

## METAL ACCUMULATION IN A POTENTIAL WINTER VEGETABLE MUSTARD (*BRASSICA CAMPESTRIS* L.) IRRIGATED WITH DIFFERENT TYPES OF WATERS IN PUNJAB, PAKISTAN

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### Abstract

Considering the harmful effects of metal-enriched vegetables a comprehensive study was conducted to appraise the extent of accumulation of different metals in mustard (*Brassica campestris* L.). The vegetable was treated with ground water, sewage water and canal water irrigation in areas of Punjab, Pakistan. Metals and metalloids observed in all three sites treated with sewage, canal and ground water were As, Cu, Fe, Ni, Pb, Mo, Se and Zn were observed in the sites treated with ground, sewage and canal waters as well as the vegetable grown therein. The metal concentration observed in water samples was: Fe>Zn >Pb> Ni> Mo> Cu> As> Se, the order in the soil was: As >Pb> Fe > Ni > Mo > Cu > Zn > Se, while the order in the vegetable was: Zn > Fe> Cu> Ni> Mo>Pb> As> Se. The values of bio-concentration factor varied from 0.09-15.47 mg kg<sup>-1</sup>. Correlation was positively significant for *Brassica campestris* and soil except Ni and Se which showed positive non significant correlation. Pollution load index was observed to be in the following order: As >Pb> Ni > Mo >Fe > Cu > Se > Zn in the sites GWI, CWI and CWI. Fe and Zn (0.169) showed highest value of daily intake of metal (DIM), while Se (0.003) showed lowest value in crop of all three sites GWI, CWI and CWI. The health risk index and EF ranged from 0.24-69.86 mg day<sup>-1</sup> and 0.134-14.12 mg day<sup>-1</sup>, respectively. Overall, the vegetable treated with sewage water may have considerable impact on food quality and in turn on the health of people consuming it.

**Key words:** *Brassica campestris*, Metals and metalloids, Pollution load index, Health risk index.

### Introduction

Although the use of domestic waste water is indispensable for peri-urban agriculture, it causes a variety of public and legal issues, because being rich in metals can easily transfer these metals to the food chain. Thus, it has straightforwardly or ultimately unfavorable possessions on human health (Wang *et al.*, 2003; Singh & Agrawal, 2010). Furthermore, sewage water can easily transfer metals to soil and then ultimately to plants (Dai *et al.*, 2006). However, specifically the plants used for vegetables can accumulate metals in the edible portion (Khan *et al.*, 2010). Metals have a higher tendency to be built-up in plants than in soil (McGrath *et al.*, 2001). Human beings when consume such metal enriched plants the metals are built-up in human body (Sharma *et al.*, 2008).

Mustard (*Brassica campestris* L.) is one of the important oil crops of the world, although it is used as a very common vegetable in some countries (Sood *et al.*, 2010). For vegetable purpose this crop is mostly grown in peri-urban areas wherein sewage water is commonly used for irrigation purpose. Thus, it is naïveto expect that this crop uptakes and accumulates large quantity of metals from the soil irrigated with sewage water containing high amounts of a variety of metals. With this in mind the present study was conducted to discover the metal concentration in soil and vegetable and in this vegetable was treated with different types of waters including sewage water, and ascertains whether this vegetable is suitable for edible purpose or not.

### Materials and Methods

**Study area:** Khushab City of Punjab, Pakistan was selected for current study. The present area was located at the coordinate of 32.30°N 72.34°E. In summer the temperature is 49°C and in winter 5-23°C and the rainfall is 526 mm approximately annually. Samples of water, soil and plants were taken from the sites in Joiya, Talokar and Khushab irrigated with ground, canal and sewage water. Samplings of the vegetable and soil from the three sites were completed in January 2015. Collection of soil and vegetable replicate samples was done for each site. The Site-I was irrigated with groundwater, Site-II with canal water, and the Site-III with wastewater.

### Sampling

**Water:** Samples (each 100 ml) of groundwater, canal water and wastewater were taken in well washed plastic bottles. 1 ml of conc. HNO<sub>3</sub> to each water sample was added to avoid growth of microbes.

**Soil:** The soil samples for each site in which vegetables were grown were collected haphazardly and the depth of soil was considered as 20cm. The estimated amount of 1 kg of soil was selected for analysis and collected for each type of soil treated with sewage, canal and ground water treated sites. First of soil was dried under the sun light and in this way moisture is evaporated and further moisture is removed by placing the soil samples in air forced oven at 72°C for 4 to 5. When the moisture is thoroughly removed the samples were subjected to be grinded and stored in plastic bags.

**Vegetable:** Randomly samples of edible portions of vegetables were collected from three different sites. Distilled water was used to wash vegetables. The vegetable samples were dried under sunlight and then placed in convection oven for 6 days at temperature was maintained at about 70°C for drying. After drying samples were crushed for further analysis.

