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ASSESSMENT OF HEAVY METAL AND METALLOID LEVELS IN SPINACH (*SPINACIA OLERACEA* L.) GROWN IN WASTEWATER IRRIGATED AGRICULTURAL SOIL OF SARGODHA, PAKISTAN

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Abstract

Heavy metal-induced pollution causes an adverse effect on the agricultural production system. In the present study we studied the pattern of accumulation of different metals in spinach (*Spinacia oleracea)* irrigated with wastewater. Considerably elevated concentrations of metals were observed in the wastewater irrigated soil and vegetable. For example, the levels Mo, Cd and Pb in the vegetable exceeded the permissible limit suggested by the World Health Organization. A significant and positive correlation was observed between the soil and vegetable in terms of levels of different metals. Transfer coefficients for Cr, Zn and Cu were above 0.5 which indicated high level of metal contamination mainly due to anthropogenic activities in the study site. The pollution load index was also considerably high for the spinach plants growing on the metal contaminated soil suggesting that proper management of the study area is required to protect all vegetables being grown therein from the accumulation of high levels of different metals examined in the present study.

Key words: Heavy metal, Metalloid, *Spinacia oleracea*, Wastewater, Soil.

Introduction

As compared with major commercial crops, vegetables are grown on a small scale, but the vegetable productivity entirely depends on the availability of good quality water. However, the area of vegetable cultivation in Pakistan is increasing consistently with time because of the reason that several vegetables are now being exported to other countries. For example, during 2007-08 vegetables were cultivated at about 253,800 ha (Perveen *et al.,* 2010). However, most of the commonly used vegetables are grown in peri-urban areas wherein farmers use polluted water coming from sewage for irrigating all vegetables. Thus, it is naïve to expect that all vegetables from peri-urban areas accumulate considerable amount of different heavy metals the source of which being the sewage water (Tahir *et al.,* 2011).

Wastewater is believed to contain a variety of toxic metals, and accumulation of such metals in plants may take place due to extensive use of waste-water for irrigation of crops (Singh *et al*., 2004). Since the removal of metals from soils is very hard, so they easily move to the food cycle (Wilson and Pyatt, 2007).

Spinach (*Spinacia oleracea*) is a potential leafy vegetable of many countries (Chopra *et al*., 1986). Compared with the root or stem vegetables, leafy vegetables including *Brassica oleracea* and *Spinacia oleracea* can accumulate considerably higher amounts of different metals in their leaves if grown on metal-contaminated soils (Al-Jassir *et al.,* 2005).

The present investigation was carried out to explore soil and vegetable metal pollution load, ascertain the suitability of wastewater for irrigating soils for the cultivation of spinach, a leafy vegetable grown round the year in most parts of Pakistan, and evaluate the pattern of accumulation of metals in this crop.

Materials and Methods

Study area: The present study was carried out at two different sites Phularwan and Chaba Purana, within District Sargodha. The experimental area is located at coordinates 32° 21' 20.52" N, 73° 00' 44.67" E. Site-I, Phularwan, is located at 50 km from Sargodha city, irrigated with canal water, whereas Site-II, Chaba Purana, is located at 67 km from Sargodha city, irrigated with wastewater.

Sample collection: Ten samples each of soil and vegetable leaves were collected from each of the two selected sites. The vegetable samples were washed using distilled water and diluted HCl so as to remove air-born contaminants, if any. These samples were dried first in sunlight and then oven-dried at 65°C for 48 h. Thereafter, all samples were kept in clean plastic bags before their analysis.

Sample preparation: Well ground vegetable and soil samples (each 2 g) were digested in H_2O_2 and H_2SO_4 in 2:1 on a hot plate for one hour, and then 2 ml of H_2O_2 were added to each sample. The digestion process continued until the digestion mixture of each sample became transparent. The digested mixture, after filtration, was diluted to 50 ml using distilled water.

