Research Journal of Chemical Sciences ISSN 2231-606X
Vol. **13(3),** 9-15, October **(2023)** *Res. J. Chem. Sci.*

Determination of Bio-accumulated Cadmium, Chromium, Copper, Nickel and Lead in some common Vegetables and Quantification of Consumer Health Risk due to their long term dietary consumption

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Available online at: www.isca.in, www.isca.me
Received 5th May 2023, revised 23rd July 2023, accepted 6th August 2023

Abstract

Due to the ability of bio-accumulation, several plants selectively accumulate heavy metals in their edible parts via the soil-plants transfer mechanism. Regular intake of such metal contaminated vegetables as dietary component; heavy metals are deposited in human organs, sometimes beyond their safe limits that may initiate various human health implications. This study was undertaken to estimate the human health risk on regular consumption of five common vegetables- potato, onion, bottle gourd, pumpkin and spinach for long by the inhabitants of Rishikesh municipal area, Dehradun. The levels of studied metals in selected vegetable samples were determined on Atomic Absorption Spectrophotometer. Based on these observed levels of metals, "the daily intake of metals" for the studied vegetables was computed. Finally, the health consequences of regular consumption of vegetables under study for long were quantified in terms of the consumer health risk index (HRI). The overall order of levels of tested metals in vegetables was: lead > chromium > copper > nickel >cadmium. The order of daily intake of metals (DIM) on regular consumption of studied vegetables was: spinach (1.828) > bottle gourd (1.747) > pumpkin (1.607) > potato (1.556) > onion (1.543). The human health risk index (HRI) was evaluated as - Pb (123.3-179.6) > Cd (80.75- 144.5) > Ni (9.14- 12.54) > Cu (4.57- 7.12) > Cr (0.252 - 0.405).

Keywords: Cadmium, chromium, copper, nickel and lead in vegetables, DIM, HRI

Introduction

During last few decades, intensive industrialization, urbanization, use of chemical fertilizers, pesticides, waste water, street runoff and other anthropogenic activities have contaminated agri-soil and water bodies beyond reversal[1]. Metals cannot breakdown in nature that let metals to be carried long distances through air and water[2]. Toxic metal contamination of air, soil and aquatic system has caused a serious threat to the biosphere and humanity, in various ways[3]. Large numbers of metals are introduced to the water bodies during their mining, extraction and weathering of rocks, however the role of domestic and industrial wastes is of much concern[4].

The agri- soil has been perturbed greatly by heavy metals from thermal power plants, coal mines, smelting, electroplating, e-waste processing and agricultural runoff etc[5]. In Indian sub-continent fresh vegetables and fruits are essential constituents of the balanced diet of the habitants that fulfill the requirements of nutrients, fiber, vitamins and antioxidants[6].

The fertile agri-soil is the fundamental basis of supporting the growing crops, which provides root support, several essential nutrients, water etc. Due to their unique tendency of bio-concentration in food grain and vegetables plants, contaminants and toxic metals may find their ways in food chain and thereby hazardous consequences of pollutants and toxic metals on the quality of crops and vegetables bound to affect greatly, the food production and consumer health[5].

On consuming metal contaminated fruits, vegetables and other farm produces as dietary component for long may initiate adverse effects on human lives. Further, the human body cannot disperse off the metals from human body; therefore these metals get stored in our important sensitive internal organs[2]. Higher levels of metals in human organs may initiate adverse health hazards, which may affect human sensitive organs, such as CNS, liver, kidney, lungs etc[7].

Due to the tendency of bio-accumulation, heavy metals from agri-soils are absorbed by the roots of the vegetable plants and stored selectively in the various edible parts of the plants during its life cycle. In addition to this, the contamination of irrigation water contributes in raising the levels of toxic metals in soil; consequently, these metals through farm soil and irrigation water enter to farm produces and vegetables[3]. On regular consumption of edible parts of metal contaminated vegetables as dietary component, the accumulated levels of these metals in human organs may reach beyond their recommended safe limits that are bound to initiate various health related implications[6].

