

Assessment of Toxic Heavy Metals, Trace Elements, and Essential Mineral Levels in Traditional Herbal Medicines Widely Used in Khyber Pakhtunkhwa, Pakistan

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ABSTRACT

Traditional herbal remedies and nutritional supplements are widely used to manage various health conditions, but many lack proper standardization and scientific validation for safety and effectiveness. In this research, multiple forms of traditional herbal medicines prescribed for specific diseases were collected from practitioners in various districts of Khyber Pakhtunkhwa, Pakistan. The samples were analyzed using atomic absorption spectroscopy to determine concentrations of heavy metals, trace elements, and essential minerals. All products contained detectable levels of heavy metals, trace elements, and minerals. Toxic metals such as arsenic (As), cadmium (Cd), and lead (Pb) were present in every sample, while trace elements including cobalt (Co), iron (Fe), zinc (Zn), and chromium (Cr) were generally within safe limits. Essential minerals like sodium (Na), magnesium (Mg), and calcium (Ca) were found at levels beneficial for bodily functions. Hazard quotient (HQ) analysis indicated that arsenic levels exceeded safe thresholds in all samples, and Cd and Pb exceeded limits in roughly half of the products tested. The presence of toxic metals above recommended levels raises concerns about the quality and safety of these herbal medicines. This study underscores the importance of routine monitoring and standardization of traditional herbal products to safeguard public health and ensure product reliability.

Keywords: Standardization, Hazard quotient, Traditional herbal medicines, Minerals, Heavy metals

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Introduction

Traditional medicines are therapeutic products consumed either directly by individuals or prescribed by local practitioners, often presented in forms such as powders, tablets, capsules, pellets, emulsions, suspensions, mixtures, or decoctions [1]. These remedies are also referred to as “complementary,” “alternative,” or “non-conventional” medicines in the healthcare systems of many countries. Examples include herbal formulations, vitamins, nutritional supplements, as well as Ayurvedic, Chinese, and Homeopathic preparations [2]. While complementary medicines and health supplements have been traditionally used to manage various health conditions, most have not undergone formal safety evaluations [3]. The widespread use of such unregulated products exposes populations to multiple health risks, raising concerns among regulatory and public health agencies at both national and international levels due to reports of adverse effects, which in some cases can be life-threatening [4].

Certain Ayurvedic and traditional formulations intentionally contain heavy metals for therapeutic purposes; thus, assessing the safety of these metals is critical to ensure that their levels remain within permissible limits and do not pose toxic risks [5]. Several studies have reported hazardous concentrations of heavy metals in traditional medicines, with arsenic, cadmium, and lead identified as major health threats [6].

In addition to toxic heavy metals, conventional herbal medicines in Asia may contain trace metals such as copper, zinc, nickel, cobalt, iron, and manganese, as well as essential minerals including sodium, potassium, calcium, and

magnesium. While these elements are beneficial within safe ranges, exceeding the recommended limits established by WHO and other authoritative bodies can lead to toxicity [5, 7].

Unexpected toxic effects can also result from contaminants or misidentified constituents in herbal products [8]. The severity of heavy metal toxicity depends on factors such as the type of metal, route and duration of exposure, and the age of the individual, with children being particularly vulnerable due to their developing physiological systems [9]. Therefore, preclinical safety evaluations are crucial for ensuring safe therapeutic use. Regulatory authorities must oversee the registration, marketing, and monitoring of both raw materials and finished herbal products, requiring submission of efficacy and safety data [10].

This study aims to quantify heavy metals, trace metals, and essential minerals in traditional herbal medicines supplied by local vendors (Hakims) in Khyber Pakhtunkhwa, Pakistan, and to calculate the hazard quotient (HQ) values. The findings will contribute to validating the safety of these widely used remedies.

Materials and Methods

Chemicals and reagents

Nitric acid (HNO₃), perchloric acid (HClO₄), and standard reference materials for arsenic, cadmium, lead, iron, zinc, cobalt, chromium, sodium, potassium, calcium, and magnesium were used in this study. Stock solutions of each metal (1000 µg/mL) were prepared and subsequently diluted with deionized water for calibration purposes. All standard solutions were procured from Sigma-Aldrich, and all reagents were of analytical grade.

Instrumentation

Analysis was performed using an Atomic Absorption Spectrophotometer (Analyst 700, PerkinElmer, USA) equipped with appropriate hollow cathode lamps at COMSATS University, Abbottabad Campus. The operational parameters for the atomic absorption analysis are detailed in **Table 1**.

Table 1. Operating conditions

Element	Fuel (Acetylene/air)	Wavelength (nm)	Sensitivity (µg/g)	LOD (µg/g)	LOQ (µg/g)
Mg	–	285.2	0.3	0.002	0.004
Ca	–	422.7	4.0	0.015	0.040
Zn	–	213.9	1.0	0.015	0.040
Na	–	589.0	0.5	0.003	0.009
K	–	766.5	2.0	0.030	0.009
Fe	–	248.3	6.0	0.005	0.015
Cr	–	357.9	4.0	0.003	0.009
Co	–	240.7	7.0	0.009	0.030
As	–	224	1.5	0.007	0.040
Cd	–	228.8	1.5	0.008	0.024
Pb	–	283.3	20	0.015	0.040

LOD: Limit of detection.

LOQ: Limit of quantification.

Sample collection

A total of 100 samples were obtained with the assistance of volunteer groups who visited local practitioners (quacks/hakims) as patients. The selection of disease conditions was guided by the common treatment practices in the region and the specialization of the respective practitioners. The collected products included mixed, compounded, or locally prepared herbal formulations, as well as powdered herbs encapsulated for oral use. These were available in various forms such as pastes (kushtay), powders, tablets, creams, and hand-prepared capsules, intended for the treatment of a wide array of conditions, including gastrointestinal disorders, hemorrhoids, sexual dysfunction, arthritis, diabetes mellitus, hypertension, leukemia, and kidney stones. Detailed information about the collected samples is presented in **Table 2**. From the total collection, samples were randomly selected for subsequent analysis.

