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MULTIVARIATE ANALYSIS OF HEAVY METALS CONTENTS AND ASSOCIATED HEALTH HAZARDS IN COMMERCIALLY AVAILABLE VEGETABLES IN FAISALABAD, PAKISTAN

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The use of wastewater for growing vegetables has become a common practice in peri-urban areas of big cities of developing world including Pakistan. In addition to providing essential elements and organic matter for plant growth, wastewater may contain potentially toxic trace elements that could pose serious threat to animals and humans, if their concentrations in edible parts increase the permissible limits. To monitor the heavy metals contents in commercially available vegetables, vegetable samples were collected from three main vegetable stores designated as A, B and C, respectively of Faisalabad, Punjab, Pakistan. These samples were analyzed for dietary contents (protein, fat, fiber, and carbohydrates) and heavy metals such as cadmium (Cd), lead (Pb), copper (Cu), and nickel (Ni). The results revealed that among the three vegetables, spinach accumulated higher contents of heavy metals followed by lettuce and fenugreek. Multivariate analysis indicated that the vegetables collected from locations A and C had higher metal contents than the permissible limits. The samples collected from location B were safe for human consumptions. Due to high metal contents, vegetables collected from locations A and C could potentially pose carcinogenic and non-carcinogenic health hazards to human beings. It is concluded that proper monitoring and regulatory measures should be done regarding the source of irrigation and post-harvest handling of vegetables in order to limit the entry of toxic metals into human food chain.
Keywords: Heavy metals, wastewater, vegetables, dietary contents, health risk

INTRODUCTION

Water is an essential natural resource and it is crucial for sustaining life on earth. About 90% of the total accessible water is being used for irrigation purposes; whereas, only 6% and 3% are being utilized for domestic and industrial purposes, respectively (Barakat *et al*., 2020). In Pakistan, the supply of surface water is limited for domestic and agricultural use. Therefore, the farmers are using untreated wastewater in the peri-urban areas for growing vegetables, fodder and cereal crops (Barakat *et al*., 2020; Sabeen *et al*., 2020; Anwar *et al.,* 2021). Wastewater irrigation provides organic matter and inorganic elements that are essential for proper plant growth and development (Nawaz *et al*., 2020). Due to continuous and excessive use of raw wastewater, heavy metals are transferred and accumulate to variable levels in vegetables and cause serious health hazards such as cancer, developmental toxicity, and genetic mutations (Singh *et al*., 2004; Sarwar *et al*., 2019; Anwar *et al.,* 2021).;
Soils can be contaminated with heavy metals including Pb, Cd, Cu, and Ni from numerous sources (Anwar *et al.,* 2021). These sources include industrial effluents, mining activities, improper disposal of wastes, use of leaded paints and gasoline, use of fertilizers in agriculture, improper handling, and disposal of sewage sludge and animal manures, unrestricted use of pesticides, non-regulated use of wastewater irrigation, combustion of coal, petrochemical spills, and atmospheric deposition (Carolin *et al*., 2017; Mehmood *et al.,* 2020). Heavy metals are extremely toxic for living beings due to their high solubility in water, long half-lives, and non-biodegradability. Elimination of toxic heavy metals from an organism's body is not possible hence, even minute concentrations of the heavy metals have devastating effects on the health of living organisms (Mehmood *et al.*, 2020).
The heavy metals accumulation in soil has serious effects on agriculture production and food quality (Naser *et al*., 2011; Rezaeian *et al*., 2020). The heavy metals absorption capacity of vegetables relies on the nature of vegetables and concentrations of the heavy metals in the soils (Anwar *et al.*, 2021; Natasha *et al.,* 2021).
Some heavy metals (i.e., Cu, Ni, Fe, and Zn) are essential for proper plant growth under certain concentrations, while others such as Pb and Cd are toxic for plants regardless of their initial concentration. These heavy metals are moved to the food chain and induce damaging effects in plants like growth inhibition and oxidative stress (White and Pongrac, 2017; Natasha *et al*., 2021). Plants have adopted various

mechanisms to mitigate the heavy metal-induced toxicity including activation of different antioxidants, sequestration of heavy metals into vacuoles, limited uptake of heavy metals, and binding to metallothioneins/phytochelatins (Shahid *et al*., 2015).

