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Assessment of heavy metal contents of green leafy vegetables

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Summary

Vegetables are rich sources of vitamins, minerals, and fibers, and have beneficial antioxidative effects. Ingestion of vegetables containing heavy metals is one of the main routes through which these elements enter the human body. Slowly released into the body, however, heavy metals can cause an array of diseases. In this study we investigated the concentrations of copper, chromium, zinc, and lead in the most frequently consumed vegetables including *Pimpinella anisum*, *Spinacia oleracea*, *Amaranthus viridis*, *Coriandrum sativum*, and *Trigonella foenum graecum* in various sites in Raipur city, India. Atomic absorption spectrophotometry was used to estimate the levels of these metals in vegetables. The mean concentration for each heavy metal in the samples was calculated and compared with the permissible levels set by the Food and Agriculture Organization and World Health Organization. The intake of heavy metals in the human diet was also calculated to estimate the risk to human health. Our findings indicated the presence of heavy metals in vegetables in the order of Cr > Zn > Cu > Pb. Based on these findings, we conclude that the vegetables grown in this region are a health hazard for human consumption.

Keywords: vegetables, contamination, potentially toxic element, daily intake

Introduction

Green leafy vegetables are popular around the world. Heavy metal contamination of vegetables cannot be underestimated as these foodstuffs are important components of human diet. Heavy metal contamination of the food items is one of the most important aspects of food quality assurance (Khan et al., 2009; Radwan and Salama, 2006; Wang et al., 2005). These vegetables are valuable sources of vitamins A and C, iron, calcium, folic acid, and dietary fibre. In recent years their consumption is increasing gradually, particularly among the urban community. Heavy metals ranks high among the chief contaminants of leafy vegetables. A number of studies have shown heavy metals as important contaminants of the vegetables (Sinha et al., 2006; Singh and Kumar, 2006; Sharma et al., 2006, 2007, 2008 a, b; Mapanda et al., 2005). Vegetables take up metals by absorbing them from contaminated soils, as well as from deposits on different parts of the vegetables exposed to the air from polluted environments (Sobukola et al., 2010). However, intake of heavy metal contaminated vegetables may pose a risk to the human health. Prolonged human consumption of unsafe concentrations of heavy metals in food stuffs may lead to the disruption of many biological and biochemical processes in the human body (WHO, 1992; Jarup, 2003). Intake of vegetables is an important path of heavy metal toxicity to human being. Dietary intake of heavy metals through contaminated vegetables may lead to various chronic diseases. Regular monitoring of these metals in vegetables and in other food materials is essential for preventing excessive buildup of the metals in the food chain. The aim of this study was to find the concentrations of heavy metals in vegetables in Raipur city Chhattisgarh, India and to estimate their contribution to the daily intake of the metals.

Materials and methods

Sampling and sample preparation

Samples of edible portions of vegetables (1 kg each) at a height above 10 cm from the soil surface were collected. The samples were collected once in a month and were kept in pre-distilled water rinsed

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polyethylene bags and pined up to avoid the excess deposition. Then the samples were brought back to laboratory, chopped into small pieces and oven dried at 80 °C till the constant weight was achieved. To assess the effects of washings on the removal of heavy metals from the vegetable surfaces, samples of vegetables were collected from selected sampling locations. The sample of each sampling location was separately divided into two groups. First group was kept as such for oven drying, whereas other group was washed with clean tap water according to the normal household technique. After draining the excess water samples were chopped into small pieces and oven dried at 80 °C till the constant weight was achieved. The dry vegetable samples were powdered with a stainless steel blender and passed through a 2 mm size sieve. The samples were then kept at room temperature for further analysis.

