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Original Article

Atomic absorption spectrophotometric analysis of toxic and essential heavy metals in some Pakistani medicinal and nutritional herbs along with their health risk assessment upon human consumption

[Análisis espectrofotométrico de absorción atómica de metales pesados tóxicos y esenciales en algunas hierbas medicinales y nutricionales paquistaníes junto con su evaluación de riesgos para la salud en caso de consumo humano]

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Abstract

Context: Medicinal herbs have gained worldwide popularity as natural raw materials, not only for herbal formulations and nutraceuticals but also in pharmaceutical and cosmeceutical products, while food and culinary products are available for the general population, having great benefits. However, heavy metal contamination in medicinal herbs is a dreadful health issue in the current scenario due to ever-increasing levels of environmental pollution and malagriculture practices.

Aims: To investigate the existence of some metal impurities, either toxic (Pb, Hg, Cd) or essential (Ni, and Cu), along with their concentration in thirteen selected medicinal herbs that are generally utilized in Pakistan as food and medicine.

Methods: The wet acid digestion method was employed for the atomic absorption spectroscopic technique to determine heavy metal contents. The system was calibrated first by preparing a standard solution for each metal in various concentrations, and then the samples were validated.

Results: Different concentrations of particular metal impurities were obtained in different herbs ranging from 0.98-2.39, 2.25-3.75, 0.42-1.17, 0.87-2.39, and 2.03-4.96 ppm for lead (Pb), mercury (Hg), cadmium (Cd), nickel (Ni), and copper (Cu), respectively. Moreover, the severity of health risk was assessed by calculating the estimated daily intake, targeted hazard quotient, target cancer risk and hazard index, which were found to be less than 1 value.

Conclusions: This study revealed that all medicinal and nutritional herbs are almost within limits according to USP and USEPA, but the regular monitoring of heavy metals in edible herbs and the implementation of mitigation procedures can help in the control and prevention of adverse health consequences in the human population.

Keywords: atomic absorption spectroscopy; heavy metal toxicity; medicinal plants; quality control; trace elements.

Resumen

Contexto: Las hierbas medicinales han ganado popularidad en todo el mundo como materias primas naturales, no sólo para formulaciones a base de plantas y nutracéuticos, sino también en productos farmacéuticos y cosmecéuticos, mientras que los alimentos y productos culinarios están disponibles para la población en general, teniendo grandes beneficios. Sin embargo, la contaminación por metales pesados en hierbas medicinales es un terrible problema sanitario en el escenario actual debido a los niveles cada vez mayores de contaminación ambiental y a las prácticas de malagricultura.

Objetivos: Investigar la existencia de algunas impurezas metálicas, ya sean tóxicas (Pb, Hg, Cd) o esenciales (Ni y Cu), junto con su concentración en trece hierbas medicinales seleccionadas que se utilizan generalmente en Pakistán como alimento y medicina.

Métodos: Se empleó el método de digestión ácida húmeda para la técnica espectroscópica de absorción atómica para determinar el contenido de metales pesados. El sistema se calibró primero preparando una solución estándar para cada metal en diversas concentraciones, y luego se validaron las muestras.

Resultados: Se obtuvieron distintas concentraciones de determinadas impurezas metálicas en diferentes hierbas, que oscilaban entre 0,98¬-2,39, 2,25-3,75, 0,42-1,17, 0,87-2,39 y 2,03-4,96 ppm para el plomo (Pb), el mercurio (Hg), el cadmio (Cd), el níquel (Ni) y el cobre (Cu), respectivamente. Además, la gravedad del riesgo para la salud se evaluó calculando la ingesta diaria estimada, el cociente de peligrosidad objetivo, el riesgo de cáncer objetivo y el índice de peligrosidad, que resultaron ser inferiores al valor 1.

Conclusiones: Este estudio reveló que todas las hierbas medicinales y nutricionales están casi dentro de los límites de acuerdo con USP y USEPA, pero el monitoreo regular de metales pesados en hierbas comestibles y la implementación de procedimientos de mitigación pueden ayudar en el control y prevención de consecuencias adversas para la salud en la población humana.

Palabras Clave: control de calidad; espectroscopia de absorción atómica; oligoelementos; plantas medicinales; toxicidad por metales pesados.

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Abbreviations: AAS: atomic absorption spectroscopy; HMs: heavy metals; EDI: estimated daily intake; THQ: targeted hazard quotient; TCR: target cancer risk; HI: hazard index.

INTRODUCTION

Natural flora and fauna of any country play a noteworthy role in the field of pharmaceuticals, nutritional or food supplements, cosmeceuticals, and agrochemicals (Evans, 2002; Hina et al., 2011). As per the World Health Organization (WHO), 80% of the population living in rural areas of developing countries use plants and their parts as traditional medicine. Apart from the various nutritional and medicinal benefits of the herbal species, their contamination with heavy metals is more likely to cause major health risks for living beings by producing drastically toxic effects on human health (Koh et al., 2009; Liu et al., 2005; Ross, 2005; Schimmer et al., 1994). To control this alarming situation, it is important to ensure the presence of heavy metals contamination in all herbal raw materials that are being used as food, medicine, or cosmetics. Generally, heavy metals (natural components of the earth's crust) are not biodegradable and have a longer biological shelf life (Li et al., 2017). Their accumulation in vital organs of the human body has a potential adverse effect, which leads to human poisoning and cell death.