#### Digestion of water, soil and vegetable samples

**Water:** All different types of water with concentrated HNO<sub>3</sub> each with 10 ml at 80°C. After the digestion was complete the final volume of each sample was raised to 50 ml by adding distilled water and stored in plastic bottles.

**Soil:** For digestion 1 gram of soil is used and for this purpose; 4 ml of H<sub>2</sub>SO<sub>4</sub> and 8 ml of H<sub>2</sub>O<sub>2</sub> were added to it, and then subjected to heating in a digestion block. When the fumes from each sample were stopped and H<sub>2</sub>O<sub>2</sub> were added to each sample in quantity of 2ml and digested once again the heating block until a colorless solution resulted. The final volume of each digested sample was brought to 50 ml by adding distilled water and analyzed. Soil physico-chemical properties such as Anne method (modified walkey-Black method) was used for determination of soil organic matter (McLean, 1982). Soil pH and electrical conductivity were measured on 1:2 extract (Soil: Water) (Mathieu & Pieltain, 2003).

**Vegetable:** For digestion of vegetable 1 gram of each sample was used and 15ml mixture of HNO<sub>3</sub>, HClO<sub>4</sub>, and H<sub>2</sub>SO<sub>4</sub> in the ratio 5:1:1 was added at 80°C for 2 h, the process of digestion was carried out till the evaporation ended and then transparent sample is taken out, filtered using Whatman filter paper # 42 and made the volume upto 50 ml and collected in plastic bottles and for identification of samples numbered the bottles.

In all digested samples As, Mo, Cu, Zn, Pb, Fe, Ni, and Se were determined using an atomic absorption spectrophotometer (AASP) Perkin-Elmer AAS-5000 (Perkin-Elmer Corp. 1980). To find out Mo contents in soil and vegetable graphite furnace and D<sub>2</sub> corrector (Perkin-Elmer Model 503) equipped atomic absorption spectrophotometer was used. Fluorometric method following Watkinson (1966) was used to determine Selenium (Se) and As in samples of soil and vegetable. Using arsenate as a standard total arsenic was determined by a flow injection hydride generation AAS (Perkin

Elmer Analyst 400). Repeated sample analysis for all metals and metalloids was done to maintain precision and accuracy of analyses against the National Institute of Standard Technology, Standard Reference Material (SRM 1570) detected in the present study.

**Statistical analysis:** The SPSS software version 20 was used for statistical analysis of data. One-way ANOVA for soil and two-way ANOVA for metals of vegetable were used. With respect to all metals correlations between the vegetable and soil were also determined.

Due to the type of irrigation used for the accumulation of metals in the edible part of the vegetable the bio-concentration factor was worked out to evaluate the exposure and associated revelation (Cui *et al.*, 2004). The method of Liu *et al.* (2005) was used to calculate the approximate pollution load index, as the ratio of predictable daily intake of metal and oral reference dose (Cui *et al.*, 2004) the health risk index was calculated (Anon., 2002). The metal enrichment factor (EF) was determined according to Buat-Menard & Chesselet, 1979).

#### Results

**Water:** Analysis of variance showed significant ( $p < 0.05$ ) As, Cu, Fe, Mo, Ni, Pb, Se and Zn. The SWI contain higher concentrations of metals and metalloids than site of GWI and CWI (Table 1). In present investigation at three sites recorded mean concentrations were below the permissible limits in As, Cu, Fe, Ni, Se and Zn except Mo and Pb. The heavy metal concentration in vegetable was in the order: Fe > Zn > Pb > Ni > Mo > Cu > As > Se (Table 2).

**Table 1. One-way ANOVA of metal concentrations in water at three different sites.**

Metals and metalloids	Sites
As	0.002*
Cu	0.001**
Fe	0.03***
Mo	0.001**
Ni	0.003**
Pb	0.006**
Se	0.001**
Zn	0.008***

\*, \*\*, \*\*\* significant at 0.05, 0.01, and 0.001 levels respectively

**Table 2. Metal and metalloid concentrations (mg L<sup>-1</sup>) in irrigation waters from three sites.**

Metals	Mean ± S.E.			Maximum permissible level in (µg/g)
	GWI (Site-I)	CWI (Site-II)	SWI (Site-III)	
As	0.015 ± 0.001	0.016 ± 0.002	0.023 ± 0.001	0.1
Cu	0.017 ± 0.001	0.022 ± 0.001	0.025 ± 0.001	0.2
Fe	0.656 ± 0.008	0.732 ± 0.030	0.854 ± 0.012	5
Mo	0.041 ± 0.006	0.069 ± 0.011	0.081 ± 0.007	0.01
Ni	0.091 ± 0.012	0.108 ± 0.010	0.154 ± 0.007	0.2
Pb	0.214 ± 0.017	0.245 ± 0.016	0.301 ± 0.008	0.1
Se	0.012 ± 0.001	0.014 ± 0.001	0.021 ± 0.001	0.02
Zn	0.574 ± 0.006	0.593 ± 0.002	0.671 ± 0.011	2