Analytical procedure for heavy metal analysis

A 5g dry weight (DW) subsample was crushed in a mortar and ashed in a muffle furnace at 450 °C for 6 h. If the ashes were not completely white, 2 mL of concentrated HNO_3 were added and the mixture was heated to boiling point on an electric plate heater until the formation of nitrous fumes had stopped. Then, the ashes were returned to the muffle at 450 °C for a further 2 h. Finally, the white ashes were digested with 3.60 mL of concentrated HNO_3, filtered through 2 mm filter paper, transferred into a 25 mL flask and brought to volume with ultrapure water to a final concentration of 10 % HNO_3. Analytical blanks were prepared in the same way, but without the addition of any sample. Vegetable samples were measured in duplicate in a Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS) (Perkin-Elmer Analyst 600) for Cu, Ni and Pb quantification, whereas Fe and Mn concentrations were determined using a Flame Atomic Absorption Spectrophotometer (FAAS) (Perkin-Elmer AA3110).

Quality control and quality assurance

Blanks and quality control standards were measured at every five samples to detect contamination and drift. The elemental concentrations of procedural blanks were generally < 5 % of the mean analyte concentrations for all the metals. Precision and accuracy of analyses were also ensured through replicate analyses of samples against standards reference material for all the heavy metals. The results were found to be within ± 2 % of certified values, thus demonstrating the accuracy of our findings.

Heavy metals in soils

A total of 15 top soil composite samples were collected in the study zone at a depth of 0-10 cm. The total multi element concentrations were and expressed as μgg^{-1}DW. In addition, with the aim of analyzing labile metals in top soils, a 0.5 M-hydrochloric acid extraction was performed. The concentrations of Cr, Mn, Fe, Ni, Cu, Zn and Pb were determined using a FAAS (Perkin-Elmer AA3110).

Results and discussion

Levels of heavy metals in vegetables

Heavy metal concentrations showed variations among different vegetables collected in the vicinity of Raipur city are shown in Table 1. The variations in heavy metal concentrations in vegetables of the same site may be ascribed to the differences in their morphology and physiology for heavy metal uptake, exclusion, accumulation and retention (Carlton-Smith and Davis; 1983, Kumar et al., 2009). Among leafy vegetables Mn concentration was highest in *Amaranthus virdis* while Zn concentration was highest in *Spinacia oleracea* (53.44 μgg^{-1}). The observed value of Zn during the present study was lower than the value (130.14 μgg^{-1}) recorded by Sharma et al. (2006). Among all the heavy metals, Zn showed maximum and Ni showed minimum concentration in all the vegetables. Sharma et al. (2009) have also found highest concentration of Zn as compared to Cu, and Pb in the vegetables collected from market as well as production sites of Varanasi city, India. The variations in the metal concentrations of vegetables may also be ascribed to the variability with absorption of metals in plants and their further translocation within the plants (Vousta et al., 1996).
The mean concentrations of Cr and Pb in all vegetables, *Amaranthus virdis* (AV) (1.1 μgg^{-1}, 2.56 μgg^{-1}), *Spinacia oleracea* (0.31 μgg^{-1}, 2.78 μgg^{-1}), *Trigonella Foenum Graecum* (1.1 μgg^{-1}, 1.55 μgg^{-1}) and *Coriandrum Sativum* (1.1 μgg^{-1}, 2.49 μgg^{-1}) were higher than the values 0.38 μgg^{-1} for Cr and 0.47 μgg^{-1} for Pb obtained from a suburban area of Zhengzhou city, Henan Province, China (Liu et al., 2006) and 78.02 μgg^{-1} for Cr and 63.1 μgg^{-1} for Pb reported in radish collected from treated wastewater irrigated suburban area of Titagarh (Gupta et al., 2008). Among all the vegetables, the concentration of Cu was maximum (47.961 μgg^{-1}) in *Coriandrum Sativum* which is lower then 201.751 μgg^{-1} reported by Liu et al. (2006). When the present concentrations of metals were compared with permissible limits of Indian Standard

(Awashthi, 2000) and safe limits given by WHO/FAO (WHO/FAO, 2007), then it was found that Cr, Mn, Ni, Cu, Zn and Pb concentrations were higher in all the vegetables. Cr and Pb are non essential metals causing adverse health effects even at very low concentrations (Ikeda et al., 2000).