Determination of metals and metalloids: For determining the concentrations of metals and metalloids in the digested soil and vegetable samples, an atomic absorption spectrophotometer was used. Particularly for the analysis of As and Se, a graphite furnace attached to an atomic absorption spectrophotometer (GFAA) was used. A standard calibration curve was drawn for each metal and metalloid. All standard quality assurance procedures were adopted to attain precise data for each metal.

Statistical analysis: The average metal and metalloid concentrations in the soil and vegetable were worked out. One-way ANOVA was applied for both soil and vegetable data using the SPSS package. Correlation between soil and vegetable with respect to each metal concentration was also worked out. The differences among mean values of each soil or vegetable metal were determined using the Least Significance Difference (LSD) test at 0.05, 0.01 and 0.001 probability levels following Steel & Torrie (1980).

Bioconcentation factor (BCF): To evaluate the transfer of metal contents (mg kg^{-1}) from soil to vegetable, a bioconcentration factor was worked out following Cui *et al*., (2004).

BCF= Vegetable metal level / soils metal level

Pollution load index (PLI): The pollution load index for each sampling site was worked out following Liu *et al*., (2005).

PLI = Metal level determined in investigated soil/ reference value of soil metal

Results

Analysis of variance showed a non-significant effect (p<0.05) of sites on soil Cr and Cu, while the reverse was true in case of Mo, Fe, , Ni, Co, Zn, As, Mo, Se, Cd and Pb. From the analysis of variance it was revealed that there was a significant effect (p<0.05) of the sites on Cr, Mn, Fe, Ni, Co, Zn, Cu, As, Se, Cd, Mo, and Pb concentrations in *S. oleracea* (Table 1).

Table 1. Analysis of variance of data for metal contents in soil and *S. oleracea* at two different sites.

Metals and metalloids	Soil grown with *S. oleracea*	*S. oleracea*
Cr	0.001^{ns}	475.5^{***}
Mn	560.8^{***}	543.8^{***}
Fe	63.65^{**}	45.75^{***}
Co	291.3^{***}	0.55^{***}
Ni	9.77^{***}	78.09^{***}
Cu	1.42^{ns}	229.8^{**}
Zn	15.02^{***}	907.9^{***}
As	34.83^{*}	36.64^{***}
Se	2.00^{**}	0.27^{***}
Mo	7.29^{**}	396.5^{***}
Cd	51.03^{***}	0.45^{***}
Pb	119.4^{***}	217.5^{***}

, **, ** significant at 0.05, 0.01, and 0.001 levels; ns, non-significant

Metal and metalloid concentrations (mg/kg) in soil: At both sites, oils metal and metalloid levels were recorded lower than the critical values, while the reverse was true for As and Cd at both sites (Table 2). At both sites, concentration of metals and metalloids in soil grown with *S. oleracea* was in the order: As> Fe> Pb> Mn> Co> Cd> Mo>Zn> Cu>Ni> Se as shown in Fig. 1.

Metal and metalloid concentrations (mg/kg) in vegetable: The mean concentrations of metals and metalloids (Cr, Mn, Fe, Co, Zn, Cu, Ni, and Se) in the vegetable recorded in the present study at both sites and As at site-I were lower, while concentrations of Mo, Cd, and Pb at both sites and As at site-II were higher as compared to the permissible limits (Table 3). The order of accumulation of metals and metalloids in *S. oleracea* at Site-I was: Mn> Zn > Fe > Cu > Cr > Mo > Ni >Pb> As > Cd > Co > Se, while at Site-II: Se < Co < Cd < As < Ni <Pb< Mo < Cu < Cr < Fe < Zn <Mn (Fig. 2).

Bioconcentration factor: The bioconcentration factor was calculated as the metal and metalloid concentrations in the edible part of the vegetable related to the concentrations of the respective metals in the soil. Highest BCF was observed at site-II irrigated with wastewater. The order of bioconcentration factor at site-I was Co < Cd < As < Pb < Se < Fe < Mo < Ni < Mn < Cu < Zn < Cr, while at site-II it was Co < Cd < As < Se < Pb < Fe < Mn < Mo < Ni < Cu < Zn < Cr (Table 4).