A lot of other contributing factors are involved in the metal contamination of herbal commodities. Mostly natural habitats of medicinal and nutritional beneficial herbs are polluted with heavy metals (HMs) through land, air, and water, where the environmental conditions are not as good, such as heavy emissions from automobile vehicles, acidic rain, industrial spills (chemical waste) contaminating irrigation water, agricultural (pesticidal sprays) and domestic waste (biohazards) (WHO/FAO, 1996; Kabata-Pendia, 2001). Metals may be either essential HMs (such as copper and nickel play a vital role in biochemical activity in the body, but their excess intake from the permissible limit may cause some serious health issues and dysfunction in the vital organs) or maybe nonessential HMs (such as lead, mercury, and cadmium have drastic effects in the body even in minute quantities, causing acute and chronic toxicity in humans) (Bradl, 2005; He et al., 2005). In addition, the intoxication of heavy metals is involved in the binding of proteins and enzymes, which causes tissue damage and generates free radicals to promote oxidative stress on lipids, proteins, and DNA molecules for damage which tends to be carcinogenic, too (Abadin et al., 2007; Florea et al., 2004; Hu, 2002).

In Pakistan, not only is the traditional system of medicines utilizing medicinal herbs very popular, but the degree of environmental pollution is also very high. This may result in contamination of herbs with heavy metals and other minerals. In this regard, the objective of this current research study was to conduct a qualitative and quantitative estimation of heavy metals in commonly used herbs through atomic absorption spectroscopy as well as to assess the major health effects by various factors such as estimated daily intakes (EDI), target hazard quotient (THQ), target cancer risk (TCR) and hazard index (HI) of these herbs upon utilization. This study contributes to gather information regarding the safety of medicinal herbs upon human consumption and developing effective mitigation approaches (Elbagermi et al., 2012; Njoga et al., 2021).

MATERIAL AND METHODS

Plant materials

A total of 13 selected herbal species (*Cassia angustifolia, Foeniculum vulgare, Nigella sativa, Strychnos nux-vomica, Coriandrum sativum, Plantago ovata, Cymbopogon citratus, Onosma bracteata, Withania somnifera, Trigonella foenum-graecum, Cinnamomum zeylanicum, Cuminum cyminum, Piper nigrum*) were procured from local herbal market of Karachi, Pakistan to carry out the study Table 1. All samples were identified by Prof. Dr. Ghazal H Rizwani, and their voucher specimens were kept in the herbarium of the Pharmacognosy Department, University of Karachi, as voucher specimen no. A00163, A00164, A00165, A00166, A00167, A00168, A00169, A00170, A00171, A00173, A00174, A00175, and A00176, respectively.

Chemicals

Nitric acid (HNO_3), hydrogen peroxide (H_2O_2) (Merck, Germany), lead (Pb) stock solution, mercury stock solution (Hg), cadmium (Cd) stock solution, nickel (Ni) stock solution were purchased from Fisher Scientific, United Kingdom, copper (Cu) stock solution (Merck/ Sigma Aldrich), acetylene (C_2H_2), and argon (Ar) (Prim Gas, Pakistan). A separate hollow cathode lamp (Perkin Elmer, USA) was used to determine each element in atomic absorption spectrophotometry (AAS).

Sample treatment

After garbling (cleaning/segregating), each sample was powdered using a grinding machine (National, Japan) and passed through sieves with mesh no. 60 (ASTM Standard, Endecotts, UK). Plant powders were individually stored in clean, airtight, labeled vials, and cleanliness was ensured during the whole

Table 1. Description of Medicinal plants used in the study.

Herbal species	Family	Parts used	Medicinal and nutritional benefits
Cassia angustifolia M.Vahl. *(C.A)*	*Fabaceae*	Leaves	Cathartic, purgative, boils and abscesses, and rheumatism*
Foeniculum vulgare Mill. *(F.V)*	*Apiaceae*	Fruits	Carminative, diuretic, purgative, stimulant, vermicide, and aromatic*
Nigella sativa L. *(N.S)*	*Runnunculaceae*	Seeds	Condiment, purgative, oil in skin disease, diuretic, carminative, and mercury poisoning*
Strychnos nux-vomica L. *(S.Nv)*	*Longaniaceae*	Seeds	Respiratory and cardiac stimulant, and also given in colic*
Coriandrum sativum L. *(C.S)*	*Apiaceae*	Fruits	Embrocating for rheumatism, dysentery, piles, flatulence, hernia, measles, nausea, and toothache*
Plantago ovata Forssk. *(P.O)*	*Plantaginaceae*	Seeds	Laxative, dysentery, and constipation*
Cymbopogon citratus (DC.) Stapf *(C.C)*	*Poaceae / Germinaceae*	Leaves	Anti-depressant, anti-fungal, and anti-microbial*
Onosma bracteata Wall. *(O.B)*	*Boraginaceae*	Leaves	Demulcent, syphilis, rheumatism, leprosy, tonic, thirst, and restlessness*
Withania somnifera (L.) Dunal *(W.S)*	*Solanaceae*	Roots	Anti-inflammatory*
Trigonella foenum-graecum L. *(T.Fg)*	*Fabaceae*	Seeds	Condiment, tonic, flatulence, anti-diarrheal, emmenagogue, and laxative*
Cinnamomum zeylanicum Blume. *(C.Z)*	*Lauraceae*	Bark	anti-diabetic properties, anti-inflammatory, antioxidant, cough, flatulence, dyspepsia, carminative, diuretic, galactagogue, diaphoretic, and antiseptic*
Cuminum cyminum L. *(C.Cy)*	*Apiceae*	Fruits	Aromatic, diuretic, carminative, condiment, Stomachic, astringent, anti-diarrheal, dyspepsia, antiseptic, and flavoring*
Piper nigrum L. *(P.N)*	*Piperaceae*	Fruits	Anti-cholerine, antibacterial, anti-inflammatory properties, functional food, bronchitis asthma, chest congestion, and declog nose*

(*Ali and Peter, 2009; Bown, 1995-2002; Duke et al., 2002; Said, 1969; WHO, 1999; WFO, 2024).

process to minimize cross-contamination among the species (Perkin Elmer, 1996).