Table 2. Samples collected for various diseases from KP.

Disease	Sample ID	Dosage Form	District
Piles	H-2	Powder	Mardan
	H- 9	Capsule	Mardan
	H- 20	Capsule	Swabi
	H-24	Powder	Bannu
	H-31	Powder	Bannu
	H-48	Powder	Buner
	H-60	Pills	Swabi
	H-83	Capsule	Mardan
Stomach Disorder	H-3	Capsule	Mardan
	H-18	Powder	Swabi
	H-25	Powder	Bannu
	H-37	Tablets	Dir (L)
	H-41	Powder	Dir (L)
	H-44	Powder	Swat
	H-54	Powder	Buner
	H-55	Powder	Buner
	H-56	Powder	Buner
	H-63	Powder	Swabi
	H-65	Tablets	Swabi
	H-68	Powder	Swabi
	H-84	Powder	Mardan
	H-89	Tablets	Charsadda
Sexual Dysfunction	H-7	Capsule	Mardan
	H-11	Tablets	Swabi
	H-12	Tablets	Swabi
	H-28	Powders	Bannu
	H-58	Powders	Buner
	H-71	Capsules	Swabi
	H-73	Powders	Swabi
	H-76	Powders	Swabi
	H-79	Powders	Buner
	H-90	Capsules	Mardan
Arthritis	H-5	Capsule	Mardan
	H-15	Powders	Swabi
	H-16	Powders	Swabi
	H-23	Powders	Bannu
	H-30	Powders	Bannu
	H-33	Powders	Dir (L)
	H-35	Powders	Dir (L)
	H-36	Powders	Dir (L)
	H-39	Capsule	Dir (L)
	H-43	Powders	Dir (L)
	H-74	Capsule	Swabi
Hypertension, Leukemia and Diabetes mellitus	H-29	Powder	Bannu
	H-32	Pellets	Bannu
	H-61	Powder	Swabi
	H-62	Powder	Swabi
	H-50	Powder	Shangla
	H-26	Powder	Bannu
H-69	Powder	Swabi	

Samples and standard preparation

To quantify heavy metals and mineral elements, 0.5 g of the powdered material from each formulation was subjected to acid digestion. The samples were placed in Teflon digestion vessels and treated with 20 mL of an acid mixture consisting of concentrated HNO₃ and HCl in a 3:1 ratio, followed by heating at 85 °C for three hours. Subsequently, 1 mL of perchloric acid (HClO₄) was added to accelerate oxidation during the digestion process. After cooling, each digest was filtered and diluted to a final volume of 50 mL using distilled water. A reagent blank, prepared using the same protocol but without sample material, served as a control.

Standard solutions representing three concentration levels for each metal were prepared as outlined in **Table 3** and used for calibration. Metal and mineral concentrations in all herbal samples were quantified in mg/L. For accurate quantification, each standard concentration was measured in triplicate, and the optimal value from these replicates was selected for calibration. Sample measurements were then performed using these finalized standard values.

Table 3. Calibration levels and corresponding optimal readings for metals and minerals

S/NO	Metal Standards (Applied Concentrations)	Optimal Measurement Obtained	Mineral Standards (Applied Concentrations)	Optimal Measurement Obtained
1	Co prepared at 2, 4, and 6	Co: 240.7	Mg prepared at 1, 5, and 15	Mg: 285.2
2	As prepared at 1, 5, and 15	As: 193.7	Ca prepared at 10, 15, and 30	Ca: 422.7
3	Cd prepared at 1, 5, and 10	Cd: 228.8	Na prepared at 2, 5, and 10	Na: 589.0
4	Fe prepared at 10, 20, and 30	Fe: 248.3	K prepared at 2, 4, and 6	K: 769.9
5	Zn prepared at 5, 10, and 25	Zn: 213.9	—	—
6	Cr prepared at 1, 5, and 15	Cr: 357.9	—	—
7	Pb prepared at 1, 5, and 10	Pb: 283.3	—	—

Health risk assessment

Because Arsenic (As), Cadmium (Cd), and Lead (Pb) pose toxicity risks even at minimal exposure, an additional safety evaluation was undertaken to quantify potential health hazards associated with these metals. Instrumental measurements initially expressed in mg/L were converted to mg/kg using the formula:

Element (mg/kg) = (mg/L in solution × solution volume × D.F) ÷ sample weight (g),

where the dilution factor (D.F) followed the method described in reference [11].

For calculating health risk indices, a daily intake of 100 mg of the traditional medicine for an adult weighing 70 kg was assumed, consistent with dosages commonly recommended by local healers. Using these assumptions, hazard quotients (HQs) were computed for metals with internationally recognized maximum permissible levels. The calculation followed [12]:

HQ = (Daily dosage × metal concentration in sample (mg/kg)) ÷ (Rf × body weight),

with Rf representing the regulatory safety threshold of the metal.

An HQ < 1 signifies an acceptable exposure level, whereas HQ ≥ 1 denotes potential health risk.

Statistical interpretation

Mean concentrations and standard deviations for all quantified metals and minerals were determined. To evaluate whether measured values deviated significantly from established reference ranges, a Student's t-test was applied.

Results and Discussion

Traditional herbal remedies collected from local practitioners underwent quantitative evaluation for eleven metals and minerals using atomic absorption spectroscopy. The findings were grouped into toxic heavy metals, trace elements, and essential minerals.

Toxic heavy metals

Arsenic (As)

Arsenic was measurable in eight of the analyzed samples, while the remaining formulations showed no detectable levels. The highest arsenic concentration identified was 0.980 mg/L (**Table 4**). For comparison, the recommended adult intake ranges from 15–25 µg/day, whereas consuming 3 mg/day for 2–3 weeks reaches the toxic threshold [13]. Health risk estimates demonstrated that all samples containing arsenic exceeded the acceptable HQ range (**Table 5**).

