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NON-CARCINOGENIC HEALTH RISK ASSESSMENT OF HEAVY METALS IN VEGETABLES CULTIVATED IN DISTRICT GHOTKI, SINDH, PAKISTAN

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Abstract

The cultivation of vegetables in District Ghotki, Sindh, Pakistan, is a cornerstone of the local economy but faces significant food safety challenges due to heavy metal contamination. This study conducted a non-carcinogenic health risk assessment of seven metals—Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu), Tin (Sn), Cobalt (Co), and Arsenic (As)—across 15 vegetable varieties. Utilizing Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), the research quantified metal concentrations and evaluated human exposure through the Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI). While essential micronutrients (Fe, Zn, Mn, Cu) and trace elements (Sn, Co) remained within safe WHO/FAO limits, Arsenic concentrations in several vegetables, including Lotus, Cauliflower, and Mustard, exceeded permissible thresholds. The THQ for Arsenic surpassed 1.0 in five varieties, identifying it as the primary toxic contributor. Furthermore, the cumulative Hazard Index exceeded 1.0 for 10 out of 15 vegetables, indicating potential long-term health risks for the local population. Principal Component Analysis (PCA) and Pearson correlation confirmed that while essential minerals often co-occur, Arsenic contamination likely originates from independent external sources.

Keywords: *Non-carcinogenic Health Risk Assessment , Heavy Metals , Vegetables , District Ghotki , Estimated Daily Intake (EDI), Hazard Index (HI), Arsenic (As) Principal Component Analysis (PCA).*

INTRODUCTION

In study area of district Ghotki, Sindh, Pakistan, vegetable farming plays a noteworthy role in the local agricultural financial system; however heavy metal contamination and food safety are major issues [1]. Heavy metals are constant environmental toxins that can build up in edible plant parts owing to soil absorption and polluted irrigation water [2]. Because vegetables are a main source of nutrition for most of the population, the transport of heavy metals from the environment to the food chain presents a serious path for human exposure [3,4]. Evaluating the non-carcinogenic health alarms related with these metals is required to determine the safety of the local products [5,6]. This study particularly spotlights on the level of iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), tin (Sn), cobalt (Co) and arsenic (As) in fifteen different varieties of vegetables like, tomatoes, bitter gourd, mustard green, fennel bulb, sunflower head, eggplant, bell peppers, and cluster bean. Some of these metals are essentials in trace levels for human health, but in excess they can be destructive to the body. To calculate these risks, the study uses standardized measures for instance, Estimated Daily Intake (EDI), which determines the daily level of metal ingested per kilogram of body weight [7]. Additionally, the Hazard Index (HI) offers a cumulative evaluation of the overall non-carcinogenic risk, whereas the Target Hazard Quotient (THQ) is used to assess the risk of individual metals [8]. To determine the distribution patterns and possible sources of these heavy metals among the different vegetable species gathered from the district, statistical techniques like Principal Component Analysis (PCA) are also utilized [9].

The purpose of this study is to assess the non-carcinogenic health risks related with heavy metals in vegetables cultivated in District Ghotki, Sindh, Pakistan. To determine whether fifteen different vegetable varieties are harmless for human use, the study attempts to calculate the quantity of the particular metals, for example Fe, Zn, Mn, Cu, Sn, Co, and As. The present work aims to evaluate the probable health exposure amounts for the local people by calculating the EDI, and THQ. Moreover, the study emphasizes particular vegetables that might be a severe continuing health risk by using the HI to conclude whether the collective occurrence of these metals exceeds safe thresholds. Eventually, to imagine and understand the correlation and distribution patterns of these metal contents among the various vegetable varieties, the study uses Principal Component Analysis (PCA)