GWI, Ground water irrigation; CWI, Canal water irrigation; SWI, Sewage water irrigation

**Table 4. One-way ANOVA of metal concentrations in soil at three different sites.**

Metals and metalloids	Sites
As	29.70**
Cu	2.893***
Fe	373.4**
Mo	9.985***
Ni	3.428**
Pb	94.16***
Se	1.870***
Zn	2.294 <sup>ns</sup>

\*\*\* = Significant at 0.001 level, \*\* = Significant at 0.01 level,  
\* = Significant at 0.05 level, ns = Non-significant

**Physico-chemical parameters of soil:** At all three sites loamy soil was found (Table 3). pH value in the soil at three sites was 7.17-8.18. At site-I the mean value of pH was 8.18, 7.23 at site-II, and 7.17 at site-III. Soil EC at three sites was ranged from 0.82-0.99 dS m<sup>-1</sup> with mean EC 0.82 of site-I, 0.93 of site-II, and 0.99 of site-III. The organic matter present in soil of all three sites ranged from 0.52-0.71 % with mean concentration of organic matter at site-I was 0.51, 0.63 at site-II, and 0.71 at site-III (Table 3).

**Soil metals:** In soil of *Brassica campestris* As, Cu, Fe, Mo, Ni, Pb and Se concentrations were significantly (p<0.05) affected as shown by analysis of variance while non-significant affect was shown by Zn (Table 4). The SWI showed high concentration of metals and metalloids in soil as compared to sites of GWI and CWI. In the current investigation of soil treated with ground water irrigation, canal water irrigation and sewage water irrigation the value of mean concentrations of metal and metalloid (Cu, Fe, Mo, Ni, Pb, Se, Zn) were lower the permissible limits except As (Table 5). The concentration of heavy metals in soil samples was in following order: As > Pb > Fe > Ni > Mo > Cu > Zn > Se (Fig. 1).

**Vegetable metals:** Significant (p<0.05) effect was shown by analysis of variance of sites by metals of vegetable such as As, Cu, Fe, Ni, Pb, Se and Zn, while Mo showed non-significant effect (Table 6). As compared to GWI and CWI the concentration of metals and metalloids in SWI was higher. While comparing the mean concentrations of metals and metalloids with permissible limits particularly those of As, Cu, Fe, Ni, Se and Zn then the value were observed to be lower in present investigation (Table 7). In the vegetable order of heavy metals accumulation was: Zn > Fe > Cu > Ni > Mo > Pb > As > Se (Fig. 2).

**Bio-concentration factor:** The bio-concentration factor was expected as the assessment of heavy metals appropriate for eating portion of a vegetable associated with the concentrations in the soil. The calculated value of bio-concentration observed variation from 0.06-18.87 mg kg<sup>-1</sup> (Table 8). The sequence observed for CWI and GWI was: Zn > Cu > Mo > Fe > Ni > Se > Pb > As. While order observed in case of sewage water irrigation was: Zn > Cu > Mo > Ni > Fe > Se > Pb > As.

**Correlations:** Correlations between the soil and vegetable with respect to Fe, As, Mo, Cu, Pb and Zn were positively significant, whereas positive and non-significant correlation was noticed in case of Ni and Se (Table 9).

**Pollution load index:** Range of metal pollution index in the soils which was irrigated with canal, sewage and ground water irrigation ranged in following sequence: As > Pb > Ni > Mo > Fe > Cu > Se > Zn and in all three sites same order was observed (Table 10).

**Health risk index and Daily intake of metal in Mustard (*Brassica campestris*):** Daily intake of metal (DIM) at all three sites GWI, CWI and CWI was observed to be highest in Fe and Zn (0.169) whereas in case of Se (0.003) its value was observed to be lowest. While highest value was observed in SWI among all three sites. The associated health risk index by the intake of *Brassica campestris* for metals and metalloids ranged from 0.24-69.86 mg day<sup>-1</sup>. The following order was observed for index of risk: As (47.88) > Pb (8.668) > Mo (4.346) > Ni (1.965) > Cu (1.548) > Se (0.531) > Zn (0.458) > and Fe (0.242) at GWI, while at CWI the order was: As (61.33) > Pb (9.734) > Mo (4.848) > Ni (2.331) > Cu (2.056) > Se (0.918) > Zn (0.553) > and Fe (0.279) and for SWI the order was: As (69.86) > Pb (10.95) > Mo (5.516) > Cu (2.732) > Ni (2.447) > Se (1.033) > Zn (0.671) > and Fe (0.346). The health risk was observed to be greater than 1 due to As, Mo, Ni, Cu and Pb. While for Se, Fe, and Zn health risk was less than 1 whereas in SWI the value of Se was higher than 1 (Table 11).