Historically, arsenic has been incorporated into medical treatments for centuries. Before penicillin, arsenic-based compounds were widely used for syphilis therapy, and Hippocrates reportedly applied arsenic sulfide preparations for dermatologic conditions [14]. By the nineteenth century, 1% arsenic trioxide (Fowler’s solution) had become a frequently prescribed remedy for multiple ailments [15]. Today, the International Agency for Research on Cancer identifies arsenic as a Group 1 human carcinogen [16], although arsenic trioxide remains clinically relevant for inducing remission in acute promyelocytic leukemia [17].

Arsenic contamination in traditional herbal preparations can arise from geological sources, polluted irrigation water, pesticide exposure, or industrial activity. Notably, widespread arsenic toxicity linked to contaminated drinking water has been documented in Bangladesh and India [18]. Acute poisoning is most often associated with ingestion of arsenic-containing pesticides, either through accidental exposure or intentional self-harm [19].

While ingestion is the primary route of entry, arsenic can also penetrate via inhalation or dermal absorption. Acute poisoning typically manifests as severe gastrointestinal distress, neurological impairment, and peripheral neuropathy, whereas chronic exposure can disrupt multiple organ systems [20]. The central nervous system is particularly vulnerable since arsenic readily crosses the blood–brain barrier, impairing cognitive functions such as memory and learning [21]. Long-term exposure is implicated in autoimmune disorders, diabetes, atherosclerosis, and dermatological cancers, and may also contribute to male reproductive dysfunction by suppressing testosterone synthesis [22].

Table 4. Concentrations of toxic heavy metals in analyzed samples.

Sample ID	As (mean ± SD) mg/L (n = 3)	Pb (mean ± SD) mg/L (n = 3)	Cd (mean ± SD) mg/L (n = 3)
2	0	1.31 ± 0.073	0.01 ± 0.003
3	0.07 ± 0.001	0.89 ± 0.033	0.02 ± 0.001
5	0	1.09 ± 0.065	0.02 ± 0.011
7	0	0.92 ± 0.026	0.02 ± 0.013
9	0	0.02 ± 0.001	0.02 ± 0.001
11	0	0.73 ± 0.044	0.05 ± 0.016
12	0.02 ± 0.002	0.21 ± 0.013	0.01 ± 0.002
15	0	0.13 ± 0.004	0.02 ± 0.015
16	0	0.18 ± 0.003	0.03 ± 0.017
18	0.98 ± 0.032	0.22 ± 0.021	0.01 ± 0.000
20	0	0.13 ± 0.008	0.03 ± 0.002
23	0.11 ± 0.031	0.13 ± 0.007	0.01 ± 0.001
24	0.03 ± 0.002	0.21 ± 0.032	0.02 ± 0.012
25	0	0.19 ± 0.016	0.07 ± 0.016
26	0	0.16 ± 0.009	0.02 ± 0.013
28	0	0.14 ± 0.005	0.03 ± 0.001
29	0	0.25 ± 0.015	0.01 ± 0.00
30	0	0.73 ± 0.045	0.04 ± 0.001
31	0	0.23 ± 0.032	0.02 ± 0.012
32	0	0.66 ± 0.047	0.04 ± 0.011
33	0	0.06 ± 0.001	0.07 ± 0.002
35	0	1.09 ± 0.078	0.02 ± 0.003
36	0	0.06 ± 0.003	0.05 ± 0.013
37	0	0.05 ± 0.002	0.08 ± 0.001
39	0	0.08 ± 0.003	0.03 ± 0.015
41	0.93 ± 0.031	0.04 ± 0.001	0.05 ± 0.014

43	0	0.05 ± 0.002	0.03 ± 0.001
44	0	0.05 ± 0.002	0.10 ± 0.025
48	0	1.02 ± 0.043	0.02 ± 0.002
50	0	0.09 ± 0.001	0.03 ± 0.012
54	0	0.89 ± 0.002	0.02 ± 0.004
55	0	0.53 ± 0.023	0.04 ± 0.005
56	0	0.07 ± 0.003	0.06 ± 0.001
58	0.82 ± 0.013	0.02 ± 0.001	0.09 ± 0.003
60	0	0.63 ± 0.005	0.04 ± 0.013
61	0	0.06 ± 0.03	0.02 ± 0.002
62	0	0.92 ± 0.002	0.08 ± 0.001
63	0	0.09 ± 0.006	0.06 ± 0.014
65	0	0.31 ± 0.002	0.06 ± 0.012
68	0	0.82 ± 0.008	0.07 ± 0.003
69	0	0.08 ± 0.006	0.08 ± 0.001
71	0	0.01 ± 0.001	0.01 ± 0.002
73	0	1.00 ± 0.042	0.06 ± 0.004
74	0	0.06 ± 0.003	0.04 ± 0.002
76	0	0.06 ± 0.002	0.05 ± 0.015
79	0	0.04 ± 0.001	0.01 ± 0.001
83	0	0.01 ± 0.000	0.01 ± 0.001
84	0	0.07 ± 0.017	0.05 ± 0.014
85	0	0.02 ± 0.023	0.10 ± 0.021
89	0	1.07 ± 0.051	0.03 ± 0.004
90	0.92 ± 0.002	0.03 ± 0.001	0.01 ± 0.001
Mean	0.08	0.35	0.04
STD	0.245	0.388	0.025