MATERIAL AND METHODS

Study Area

District Ghotki's subtropical desert climate is differentiated by intense temperature variations and minimum precipitation. In summer days the temperature frequently exceeds 45 ºC, with June usually the hottest month of the year. Winter is comparatively short as well as pleasant, with temperature ranging from 10 ºC – 20 ºC. Rainfall is uncommon, frequently averaging between 150 and 200 mm yearly, with the mainstream happening during the monsoon season from July to September. The area is also familiar for its recurrent dust storms during dry spells and high monsoon levels of humidity. The cropland of Ghotki is highly fruitful because it receives irrigation water from the Guddu Barrage system. The district's primary cash crops are wheat, cotton, and sugarcane. The region is also one of the top producers of sugarcane and wheat in the province. Rice, corn, tobacco, and oilseeds like mustard and rapeseed are also important crops grown in the area. The district is also known for its horticulture, especially its date palms, guavas, mangoes, and bananas. The study also looked at the risk of heavy metals in a number of seasonal vegetables, such as tomatoes, bitter gourd, mustard green, fennel bulb, sunflower head, eggplant, bell peppers, and cluster bean [10].

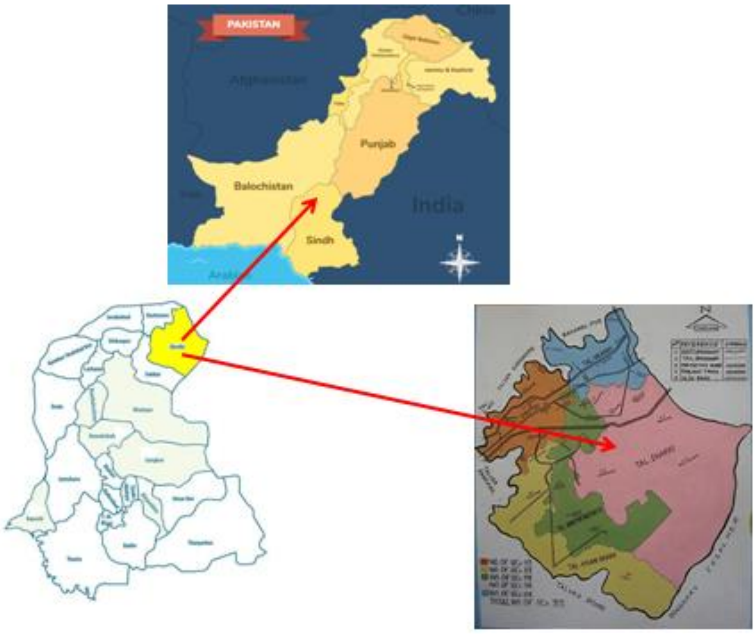

Figure: 1 Map of Study area of district Ghotki

Sample Collection

The replicate three samples of each vegetable including tomatoes, bitter gourd, mustard green, fennel bulb, sunflower head, eggplant, bell peppers, and cluster bean were collected from each study. First of all, tap water was used to clean all vegetable samples and the double distilled water was employed to eliminate surface contaminants. - In this way 45 samples were collected from agricultural locations. After washing all samples were stored in clean air tight polyethylene bags and transported to laboratory for analysis. The samples were dried in an oven at controlled temperature to remove moisture. All samples were properly labeled with information such as, Sample ID, sampling location, date of collection and vegetable type [11].

Sample Preparation

Wet Digestion

Vegetable samples were washed first with tap water and then with double-distilled water and stainless-steel blade was used to cut them into pieces for preparation process. The resulting samples were kept in an oven at 100 ℃ to remove moisture content, and mortar was used to ground them. The modified - wet digestion process are applied-using an acid mixture (1:4 of HNO_3: H_2SO_4; v: v). 5 mL of the acid mixture was introduced to a digestion tube containing 2 g of each homogenized dried sample. After that, the mixture was heated for an hour between 130 and 170 °C until a transparent solution was visible. Two mL of 30% concentrated H_2O_2 were added after cooling, and the mixture was heated until a clear solution was obtained. Following filtration using Whatman #42 filter paper, the clear solution was diluted with 250 mL of de-ionized water. In order to prepare the samples for analysis using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), they were then collected in falcon tubes [12, 13].