**Enrichment coefficient in *Brassica campestris*:** For the duration of ingestion of *Brassica campestris* at all three sites the enrichment coefficient of metals and metalloids ranged from 0.134-14.12. The practiced sequence of EF for GWI and CWI was: Fe > As > Pb > Mo > Cu > Ni and > Zn whereas that for SWI was Fe > Pb > As > Mo > Cu > Ni and > Zn. EF greater than 1 was observed for Cu, Ni and Zn while Mo, Pb, As and Fe showed less than 1 at all three sites while Mo level was observed to be higher than 1 in case of GWI (Table 12).

**Table 3. Physico-chemical parameters of soil.**

Physico-chemical parameters	pH	EC (dS m <sup>-1</sup> )	Organic matter (%)	Textural class
GWI (Site-I)	8.18 ± 0.195	0.82 ± 0.01	0.51 ± 0.01	Loamy
CWI (Site-II)	7.23 ± 0.141	0.93 ± 0.02	0.63 ± 0.02	Loamy
SWI (Site-III)	7.17 ± 0.196	0.99 ± 0.01	0.71 ± 0.01	Loamy
Mean Squares	1.01**	1.01**	0.03***	

**Table 5. Analysis of variance for metals and metalloids in soil ( $\mu\text{g/g}$ ) grown with *Brassica campestris* irrigated with canal, ground or sewage water.**

Metals	Mean $\pm$ S.E.			Maximum permissible level in soil ( $\mu\text{g/g}$ )
	GWI (Site-I)	CWI (Site-II)	SWI (Site-III)	
As	37.71 $\pm$ 971	40.74 $\pm$ 0.361	44.01 $\pm$ 0.609	20
Cu	2.335 $\pm$ 0.101	2.794 $\pm$ 4.219	4.219 $\pm$ 0.522	100
Fe	21.65 $\pm$ 0.393	24.21 $\pm$ 1.131	42.12 $\pm$ 2.551	50000
Mo	3.793 $\pm$ 0.098	5.348 $\pm$ 0.192	7.429 $\pm$ 0.182	40
Ni	6.161 $\pm$ 0.068	7.041 $\pm$ 0.245	8.288 $\pm$ 0.289	50
Pb	25.68 $\pm$ 0.737	28.16 $\pm$ 0.546	36.38 $\pm$ 0.972	100
Se	1.671 $\pm$ 0.025	1.733 $\pm$ 0.013	3.068 $\pm$ 0.196	10
Zn	1.561 $\pm$ 0.096	2.459 $\pm$ 0.059	3.311 $\pm$ 0.151	300

PML = Permissible maximum limit (Chiroma *et al.*, 2014); S.E = Standard Error, GWI= Ground water irrigation, CWI= Canal water irrigation, SWI= Sewage water irrigation

**Table 6. One-way ANOVA of metal concentration in vegetable at three different sites.**

Metals and metalloids	Sites
As	1.003**
Cu	51.17***
Fe	123.4**
Mo	2.533 <sup>ns</sup>
Ni	2.301**
Pb	1.455**
Se	0.157***
Zn	141.4***

\*\*\* = Significant at 0.001 level, \*\* = Significant at 0.01 level, \* = Significant at 0.05 level, ns = Non-significant

## Discussion

The highest permissible limits of different metals (mg/l) in water reported by WWF (2007) are: Cu (0.2), Se (0.02), As (0.1), Mo (0.01), Fe (5), Pb (0.1), Ni (0.2) and Zn (2.0) and the values of the current investigation were found below the permissible levels except those of Mo and Pb. Our results of sewage water for Cu, Fe, Pb and Ni

were less than the findings of obtained by Lone *et al.* (2003) while for Zn the values were greater. In present investigation for ground water irrigation the concentrations of Cu and Pb were less while for Ni, Fe and Zn were observed to be greater than the standards recommended by Lone *et al.* (2003).

In the soil irrigated with ground, canal and sewage waters at all three sites levels of metals and metalloids were found below the permissible levels while As showed higher value than the permissible values (Chiroma *et al.*, 2014). The levels of soil Ni, Fe, Cu, Pb and Zn observed in the present investigation were minor as contrast to the values reported in another study (Bigdeli & Seilsepour, 2008) which were as (in mg/kg): Ni(32), Fe(532), Cu(54), Pb(60) and Zn(190). The levels metals particularly Mn, Fe, Cu and Zn in soil observed in the current investigation were also lower than those reported by Naser *et al.* (2009) in waste water in Bangladesh.

Lower permissible levels were observed at all three sites irrigated with canal, ground and sewage waters by the determined amount of different metals and metalloids in the *Brassica* vegetable while Mo and Pb showed values higher than permissible limits (Chiroma *et al.*, 2014).