Table 5. Concentration in mg/kg and hazard quotients of toxic heavy metals

Sample ID	Pb (mg/kg)	Pb HQ	Cd (mg/kg)	Cd HQ	As (mg/kg)	As HQ
2	130.6	18.657	1.1	0.524	0	0.000
3	89	12.714	2.3	1.095	7.1	33.810
5	109.4	15.629	1.8	0.857	0	0.000
7	92	13.143	1.9	0.905	0	0.000
9	1.9	0.271	2.1	1.000	0	0.000
11	73.2	10.457	5.2	2.476	0	0.000
12	21.3	3.043	0.9	0.429	1.9	9.048
15	12.9	1.843	2.1	1.000	0	0.000
16	17.6	2.514	2.9	1.381	0	0.000
18	22.3	3.186	0.8	0.381	98	466.667
20	13.1	1.871	2.8	1.333	0	0.000
23	12.9	1.843	1.3	0.619	10.9	51.905
24	21.3	3.043	1.9	0.905	2.7	12.857
25	18.7	2.671	7.1	3.381	0	0.000
26	16.1	2.300	2.3	1.095	0	0.000
28	13.6	1.943	2.8	1.333	0	0.000
29	25.4	3.629	1.1	0.524	0	0.000
30	73.4	10.486	4.2	2.000	0	0.000
31	23.1	3.300	2.2	1.048	0	0.000
32	66.1	9.443	3.9	1.857	0	0.000
33	6.2	0.886	7.2	3.429	0	0.000
35	109.1	15.586	2.4	1.143	0	0.000
36	6.2	0.886	5.1	2.429	0	0.000
37	5.3	0.757	7.8	3.714	0	0.000
39	7.8	1.114	2.7	1.286	0	0.000

41	4.2	0.600	4.6	2.190	92.8	441.905
43	5.3	0.757	2.6	1.238	0	0.000
44	5.5	0.786	9.8	4.667	0	0.000
48	102.1	14.586	1.7	0.810	0	0.000
50	8.8	1.257	3.1	1.476	0	0.000
54	89.1	12.729	2.5	1.190	0	0.000
55	52.9	7.557	4.1	1.952	0	0.000
56	7.2	1.029	5.6	2.667	0	0.000
58	1.7	0.243	9.2	4.381	82.1	391.000
60	62.8	8.971	4.1	1.952	0	0.000
61	6.3	0.900	2.1	1.000	0	0.000
62	91.9	13.129	8.1	3.857	0	0.000
63	8.8	1.257	6.5	3.095	0	0.000
65	31	4.429	6.2	2.952	0	0.000
68	82	11.714	7.5	3.571	0	0.000
69	8.1	1.157	8.3	3.952	0	0.000
71	1.5	0.214	1.3	0.619	0	0.000
73	100.5	14.357	6.5	3.095	0	0.000
74	5.9	0.843	4.3	2.048	0	0.000
76	6.3	0.900	5.4	2.571	0	0.000
79	3.8	0.543	1.2	0.571	0	0.000
83	1.1	0.157	0.9	0.429	0	0.000
84	7.2	1.029	5.4	2.571	0	0.000
85	2.3	0.329	9.8	4.667	0	0.000
89	106.7	15.243	3.4	1.619	0	0.000
90	2.6	0.371	1.1	0.524	92.1	438.600
Mean	35.18	5.02	3.94	1.88	7.60	36.19
STD	39.23	5.60	2.58	1.23	24.77	117.94

Cadmium (Cd)

Cadmium was present in every formulation examined for metal contamination. The highest concentration observed was 0.098 mg/L, which remains below the 3 mg/L ceiling set by the Ayurvedic Pharmacopoeia for herbal preparations (**Table 4**). Using the established risk-assessment criteria, HQ values indicated that only half of the samples posed no immediate concern, whereas the remaining formulations showed HQ values that surpassed acceptable exposure thresholds (**Table 5**).

Cd is a toxic, non-essential element and has been implicated in numerous poisoning cases due to its occurrence in water, dairy products, and traditional medicinal preparations. It frequently appears as an unintentional adulterant in herbal remedies and is associated with renal injury, cardiovascular dysfunction, and respiratory damage when consumed above recommended limits [23]. Even minimal exposure may compromise kidney function. Cadmium tends to accumulate in the body by displacing zinc, contributing to hypertension and hepatotoxicity. Severe intoxication has been linked to the condition known as “Itai-itai disease,” characterized by bone demineralization, kidney failure, profound anemia, and eventual mortality [24].

Lead (Pb)

Lead was also detected in all analyzed samples, with the highest concentration measured at 1.306 mg/L (**Table 4**). Although this value remains below the WHO upper limit of 10 mg/L for medicinal plants [25], the HQ analysis showed that most samples exceeded safe intake levels (**Table 5**).

Pb contamination is frequently reported in traditional herbal products and is classified among the most harmful heavy metals. As early as 1763, Franklin documented abdominal colic and neuropathy as hallmark outcomes of prolonged lead exposure. Entry into the body occurs through inhalation, ingestion, or dermal absorption, and toxicity manifests when internal lead levels rise beyond physiologically tolerated limits. Clinical features include severe abdominal pain, anemia, nephritis, seizures, and disorders of the central nervous system [26].

Respiratory absorption is the dominant pathway in occupational settings, yet cultural and folk remedies—such as azarcon and greta used among certain Hispanic populations—also represent major exposure sources [9]. Lead

poisoning commonly presents with hypertension, but patients may additionally experience renal impairment, abdominal complaints, joint and muscle pain, anemia, and peripheral motor neuropathy [27].

All evaluated formulations contained the targeted toxic heavy metals. **Figure 1** displays the mean concentrations of these elements. Given the toxicity of heavy metals even at very low levels, HQ values were calculated, revealing that arsenic exceeded the safety threshold in every sample, whereas cadmium and lead surpassed acceptable limits in half of the analyzed formulations. A consolidated overview of detected toxic metals, their quantified concentrations, and associated HQ values is provided in **Table 6**.

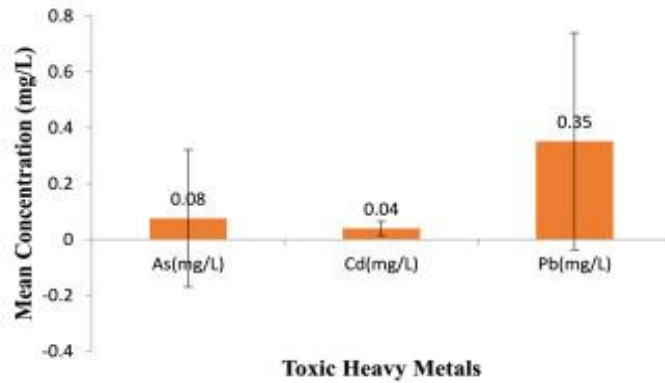


Figure 1. Average levels measured for each toxic heavy metal.

