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HEAVY METAL CONTENTS AND THEIR DAILY INTAKE IN VEGETABLES UNDER PERI-URBAN FARMING SYSTEM OF MULTAN, PAKISTAN

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Irrigating soil with waste water results in accumulation of heavy metals in the food chain. To examine the possible health hazards of heavy metals in local population, quantification of accumulation potential and daily intake values of heavy metals in two commonly grown vegetables of Pakistan; okra (*Abelmoschus esculentus*) and brinjal (*Solanum melongena*) was assessed. Three major sites of Multan were selected: Kot Abdul Fateh, Hamroot and Mozu Alamgir, irrigated by waste, tube-well and underground water respectively. Total 30 soil samples, 30 irrigation water samples and 60 vegetables samples were analysed. All samples of irrigation water, soil and vegetables were contaminated with heavy metals (Ni, Cd, Cu, Pb, Mn and Co). Out of contaminated, all irrigation water samples exceeded their maximum residual limits (MRLs) except Cu and Co while for soil only Cd exceeded its MRL. Documentation of accumulated heavy metals in vegetables suggests that the concentration of lead and cadmium in both vegetables is higher than their respective MRLs. However, the daily intake values of these metals in both children and adults were below their recommended values according FAO/WHO. The study suggests that regular monitoring of trace-metals may be helpful in controlling unnecessary build-up of trace metals in the food chain.
Keywords: Daily intake, heavy metals, MRLs, Okra

INTRODUCTION

Safe and hygienic food is the first preference of consumer all over the world. Demand of risk-free food is increasing day by day. End users are more conscious about their health and in consumption of safe food. To meet the need of the day, scientists are conducting various experimental works to provide safe, nutritious and wholesome food to the world (Wei and Yang, 2010).

Metals having a specific density of more than 5 g/cm^3 are categorized as heavy metals (Jarup, 2003). These are the most toxic food pollutants and have gained importance due to their hazardous effect to nature (Zaidi *et al.,* 2005). Toxic metal contamination of soil, aqueous waste streams and groundwater causes major environmental and human health problems (Kibassa *et al.,* 2013). The major threat to the health of individual from heavy metals is linked with contact of cadmium, lead, arsenic and mercury (Jarup, 2003). Toxic elements are regarded as non-biodegradable materials, having prolonged half-life. They cause serious health concerns due to their ability to get store in various body parts. This issue is becoming worse day by day with special reference to third world countries like Pakistan, India and Bangladesh (Singh *et al.,* 2010).

Trace metals are extensively stored in the edible parts of vegetables as compare to fruits and grain crops (Mapanda *et al.,* 2005). Vegetables absorb toxic elements and gather them in proportion to elevate amounts that are enough bases for causing medical complications both in human beings and animals on consuming these contaminated plants (Alam *et al.,* 2003; Sobukola *et al.,* 2010; Lone, 1995). The high concentration of Pb, Cu, Co and Cd in vegetables and fruits is the cause of high prevalence of upper intestinal cancer, bone cancer; renal failure affects reproductive systems and hypertension (Turkdogan *et al.,* 2003). Long-term exposure even in a minute quantity to these heavy metals has lethal effects on human beings and other living organisms (Qadir *et al.,* 1997). The Toxicity studies authenticate that these metals can directly harm human beings by damaging mental and neurological function, by influencing the production of neurotransmitter and by modifying numerous metabolic body processes. Induce impairment by the toxic elements could be found in cardiovascular system, detoxification pathways (colon, liver, kidneys, skin), endocrine system (hormonal), energy production pathways, enzymatic system, gastrointestinal tract, immune system, nervous system (central and peripheral), reproductive system, and urinary system (Jarup, 2003). The main objectives of the current study are to document the concentration of heavy metals (zinc , iron, manganese, cobalt, nickel, cadmium, copper and lead), in commonly grown vegetables of Multan, Pakistan and to examine the effect of irrigation water in accumulation potential of trace-metals; and to evaluate the health risks of trace-metals by determining their daily intake values.