**Table 7. Analysis of variance of data for metals and metalloids ( $\mu\text{g/g}$ ) in *Brassica campestris* treated with canal, ground or sewage water.**

Metals	Mean $\pm$ S.E.			Maximum permissible level in ( $\mu\text{g/g}$ )
	GWI (Site-I)	CWI (Site-II)	SWI (Site-III)	
As	2.498 $\pm$ 0.195	3.201 $\pm$ 0.117	3.645 $\pm$ 0.303	7
Cu	10.77 $\pm$ 0.128	14.31 $\pm$ 0.304	19.01 $\pm$ 0.415	73
Fe	29.42 $\pm$ 0.903	33.98 $\pm$ 1.845	42.08 $\pm$ 2.266	425
Mo	6.803 $\pm$ 0.325	7.588 $\pm$ 0.122	8.635 $\pm$ 0.115	5
Ni	6.833 $\pm$ 0.468	8.106 $\pm$ 0.166	8.512 $\pm$ 0.116	67
Pb	5.276 $\pm$ 0.198	5.925 $\pm$ 0.151	6.668 $\pm$ 0.149	0.30
Se	0.461 $\pm$ 0.029	0.798 $\pm$ 0.024	0.898 $\pm$ 0.221	-
Zn	29.47 $\pm$ 0.312	35.57 $\pm$ 1.106	43.17 $\pm$ 2.016	100

PML = Permissible maximum limit (Chiroma *et al.*, 2014); S.E = Standard Error  
 GWI= Ground water irrigation, CWI= Canal water irrigation, SWI= Sewage water irrigation

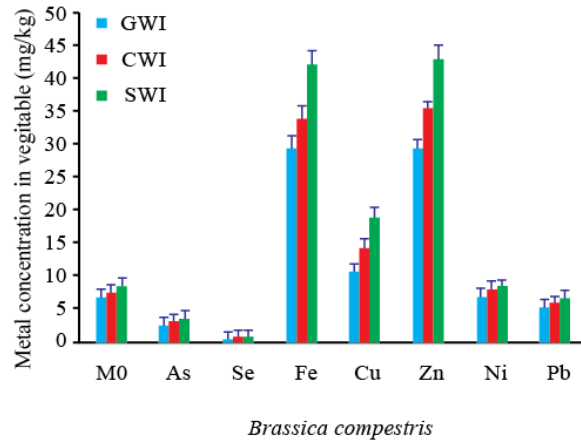
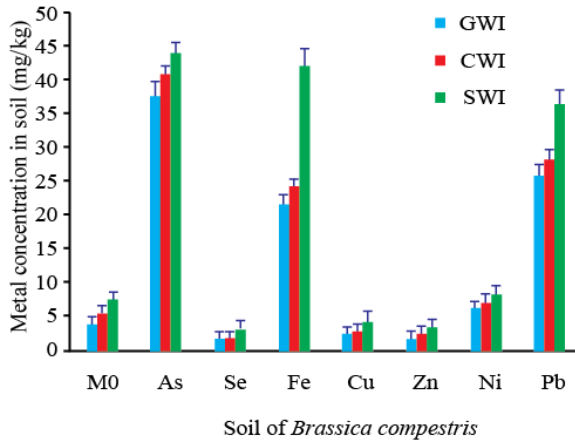


Fig. 1. The fluctuation in soil grown with *Brassica campestris* irrigated with ground, canal and sewage water.

Fig. 2. The fluctuation in *Brassica campestris* concentration during Ground, Canal and Sewage water irrigation.

**Table 8. Bio-concentration factor for vegetable/soil system with reference to *Brassica campestris*.**

Study sites	Bio-concentration factor							
	Mo	As	Se	Fe	Cu	Zn	Ni	Pb
GWI (Site-I)	1.793	0.066	0.276	1.358	4.611	18.87	1.109	0.205
CWI (Site-II)	1.419	0.078	0.461	1.404	5.118	14.46	1.152	0.211
SWI (Site-III)	1.162	0.082	0.293	0.999	4.504	13.04	1.026	0.183

**Table 9. Correlation between the soil and vegetable with respect to metal concentration.**

Metals and metalloids	As	Cu	Fe	Mo
Soil-vegetable	0.949**	0.853**	0.853**	0.933**
Metals and metalloids	Ni	Pb	Se	Zn
Soil-vegetable	0.787 <sup>ns</sup>	0.790*	0.650 <sup>ns</sup>	0.913**

**Table 10. Pollution load index for metals and metalloids in the soil and vegetable.**

Study sites	Pollution load index							
	Mo	As	Se	Fe	Cu	Zn	Ni	Pb
GWI (Site-I)	0.416	12.56	0.057	0.381	0.278	0.035	0.680	3.151
CWI (Site-II)	0.587	13.58	0.059	0.425	0.333	0.055	0.777	3.455
SWI (Site-III)	0.816	14.66	0.105	0.740	0.502	0.074	0.914	4.464
Ref. values mg kg <sup>-1</sup>	3.0	29.0	0.7	56.90	8.39	44.19	9.06	8.15

(Anon., 2000); (Singh *et al.*, 2010b); (Dosumu *et al.*, 2005);(Singh *et al.*, 2010a)

**Table 11. Health risk intake (HRI) and daily intake of metals (DIM) of metal contents via intake of *Brassica campestris* from wastewater irrigated sites.**

