

Heavy Metals Bioconcentration from Soil to Vegetables and Assessment of Health Risk Caused by Their Ingestion

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Abstract The present study was undertaken to assess the non-carcinogenic human health risk of heavy metals through the ingestion of locally grown and commonly used vegetables viz. *Raphanus sativus* (root vegetable), *Daucus carota* (root vegetable), *Benincasa hispida* (fruit vegetable) and *Brassica campestris* leaves (leafy vegetable) in a semi-urbanized area of Haryana state, India. Heavy metal quantification of soil and vegetable samples was done using flame atomic absorption spectrophotometer. Lead, cadmium and nickel concentration in vegetable samples varied in range of 0.12–6.54 mg kg⁻¹, 0.02–0.67 mg kg⁻¹ and <0.05–0.41 mg kg⁻¹, respectively. Cadmium and lead concentration in some vegetable samples exceeded maximum permissible limit given by World Health Organization/Food and Agriculture Organization and Indian standards. Much higher concentrations of Pb (40–190.5 mg kg⁻¹), Cd (0.56–9.85 mg kg⁻¹) and Ni (3.21–45.87 mg kg⁻¹) were reported in corresponding vegetable fields' soils. Correlation analysis revealed the formation of three primary clusters, i.e. Cu–Cd, Cd–Pb and Ni–Zn in vegetable fields' soils further supported by cluster analysis and principal component analysis. Bioconcentration factor revealed that heavy metals' uptake was more by leafy vegetable than root and fruit vegetables. Hazard index of all the vegetables was less than unity; thus, the

ingestion of these vegetables is unlikely to pose health risks to the target population.

Keywords Heavy metals · Hazard quotient · Hazard index · Health risk · Bioconcentration factor · Reference oral dose

Introduction

Heavy metals are omnipresent due to their non-biodegradable and persistent nature and potential to accumulate in different parts of the body. Water solubility of heavy metals and lack of proper mechanism for their removal from the body makes most of the heavy metals extremely toxic even at trace concentrations [1]. Due to rapid industrialization during the last five decades, soil contamination with heavy metals arises as a serious environmental problem on a global scale [2]. Agricultural soils to food transfer of heavy metals via crops is the major pathway of human exposure to them. A number of factors such as local climate, atmospheric dry depositions, physicochemical properties of soil and the degree of maturity of plants at the time of harvest influence uptake and bioaccumulation of heavy metals in crops including vegetables [3, 4]. Long-term wastewater irrigation practices also contribute to higher uptake to heavy metals by vegetables [5]. Anthropogenic sources of heavy metals in agricultural fields include the addition of manures, sewage sludge, agrochemicals and may also affect the uptake of heavy metals by modifying the physico-chemical properties of the soil such as pH, organic matter and bioavailability of heavy metals in the soil [6].

Vegetables with antioxidative properties are rich source of carbohydrates, proteins, vitamins, fibres, minerals, trace elements, etc. and are important components of the human diet [7]. Dietary intake through contaminated food including vegetables is the main route for human intake of heavy metals

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except occupational exposure [8]. Heavy metal-contaminated food consumption results in depletion of some essential nutrients from the body that can further result in intra-uterine growth retardation and decreased immunological defences [9]. Therefore, to assure food quality various international and national regulatory bodies had laid down maximum permissible levels of toxic metals in different food items due to an increased awareness of the risk for these metals [10].

Various studies have been undertaken to monitor heavy metal concentration in edible vegetables. Heavy metals in soil and vegetables had been quantified in China [11]. Heavy metals contamination of various vegetables collected from Gujarat (India) had been monitored [12]. The results showed that different heavy metals had different uptake pattern by different vegetables. Cadmium was highest in onion, but Co and Cu were highest in cauliflower. Distribution of heavy metals in contaminated agriculture soil and their availability to several vegetables species in Northern Greece had been studied [13]. The results showed that highest concentration factor of heavy metal was in lettuce and lowest in cabbage. Heavy metals in agricultural soil and vegetables in Dongguan City, Guandong, China, had also been monitored [14]. The results showed that there was higher accumulation of heavy metals in the vegetables and crops. The order of concentration factor in vegetable was $Cd > Zn > Cu > As > Ni > Hg > Cr > Pb$.

Based upon the bibliographic survey, it was concluded that no work has been undertaken on non-carcinogenic risk assessment of heavy metals via dietary intake of vegetables grown in Haryana State, India. In the Indian subcontinent where a majority of the population is vegetarian, vegetables are important constituents of the human diet both in terms of quantities consumed and nutritional value [15]. Keeping this in view, this study was undertaken to quantify the levels of different heavy metals in edible portions of vegetables and to assess the health risk associated with them to target population.