This study was aimed to quantify the consequences of the regular intake of heavy metal polluted vegetables for long, in terms of the consumer health risk index (HRI), by evaluating the accumulated levels of Cd, Cr, Cu, Ni and Pb in the eatable parts of the vegetables under study. The vegetables most consumed by the inhabitants of Rishikesh municipal area, district Dehradun were selected for this study and compiled in Table-1. The determination of observed levels of accumulated Cd, Cr, Cu, Ni and Pb in samples of *potato*, *onion* (young bulb with green leaves), *bottle gourd*, *pumpkin* and *spinach* were done on Perkin Elmer Atomic Absorption Spectrometer. The concentration of tested heavy metals in vegetables were used to compute the daily intake of metals (DIM) as per FAO/WHO Tech Reports[8,9] and as reported earlier[10,11]. Finally, the quantum of health risk on regular consumption of the studied vegetables was computed in terms of health risk index (HRI).

Materials and methods

Selection of vegetables: The vegetables selected for evaluation of the daily uptake of metals are presented in Table-1.

Table-1: Vegetables selected for this study and their importance.

Common name	Botanical name, family	Importance due to presence of
Potato	*Solanum tuberosum*, Solanaceae	Dietary fibre, vitamin C and B6 etc.
Onion	*Allium cepa*, Amaryllidaceae	Vitamin E and immunity booster
Bottle gourd	*Lagenaria Oleracea,* Cucurbitaceae	Vitamin C, riboflavin, antioxidants
Pumpkin	*Cucurbita maxima,* Cucurbitaceae	Vitamin C, E, folate, immune booster
Spinach	*Spinacia oleracea,* Amaranthaceae	Helps in diabetes, immune booster

Collection and treatment of vegetable samples: The vegetable samples were collected from five local venders during October 2016 to January 2017. The samples were cleaned separately 3- 4 times with tap water and finally by using double distilled water. The cleaned plant samples were chopped into smaller sizes by using a sharp stainless-steel knife and air- dried for 4-5 days in dust free glass chamber and finally in an electric oven for few hours, at 70^0C. The dried plant samples were grinded and kept in labeled glass sample bottles, separately.

Reagents and Chemicals: In this atomic absorption spectrometric analysis, all the required standard stock solutions of metal ions used were procured from Sigma-Aldrich. For preparing working standards the Sigma-Aldrich standards are diluted to required concentrations. All the other reagents, chemicals and solvents used were of Analytical grade[12]. Double distilled water was used for all purposes.

Determining Metals in Vegetables: The grinded samples (1.0 gm) were digested with $HClO_4$: HNO_3 (1:5 mixture) for several hours by following the standard methods[13] to get transparent and light colored liquid, that was filtered and diluted with double distilled water to 100 ml. By using Perkin Elmer *Atomic Absorption Spectrometer*, Analyst 200, using air-acetylene flame, the accumulated concentrations (mg/kg, dw) of Cd, Cr, Cu, Ni and Pb in these solutions were determined, in accordance to standard methods[13], using Sigma Aldrich standard stock solutions of metal ions, diluted to prepare working standards of required concentrations. All the AAS analysis was run in triplicate.

Consumer health risk on dietary intake of vegetables: The consumer health risk due to dietary consumption of vegetables grown on contaminated farm soil is usually carried out in terms of the health risk index[14]. The health risk index was evaluated by dividing the computed values of DIM[10] with the concerned value of RfD, as described by Jan[15] and US-EPA[16,17,18], where, DIM is the daily intake of metals on dietary consumption of selected vegetables and RfD signifies the oral reference dose as follow-

HRI=Daily intake of metals (DIM)/Oral reference dose (Rf D) (1)

Where, $DIM = C_{Metal} \times C_{Factor} \times D_{Food\ Intake} / B_{Average\ Body\ Weight}$ (2)[10,19]
and
RfD, approximates the daily tolerable exposure to the consumer as reported earlier by US-EPA, Jan and Khan[14,15,17-19].

In equation (2), C_{Metal} signifies levels of tested metal (mg/Kg) in vegetables, $C_{ofactor}$ is the conversion factor (0.085) to convert fresh weight of vegetables to dry weight[11,19]. $D_{Food\ Intake}$ signifies the consumption of vegetables per day. The $A_{verage\ Body\ Weight}$ is the average weight of the consumer. The average daily intake of vegetables amounting 300 to 350g in diet for an adult was suggested by FAO/WHO[8] and the average body weight of an adult consumer is taken 60kg as suggested by FAO/ WHO[9]. The oral reference dose of the metals for cadmium, lead, copper, manganese, zinc, chromium and iron reported was 0.001, 0.004, 0.04, 0.14, 0.3, 1.5, 15mg/kg/day, respectively, suggested by US -EPA[15] and for Ni was 0.02mg/Kg/day suggested by US-EPA[17]

Computation of DIM and HRI: DIM due to regular intake of metal contaminated vegetable for long was computed by putting appropriate values in above equation (2) and the consumer health risk index (HRI) was obtained on dividing DIM by RfD as per equation (1)[10].