Leafy vegetables (i.e., spinach, lettuce, and fenugreek) contain different minerals, vitamins, proteins, and carbohydrates, so they constitute a significant part of the human diet. Vegetables have nutritious values for humans and play a very important role in the maintenance of normal metabolism and the prevention of several diseases (Chauhan *et al*., 2020).

A great deal of literature show that use raw wastewater is on the rise in peri-urban areas of Faisalabad metropolitan and farmers use untreated wastewater to irrigate agricultural fields for the cultivation of crops especially vegetables (Farid et al., 2015; Natasha *et al.,* 2021). A substantial quantity of wastewater irrigated vegetables is being sold in the local markets. Consumption of vegetables grown under wastewater irrigation can lead to the accumulation of heavy metals in human beings. We hypothesized that commercially available vegetables in different stores of Faisalabad could be contaminated with heavy metals. Thus, the current study was carried out to evaluate the heavy metals accumulation in vegetables commonly available in local markets, and the associated health risks for human beings via consumption of those vegetables.

MATERIALS AND METHODS

Collection and preparation of vegetable samples: Samples of three leafy vegetables viz. spinach, fenugreek, and lettuce were collected from three main vegetable stores of the Faisalabad metropolitan, Punjab, Pakistan. These three stores were located in three different areas of the metropolitan city and were named as A, B and C (Fig. 1). After collection of four samples of each vegetable from each location, the samples were transferred to the laboratory and washed using tap water for the removal of dust particles. Later, the samples were washed twice with distilled water and dried with blotting paper. Following air drying, the samples were cut into small pieces and oven-dried at 65^{0}C till constant weight. The samples were ground to a fine powder with the help of porcelain mortar and pestle.

Metals analyses: Collected vegetable samples were digested by using a di-acid mixture (HNO_3 + $HClO_4$ in 2:1 ratio) to determine the heavy metals (Cd, Pb, Cu, and Ni) concentrations. After digestion, the samples were allowed to cool and then they were filtered through Whatman filter paper No. 42. Following filtration, the volume of the samples was brought up to 100 mL with deionized water and they were analyzed on Atomic Absorption Spectrophotometer (PerkinElmer Model: PinAAcle 900F, Inc. USA) to estimate the concentrations of heavy metals. For each metal calibration, standards were prepared from the dilution of the respective metal stock solution (CPAchem, Bulgaria) using deionized water.

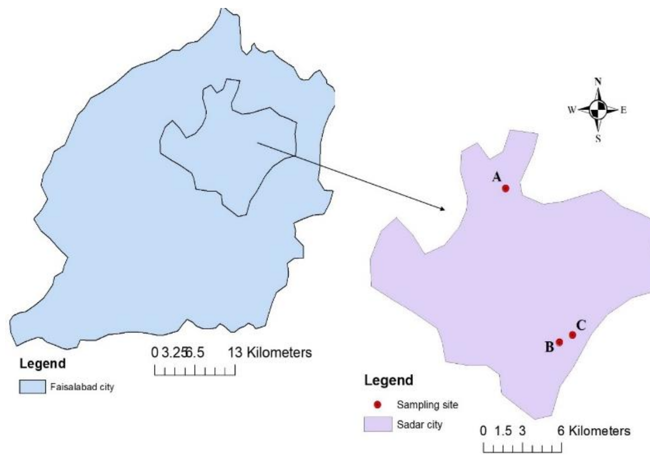

Figure 1. GIS map of Faisalabad city indicating different locations of vegetables sampling.

Crude protein: The determination of crude protein contents in vegetable samples was carried out by using the micro Kjeldahl method AOAC (2005). Following equation was used for calculation of percentage of nitrogen (N):

$$\text{Nitrogen (\%)} = \frac{(S-B)\times N\times 0.014\times D\times 100}{\text{Weight of sample}} \quad (1)$$

Where, D indicates the dilution factor, N represents the normality of the acid, S is volume of acid, B represents the volume of blank used, and 0.014 is the constant value.

