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# BIO-ACCUMULATION OF HEAVY METALS IN SOME NORTH INDIAN LEAFY VEGETABLES AND QUANTIFICATION OF CONSUMER HEALTH RISK DUE TO THEIR DIETARY INTAKE

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**ABSTRACT**

**In this study bio-accumulated levels of Cd, Cr, Cu, Ni and Pb in seven most popular North Indian leafy vegetables, spinach (*Spanacia oleracea*), bathua (*Chenopodium album*), chaulayi (*Amranths pinosus)*, mustard (*Brassica nigra*), rayi (*Brassica juncea)*, radish leaves (*Raphanus sativus)* and fenugreek (*Trigonella foenum-graecum)* collected from local venders were evaluated. The daily intakes of metals (DIM) through consumption of these leafy vegetables were computed for tested metals to quantify consumer health risk index (HRI). For the purpose of comparison, vegetables cultivated under ideal conditions in a walled garden were also tested for studied metals. The order and accumulated levels of metals in leafy vegetables varied as: Pb (2.16-3.16) > Cr (1.54-2.84) > Cu (0.39- 0.94) > Ni (0.38-0.78) > Cd (0.33-0.51). However, the accumulated levels of tested metals in vegetables cultivated in the walled garden were much lower and varied as: Pb (1.11-1.46) > Cr (0.66-1.22) > Cu (0.21- 0.42) > Ni (0.22-0.33) > Cd (0.19- 0.29). The order of computed consumers health risk index (HRI) due to tested metals on dietary intake of studied vegetables collected from local venders varied as: Pb (229.5 – 335.7) > Cd (140.2 – 216.7) > Ni (8.74- 14.73) > Cu (4.49- 10.82) > Cr (0.473- 0.872).**

**KEY WORDS:** Heavy metals in Leafy Vegetables, Dietary intake of metals, Consumer health risk

## INTRODUCTION

Industrial activities, mining cum metallurgical operations and finishing of metals and metal products have increased the incidences of percolation of toxic metals to the soil and water bodies (Singh, 2005). The non-biodegradable characteristic of heavy metals makes them persistent for long in soil and water. Long term waste water irrigation of crops may lead to the accumulation of heavy metals in agricultural soils. (Sharma *et al*., 2007; Singh *et al*., 2010). Due to the in-built bio-accumulation mechanism root system of the plants usually absorb the bio-available fractions of the nutrients and heavy metals in varying proportions from the farm soil. Once entered in the plant root cells, these are accumulated in plant tissues of tubers and also translocated to the above ground parts of vegetables (Agrawal *et al.,* 2007). Usually, the observed bio-accumulated order of both essential and non-essential heavy metals in vegetables was as: *leafy vegetables >tubers >fruity vegetables* (Pandey and Pandey, 2009; Luo *et al*., 2011; Singh *et al*., 2012). Many plants are known to selectively accumulate higher levels of metals than concerned plant's biological requirement and their bio-availability levels in the soil (Singh, 2005).

Varieties of fresh fruits and vegetables are integral constituent of balanced diet and fulfill the human requirements of essential micro-nutrients, vitamins, fiber, poly-phenols, sugars and antioxidants (Agrawal *et al*., 2007; Ametepey *et al*., 2018). However, vegetables grown on heavy metal contaminated soil accumulate heavy metals in varying proportions, sometimes much higher than their safe limits. Heavy metals even in low concentration can be harmful to human body (Singh *et al*., 2010). Prolong intake of metal contaminated vegetables may lead to the deposition of heavy metals in the human tissues and sensitive internal organs (Tripathi *et al*., 1997). When, the accumulated levels of heavy metals in human body exceed the recommended permissible limits for human, it may affect the functioning of vital organs like, kidneys, bones, liver and blood (Agrawal *et al.,* 2007; Singh *et al.*, 2010). Since, dietary consumption of vegetables being the main route of human exposure of heavy metals, to evaluate the quantum of human health risk through dietary intake of vegetables, has gained importance (Tripathi *et al*., 1997; Zhuang *et al*., 2009; Singh *et al.*, 2010).

