴	1.6 x10 ⁻²	3.2 x10 ⁻¹
Black pepper	5.3 x10 ⁻⁵	1.3 x10 ⁻²	B D	B D	4.3 x10 ⁻⁴	1.4 x10 ⁻¹	3.4 x10 ⁻⁵	1.7 x10 ⁻³	7.9 x10 ⁻⁴	2.0 x10 ⁻²	1.8 x10 ⁻¹

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			L	L							1
Fenugreek	4.1	1.0	B	B	8.1	2.7	3.6	1.8	6.2	1.6	3.0
	$\times 10^{-5}$	$\times 10^{-2}$	D	D	$\times 10^{-4}$	$\times 10^{-1}$	$\times 10^{-5}$	$\times 10^{-3}$	$\times 10^{-4}$	$\times 10^{-2}$	$\times 10^{-1}$
			L	L							1

Table 4f: ADI, THQ and HI values of heavy metals in packed spice

S. No	Pb		Hg		Cr		Ni		Cu		HI
	ADI	THQ	ADI	THQ	ADI	THQ	ADI	THQ	ADI	THQ	
Cinnamon	1.2	3.1	B	B	1.7x	8.5x	1.7x	5.6x1	1.7x	5.6x	5.4x
	$\times 10^{-4}$	$\times 10^{-2}$	D	D	10^{-4}	10^{-3}	10^{-5}	0^{-3}	10^{-5}	10^{-3}	10^{-1}
Dried Ginger	9.5	2.4	B	B	3.7x	1.8x	1.9x	1.9x1	1.9x	6.3x	6.9x
	$\times 10^{-5}$	$\times 10^{-2}$	D	D	10^{-4}	10^{-2}	10^{-5}	0^{-3}	10^{-5}	10^{-3}	10^{-1}
Turmeric	1.2	3.0	B	B	5.4x	2.7x	2.3x	1.6x1	2.3x	7.7x	7.8x
	$\times 10^{-4}$	$\times 10^{-2}$	D	D	10^{-4}	10^{-2}	10^{-5}	0^{-3}	10^{-5}	10^{-3}	10^{-1}
Black pepper	4.6	1.1	B	B	2.8x	1.3x	3.4x	1.7x1	3.4x	1.1x	5.8x
	$\times 10^{-5}$	$\times 10^{-2}$	D	D	10^{-4}	10^{-2}	10^{-5}	0^{-3}	10^{-5}	10^{-2}	10^{-1}
Fenugreek	5.1	1.2	B	B	3.7x	1.8x	3.2x	1.8x1	3.2x	1.1x	5.7x
	$\times 10^{-5}$	$\times 10^{-2}$	D	D	10^{-4}	10^{-2}	10^{-5}	0^{-3}	10^{-2}	10^{-2}	10^{-1}

3.4. Correlation analysis

Pearson correlation was used to calculate the successive combinations of the original variables and the results are presented in matrix form (Ahmed et al., 2023). Thus, the Pearson correlation coefficient was assigned to evaluate whether the different heavy metal levels in different spices were correlated. Table 5 demonstrated the values of the Pearson correlation test, which illustrates the correlation between the following metals Ni with Cr (0.8) in Cinnamon, Cr-Pb (0.8) in Dried Ginger, Cr-Pb (0.8), Cu-Pb (0.9), Cu-Cr (0.8), Cu-Ni (0.8) in Fenugreek. The highest significantly positively correlated heavy metals in spices and herbs show that these metals were derived from the same source and have the same origin.

Table 5: Pearson correlation matrix for different samples of spices.

		Pb	Hg	Cr	Ni	Cu
Cinnamon	Pb	1				
	Hg	0.1786	1			
	Cr	0.1090	0.0734	1		
	Ni	-0.3164	-0.2245	0.8708	1	
	Cu	0.1619	0.1429	-0.2210	-0.4370	1
Dried ginger	Pb	1				
	Hg	0.7149	1			
	Cr	0.8645	0.5166	1		
	Ni	0.2751	0.1069	0.6275	1	
	Cu	0.1619	0.1429	-0.2210	-0.4370	1

Turmeric	<i>Cu</i>	-0.4811	-0.2524	-0.2251	-0.2304	1
	<i>Pb</i>	1				
	<i>Hg</i>	-0.3360	1			
	<i>Cr</i>	0.4969	0.3071	1		
	<i>Ni</i>	0.04870	-0.4025	0.2428	1	
	<i>Cu</i>	0.2080	0.6041	0.5275	-0.6808	1
Black pepper	<i>Pb</i>	1				
	<i>Hg</i>	-0.3091	1			
	<i>Cr</i>	0.7626	0.2887	1		
	<i>Ni</i>	0.0221	-0.1470	0.0983	1	
	<i>Cu</i>	0.0192	0.6146	0.1391	-0.1748	1
	<i>Pb</i>	1				
Fenugreek	<i>Hg</i>	0.0563	1			
	<i>Cr</i>	0.8272	0.3916	1		
	<i>Ni</i>	-0.8784	-0.2587	-0.7855	1	
	<i>Cu</i>	0.9045	0.4740	0.8809	-0.8988	1

4. Conclusion

In conclusion, this study surveyed the concentrations of heavy metals Pb, Hg, Cr, Ni, and Cu in spices and herbs from five areas of Karachi, finding that these levels are mostly below the maximum acceptable limits for human consumption set by the WHO/FAO but higher from the accumulations from the packet spices and herbs. However, spices from Jona Market originated to accumulate significantly higher levels of heavy metals compared to those from other regions. The variation in metal content across the different spices can likely be attributed to differing local environmental pollution levels. Among the metals tested, chromium (Cr) was the only one that showed a marginal increase in non-carcinogenic risk, as indicated by the Total Hazard Quotient (THQ) and total Cancer Risk Index (TCRI) values. Strong correlations were observed between certain metal pair: Cu-Pb in Fenugreek. Based on these findings, it can be speculated that while the overall health risk from heavy metals may be mild, prolonged and regular consumption of these contaminated spices could pose a potential health risk to the population.

Credit authorship contribution statement

Faiza Nayyar : Writing - original draft, Formal analysis, Investigation, Data curation, **Nasreen Fatima**: Supervision, conceptualization, Methodology, **Saima Imad**: Methodology, Software, **Kanwal Zahid**: Review & Editing, Formatting. **Sheraz Shafiq** : Validation, Data curation. **Faisal Ghazanfer**: Visualization, Software, **Shazia Nisar**: Resources, Review & Data curation.

Author agreement statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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