MATERIALS AND METHODS

***Area description*:** The study area, Multan is located within the Southern Punjab, Pakistan. It covers an area of 3,721 Km^2 with latitude from 30° to 12′0’ N and longitude from 71° to 25′0’ E. The area around the city is a flat, alluvial plain and is ideal for agriculture, with many citrus and mango farms. The most commonly produced vegetables in the study sites are radish (*Raphanus sativus*), tomato (*Lycopersicum esculentum*), okra (*Abelmoschus esculentus*), cauliflower (*Brassica oleracea* var. acephala), cabbage (*Brassica oleracea* var. capitata), carrots (*Daucus carota*) and brinjal (*Solanum melongena*). These vegetable crops are mainly cultivated for domestic consumption and to be sold in the local market of Multan and other parts of Southern Punjab. There are many canals that flow in the Multan district and provide water for irrigation purposes to nearby farms. This makes the land very fertile. Multan features an arid climate with very hot summers and mild winters. The city witnesses some of the most extreme weather in the country. The highest recorded temperature is approximately 54°C (129°F), and the lowest recorded temperature is approximately −1°C (30°F). The average rainfall is approximately 127 millimeters (5 inches). Dust storms are a common occurrence within the city.

The study field is situated in peri-urban areas of Multan, Pakistan. A reconnaissance survey was conducted to categorize the localities where waste-water from industries or municipal/domestic sewage is used for irrigation of vegetable crops. Interviews with official authorities and farmers facilitated to pinpoint areas of waste-water irrigation. To fulfill the irrigation needs the water from tube-well or from underground sources has been common practice for many years. Three major vegetable production sites including Kot Abdul Fateh, Hamroot and Mozu Alamgir were selected. Vegetable sites of Kot Abdul Fateh are irrigated with industrial run off while that of Hamroot and Mozu Alamgir are irrigated with tube-well and underground source, respectively.

***Soil sampling and preparation*:** In the study area, 10 samples were collected from each sampling site (total 30 samples) at a depth of 0–15cm with stainless steel auger. Each sample was collected in the form of sub-samples at a distance of about 20 m each from the first sub-sample in different directions, at each sampling site. These sub-samples were thoroughly mixed to a combined sample. The samples were brought to laboratory in polyethylene bags and air-dried covered with cloth to prevent contamination. After drying, the soil samples were mechanically ground and passed through a sieve of 2 mm and properly stored for analysis (Turkdogan *et al.,* 2003).

***Vegetable sampling and preparation*:** The vegetable samples, each of brinjal and okra were collected from each site, the total number of vegetable samples were 60. Samples were washed with double deionized water to remove the soil particles and then placed in separate paper bags properly marked. The vegetable samples were then kept in oven at 70°C till complete dryness. Dried vegetable samples, were grounded using clean electronic grinder and then stored in paper bags at room temperature for further analysis.

***Soil digestion*:** Soil samples were digested according to the method adopted by FAO (FAO, 1985). 0.5 g air-dried and powdered soil sample was taken into a Pyrex beaker and 15 mL of aqua regia was added. It was kept overnight and then heated on the hot plate till production of brown fumes stopped. Concentrated $HClO_4$ (5 mL) was added and heated again on low heat until the solution was evaporated near to dryness. The extracts were filtered and the final volume of 25 mL was made with double de-ionized water in a clean volumetric flask.

***Vegetable digestion*:** The vegetable samples were kept in oven at 70°C till complete dryness. Powdered vegetable sample (0.5 g) was taken in a Pyrex beaker; 10 mL of concentrated HNO_3 was added in it and was kept overnight without heating. It was then heated on a hotplate, and after evaporation near to dryness, the sample was cooled and 5 mL $HClO_4$ was added and heated again. After complete digestion, the sample was filtered into a clean volumetric flask and diluted to 50 mL with double de-ionized water (AOAC, 2000).

***Water sampling and preparation*:** Plastic bottles of 500 mL capacity were used to collect the samples. The sampling bottles for heavy metal determination were pre-soaked overnight with 10% HCl solution, rinsed with distilled water to avoid cross contamination. 1-2 drops of concentrated HNO_3 were added in each sample to avoid microbial utilization of heavy metals and were stored below 4°C until analyzed.

***Heavy metal analysis*:** In the acid extracts, the concentrations of heavy metals including Cd, Cu, Ni, Pb and Zn were determined using Varian GTA 120 AA 240 Graphite Atomic Absorption Spectrometer following the instructions according to the operating manual of National Institute and Technology .The analysis was performed in triplicates under standard optimize conditions. The blank reagent and standard reference soil (NIST, 2709 San Joaquin) and plant materials (NIST,1547 Peach leave) of National Institute of Science and Technology were contained within in each sample batch to authenticate the accuracy and precision of the breakdown process and for ensuing analyses. The analyses were within the confidence limit of 95% (AOAC 2000).