Sample preparation

Samples were prepared using the wet digestion method with slight modifications. Acid digestion of each plant sample was done using 7 mL of concentrated HNO_3, followed by the addition of 1 mL of hydrogen peroxide in a polytetrafluoroethylene digestion vessel, and the solution was heated in a water bath (Memmert, Germany) for a few hours until the fume was emitted from the digestion vessel. After being allowed to cool after digestion, each solution was evaporated to 2 mL and filtered through Whatman No. 41 filter paper in a 50 mL volumetric flask, and the volume was brought up with distilled water until marked (Perkin Elmer, 1996).

Stock solution

Stock solutions of 1000 ppm concentration were purchased from Merck/Sigma Aldrich chemicals and Fisher Scientific for Pb, Hg, Cd, Ni, and Cu as AAS analytes (Perkin Elmer, 1996).

Standard solution

Standard solutions of each element were prepared in five different concentrations to obtain the standard calibration curve by diluting a stock solution of 1000 ± 5 ppm (Nkansah et al., 2016).

Instrumentation

Perkin Elmer atomic absorption spectrophotometer 100 US was used for heavy metals analysis of all herbal species. All operating parameters were set according to standards, as mentioned in Table 2. The complete procedure of all the samples was replicated thrice to validate the process. Acetylene with supporting gas (air) was used as fuel in the combination of 2.5:15.0 L/min and was found to be suitable for the separation of cadmium, nickel, copper, and lead, while the fuel gas (argon) with supporting gas (air) in the combination of 5.5:15.0 L/min was robust for the separation of mercury (Lakshmi et al., 2015).

Table 2 Parameter set for AAS for a particular element.

Parameter	Lead (Pb)	Mercury (Hg)	Cadmium (Cd)	Nickel (Ni)	Copper (Cu)
Wavelength (nm)	283.5	253.7	228.8	232	324.8
Slit width (nm)	0.5	0.5	0.5	0.2	0.5
Lamp current (mA)	5	4	6	10	7
Atomization (°C)	2000	2400	2000	2300	2300
Sample solution flow rate (mL/min)	7	7	7	7	7
Drying temperature (°C)	110	110	110	110	110

Table 3 Absorbance of blank samples and concentration of the standard solution was used for the analysis of lead (Pb), mercury (Hg), cadmium (Cd), nickel (Ni), and copper (Cu).

Element	Absorbance of blank (ppm)	Standard solution prepared (ppm)
Pb	0.005	6
Hg	0.003	9
Cd	0.008	2
Ni	0.009	3
Cu	0.004	6

Table 4 Equation and correlation coefficient for the standard curves of lead (Pb), mercury (Hg), cadmium (Cd), nickel (Ni), and copper (Cu).

Element	Equation	Fitting method	Correlation coefficient (R^2)
Pb	Y= 0.0928X-0.0029	Linear	0.9999
Hg	Y= 0.0846X+0.0003	Linear	1.0000
Cd	Y= 0.009X+4E-05	Linear	0.9997
Ni	Y= 0.1163X+0.0034	Linear	0.9998
Cu	Y= 0.066X+0.005	Linear	0.9998

Linear correlation coefficient

Before the sample run, standard solutions of each metal in various concentrations were made from freshly prepared stock solutions with a blank (distilled water) to optimize AAS in Table 3 to plot calibration curves (Perkin Elmer, 1996). The linearity of the calibration curves based on the correlation coefficient (R^2) is evaluated in Table 4. Concentrations of metal impurities in all samples were determined by plotting graphs in accordance with the absorbance of each sample, as shown in Table 5 and Fig. 1A-E.

Health risk assessment

Human health risk assessment associated with the consumption of heavy metal-contaminated food and drugs was estimated by measuring four parameters, including estimated daily intake, target hazard quotient, hazard index, and target cancer risk (Njoga et al., 2021).

Estimated daily intake (EDI)

The evaluation of EDI for Pb, Hg, Cd, Ni, and Cu were computed by using the formula provided by the United States Environmental Protection Agency (USEPA) [1] (Smith, 1995).

$$EDI = \frac{(C \times QMC)}{BW} \quad [1]$$

Whereas C is the average concentration of specific heavy metals in herbal species, QMC is the average quantity of herbal species consumed daily (g), and BW is the average body weight set by USEPA. The estimated average daily ingestion of herbal species as foodstuffs and medicines is 0.01 kg/day, and the average body weight for adults is 70 kg (FAO/WHO, 2011).

Table 5. Statistical comparison of mean heavy metal content among herbs.