Crude protein (%) was calculated by multiplying the corresponding total nitrogen content by a conversion factor of 6.25.

$$\text{Crude protein (\%)} = \text{\% of N} \times 6.25 \quad (2)$$

Crude fat: For the determination of crude fat, the Soxhlet extraction technique was used in following steps (AOAC, 2005). First, the vegetable sample was properly packed in a filter paper and then it was placed in the thimble. Next, the vapors produced by a fresh solvent in a distillation flask were directed to the thimble where samples were placed and after that the vapors were liquefied in the condenser. Extraction of fat content from dried vegetable samples was performed by using an organic solvent (i.e., petroleum ether) at 40 to 60 ^{0}C for 6 h.

Crude fiber and carbohydrates: Crude fiber is the bulk of roughage in vegetable. The vegetable samples were milled, dried, defatted by using ethanol-acetone mixture, and analyzed to determine crude fiber contents by following the standard method (AOAC, 2005). Whereas, the carbohydrate contents in vegetable samples were estimated by using the different method as described by AOAC (2005).

Health risk assessment: The calculation of estimated daily intake (EDI) values of Cd, Pb, Cu, and Ni was carried out by using the following equation (Murtaza *et al*., 2020).

$$EDI = \frac{C \times IR \times EF \times ED}{AT \times BW} \quad (3)$$

In the above equation, C represents the concentration (mg/kg) of each metal in vegetables samples, ingestion rate is indicated by IR, ED specifies the exposure duration (64.4 years), EF indicates exposure frequency (365 days/year), AT represents the average life expectancy (23,506), while BW indicates the body weight of individuals (70 kg).

Hazard quotients of Cd, Pb, Cu, and Ni were calculated as described by Murtaza *et al*. (2020) by using the following equation.

$$HQ = \frac{EDI}{RfD} \quad (4)$$

Where; HQ is the hazard quotient while, EDI represents the estimated daily intake of each metal, and R_fD is the oral reference dose of each metal. Rfd values for Cd, Ni, Cu and Pb are 0.001, 0.02, 0.04, 0.004, (mg/kg bw/day), respectively (US-EPA IRIS, 2006).

The possible cancer risk by consuming Cd, Pb, Cu and Ni was calculated by the following equation given by Murtaza *et al*. (2020).

$$CR = EDI \times CS \quad (5)$$

Where; CR denotes cancer risk, EDI is the estimated daily intake and CSF stands for “cancer slope factor” of each metal.

Quality control and quality assurance: Heavy metals concentrations in the vegetable samples were measured on atomic absorption spectrophotometer (PerkinElmer Model: PinAAcle 900F, Inc. USA). Calibrated glassware, analytical grade chemicals (Merck, Germany), and deionized water were used for the analyses. The certified reference materials, internal standards, and reagent blanks for each metal were used for standardization, quality control and quality assurance in the metal analyses.

Statistical analysis: The data were statistically examined using the SPSS statistical package (SPSS Statistics, Ver 20, IBM). A two-way analysis of variance (ANOVA) was used to compare different locations and vegetables. Least significant difference (LSD) test at 5% significance level was used for further comparison of the variables (Steel *et al*., 1997). The data were presented as mean values of four replicates ± standard error. Principal component analysis (PCA) and Pearson correlation matrices were drawn using XLSTAT 2014 version.

RESULTS

Heavy metals contents: The results presented in Table 1 showed that the metals contents were variable with respect to three vegetables as well as the locations. The highest Cd contents were found in spinach followed by lettuce and fenugreek, respectively. Among locations, the highest contents of Cd were observed for location A, followed by C and B, respectively. For locations A and C, the Cd contents in all the three vegetables were higher than the threshold (0.2 mg kg^{-1}) set by European Union Standards (European Union, 2006). Vegetables collected from location B accumulated least amount of Cd that was within the safe limits. Lead contents were the highest in spinach for locations A followed by C and B, respectively. The lowest Pb contents were found in fenugreek for all three locations. The accumulation pattern of Pb was similar to Cd i.e. spinach accumulated a greater amount of Pb than lettuce and fenugreek, and the vegetables collected from locations A and C had higher Pb contents than its threshold level (0.43 mg kg^{-1}) (European Union, 2006). The highest Cu and Ni contents were found in spinach followed by lettuce and fenugreek, respectively. Among the locations, the highest contents of Cu and Ni were observed for location A, followed by C and B, respectively. For locations A and C, all the three vegetables accumulated higher amount of Cu than its permissible limit of 20 mg kg^{-1} (European Union, 2006). However, the Ni contents were within the safe limit of 66 mg kg^{-1} (European Union, 2006) for all the three vegetables and locations.