ICP-OES Analysis

Standard solutions of known concentrations were prepared for Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu), Tin (Sn), Cobalt (Co), and Arsenic (As) to calibrate the ICP-OES instrument. The digested and diluted vegetable samples were then introduced into the *Thermo Scientific iCAP 7000 Series, ICP Spectrometer*, *Thermo Fisher Scientific*, United States, Name: *Teledyne CETAC Technologies ASX-280* Model: ASX-280, Nebraska, USA ICP-OES. The instrument measured the intensity of light emitted by excited metal atoms in the plasma, which is directly proportional to the metal's concentration in the sample. The ICP-OES software then calculated the metal concentrations in the samples based on the established calibration curves.

Risk Assessment

Human health risk assessment from vegetable consumption is a scientific process used to evaluate the potential for adverse health effects caused by consuming vegetables contaminated with toxic substances, such as, EDI of HMs, THQ, and HI (Table: 1)

Table: 1 Risk assessment parameters, their formulae and description

Risk Assessment Parameter	**Formula**	**Description**
Estimated Daily Intake (EDI)	$EDI = \frac{C \times IR \times EF \times ED}{BW \times AT}$	Calculates the daily exposure to a specific metal per kilogram of body weight.
Target Hazard Quotient (THQ)	$THQ = \frac{EDI}{RfD}$	Determines the non-carcinogenic risk of an individual heavy metal relative to its oral reference dose.
Hazard Index (HI)	$HI = \sum THQn$	Represents the total non-carcinogenic risk by summing the THQ values of all metals studied.

Oral Reference Dose (RfD) (mg/kg/day) of metals utilized in this study were as follows: Fe (0.7), Zn (0.3), Mn (0.14), Cu (0.04), Sn (0.6), Co (0.43), As (0.0003).

Figure: 2 Images of various vegetables/fruits under study

RESULTS AND DISCUSSIONS

Essential Micronutrients: Iron, Zinc, and Manganese

The WHO/FAO generally sets the permissible limit for Iron (Fe) in vegetables at 425 mg/kg [14], which means all vegetables in this dataset, ranging from Sweet potato (0.177 mg/kg) to Brinjal (1.248 mg/kg), are well within safe consumption limits. Similarly, Zinc (Zn) levels are far below the standard threshold of 60 mg/kg [15], with Bitter Gourd showing the highest concentration at 6.11 mg/kg. Manganese (Mn) also remains within safe margins; although Bitter Gourd and Sunflower exhibit higher accumulations at 9.28 mg/kg and 8.47 mg/kg respectively, they do not exceed typical safety guidelines which often allow up to 500 mg/kg depending on the specific food category (Table: 2 & 3, Figure: 3).

Copper and Tin Concentrations

The highest permissible level of Cu as given by the WHO/FAO is typically 40 mg/kg [16]. However, extremely low values of Cu are measured from vegetables of district Ghotki. The maximum values of 2.087 mg/kg and 2.012 mg/kg were found in Methi and Bitter Gourd respectively. These results demonstrate that there is no chance of Cu contamination in vegetable samples under study. Tin (Sn) shows an extremely high WHO/FAO level of 200 mg/kg for canned goods and less for fresh vegetable samples [17]. Therefore, level in cluster beans and peas was found as 0.091 mg/kg and 0.096 mg/kg, showing that these don’t pose a health hazard (Table: 2 & 3, Figure: 3).

Toxic Elements: Cobalt and Arsenic

Co levels are usually low, with capsicum (0.126 mg/kg), being the most visible, although there is not a definite guideline like for Co [18]. The WHO and FAO frequently state that certain food crops should not have more than 0.1 mg/kg of arsenic [19], making this assessment critical. Lotus, cauliflower, Mustard, and Green Chilli displayed their levels as 0.131 mg/kg, 0.12 mg/kg, 0.117 mg/kg and 0.107 mg/kg respectively exceed the permissible limit as given in the Table: (). Consequently, it may be essential to keep a closer eye on certain specific vegetables to make sure that consuming them doesn't lead to continuing heavy metal contamination (Table: 2 & 3, Figure: 3).