Herb species	Heavy metals content (ppm)				
	Pb	Hg	Cd	Ni	Cu
C. angustifolia	0.983 ± 0.005^{a}	2.513 ± 015^{c}	0.953 ± 0.02^{f}	1.123 ± 0.025^{bc}	2.030 ± 0.01^{a}
F. vulgare	1.120 ± 0.01^{b}	3.050 ± 0.02^{e}	0.743 ± 0.011^{d}	1.146 ± 0.015^{cd}	2.060 ± 0.02^{a}
N. sativa	2.236 ± 0.01^{fg}	2.750 ± 0.03^{d}	0.530 ± 0.02^{b}	1.203 ± 0.025^{d}	4.900 ± 0.025^{hi}
S. nux-vomica	2.290 ± 0.01^{gh}	2.380 ± 0.02^{b}	0.640 ± 0.01^{c}	2.033 ± 0.035^{f}	4.960 ± 0.02^{i}
C. sativum	1.973 ± 0.01^{e}	2.250 ± 0.03^{a}	1.060 ± 0.02^{g}	2.003 ± 0.005^{f}	4.980 ± 0.01^{i}
P. ovata	2.120 ± 0.02^{f}	3.576 ± 0.03^{f}	1.170 ± 0.01^{h}	2.293 ± 0.015^{g}	2.176 ± 0.015^{b}
C. citratus	2.390 ± 0.01^{h}	3.753 ± 0.01^{h}	0.420 ± 0.02^{a}	2.393 ± 0.025^{h}	2.353 ± 0.015^{c}
O. bracteata	1.210 ± 0.01^{c}	3.083 ± 0.05^{e}	0.526 ± 0.005^{b}	1.743 ± 0.025^{e}	3.423 ± 0.015^{d}
W. somnifera	1.330 ± 0.015^{c}	3.623 ± 0.015^{fg}	0.736 ± 0.025^{d}	0.913 ± 0.015^{a}	4.820 ± 0.02^{h}
T. foenum-graecum	1.120 ± 0.01^{b}	3.680 ± 0.03 ^{g}	0.950 ± 0.03^{f}	0.870 ± 0.02^{a}	4.960 ± 0.03^{i}
C. zeylanicum	1.640 ± 0.01^{d}	3.023 ± 0.015^{e}	1.060 ± 0.02 ^{g}	1.107 ± 0.02^{b}	3.790 ± 0.06^{e}
C. cyminum	1.532 ± 0.005^{d}	2.723 ± 0.015^{d}	1.170 ± 0.02^{h}	1.120 ± 0.02^{bc}	4.313 ± 0.055^{g}
P. nigrum	1.320 ± 0.01^{c}	2.243 ± 0.015^{b}	0.853 ± 0.01^{e}	1.106 ± 0.01^{bc}	4.010 ± 0.065^{f}

Each value represents mean ± SD heavy metal contents of three replicates, and values represented by the same alphabet in a column represent no statistically significant difference using ANOVA (p≤0.05) followed by Tukey HSD *posthoc* test, while statistical comparisons are done among herbs for each heavy metal.