Study sites	Hazard quotient	Metals and metalloids							
		Mo	As	Se	Fe	Cu	Zn	Ni	Pb
GWI (Site-I)	DIM	0.039	0.014	0.003	0.169	0.062	0.169	0.039	0.031
	HRI	4.346	47.88	0.531	0.242	1.548	0.458	1.965	8.668
CWI (Site-II)	DIM	0.043	0.0184	0.005	0.195	0.082	0.204	0.047	0.034
	HRI	4.848	61.33	0.918	0.279	2.056	0.553	2.331	9.734
SWI (Site-II)	DIM	0.049	0.021	0.005	0.242	0.109	0.248	0.048	0.038
	HRI	5.516	69.86	1.033	0.346	2.732	0.671	2.447	10.95

**Table 12. Enrichment coefficient of metals and metalloids in *Brassica campestris*.**

Study sites	Enrichment Factor (EF)						
	Mo	As	Fe	Cu	Zn	Ni	Pb
GWI (Site-I)	1.076	0.274	0.181	3.868	14.12	6.699	0.334
CWI (Site-II)	0.851	0.325	0.187	4.294	10.82	6.955	0.342
SWI (Site-III)	0.697	0.343	0.134	3.779	9.761	6.202	0.298
Ref <sub>soil</sub>	3 <sup>c</sup>	29 <sup>e</sup>	56.9 <sup>f</sup>	8.39 <sup>g</sup>	44.19 <sup>g</sup>	9.06 <sup>g</sup>	8.15 <sup>g</sup>

Source <sup>a</sup> Chiroma *et al.* (2014); Source <sup>b</sup> Anon. (2007); Source <sup>c</sup> Ministry of Health of China, (1994); Source <sup>d</sup> Anon., (2011); Source <sup>e</sup> Anon. (2000); Source <sup>f</sup> Dosumu *et al.* (2005); Source <sup>g</sup> Singh *et al.* (2010)

Anon. (2011) suggested the permissible limit of (50 mg/kg) for Cu, in case of present investigation the mean concentration of copper was lower than this permissible limit. As recommended by Kharub (2012) the levels of vegetable Cu and Ni in the current investigation were found to be lower while those of Fe, Pb and Zn were higher than the permissible limits. The levels of As, Mo and Pb in the vegetable were lower in the current investigation while those As, Cu, Fe, Ni, Se and Zn were almost within the same range as suggested by Ahmad *et al.* (2014a, b) and Ashfaq *et al.* (2015).

The present study shows that wastewater treatment caused a considerable increase in heavy metal concentrations in soils and then in the edible portion of the vegetable. As suggested by Schmidt (1997) waste water contains high amount of Pb, Cd, Ni, Zn and Cu. The accumulations of heavy soil receiving irrigation with waste-water are then transferred from the soil to all parts of plant and they cause main troubles human beings and animals health who feed on these vegetables enriched with metals. Due to alkaline nature of most of the soils in Pakistan high uptake of Zn is expected as suggested by Hornburg *et al.* (1995). Likewise, in the present study, the Zn uptake in the *Brassica* vegetable was reasonably high.

In an earlier study, for metals Rahman *et al.* (2012) recommended that pollution load index values in soil should be as following: Pb(1.24-1.38), As (1708-2715), Fe (0.36-0.65), Zn (2.72-2.95), Cu (4.68-7.72), and Ni (1.77-2.4). However, the pollution load indices observed in the current study for all metals except Pb were lower than those reported by Rahman *et al.* (2012). For the metals such as Mn and Cr the pollution load indices were lower than 1, whereas those for Mo, As and Zn being greater than 1.

In relation to set values of Integrated Risk Information System, the values of R<sub>FD</sub> reported were 0.70, 0.009, 0.04, 5×10<sup>-3</sup>, 0.02, 3×10<sup>-4</sup>, and 0.37 mg kg<sup>-1</sup> day<sup>-1</sup> for Fe, Mo, Cu, Se, Ni, As, and Zn (Anon., 2010). The R<sub>FD</sub> value for Pb reported was 0.0035 mg kg<sup>-1</sup> day<sup>-1</sup> (Anon., 1993). For grown-up humans, the standard daily metal intake was reported to be 0.345kg of a vegetable, while 60 kg of human body weight was considered as average (Ge, 1992; Wang *et al.*, 2005).

Zinc is an essential constituent varying from 5 mg for newborn to 15 mg for adults (Anon., 2008). It is pertinent to note that a small amount of Zn is believed to affect healthiness and its excessive amount is inhibitory but according to Anon. (2008) harmful effects generally begin at levels in the 100 to 250 mg/day range. Adults when

exposed to Pb dose higher than R<sub>FD</sub> they usually experience nausea, loss of remembrance, anorexia, insomnia and weakness of the joints (Anon., 2009).