Results and discussion

Table-2 presents the levels of cadmium, chromium, copper, nickel and lead, mg/Kg dry weight of vegetables, found in the studied vegetables: *potato, onion, bottle gourd, pumpkin and spinach,* collected from local venders with in Rishikesh municipal area of district Dehradun. Observed concentration of studied metals in analysed samples of vegetables was compared with the safe limits recommended[19,20].

Bio-accumulation of metals in Vegetables: The extent of bio-accumulation of a metal in a plant is the plant specific phenomenon and varies species to species that usually depends on the morphology and metal uptake potentials of the plant[6], metal levels in agri-soil and soil characteristics[7,21]. The observed levels of cadminm, chromium, copper, nickel and lead in studied vegetables presented in Table-2, Figure-1, were below the safe limits recommended for vegetables[19,20]. The overall order of accumulated metals in studied vegetables were, Pb > Cr >Cu > Ni > Cd. Cadmium is one of the toxic metals, when accumulated in human liver and kidney via dietary intake of vegetables and other farm produces, it initiates several health implications such as damage to endocrine system and DNA[14,22]. The order of accumulated Cd in vegetables was: *bottle gourd* (0.34± 0.019) > *pumpkin* (0.31±0.016) > *spinach* (0.26±0.014) > *onion* (0.23±0.013) > *potato* (0.19± 0.009). Chromium is an essential mineral for plant growth up to the safe limit of 5.0 mg/kg, dw[5,20]. Chromium has specific role in transcription of DNA and insulin activity[23]. However, its occupational exposure causes dermatitis on hands and liver inflammation[3,12 21]. The order of Cr in vegetables under study was found as *spinach* (1.43±0.062) > *bottle gourd* (1.28±0.059) > *onion* (1.19±0.036) > *potato* (0.97±0.031) > *pumpkin* (0.89±0.026 mg/ Kg, dw). Copper is essential for body pigmentation, its higher concentration may result brain and kidney damage, anemia and liver cirrhosis[23]. The order of Cu levels in vegetables were, *bottle gourd* (0.68±0.028) >*pumpkin* (0.61±0.031) >*spinach* (0.49±0.023) > *onion* (0.46±0.019) > *potato* (0.43±0.021mg/Kg, dw). Nickel facilitates bio-synthesis of urease in plants, may cause lungs cancer and affect liver, intestine and respiratory tract [3]. Observed concentration of Ni in vegetables was, *onion* (0.59±0.027) > *pumpkin* (0.56±0.023) > *bottle gourd* (0.48±0.021) > *potato* (0.46±0.022) > *spinach* (0.43± 0.019). Lead is a neurotoxic and lowers IQ level and may cause risk of cardiovascular diseases (3). Accumulated order of Pb in vegetables was, *spinach* (1.69±0.071 > *potato* (1.57±0.049) >*pumpkin* (1.41±0.053) >*bottle gourd* (1.34±0.062) >*onion* (1.16±0.034, mg/Kg, dw).

Table-2: The concentration (mg/ Kg, dw) of tested metals found in vegetable samples.

Vegetables (n = 5)	Cadmium	Chromium	Copper	Nickel	Lead
Potato	0.19± 0.009	0.97±0.031	0.43±0.021	0.46±0.022	1.57±0.049
Onion	0.23±0.013	1.19±0.036	0.46±0.019	0.59±0.027	1.16±0.034
Bottle gourd	0.34± 0.019	1.28± 0.059	0.68± 0.028	0.48± 0.021	1.34± 0.062
Pumpkin	0.31±0.016	0.89±0.026	0.61±0.031	0.56±0.023	1.41±0.053
Spinach	0.26± 0.014	1.43±0.062	0.49 ± 0.023	0.43±0.019	1.69± 0.071
Safe limits[19, 20]	1.5	5.0	30	1.5	2.5