Table 6. Summary of toxic metals detected and Hazard Quotient (HQ) values

Toxic metal	% Sample detection	Mean value (mg/L)	Safe limit	Max HQ value	Risk
Lead	100	0.35	10 mg/L	18	High
Cadmium	100	0.04	3 mg/L	4.6	High
Arsenic	16	0.08	25 µg/day	466	Very high

Trace elements

Chromium (Cr)

All analyzed samples contained detectable amounts of Cr. Among them, sample ID S23 showed the highest measurement, reaching 0.073 mg/L (**Table 7**). According to WHO guidelines, the permissible concentration of chromium in unprocessed herbal materials is 2.0 mg/L [25]. The Cr values identified in every sample were well below this WHO threshold.

Chromium is an essential trace element involved in maintaining normal glucose homeostasis, particularly through its role in supporting insulin function. However, excessive exposure to Cr can produce adverse effects, including skin irritation, nasal discomfort, gastrointestinal disturbances, hepatic and renal injury, and, in severe cases, lung cancer. Potential environmental sources of Cr contamination include waste from paint production, steel manufacturing activities, and the application of sewage sludge [28].

Table 7. Concentration of trace metals in analyzed samples.

Sample ID	Cr (mean ± SD) mg/L (n = 3)	Zn (mean ± SD) mg/L (n = 3)	Co (mean ± SD) mg/L (n = 3)	Fe (mean ± SD) mg/L (n = 3)
2	0.03 ± 0.007	40.24 ± 2.092	0.13 ± 0.099	0.03 ± 0.021
3	0.07 ± 0.008	17.93 ± 0.870	0.16 ± 0.010	0.10 ± 0.005
5	0.03 ± 0.001	27.72 ± 0.987	0.21 ± 0.107	0.03 ± 0.004
7	0.04 ± 0.003	24.55 ± 0.812	0.12 ± 0.009	0.01 ± 0.000
9	0.05 ± 0.006	21.82 ± 0.921	0.32 ± 0.121	0.10 ± 0.031
11	0.04 ± 0.003	22.89 ± 1.129	0.12 ± 0.090	0.03 ± 0.003
12	0.04 ± 0.002	19.91 ± 0.729	0.21 ± 0.043	0.02 ± 0.009
15	0.07 ± 0.005	25.26 ± 1.218	0.13 ± 0.007	0.03 ± 0.040
16	0.03 ± 0.001	11.21 ± 0.851	0.18 ± 0.009	0.07 ± 0.005

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18	0.02 ± 0.001	14.98 ± 0.987	0.22 ± 0.098	0.06 ± 0.001
20	0.06 ± 0.004	16.20 ± 1.679	0.13 ± 0.087	0.03 ± 0.013
23	0.07 ± 0.006	09.72 ± 0.709	0.13 ± 0.067	0.03 ± 0.010
24	0.04 ± 0.002	21.78 ± 1.224	0.21 ± 0.029	0.02 ± 0.003
25	0.03 ± 0.001	27.35 ± 1.429	0.13 ± 0.009	0.02 ± 0.010
26	0.02 ± 0.001	19.22 ± 0.998	0.12 ± 0.066	0.02 ± 0.025
28	0.03 ± 0.002	23.92 ± 0.893	0.12 ± 0.100	0.04 ± 0.031
29	0.04 ± 0.002	21.77 ± 0.926	0.15 ± 0.072	0.03 ± 0.010
30	0.03 ± 0.001	15.15 ± 0.773	0.13 ± 0.082	0.01 ± 0.000
31	0.02 ± 0.001	39.18 ± 1.614	0.23 ± 0.067	0.01 ± 0.000
32	0.01 ± 0.000	21.30 ± 0.997	0.36 ± 0.107	0.05 ± 0.019
33	0.03 ± 0.002	22.28 ± 0.833	0.21 ± 0.099	0.06 ± 0.021
35	0.01 ± 0.000	21.72 ± 1.049	0.20 ± 0.098	0.06 ± 0.012
36	0.02 ± 0.001	21.82 ± 1.067	0.22 ± 0.055	0.04 ± 0.002
37	0.04 ± 0.002	23.50 ± 1.012	0.14 ± 0.031	0.02 ± 0.001
39	0.03 ± 0.001	15.91 ± 0.709	0.12 ± 0.009	0.06 ± 0.012
41	0.05 ± 0.004	22.71 ± 1.045	0.13 ± 0.010	0.04 ± 0.001
43	0.04 ± 0.003	33.21 ± 1.543	0.18 ± 0.043	0.04 ± 0.011
44	0.02 ± 0.001	36.90 ± 1.240	0.16 ± 0.086	0.03 ± 0.020
48	0.03 ± 0.002	31.59 ± 1.391	0.20 ± 0.093	0.02 ± 0.001
50	0.02 ± 0.002	37.55 ± 2.020	0.12 ± 0.069	0.05 ± 0.011
54	0.05 ± 0.004	37.27 ± 1.390	0.11 ± 0.043	0.01 ± 0.002
55	0.01 ± 0.000	33.89 ± 1.876	0.19 ± 0.059	0.02 ± 0.010
56	0.05 ± 0.006	28.31 ± 1.511	0.11 ± 0.068	0 ± 0
58	0.03 ± 0.005	25.28 ± 0.923	0.12 ± 0.077	0.09 ± 0.004
60	0.02 ± 0.001	26.23 ± 0.990	0.18 ± 0.090	0.01 ± 0.001
61	0.04 ± 0.006	29.11 ± 1.034	0.15 ± 0.042	0.02 ± 0.002
62	0.03 ± 0.001	26.17 ± 1.009	0.13 ± 0.081	0.06 ± 0.019
63	0.02 ± 0.003	24.87 ± 0.820	0.21 ± 0.075	0.01 ± 0.003
65	0.03 ± 0.003	26.56 ± 1.012	0.20 ± 0.069	0.05 ± 0.002
68	0.05 ± 0.002	27.87 ± 2.087	0.10 ± 0.037	0.10 ± 0.019
69	0.02 ± 0.001	28.90 ± 0.992	0.12 ± 0.050	0.07 ± 0.014
71	0.05 ± 0.002	40.29 ± 2.012	0.10 ± 0.049	0.01 ± 0.000
73	0.04 ± 0.006	20.21 ± 1.019	0.21 ± 0.051	0.02 ± 0.001
74	0.06 ± 0.008	22.98 ± 0.918	0.20 ± 0.043	0.06 ± 0.012
76	0.04 ± 0.007	19.88 ± 0.872	0.19 ± 0.021	0.10 ± 0.002
79	0.02 ± 0.003	18.32 ± 0.998	0.18 ± 0.009	0.06 ± 0.033
83	0.03 ± 0.002	16.28 ± 1.108	0.16 ± 0.021	0.09 ± 0.010
84	0.05 ± 0.006	40.03 ± 1.901	0.19 ± 0.020	0.01 ± 0.010
85	0.04 ± 0.007	39.80 ± 1.976	0.18 ± 0.031	0.08 ± 0.003
89	0.03 ± 0.006	20.01 ± 0.965	0.13 ± 0.026	0.06 ± 0.001
90	0.03 ± 0.007	33.51 ± 1.388	0.21 ± 0.020	0.03 ± 0.002
Mean	0.04	25.39	0.17	0.04
STD	0.014	7.748	0.051	0.027