This study was undertaken to quantify the health risk due to dietary intake of seven leafy vegetables most consumed in North India, in terms of consumer health risk index (HRI) by computing dietary intake of metals (DIM) (Chary *et al*., 2008). DIM was evaluated (FAO/ WHO Tech. Report, 1989, 1993; Rattan *et al*., 2005) by estimating the levels of bio-accumulated Cd, Pb, Cr, Ni and Cu in studied vegetables collected from local venders. For comparison purpose, leafy vegetables cultivated in a walled garden were also tested to find the levels of bio-accumulated metals in the studied vegetables. In this walled garden, crops are grown under ideal conditions, without using chemical fertilizers, waste water and street runoff. The description and dietary importance of the leafy vegetables selected for this study are presented in Table 1.

## MATERIALS & METHODS

### Sample Collection

The samples of selected leafy vegetables were collected during July 2015 to March 2016 from five different venders of Dehradun City area. Farmers of Doiwala, Premnagar, Biharigarh and Chutmalpur fulfill the vegetable requirement of Dehradun City. These places are sub-urban settlements devoid of proper sewage and waste water disposal facilities. Farmers of the area mostly use dug wells, hand pumps and domestic cum other waste water to irrigate the vegetable crops (Kulshrestha, 2021). For comparison purpose, samples of leafy vegetables were also collected from a walled garden where vegetable crops are grown under ideal conditions for last two decades using tube well water besides open sky rain water, without chemical fertilizers. The young, soft and fresh samples of *spinach, bathua, chaulayi, mustard, rayi, radish leaves* and *fenugreek* were collected much before the flowering stage. After discarding damaged and decayed leaves, samples were cleaned 2-3 times with tap water and then finally with distilled water to remove dust, mud and soil particles. The cleaned samples were spread over filter paper sheets separately, at room temperature in a dust free chamber for few days to make samples moisture free. The air dried samples were cut into small pieces and oven dried for 2-3 days at $80^0$C to a constant weight. The dried samples were grinded to powder and kept in labeled sample bottles (Kulshrestha, 2021).

### Estimation of accumulated metals in leafy vegetable samples

To determine the accumulated metals in vegetables, grinded samples (1.0 g) were subjected to $HNO_3$ –

**Table 1.** Selected Leafy Vegetables and their dietary importance

| Scientific Name, Family | Common Name | Dietary importance |
|---|---|---|
| *Spanacia oleracea, Amaranthaceae* | Spinach | Fresh leaves are rich in flavonoids, anti-oxidants, vitamins and poly-phenolics. It regulates blood pressure. |
| *Chenopodium album,* Amaranthaceae | Bathua | Rich in vitamin A and fibers. It is laxative and helps in helminthiasis, peptic ulcer and improves hemoglobin. |
| *Amranthspinosus L., Amaranthaceae* | Chaulayi | Store house of phyto-nutrients, vitamins A, C, fibers and antioxidants. It is anti-cancer and immunity booster. |
| *Brassica nigra, Brassicaceae* | Mustard (Sarsno) | Rich in folic acid, antioxidants, vitamin A and C. Good for eyes, heart, asthma and migraine. It lowers BP. |
| *Brassica juncea Brassicaceae* | Rayi | Rich in vitamins C and E, carotenoids and anti-oxidants. It has antimicrobial and anticancer characteristics. |
| *Raphanus sativus, Cruciferae* | Radish Leaves | Rich in dietary fibers and antioxidants. Strengthens immune system. It is diuretic, digestive and laxative |
| *Trigonella foenum-graecum L., Fabaceae* | Fenugreek | Improves liver functioning, lowers blood sugar, excellent source of antioxidants and dietary fibers. |

$HClO_4$ (5:1, v/v) digestion at nearly $80^0C$ for several hours following the standard methods (APHA, 2005) to get a transparent light colored liquid, which was then filtered in 100 mL volumetric flasks and filled up to the mark with double distilled water. The concentration (mg/kg, dw) of Cd, Cr, Cu, Ni and Pb in vegetable samples were determined on Atomic Absorption Spectrometer, *Analyst 200* (Perkin Elmer), using air-acetylene flame, in accordance to standard methods (APHA, 2005). The standard stock solutions of metal ions used were from *Sigma Aldrich*, which were diluted to required concentrations for preparing working standards. Other reagents, chemicals, and solvents used were of analytical grade (Kulshrestha *et al.*, 2012). Doubly-distilled water was used for all purposes.