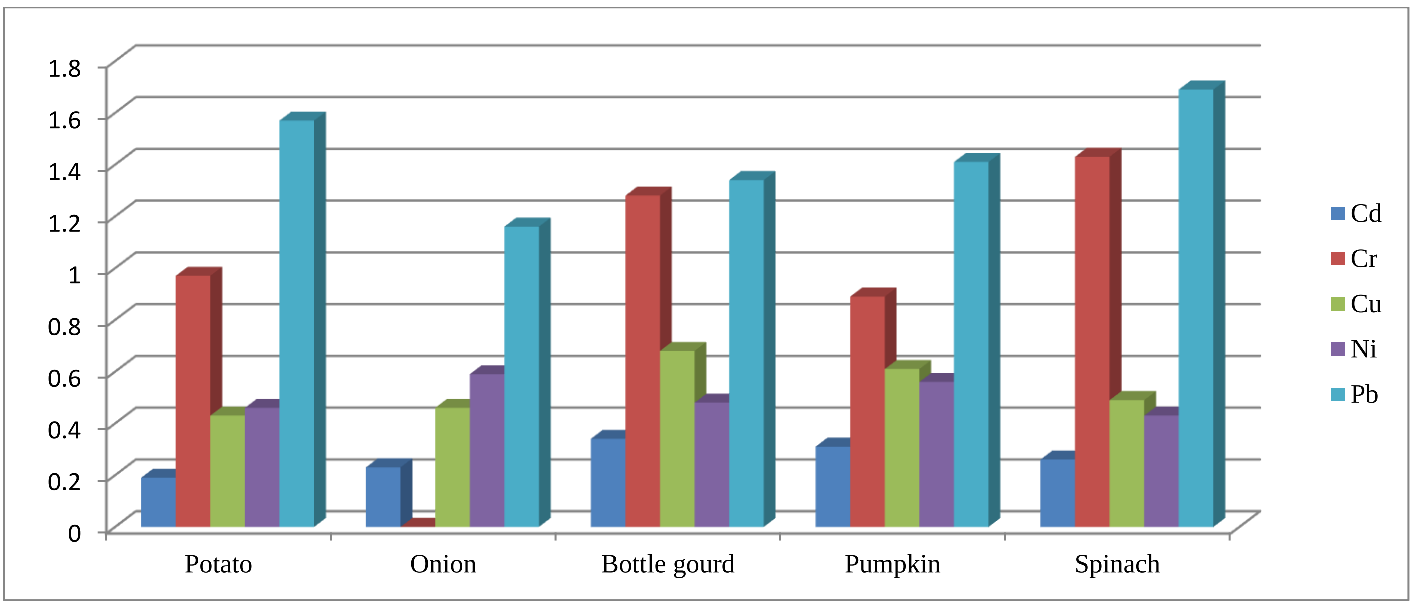

Figure-1: Concentration (mg/Kg,dw) of metals fund in vegetable samples.

On comparing these results with earlier investigations, the levels of Cu and Pb in *Potato* reported in samples collected from *Katihar* by Kumar were comparatively much higher, while levels of Cd and Cr were comparable[24]. On the other hand, the concentrations of cadmium, chromium, copper, nickel and lead reported in samples collected from *Jwalapur* by Kumar were higher and of Cr and Pb were comparatively lower than this findings[25]. The levels of Cd, Cr, Cu, Ni and Pb reported in samples of *Onion* grown on agricultural farm of ICAR, New Delhi by Singh were much higher than our result[22]. However, the concentrations of Cd, Cr, Cu, Ni and Pb in samples collected from *Sao Paulo, Brazil* by Fernando were much lower than present results[23]. In the samples of *Bottle gourd* collected from *Varanashi* by Singh[1], the levels of tested metals except Cd were much lower[1]. The levels of cadmium, chromium, copper, nickel and lead reported by Kulshrestha in samples from Dehradun were slightly higher than the present results[26]. In the samples of *Pumpkin* collected from Varanashi by Singh, the levels of Cd and Cu were comparable and levels of Cr, Ni and Pb were much lower than results of present findings[1]. The levels of cadmium, chromium, copper and lead in *Spinach samples* reported from *Korba by* Ramtek and the levels of all the five tested metals collected from Katihar by Kumar were much higher than this investigation[27,24]. In *Spinach* the levels of all the tested metals in samples collected from *Dehradun* by Kulshrestha were comparable, on the other hand the levels of chromium, nickel and lead in spinach reported from *Varanashi* by Singh were much lower and the levels of Cd and Cu were comparable with present findings[28,1]. Although, in all the studied vegetables the observed levels of Cd, Cr, Cu, Ni and Pb were below the recommended safe limits for human consumption[19, 20], yet their long term dietary consumption is bound to enrich the heavy metal levels in kidney, liver and spleen that may initiate several human health risks in due course[2].

