

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

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Abstract.- Accumulation of six different metals such as Cr, Mn, Fe, Mo, Pb, and Cd in a potential vegetable crop, pumpkin (*Cucurbita maxima*), and its subsequent human exposure risks were determined at two peri-urban sites (Sahiwal and Shahpur) within the Sargodha city, Pakistan, where wastewater is used for irrigating most of the vegetables grown therein. The results demonstrated that the metal levels in the soil samples were relatively below the respective maximum permissible limits of various metals analyzed. The pattern of total metal concentration in vegetable was Mn > Fe > Cr > Mo > Pb > Cd. At both the peri urban sites, the transfer factor ranged from 0.01 to 71.295, with Cr having the highest transfer factor. The differences in uptake of these heavy metals can be ascribed to difference in tolerance to these metals by the vegetable species. The studies also showed dietary intake of Pb, Cd, Mn, and Mo via *Cucurbita maxima* was not free of risk for residents of investigated sites consuming this vegetable.

Key words: Heavy metals, wastewater, maximum permissible limit, *Cucurbita maxima*, health risk index.

INTRODUCTION

Wastewater irrigation is very common due to scarcity of canal/fresh water and/or occasionally due to its nutritive value (Jagtap *et al.*, 2010). Water pollution is a great problem throughout the world and ground water pollution occurs due to release of industrial effluents and domestic sewage into watercourses (Mashiatullah *et al.*, 2005). Wastewater not only provides the supplemental irrigation but also provides the useful nutrients, especially organic matter phosphorus and nitrogen to improve physical properties and fertility of soil (Gibbs *et al.*, 2006). The continuous utilization of wastewater for irrigation of leafy and non-leafy vegetables results in metal deposition in soil as well as in under-cultivated crops well over the maximum permissible level (Nrgholi, 2007).

Consequently the soil is heavily contaminated with metal contents (Mapanda *et al.*, 2005) due to excessive irrigation which accumulate in vegetables via root absorption (Cui *et al.*, 2004; Zheng *et al.*,

2007; Malla *et al.*, 2007; Arora *et al.*, 2008; Bibi *et al.*, 2014).

Cucurbita maxima, an important summer vegetable, is cultivated both as vegetable and medicine all over the world. Its edible portion is used as antidiabetic, antihypertensive, anti-inflammatory, immunomodulatory, antitumor, and antibacterial agent (Khan *et al.*, 2013). Like most common vegetables, it is grown in peri-urban areas that are usually irrigated with municipal wastewater. Since most municipal wastewaters contain heavy metals, it is highly likely that such vegetables accumulate appreciable amounts of these metals.

The toxic metals in contaminated soil are taken up by the plants, exposing humans to these contaminants (Demirezen and Ahmet, 2006; Khan *et al.*, 2008). Parashar and Prasad (2013) assessed the impact of sewage irrigation on heavy metal contamination of vegetables consumed by urban India. The study concluded that the use of untreated and treated wastewater for irrigation had increased the contamination of Pb and Cd in fruits and vegetables causing health risks. Orisakwe *et al.* (2012) evaluated the dietary toxicity due to Cd, Hg, Ni and Pb in vegetable samples collected from sites in Nigeria exposed to variety of chemicals. Results

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indicated that Cd, Pb, and Ni levels were higher in vegetables and among all, Pb accumulated heavily in the body of consumers.

This study was therefore, conducted to determine the bioaccumulation of Cr, Mn, Fe, Mo, Pb, and Cd in *Cucurbita maxima* and rhizospheric soil irrigated with domestic wastewater. Moreover, this study was aimed to assess the distribution of heavy metals in soil and vegetable and to identify the transfer factor and pollution load index of metals in soil and to estimate the possible health risk due to metals via consumption of vegetable.

MATERIALS AND METHODS

Study area

The study area was in Sargodha district in the central Punjab. Sargodha is an agricultural district being largest citrus growing area in the world. A reconnaissance survey was conducted in the peri-urban sites, irrigated with municipal wastewater in the region of Sargodha, during July to August 2012. The samples of *Cucurbita maxima* (edible portion) and rhizospheric soil were collected from two sites (Sahiwal and Shahpur). In these areas wastewater (sewage and industrial) is used by farmers for irrigation purpose.

