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ORIGINAL ARTICLE

Assessment of toxicological health risk of trace metals in vegetables mostly consumed in Punjab, Pakistan

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Abstract In the present study, levels of trace metals in two commonly consumed vegetables (*Spinacia oleracea* and *Brassica campestris*) were assessed. Both vegetables are cultivated in a semi-arid area which receives effluents from various sources. Leafy parts and tender stems are used for human consumption as well as for farm animals as a source of nourishment. However, the aim of the present study was to appraise the concentrations of trace metals in different tissues of both vegetables at the time of harvesting when they become accessible to the humans. The analysis showed that mean trace metal levels in the stem of *S. oleracea* showed the highest bioconcentration of Zn (25.43 mg kg^{-1}), while in the stem (28.56 mg kg^{-1}) and roots (22.40 mg kg^{-1}), Fe level was the highest. In case of *B. campestris,* Fe level was highest (18.45 mg kg^{-1}) in the stems. Copper (Cu) has the highest bioconcentration factor values (BF = 1.030) in the stem of *B. campestris,* whereas Mn (BF = 0.010) was the least accumulated element in the leaves and roots of *B. campestris.* Collectively, bioconcentration of trace metals in plant tissues exceeded the standard values set by the World Health Organization (WHO) and the US Environmental Protection Agency. So, the vegetables cultivated in effluent-impacted areas may stance a potential public health risk for end-consumers.

Keywords Bioconcentration · Trace metals · *Spinacia oleracea* · *Brassica campestris*

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Introduction

Heavy metals are natural ingredients in the outer layer of earth and cannot be besmirched nor damaged (non-biodegradable). They have long-term residual effects on various food products which require immediate remedial strategies (Lim et al. 2005). Due to their snowballing behavior, heavy metals are harmful even at a very low concentration. In the living systems, the metals react with the proteins and stop the indispensable reactions (Biddle 1982).

Waste-water irrigation is believed to contribute considerably to the heavy metal contamination of soils (Mapanda et al. 2005), which ultimately affects the crops growing therein and the organisms consuming them. Soils which are irrigated with sewage water quite often show elevated levels of heavy metals like cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn) (Singh and Kumar 2006). When vegetables are grown on these soils, they absorb heavy metals from the soil resulting in contamination of the food chain. Metals such as lead, mercury, cadmium and copper are highly toxic and cause environmental hazards (Yargholi et al. 2008). Cadmium and zinc are also toxic to all organisms (Bonnet et al. 2000; Dong et al. 2005). The increased cadmium concentrations in soil may impose toxic effects on plants such as retarded growth (of roots usually) (Weigel and Jäger 1980). Crop plants can accumulate high concentration of trace elements when grown on contaminated soil. So, contaminated soil may cause serious health problems for the consumers (Long et al. 2003).

As heavy metals are not easily degraded in the living systems, they may accumulate in vital organs of those individuals, consuming contaminated food (Demirezen and Aksoy 2006). Heavy metals have lasting physiological effects on humans and animals as they interrupt the central nervous system, leading to psychotic disorders and other ailments (Lewis et al. 1992; Dolk and Vrijheid 2003).

The present study was conducted with the aim to investigate and compare the concentrations of metals including cadmium (Cd), nickel (Ni), lead (Pb), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and chromium (Cr) as well as their accumulation potential in commonly grown vegetables *Spinacia oleracea* and *Brassica campastris* irrigated with sewage water enriched with heavy metals. In the present study, the levels of trace metals were determined in various parts (i.e., roots, stem and foliage) of *S. oleracea* and *B. campestris* cultivated in area contaminated with untreated industrial and domestic effluents containing metals. The status of trace metals in soil and plant tissues would provide data for comparison with national and international guideline standards for public health safeguard policies.

Materials and methods

Study area

The study area was confined to the vegetable beds along roadside and canal side of Sargodha city, Punjab, Pakistan. These vegetable beds are liable to be irrigated with effluent sewage water and sludge during the season.

Collection of plant samples

The vegetables and other plant parts were obtained directly from the fields. Soil and dust particles were removed by a thoroughly washing with tapwater followed by a wash with distilled water to remove metal contamination of the tapwater used for washing. Plants and vegetables were air-dried and cut into various parts, i.e., leaves, stems, and roots. Vegetables and other parts of plants were wrapped in an aluminum foil and oven-dried at 105 °C for 6 h. Once drying was complete, the samples were pulverized into fine powder and labeled. Ground sample (0.5 g of each type) was digested in Teflon tubes on a dry heating block by adding 2 ml trace metal grade HNO_3 and boiled. After that, one ml H_2O_2 was added to each tube. Distilled water was added to the sample to make a final volume up to 10 ml. The samples were then subjected to analysis on an atomic absorption spectrometer. The method described by Tyokumbur and Okorie (2011) was followed.