Materials and Methods

Study Area

Haryana state is located in North-west of India between a latitude of 30.30° North and longitude of 74.60° East (Fig. 1), sharing its boundaries with Rajasthan in south and west, Himachal Pradesh and Punjab in the north and the territory of Delhi in the east. Vegetables are grown seasonally in different parts of the state. The study area is located in Southwestern part of state. Climate is tropical with wide variation in temperature ranging from 47°C in summer to

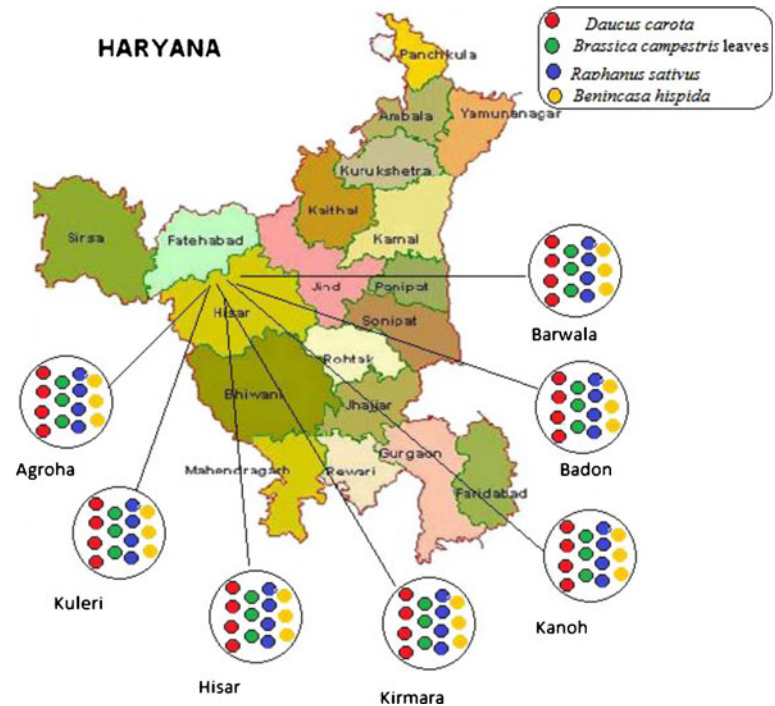
2°C in winter. The area receives an annual average rainfall of 395.6 mm. About 71 % of the annual normal rainfall is received during the short south-west monsoon period, July to September. Study area is an alluvial plain of Indo-Gangetic basin with sandy, sandy loam and clay soil types. As a whole, the topography of the region is flat plain with an average elevation of 215 m gently sloping from north-east to south-west.

Sample Collection and Processing

Vegetables Sampling and Analysis

Samples of *Raphanus sativus* (root vegetable), *Daucus carota* (root vegetable), *Benincasa hispida* (fruit vegetable) and *Brassica campestris* leaves (leafy vegetable) were collected from seven different villages of district Hisar, Haryana based upon the availability directly from the agricultural fields. The collected samples were sealed in plastic bags and taken to the laboratory for further analysis. Vegetables were washed thoroughly with running tap water to remove dust and extraneous matter and chopped into small pieces after discarding the non-edible portions. The samples were dried in drying oven at 90°C until a constant weight was achieved. The dried samples were powdered, homogenized and stored in air tight plastic containers for further use. After that, 100 g dry vegetable sub-sample was ashed in muffle furnace at $350\text{--}400^\circ\text{C}$. Of each vegetable ash samples, 0.2 g was mixed with 5.0 ml diacid (HNO_3 and HClO_4) mixture in the ratio of 9:1 (v/v). Sample mixture was then taken in pre-cleaned Teflon vessels, left open overnight at room temperature and digested in a microwave digester (CEM Mars X). The operating program for microwave digestion system for each digestion set up was optimized at a power of 800 W and at maximum operable temperature of 170°C . When digestion was complete, vessels were cooled at room temperature and digests were quantitatively transferred into glass beakers. Then, the digests were evaporated to dryness on hot plate at a temperature of $130\text{--}150^\circ\text{C}$, and residue was dissolved in double-distilled water to make desired volume. Extracted solutions were transferred to polypropylene bottles and refrigerated until analysis. All working standards used for analysis were prepared by diluting 1,000 mg/L certified standard solutions. Acetylene gas was used as fuel and air as support in FAAS. An oxidizing flame was used in all the cases except chromium, where reducing nitrous oxide flame was used for metal quantification. Detailed instrumental analytical conditions for analyses of selected heavy metals are given in Table 1. The extracts were analyzed for eight heavy metals viz. Fe, Cu, Cd, Ni, Zn, Cr, Co and Pb using flame atomic absorption spectrophotometer (Shimadzu AA-6300), and concentrations were finally expressed in milligrams per kilogram on dry weight basis.

Fig. 1 Map of the study area showing different sampling sites and type of samples collected



Soil Sampling and Analysis

In order to evaluate bioconcentration factor of heavy metals from soil to edible parts of vegetables, corresponding sub-surface soil samples from rooting zone were also collected. From a depth of 5–10 cm, four sub-samples of soil from rectangular grid of 0.5 m² area were collected and then mixed together to obtain a representative sample. The soil samples were collected using non-metallic spade to get rid of any metal contamination. Samples were stored in clean plastic bags after removing foreign bodies. Soil samples were air-dried, ground and passed through a 2.0-mm sieve prior to analysis. Heavy metals' concentration was determined in soil samples as given for vegetable samples.