Sample collection

From each site of sampling, the composite soil of almost 1 kg was collected under the vegetable. The composite soil consisted of 5 subsamples. To attain randomness, the soil was dug up to 20 cm depth in a zigzag path by an auger containing all layers (Sanchez, 1976). Firstly, the soil samples were dried in an air and then dried at 72°C in an oven for three consecutive days (Campbell and Planks, 1992). The dried soil was pulverized and mashed into powder form by using a grinder, sieved and then packed in polythene bags.

Six replicates of the vegetable (edible part) were collected from each site. Vegetable samples were washed once with distilled water to remove air-borne contaminants. Edible portions were cut into small pieces with a knife and dried in sunlight to remove moisture and then placed in an oven at 72°C for 48 h. Dried samples were ground to pass through a 1mm mesh sieve and stored in clean plastic bags before analysis.

Digestion of soil and vegetable samples

The soil samples were digested by the wet digestion method. One g soil sample was taken in a flask and then added to 8 mL of H₂O₂ and 4 mL of H₂SO₄. The samples were placed for 30 min in a digestion chamber at appropriate temperature. After the digestion was completed, 2 mL of H₂O₂ were added to each flask. All samples were heated again until the samples became colorless. After filtering the digests, the final volume of each sample was raised to 50 mL and then stored in plastic bottles until analysis.

One g dried sample of the vegetable was taken in a flask and 4 mL of H₂O₂ and 2 mL of H₂SO₄ were added to it. Further course of the protocol was same as that adopted for soil analysis (Naeem *et al.*, 2012).

Detection of metals in samples

To analyze the metal concentration in vegetable and soil samples, atomic absorption spectrophotometer was used (Lindsay and Norvell, 1978). The standard solutions of Cr, Mn, Fe, Mo, Pb, and Cd were prepared to get the accurate values. From a standard stock solution, a calibration curve was constructed by different metal concentrations (Naeem *et al.*, 2012).

Statistical analysis

Biostatistics was applied on data using MSTAT computer package (MSTAT Development Team, 1989). One-way and two-way analyses of variance (ANOVA) were applied on data of heavy metals in the soil and the vegetable. Correlation between soil and vegetable with respect to each metal was worked out. Significance of means was tested at probability levels of 0.001, 0.01 and 0.05 (Steel and Torrie, 1980).

Transfer factor for vegetable/soil system

To determine the metal accumulation from soil to vegetable, transfer factor was determined (Cui *et al.*, 2004).

$$TF = [M]_{\text{vegetable}} / [M]_{\text{soil}}$$

where [M]_{vegetable} is the concentration of metal in vegetables (mg/kg) and [M]_{soil} is the concentration of metal in soil (mg/kg).

Pollution load index

Pollution load index (PLI), for a particular site, was evaluated (Liu *et al.*, 2005).

PLI = Metal concentration in investigated soil /
reference value of the metal in soil

Health risk index

Health risk index (HRI) was considered as the ratio of daily intake of metal (DIM) and oral reference dose (Cui *et al.*, 2004).

$DIM = C_{\text{metal}} \times D_{\text{food intake}} / B_{\text{average weight}}$,

$HRI = DIM / R_{\text{d}}D$ (USEPA, 2002)

C_{metal} represents metal concentration in the vegetable (mg/kg), $D_{\text{food intake}}$ is daily intake of vegetable (kg/day), and $B_{\text{average weight}}$ is the body weight (kg). From the integrated risk information system, $R_{\text{d}}D$ values for Cd, Cr, Mn, Fe, and Mo were 0.001, 1.5, 0.041, 0.70, and 0.009 mg/kg/day (USEPA, 2010). The $R_{\text{d}}D$ value for Pb was 0.0035 mg/kg/day (WHO, 1993). Sixty kg of average body weight and daily intake of metal as 0.345 kg of the vegetable were considered for adult per day (Ge, 1992; Wang *et al.*, 2005).

RESULTS*Concentration of heavy metal in soil*

In soil samples, the concentrations of heavy metal ranged from 0.174 to 0.257 mg/kg for Cr, 18.83 to 21.62 for Mn, 24.23 to 29.44 for Fe, 11.84 to 14.09 for Mo, 22.33 to 23.16 for Pb, and 16.89 to 17.89 for Cd (Table I), respectively. Total concentration of heavy metal detected in the soil irrigated with wastewater at both sites was lower than the maximum permissible level except Cd (USEPA Standards). The concentrations of metals showed variations among sites.