Collection of soil samples

Soil samples were collected after harvesting the vegetables from the same fields. Five patches were selected randomly for collection of soil samples, and the soil was taken at the depth of 0–20 cm with the help of a hand trowel. The samples were air-dried and ground into fine powder with the help of a mortar and stored in labeled sealed bags. Each soil sample (0.5 g) was digested for 1 h in 2 ml of technical grade HNO_3 at 95 °C. Later on 2 ml H_2O_2 were added. For further atomic absorption spectrometry (AAS) analysis, distilled water was added to make the final volume of 10 ml (Tyokumbur and Okorie 2011).

Quality control

To evaluate the accuracy and precision of the results obtained from the AAS facility, the obtained results were compared with that of San Joaquin soil NIST SRM 2709 for soil samples, apple leaves NIST SRM 1515 as Standard Reference Material (SRM) for the vegetable samples. Distilled water was used as the blank to correct the results for errors.

Statistical analysis

Analysis of variance of data (ANOVA) was carried-out to assess the level of significance using a Costat program, Cohert version 6.3.

Results and discussion

Trace metals concentrations in vegetables

Bioconcentration values of trace metals in the cultivated soil, floodplain and vegetable tissues were expressed as parts per million (ppm). Table 1 depicts tissue level bioconcentration of different elements in plant tissues. Bioconcentration of trace metals in the vegetable tissue was higher than the values prescribed by the FAO/WHO (1989)

Table 1 Trace metals concentrations in vegetables tissue of *Spinacia oleracea* and *Brassica campestris* expressed as mg kg^{-1}

Metals	*Brassica campestris*			*Spinacia oleracea*		
	Leaves	Roots	Stems	Leaves	Roots	Stems
Cr	0.16 ± 0.051	1.27 ± 0.35	2.66 ± 0.24	0.82 ± 0.358	1.20 ± 0.23	3.67 ± 0.76
Mn	1.22 ± 0.38	2.34 ± 0.39	3.86 ± 0.76	5.82 ± 0.917	6.92 ± 1.04	10.65 ± 2.80
Fe	11.7 ± 1.36	14.98 ± 1.42	18.45 ± 1.65	11.20 ± 0.732	22.40 ± 4.97	28.56 ± 3.45
Ni	0.42 ± 0.129	0.42 ± 0.83	1.32 ± 0.76	0.28 ± 0.45	0.37 ± 0.57	0.56 ± 0.23
Cu	3.42 ± 0.58	4.84 ± 0.56	8.76 ± 0.24	1.79 ± 0.255	4.50 ± 0.72	7.89 ± 1.45
Zn	7.33 ± 0.689	13.71 ± 1.66	20.30 ± 2.34	17.82 ± 1.609	19.80 ± 1.77	25.43 ± 3.12
Cd	2.1 ± 0.294	3.36 ± 0.35	4.54 ± 0.42	2.67 ± 0.33	4.60 ± 0.25	5.65 ± 0.45
Pb	1.20 ± 0.300	2.13 ± 0.31	4.67 ± 1.25	2.01 ± 0.425	1.86 ± 0.16	4.25 ± 1.68

Table 2 Allowable and acceptable limits of trace elements in plant food sources (FAO/WHO 1989; FEPA 1991)

Standards	Elements						
WHO standards (mg kg^{-1} limit)	Cr	Ni	Zn	Cu	Mn	Pb	Cd
Maximum acceptable	0.05	0.05	5.0	1.0	0.05	0.05	–
Maximum allowable	0.05	0.05	15.0	1.5	0.5	0.05	–
Spirulina food safety: US and Japan growers	–	–	–	–	–	<1.0	<0.05

and the Spirulina Food Safety Guidelines for the US and Japan growers and the Federal Environmental Protection Agency (FEPA 1991) i.e., <0.05–1.0 ppm (Table 2). The leaves of *B. campestris* had the peak concentration of Cr (0.16 ± 0.051), Mn (1.22 ± 0.38), Fe (11.7 ± 1.36), Ni (0.42 ± 0.129), Cu (3.42 ± 0.58), Zn (7.33 ± 0.689), Cd (2.1 ± 0.294) and Pb (1.20 ± 0.300) while in the leaves of *S. oleracea,* Cr (0.82 ± 0.36), Mn (5.85 ± 0.92), Fe (11.20 ± 0.73), Ni (0.28 ± 0.045), Cu (1.79 ± 0.24), Zn (17.8 ± 4.6), Cd (2.67 ± 0.33), and Pb (2.0 ± 0.43) were recorded. The high contents of trace metals in the leafy parts may be due to rapid transportation after the uptake from the soil as a result of ambient drift into stomatal and lenticel openings. Collectively, in the leaves of *S. oleracea,* concentrations of the examined elements were considerably high. The roots of *B. campestris* bio-accumulated higher levels of Cr, Cu, Pb, and Ni, and lower levels of Mn, Fe, and Zn than those of *S. oleracea*. Copper accumulation was at par in the roots of both vegetables. The concentrations of Cr, Mn, Fe, Zn, and Cd in the stem of *S. oleracea* were higher than those in *B. campestris*. However, concentrations of Ni, Cu, and Pb were lower in *S. oleracea* compared to those in *B. campestris*. The leaves of *B. campestris* accumulated higher concentrations of Ni, Cu, Zn, Cd, and Pb than those in *S. oleracea,* while the reverse was true for leaf Mn and Fe. Bioavailability of certain trace metals in soil is proportional to the uptake of these metals by plants (Table 3).