Analytical Quality Control

During processing and analysis of samples, quality assurance and control measures were adopted to ensure the reliability of results. To avoid sample contamination, high purity deionized water was used for rinsing glassware, preparation of standards and dilution of samples. Reagent blanks were analyzed, and the data were subsequently blank corrected to remove analytical bias. Relative standard deviations of replicate measurements were <10 % heavy metals. The calibration curves were linear within the concentration range, with the regression coefficients (R^2) >0.999. To ensure the reliability of results, standards of respective metals were run after every ten samples analyzed.

Table 1 Instrumental analytical condition for analyses of selected heavy metals in the study area

Heavy metals	Flame type	Wavelength (nm)	Slit width (nm)	Gas flow (L min ⁻¹)	Lamp current (m A)	Detection limits (mg L ⁻¹)
Pb	Air-acetylene	217.0	0.7	2.0	12	0.06
Ni	Air-acetylene	232.0	0.2	7.0	12	0.04
Cd	Air-acetylene	228.8	0.7	1.8	8	0.01
Zn	Air-acetylene	213.8	0.2	7.5	8	0.01
Cu	Air-acetylene	324.7	0.7	1.8	6	0.025
Fe	Air-acetylene	248.3	0.2	2.2	12	0.45
Cr	Nitrous oxide-acetylene	357.9	0.2	2.0	6	0.05
Co	Air-acetylene	240.7	0.2	2.2	6	0.05

Statistical Analysis

The bivariate correlation analysis with the Pearson’s correlation coefficient (*r*) at two-tailed significance level (*P*), principal component analysis (PCA) and hierarchical cluster analysis (CA) using complete linkage method with Pearson correlation interval were applied using SPSS software package (version 16.0).

Bioconcentration Factor of Heavy Metals from Soil to Vegetables

To evaluate the heavy metals’ uptake efficiency by edible portions of vegetables, bioconcentration factors (BCF) were calculated. It is computed as the ratio of concentration of the heavy metal vegetables edible portion to their concentration in respective soils.

$$BCF_{\text{Edible}} = \frac{C_{\text{vegetable}}}{C_{\text{soil}}} \tag{1}$$

Where $C_{\text{vegetable}}$ and C_{soil} are the concentration of heavy metal in vegetables edible portion and rooted soils on dry weight (DW) basis, respectively.

MPI

To compare the total heavy metals content at different sampling sites, metal pollution index (MPI) was calculated using the equation given by [16].

$$MPI = (Cf_1 \times Cf_2 \dots \dots Cf_n)^{1/n} \tag{2}$$

Where Cfi is the concentration for the metal *i* in the vegetable sample and *n* is the total number of metals.

Health Risk Assessment from Consuming Vegetables

Chronic daily intake (CDI) is the exposure to the population expressed as the mass of a substance per unit body weight per unit time averaged over a long period of time (a lifetime). Chronic daily intake through vegetable ingestion was calculated according to formula given by [17].

$$CDI_{\text{ingestion}} = \frac{[C_M \times C_F \times F_{IR} \times E_F \times E_D]}{[W_{AB} \times T_A]} \tag{3}$$

Where, C_M is the concentration of a heavy metal in vegetable (milligrams per kilogram DW), C_F is conversion factor

(0.085) used to convert fresh crops into dry weight [18], F_{IR} is the ingestion rate (0.240 kg day⁻¹) of vegetables for an adult person living in the study area, E_F is the exposure frequency (365 days year⁻¹), E_D is the exposure duration (70 years for adults), W_{AB} is the average body weight (60 kg for Indian adults) [19] and T_A is the average exposure time for non-carcinogenic effects (ED×365 days year⁻¹).

Hazard quotient (HQ) has been calculated as the ratio between the estimated dose of a contaminant and the dose level below which there will not be any appreciable risk, i.e. the reference oral dose.

$$HQ = \frac{CDI}{RfDo} \tag{4}$$

Where RfDo is the oral reference dose (milligrams per kilogram per day) and is an estimation of the daily exposure to which human population is likely to be exposed without any appreciable risk of deleterious effects during a lifetime [20]. The RfDo values used were 1.0×10⁻³, 4.0×10⁻², 2.0×10⁻², 7.0×10⁻¹, 3.0×10⁻¹, 4.0×10⁻³ and 1.5 mg kg⁻¹ day⁻¹ for Cd, Cu, Ni, Fe, Zn, Pb and Cr, respectively [20]. If HQ exceeds unity, potential non-cancer effects may be a concern [20].

Hazard index (HI) approach is used to assess the overall potential non-carcinogenic effects posed by more than one heavy metal based on the EPA’s Guidelines for Health Risk Assessment of Chemical Mixtures [17]. Hazard index is calculated as the sum of the Hazard quotients due to individual heavy metal, as described in the following equation [21].

$$HI = \sum HQ = \frac{CDI_1}{RfDo_1} + \frac{CDI_2}{RfDo_2} + \dots \dots + \frac{CDI_i}{RfDo_i} \tag{5}$$

Results and Discussion

Heavy Metal Concentration in Vegetables

Being the important constituents of daily human diet, it is essential to ensure the quality of vegetables as they can accumulate such quantities of heavy metals which may cause clinical problems both to animals and human beings after consumption [22]. As compared to fruit and grain crops, heavy metals get easily accumulated in edible portions of vegetables and mainly in leafy vegetables [23]. Leafy vegetables exhibit higher accumulation of heavy metals because they absorb them in their broad leaves with larger surface area and favour accumulation of metals from particles suspended from

contaminated soil or from foliar intake [4, 24]. Higher uptake of heavy metals by spinach (leafy vegetable) has been attributed due to climatic conditions, heavy metals concentrations in atmosphere and consequently increased foliar uptake by deposition [25].