Table: 2 Heavy metal concentration (mg/kg) in vegetable samples collected from district Ghotki

Metals → / Vegetables ↓	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Sn (mg/kg)	Co (mg/kg)	As (mg/kg)
Peas	1.189	1.34	3.2	0.92	0.096	0.014	0.026
Capsicum	0.381	2.73	2.23	0.99	0.054	0.126	0.026
Spinach	0.971	2.29	2.13	0.227	0.022	0.013	0.08
Tomato	1.207	3.64	3.19	1.113	0.076	0.024	0.046
Sweet potato	0.177	1.35	4.87	1.233	0.022	0.036	0.033
Brinjal	1.248	5.72	7.02	0.95	0.017	0.011	0.013
Cauliflower	0.325	3.2	1.96	1.22	0.031	0.013	0.12
Lotus	0.939	5.11	3.19	1.045	0.022	0.013	0.131
Bitter Gourd	0.888	6.11	9.28	2.012	0.01	0.02	0.052
Mustard	0.461	1.22	2.99	1.13	0.012	0.061	0.117
Green Chilli	0.987	3.23	1.17	1.023	0.012	0.012	0.107
Fennel	0.956	2.14	3.16	1.317	0.031	0.028	0.039
Sunflower	0.466	1.34	8.47	1.025	0.036	0.015	0.022
Cluster Beans	0.539	4.23	2.19	1.123	0.091	0.011	0.046
Methi	0.513	3.21	4.13	2.087	0.086	0.031	0.047

Table: 3 Data Comparison with WHO/FAO Permissible Limits (mg/kg)

Metal	WHO/FAO Limit (mg/kg)	Present Data Range	Status	Highest Source
Fe	425.0	0.177 – 1.248	Safe	Brinjal
Zn	60.0	1.22 – 6.11	Safe	Bitter Gourd
Mn	500.0	1.17 – 9.28	Safe	Bitter Gourd
Cu	40.0	0.227 – 2.087	Safe	Methi
Sn	250.0	0.01 – 0.096	Safe	Peas
Co	50.0	0.011 – 0.126	Safe	Capsicum
As	0.1	0.013 – 0.131	Caution	Lotus