Computation of the daily intake of metals (DIM): By putting the appropriate values of the concerned terms in following equation (2), the various values of DIM[10] were evaluated due to dietary intake of studied vegetables.

Daily intake of metals = $C_{Metal} \times C_{Factor} \times D_{Food\ Intake} / B_{Average\ Body\ Weight}$ ………… (2)[10,18a]

In the equation (2) C_{Metal} signifies the observed levels of heavy metals (mg/Kg) found in tested vegetables. The significances of other terms are presented earlier. The value of C_{Factor} of 0.085 was used to obtain the dry weight of vegetable from the fresh weight[11,19], The $D_{Food\ Intake}$ the mean daily intake of vegetables for human diet suggested by FAO/WHO[8,9] was 300 to 350g, in this study, the minimum of the suggested values i.e. 300 g per adult person was used and the average body weight of an adult consumer was taken 60kg as suggested by FAO/WHO[8,9]. The computed daily intake (DIM) of cadmium, chromium, copper nickel and lead due to regular dietary consumption of studied vegetables are tabulated in Table-3.

The daily intake of metals (DIM) usually varied with vegetable plant species, heavy metal levels in soil, agri-soil profile and quality of water used to irrigate the vegetable crops[24,29]. Among the studied vegetables, the overall highest DIM was found in *Spinach* (1.8277) followed by *Bottle gourd* (1.7468), *Pumpkin* (1.6067), *Onion* (1.5429) and lowest in *Potato* (1.5386). On the other hand, the order of DIM due to Cd *was, Bottle gourd* (0.1445) > *Pumpkin* (0.1318) > *(Spinach (*0.1105) > *Onion* (0.0978) > *Potato* (0.0807) *and due to Cr was, Spinach (*0.6078) > *Bottle gourd (*0.544) > *Onion (*0.5058) >*Potato* (0.4123) > *Pumpkin (*0.3783) *and due to Cu was, Bottle gourd (*0.2848) > *Pumpkin* (0.2593) > *Spinach* (0.2083) > *Onion (*0.2508) > *Potato* (0.1828). *While, DIM due to Ni was, Onion* (0.2508) > *Pumpkin* (0.238) > *Bottle gourd* (0.204) > *Potato* (0.1955) > *Spinach* (0.1828) *and due to Pb was, Spinach* (0.7183) > *Potato* (0.6673) > *Pumpkin* (0.5993) > *Bottle gourd* (0.5695) > *Onion* (0.493 mg/Kg/day/person).

Quantification of health risk index (HRI) on the basis of DIM: The health risk index (HRI) due to dietary intake of metal contaminated vegetables was evaluated simply on dividing the various values of daily intake of metals (DIM, mg/Kg/ day /person) by the concerned reference doses (RfD, mg/Kg/day), which approximates the daily tolerable exposure to the consumer as reported earlier by US-EPA and Jan[14,15,17-19]. The values of oral reference doses (RfD) suggested by US- EPA for cadmium, chromium, copper, and lead were 0.001, 0.004, 0.04 and 1.5mg/kg/ day, respectively[15]. While the R_fD for Ni was 0.02 mg/Kg/day by US-EPA[17]. The health risk index (HRI), due to dietary intake of tested metals, were evaluated by putting the computed values of DIM (Table-3) and corresponding RfD values, in the above relation (1). Table-4 and Figure-2 present the metal wise values of HRI for studied vegetables.

Table-3: Computed values of DIM (mg/Kg/day/person) due to intake of studied vegetables.