This small bioconcentration could be attributed to the stem because it has well-developed transportation system containing xylem and phloem, which in turn facilitates movement of metals either from root to foliage via xylem, along with water or from foliage to roots via phloem, bound with manufactured food molecules. It is evident from some previous studies that the cells of stem tissue receive food from foliage resulting in bioconcentration of trace metals through this pathway (Tyokumbur and Okorie 2011). The bioconcentrations of metals in *S. oleracea,* and *B. campestris,* recorded in the present study were significantly higher than those recorded by Ademoroti (1996) for different vegetables. The difference in the results between these two studies may be due to the lack of a point source effluent impact in the studied areas.

Table 3 Bioconcentration factors of trace metals from soil to vegetable parts

Metals	*Brassica campestris*			*Spinacia oleracea*		
	Leaves	Roots	Stems	Leaves	Roots	Stems
Cr	0.015	0.12	0.25	0.077	0.12	0.35
Mn	0.005	0.011	0.016	0.025	0.03	0.05
Fe	0.018	0.023	0.029	0.017	0.035	0.045
Ni	0.091	0.103	0.325	0.068	0.091	0.138
Cu	0.402	0.569	1.030	0.21	0.53	0.93
Zn	0.14	0.25	0.37	0.34	0.38	0.45
Cd	0.43	0.69	0.93	0.55	0.94	1.16
Pb	0.091	0.161	0.36	0.15	0.14	0.33

As far bioconcentration factor (BF) for the transfer of trace metals from soil to the tissues showed an increasing tendency of bioaccumulation

Soil levels of trace metals and bioconcentration factors

Levels of trace metals in the soil of the vegetable bed were as follows: Cr: 10.52, Mn: 230, Fe: 634, Ni: 4.06, Cu: 8.5, Cd: 4.88, and Pb: 13.15 mg kg^{-1}. Mean values of metal concentrations were ordered as mentioned, Fe > Mn > Zn > Pb > Cr > Cu > Cd > Ni. Noticeably these values were higher than the prescribed safe values reported by FEPA (1991). During the course of a growing season, deposition of trace metals increases as they are irrigated with contaminated effluent water. The bioconcentration factor can be explained as the ratio of the trace elements between plant tissue and their immediate environment. BF was calculated from the results shown in Table 3 by using the following formula:

$$BF: \frac{\text{Trace element concentration in vegetable tissues}}{\text{Trace element concentration in soil}}$$

The transfer rate of different metals from soil to plant tissue has already been established (Chamberlain 1983; Harrison and Chirgawi 1989), but Voutsa et al. (1996) have also evaluated air accumulation factors (AAF) and concentration factors (CF) in relation to soil and air particulate matter. Our calculated BF values (Table 3) depict that the leaves of *S. oleracea* more efficiently accumulated soil Cr, Mn, Zn, Cd and Pb as compared to the leaves of *B. campestris.* Higher BF values of Fe, Zn, and Cd in the roots and those of Cr, Mn, Fe, Zn, and Cd in stem of *S. oleracea* were also observed. Fe (0.018), Ni (0.091) and Cu (0.402) were more accumulated in the leaves of *B. campestris*. Likewise, BF values for Mn, Ni, Cu and Pb in the roots and Ni, Cu and Pb in the stem were also higher in *B. campestris.* Mn was accumulated less efficiently in the leaves and roots of *B. campestris* (BF-0.01), while Cu levels were highest in the stem (BF-1.03). Variations in the bioconcentration factor value of trace metals among different plant tissues may be due to difference in soil properties like, trace metal deposition in soil, their form and may be due to plant physiological phenomena like uptake efficiency and dynamics of growth factors (Tyokumbur and Okorie 2011). Concentration values of metals in the vegetables reported in the present study were lower than those found in the previous investigation (Voutsa et al. 1996). This investigation shows lower bioaccumulation which may be ascribed to the variation in geographical location of the sampling sites, soil mineral contents and in addition to different bio-accumulation potential of different vegetables.

Conclusion

It is concluded from the present study that foliage levels of trace metals were high in *S. oleracea* and *B. campestris*. The levels of trace metals that are critical components of some enzymes and body including Mn, Cu, Zn, Pb and Ni were also unacceptably high in the stems and roots. Although the consumption of *S. oleracea* and *B. compestris* is beneficial to human health because of their nutritional value, the consumption of contaminated vegetables may pose a long-term public health risk due to ease of bioconcentration of toxic trace metals by these vegetables.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflict of interests regarding the publication of this paper.

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