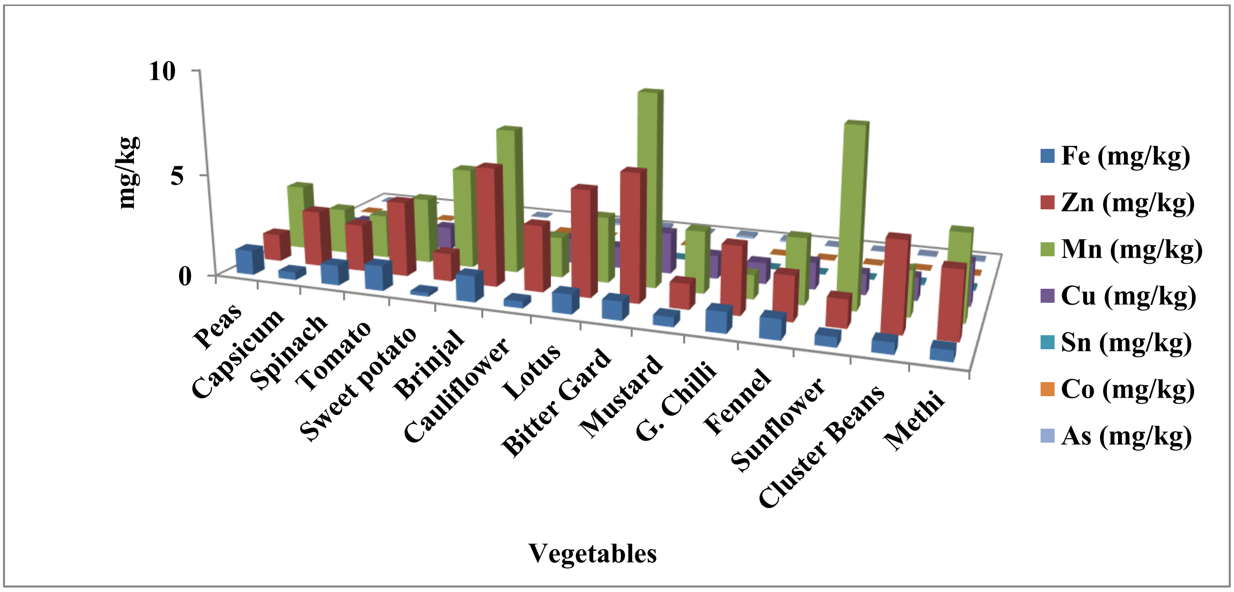

Figure: 3 Concentration of heavy metals in different vegetable samples collected from District Ghotki

Estimated Daily Intake (EDI)

By comparing the estimated heavy metal intake values to the Oral Reference Dose (RfD) recommendations established by international organizations such as the WHO/FAO, the Estimated Daily Intake (EDI) is used to determine health hazards. The findings indicate that consuming these vegetables is still safe for some trace metals and essential minerals. All vegetables have EDI values for Fe that is far lower than the RfD of 0.7 mg/kg/day. For instance, the EDI for Fe in sweet potatoes is 0.00102 mg/kg/day, whereas the EDI for Fe in brinjal is 0.00718 mg/kg/day. Furthermore, the EDI for Mn and Zn for the entire results did not exceed their respective RfD limits of 0.14 mg/kg/day and 0.3 mg/kg/day respectively. The highest quantities of Mn (0.05336 mg/kg/day) and Zn (0.03513 mg/kg/day) were found in bitter gourd; however, these levels are still well below the safety limits. Cu EDI values in spinach are between 0.00131 mg/kg/day and 0.01200 mg/kg/day in methi. These results are all less than the RfD of 0.04 mg/kg/day. In comparison to their high safety limits of 0.6 mg/kg/day and 0.43 mg/kg/day, respectively, the amounts of Sn and Co that individuals consume are likewise extremely low. This indicates that consuming these particular vegetable crops doesn’t immediately put people’s health at risk. However, analysis of As reveals potential health hazards. The RfD for As is extremely low at 0.0003 mg/kg/day due to its extreme toxicity. The EDI data shows that several vegetables are beyond this safety threshold. The WHO/FAO recommended reference dose of As is exceeded in lotus (0.00075 mg/kg/day), cauliflower (0.00069 mg/kg/day), mustard (0.00067 mg/kg/day), green chilli (0.00062 mg/kg/day), and spinach (0.00046 mg/kg/day). Bitter Gourd is exactly at the 0.00030 mg/kg daily limit. These findings suggest that long-term intake of these particular vegetables may raise the cumulative health risk linked to exposure to As, but given current consumption patterns, the other metals under investigation do not pose such a risk (Table: 4).

Table: 4 Estimated Daily Intake (EDI) (mg/kg/day) of heavy metals in vegetables of district Ghotki

RfD Values → Vegetable ↓	Fe	Zn	Mn	Cu	Sn	Co	As
	0.7	0.3	0.14	0.04	0.6	0.43	0.0003
Peas	0.00684	0.00771	0.01840	0.00529	0.00055	0.00008	0.00015
Capsicum	0.00219	0.01570	0.01282	0.00569	0.00031	0.00072	0.00015
Spinach	0.00558	0.01317	0.01225	0.00131	0.00013	0.00007	0.00046
Tomato	0.00694	0.02093	0.01834	0.00640	0.00044	0.00014	0.00026
Sweet Potato	0.00102	0.00776	0.02799	0.00709	0.00013	0.00021	0.00019
Brinjal	0.00718	0.03289	0.04037	0.00546	0.00010	0.00006	0.00007
Cauliflower	0.00187	0.01840	0.01127	0.00702	0.00018	0.00007	0.00069
Lotus	0.00540	0.02938	0.01834	0.00601	0.00013	0.00007	0.00075
Bitter Gourd	0.00511	0.03513	0.05336	0.01157	0.00006	0.00012	0.00030
Mustard	0.00265	0.00702	0.01719	0.00650	0.00007	0.00035	0.00067
Green Chilli	0.00568	0.01857	0.00673	0.00588	0.00007	0.00007	0.00062
Fennel	0.00550	0.01231	0.01817	0.00757	0.00018	0.00016	0.00022
Sunflower	0.00268	0.00771	0.04870	0.00589	0.00021	0.00009	0.00013
Cluster Beans	0.00310	0.02432	0.01259	0.00646	0.00052	0.00006	0.00026
Methi	0.00295	0.01846	0.02375	0.01200	0.00049	0.00018	0.00027