## RESULTS & DISCUSSION

The observed levels of accumulated Cd, Cr, Cu, Ni and Pb, mg/Kg dry weight of leafy vegetables collected from local market, namely *Spinach, Bathua, Chaulayi, Mustard, Rayi, Radish leaves* and *Fenugreek* are presented in Table-2. The levels of tested metals in samples of leafy vegetables collected from walled garden are given in parenthesis.

### Bio-accumulated levels of studied metals in leafy vegetables

Root system of the plants bio-accumulates certain useful nutrients along with other metals from farm soil which depends upon their availability in soil system near the plant roots, characteristics of farm soil and plant age (Singh *et al.*, 2012). The metal bio-accumulation potential of plants differs widely (Singh *et al*., 2010). The prolonged consumption of foodstuff with unsafe concentrations of heavy metals may lead the accumulation of heavy metals in the kidney and liver of human beings leading to cardiovascular, bone, kidney and CNS diseases (Ametepey *et al.*, 2018). Leaf vegetables generally grow faster due to higher transpiration rate than non-leaf vegetables (Luo *et al*., 2011). Thus, metal uptake by plant roots is higher in leaf vegetables which also results in the efficient translocation of metals from roots to above ground parts (Zheng *et al*., 2007). The leafy vegetables have great tendency to accumulate higher concentration of heavy metals that might be linked with the high absorption rates of leafy vegetables bearing a large surface area (Chary *et al*., 2008). Due to the broad leaf area, leaf vegetables are more susceptible to accumulate pollutants through leaf surface out of the road dust, soil and rainwater (Chang *et al*., 2014).

In all the studied leafy vegetables collected from local venders, the levels of all tested metals were found below the recommended safe limits (Awasthi, 2000; WHO, 2007). However, all the vegetables under study differ much in their ability to bio-accumulate metals from soil. Thus, the order of bio-accumulated level of Cadmium in studied vegetables (Table-2, Fig.1) was: *Chaulayi* (0.51±0.020) > *Rayi* (0.49±0.018) > *Spinach* (0.47± 0.018) > *Mustard* (0.46±0.020) >*Radish* (0.44±0.018) >*Bathua*

**Table 2.** Bio-accumulated levels (mg/ Kg, dw) of Cd, Cr, Cu, Ni and Pb found in Leafy Vegetable Samples (n = 5) collected from local venders and walled garden

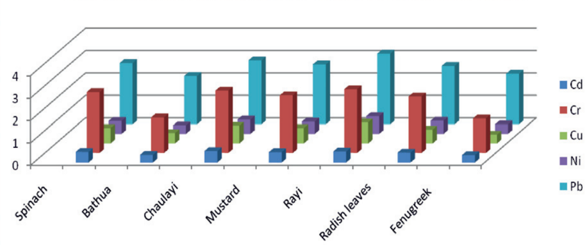
| Leafy Vegetables | Cadmium | Chromium | Copper | Nickel | Lead |
|---|---|---|---|---|---|
| Spinach | 0.47±0.018<br>(0.26± 0.014) | 2.71±0.032<br>(1.09±0.019) | 0.68±0.024<br>(0.42±0.020) | 0.58±0.016<br>(0.31±0.014) | 2.74±0.033<br>(1.26±0.024) |
| Bathua | 0.34±0.016<br>(0.22± 0.013) | 1.58±0.027<br>(0.71±0.023) | 0.46±0.016<br>(0.27±0.016) | 0.38±0.015<br>(0.24±0.011) | 2.16±0.028<br>(1.17±0.021) |
| Chaulayi | 0.51±0.020<br>(0.29± 0.016) | 2.78±0.038<br>(1.21±0.021) | 0.79±0.026<br>(0.34±0.018) | 0.64±0.016<br>(0.29±0.013) | 2.86±0.030<br>(1.31±0.024) |
| Mustard | 0.46±0.020<br>(0.24± 0.015) | 2.57±0.034<br>(1.06±0.018) | 0.68±0.023<br>(0.31±0.017) | 0.55±0.018<br>(0.24±0.013) | 2.68±0.028<br>(1.34±0.024) |
| Rayi | 0.49±0.018<br>(0.28± 0.017) | 2.84±0.035<br>(1.22±0.018) | 0.94±0.021<br>(0.37±0.018) | 0.79±0.026<br>(0.33±0.014) | 3.16±0.040<br>(1.46±0.021) |
| Radish leaves | 0.44±0.018<br>(0.23± 0.015) | 2.52±0.028<br>(0.98±0.023) | 0.61±0.018<br>(0.26±0.014) | 0.59±0.018<br>(0.27±0.014) | 2.61±0.028<br>(1.27±0.023) |
| Fenugreek | 0.33±0.014<br>(0.19± 0.015) | 1.54±0.030<br>(0.66±0.021) | 0.39±0.014<br>(0.21±0.013) | 0.43±0.014<br>(0.22±0.012) | 2.26±0.024<br>(1.11±0.019) |
| Safe limits (Awasthi, 2000; WHO, 2007) | (1.5) | (5.0) | (30) | (1.5) | (2.5) |