The heavy metals' concentration in different vegetables from the study area is given in Table 2. Among all the studied heavy metals, the mean concentration of Fe ($41.43 \pm 24.70 \text{ mg kg}^{-1}$) was maximum followed by Zn ($8.55 \pm 6.59 \text{ mg kg}^{-1}$), Pb ($1.95 \pm 1.83 \text{ mg kg}^{-1}$), Cu ($1.43 \pm 0.81 \text{ mg kg}^{-1}$), Cd ($0.26 \pm 0.19 \text{ mg kg}^{-1}$) = Co ($0.26 \pm 0.09 \text{ mg kg}^{-1}$), Ni ($0.25 \pm 0.26 \text{ mg kg}^{-1}$) and Cr ($<0.05 \text{ ppm}$) in all the test vegetables. Iron, an essential element for plant growth [26] was found minimum in *B. campestris* leaves (10.35 mg kg^{-1}) and maximum in 89.10 mg kg^{-1} in *B. hispida*. Deficiency of iron in diet results into anaemia while overdose may cause an accumulation of iron in body which in turn may damage liver, heart and pancreas into hemochromatosis patients [27]. A man needs an average daily intake of 7.0 mg of iron and a woman 11.0 mg.

Cobalt and nickel concentration in vegetables varied from <0.05 – 0.41 to 0.03 – 0.98 mg kg^{-1} , respectively, both were minimum in *B. hispida* and maximum in *B. campestris* leaves. Cobalt is an essential element as it is the key constituent of cobalamine (B_{12}) which is required for normal functioning of the nervous system. Deficiency of cobalt in the diet may result into pernicious anaemia, severe fatigue, shortness of breath

and hypothyroidism, while overdose may lead to angina, asthma, cardiomyopathy, polycythemia and dermatitis. The safety limit for human consumption of cobalt is 0.05 to 1.0 mg day^{-1} in humans [28]. The observed cobalt concentrations in the vegetables were in the safety limit in this study. Deficiency of nickel has been linked with hyperglycemias, depression, sinus congestion, fatigue, reproductive failure and growth problems in humans. Hyper-intake of nickel may lead to hypoglycemia, asthma, nausea and headache. The permissible safe limit of Ni is 3.0 to 7.0 mg day^{-1} in man [29].

Zinc and Cu concentrations of all the tested vegetables were in a range of 1.56 – 23.76 mg kg^{-1} and 0.36 – 2.99 mg kg^{-1} , respectively. Maximum accumulation of zinc and copper was in *B. campestris* leaves and minimum in *R. sativus*. The Zn and Cu concentrations were within maximum permissible limit (60 and 40 mg kg^{-1}) and (50 and 30 mg kg^{-1}) as suggested by [30, 31], respectively. Zinc is required to maintain the functioning of the immune system; its deficiency in the diet may be highly detrimental leading to diarrhoea, hair loss, poor wound healing, reduced work capacity of respiratory muscles, immune dysfunction, anorexia and depression. Copper and zinc concentrations reported by [32] were within permissible limits in edible parts of vegetables which were irrigated with different water sources at Sri Ganganagar, Rajasthan.

Cadmium is a non-essential metal and its concentration in the studied vegetable samples varied from 0.02 to 0.67 mg kg^{-1} . Its maximum concentration was 0.67 mg kg^{-1}

Table 2 Heavy metals' concentration (mg kg^{-1}) of different vegetables–soil system of the study area