Table 1. Metal average concentration in different vegetable ($\mu g g^{-1}$DW)

Metal	AV	C S	TG	SO	PA	Average
Cr	1.10	2.07	1.09	0.31	2.10	1.33 ± 0.76
Mn	1.23	0.75	0.78	1.08	0.96	0.96 ± 0.20
Ni	0.44	0.49	0.40	0.32	0.34	0.40 ± 0.10
Cu	33.46	47.96	29.57	16.31	28.46	1.15 ± 11.38
Zn	35.01	42.97	51.54	53.44	45.01	45.59 ± 7.35
Pb	2.56	2.49	1.55	2.78	1.84	2.24 ± 0.52

AV - *Amaranthus viridis*, CS - *Coriandrum sativum*, TG - *Trigonella foenum graecum*, SO - *Spinacia oleracea*, PA - *Pimpinella anisum*

Toxic elements such as Pb and Ni are natural constituents of the earth's crust, which are taken up from the soil by plants and transferred to the food chain. According to Kabata-Pendias and Pendias (1984), surveys of trace elements in soil have demonstrated that their composition is extremely varied and soil types must be taken into account. Although metal concentrations of plant tissues are generally results of the metal concentration in the growth solution or soil, the relationship differs according to the plant species and tissues (Kabata-Pendias and Pendias, 1984). The average values posted for Pb in this study was higher than the posted corresponding values for lead (45.75 $\mu g g^{-1}$) in Chile samples (Queirolo et al., 2000).

Enrichment factor

Higher values of enrichment factor (EF) suggest poor retention metals in soil and/or more translocation in plants. Within the plants, *Trigonella Foenum Graecum* and *Spinacia oleracea* (leafy vegetable) showed highest EF value 27.1 and 26.4 for Ni (Table 2). Enrichment factor of other metals like Pb (28.7) and Cr (10.2) was highest in *Trigonella Foenum Graecum* while Zn (2.64) EF was highest for *Spinacia oleracea* (Table 2). EF for Pb is higher in all vegetable with order: CS (50 %) > AV (44 %) > TG (42 %) > SO (41 %) > PA (42 %) following Ni with order: SO (46 %) > PA (44 %) > CS (41 %) > TG (40 %) > AV (37 %), and for Cr it follows order: AV (16 %) > TG (15 %) > PA (8 %) > CS (6 %) = SO (6 %). The average EF value of metals in different vegetable is found to be in the order of Pb (44 %) > Ni (37 %) > Cr (16 %). It suggests that transfer of Pb from soil to plant is higher in all vegetable following Ni and Cr. Sridhara Chary et al. (2008) also reported highest EF for heavy metals through leafy vegetables. EF of heavy metals depends upon bioavailability of metals, which in turn depends upon its concentration in the soil, their chemical forms, difference in uptake capability and growth rate of different plant species (Tinker, 1981). The higher uptake of heavy metals in leafy vegetables may be due to higher transpiration rate to maintain the growth and moisture content of these plants (Tani and Barrington, 2005). As these vegetables are widely consumed by human, through these plants toxic metals can be transferred to human body creating disruption in various biological systems. Therefore the residents of this area are in high health risks of toxic metals exposure.

Table 2. Enrichment factor of heavy metals in foodstuffs ($\mu g g^{-1}$DW)

Foodstuff	Cr	Ni	Cu	Zn	Pb
AV	8.75	20.10	1.10	0.75	24.10
CS	3.14	21.20	0.72	0.68	25.60
TG	10.20	27.10	0.98	1.42	28.70
SO	3.42	26.40	0.94	2.64	23.40
PA	4.12	21.21	1.23	1.67	20.24

AV - *Amaranthus viridis*, CS - *Coriandrum sativum*, TG - *Trigonella foenum graecum*, SO - *Spinacia oleracea*, PA - *Pimpinella anisum*

Daily intake of heavy metals

In order to observe the health risk of any pollutant, it is very important to estimate the level of exposure, by detecting the routes of exposure to the target organisms. There are several possible pathways of exposure to humans but amongst them the food chain is the most important pathway. The daily intake of metals was estimated according to the average vegetable consumption for adults (Table 3). Daily intake of heavy metals through the consumption of the vegetables tested was calculated according to the given equation Eq. 1 (Cui et al., 2004).