Concentration of heavy metal in vegetable

The mean values of heavy metals in *Cucurbita maxima* irrigated with wastewater are presented in Table II. The mean values of Cr in *C. maxima* ranged from 12.40 to 13.52 mg/kg at both sites. The results showed that the values observed during current study were lower than the

permissible limit (50 mg/kg) reported by the WHO (1993, 1996).

Table I.- Concentration (mg/kg dry soil) of some heavy metals in soil samples obtained from two different sites of District Sargodha, Pakistan.

Heavy metals	Sampling sites		Permissible maximum limit (WHO, 1993, 1996)
	Sahiwal (Means±SE)	Shahpur (Means±SE)	
Cr	0.174±0.022	0.257±0.031	400
Mn	18.83±0.107	21.62±2.907	80
Fe	24.23±1.181	29.44±0.820	21000
Mo	11.84±0.210	14.09±0.457	40
Pb	23.16±0.268	22.33±0.609	300
Cd	16.89±0.386	17.97±0.338	3

PML, permissible maximum limit (USEPA Standards); SE, Standard error

Table II.- Concentration (mg/kg dry soil) of some heavy metals in *Cucurbita maxima* obtained from two different sites of District Sargodha.

Heavy metals	Sampling sites		Permissible maximum limit (WHO, 1993, 1996)
	Sahiwal (Means±SE)	Shahpur (Means±SE)	
Cr	12.40±0.262	13.52±0.271	50
Mn	53.54±0.143	44.73±1.275	30
Fe	42.23±0.259	47.33±0.028	1000
Mo	7.970±0.028	7.740±0.260	5
Pb	1.797±0.139	2.044±0.060	10
Cd	0.365±0.015	0.244±0.028	0.5

PML, permissible maximum limit (WHO Standards); SE, Standard error

The mean Mn values ranged from 41.72 to 54.34 mg/kg. The mean Mn concentration showed variations among sites. All the mean Mn soil values were above the permissible level (30 mg/kg) suggested by the WHO (1993).

At site-II, higher value of Fe in the vegetable was observed. The values observed at site-I ranged from 41.31 to 43.160 mg/kg and 41.74 to 49.60 mg/kg at site-II. All the Fe concentrations were lower than the permissible limit (1000 mg/kg) already reported by the WHO. The mean values of Mo in *C. maxima* varied from 7.37 to 7.97 mg/kg. The concentration of Mo at both sites was almost

similar. The Mo concentrations observed in current study were higher than the permissible limit (5 mg/kg) given by the WHO. The level of Pb in *C. maxima* varied from 1.79 to 2.04 mg/kg. The mean Pb level investigated at both sites was lower than the permissible limit (10 mg/kg) reported by the WHO. The mean Cd values ranged from 0.14 to 0.345 mg/kg. The mean Cd level was almost similar at both sites. Soil mean Cd values were lower than the permissible level (0.5 mg/kg) reported by the WHO.

Transfer of metal

The average transfer factor for Cr, Mn, Fe, Mo, Pb, and Cd at both sites differed. Transfer factor for vegetable to soil system ranged from 0.013 to 71.29 (Table III). The order of transfer factor for metals was Cr (71.29) > Mn (2.84) > Fe (1.74) > Mo (0.67) > Pb (0.07) > Cd (0.02) at Sahiwal and Cr (52.60) > Mn (2.07) > Fe (1.60) > Mo (0.54) > Pb (0.09) > Cd (0.01) at Shahpur, respectively. There were positive and non-significant correlations of Fe ($r = 0.319$) and Mo ($r = 0.044$) between the soil and the vegetable while reverse was true for Cd ($r = -0.672$) and Mn ($r = -0.578$). Cr ($r = -0.282$) and Pb ($r = -0.242$) correlations were non-significant and positive between soil and vegetable.

Table III.- Transfer factor (TF) for vegetable/soil system.

Metals	Transfer factor	
	Sahiwal	Shahpur
Cr	71.29	52.60
Mn	2.84	2.0
Fe	1.74	1.60
Mo	0.67	0.54
Pb	0.07	0.09
Cd	0.02	0.01

Pollution load index

Pollution load index was determined to analyze the contamination status along sites (Table IV). The degree of contamination was greater at site-II than those found at site-I. In soil, the reference values for Cd, Pb, and Cr were 1.49, 8.15, and 9.07 µg/g (Singh *et al.*, 2010a). Mo was at elevated level at both sites as compared to the reference value which was 3.0 mg/kg dry matter.