HM	Statistics	Vegetables				Vegetable fields' soil			
		<i>Brassica campestris</i> leaves	<i>Daucus carota</i>	<i>Raphanus sativus</i>	<i>Benincasa hispida</i>	<i>Brassica campestris</i> leaves	<i>Daucus carota</i>	<i>Raphanus sativus</i>	<i>Benincasa hispida</i>
Fe	Range	30.11–89.10	20.35–82.70	23.10–83.24	10.35–24.89	1,539–3,605	1,917–7,314	1,848–7,645	1,876–3,098
	Mean \pm SD	59.2 \pm 21.37	51.47 \pm 24.39	37.89 \pm 22.07	17.18 \pm 4.53	2,139 \pm 673.08	3,757. \pm 2172	4,068 \pm 2473	2,273 \pm 508.7
Cu	Range	1.42–2.99	0.51–2.63	0.36–1.49	0.56–1.98	18.5–45.54	14.39–48.3	19.85–45.03	23.81–45.03
	Mean \pm SD	2.32 \pm 0.58	1.50 \pm 0.71	0.66 \pm 0.41	1.23 \pm 0.54	30.59 \pm 10.94	30.91 \pm 11.30	27.59 \pm 9.62	34.61 \pm 8.47
Cd	Range	0.10–0.61	0.03–0.67	0.02–0.19	0.23–0.47	0.56–9.04	0.62–8.23	0.84–9.85	3.89–8.86
	Mean \pm SD	0.33 \pm 0.19	0.23 \pm 0.24	0.10 \pm 0.06	0.36 \pm 0.09	6.05 \pm 3.74	3.89 \pm 2.54	5.28 \pm 3.43	5.20 \pm 1.78
Pb	Range	2.34–6.54	0.30–3.65	0.12–0.97	0.23–2.86	50.91–175.47	40.02–58.81	46.7–190.5	43.6–157.32
	Mean \pm SD	4.41 \pm 1.56	1.12 \pm 1.16	0.63 \pm 0.37	1.45 \pm 0.86	96.96 \pm 51.88	50.80 \pm 7.10	76.48 \pm 50.78	74.98 \pm 41.17
Ni	Range	0.08–0.98	0.12–0.94	0.07–0.28	0.03–0.21	3.21–45.87	19.23–32.77	13.46–34.29	12.24–43.32
	Mean \pm SD	0.38 \pm 0.39	0.33 \pm 0.28	0.17 \pm 0.08	0.12 \pm 0.08	31.32 \pm 14.12	25.0 \pm 5.54	24.24 \pm 6.10	24.72 \pm 10.41
Zn	Range	7.58–21.65	3.24–23.76	1.56–5.72	3.65–5.24	67.35–223.9	93.85–153.6	72.65–140.5	78.33–140.8
	Mean	15.06 \pm 5.33	11.0 \pm 7.64	3.71 \pm 1.55	4.43 \pm 0.70	107.17 \pm 55.52	114.27 \pm 23.28	103.75 \pm 24.56	101.77 \pm 23.80
Co	Range	0.24–0.41	<0.05–0.32	<0.05–0.16	<0.05	6.25–14.5	4.54–17.6	2.36–17.2	7.09–18.65
	Mean \pm SD	0.31 \pm 0.05	0.25 \pm 0.07	0.13 \pm 0.03	–	10.61 \pm 6.25	12.89 \pm 4.91	9.46 \pm 4.51	1531 \pm 4.04
Cr	Range	<0.05	<0.05	<0.05	<0.05	2.3–9.4	2.6–8.23	3.89–9.1	3.1–1.82
	Mean \pm SD	–	–	–	–	5.61 \pm 2.43	5.67 \pm 2.11	5.64 \pm 1.74	4.91 \pm 1.82

Table 3 Inter-metal Pearson’s correlation of vegetable field soils

	Fe	Cu	Cd	Pb	Ni	Zn	Co	Cr
Fe	1							
Cu	0.05	1						
Cd	0.192	0.406 ^a	1					
Pb	0.176	0.538 ^b	0.607 ^b	1				
Ni	-0.111	-0.045	-0.132	-0.042	1			
Zn	-0.112	0.147	-0.583 ^b	-0.294	0.382 ^a	1		
Co	-0.425 ^a	0.04	-0.21	-0.169	0.051	0.124	1	
Cr	-0.178	-0.104	0.04	-0.037	0.054	-0.197	0	1

^a Correlation is significant at the 0.05 level (two-tailed)

^b Correlation is significant at the 0.01 level (two-tailed)

in *D. carota* while minimum concentration was 0.02 mg kg⁻¹ in *R. sativus*. An acute dose of cadmium (10–30 mg kg⁻¹ day⁻¹) may lead to severe gastrointestinal irritation, vomiting, diarrhoea and excessive salivation and a dose of 25 mg kg body weight⁻¹ can cause death. All the test vegetables had cadmium concentration within maximum permissible Indian limit of 1.5 mg kg⁻¹. However 57, 43.8 and 57 % of *B. campestris* leaves, *D. carota* and *B. hispida* samples, respectively, exceeded the FAO/WHO permissible limit of 0.3 mg kg⁻¹ for cadmium [30].

Lead concentration in test vegetables varied from 0.12 mg kg⁻¹ in *R. sativus* to 6.54 mg kg⁻¹ in *B. campestris* leaves. Among all the vegetables, *B. campestris* leaves had higher lead content than international and national permissible limits for Pb. Of the *B. campestris* leaf samples, 28 and 85 % had Pb concentration higher than FAO/WHO (5 mg kg⁻¹) and Indian (2.5 mg kg⁻¹) maximum permissible limits [30, 31]. Of *D. carota* and *B. hispida*, 14.28 % samples were also having Pb concentration higher than the Indian safe limit. Lead a serious cumulative body poison finds its way to the body through inhalation and ingestion of water and food and cannot be removed by washing fruits and vegetables [8].

It is evident from the results that quantitative distribution of heavy metals in different types of vegetables was different. Differences in degrees of accumulation of heavy metals in different vegetable species and medicinal plants have previously also been reported [25, 33]. The differences in degree of accumulation of heavy metals by individual vegetables

species may be attributed to their different uptake efficiencies for heavy metals from soil solution and intakes rate from the foliage [25]. Uptake of heavy metals from the soil is influenced by physico-chemical properties of soil (pH, organic matter, nutrient status, soil texture, moisture, etc.) in the agricultural field, age and part of the bioavailable concentration and nature of metallic species [8]. Atmospheric deposition and foliar intake of heavy metals are controlled by environmental factors such as temperature, moisture and wind velocity and nature of the vegetables, i.e. leafy, root, fruit, exposed surface area, hairy or smoothness of the exposed parts [34].