$$\text{Daily intake of heavy metals (mgday}^{-1}\text{)} = \text{(Daily vegetable consumption)} \times \text{(Vegetable heavy metal concentration)} \quad (1)$$

Table 3. Metal average concentration in different vegetable (μgg^{-1}DW) and Estimated Daily Intake of metals (DIM) through vegetables

Vegetables	DIM (Cr)	DIM (Mn)	DIM (Ni)	DIM (Cu)	DIM (Zn)	DIM (Pb)
AV	0.38	0.42	0.15	11.54	12.08	0.88
C S	0.71	0.26	0.17	16.55	14.82	0.86
TG	0.38	0.27	0.14	10.2	17.78	0.53
SO	0.11	0.37	0.11	5.63	18.44	0.96
PA	0.72	0.33	0.12	9.82	15.53	0.63
Average(DIM)	0.46	0.33	0.14	10.75	15.73	0.77

Daily vegetable consumption was obtained through a formal survey conducted in the study area. An interview of 100 persons of 30-50 years age group and of 55-70 kg body weight was conducted at each market site regarding their daily consumption rate of vegetables tested. Each person represents a household having ≥ 5 individuals, and thus a total of 100 persons were effectively interviewed. An average consumption rate of each vegetable per person per day was calculated. The percent contributions to dietary intake of heavy metals by the urban population through the consumption of the vegetables tested in this study were calculated by dividing the daily consumption rates of the heavy metals with the values of provisional tolerable daily intake (TDI) (Joint FAO/WHO Expert Committee on Food Additives, 1999). The required amount of vegetables in our daily diet must be 300 to 350 g per person has been suggested by WHO guidelines (WHO, 1989).

Cr is an important element for the insulin activity and DNA transcription. However an intake below 0.02 mgday^{-1} could reduce cellular responses to insulin (Kohlmeier, 2003). The daily intake, estimated as 0.47 mg, was lower than the R_fD established at 1.5 mgkg^{-1}day^{-1} (US EPA, 2010). This value was also lower than that reported by Santos et al. (2004). The greatest contribution for Cr intake came from *Coriandrum sativum* (0.71 mgday^{-1}), *Pimpinella anisum* (0.72 mgday^{-1}) followed by *Trigonella foenum graecum* (0.38 mgday^{-1}), *Amaranthus viridis* (0.38 mgday^{-1}), followed by *Spinacia oleracea* (0.0018 mgday^{-1}), accounting for 2.0 % of the total daily intake.

Mn activates numerous essential enzymes. Food contains trace amounts of manganese (Colaket et al., 2005). The daily intake, estimated as 0.33 mgday^{-1}, was less than R_fD established (US EPA, 2010).

Ni does not have a specific function in humans; however, it is a co-factor for some microbial intestine enzymes. Ni content in the adult human body should remain below 0.1 mg day^{-1} and excess may cause damages to DNA and cell structures (Kohlmeier, 2003). The daily intake of Ni was estimated as 0.14 mg, which represents < 1 % for a 60 kg adult (WHO, 1993). The daily intake was below the TDI of 1.4 mgday^{-1}and lower than that reported in literature (0.231 mgday^{-1}) (Santos et al., 2004). The greatest contribution for the Ni intake came from *Corianderum sativum* (0.17 mgday^{-1}).

Cu is an essential substance to human life, but in high doses it can cause anemia, liver, kidney damage, stomach and intestinal irritation. People with Wilson's disease are at greater risk for health effects from overexposure to copper. Cu accumulates easily in the body; hence, chronic low level intakes of heavy metals have damaging effects on human beings and other animals (Bermudez et al., 2011). The daily intake of Cu was estimated as 10.75 mg, which represents 38 % for a 60 kg adult (WHO, 1993). The order of contribution for the Cu intake follows order: *Corianderum sativum* (16.55) > *Amaranthus viridis* (11.54) > *Trigonella foenum graecum* (10.2).