In leafy vegetables for heavy metals highest values of enrichment factor was observed as reported by Sridhara *et al.*, (2008). In lady finger, pumpkin and rice transfer factor for Pb, Zn and Cr was higher (Singh *et al.*, 2010). Due to higher transpiration rate leafy vegetables uptake in this way plant's growth and moisture content is maintained. (Tani & Barrington, 2005).

The present investigation showed that transfer of metals and metalloids from soil are greatly accumulated *Brassica*. In the soil and vegetable irrigated with ground, canal and sewage waters at all three sites levels of metals and metalloids were found below the permissible levels while As showed higher value than the permissible values. In sewage-water highest pollution load index was observed so health hazardous is caused by the intake of vegetables effecting humans. This study discovered the certainty that increased heavy metal concentrations was observed by the wastewater treatment in soils and in this way edible portion of the vegetables is accumulated with heavy metals. Immediate acts ought to be taken to examine the environmental value by monitoring standards of metals and metalloids in soils and waters.

## References

- Ahmad, K., Z.I. Khan, A. Ashfaq, M. Ashraf and S. Yasmin. 2014b. Assessment of heavy metal and metalloid levels in spinach (*Spinacia oleracea* L.) grown in wastewater irrigated agricultural soil of Sargodha, Pakistan. *Pak. J. Bot.*, 46(5): 1805-1810.
- Ahmad, K., Z.I. Khan, S. Yasmin, M. Ashraf and A. Ishfaq. 2014a. Accumulation of metals and metalloids in turnip (*brassica rapa* L.) irrigated with domestic wastewater in the peri-urban areas of Khushab city, Pakistan. *Pak. J. Bot.*, 42(2): 511-514.
- Anonymous. 1993. Trace elements in human nutrition and health. World Health Organization (WHO), Geneva.
- Anonymous. 2000. Dutch Standards. Circular on target values and intervention values for soil remediation.
- Anonymous. 2002. USEPA (US Environmental Protection Agency). Region 9, Preliminary Remediation Goals. <http://www.epa.gov/region09/waste/sfund/prg>
- Anonymous. 2007. FAO/WHO. *Expert Committee on Food Additives*. Cambridge University Press, Cambridge. pp. 329-336.
- Anonymous. 2008. The Risk Assessment Information System (RAIS), 2007. [http://rais.ornl.gov/tools/tox\\_profiles.html](http://rais.ornl.gov/tools/tox_profiles.html) (accessed 04 September, 2012).