Heavy Metal Concentration of Vegetable Fields’ Soils

Heavy metal concentrations of different vegetable fields’ soils varied considerably and are given in Table 2. The heavy metals concentration in the soil were in the following order: Fe (3,059.82±1,826.63 mg kg⁻¹) > Zn (106.74±33.0 mg kg⁻¹) > Pb (74.81±42.55 mg kg⁻¹) > Cu (30.92±9.89 mg kg⁻¹) > Ni (26.32±9.60 mg kg⁻¹) > Co (12.07±4.58 mg kg⁻¹) > (5.48±1.95 mg kg⁻¹) > Cd (5.11±2.92 mg kg⁻¹). With reference to Indian standards [31], mean heavy metal concentrations of vegetable fields’ soils were well within permissible limits for Cu (135–270 mg kg⁻¹), Cd (3–6 mg kg⁻¹), Pb (250–500 mg kg⁻¹), Ni (75–150 mg kg⁻¹) and Zn (300–600 mg kg⁻¹). Higher iron content in the fields’ soils may be attributed to its relative abundance in the earth’s crust while that of Zn might be due to application of Zn fertilizers.

Inter-metal Correlation, CA and PCA

Inter-metal correlation technique was applied to soil heavy metal results to explore any association among the heavy metals in vegetable fields’ soils (Table 3). Copper was found positively and significantly correlated with Cd ($r=0.406^*$; $P<0.05$) and Pb ($r=0.538^{**}$; $P<0.01$). Cadmium showed a higher degree of positive correlation with Pb ($r=0.607^{**}$; $P<0.01$), but correlated negatively with Zn ($r=-0.583^{**}$; $P<0.01$). Among all the heavy metals, Zn was having positive correlation only with Ni ($r=0.382^*$; $P<0.01$). Results of the present study revealed that Fe was most abundant among the heavy metals in the vegetable soil system. In spite of its abundance, Fe did not show association with other metals as

Fig. 2 Dendrogram of selected heavy metals in vegetable fields’ soil using complete linkage method

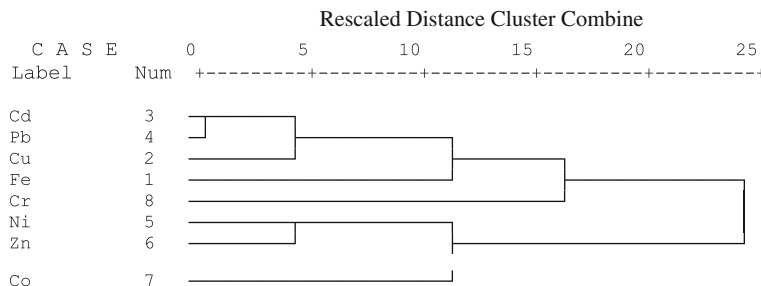


Table 4 Factor loading for selected heavy metals of vegetable fields' soils

Heavy metals	Factor 1	Factor 2	Factor 3	Factor 4
Fe	0.422	-0.115	-0.723	0.047
Cu	<i>0.492</i>	<i>0.727</i>	0.144	-0.087
Cd	<i>0.867</i>	0.024	0.21	0.086
Pb	<i>0.786</i>	0.362	0.131	0.106
Ni	-0.329	<i>0.430</i>	-0.044	0.703
Zn	-0.617	<i>0.613</i>	-0.298	0.081
Co	-0.396	0.251	0.614	-0.387
Cr	-0.012	-0.377	0.514	0.599
Eigen value	2.435	1.439	1.337	1.038
% of variance	30.432	17.984	16.714	12.969
Cumulative %	30.432	48.416	65.13	78.099

Values of dominant heavy metals in each factor are in italics

no significant positive correlations of the metal were observed with other metals (Table 3). Results of the correlation matrix suggesting formation of some primary cluster pairs which were further supported by cluster analysis dendrogram. Three primary clusters found were Cu–Cd, Cd–Pb and Ni–Zn in vegetable fields' soils (Fig. 2).

PCA, a multivariate statistical technique, was applied to analyze the inter-dependencies within soil heavy metals and for their qualitative evaluation of clustering behaviour, and the results are given in Table 4. Four factors having a total variance of 78.009 % were obtained. Factor-1 contributed 30.432 % to the total variance with a high loading on Cd ($r=0.867$), Pb ($r=0.786$) and Cu ($r=0.492$). Thus, factor-1 supported purely two primary cluster pairs, i.e. Cd–Pb and Cu–Cd. Factor-2 contributed 17.984 % to the total variance with a high loading on Zn ($r=0.613$) and Cu ($r=0.727$) and Ni ($r=0.430$), supporting Ni–Zn cluster pair.