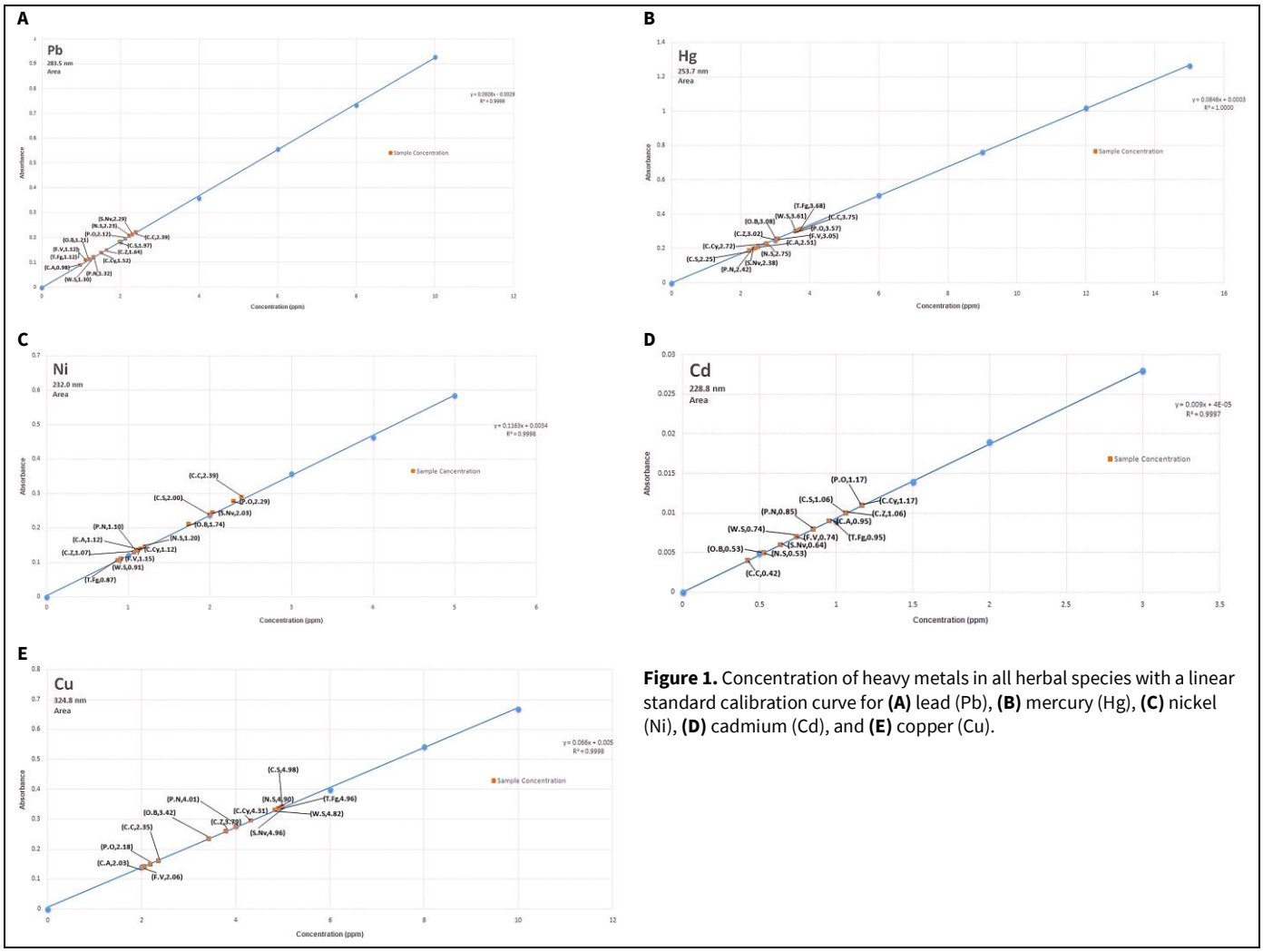

Figure 1. Concentration of heavy metals in all herbal species with a linear standard calibration curve for **(A)** lead (Pb), **(B)** mercury (Hg), **(C)** nickel (Ni), **(D)** cadmium (Cd), and **(E)** copper (Cu).

Target hazard quotient (THQ)

THQ is a non-carcinogenic health risk that occurs during the consumption of HMs, and it was determined using the formula according to USEPA [2] (Smith, 1995).

$$THQ = \frac{(Ef \times Ed \times QMC \times C)}{(RfD \times BW \times Et)} \times 10^{-3} \quad [2]$$

Where Ef is the frequency of exposure in days (i.e., 365), Ed is the duration of exposure in a lifetime (i.e., 60 years), QMC is the amount or quantity of herbal species consumed (g), and C is the average concentration of HMs (mg/kg), while RfD is the oral reference dose of the HMs set by the USEPA, as 4×10^{-3} mg/kg/day for Pb, 1×10^{-3} mg/kg/day for Cd, 4×10^{-2} mg/kg/day for Cu, 2×10^{-2} mg /kg/day for Ni, and 16×10^{-5} mg/kg/day for Hg. BW is the body weight, and Et is the lifetime exposure. If the computed value of THQ is <1, there is little probability of occurrence or non-carcinogenic health risk, and if its value is greater than or equal to 1, there is a high probability of non-carcinogenic adverse effects (FAO/WHO, 2011; Njoga et al., 2021).

Hazard index (HI)

There is more possibility that one herbal species may be contaminated with one or multiple HMs, and their consumption is necessary to determine the HI value. HI is the sum of THQ of HMs detected in herbal species, which was calculated as [3].

$$HI = THQPb + THQCd + THQCu + THQNi + THQHg \quad [3]$$

If the HI value is <1, there is no non-carcinogenic health effect, and if the value is ≥ 1, there is a possible risk of non-carcinogenic adverse effects (FAO/WHO, 2011; Njoga et al., 2021; Smith, 1995).

Target cancer risk (TCR)

The TCR evaluates the influence of carcinogenic risk related to lifetime exposure to carcinogenicity and was calculated by the formula [4].

$$TCR = CSF \times EDI \quad [4]$$

Whereas CSF is the cancer slope factor of specific HMs, set by the USEPA, the CSF for Pb is 0.008 mg/kg/day, 0.38 mg/kg/day for Cd, and 0.84 mg/kg/day for Ni (FAO/WHO, 2011; Smith, 1995).

Statistical analysis

Statistical analysis of the studied herbs was computed by using the tool Statistical Package for Social Sciences (SPSS) version 20 in order to find significant differences in heavy metal contents among various tested herbs using ANOVA followed by Tukey HSD (honestly significant difference) *posthoc* test at a significant level of ≤0.05 (Abdi and Williams, 2010; Nanda et al., 2021).

RESULTS

Heavy metal contamination of 13 herbal species is exhibited in Table 5, and concentrations of Pb, Hg, Cd, Ni, and Cu in all medicinal plants are graphically presented in Fig. 1A-E, respectively. The intensity of health hazards was determined by evaluating the average daily intake (EDI), target hazard quotient (THQ), and hazard index (HI) that are shown in Figs. 2-4, respectively.

Lead (Pb)

As a result of the atomic absorption of examined samples, a higher concentration of lead was observed in *Cymbopogon citratus* at 2.39 ppm, while a lower concentration was found in *Cassia angustifolia* at 0.98 ppm, as shown in Table 5.

Mercury (Hg)

In the tested samples of *Cymbopogon citratus, Withania somnifera,* and *Trigonella foenum-graecum,* 3.75 ppm, 3.61 ppm, and 3.68 ppm were observed high concentrations of mercury, respectively, whereas the minimum concentration of mercury found in *Coriandrum sativum* was 2.25 ppm as revealed in Table 5.

Cadmium (Cd)

The maximum concentration of cadmium content depicted in the studied herbs of *Plantago ovata* and *Cuminum cyminum,* i.e., 1.17 ppm for both, and both herbs are very demanding and have great potential in GIT aliments. However, the minimum concentration of cadmium content was found in *Cymbopogon citratus* 0.42 ppm, as exhibited in Table 5.