The values observed for Mn and Fe were lower than reference values which were 46.75 mg/kg (Singh *et al.*, 2010b) and 56.90 mg/kg (Dosumu *et al.*, 2005). At one site, pollution load index was in sequence: Cd (11.38) > Mo (3.94) > Pb (2.84) > Mn (0.87) > Fe (0.80) > Cr (0.02) at Sahiwal site and Cd (12.06) > Mo (4.69) > Pb (2.74) > Mn (1.00) > Fe (0.98) > Cr (0.03) at Shahpur site.

Table IV.- Pollution load index (PLI) for metals in soil.

Metals	Pollution load index	
	Sahiwal	Shahpur
Cr	0.02	0.03
Mn	0.87	1.00
Fe	0.08	0.98
Mo	3.94	4.69
Pb	2.84	2.74
Cd	11.33	12.06

Health risk

The estimated health risk, due to daily intake (mg/day) of commonly grown vegetable *C. maxima* in Sargodha city for Cr, Mn, Fe, Mo, Pb, and Cd was 0.04, 7.51, 0.34, 5.09, 2.95, and 2.09 at Sahiwal and 0.05, 6.27, 0.39, 4.94, 3.36, and 1.40 mg/day at Shahpur, respectively (Table V). The risk index at both sites was in the order: Mn > Mo > Pb > Cd > Fe > Cr. Health risk observed for Mn was maximum and due to Cr was minimum.

Table V.- Health risk intake (HRI) of heavy metals via intake of *Cucurbita maxima* from wastewater irrigated sites.

Metals	Health risk intake (mg/day)	
	Sahiwal	Shahpur
Cr	0.04	0.05
Mn	7.51	6.27
Fe	0.34	0.39
Mo	5.09	4.94
Pb	2.95	3.36
Cd	2.09	1.40

DISCUSSION

Heavy metal in soil

Constant utilization of untreated and treated wastewater for irrigation purpose results in metal

accumulation in soil. At both sites, the concentration of Fe (24.23-29.44 mg/kg) and Pb (22.33-23.16 mg/kg) was maximum while Cr (0.174-0.26 mg/kg) was minimum in soil (Table I). These differences could be ascribed to variation in agricultural practices and other environmental factors prevalent on the two sites. The concentrations of all metals found in the soil were lower than the permissible level except Cd. The regular cultivation and standard monitoring of plants at wastewater irrigated sites keeps the metal concentration within acceptable limit (Singh *et al.*, 2010a). The concentration of Cr and Pb was low and Mn level was high in present study in soil while Cd level was similar to that analyzed by Hussain *et al.* (2006) at Uchkera, Faisalabad, Pakistan. The mean Fe and Cd contents in rhizospheric soil were above the values, whereas Pb concentration was within range as determined by Parashar and Prasad (2013) in India. Fe and Cd concentrations in soil were found to be higher in current study while the results for Pb were very close to the findings of Mosleh and Almagrabi (2013) in Jeddah.

Concentration of heavy metals in C. maxima

The concentrations of Mn (44.73-53.54 mg/kg) and Fe (42.23-47.33 mg/kg) in *C. maxima* were above the prescribed limit of the WHO. The accumulation of metals in vegetable is a major cause of public health risk (Cui *et al.*, 2005; Bi *et al.*, 2006). The heavy metal concentrations in the vegetable varied between the two sites. This may be due to translocation of metals in different parts of plants or uptake capacity of the vegetable through roots (Vousta *et al.*, 1996). Khimpa *et al.* (2011) determined the heavy metal concentration in vegetables grown in vicinity of closed dumpsite, Tanzania. Results concluded that Cr level was 13.59 mg/kg, which was almost similar to the present study at some sites but Cd level was lower in present study in pumpkin. The level of Cd and Pb in pumpkin in current study was similar to the findings of Parveen *et al.* (2003) in Pakistan. The present study also revealed that the mean Cd and Pb level measured in vegetable from Sargodha region was lower whereas the upper concentration of Fe was found very similar to the values reported by Yadav *et al.* (2002) for vegetables irrigated with industrial

effluents in India. The present study revealed that the mean Cd measured in vegetable from Sargodha region was lower than the vegetables from Titagarh West Bengal, India (10.37-17.79 mg/kg) (Gupta *et al.*, 2008), but similar to the vegetables from China (0.03-0.73 mg/kg) (Liu *et al.*, 2005) and significantly more than the vegetables from Egypt (0.002-0.08 mg/kg) (Dogheim *et al.*, 2004) whereas it was very close to the findings of Sharma *et al.* (2007) (0.5-4.36 mg/kg) in vegetables from Varanasi, India. The results showed irregular pattern of metal availability. The concentration of metals is greater in vegetable while lower in soil. For example Cr, Mn, and Fe concentration in edible portion was several fold greater than soil. The difference in metals in soil and plants is due to variation in sources of metals (Tsafé *et al.*, 2012).