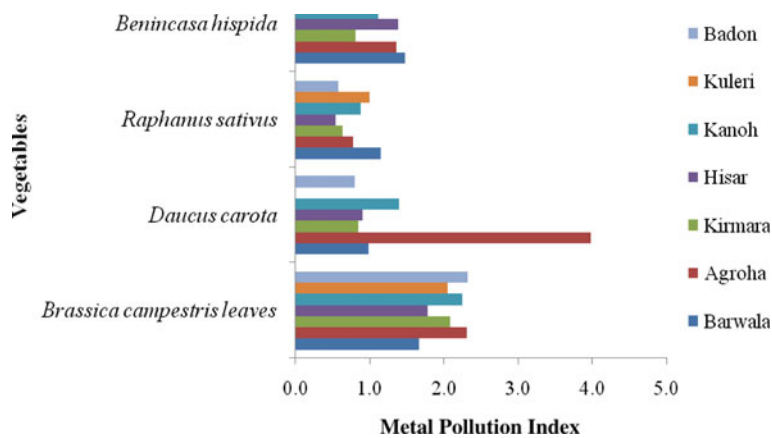
Bioconcentration Factor of Heavy Metals from Vegetable Fields' Soils to Edible Portions of Vegetables

Bioconcentration is the accumulation of a contaminant in a living system including flora or fauna from the ambient environment. Traditionally, this term is used to study the toxicity in aquatic organisms from water, but in recent years, this term has been adapted to express the accumulation of soil contaminants in food crops and expressed as BCF. BCF value of a heavy metal depends upon soil characteristics, metal chemistry and also on the plant species. BCF values of the studied heavy metals in different vegetables were calculated (on dry

Table 5 Bioconcentration factor of heavy metals in test vegetables from the study area

Heavy metals	Statistics	Transfer factor			
		<i>Brassica campestris</i> leaves	<i>Daucus carota</i>	<i>Raphanus sativus</i>	<i>Benincasa hispida</i>
Fe	Range	0.014–0.045	0.007–0.039	0.004–0.045	0.004–0.013
	Mean	0.029	0.016	0.013	0.008
	SD	0.011	0.012	0.014	0.003
Cu	Range	0.054–0.148	0.022–0.091	0.011–0.070	0.014–0.068
	Mean	0.083	0.051	0.027	0.037
	SD	0.033	0.023	0.021	0.017
Cd	Range	0.031–0.179	0.006–0.132	0.013–0.036	0.028–0.121
	Mean	0.083	0.064	0.021	0.077
	SD	0.058	0.045	0.009	0.031
Pb	Range	0.034–0.080	0.005–0.062	0.000–0.019	0.002–0.036
	Mean	0.051	0.021	0.009	0.022
	SD	0.017	0.019	0.008	0.011
Ni	Range	0.003–0.034	0.006–0.029	0.003–0.011	0.001–0.013
	Mean	0.014	0.012	0.007	0.005
	SD	0.012	0.008	0.003	0.004
Zn	Range	0.078–0.286	0.024–0.197	0.015–0.058	0.026–0.064
	Mean	0.158	0.101	0.037	0.046
	SD	0.075	0.070	0.016	0.014
Co	Range	0.017–0.051	BDL–0.023	BDL–0.019	–
	Mean	0.032	0.018	0.015	–
	SD	0.012	0.004	0.004	–
Cr	Range	–	–	–	–

Fig. 3 Metal pollution index of different vegetables from sampling sites



weight basis) using Eq. 1 and are given in Table 5. In all the test vegetables, mean BCF of Zn (0.086±0.07) was highest and that of Ni was lowest (0.010±0.01). The order of BCF of iron and nickel in different vegetables was *B. campestris* leaves > *D. carota* > *R. sativus* > *B. hispida* (Table 5). *B. campestris* leaves had highest BCF for all the heavy metals while *R. sativus* had lowest BCF for Cu, Cd, Pb and Zn (Table 5). The mean bioconcentration factors values of heavy metals in vegetables in the study area followed the following uptake order: Zn (0.086±0.07) > Cd (0.061±0.02) > Cu (0.049±0.03) > Pb (0.026±0.02) > Co (0.024±0.01) > Fe (0.017±0.01) > Ni (0.010±0.01), respectively. As the Cr concentrations were BDL (<0.05 mg kg⁻¹) in vegetable samples, therefore, BCF values for the metal could not be

calculated. Higher BCF for Zn have also been reported [35] in vegetables and fish samples in Tianjin, China.

MPI and Health Risk Assessment

To evaluate whether the studied agricultural fields have heavy metals contamination or not, the MPI was calculated using Eq. 2. The MPI provides information about overall contamination at a site. The MPI for the vegetables were calculated, and the results are presented in Fig. 3. Among the different vegetables, higher MPI values were exhibited by leafy vegetable, i.e. *B. campestris* leaves and root vegetable, i.e. *D. carota*. Another root vegetable, i.e. *R. sativus* had least

Table 6 Chronic daily intake (mg kg⁻¹ day⁻¹) and hazard quotient of heavy metals via ingestion of vegetables to target population