Table 2. Metal and metalloid concentrations (mg kg^{-1} ± SE) in soil samples at two different sites.

Metals and metalloids	Sampling sites		PML (µg/g)[a]
	Site-I	Site-II	
Cr	0.08 ± 0.013	0.09 ± 0.01	100
Mn	18.5 ± 1.02	33.4 ± 1.87	2000
Fe	43.5 ± 0.47	48.4 ± 1.07	50000
Co	14.4 ± 0.91	25.2 ± 0.89	50
Ni	2.51 ± 0.15	4.48 ± 0.41	50
Cu	3.86 ± 0.24	4.62 ± 0.31	100
Zn	7.23 ± 0.43	9.66 ± 0.49	300
As	56.4 ± 0.77	60.2 ± 1.16	02020
Se	2.42 ± 0.06	3.32 ± 0.21	10
Mo	7.34 ± 0.43	9.04 ± 0.11	40[b]
Cd	11.1 ± 0.62	15.6 ± 0.45	3
Pb	39.5 ± 1.04	46.4 ± 0.37	100

PML = Permissible maximum limit; S.E = Standard Error
[a]Source: Chiroma *et al*., (2014); [b]Source: Anon., 1997

Table 3. Metal and metalloid concentrations (mg kg^{-1} ± SE) in *Spinacia oleracea* collected from two different sites.

Metals and metalloids	Sampling sites		PML (µg/g)[a]
	Site-I	Site-II	
Cr	16.5 ± 0.52	30.7 ± 0.39	50[b]
Mn	68.1 ± 1.33	82.8 ± 1.07	500
Fe	46.9 ± 2.47	71.9 ± 2.66	425
Co	0.64 ± 0.03	1.11 ± 0.04	50
Ni	8.97 ± 0.25	14.6 ± 0.45	67
Cu	22.8 ± 2.02	31.8 ± 0.74	73
Zn	62.6 ± 1.05	81.9 ± 1.11	100
As	4.48 ± 0.33	8.31 ± 0.35	7[b]
Se	0.62 ± 0.04	0.93 ± 0.02	-
Mo	13.7 ± 1.71	26.2 ± 0.81	5[b]
Cd	0.74 ± 0.04	1.16 ± 0.07	0.10
Pb	8.58 ± 0.53	17.9 ± 0.51	0.3

PML = Permissible maximum limit; S.E = Standard Error
[a]Source: Chiroma *et al*., (2014); [b]Source: Anon., 1996

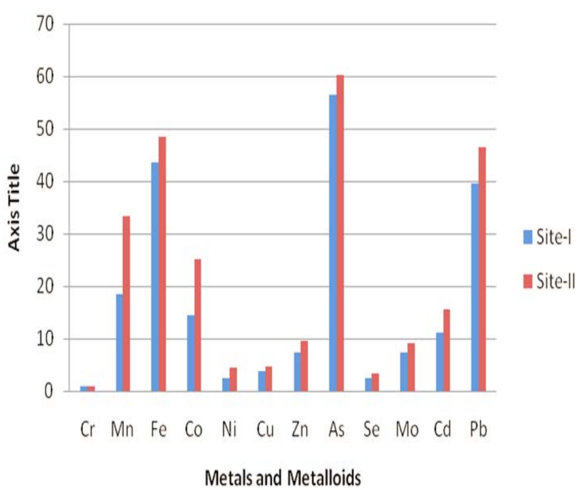

Fig. 1. The fluctuation in metal and metalloid concentrations (mg/kg) in soil at both sites.