Zn is one of the important metals for normal growth and development in human beings. Deficiency of zinc can result from inadequate dietary intake, impaired absorption, excessive excretion or inherited defects in zinc metabolism (Narin et al., 2005). The daily intake of Zn was estimated as 15.73 mg, which represents 56 % for a 60 kg adult (WHO, 1993). The order of contribution for the Zn intake came from *Spinacia oleracea* (18.44 $mgday^{-1}$) > *Trigonella foenum graecum* (17.78) > *Pimpinella anisum* (15.53).

High Pb concentrations observed in many vegetables, although they do not pose a risk to human health, may be attributed to crops located near roads of heavy traffic. The main sources of this element to humans are inhalation of airborne Pb from vehicle emissions and from direct atmospheric deposition on soils, water and crops, constituting the gateway into the food chain (Baird, 2002). The toxic effects of Pb focus on several organs, such as liver, kidneys, spleen and lung, causing a variety of biochemical defects. The daily intake of Pb was estimated at 0.77 mg, which represents 3 % of total intake of metals 60 kg adult. Although Pb concentrations were high in some vegetables, low consumption of vegetables results in low intake of this element. This value is higher than those reported in literature (0.521 mg per day) (Tripathi et al., 1997).

Health risk from consuming vegetable

Hazard Quotient Calculation

The health risks associated with metals ingested through vegetable consumption were assessed using hazard quotient (HQ) (Bermudez et al., 2011). This is a ratio of determined dose to the reference dose (R_fD) (Eq. 2).

$$HQ = (Div) \times (C_{metal}) / R_fD \times B_o \qquad (2)$$

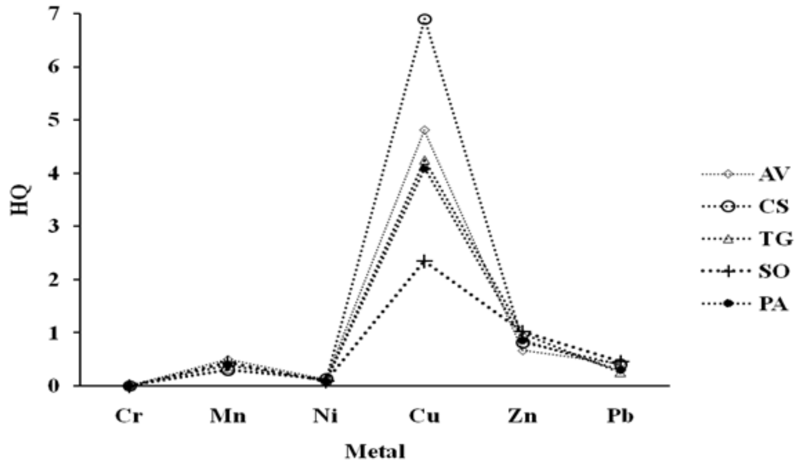
where Div is the daily intake of vegetables (kg $person^{-1}day^{-1}$), C_{metal} is the concentration of metal in the vegetable ($mgkg^{-1}$), R_fD is the oral reference dose for the metal ($mgkg^{-1}day^{-1}$) and B_o is the human body mass (kg). R_fD is an estimation of the daily exposure to which the human population is likely to be without any appreciable risk of deleterious effects during a lifetime. The values of R_fD for heavy metals were taken from Integrated Risk Information System (2003) and Department of Environment, Food and Rural Affairs (1999). The HQ is a highly conservative and relative index. When HQ is < 1, there is no obvious risk from the substance over a lifetime of exposure, while HQ is > 1, the toxicant may produce an adverse effect. The average value of HQ in different vegetables follows the order: Cu (72 %) >Zn (14 %)> Pb (6 %) = Mn (6 %) > Ni (2 %). The probability of experiencing long-term carcinogenic effects increases with the HQ value. This risk assessment method has been used by researchers (Tsafe et al., 2012; Zhuang et al., 2004; Jena et al., 2012) and proved to be valid and true.