Transfer of metals

Transfer factor gives useful information on the availability of metals from soil to edible portion of vegetable (Alloway *et al.*, 1988). Highest transfer factor for Cr was observed at both sites. Transfer factor for Cr, Mn, and Fe was greater than 1, which indicates that these metals retain less in soil and more mobile to aerial parts. Lowest transfer factor for Cd showed that it binds to the soil particles and is less mobile and has greater ability to retain in soil. The metal concentration increases in plant due to increase in the concentration of metals in soil. Transfer factor varies from vegetable to vegetable because it depends on metal concentration in soil and metal uptake in vegetable (Cui *et al.*, 2004; Zheng *et al.*, 2007). Transfer factor does not indicate the risk due to these metals in any form. The extent of metal toxicity in humans depends on their daily intake (Anita *et al.*, 2010). Transfer factor for Pb and Cd was lower in current study as compared to those reported by Ullah *et al.* (2012). Transfer of Pb from soil to vegetable observed in current study was similar to those reported by Chao *et al.* (2007) in vegetables of Nanjing, China.

To establish the metal association between variables such as in the soil and the vegetable, Pearson correlation was worked out (Pentecost, 1999). The Mn and Cd showed negative and significant correlations while non-significant negative correlations were observed due to Cr and

Pb between the soil and the vegetable. The negative sign indicates weak correlation between the soil and the vegetable. These results indicated imbalance of metals among soil and vegetable. Fe and Mo reveal positive non-significant correlation in between the soil and the vegetable. This indicated a strong association of soil and vegetable.

Pollution load index

To estimate the contamination status of metals in soil, pollution load index was determined. Pollution load index indicates whether the quality of soil is suitable for vegetable growth and agricultural use (Liu *et al.*, 2005). Due to anthropogenic inputs, agricultural runoff, and industrial activities, the pollution load due to Cd, Pb, and Mo was greater than 1 at both sites. Pollution load index greater than 1.0 indicates that these metals can cause environmental risk and investigated sites need proper monitoring and necessary steps should be taken to overcome it. The pollution load index due to Cd was higher but due to Cr was lower in present research whereas Pb concentration was similar to the findings of Oti-Wilberforce and Nwabue (2013). The sites indicating pollution load index greater than 1.0 need suitable supervision and legislative measures to control the rate of contamination in affected sites.

Health risks

The intake of metal contaminated vegetables poses direct impact on consumer's health. The population is therefore at greater risk of Mn, Cd, Pb, and Mo since their values were greater than 1.0. The population will be at greater risk if it is equal to or greater than 1 (Sajjad *et al.*, 2009). Health risk index depends on vegetable type, consumption rate of vegetable, physical characteristics, and chemical composition of soil. The values observed for Pb and Cd, which are considered as important metals affecting vegetables were greater (Kachenko and Singh, 2006). To reduce the accumulation of metals in food chain, regular monitoring of metals in vegetables is necessary. Dietary intake of metal may cause disorders, so monitoring of these substances is essential in human diet (Zukowska and Biziuk, 2008). To avoid the metal accumulation in body, it is suggested that inhabitants living in metal polluted

areas should not consume large amount of vegetables. The risk index due to Cd and Fe was lower whereas due to Pb was higher in current study as compared to findings of Cui *et al.* (2004). Health risk due to Cd, Pb, and Cr was higher in present investigation as compared to Singh *et al.* (2010a). Our results indicate that the inhabitants of the area under study were at risk due to the intake of Mo, Mn, Pb, and Cd via consumption of *C. maxima* grown therein.

CONCLUSIONS

Soil is an important constituent of biosphere and acts as reservoir for various pollutants. This study showed that metals are transferred from soil to vegetable at higher level. It was concluded that the dietary intake of *C. maxima* was not free of risk for inhabitants around the sampling sites. To reduce the health risk effects, it is suggested to treat the industrial wastes properly and phytoextract the overload of heavy metals from polluted sites. Legislative measures should be taken by forbidding the expulsion of the untreated industrial effluents and municipal waste.

Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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