Vegetables/ Metals	Cadmium	Chromium	Copper	Nickel	Lead	Total
Potato	0.0807	0.4123	0.1828	0.1955	0.6673	1.5386
Onion	0.0978	0.5058	0.1955	0.2508	0.493	1.5429
Bottle gourd	0.1445	0.5440	0.2848	0.204	0.5695	1.7468
Pumpkin	0.1318	0.3783	0.2593	0.238	0.5993	1.6067
Spinach	0.1105	0.6078	0.2083	0.1828	0.7183	1.8277

The quantification of health risk due to cadmium, chromium, copper, nickel and lead present in vegetables under study was done in terms of consumer health risk index (HRI), which was obtained on dividing the computed value of DIM via dietary ingestion of vegetables by local inhabitants and reference oral dose reported for the concerned metals. However, the health risk consequences of all tested metals individually contribute to the overall HRI, therefore the overall health risk index, HRI is the sum total of all the fractional health risks, contributed individually by each heavy metal under study. As per US-EPA, when the computed health risk index value is found < 1, the concerned vegetable was graded as safe for human intake, as established by US-EPA-IRIS[16]. If, the evaluated value of health risk index >1 the vegetable is considered unsafe for human consumption[10].

Dietary consumption of farm produces including vegetables is the most prominent route of exposure of local inhabitants to heavy metals that ultimately enrich their levels in human organs and may initiate health implications, depending on the magnitude of exposure[1,3]. The magnitude of the consumers' exposure to heavy metals can be related roughly to the levels of toxic metals in agriculture fields, farm produces and duration of regular consumption of metal contaminated vegetables[1,22]. The evaluated values of HRI presented in Table-4, Figure-2, revealed that the HRI values due to cadmium, chromium, copper, nickel and lead in *Potato* varied as: Pb (166.8) > Cd (80.75) > Ni (9.78) > Cu (4.57) > Cr (0.275); in *Onion HRI values varied as:* Pb (123.3) > Cd (97.75) > Ni (12.54) > Cu (4.88) > Cr (0.337); in *Bottle gourd* HRI varied as: Cd (144.4) > Pb (142.4) > Ni (10.20) > Cu (7.12) > Cr (0.363); in *Pumpkin* HRI varied as: Pb (149.8) > Cd (131.75) > Ni (11.91) > Cu (6.48) > Cr (0.252); and in *Spinach* the order of HRI was: Pb (179.6) > Cd (110.5) > Ni (9.14) > Cu (5.20) > Cr (0.405). Thus, among all the tested metals, HRI values recorded due to *Lead* in all the vegetables, except *Bottle guard* were higher and the range was between 123.3 (Onion) and 179.6 (Spinach). However, Chromium recorded lowest HRI values among the tested metals and the range was between 0.252 (*Pumpkin*) and 0.405 (*Spinach*). Almost all the evaluated values of HRI of present findings are either lower or comparable with some earlier findings[7,24,26-28].

Table-4: Health risk index (HRI), due to tested metals through regular dietary intake of vegetables.

Vegetables	Cd	Cr	Cu	Ni	Pb
Potato	80.75	0.275	4.57	9.78	166.8
Onion	97.75	0.337	4.88	12.54	123.3
Bottle gourd	144.50	0.363	7.12	10.2	142.4
Pumpkin	131.75	0.252	6.48	11.91	149.8
Spinach	110.5	0.405	5.20	9.14	179.6