Cobalt (Co)

Every sample assessed for trace metals contained measurable amounts of Co, with concentrations spanning from 0.130 mg/L at the lowest to 0.321 mg/L at the highest (**Table 7**). According to WHO guidelines, cobalt levels in medicinal plants should not exceed 0.48 mg/L [25], and all analyzed samples fell comfortably within this allowable limit.

Cobalt is closely linked to vitamin B12, playing a vital part in metabolic pathways, red blood cell formation, and protection against anemia. However, excessive intake of Co may lead to respiratory slowing, cardiomyopathy, skin inflammation, elevated blood glucose, and pulmonary complications, whereas insufficient Co can produce weight loss, impaired growth, and anemia [29].

Zinc (Zn)

FAO/WHO (1984) recommends 27.4 mg/kg as the acceptable upper limit of Zn in edible plant materials. In the present evaluation, Zn was detected in all tested samples, with the highest concentration—40.29 mg/L—observed in sample H-71, which is traditionally used as a sexual tonic (**Table 7**). Fourteen samples exceeded the WHO-recommended safe level, indicating that Zn was the only trace element consistently surpassing permissible limits. Zn is indispensable for multiple physiological processes, including normal growth, thyroid activity, coagulation mechanisms, and protein biosynthesis. Nonetheless, excessive Zn exposure can disrupt lipoprotein metabolism, compromise immune function, and produce gastrointestinal distress [30]. Globally, millions are exposed to Zn through dietary supplements, medications, disinfectants, antiseptic formulations, and dental materials. Daily Zn intakes of 100–300 mg/d have become common among users of Zn-rich supplements and herbal formulations, raising concerns about chronic toxicity. Long-term overconsumption can trigger secondary copper deficiency, manifesting as hypocupremia, anemia, neutropenia, and leucopenia [31].

Iron (Fe)

The FAO/WHO (1984) permissible limit for Fe in edible plant products is 20 mg/kg [32]. Iron was detected in every sample screened, with concentrations ranging between 0.008 mg/L and 0.098 mg/L (**Table 7**). All Fe measurements remained safely below the recommended threshold.

Fe forms the core of hemoglobin, contributing to oxygen transport, electron transfer, metabolic reactions, and neural functioning [11]. Despite its biological importance, Fe contamination in traditional medicinal products has been frequently documented. Fe deficiency culminates in anemia, while excessive accumulation—particularly in children—can result in toxicity [23].

Overall, all examined trace metals were quantified in the analyzed samples. Mean trace element concentrations are illustrated in **Figure 2**. With the exception of Zn, all detected values adhered to WHO safety recommendations. Detailed concentrations, WHO reference limits, and associated risk estimations are presented in **Table 8**.

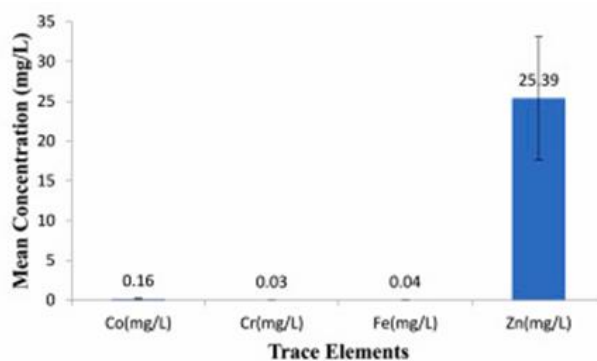


Figure 2. Average levels of the measured trace elements.

Table 8. Summary of analyzed samples for trace metals

Trace metal	% Sample detection	Mean value	Safe limit	Risk
Chromium	100	0.04 mg/L	2.0 mg/L	Low
Iron	100	0.04 mg/L	20 mg/kg	Low
Cobalt	100	0.17 mg/L	0.48 mg/L	Low
Zinc	100	25.39 mg/kg	27.4 mg/kg	Low

Essential minerals

Sodium (Na) and Potassium (K)

Both Na and K were present in every sample assessed for essential minerals, and their measured concentrations complied with the WHO-recommended limits (**Table 9**).