(0.34±0.016) >*Fenugreek* (0.33±0.014, mg/Kg, d.w.). The order of accumulated level of Chromium was: *Rayi* (2.84±0.035) > *Chaulayi* (2.78±0.038) > *Spinach* (2.71±0.032) > *Mustard* (2.57±0.034) > *Radish* (2.52±0.028) > *Bathua* (1.58±0.027) > Fenugreek (1.54±0.030 mg/Kg, d.w.). The order of accumulated Copper was: *Rayi* (0.94±0.021) > *Chaulayi* (0.79±0.026) > *Spinach* (0.68±0.024) > *Mustard* (0.68±0.023) >*Radish* (0.61±0.018) > *Bathua* (0.46±0.016) > Fenugreek (0.39±0.014 mg/Kg, d. w.). The order of accumulated Nickel in studied vegetables was: *Rayi* (0.79±0.026) > *Chaulayi* (0.64±0.016) > *Radish* (0.59±0.018) > *Spinach* (0.58±0.016) > *Mustard* (0.55±0.018) >*Fenugreek* (0.43±0.014) > *Bathua* (0.38±0.015 mg/Kg, d.w.). The order of Lead in vegetables was: *Rayi* (3.16±0.040) > *Chaulayi* (2.86±0.030) > *Spinach* (2.74±0.033) > *Mustard* (2.68±0.028) > *Radish* (2.61±0.028) >*Fenugreek* (2.26±0.024) > *Bathua* (2.16±0.028mg/Kg, d. w.). Among all the heavy metals, Lead has shown highest and Cadmium recorded lowest accumulation in studied leafy vegetables (Table 2). The overall range of studied metals in vegetables collected from local venders varied as: Cd from 0.33 to 0.51, Cr from 1.54 to 2.84, Cu from 0.39 to 0.94, Ni from 0.38 to 0.79 and Pb varied from 2.16 to 3.16 mg /Kg, dry weight. The pattern of metals accumulated in studied vegetables was: Rayi ~ *Chaulayi* > *Spinach* > *Mustard* ~*Radish* > *Bathua* > *Fenugreek.* This observed pattern can be related roughly to the leaf area of vegetables (Chang *et al*., 2014) as first three vegetables (*Rayi*, *Chaulayi* and *Spinach*) have broad leaf area, followed by mustard and radish leaves, while, Bathua and Fenugreek have smallest leaves.