Target Hazard Quotient (THQ) and Hazard Index (HI)

The non-carcinogenic health concerns connected to eating these veggies are thoroughly examined by the Target Hazard Quotient (THQ) and Hazard Index (HI). A THQ or HI value less than 1.0 implies that there is no major health risk to consumers, while values greater than 1.0 indicate possible health problems, according to WHO/FAO and the United States Environmental Protection Agency (USEPA).

$$HI = THQFe + THQZn + THQMn + THQCu + THQSn + THQCo + THQAs$$

All vegetables stay well within the safety threshold when considering the individual THQ values for important metals such as iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), tin (Sn), and cobalt (Co). For example, the THQ for Iron is incredibly low throughout, reaching a peak of just 0.01026 in Brinjal. Even manganese, which has the highest THQ in bitter gourd (0.38114), stays well below the dangerous threshold. However, the THQ for Arsenic (As) presents a completely different picture. Many vegetables have arsenic-specific THQ levels greater than 1.0, including as lotus (2.50), cauliflower (2.30), mustard (2.23), green chilli (2.07), and spinach (1.53). This recommends that As is the main source of the potential toxicity in these samples. The HI, which exhibits the overall risk of all metals combined, shows severe concerns for the majority of the vegetables assessed. Among the 15 vegetable varieties analyzed, 10 samples possessed greater HI than 1.0, showing the long – term intake may be detrimental to someone's health. The highest cumulative risks are declared by mustard (2.55), cauliflower (2.62) and lotus (2.89). Interestingly, even crops such as tomato (1.24), sweet potato (1.04), and fennel (1.10) displayed HI values above the safety threshold because of the combined effects of As and trace metals. On the other hand, sunflower (0.96), brinjal (0.78), capsicum (0.81), and peas (0.80) are the only vegetables which remained below safety cumulative range. Whereas, most individual metals are at tolerable limits, our work highlights that existence of As significantly increases the overall health risk profile for these crops (Table: 5).

Table: 5 Target Hazard Quotient (THQ) calculated for each vegetable and Hazard Index (HI)

Vegetable	Fe-THQ	Zn-THQ	Mn-THQ	Cu-THQ	Sn-THQ	Co-THQ	As-THQ	**HI**
Peas	0.00977	0.02570	0.13143	0.13225	0.00092	0.00186	0.50000	0.80193
Capsicum	0.00313	0.05233	0.09157	0.14225	0.00052	0.01674	0.50000	0.80654
Spinach	0.00797	0.04390	0.08750	0.03275	0.00022	0.00163	**1.53333**	**1.70730**
Tomato	0.00991	0.06977	0.13100	0.16000	0.00073	0.00326	0.86667	**1.24134**
Sweet Potato	0.00146	0.02587	0.19993	0.17725	0.00022	0.00488	0.63333	**1.04294**
Brinjal	0.01026	0.10963	0.28836	0.13650	0.00017	0.00140	0.23333	0.77965
Cauliflower	0.00267	0.06133	0.08050	0.17550	0.00030	0.00163	**2.30000**	**2.62193**
Lotus	0.00771	0.09793	0.13100	0.15025	0.00022	0.00163	**2.50000**	**2.88874**
Bitter Gourd	0.00730	0.11710	0.38114	0.28925	0.00010	0.00279	**1.00000**	**1.79768**
Mustard	0.00379	0.02340	0.12279	0.16250	0.00012	0.00814	**2.23333**	**2.55407**
Green Chilli	0.00811	0.06190	0.04807	0.14700	0.00012	0.00163	**2.06667**	**2.33350**
Fennel	0.00786	0.04103	0.12979	0.18925	0.00030	0.00372	0.73333	**1.10528**
Sunflower	0.00383	0.02570	0.34786	0.14725	0.00035	0.00209	0.43333	0.96041
Cluster Beans	0.00443	0.08107	0.08993	0.16150	0.00087	0.00140	0.86667	**1.20587**
Methi	0.00421	0.06153	0.16964	0.30000	0.00082	0.00419	0.90000	**1.44039**