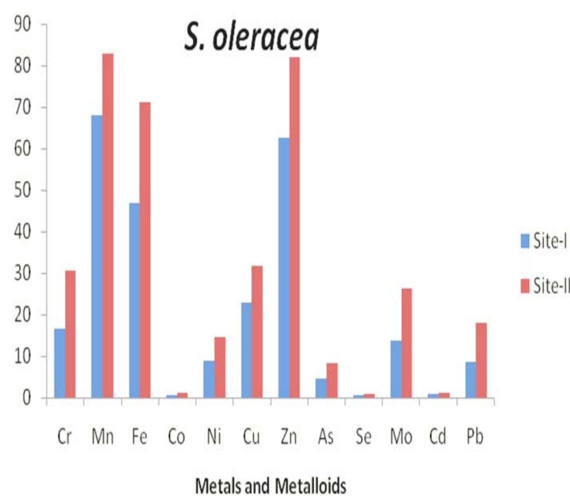

Fig. 2. The fluctuation in metal and metalloid concentrations in *S. oleracea* at both sites.

Table 4. Bioconcentration factor for vegetable/soil system.

Study sites	Bioconcentration factor											
	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Pb
Site-I	216.8	3.68	1.08	0.04	3.59	5.75	8.69	0.08	0.25	1.86	0.06	0.22
Site-II	336.3	2.47	1.48	0.04	3.25	6.88	8.46	0.14	0.28	2.9	0.08	0.39

Correlation between soil and vegetable with respect to metal levels: The correlation between soil and vegetable was significant and positive for Fe, Mn, Ni, Co, Ni, Mo, Se, As, Pb and Cd, while non-significant for Cr and Cu (Table 5).

Pollution load index: Contamination level in soils can be analyzed using pollution load index (Angula, 1996). The order of pollution load index for heavy metals in soil was: Pb > Mo > As > Cr > Co > Ni > Cd > Se > Fe > Cu > Zn > Mn (Table 6).

Discussion

High levels of metals and metalloids in water are believed to raise the levels of these metals in soil irrigated with wastewater. Lead (Pb) showed highest concentration in the areas irrigated with the sewage water as compared to the soil irrigated with fresh water.At wastewater irrigated areas, low concentrations of heavy metals and metalloids suggest their continuous removal from vegetables and other crops as well as due to the discharge of heavy metals in the deeper sediments of soil (Beausse, 2004).Leaves of the vegetables contain more Ni, Fe than their edible parts (Rehman *et al.,* 2013). Edible portions of different vegetables have an average concentration of Ni, Pb, Cd and Cr as reported by Perveen *et al.,* (2012). In the present investigation, mean concentrations of Zn, Cu and Cr in the soil were higher while those of Ni and Pb lower as reported by Sharma *et al*., (2007). Mo concentrations were low, whereas those of As and Cd high at both sites. The pH and organic matter of soil are responsible for high concentrations of heavy metals. High soil Zn concentrations were recorded from Dinapur area, India (Singh *et al*., (2004)) and in the soil of Najafgarh, Delhi (Singh & Kumar, 2006) wherein the principal factor of metal contamination was wastewater irrigation. The mean soil Cd concentration recorded in the present investigation was much over 3µg/g, a critical value reported by Indian Standards (Awashthi, 2000).

Considerable variation was found among the mean concentrations of metals and metalloids from canal and wastewater irrigated *S. oleracea* samples. Considerable variation in the levels of heavy metals in the vegetable samples of the same site could be attributable to a variety of factors including the differences in uptake, retention, and exclusion of a specific metal (Kumar *et al.,* 2009). Mean concentrations of metals and metalloids at site-II were many-fold higher than those at site-I. Continuous use of wastewater at site-II increased the intrinsic levels of heavy metals in *S. oleracea*. Presence of high amounts of toxic metals in wastewater irrigated vegetables was also reported by Kawatra & Bakhetia (2008). In the present investigation, mean levels of Cd, Pb, Cr and Cu were higher and those of Cu lower as reported by Liu *et al*., (2006). Among different leafy vegetables commonly used by humans (spinach, amaranthus and cabbage), spinach is believed to accumulate high amounts of metals in its leaves. The levels metals reported here are greater than those suggested by Sinha *et al*., (2006). However, the Se levels at both sites were good enough. These results are parallel to those of Tisdale *et al*., (1993) in which it was reported that soils with high pH and calcium receiving low rainfall usually possess high amount of Se.