***Standards*:** Standard solutions of heavy metals (1000 mg L^{-1}) namely manganese (Mn), cobalt (Co), nickel (Ni), cadmium (Cd), copper (Cu) and lead (Pb) were acquired from *Fisher* Scientific. By diluting the standards solutions of variable concentrations were prepared for all the metals.

***Data analysis*:** Using the following equation, the daily intake of metals (DIM) was calculated:

$$DIM = \frac{M \times K \times I}{W}$$

Where M = Concentration of heavy metals in plants (mg Kg^{-1}); K = Conversion factor; I = Daily intake of vegetables W = Average body weight

The conversion factor used to convert fresh green vegetable; weight to dry weight is 0.085, as described by Rattan *et al.* (2005). The typical body weight taken for child and adult were 5.9 and 32.7 Kg, respectively, while average daily vegetable intakes taken for children and adults were 0.345 and 0.232 Kg/person/day, respectively, as reported in the literature (Ge, 1992; Wang *et al.,* 2005).

RESULTS AND DISCUSSION

Residues in water samples: The use of waste water usually led to changes in the physicochemical properties of soil and, as a result, heavy metal uptake by vegetables. For this purpose, concentration of heavy metals was observed across the study area in water samples used for irrigating okra and brinjal samples. The mean concentration was highest for Mn followed by Pb, Ni, Cd, Cu and Co (Fig. 1).

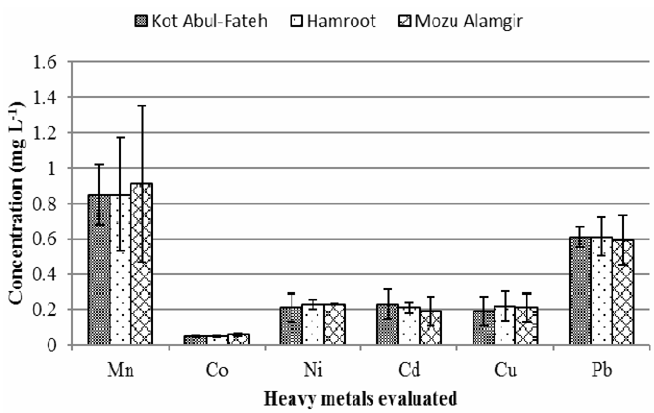

Figure 1. Graphical representation of evaluated of heavy metals (mg L^{-1}) in irrigated water samples, collected from peri-urban farming system of Multan, Pakistan

The heavy metal concentration varied between irrigation sites. The level of Mn in irrigation water varied from 0.85 to 0.91, Co from 0.05 to 0.061, Ni from 0.21 to 0.23, Cd from 0.19 to 0.23, Cu from 0.191 to 0.219 and Pb from 0.59 to 0.61 mg Kg^{-1} (Table 1).

The results indicated that residual values of all heavy metals in water samples exceed their respective Maximum Residual Limits (MRL) except copper (Cu) at one sites and cobalt at two sites. Elevated levels of heavy metals in sewage and industrial wastes have been reported by various scientists in Pakistan. Dewani *et al.,* (1997) reported the raised levels of heavy metals in sewage samples of Hyderabad city. Rensink *et al.,* (2007) reported the same in industrial effluents of Faisalabad and sewage of Haroonabad areas. Farid (2003) reported the polluted sewage samples of Attock area. Khattak *et al.* (2012) reported in his study that 79% ground water samples taken from adjoining areas of Hudiara industrial drain were unfit for irrigation purpose.

Residues in soil samples: A wide range of heavy metals were detected in soil samples at sites of vegetables cultivation. The mean concentration was highest for Mn followed by Cu, Ni, Co, Pb and Cd (Fig. 2).