The wide variations in accumulated levels of heavy metals were observed among the studied vegetables which reflect the differences in their uptake capabilities and their translocation to edible portion of the plants (Singh *et al.*, 2010). The order of observed levels of studied metals in *Spinach* (Table 2, Fig.1) was: Cd (0.47±0.018) < Ni (0.58±0.016) < Cu (0.68±0.024) < Cr (2.71±0.032) < Pb (2.74±0.033); in *Bathua: Cd* (0.34±0.016) <*Ni* (0.38±0.015) < Cu (0.46±0.016) < Cr (1.58± 0.027) < (2.16±0.028); in *Chaulayi, Cd* (0.51±0.020) < Ni (0.64±0.016) < Cu (0.79±0.026) < Cr (2.78±0.038) < Pb (2.86±0.030); in *Mustard,* Cd ((0.46±0.020) < Ni (0.55±0.018) < Cu (0.68±0.023) < Cr (2.57± 0.034) < Pb (2.68±0.028); in *Rayi*, Cd (0.49±0.018) < Ni (0.79±0.026) < Cu (0.94±0.021) < (2.84±0.035) < Pb (3.16±0.040); in *Radish leaves*, Cd (0.44±0.018) < Ni (0.59±0.018) < Cu (0.61±0.018) < Cr (2.52±0.028) < (2.61±0.028) and in *Fenugreek*, Cd (0.33±0.014) < Cu (0.39±0.014) < Ni (0.43±0.014), Cr (1.54±0.030) < Pb (2.26±0.024). These results were lower or comparable with earlier findings carried out on leafy vegetables grown on North Indian soil e.g. Cd and Cu in *Spinach* and *Radish leaves* (Singh *et al*., 2010); Cu and Pb in *Mustard*, *Spinach*, *Radish leaves and Fenugreek* (Singh *et al.*, 2012); Ni and Pb in *Spinach* (Tamrakar and Richhariya, 2012). On the other hand, the observed Cr and Pb in *spinach* were much higher than reported earlier (Singh *et al*., 2010). However, the reported results of Cd in *Spanich* (Tamrakar and Richhariya, 2012); Cd, Cu, Ni and Pb in *Spinach* (Yadav *et al*., 2013); Cd in *Spinach* and *Fenugreek* (Sharma *et al*., 2016) and Cd, Cu, Cr and Pb in *Bathua* (Ramteke *et al*., 2016) were much higher than reported in this study.

The studied metals in vegetable samples grown in walled garden were much lower than found in samples collected from local venders, which varied as: Cd from 0.19 to 0.29, Cr from 0.66 to 1.22, Cu from 0.21 to 0.42, Ni from 0.22 to 0.33 and Pb varied from 1.11 to 1.46 mg /Kg, dry weight (Table 2, Fig.2). Such a lower levels of studied heavy metals in tested vegetables grown in walled garden may be related to the adoption of good agricultural practices for last two decades, which includes no use of chemical fertilizers and no entry of waste water, street runoff and even domestic water in walled garden. These results are comparable with earlier results (Kumar *et al*., 2017) reported in leafy vegetables from ground water irrigated zone.

**Fig.1 Levels of tested metals in Leafy Vegetables collected from Venders**



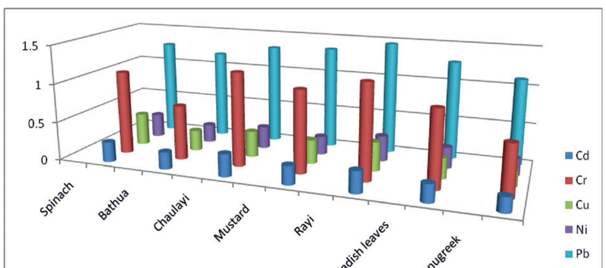




**Fig.2 Levels of tested metals in Leafy Vegetables collected from Walled Garden**

**Quantification of consumer health risk due to dietary intake of leafy vegetables**

Application of chemical fertilizers and regular use of waste water of diverse sources for long to irrigate crops may lead to higher levels of heavy metals in agri-soil (Agrawal *et al.,* 2007). As a consequence, the chances of accumulation of these metals in roots, tubers and other edible parts of the vegetables increase (Singh *et al.,* 2010). Through regular dietary intake of heavy metal contaminated vegetables for long, these metals ultimately get deposited in various human organs causing serious health problems to the inhabitants (Zhuang *et al*., 2009). In this study the consumer's health risk on regular intake of seven leafy vegetables most popular in North India, was quantified in terms of health risk index (HRI) (US -EPA. Tech. Report, 1989).