Table 5. Correlation between soil and *Spinacia oleracea* with respect to different metals.

Metals and metalloids	Correlation: Soil-vegetable
Cr	0.304^{ns}
Mn	0.885^{**}
Fe	0.881^{**}
Co	0.893^{**}
Ni	0.860^{**}
Cu	0.583^{ns}
Zn	0.763^{*}
As	0.748^{*}
Se	0.733^{*}
Mo	0.849^{**}
Cd	0.872^{**}
Pb	0.842^{**}

^{ns} = Non-significant, * = Correlation is significant at the 0.05, ** = Correlation is significant at the 0.01

Table 6. Pollution load index for metals and metalloids in soil.

Metals and metalloids	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Mo	Cd	Pb
Soil	1.84	1.22	1.52	1.73	1.62	1.43	1.3	1.85	1.55	1.92	1.57	2.09

The present investigation showed high bioconcentration factor for Cr and low for Co. Soil pH is believed to be one of the critical soil properties that ascertain Cr and Co availability to vegetable crops (Adriano & Page, 1981). Metal uptake by plants is affected by a number of factors such as soil pH, soil metal levels, soil organic matter content, soil cation exchange capacity, plant age, and types and crop varieties (Barancikova *et al*., 2004), however, soil metal concentration is reported to be the dominant factor (Adriano, 1986). BCF was high for Cr, Zn and Cu and low for Cd, As and Se at both sites. Such a difference could be due to varied uptake rate for different metals under different conditions by different vegetables. Leafy vegetables especially *Spinacia oleracea* showed strong accumulation capacity for respective metals. Liu *et al*., (2005) have also reported low BCF values for Zn, Pb and Cd and high for Cr in vegetables.

Correlation between the soil and vegetable with respect to different metals was highly significant in the current investigation. A positive correlation was found for Fe, Mn, Ni, Co, Cd, Mo, and Pb which indicates that these metals might have been derived from a collective source i.e. wastewater. The strong association of Zn, As and Se indicated that these metals might have been originated from man-made sources such as paint industry and sewage waste-water. In another study, a positive non-significant correlation with Cu and a significant correlation with Ni and Mn were reported (Szabo & Czeller, 2009). In the present study, a positive non-significant correlation of Cr and Cu was due to a weak relationship between the vegetable and the soil. Ekmekyapar *et al*., (2012) have also found a non-significant correlation between concentrations of heavy metals and distance.

Pollution severity and its inconsistency in soil were appraised using the pollution load index (PLI). PLI values greater than 1 are considered as contaminated, whereas PLI values lower than 1 as uncontaminated (Harikumar *et al.,* 2009). The contamination factor observed for Mn, Fe, Zn, Cu, Cr, Co, Ni, Se, As, Mo, Pb and Cd were greater than 1. High PLI was due to different factors including industrial activities, agricultural runoff and several man-made activities (Maiz *et al*., 2000; Usman & Ayodele, 2002; Uwah *et al*., 2009).

However, relatively higher pollution load index values at sewage treated sites could be due to increased human activity in such populated municipal areas. As in spinach, Singh *et al*., (2010) also recorded higher metal pollution index in *Momordica charantia* L., so the consumption of such vegetables with high metal accumulation may cause hazardous effects in humans. Thus, the vegetables grown on wastewater-irrigated soils may contain large quantity of heavy metals, and thus are of primary concern for human health.

Conclusion

The wastewater irrigation for a long time period led to contaminate soils with a variety of toxic metals as well as and *Spinacia oleracea* growing thereon. Considerable variation was found in the mean metal concentrations of soil and the vegetable samples analyzed. Such variations were observed due to different geological and ecological conditions of the area under study. The PLI values in the present investigation indicated that the waste-water irrigated soil was highly contaminated. Thus, a regular consumption of this vegetable along with other such vegetables accumulating high levels of different metals should be avoided.

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