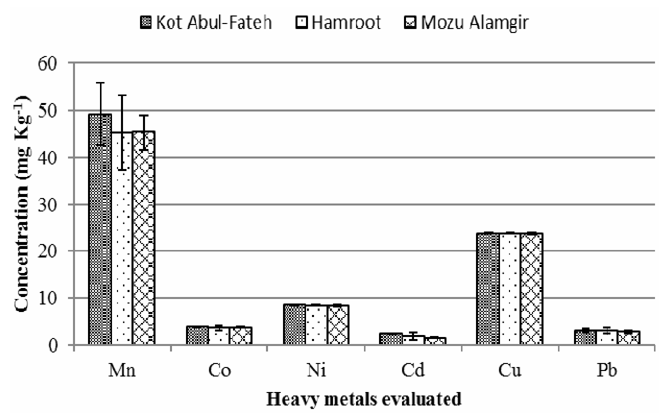

Figure 2. Graphical representation of evaluated heavy metals (mg Kg^{-1}) in soil samples, collected from peri-urban farming system of Multan, Pakistan

Soil analysis for heavy metals have revealed that concentration of Mn, Cu, Ni, Co and Pb in soil was within

Table 1. Concentration of heavy metals (mg Kg^{-1}) in water samples, used for irrigation purpose, collected from different peri-urban farming system of Multan, Pakistan

Elements (Heavy metals)	Area			MRLs
	Kot Abdul Fateh Mean ± S.D	Hamroot Mean ± S.D	Mozu Alamgir Mean ± S.D	
Manganese (Mn)	0.85±0.170	0.85±0.320	0.91±0.440	0.20
Cobalt (Co)	0.05±0.006	0.05±0.005	0.06±0.009	0.05
Nickel (Ni)	0.21±0.083	0.23±0.030	0.23±0.003	0.20
Cadmium (Cd)	0.23±0.083	0.21±0.030	0.19±0.080	0.01
Copper(Cu)	0.191±0.08	0.219±0.08	0.21±0.083	0.20
Lead (Pb)	0.61±0.060	0.61±0.110	0.59±0.140	0.10

their Maximum Residual Limit (MRL) established by FAO except Cd (Table 2). The mean concentrations of cadmium ranged from 1.57 to 2.48 mg kg^{-1}. The concentration of Cadmium was found highest in Kot Abdul Fateh (2.48 mg Kg^{-1}) followed by Hamroot (1.84 mg Kg^{-1}) and Mozu Alamgir (1.57 mg Kg^{-1}) (Table 2). This elevated level of the Cd and presence of other heavy metals may be due to irrigation with sewage water. It is common practice in Pakistan to irrigate soils with sewage and untreated industrial water as it is available free of cost to farmers, without giving consideration to its consequences. Government needs to establish rule regarding use of sewage and industrial waste water.

Soils irrigated with sewage and untreated industrial effluent containing high level of heavy metals especially Cd has been reported in different studies. Jawahar and Javed (1997) reported Cd and Pd contamination in sewage water irrigated soils in Lahore, Sheikhupura and Muridke area. Soil contamination with heavy metals irrigated with industrial effluent in Islamabad was reported by Mian and Ahmed (1997). Khan *et al.,* (2003) also reported contamination of soil with heavy metals irrigated with sewage water. Farmers use sewage water as a source of irrigation and nutrients in Faisalabad Pakistan (Ghafoor *et al.*,1994). Soil irrigated with sewage and industrial effluent were found higher in Cd, Fe and Ni (Mushtaq and Khan, 2010)

***Residues in vegetable samples*: ** Concentration of heavy metals was estimated in okra and brinjal samples across the study area. The heavy metal concentration varied between production sites and vegetables (Fig. 3).

Within production sites, mean concentration of Mn in brinjal was 1.02, 0.87 and 0.89 mg Kg^{-1} while in okra was 1.39, 0.95 and 1.35 mg Kg^{-1}. The mean concentration for Co in brinjal and okra was 0.52, 0.55 and 0.47; 0.55, 0.56 and 0.56 mg Kg^{-1} respectively. The mean concentration of Ni 1.23, 1.26, and 1.02; 0.95, 0.93 and 0.85 mg Kg^{-1} was calculated in brinjal and okra respectively. The mean concentration of Cd in brinjal 0.28, 0.61 and 0.40 while 0.56, 0.48 and 0.47 mg Kg^{-1} found in okra. The mean concentration of Cu and Pb in brinjal was 1.77, 1.26 and 1.31; 1.68, 1.58 and 1.48 mg Kg^{-1}. Similarly okra contained Cu and Pb at level of 0.93, 1.45 and 1.65; 0.76, 2.35 and 2.29 mg Kg^{-1}. Cadmium and lead were the main pollutants, making the vegetables unfit for human consumption. Cadmium and Lead exceeded their

Table 2. Concentration of heavy metals (mg Kg^{-1}) in soil samples, used for okra and brinjal cultivation, collected from peri-urban farming system of Multan, Pakistan