Dietary contents of vegetables: Dietary contents of vegetables varied with respect to location and type of vegetables (Table 2). The highest contents of protein, fiber, fat, and carbohydrates were found in fenugreek followed by

Table 1. Heavy metals contents (mg kg^{-1}) in vegetables collected from different locations of Faisalabad city

Vegetables	Locations	Cadmium	Lead	Copper	Nickel
Spinach	A	12.00±0.58a	4.20±0.15a	66±3.79a	36±3.20b
	B	0.19±0.02f	0.25±0.02e	15±1.50f	23±1.70c
	C	2.60±0.20d	3.60±0.29ab	50±3.61bc	45±3.61a
Lettuce	A	9.00±0.57b	3.90±0.24ab	54±3.47b	35±3.22b
	B	0.18±0.01f	0.20±0.02e	11±1.50fg	14±0.58d
	C	1.60±0.15e	2.85±024c	38±2.65de	36±2.89b
Fenugreek	A	4.30±0.35c	3.40±0.23bc	42±3.47cd	24±2.00c
	B	0.15±0.01f	0.18±0.015e	6±0.57g	10±1.00d
	C	1.20±0.10e	2.20±0.31d	30±3.47e	33±2.89b
		12.37*	15.58*	-	30.38*

Data are mean of four replications ± SE. For each parameter, values having different letters indicate significant difference at 5% probability level. Values with asterisk (*) are the mean values of heavy metals in wastewater irrigated vegetables as reported by Nawaz *et al.*, (2020).

lettuce and spinach, respectively. Among the location, these dietary contents were higher for location B followed by C and A, respectively.

Health risk assessment: The health risk parameters were calculated to determine the carcinogenic and non-carcinogenic health risks for adults by the consumption of heavy metals contaminated vegetables (Table 3). The HQ value for Cd was greater than 1 (limit hazard value) for location A for spinach and lettuce. For other two locations, HQ was lower than its threshold limit for all the three vegetables. The cancer risk factor (CR) for Cd showed values higher than threshold limit (0.0001) for all the locations and vegetables. The HQ and CR values for Cu and Pb remained below the threshold levels for all the locations and all the three vegetables. The HQ values for Ni were lower than 1 in all the three vegetables for all the three locations. However, the CR values for Ni were higher than threshold limit for all the locations and vegetables.

Multivariate analyses: The correlations between different observations and response variables were determined using Principal component analysis (PCA) and Pearson correlation matrix (Fig. 2, Table 4). All the variables were clustered in two groups. The contents of Cd, Pb, Cu and Ni were grouped together; whereas, the dietary contents were grouped together. Pearson correlation showed that all the metals had strong positive correlation with each other, and negative correlations with carbohydrates contents of all the vegetables (Table 4). The response of different locations and vegetables was also illustrated by PCA (Fig.1). Locations A and C had similar effect; therefore, they were scatted in the same axis. Location

Table 2. Dietary contents (%) in vegetables collected from different locations of Faisalabad city

Vegetables	Locations	Protein	Fat	Fiber	Carbohydrate
Spinach	A	0.47±0.18f	0.48±0.03c	0.40±0.12e	1.63±0.06g
	B	2.67±0.24bc	0.19±0.01d	1.50±0.15c	2.03±0.24fg
	C	1.72±0.1168d	0.25±0.02c	0.87±0.17d	2.27±0.15f
Lettuce	A	0.60±0.17f	0.77±0.06b	0.27±0.07e	2.83±0.09e
	B	3.21±0.49b	0.16±0.03d	2.10±0.14b	3.00±0.06de
	C	1.50±0.15de	0.40±0.03c	1.60±0.09c	3.30±0.12d
Fenugreek	A	0.77±0.17ef	0.99±0.06a	0.40±0.089e	4.60±0.17c
	B	4.97±0.29a	0.26±0.03d	2.90±0.58a	5.10±0.23b
	C	2.00±0.21cd	0.82±0.03b	1.97±0.03b	5.97±0.15a

Data are mean of four replications ± SE. For each parameter, values having different letters indicate significant difference at 5% probability level.