- Anonymous. 2009. NSC Lead Poisoning. *National Safety Council*. [http://www.nsc.org/news\\_resources/Resources/Documents/Lead\\_Poisoning.pdf](http://www.nsc.org/news_resources/Resources/Documents/Lead_Poisoning.pdf). (Accessed on 4th September, 2012).
- Anonymous. 2010. U.S. Environmental Protection Agency (USEPA). Integrated risk information system.
- Anonymous. 2011. Joint FAO/WHO food standards programme codex committee on contaminants in foods, fifth session pp 64-89.
- Ashfaq, A., Z.I. Khan, Z. Bibi, K. Ahmad, M. Ashraf, I. Mustafa, N.A. Akram, R. Perveen and Sumaira Yasmeen. 2015. Heavy metals uptake by *Cucurbita maxima* grown in soil contaminated with sewage water and its human health implications in Peri-urban Areas of Sargodha City. *Pakistan J. Zool.*, 47(4): 1051-1058.
- Bigdeli, M. and M. Seilsepour. 2008. Investigation of metals accumulation in some vegetables irrigated with waste water in Shahre Rey-Iran and toxicological implications. *American-Eurasian. J. Agri. & Environ. Sci.*, 4(1): 86-92.
- Chiroma, T.M., R.O. Ebebele and F.K. Hymore. 2014. Comparative assessment of heavy metal levels in soil, vegetables and urban grey wastewater used for irrigation in Yola and Kano. *Internat Refereed J. Eng. Sci. (IRJES)*, 3: 01-09.
- Cui, Y.L., R.H. Zhu, R.H. Zhi, D.Y. Chen, Y.Z. Huang and Y. Qiu. 2004. Transfer of metals from soils to vegetables in an area near a smelter in Nanning, China. *Environ. Int.*, 30: 785-791.
- Dai, J.Y., H.G. Chen, Y.Y. Leng, Y. Zhao, W. Jian-Fu and N.A. Ma. 2006. Characteristics of sewage sludge and distribution of heavy metals in plants with amendment of sewage sludge. *J. Environ. Sci.*, 18:1094-1100.
- Dosumu, O.O., N. Abdus-Salam, S. Oguntoye and F.A. Afdekale. 2005. Trace metals bioaccumulation by some Nigerian vegetables. *Centrepint.*, 13(1): 23-32.
- Ge, K.Y. 1992. The Status of Nutrient and Meal of Chinese in the 1990s. Beijing People's Hygiene Press. pp. 415-434.
- Hornburg, V., G. Welp and G.W. Brummer. 1995. Availability of heavy metals in soils and their uptake by vegetable species. *Soil Geology*, 158: 137-145.
- Khan, S., S. Rehman, AZ. Khan, M.A. Khan and M.T. Shah. 2010. Soil and vegetables enrichment with heavy metals from geological sources in Gilgat, Pakistan. *Ecoto. & Environ. Safty*, 73: 1820-1827.
- Kharub. M. 2012. Investigation of heavy metal content in vegetables in and around Fatehabad city. *Int. J. Environ. Bio.*, 2(2): 84-87.
- Liu, W.H., J.Z. Zhao, Z.Y. Ouyang, L. Soderlund and G.H. Liu. 2005. Impacts of sewage irrigation on heavy metals distribution and contamination in Beijing, China. *Environ. Int.*, 31: 805-812.
- Lone, M.I., S. Saleem, T. Mahmood, K. Saifullah and G. Hussain. 2003. Heavy metal contents of vegetables irrigated by Sewage/Tubewell water. *Int. J. Agri. & Bio.*, 1560 (4): 533-535.
- Mathieu, C. and F. Pieltain. 2003. Chemical Analysis of Soils. *Selected Methods*, France, pp. 387.
- McGrawth, S.W., F.J. Zhao and E. Lombi. 2001. Plant and rhizosphere processes involved in Photoremediation of metal-contaminated soil. *Plant & Soil*, 232: 207-214.
- McLean, E.O. 1982. Soil pH and Lime Requirement, In: *Methods of Soil Analysis, Chemical and Microbiological Properties*, (Ed.): Keeney, 2nd Edition, American Society of Agronomy, Madison. pp. 199-224.
- Ministry of Health of China. 1994. Sanitary standard of copper level for vegetables GB15199-94.
- Naser, H.M., N.C. Shil, N.U. Mahmud, M.H. Rashid and K.M. Hossain. 2009. Lead, cadmium and nickel contents of vegetables grown in industrially polluted and non-polluted areas of Bangladesh. *Bangladesh J. Agri. Res.*, 34(4): 545-554.
- Rahman, S.H., D. Khanam, T.M. Adyel, M.S Islam, M.A. Ahsan and M.A. Akbor. 2012. Assessment of heavy metal contamination of agricultural soil around Dhaka Export Processing Zone (DEPZ), Bangladesh: Implication of Seasonal Variation and Indices. *App. Sci.*, 2: 584-601.
- Schmidt, J.P. 1997. Understanding phytotoxicity threshold for trace elements in land-applied sewage sludge. *J. Environ. Quality*, 26: 4-10.
- Sharma, AP. and B.D. Tripathi. 2008. Magnetic mapping of fly-ash pollution and heavy metals from soil samples around a point source in a dry tropical environment. *Environ. Monit. Assess.*, 138:31-39.
- Singh, A., R.K. Sharma, M. Agarwal and F.M. Marshall. 2010a. Health risk assessment of heavy metals via dietary intake of foodstuffs, from waste water irrigated site of dry tropical area of India. *Food Chem. Toxicol.*, 48: 611-619.
- Singh, R., D.P. Singh, N. Kumar, S.K. Bhargava and S.C. Barman. 2010b. Accumulation of translocation of heavy metals in soil and plants from fly ash contaminated area. *J. Environ. Biol.*, 31: 421-430.
- Singh, R.P. and M. Agrawal. 2010. Variations in heavy metals accumulation, growth and yield of rice plants grown at different sewage sludge amendment rates. *Ecotox. & Environ. Safety*, 73: 632-641.
- Sood, S.K., D. Sharma, S. Kumar and T.N. Lakhnarpal. 2010. (*Healing Herbs: Traditional Medications for Wounds, Sores and Bones*. Pointer Publishers, Jaipur, India).
- Sridhara, N. Chary, C.T. Kamala and R.D. Samuel. 2008. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotox. & Environ. Safety*, 69(3): 513-524.
- Tani, F.H. and S. Barrington. 2005. Zinc and copper uptake by plants under two transpiration ratios Part I. Wheat (*Triticum aestivum* L.). *Environ. Poll.*, 138: 538-547.
- Wang, Q.R., YS. Cui, X.M. Liu, YT. Dong and P. Christie. 2003. Soil contamination and plant uptake of heavy metals at polluted sites in china. *J. Environ. Sci. & Health (A)*, 38: 823-838.
- Wang, X., T. Sato, B. Xing and S. Tao. 2005. Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. *Sci. Total Environ.*, 350: 28-37.
- Watkinson, J.H. 1966. Fluorometric determination of selenium in biological material with 2, 3-diaminonaphthalene. *Analytical Chemistry*, 38: 92-97.
- Welsch, F.P, J.G. Crock and R. Sanzalone. 1990. Trace elements determination of arsenic and selenium using continuous flow hydride generation atomic absorption spectrophotometry (HG-AAS). In: *Quality Assurance Manual for the Branch of Geochemistry*, (Ed.): B.F. Arbogast, pp. 38-45.