Nickel (Ni)

The high concentration of nickel content was found in the herbal species of *Cymbopogon citratus* at 2.39 ppm, and the low concentration was detected in *Trigonella foenum-graecum* (0.87 ppm), as shown in Table 5.

Copper (Cu)

The high concentration of copper was found in most of the examined medicinal plants such as *Coriandrum sativum, Strychnos nux-vomica, Nigella sativa, Trigonella foenum-graecum, Withania somnifera, Cuminum cyminum,* and *Piper nigrum* as 4.98 ppm, 4.96 ppm, 4.90 ppm, 4.96 ppm, 4.82 ppm, 4.31 ppm and 4.01 ppm, respectively, while the low concentration of

copper was presented in *Cassia angustifolia* (2.03 ppm) as revealed in Table 5.

Statistical analysis

The significant differences in heavy metal contents among 13 herbal species were determined by ANOVA followed by Tukey HSD (honestly significant difference) *posthoc* test using statistical package for social sciences (SPSS) version 20 tool. In this statistical analysis, the p-value showed $p \leq 0.05$, which indicated that *Cymbopogon citratus* and *Strychnos nux-vomica* showed a maximum concentration of Pb that was statistically significant compared to other herbs. Similarly, statistically highest concentrations of Hg, Cd, Ni, and Cu were detected in *Cymbopogon citratus*, *Cuminum cyminum*, and *Coriandrum sativum*, respectively, in Table 5 (Abdi et al., 2010; Nanda et al., 2021).

DISCUSSION

Heavy metal toxicity is a burning issue in the current scenario and needs to be addressed regarding the safety of general public health from dreadful effects on metals. Direct or indirect exposure to heavy metal contamination poses serious health hazards as traces of metals are ingested through foods, drinking water, and beverages via soil and air (He et al., 2005; Hu, 2002).

Medicinal and nutritional herbs from nature have been of great importance for use as remedies and foodstuffs for promoting health since prehistoric times (Liu et al., 2005). However, contaminated herbs may produce heavy metal toxicity upon human exposure. In this research study, we have determined three toxic heavy metals (Pb, Hg, and Cd) and two essential metals (Ni and Cu) concentrations in thirteen selected most commonly used medicinal plants that are consumed as drugs as well as food condiments in the daily routine of Asian cuisines. In addition, we have calculated the estimated daily intakes, target hazard quotient, hazard index, and target cancer risk expected from the utilization of all these herbs (Njoga et al., 2021).

Lead (Pb)

The concentration of Pb was found in the range of 0.98-2.39 ppm in all tested samples of medicinal plants, as mentioned in Table 5. Similarly, EDI values of all herbs were in the range from 1.4×10^{-4} to 3.91×10^{-4} µg/day, which was in the recommended limit of daily intake of USP, i.e., 10 µg/day for Pb in Table 6 and Fig. 2. Whereas, their THQ, TCR, and HI were found less than 1.0, as shown in Table 6 and Figs. 3-4.

Pb is one of the most toxic metals, which causes adverse health effects as a result of acute and chronic exposure above 20 µg/dL in blood level, such as their effect on the nervous system causing poor concentration, confusion, headache, and memory loss. It may also cause kidney dysfunction and damage the liver, cardiac and other body tissues (Harvey, 2002; Wani et al., 2015; Yedjou and Tchounwou, 2008).

Mercury (Hg)

Hg is another toxic heavy metal that was detected in the range of 2.24-3.75 ppm, as exhibited in Table 5. The estimated daily intake was also calculated for health risk assessment, which was in the range from 3.21×10^{-4} to 5.36×10^{-4} µg/day, which was found within the permissible limit of USP 15 µg/day for Hg. Furthermore, the target hazard quotient and hazard index of examined species were found to be less than 1.0, as shown in Table 6 and Figs. 2-4. However, the target cancer risk was not calculated as USEPA did not provide CSF for Hg.

Mercury can be inhaled as vapor and transmitted to humans through municipal sewage water discharge, agriculture, incineration, and industrial effluent (Tchounwou et al., 2003; Unaegbu et al., 2016). It may cause certain complications in humans if their blood mercury level is higher than 20 µg/dL, which is associated with neurological tissue damage, nephropathy, neuropathy, and hepatopathy (Engwa et al., 2015; Tchounwou et al., 2003).

Cadmium (Cd)

The concentration of Cd in all the studied samples was found in the range of 0.42-1.17 ppm, as shown in Table 5. Whereas the EDI for Cd was estimated during the study from 9.10×10^{-5} to 1.67×10^{-4} µg/day, that was lie in the accepted limit of USP, i.e., 5 µg/day. Besides that, THQ, HI, and TCR were also found to be minimal in examined species as their values were below 1.0, as revealed in Table 6 and Figs. 2-4.

Acute and chronic toxicity of Cd includes lung, respiratory, and stomach irritation, osteoporosis, and kidney problems if its level exceeds 5 µg/dL in blood (Ashizawa et al., 2008; Rahimzadeh et al., 2017; Satarug et al., 2003).