Table 3. Estimated daily intake (EDI), health hazards (HQ), and cancer risk (ILTCR) of heavy metals (Cd, Pb, Ni, Cu) via consumption of contaminated vegetables.

			Cd			Cu	
Vegetable	**Locations**	**EDI**	**HQ**	**ILTCR**	**EDI**	**HQ**	**ILTCR**
	A	0.00146	1.46	0.00892	0.00805	0.2012	0
Spinach	B	0.00002	0.02	0.00014	0.00183	0.0457	0
	C	0.00032	0.32	0.00193	0.00610	0.1524	0
	A	0.00110	1.10	0.00669	0.00658	0.1646	0
Lettuce	B	0.00002	0.02	0.00013	0.00134	0.0335	0
	C	0.00020	0.20	0.00119	0.00463	0.1158	0
	A	0.00052	0.52	0.00320	0.00512	0.1280	0
Fenugreek	B	0.00002	0.02	0.00011	0.00073	0.0183	0
	C	0.00015	0.15	0.00089	0.00366	0.0914	0
			Pb			**Ni**	
	A	0.00051	0.13	0	0.00439	0.22	0.00369
Spinach	B	0.00003	0.01	0	0.00280	0.14	0.00236
	C	0.00044	0.11	0	0.00549	0.27	0.00461
	A	0.00048	0.12	0	0.00427	0.21	0.00358
Lettuce	B	0.00002	0.01	0	0.00171	0.09	0.00143
	C	0.00035	0.09	0	0.00439	0.22	0.00369
	A	0.00041	0.10	0	0.00293	0.15	0.00246
Fenugreek	B	0.00002	0.01	0	0.00122	0.06	0.00102
	C	0.00027	0.07	0	0.00390	0.20	0.00328

Table 4. Correlation matrix of different variables of vegetables collected from different locations of Faisalabad city

Variables	Cd	Pb	Cu	Ni	Car	Fat	Fib	Pro
Cd	1.0000							
Pb	0.7593	1.0000						
Cu	0.8422	0.9178	1.0000					
Ni	0.4580	0.7536	0.7748	1.0000				
Car	-0.4213	-0.2412	-0.3836	-0.3450	1.0000			
Fat	0.4029	0.5611	0.4481	0.2318	0.4615	1.0000		
Fib	0.4779	0.6995	0.5981	0.3479	0.3014	0.9282	1.0000	
Pro	-0.0180	0.4044	0.3359	0.5209	0.4490	0.4741	0.4962	1.0000

Values in bold are different from 0 with a significance level alpha=0.05

Table 5. Eigenvalues of different factors in principal component analysis

	F1	F2	F3	F4	F5	F6	F7	F8
Eigenvalue	4.3644	2.1314	0.9892	0.2157	0.1215	0.0987	0.0520	0.0271
Variability (%)	54.5553	26.6419	12.3646	2.6963	1.5186	1.2340	0.6500	0.3393
Cumulative %	54.5553	81.1972	93.5618	96.2581	97.7767	99.0107	99.6607	100.0000

B behaved differently; hence, it was scattered in different axis. The PCA divided all the variables in to 8 factors (F1 to F8), but the main contribution was only from four factors. These four factors contributed 54%, 27%, 12%, and 3% variability, respectively (Table 5).