**Assessment of consumer health risk index (HRI)**

The human health risk in terms of health risk index (HRI) (US -EPA Tech. Report, 1989) was computed as the ratio of the daily intake of metals (DIM) due to long term dietary consumption of metal contaminated vegetables under study and oral reference dose (Cui *et al*., 2004, US –EPA Tech. Report, 2013) by using the following relation (1):
HRI = Daily Intake of Metals (DIM) / Reference Oral Dose ($R_f$D) …………….. (1)

**(a) Computation of daily intake of metals (DIM)**

The daily intake of metals (DIM) on taking concerned vegetable as dietary component was evaluated by putting appropriate values in following relation (Chary *et al*., 2008).

$$DIM = C_{Metal} \times C_{Factor} \times D_{Food\ Intake} / B_{Average\ Body\ Weight} \quad \ldots\ldots (2)$$

In relation (2), $\mathbf{C}_{Metal}$ signifies the metal concentration (mg/Kg, dw) in edible parts of vegetable plant, $\mathbf{C}_{Factor}$ is conversion factor (0.085) used for conversion of fresh weight of vegetable to dry weight (Rattan *et al*., 2005). $\mathbf{D}_{Food\ Intake}$ is the average daily intake of vegetables; the suggested value in WHO guidelines for human adult diet is 300 to 350g per day per person (FAO/ WHO, Tech. Report, 1989) and $\mathbf{B}_{Average\ Body\ Weight}$ is the average body weight of an adult consumer, the suggested value is 60kg (FAO/ WHO Tech. Report, 2013). In this study, the average daily intake of vegetables was taken as 300 g per day per adult consumer, as minimum vegetable requirement of 100 g vegetables for 3 times, a balanced diet recommended for Indians (Dietary Guidelines for Indians, 2011). The computed values of DIM are presented in Table 3.

The daily intake of metals (DIM), through consumption of metal contaminated vegetables depends upon the properties of soil, level of the concerned metal in soil, its bioavailability and vegetable species (Kumar *et al*., 2017). Among all studied leafy vegetables, the highest daily intake of tested metals (Table 3) was recorded in *Rayi* and lowest in *Bathua* and the overall order was*: Rayi* (3.4934) > *Chaulayi* (3.2214) > *Spinach* (3.0512) > *Mustard* (2.9492) > *Radish leaves* (2.8070) > *Fenugreek* (2.1040) > *Bathua* (2.0905). The daily intake of metals due to *Cadmium* in studied leafy vegetables varied from 0.1402 to 0.2167; due to *Chromium* from 0.6545 to 1.207, due to *Copper* from 0.1658 to 0.3995, due to *Nickel* from 0.1615 to 0.3357 and due to *Lead* varied from 0.9180 to 1.343 mg/Kg /day/person.

**(b) Computation of health risk index (HRI) based on daily intake of metals (DIM)**

The Reference Oral Dose ($R_f$D) of the metal (mg/kg/day) (US –EPA, 1997; 2010; 2013; Jan, 2010) is an approximation of daily tolerable exposure to which a person is expected to have without any significant risk of harmful effects during the life span. The oral reference dose for *Cadmium, Lead, Copper* and *Chromium* was 0.001, 0.004, 0.04 and 1.5 mg/kg/day, respectively (US –EPA, 1997; US-EPA IRIS, 2006) and the $R_f$D for *Nickel* was 0.02 mg/Kg/day

**Table 3.** Daily intake of metals (DIM, mg/Kg/day/person) through consumption of studied leafy vegetables collected from local venders

| Vegetables/ Metals | Cd | Cr | Cu | Ni | Pb | Total |
|---|---|---|---|---|---|---|
| Spinach | 0.1997 | 1.152 | 0.2890 | 0.2465 | 1.164 | 3.0512 |
| Bathua | 0.1445 | 0.671 | 0.1955 | 0.1615 | 0.9180 | 2.0905 |
| Chaulayi | 0.2167 | 1.182 | 0.3357 | 0.2720 | 1.215 | 3.2214 |
| Mustard | 0.1955 | 1.092 | 0.2890 | 0.2337 | 1.139 | 2.9492 |
| Rayi | 0.2082 | 1.207 | 0.3995 | 0.3357 | 1.343 | 3.4934 |
| Radish leaves | 0.1870 | 1.071 | 0.2593 | 0. 2507 | 1.109 | 2.8070 |
| Fenugreek | 0.1402 | 0.6545 | 0.1658 | 0.1827 | 0.9608 | 2.1040 |

(US- EPA, 2010). The health risk index (HRI) due to dietary intake of Cd, Cr, Cu, Ni and Pb bio-accumulated in studied leafy vegetables were computed by putting appropriate values of DIM from Table -3 and $R_fD$ **(**US –EPA, 1997; 2010) in equation (1) mentioned earlier. The computed values of health risk index for studied leafy vegetables are summarized in Table-4.