Nickel (Ni)

The concentration of examined herbal samples was in the range of 0.87-2.39 ppm, as exhibited in Table 5. However, the health risk was observed to be very low on the basis of hazard calculation, and the estimated daily intake of each herbal species was in the range of 1.24×10^{-4} to 3.41×10^{-4} µg/day, which

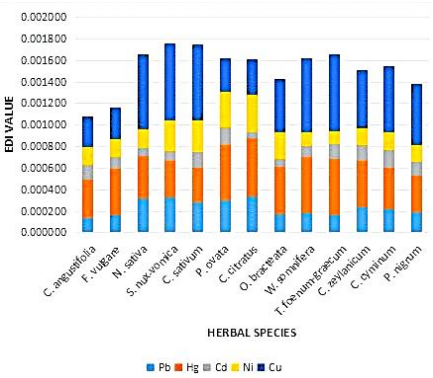

Figure 2. Estimated daily intakes of (EDI value) of selected medicinal and nutritional herbs.

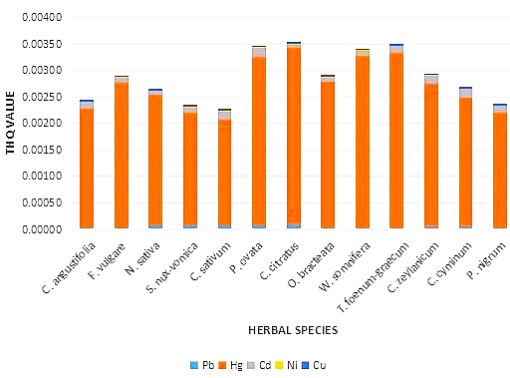

Figure 3. Target hazard quotient (THQ) of selected medicinal and nutritional herbs.

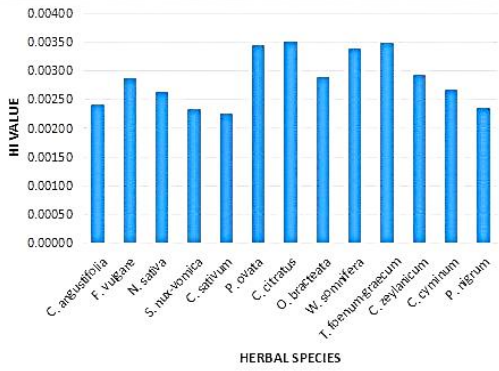

Figure 4. Estimated hazard index (HI) of selected medicinal and nutritional herbs.

was in the safe limit of Ni, i.e., 200 µg/day provided by USP. Similarly, the risk assessment for target hazard quotient, target cancer risk, and hazard index was also found to be minimal in studied herbal samples as the value was below 1.0, as shown in Table 6 and Figs. 2-4. Ni found in many products can cause toxicity in long-term exposure if their content is accumulated in blood until 1 µg/dL. It can mutate genes, generate free radicals, and regulate the expression of long-chain non-coding RNAs, microRNAs, and certain mRNAs. The generation of free radicals is involved in the promotion of carcinogenicity (Amirah et al., 2013; Zambelli et al., 2016; Zhou et al., 2017).

Copper (Cu)

Cu is administered in humans by anthropogenic activity (Dorsey et al., 2004). The concentrations of Cu in the studied herbal samples were in the range of 2.03-4.98 ppm in Table 5, while the estimated daily intake of plant species was in the range from 2.90 × 10^{-4} to 7.11 × 10^{-4} µg/day, which was in the permissible limit of daily intake of USP 3000 µg/day for Cu. In addition, the hazard value for Cu was also below 1.0, as shown in Table 6 and Figs. 2-4, whereas, the target cancer risk for copper in examined plant species was not measured due to the cancer slope factor was not available by USEPA. Chronic exposure to copper may be toxic to humans in the form of cupric (Cu^{2+}) and cuprous (Cu^{1+}), which is involved in the oxidation-reduction reaction, resulting in the generation of free radical $OH^{\cdot}$ that can distress the gene (DNA) expression and inducing oxidation of low-density lipoprotein (Burkitt, 2001).

CONCLUSION

In the current scenario, the contamination of herbal material by heavy metals is a hot topic for healthcare researchers. Crude medicinal plants are used for therapeutic purposes in traditional and complementary medicines and serve as raw materials for herbal medicinal products. Heavy metal-contaminated herbs may cause serious unwell effects, especially upon long-term use. Hence, it is a prerequisite to qualitatively and quantitatively evaluate the herbal material for heavy metal contamination. In our research study, we analyzed the concentration of Pb, Cd, Hg, Ni, and Cu in some Pakistani medicinal plants through atomic absorption spectroscopy. In addition, EDI, THQ, HI, and TCR were also assessed and were found satisfactory, but the adoption of good agriculture practices and regular monitoring of heavy metals in the environment and edible items are essential for the control and prevention of harmful health consequences in human society.

Table 6. Risk analysis of toxic heavy metals in selective herbal species.