Heavy metals	CDI/HQ	Vegetables			
		<i>Brassica campestris</i> leaves Mean ± SD	<i>Daucus carota</i> Mean ± SD	<i>Raphanus sativus</i> Mean ± SD	<i>Benincasa hispida</i> Mean ± SD
Fe	CDI	2.01E-02±7.27E-03	1.75E-02±8.29E-03	1.29E-02±7.50E-03	5.84E-03±1.54E-03
	HQ	2.88E-02±1.04E-02	2.50E-02±1.18E-02	1.84E-02±1.07E-02	8.34E-03±2.20E-03
Cu	CDI	7.88E-04±1.99E-04	5.10E-04±2.43E-04	2.23E-04±1.40E-04	4.18E-04±1.85E-04
	HQ	1.97E-02±4.97E-03	1.27E-02±6.07E-03	5.59E-03±3.49E-03	1.05E-02±4.63E-03
Cd	CDI	1.13E-04±6.57E-05	7.72E-05±8.10E-05	3.40E-05±2.03E-05	1.24E-04±3.15E-05
	HQ	1.13E-01±6.57E-02	7.72E-02±8.10E-02	3.40E-02±2.03E-02	1.24E-01±3.15E-02
Pb	CDI	1.50E-03±5.31E-04	3.81E-04±3.95E-04	1.84E-04±1.39E-04	4.93E-04±2.92E-04
	HQ	4.29E-01±1.52E-01	1.09E-01±1.13E-01	5.25E-02±3.98E-02	1.41E-01±8.36E-02
Ni	CDI	1.29E-04±1.31E-04	1.14E-04±9.58E-05	5.78E-05±2.55E-05	3.98E-05±2.62E-05
	HQ	6.44E-03±6.55E-03	5.68E-03±4.79E-03	2.89E-03±1.28E-03	1.99E-03±1.31E-03
Zn	CDI	5.12E-03±1.88E-03	3.74E-03±2.60E-03	1.26E-03±5.27E-04	1.51E-03±2.38E-04
	HQ	1.71E-02±6.27E-03	1.25E-02±8.66E-03	4.21E-03±1.76E-03	5.03E-03±7.92E-04
Co	CDI	1.06E-04±1.76E-05	8.50E-05±2.24E-05	4.31E-05±1.04E-05	<1.7E-05
	HQ	–	–	–	–
Cr	CDI	<1.70E-05	<1.70E-05	<1.70E-05	<1.70E-05
	HQ	<1.13E-0	<1.13E-05	<1.13E-05	<1.13E-05

MPI values indicating lowest accumulation of heavy metals and lesser health risks (Fig. 3).

The main routes of heavy metal exposure to human body are oral, dermal and nasal, but oral being the most important [36]. As the vegetables are important components of the human diet, therefore, health risks due to CDI of heavy metals via ingestion of vegetables to the target population were determined. Chronic daily intake of heavy metals via ingestion of different tested vegetables is given in Table 5. CDI of Fe was highest through the ingestion of all the tested vegetables from the study area and ranged from $1.29\text{E-}02 \pm 7.50\text{E-}03$ to $5.84\text{E-}03 \pm 1.54\text{E-}03 \text{ mg kg}^{-1} \text{ day}^{-1}$. Among the vegetables, chronic daily intake of cadmium was lowest via ingestion of *B. campestris* leaves ($1.13\text{E-}04 \pm 6.57\text{E-}05 \text{ mg kg}^{-1} \text{ day}^{-1}$), *D. carota* ($7.72\text{E-}05 \pm 8.10\text{E-}05 \text{ mg kg}^{-1} \text{ day}^{-1}$), *R. sativus* ($3.40\text{E-}05 \pm 2.03\text{E-}05 \text{ mg kg}^{-1} \text{ day}^{-1}$) and that of Ni through *B. hispida* ($3.98\text{E-}05 \pm 2.62\text{E-}05 \text{ mg kg}^{-1} \text{ day}^{-1}$) ingestion.

HQ index is used to assess non-carcinogenic health risks associated with the ingestion of individual heavy metal via dietary intake of vegetables, and results are encapsulated in Table 6. Mean HQ values of different heavy metals occupied following decreasing order for non-carcinogenic risks: Pb (0.183) > Cd (0.087) > Fe (0.020) > Cu (0.012) > Zn (0.010) > Ni (0.004). The results of the present study revealed that daily intake of heavy metals through the ingestion of vegetables is unlikely to pose health risks to the target population as HI for all the studied heavy metals is less than unity and ranged from 0.077 of *R. sativus* from Hisar site to 0.846 of *B. campestris* leaves from Kirmara site. Much higher MPI (7.50–11.82) and HI (>1) values have been reported for different vegetables grown Indian cities [37]. Hazard quotient for Cd (1.05–8.54) and Ni (1.17–3.89) exceeded the safe limit for the vegetables grown in the vicinity of thermal power plants at Delhi, India, indicating non-carcinogenic health risks associated with their ingestion [25]. Health risk assessment for the vegetables grown in Northern Pakistan revealed potential health risk to the consumers as HQ for cadmium was reported greater than unity [38].

Conclusion

This study concludes that edible parts of leafy vegetables can accumulate higher concentrations of heavy metals as compared to the root and fruit vegetables. Significant differences in heavy metal concentration were reported in the soil–vegetable system that varied with different metal species and vegetable types and may be due to differences in uptake capabilities and their further translocation to the edible portion of the plants. The test vegetables from the study area are safe for human consumption as HRI of all the vegetables was less than unity. However, other sources of metal exposure like dermal, nasal and ingestion of metal-contaminated soils were

not taken into account in the present study. Therefore, further detailed studies are required to completely understand the problem and risks involved.

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Conflict of Interest The authors declare that there are no conflicts of interest.

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