The degree of toxicity of heavy metals to consumer health depends upon their intake through vegetables (Mahmood and Malik, 2014). Individual contribution of studied metals in health risk index may result in aggregate risk via consumption of vegetables (Ametepey *et al.,* 2018). The computed HRI below unity are considered to be safe for human consumption (USEPA IRIS., 2006), while the health risk index >1 is unsafe for human health (U.S. EPA, 2002; Chary *et al*., 2008). Among all the studied metals, *Lead* recorded the highest health risk index (HRI) in all the studied vegetables that varied from 229.5 to 335.8, while the lowest was due to *Chromium* (0.436 to 0.805). The overall order of recorded HRI (Table 4, Fig. 3) was: Pb (229.5 - 335.8) > Cd (140.2 to 216.7) > Ni (8.08 – 16.79) > Cu (4.15 – 9.98) > Cr (0.436 - 0.805). The order of computed HRI due to tested metals in *Spinach* was: Pb (291.0) > Cd (199.7) > Ni (12.33) >Cu (7.23) > Cr (0.768); in *Bathua* was: Pb (229.5) > Cd (144.5) > Ni (8.08) > Cu (4.89) > Cr (0.447); in *Chauleyi* was: Pb (303.8) > Cd (216.7) >Ni (13.60) > Cu (8.39) > Cr (0.788); in *Mustard* was: Pb (284.8) > Cd (195.5) > Ni (11.69) > Cu (7.23) > Cr (0.728); in *Rayi* the order was: Pb (335.8) > Cd (208.2) >Ni (16.79) > Cu (9.99) > Cr (0.805); in *Radish leaves* the order was: Pb (277.3) > Cd (187.0) > Ni (12.54) > Cu (6.48) > Cr (0.714); and the order of HRI in *Fenugreek* due to tested metals was*:* Pb (240.2) > Cd (140.2) > Ni (9.14) > Cu (4.15) > Cr (0.436). The computed HRI values (Table 4.) are comparable with earlier results reported from north Indian soil (Ramteke *et al.,* 2016; Kumar *et al*., 2017; Kumar and Thakur, 2018)). Further, leafy vegetable wise order of health risk due to accumulated *Cadmium* was: *Chauleyi > Rayi > Spinach > Mustard > Radish leaves > Bathua > Fenugreek*; *Chromium* was: *Rayi > Chauleyi > Spinach > Mustard > Radish leaves > Bathua > Fenugreek; Copper* was: *Rayi > Chauleyi* **>** *Spinach ~* Mustard > *Radish leaves > Bathua > Fenugreek; Nickel* was: *Rayi > Chauleyi > Radish leaves >Spinach > Mustard > Fenugreek > Bathua* and vegetable wise order of HRI due to accumulated *Lead* was*: Rayi > Chauleyi* **>** *Spinach* > Mustard > *Radish leaves > Fenugreek >Bathua*. Table 4 reveals the potential health risk to the consumers through dietary consumption of studied leafy vegetables contaminated with Cd, Cr, Cu, Ni and Pb.

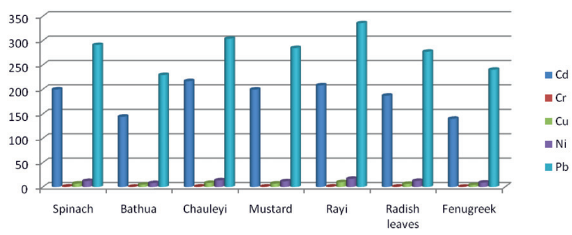
Some of the reported order of consumer health risk index (HRI) due to dietary intake of vegetables contaminated with studied metals: from *Sialkot* and *Wazirabad* (Khan *et al*., 2013) were : Pb (5.17 ) > Cr (3.50) > Cd (2.63) > Ni (1.68) due to the waste water irrigated *Spinach* and due to tube well water irrigated *Spinach* : Ni (5.42 ) > Cd (5.35) > Pb (4.07) > Cr (3.00); from *Lahore* due to *Spinach* was: *Ni* (0.33) >Cd (0.22) >Pb (0.17) >Cu (0.17) (Mahmood and Malik, 2014); from *Korba* (Ramteke *et al.*, 2016) HRI due to *Spinach* was: Cu (659) > Cd (621) > Pb (499) > Cd (2.3) and in *Bathua* was: Cd (667) > Cu (489) > Pb (244) > Cd (4.8); from *Kathiar* (Kumar *et al.*, 2017) the order of HRI due to consumption of *Spinach* grown by using sewage waste water was: Cd (2.12) > Pb (2.09) > Ni (0.50) > Cu (0.18) and from *Haridwar* (Kumar and Thakur, 2018) the reported order of HRI due to *Spinach* was: Cr (3.47) > Cd (2.63) > Cu (0.94). On the other hand the reported consumer health risk in terms of health quotient (HQ) from *Amritsar* (Sharma *et al.,* 2016) due dietary intake of *Spinach* and *Fenugreek* were Cd (1.01) > Pb (0.16) and Cd (1.9) > Pb (0.2), respectively.