Table 9. Concentration of essential minerals in analyzed samples

Sample ID	Na (mean ± SD) mg/L (n = 3)	K (mean ± SD) mg/L (n = 3)	Ca (mean ± SD) mg/L (n = 3)	Mg (mean ± SD) mg/L (n = 3)
2	5.76 ± 0.195	39.70 ± 1.285	27.91 ± 1.151	14.17 ± 0.913
3	3.29 ± 0.049	40.06 ± 2.198	19.30 ± 1.098	13.22 ± 0.902
5	6.09 ± 0.042	39.51 ± 1.120	11.87 ± 1.077	14.35 ± 1.056
7	11.09 ± 0.137	37.90 ± 1.302	09.21 ± 0.903	12.98 ± 1.034
9	9.02 ± 0.094	38.98 ± 1.088	18.65 ± 0.919	13.54 ± 1.021
11	8.22 ± 0.073	39.11 ± 1.094	13.72 ± 0.938	11.76 ± 0.956
12	5.53 ± 0.084	38.56 ± 1.078	18.09 ± 0.965	13.65 ± 0.999
15	5.71 ± 0.090	37.09 ± 1.097	12.42 ± 0.844	14.65 ± 1.087
16	5.00 ± 0.089	36.01 ± 1.033	14.47 ± 0.878	13.81 ± 1.026
18	4.90 ± 0.078	39.05 ± 1.021	19.11 ± 0.985	11.65 ± 0.909
20	9.66 ± 0.088	39.71 ± 1.099	28.78 ± 1.109	14.76 ± 1.080
23	8.96 ± 0.096	39.52 ± 1.021	26.46 ± 1.088	13.76 ± 1.522
24	7.09 ± 0.084	27.60 ± 1.090	23.30 ± 1.040	13.87 ± 0.976
25	7.92 ± 0.094	21.87 ± 1.107	29.90 ± 1.817	13.94 ± 0.923
26	6.89 ± 0.097	31.97 ± 1.088	11.54 ± 0.929	11.83 ± 0.957
28	5.09 ± 0.089	38.00 ± 1.098	06.98 ± 0.806	13.11 ± 0.978
29	6.88 ± 0.079	31.99 ± 1.187	23.67 ± 1.034	12.59 ± 0.954
30	7.09 ± 0.097	29.90 ± 1.030	17.56 ± 1.050	13.21 ± 0.921
31	5.66 ± 0.088	31.09 ± 0.967	21.88 ± 1.120	14.78 ± 0.836
32	5.03 ± 0.092	33.98 ± 1.099	13.12 ± 0.988	14.61 ± 0.998
33	5.92 ± 0.077	35.00 ± 1.087	19.73 ± 1.712	13.45 ± 1.012
35	5.07 ± 0.078	39.01 ± 1.098	14.79 ± 0.909	12.69 ± 0.098
36	5.05 ± 0.091	28.91 ± 0.956	17.67 ± 0.986	10.00 ± 0.933
37	5.54 ± 0.089	38.29 ± 0.988	14.78 ± 0.877	14.55 ± 1.064
39	7.23 ± 0.083	35.33 ± 1.044	14.25 ± 0.896	15.09 ± 1.034
41	4.89 ± 0.098	34.63 ± 1.065	13.20 ± 0.823	13.71 ± 1.211
43	3.09 ± 0.091	31.90 ± 1.098	20.12 ± 1.086	12.91 ± 1.121
44	4.98 ± 0.084	37.10 ± 1.099	12.35 ± 0.902	13.56 ± 1.079
48	4.87 ± 0.095	37.70 ± 1.023	18.35 ± 0.978	12.56 ± 1.098
50	5.44 ± 0.089	36.20 ± 0.982	19.29 ± 1.107	13.27 ± 1.068
54	5.67 ± 0.083	36.10 ± 1.088	21.87 ± 1.099	13.01 ± 1.023
55	5.72 ± 0.098	36.01 ± 1.120	23.68 ± 1.023	12.19 ± 0.990
56	5.52 ± 0.095	33.90 ± 1.200	31.79 ± 1.201	13.01 ± 0.910
58	6.93 ± 0.095	33.56 ± 1.019	20.80 ± 1.088	14.69 ± 0.912
60	7.00 ± 0.181	33.21 ± 1.098	29.33 ± 1.076	13.98 ± 1.009
61	8.01 ± 0.096	28.72 ± 1.076	16.87 ± 1.056	14.72 ± 1.089
62	5.98 ± 0.099	39.00 ± 2.045	27.91 ± 1.223	12.55 ± 1.081
63	5.11 ± 0.091	39.21 ± 1.086	26.48 ± 1.508	13.31 ± 1.071
65	5.83 ± 0.098	39.19 ± 1.090	29.12 ± 1.078	11.89 ± 1.095
68	5.83 ± 0.096	38.98 ± 1.076	23.00 ± 1.023	14.03 ± 1.034
69	5.21 ± 0.083	39.11 ± 1.069	25.81 ± 1.367	13.43 ± 0.969
71	5.82 ± 0.087	37.79 ± 1.083	26.09 ± 1.298	13.97 ± 1.088
73	5.33 ± 0.093	36.98 ± 1.081	22.00 ± 1.422	14.29 ± 1.021
74	5.83 ± 0.092	35.90 ± 1.076	24.22 ± 1.198	12.62 ± 0.910
76	6.01 ± 0.098	33.65 ± 1.109	28.34 ± 1.167	11.67 ± 0.998
79	6.88 ± 0.097	33.97 ± 1.088	31.36 ± 1.633	13.11 ± 1.019
83 in	6.77 ± 0.088	38.09 ± 2.039	28.39 ± 1.278	14.05 ± 1.018
84	6.00 ± 0.071	39.30 ± 1.966	34.11 ± 1.910	11.56 ± 1.033
85	5.23 ± 0.121	39.66 ± 1.500	21.51 ± 1.039	14.10 ± 1.056
89	5.98 ± 0.089	37.88 ± 1.908	22.58 ± 1.055	13.99 ± 1.020

90	6.10 ± 0.096	38.07 ± 1.586	19.21 ± 1.044	14.76 ± 1.107
Mean	6.15	35.96	20.92	13.39
SD	1.444	3.804	6.411	1.047

Magnesium (Mg)

Mg was present in every sample evaluated for essential minerals. Human intake of magnesium is generally considered safe up to 400 mg/day [33]. Across all examined samples, the measured Mg levels remained within the permissible range (**Table 9**).