Elements (Heavy metals)	Area			MRLs
	Kot Abdul Fateh Mean ± S.D	Hamroot Mean ± S.D	Mozu Alamgir Mean ± S.D	
Manganese (Mn)	49.12±6.68	45.24±7.93	45.31±3.68	100
Cobalt (Co)	3.97±0.10	3.75±0.53	3.82±0.12	100
Nickel (Ni)	8.52±0.16	8.49±0.13	8.44±0.19	70
Cadmium (Cd)	2.48±0.08	1.84±0.83	1.57±0.07	1.5
Copper(Cu)	23.91±0.08	23.89±0.09	23.85±0.13	100
Lead (Pb)	3.19±0.50	3.15±0.57	2.86±0.28	100

Table 3. Concentration of heavy metals (mg Kg^{-1}) in edible portion of okra and brinjal samples, collected from peri-urban farming system of Multan, Pakistan

Elements (Heavy metals)	Vegetable	Kot Abul-Fateh Mean ± S.D	Hamroot Mean ± S.D	Mozu Alamgir Mean ± S.D	MRL value
Manganese (Mn)	Brinjal	1.02±0.150	0.875±0.06	0.89±0.051	10^b
	Okra	1.39±0.250	0.95±0.190	1.35±2.010	
Cobalt (Co)	Brinjal	0.52±0.093	0.55±0.150	0.47±0.180	10^b
	Okra	0.55±0.066	0.56±0.070	0.56±0.090	
Nickel (Ni)	Brinjal	1.234±0.33	1.27±0.099	1.02±0.480	66.9^b
	Okra	0.95±0.021	0.93±0.010	0.85±0.270	
Cadmium (Cd)	Brinjal	0.28±0.500	0.61±0.550	0.40±0.120	$0.05\text{-}0.1^b$
	Okra	0.56±0.069	0.48±0.100	0.47±0.210	
Copper (Cu)	Brinjal	1.77±0.310	1.26±0.097	1.31±0.014	73.3^b
	Okra	0.93±0.590	1.45±0.040	1.65±0.080	
Lead (Pb)	Brinjal	1.688±0.76	1.58±0.304	1.48±0.390	0.1^a
	Okra	0.76±0.610	2.35±0.660	2.29±0.430	

aUSDA (United Sates Department of Agriculture) nutrient value

bFAO MRL (Maximum Residual Limit) value

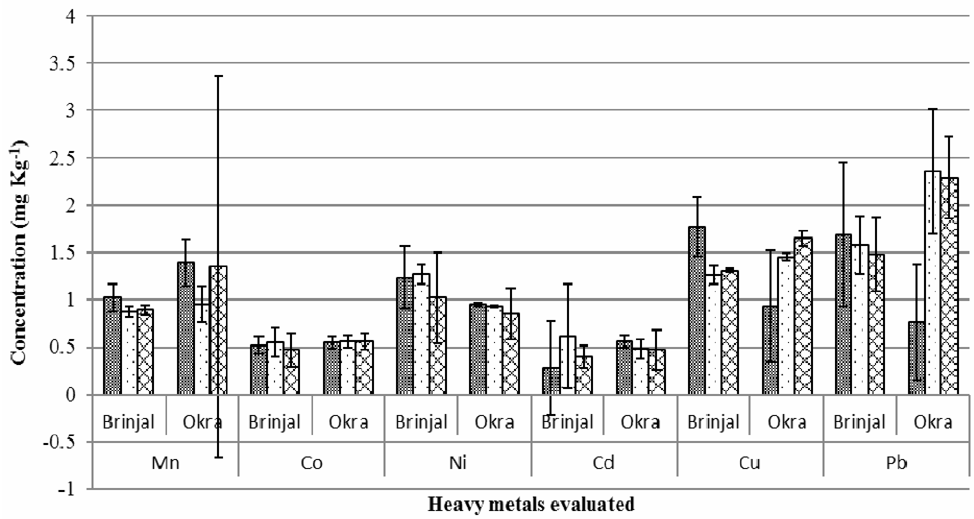

Figure 3. Graphical representation of calculated heavy metals (mg Kg^{-1}) in okra & brinjal samples, collected from peri-urban farming system of Multan, Pakistan

MRL in both okra and brinjal in all regions under study. Contamination of soil is the main source of heavy metals pollution in water and crop grown on soil. The elevated level of both Cd and Pb in vegetables can be due to number reasons. Irrigation of soils near peri urban with sewage and industrial waste water is most influencing and significant factor for contamination of soil, ground water and ultimately vegetables. Other reason can be air pollution due to exposure of peri urban soil to smoke of vehicles rich in these compounds.