Principal Component Analysis (PCA)

The Principal Component Analysis (PCA) biplot offers a diagram synthesis of the correlations between different vegetables as well as their associated heavy metal contents, with PCA component 1 (PC1) accounted for 27.5% of the overall variance and PCA component 2 (PC2) for 23.8%. The red vectors that exude from the center show the metals; their length as well as direction specifies the extent to which every metal manages the distribution of vegetables. Brinjal and bitter gourd displayed the high content of Mn, Zn, and Fe which are greatly related with an important cluster of vectors pointing to the right side of the figure. Conversely, As vector is strongly associated with vegetables including green chili, lotus, spinach, and cauliflower and point towards the upper left quadrant. This graphical association sustains the results of the prior risk assessment, which showed that these specific vegetables had the maximum THQ and HI values related with As. The Cu, Co, and Sn vectors are oriented in the lower–left and bottom regions of the plot, signifying a closer relation with plants like Sunflower, Methi and Capsicum. Vegetable samples that are closer to the origin, for instance fennel, peas, and cluster beans, generally display samples with metal contents that are near to the results average, showing a more balanced or lower overall heavy metal profile. Finally, the biplot efficiently highlights the inconsistent impact of Mn and As on the overall variation in the research samples by categorizing the vegetables into different groups based on their distinct metal – loading nature (Figure: 4).