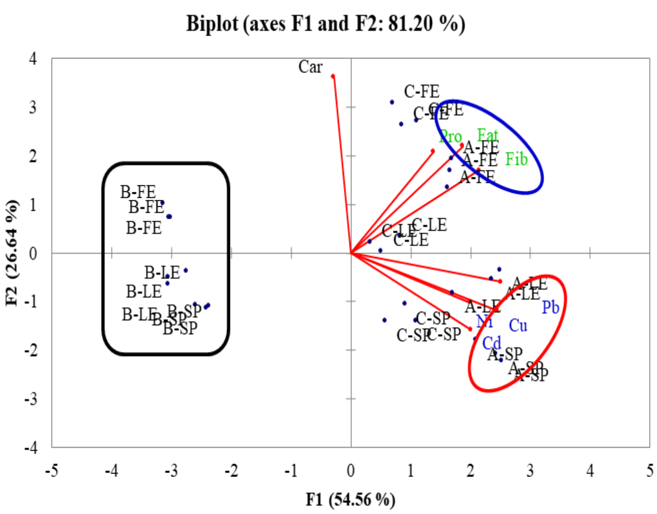

Figure 2. Comparison of different locations and vegetables for heavy metals and dietary contents using principal component analysis.

DISCUSSION

Our results showed heavy metals contaminations of leafy vegetables collected from various locations of Faisalabad city. Mahmood *et al*. (2014) also found that most of the vegetables collected from different locations in Lahore, Pakistan including spinach accumulated higher Cd contents than the permissible limits. Similarly, Nawaz *et al*. (2020) found higher levels of heavy metals including Cd, Pb and Ni in vegetables collected from surrounding of a wastewater treatment plant in Faisalabad, Pakistan. In the present study, higher levels of Cd at A and C could be attributed to higher Cd levels in soil and water/wastewater used for growing vegetables. Moreover, if the vegetables were grown along the roads, foliar and atmospheric deposition might also have played their parts (Pandey *et al*., 2012; Ahmad *et al.*, 2020). Spinach accumulated more Cd than the other two vegetables due to its higher metal uptake and accumulating potential (Pandey *et al*., 2012; Mahmood *et al*., 2014).

The contents of Pb were higher than its threshold level (0.43 mg kg^{-1}) (European Union, 2006) in vegetables collected from locations A and C. In line with our results, Khan *et al*. (2020) found higher concentration of Pb in commercially available vegetables including spinach and lettuce in Karachi, Pakistan. Similarly, Khan *et al*. (2013) observed dangerous level of Pb in leafy vegetables including spinach in peri-urban areas of Lahore district. The higher Pb contents in vegetables may be due to the use of wastewater for irrigation that may route the uptake of Pb from roots to shoot/leaves of the vegetables. Other sources may include burning of fossil fuels, traffic and industrial emission, sewage water, discharge of Pb storage batteries, pigments and paints (Murtaza *et al*., 2019; Ahmad *et al.*, 2020).

The highest Cu and Ni contents were found in spinach followed by lettuce and fenugreek, respectively. For locations A and C, all the three vegetables accumulated higher amount of Cu than its permissible limit of 20 mg kg^{-1}. Our results are supported by the findings of Khan *et al*. (2013) for peri-urban areas of Lahore district. Contrarily, Mahmood *et al*. (2014) and Khan *et al*. (2020) found Cu contents within the safe limits in wastewater irrigated vegetables. We found that the Ni contents were within the safe limit of 66 mg kg^{-1} (European Union, 2006) for all the three vegetables and locations. Similar lower values of Ni were found in leafy vegetables receiving metal contaminated water in Karachi (Khan *et al*., 2020), Lahore (Mahmood *et al*., 2014) and Faisalabad (Nawaz *et al*., 2020), respectively. Such variation in metal contents may be related to the source of irrigation. The vegetables collected from locations (A) and (C) might be irrigated with industrial wastewater and sewage water, respectively. Whereas, the vegetables collected from location (B) might be irrigated with good quality water. Faisalabad is one the leading industrial city of Pakistan. In different industries, huge quantities of heavy metals are being used in routine and discharged into wastewater streams (Ahmad *et al.,* 2020). That water is being used for vegetable production in the peri urban areas of the city. The continuous use of such polluted water might be the main reason of metals accumulation in vegetables.