In Pakistan, Heavy metals contamination of vegetables grown on waste water irrigated soil has been reported in various studies. In a study conducted in Multan reported that the vegetables and respective soils irrigated with canal water were found to have higher heavy metal contamination followed by sewerage- and tube-well-watered samples, close to our findings which suggest same results (Ismaila *et al.,* 2014). Farid (2003) collected samples of spinach, eggplant, okra, bitter gourd and pumpkin from Faisalabad city and reported that these vegetables were unsafe for eating due to heavy metals pollution. Butt *et al.* (2005) observed the same in squash, potato, spinach, turnip, tomato and coriander samples of the same area. Lone *et al.* (2003) revealed the same in sewage irrigated okra and spinach leaves and fruits in Attock area. Zaidi *et al.* (1997) collected vegetables and pulses (caramay, cuntin, spices clover, black pepper, coriander, black bean, mung bean) samples from Rawalpindi/Islamabd markets and reported the similar results as were found by Randhwa (2007). Samples of vegetables were analyzed by Ahmed *et al.* (2012) grown in surroundings of Rawalpindi revealed the same result about contamination of vegetables with heavy metals by sewage water. Samples collected from Malaysia and reported similar results as reported by Aweng (2011). In a study it was reported that highest value of Pb (33.70 mg/kg) and some other heavy metals were observed in vegetables grown on a soils irrigated with industries effluent.

Daily intake of metals (DIM): To observe the health hazards of any pollutant, it is of much concern to guess the level of contact, by identifying the possible ways of exposure to the target organisms. There are numerous potential pathways of exposure to humans. Among them, the most important one is the contamination through food chain. The daily ingestions of metals were assessed according to the regular vegetable consumption for both adults and children (Table 4).

Table 4. Daily intake of metals (mg) in adults & children

Elements (Heavy metals)	Brinjal		Okra	
	Adult	Child	Adult	Child
Manganese (Mn)	0.00063	0.00073	0.00047	0.00055
Cobalt (Co)	0.00022	0.00026	0.00026	0.00030
Nickel (Ni)	0.00068	0.00078	0.00070	0.00080
Cadmium (Cd)	0.00083	0.00095	0.00094	0.00100
Copper (Cu)	0.03400	0.03900	0.08500	0.09800
Lead (Pb)	0.05600	0.06400	0.04900	0.05600

The DIM values for heavy metals were high when based on the consumption of vegetables grown in wastewater-irrigated soils. This study tells that consumption of vegetables grown in wastewater contaminated soils, poses high risks of metals consumption as compared to tube well irrigated soils but difference is not significant. DIM study suggests that consumption of vegetables nearly free of risks, as the dietary intake limits of Mn, Co, Ni, Cd, Cu and Pb in adults can range from 2 to 15 mg, 2.0 to 20.0 mg, 0.5 to 1 mg, 0.0002 to 0.0004 mg, 1.2 to 3.0 mg and 0.0001 to

0.0005 mg, respectively (WHO, 1996). All heavy metals are free of risk due to our low average consumption of vegetables. However, there are some other possible sources like dermal contact, breathing in dust and consumption of metal-contaminated soils, which were not taken into account in the present study. Advance comprehensive studies are required to fully apprehend the harms and menaces involved in high intake of metals by an individual.

Conclusion: Accumulation of heavy metals (Mn, Pb, Ni, Cd, Cu and Co) in vegetables is due to presence of high concentration in soil and irrigation water. Brinjal and okra have varied amount of heavy metal, which show difference in their uptake capacity. Heavy metals concentration in irrigation water was above the MRL, and Cd was exceeded in soil samples and lead in accumulation of vegetables, even though only Pb and Cd exceeded permissible limits remaining were in the limits. This trend revealed that Irrigation water was a determining factor in determination of heavy metals in vegetables. On basis of results, it can be concluded irrigation with sewage water is the main factor that contribute in accumulation and make vegetables unfit for human consumption. By avoiding irrigation with sewage water, heavy metal concentration can be reduced. This research will be helpful for the country to build database for policy making to control this alarming situation in country.

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