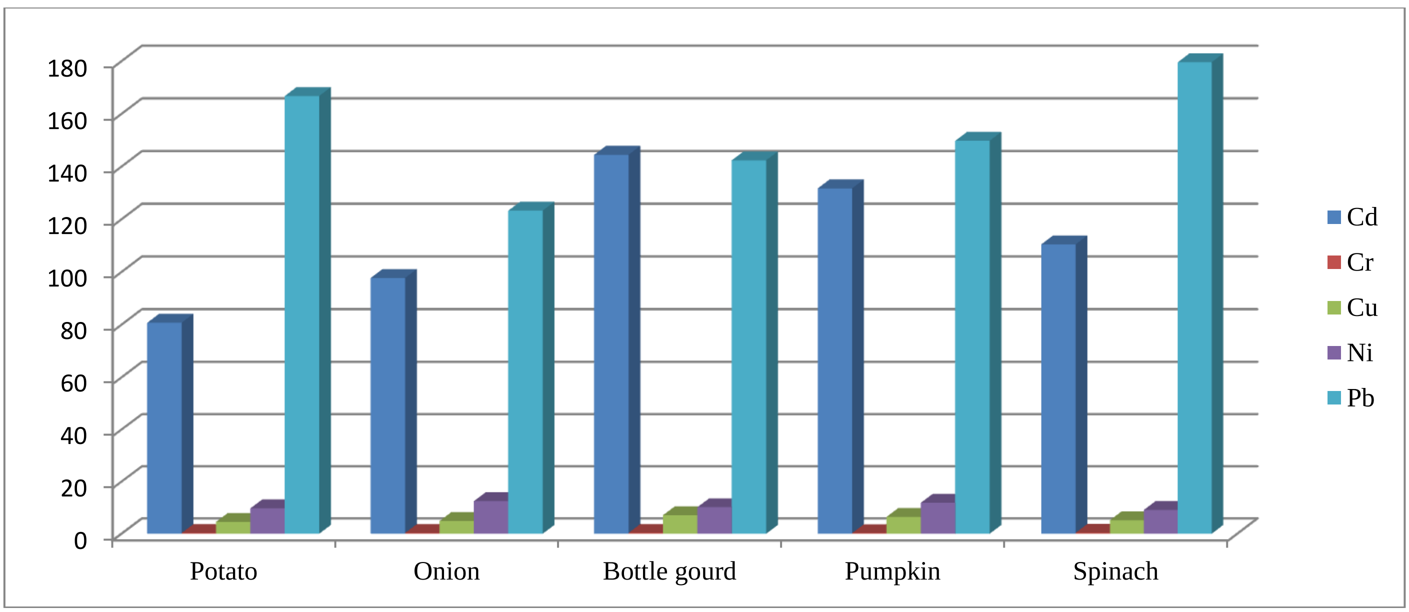

Figure-2: Various HRI values on consumption of metal contaminated vegetables in diet.

Conclusion

Due to regular dietary consumption of vegetables for long, heavy metals from agri-soil via edible parts of vegetables may find their ways to human internal organs. The deposition of heavy metals in these sensitive organs beyond safe limits may initiate several serious health implications. In this study the bio-accumulated levels of Cd, Cr, Cu, Ni and Pb were determined in *potato, onion, bottle gourd, pumpkin* and *spinach* collected from local venders within Rishikesh municipal area, Dehradun district of Uttarakhand. The observed levels of metals in studied vegetables varied as: Pb (1.16±0.034 -1.69±0.071) > Cr (0.89± 0.026-1.43±0.0.062) > Cu (0.43±0.0.021-0.68±0.028) > Ni (0.43±0.019-0.59±0.027) > Cd (0.19±0.009-0.34±0.0.019). Although, all these observed values are below the recommended safe limits for human consumption, yet regular intake of metal contaminated vegetables for long may enrich their levels in human organs. The dietary intake of metals (DIM) for individual vegetable was computed from the levels of tested metals determined in that vegetable. Finally, DIM values were used to evaluate the consumer health risk index (HRI) as a direct ratio of DIM and RfD. Among the studied vegetables, the overall highest DIM was found in *Spinach* (1.8277) followed by *Bottle gourd* (1.7468), *Pumpkin* (1.6067), *Onion* (1.5429) and lowest in *Potato* (1.5386). The values of HRI (Table-4, Figure-2) revealed that the HRI values on consuming the edible portion of the studied vegetables were highest due to lead, followed by cadmium, nickel, copper and lowest being due to chromium. The individual contribution in evaluated values of HRI was: Pb (123.3-179.6) > Cd (80.75-144.50)> Ni (9.14-12.54) > Cu (4.57-7.12) > Cr (0.252-0.405). HRI values indicated that regular dietary consumption of spinach and bottle gourd for long may pose higher health risks than pumpkin, potato and onion. Thus, regular monitoring of agri- soils for heavy metal levels and quality of water being used to irrigate vegetable crops, will be useful to keep the levels of toxic metals in vegetable crops within the permissible limits suggested for human consumption.

Acknowledgement

The author is thankful to Dr. S. K. Dabral, former head and Dr. Dayadhar Dikshit, Department of Chemistry, Pt. L. M. S. Govt. Post Graduate College (Autonomous College) Rishikesh, Dehradun, Uttarakhand, for help in the analysis of metals and valuable discussion.

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