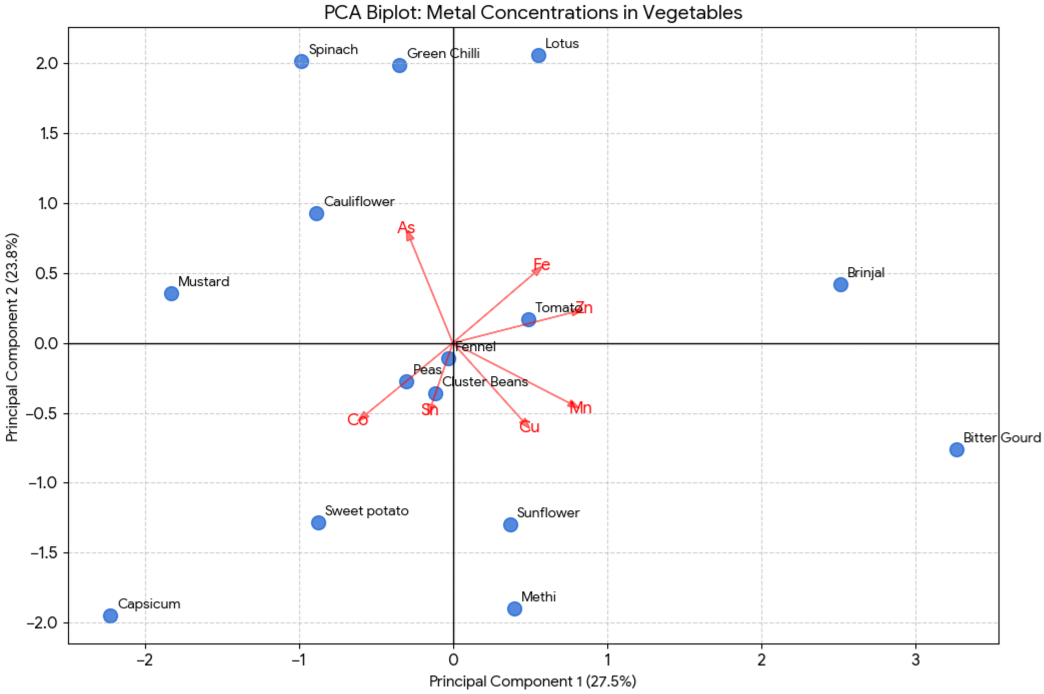

Figure: 4 The PCA (Principal Component Analysis) biplot for the vegetable metal concentration data

Pearson Correlation Matrix

The Pearson correlation matrix presents an impression of the linear correlation between the seven metals analyzed in the vegetable samples. These coefficients, which range from -1.0 to +1.0, facilitate to establish whether the presence of one metal is associated to the presence of another due to shared soil sources, similar plant uptake methods, or common agricultural inputs. With a correlation value of 0.584, Fe and Zn display the sample's maximum positive correlation. The single asterisk represents that this relation is statistically significant at the $p<0.05$ level. Since several plant species utilize equivalent transport proteins and pathways for these vital elements, vegetables that accumulate more Fe are also likely to possess more Zn. Likewise, there is a considerable positive relationship of 0.521 ($p<0.05$) between Zn and Mn, signifying that these minerals often co–occur in vegetable tissues. Other important metals for instance Mn and Cu, also declare a rather significant positive correlation (0.496), though it is not statistically significant in this sample size. This increasing inclination recommends that these micronutrients are usually more probable to be absorbed together. The absence of a positive relationship recommends that As contamination is most likely caused by independent external aspects, for instance specific arsenic–based herbicides or contaminated irrigation water, rather than being naturally related with the ingestion of essential minerals. As observed by their extremely weak or negative relationship with the other elements, the prevalence of Co, and Sn in the plants is mostly independent of the overall mineral composition (Table: 6).

Table: 6 Pearson Correlation coefficient among different metals determined from vegetables of District Ghotki

Metals	Fe	Zn	Mn	Cu	Sn	Co	As
Fe	1.000	0.584*	0.286	-0.052	0.147	-0.323	-0.176
Zn		1.000	0.521*	0.327	-0.284	-0.187	-0.014
Mn			1.000	0.496	-0.198	-0.169	-0.264
Cu				1.000	-0.106	0.042	-0.026
Sn					1.000	0.016	-0.134
Co						1.000	-0.028
As							1.000

CONCLUSION

The District Ghotki health risk assessment states that concentrations of Fe, Zn, Mn, Cu, Sn, and Co are within safe ranges, while THQ and EDI values are below critical thresholds. However, some varieties, such as lotus, cauliflower, mustard, and green chilli contain more arsenic than the WHO/FAO guideline of 0.1 mg/kg, which is dangerous for human health. Ten of the fifteen vegetables under investigation may not be carcinogenic due to the combined effects of metals, particularly arsenic, according to the Hazard Index. A statistical analysis indicates that independent factors like pesticides or tainted irrigation water are more likely to induce arsenic pollution than essential nutrient uptake mechanisms. In order to minimize long-term arsenic bioaccumulation, our findings demand stringent management and monitoring.

Author Contribution

Tahmina Fakhur-Un-Nisa Abbasi conceptualized, collected samples, designed experiments, Mubeen Ahmed Solangi and Dr. Abdul Raheem Shar prepared the draft of the article Iqra Kareem Sahito and Mohbat Ali Mahar interpreted the data, Faiqa Mukhtiar, Zahid Hussain Palh and Ghulam Hassan Wassan performed experimental analysis, Prof. Dr. Ghulam Qadir Shar and Prof. Dr. Shamroz Bano Sahito supervised the whole work, all authors read, revised, and approved the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest

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