Calcium (Ca)

Ca was also identified in all analyzed samples. According to WHO guidelines, the acceptable daily intake of calcium is 2500 mg [34]. In this study, the Ca concentrations for all samples stayed below the recommended upper limit (**Table 9**). Overall, all essential minerals quantified in the collected samples were recorded within their respective safety thresholds.

Essential elements are fundamental for sustaining normal physiological processes, and inadequate levels can impair routine bodily functions. Interest in determining essential mineral profiles in both dietary sources and traditional medicines has grown, given their crucial roles in maintaining health. These minerals contribute to enzymatic systems, often acting as cofactors that modulate biochemical reactions within cells [35]. Na and K, for instance, support nerve transmission, muscle contraction, and maintain acid–base equilibrium in intracellular and extracellular compartments [36]. Mg is vital for enzyme regulation, skeletal and muscular development, and overall structural stability of tissues [37]. Ca is indispensable for the integrity of bones, teeth, and muscles, and also contributes to controlling hypertension and alleviating premenstrual syndrome symptoms [38].

A consolidated overview of the detected essential minerals and their associated safety indicators is provided in **Table 10**. The average concentrations of these minerals are illustrated in **Figure 3**. Statistical comparison of mean values for heavy metals, trace elements, and essential minerals against their permissible limits using the student t-test (**Table 11**) showed that arsenic had no significant difference ($P = 0.113$), whereas significant differences ($p < 0.05$) were observed for the remaining metals.

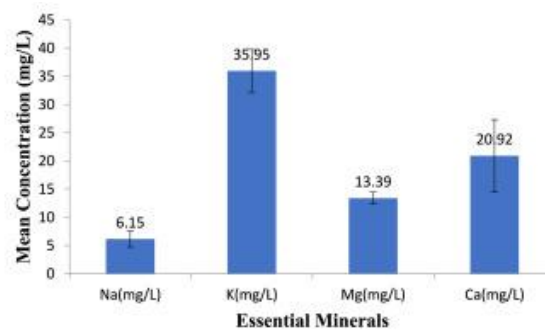


Figure 3. Average measured levels of the essential minerals.

Table 10. Summary of analyzed samples for essential minerals

Essential minerals	% Sample detection	Mean values (mg/day)	Safe limit (mg/day)	Risk
Sodium	100	6.15	–	None
Potassium	100	35.95	–	None
Calcium	100	20.92	2500	None
Magnesium	100	13.39	400	None

Table 11. Comparison of the mean values with permissible values using *t*-test.

S.No.	Parameter	Mean Value (mg/L)	T-test	Permissible limit (mg/L)	Remarks
1	Arsenic	0.08	$p = 0.113$	0.02	Non Significant
2	Cadmium	0.04	$p < 0.05$	03	Significant
3	Lead	0.35	$p < 0.05$	10	Significant
4	Chromium	0.04	$p < 0.05$	2.0	Significant

5	Cobalt	0.17	$p < 0.05$	0.48	Significant
6	Zinc	25.39	$P = 0.073$	27.4	Non Significant
7	Iron	0.04	$p < 0.05$	20	Significant
8	Magnesium	13.39	$p < 0.05$	400 mg/day	Significant
9	Calcium	20.92	$p < 0.05$	2500 mg/day	Significant
10	Sodium	163.05	$p < 0.05$	2300 mg/day	Significant
11	Potassium	35.96	$p < 0.05$	3400 mg/day	Significant

The widespread reliance on traditional remedies exposes users to considerable health hazards, largely because many of these preparations contain unsafe quantities of toxic metals. This investigation sought to characterize the burden of heavy metals, trace elements, and essential minerals in traditional herbal medicines commonly consumed in Khyber Pakhtunkhwa, Pakistan. Using atomic absorption spectroscopy, the samples were found to contain arsenic, cadmium, and lead—elements known to exert toxicity even at minimal concentrations. As reflected by the Hazard Quotient (HQ) estimates, several preparations exceeded acceptable safety thresholds, raising concerns for individuals depending on these formulations for therapeutic purposes.

Several factors likely contribute to the contamination observed, including the use of unverified plant materials, preparation of remedies in nonstandard or deteriorating utensils, and storage in containers prone to releasing metal residues. These issues reflect the broader lack of regulatory oversight and emphasize the need for proper standardization to prevent metal-induced toxicities. In addition to heavy metals, the study also quantified chromium, cobalt, zinc, and iron as trace elements, along with sodium, potassium, calcium, and magnesium as essential minerals. Collectively, these results provide insight into the elemental composition of widely used non-conventional medicinal products.

Statistical analysis using the student t-test indicated that arsenic levels did not significantly differ from its permissible reference value ($P = 0.113$), whereas the remaining metals demonstrated statistically significant deviations ($P < 0.05$). The interpretation of these values is complicated by the absence of standardized dosing practices among traditional healers; remedies may be dispensed in unmeasured quantities—from paper-wrapped portions to vague directions such as taking a “palm full.” Such inconsistency greatly increases the potential for heavy metal toxicity among users.

Conclusion

Reports of metal and mineral contamination in traditional medicines are well documented, highlighting the importance of systematically assessing heavy metals, trace elements, and essential minerals in commonly prescribed herbal formulations. Ensuring that such preparations meet safety benchmarks is crucial for protecting the public from toxic exposures. It is therefore recommended that regulatory authorities formally incorporate metal and mineral analysis into routine quality control procedures for traditional medicines distributed in Khyber Pakhtunkhwa, Pakistan. Verifying the safety, efficacy, and potency of these preparations through standardized screening is essential for safeguarding public health and ensuring responsible therapeutic use.

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Ethics Statement: The study was approved by the Ethical Committee of Department of Pharmacy, University of Swabi (Pharm/EC/003). The written consent was taken from all the participants of the study.

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