Table 4. HQ for individual heavy metals in different vegetables

Metal	AV	CS	TG	SO	PA	Mean HQ
Cr	0.0042	0.0079	0.0042	0.0012	0.0081	0.0051
Mn	0.5052	0.3080	0.3204	0.4436	0.3943	0.3943
Ni	0.1265	0.1409	0.1150	0.0920	0.0978	0.1144
Cu	4.8099	6.8943	4.2507	2.3446	4.0911	4.4781
Zn	0.6710	0.8236	0.9879	1.0243	0.8627	0.8739
Pb	0.4206	0.4091	0.2546	0.4567	0.3023	0.3687
						∑HI=6.23

HQs for individual vegetables were all below 1.0 for Cr, Mn, Ni, Zn and Pb, except Cu (HQ = 4.5) regardless the population type (Table 4). So, the consumption of these vegetables can be considered safe with no risk to human health. The sequence of HQ for adults followed the decrescent order Cu < Zn < Pb ~ Mn < Ni < Cr. Although the HQ-based risk assessment method does not provide a quantitative estimate for the probability of an exposed population experiencing a reverse health effect, it indeed provides an indication of the risk level due to exposure to pollutants (Chary et al., 2008). Many researchers consider the risk estimation method reliable (Chary et al., 2008; Khan et al., 2009; Wang et al., 2005) and it has been proven to be valid and useful. However, this HQ method considers only exposure to heavy metals via consumption of vegetables, without taking into account other routes like dermal contact, soil ingestion, and other factors such as the presence of agrochemicals and herbicide molecules. When the hazard index (HI) exceeds unity (1.0), there is concern for potential health effects (Zhuang et al., 2004). Even though there was no apparent risk when each metal was analyzed individually, the potential risk could be multiplied when considering all HMs. HI for adults was 6.23. Although HI was higher for children, neither population suffered from ingestion of vegetables contaminated with heavy metals.

Fig. 1. HQ of individual metal for different vegetables



Risk of individual elements and aggregate effects of consuming vegetables

The results of the HQs for individual elements are shown in Fig. 1. The majority of the elements had HQ values lower than unity, with the exception of Cu. The mean HQ values for consumers showed the following decreasing order of non-cancer risk: Cu (4.5) > Zn (0.87) > Mn (0.39) > Pb (0.37 > Ni (0.11) > Cr (0.005).

In order to assess the overall potential for non-carcinogenic effects posed by more than one HM, a Hazard Index (HI) approach as been developed based on the EPA's Guidelines for Health Risk Assessment of Chemical Mixtures. This Hazard Index is given by the sum of the Hazard Quotients (HQ), as described in Eq. 3.

$$HI = \sum HQ = DIM_1 / R_fD_1 + DIM_2 / R_fD_2 \text{-}+ DIM_3 / R_fD_3 + \ldots\ldots\ldots + DIM_n / R_fD_n \qquad (3)$$

When the Hazard Risk Index exceeds unity (Cu = 4.5), there may be a concern for potential health effects. Our findings (Mean HI: 6.23) were similar to those of Zheng et al. (2007) but had higher values than those reported by Wang et al. (2005).

Conclusions

Among analyzed vegetable tested: *Pimpinella anisum*, *Coriandrum Sativum* and *Spinacia oleracea* showed the extremely high accumulation tendency towards the heavy metals (i.e. Cu, Zn, Pb). It may be due to co-ordination with bio-chemicals present. This study indicated that long-term and indiscriminate application of raw sewage effluents or letting of sewage water directly to agricultural field without prior treatment which contains heavy metals. Dietary intake of food results in long- term low level body accumulation of heavy metals and the detrimental impact becomes apparent only after several years of exposure. Thus regular monitoring of these heavy metals in vegetables and in other food materials is essential, to prevent their excessive build-up in the food chain. The present study further suggested that to reduce the health risk, vegetables should be washed properly before consumption as washing can remove a significant amount of aerial contamination from the vegetable surface.

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