Herb species	Pb			Hg		Cd			Ni			Cu		HI
	EDI	THQ	TCR	EDI	THQ	EDI	THQ	TCR	EDI	THQ	TCR	EDI	THQ	
C. angustifolia	1.40×10^{-4}	4.0×10^{-5}	1.12×10^{-6}	3.59×10^{-4}	2.24×10^{-3}	1.36×10^{-4}	1.40×10^{-4}	5.16×10^{-5}	1.60×10^{-4}	1.0×10^{-5}	1.34×10^{-4}	2.90×10^{-4}	1.0×10^{-5}	2.43×10^{-3}
F. vulgare	1.60×10^{-4}	4.0×10^{-5}	1.28×10^{-6}	4.36×10^{-4}	2.72×10^{-3}	1.06×10^{-4}	1.10×10^{-4}	4.02×10^{-5}	1.64×10^{-4}	1.0×10^{-5}	1.39×10^{-4}	2.94×10^{-4}	1.0×10^{-5}	2.88×10^{-3}
N. sativa	3.91×10^{-4}	8.0×10^{-5}	2.55×10^{-6}	3.93×10^{-4}	2.72×10^{-3}	7.60×10^{-5}	8.0×10^{-5}	2.88×10^{-5}	1.71×10^{-4}	1.0×10^{-5}	1.44×10^{-4}	7.00×10^{-4}	2.0×10^{-5}	2.46×10^{-3}
S. nux-vomica	3.27×10^{-4}	8.0×10^{-5}	2.62×10^{-6}	3.40×10^{-4}	2.13×10^{-3}	9.10×10^{-5}	9.0×10^{-5}	3.47×10^{-5}	2.90×10^{-4}	1.0×10^{-5}	2.44×10^{-4}	7.09×10^{-4}	2.0×10^{-5}	2.33×10^{-3}
C. sativum	2.81×10^{-4}	7.0×10^{-5}	2.55×10^{-6}	3.21×10^{-4}	2.01×10^{-3}	1.51×10^{-4}	1.50×10^{-4}	5.75×10^{-5}	2.86×10^{-4}	1.0×10^{-5}	2.40×10^{-4}	7.11×10^{-4}	2.0×10^{-5}	2.26×10^{-3}
P. ovata	3.03×10^{-4}	8.0×10^{-5}	2.42×10^{-6}	5.10×10^{-4}	3.19×10^{-3}	1.67×10^{-4}	1.70×10^{-4}	6.35×10^{-5}	3.27×10^{-4}	2.0×10^{-5}	2.75×10^{-4}	3.11×10^{-4}	1.0×10^{-5}	3.45×10^{-3}
C. citratus	3.41×10^{-4}	9.0×10^{-5}	2.73×10^{-6}	5.36×10^{-4}	3.35×10^{-3}	6.00×10^{-5}	6.0×10^{-5}	2.28×10^{-5}	3.41×10^{-4}	2.0×10^{-5}	2.87×10^{-4}	3.36×10^{-4}	1.0×10^{-5}	3.52×10^{-3}
O. bracteata	1.73×10^{-4}	4.0×10^{-5}	1.38×10^{-6}	4.40×10^{-4}	2.75×10^{-3}	7.60×10^{-5}	8.0×10^{-5}	2.88×10^{-5}	2.49×10^{-4}	1.0×10^{-5}	2.10×10^{-4}	4.89×10^{-4}	1.0×10^{-5}	2.89×10^{-3}
W. somnifera	1.86×10^{-4}	5.0×10^{-5}	1.49×10^{-6}	5.16×10^{-4}	3.22×10^{-3}	1.06×10^{-4}	1.10×10^{-4}	4.02×10^{-5}	1.30×10^{-4}	1.0×10^{-5}	1.09×10^{-4}	6.89×10^{-4}	2.0×10^{-5}	3.40×10^{-3}
T. foenum-graecum	1.6×10^{-4}	4.0×10^{-5}	1.28×10^{-6}	5.26×10^{-4}	2.29×10^{-3}	1.36×10^{-4}	1.40×10^{-4}	5.16×10^{-5}	1.24×10^{-4}	1.0×10^{-5}	1.04×10^{-4}	7.09×10^{-4}	2.0×10^{-5}	3.49×10^{-3}
C. zeylanicum	2.34×10^{-4}	6.0×10^{-5}	1.87×10^{-6}	4.31×10^{-4}	2.70×10^{-3}	1.51×10^{-4}	1.50×10^{-4}	5.75×10^{-5}	1.53×10^{-4}	1.0×10^{-5}	1.28×10^{-4}	5.41×10^{-4}	1.0×10^{-5}	2.93×10^{-3}
C. cyminum	2.17×10^{-4}	5.0×10^{-5}	1.74×10^{-6}	3.89×10^{-4}	2.43×10^{-3}	1.67×10^{-4}	1.70×10^{-4}	6.35×10^{-5}	1.60×10^{-4}	1.0×10^{-5}	1.34×10^{-4}	6.16×10^{-4}	2.0×10^{-5}	2.67×10^{-3}
P. nigrum	1.89×10^{-4}	5.0×10^{-5}	1.51×10^{-6}	3.46×10^{-4}	2.16×10^{-3}	1.21×10^{-4}	1.20×10^{-4}	4.61×-10^{-5}	1.57×10^{-4}	1.0×10^{-5}	1.32×10^{-4}	5.73×10^{-4}	1.0×10^{-5}	2.35×10^{-3}

TCR for Hg and Cu could not be calculated because the cancer slope factor was not available from USEPA. * Minimal risk or no risk found on consumption (i.e., <1 mg/kg/day). EDI: estimated daily intake; THQ: targeted hazard quotient; TCR: target cancer risk; HI: hazard index; Pb: lead; Hg: mercury; Cd: cadmium; Ni: nickel; and Cu; copper.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Hyder Z	Rizwani GH	Hina B	Ahmed I	Ahmed K	Iqbal W	Azhar I	Shareef H
Concepts or ideas		x						
Design		x						
Definition of intellectual content	x				x		x	x
Literature search	x		x		x	x	x	x
Experimental studies	x			x			x	x
Data acquisition		x		x				
Data analysis	x		x	x	x	x		x
Statistical analysis	x		x	x	x	x	x	x
Manuscript preparation	x							
Manuscript editing	x	x	x					
Manuscript review	x	x	x	x	x	x	x	x

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