However, as the levels of Cd, Cr, Cu, Ni and Pb in vegetable samples grown in walled garden (Table -2, Fig.2) were much lower than found in samples

**Table 4.** The computed values of health risk index (HRI) due to bio-accumulated metals in studied leafy vegetables collected from local venders

| Vegetables/ Metals | Cd | Cr | Cu | Ni | Pb |
|---|---|---|---|---|---|
| Spinach | 199.7 | 0.768 | 7.23 | 12.33 | 291.0 |
| Bathua | 144.5 | 0.447 | 4.89 | 8.08 | 229.5 |
| Chauleyi | 216.7 | 0.788 | 8.39 | 13.60 | 303.8 |
| Mustard | 199.5 | 0.728 | 7.23 | 11.69 | 284.8 |
| Rayi | 208.2 | 0.805 | 9.99 | 16.79 | 335.8 |
| Radish leaves | 187.0 | 0.714 | 6.48 | 12.54 | 277.3 |
| Fenugreek | 140.2 | 0.436 | 4.15 | 9.14 | 240.2 |

Fig. 3 Health Risk Index (HRI) due to tested metals in studied Leafy Vegetables




collected from local venders therefore, dietary consumption of vegetables grown in walled garden using ground water without use of chemical fertilizers are much safer for human consumption than collected from local venders. Thus, to minimize the heavy metal contamination of vegetables and possible human health risk, it is essentially needed to avoid use of chemical fertilizers and waste water of all kind to cultivate vegetable crops.

## CONCLUSION

Globally, leafy vegetables are consumed as a dietary source of essential nutrients required by human body. Regular use of waste water for irrigation usually elevates the levels of heavy metals in farm soil and subsequently through plant roots in edible parts of the vegetables. Further, due to dietary intake of such vegetables for long, heavy metals are stored in human organs causing serious health problems. In this study, levels of accumulated Cd, Cr, Cu, Ni and Pb were evaluated in studied leafy vegetables collected from local venders and from walled garden where ideal farming conditions are being followed. The levels of metals in leafy vegetables collected from local venders varied as: Pb (2.16-3.16) > Cr (1.54-2.84) > Cu (0.39- 0.94) > Ni (0.38-0.78) > Cd (0.33-0.51). However, the levels of metals in vegetables grown under ideal conditions were much lower and varied as: Pb (1.11-1.46) > Cr (0.66- 1.22) > Cu (0.21- 0.42) > Ni (0.22-0.33) > Cd (0.19- 0.29). This study revealed that among the studied metals, *Lead* exhibited highest health risk index (HRI) in all the vegetables, while *Chromium* registered the lowest HRI. The order of contribution of metals in evaluated health risk index (HRI) was Pb (240.2 - 335.8) > Cd (140.2- 216.7) > Ni (8.08- 16.79) > Cu (4.15- 9.99) > Cr (0.436 -0.805). Among the studied vegetables, on the basis of overall HRI, dietary consumption of Rayi and *Chaulai* are expected to cause high health risks, followed by *Spinach*, *Mustard*, *Radish leaves*, *Fenugreek* and *Bathua* in decreasing order. Therefore, regular monitoring of levels of heavy metals in the farm soil, irrigation water and in vegetables, are needed to avoid the possible health risk to the consumers.

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