We found that the dietary contents of the vegetables were decreased due to heavy metals accumulation. Similar to our results, other reports have also shown that dietary content in vegetables decreased due to high levels of heavy metals in soil and consequent accumulation in plant parts (Xu *et al*., 2006; Weihong *et al*., 2009). For instance, Alia *et al*. (2015) reported that the fiber and total protein content in spinach decreased by 29 and 31% under Cd stress. While, Pb toxicity caused 22 and 23% decrease in fiber and total protein content in spinach, respectively as compared to control. Likewise, protein content in the mustard greens (*Brassica juncea*) grown under Pb stress decreased by 77% as compared to control (Weihong *et al*., 2009). The decline in protein contents under heavy metals stress can be attributed to an increase in protease activity which leads to faster degradation of protein under heavy metals stress and consequently it decreases total protein content in vegetables (Xu *et al*., 2006). Decrease in protein contents can be associated with the interference caused by heavy metals in nitrogen metabolism (Auda and Ali, 2020). Furthermore, heavy metals decrease plant biomass by disturbing photosynthetic and metabolic processes and as a result carbohydrates and fat contents are also decreased.

Results of our study showed that cancer risk (CR) values for Cd and Ni were greater than their threshold limits for all the vegetables. The values of HQ for Cd were also higher than 1, which indicated potential non-carcinogenic health risks. Heavy metals can cause various ailments in human body, and they enter into human food chain via plants grown under contaminated growth mediums (Shahid *et al*., 2013; Ahmad *et al.,* 2020; Natasha *et al.,* 2021). These heavy metals are known to have long half-lives owing to their non-biodegradability, and this problem is further aggravated by their higher bio-accumulation in various organs and tissues of human body (Shahid *et al*., 2015). Moreover, they are highly mobile and reactive, and for this reason, they can have devastating effects on human health even in low concentrations (Uzu *et al*., 2011). These negative effects include liver and lung diseases, cardiovascular problems, osteoporosis, mental disorders, and renal failure (Yargholi *et al*., 2008). Long term exposure to minute concentrations of heavy metals can induce serious damage to different human organs and in severe cases it can lead to cancer (Ahmad *et al.,* 2020; Anwar *et el.,* 2021). It is also possible that the heavy metals contents in vegetables may increase when they are being transported or when they are being marketed, and in this way, the customers are likely to be exposed to heavy metals. This is particularly true for industrial cities where safe disposal mechanisms for heavy metals contaminated water are not present (Ahmad *et al.,* 2019). In addition, heavy metal contents in air can also contaminate vegetables, either in the field or at the place where they are being sold (Ahmad *et al.,* 2020). Li *et al*. (2015) reported that the soil or growth medium is the primary source of heavy metals (Cd, Pb, Cu and Ni) in vegetables. However, they also suggested that the foliar uptake of heavy metals can also be a significant source of bioaccumulation in vegetables. The findings of the current study indicated that the consumption of vegetables grown under wastewater irrigation can be a major source of heavy metals intake in humans. Continuous use of these vegetables can cause severe health risks due to bio-magnification. Therefore, it is recommended that stringent actions should be taken against wastewater use in agriculture to ensure public health.

The variation in metal contents with respect to locations and vegetables was also verified by multivariate analyses technique. This is regarded as a very ideal approach to trace correlation and covariance among different treatments and variables (Murtaza *et al*., 2019; Natasha *et al*., 2020). In our study, Pearson correlation and PCA showed strong positive correlation among four heavy metals and negative correlation with the dietary contents particularly carbohydrate contents. It was also demonstrated that different locations and vegetables had different effects. Therefore, these variables were scatted in different axes. Locations A and C were grouped closer to each other because the vegetables collected from both of these locations had higher metals contents. The separate clustering of vegetables for locations B is due less metal accumulation. Our results were further verified by Pearson correlations which indicated strong positive correlation of four metals with each other. This indicated the multi-metal contamination of vegetables irrigated with wastewater.

Conclusion: The results of the current study showed that the vegetables including spinach, lettuce, and fenugreek collected from different stores of Faisalabad were contaminated with heavy metals such as Cd, Pb, Cu and Ni. All the vegetables posed cancer risk due to higher contents of Cd and Ni; along with non-carcinogenic health risk due to Cd accumulation. Therefore, it is recommended to implement proper monitoring and regulatory measures for the source of water being used for crops/ vegetables production. Moreover, care should be taken during post-harvest handling and transportation of vegetables to markets in order to limit the entry of toxic metals into human food chain.

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