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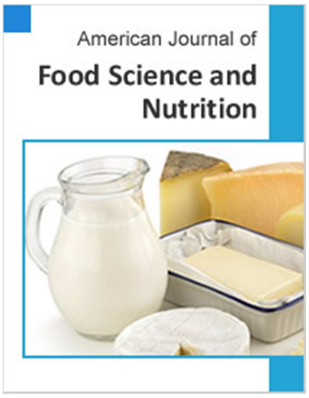

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Human Health Risk of Heavy Metals in Vegetables Grown in Contaminated Soil Irrigated with Sewage Water

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Abstract
The present study was carried out to assess the level of heavy metals in water, soil and some selected common vegetables grown at sewage water irrigated (SWI) and ground water irrigated (GWI) zone in the peri - urban area of Katihar city and also to estimate human health risk through their consumption. It was found that sewage water and soil irrigated with sewage water were highly polluted. The results revealed that the accumulation of heavy metals in the test vegetables grown in soil at SWI zone moderately enhanced with Cr, Cu, Ni and Mn while strongly enriched with Cd and Pb was observed in the order of spinach (134.45) > coriander (130.06) > cabbage (129.87) > cauliflower (128.46) > carrot (77.78) > potato (69.88). The population load index (PLI), bioaccumulation factor (BAF), daily intake of metals (DIM) and health risk index (HRI) were also studied. The maximum value of PLI was found for Cd (990.03%) and minimum for Mn (8.40%) in soil irrigated with sewage water. High BAF values greater than 1 for Cd, Mn and Pb were observed in cabbage, cauliflower, spinach and coriander at SWI zone and could be one of the possible reasons for health risk in human via their consumption. The consumption of vegetables collected from SWI zone to daily intake of Pb, Cd, Mn, Cu, Ni and Cr were 17.26%, 12.91%, 2.27%, 0.584% 8.62% and 5.67% of PTDI respectively. Thus, the consumption of average amount of these vegetables does not pose a health risk for consumer. Cd, Pb and Mn showed HRI greater than one in cabbage, cauliflower, spinach and coriander collected from SWI zone, pose severe health risk while potato and carrot were almost safe for consumption. The vegetables grown at GWI zone were entirely free from any risk. However, the regular monitoring of levels of these metals in sewage water, soil and in vegetables is essential to prevent excessive build of the metals in the food chain.

1. Introduction

Vegetables are important part of human diet and are known to have positive effect on human health (Kumar and Seema, 2016; Ibrahim *et al.*, 2013). Anthropogenic and natural processes are responsible for increasing the levels of heavy metals in the agricultural soil (Shuaibu *et al.,* 2013). The use of pesticides, fungicides, fertilizers and agro chemicals by farmers for high production of vegetables may increase the concentration of heavy metals in soil which are taken by aerial parts of plant. The accumulation of heavy metals in plant vary with factors such as climate, soil properties, atmospheric deposition, plant species, and soil to plant factors of metals (Aktauzzaman *et al.,* 2013; Hamid *et al.,* 2016). The leafy part of vegetables were found to be contaminated by heavy metals more frequently than the stem and root parts of plant (Kumar and Seema, 2016; G. E. Nwajei, 2009). This is due to higher transpiration rate to maintain the growth and moisture content of these plants (Tam and Barrington, 2005). The major sources of metals are wastewater untreated or partially treated industrial effluents, municipal wastes and vehicles (Rasheed *et al.,* 2014; Mahmood and Malik, 2014). Aerosols also contribute to high levels of toxic metals on the surface of vegetables during the harvesting, transportation and marketing that depend upon various factors such as level of the pollutants, especially dust in air, nature of road, traffic loads and period of exposure or duration in which the vegetables are exported for marketing (Al Jassir *et al.,* 2005)

Due to shortage of water source and waste water treatment cost for discharge, the use of sewage water for agricultural activities specially growing vegetables, has gained importance through the world (Lone *et al.,* 2013). Sewage water mainly contain high amount of organic matter nutrients and also contain heavy metals so that sewage water irrigation has great potential to contaminate the soil which may lead to the accumulation of heavy metals in crops and plants and may be toxic to plants and animals, when present above permissible limits (Balkhair *et al.,* 2015). Continuous human consumption of contaminated vegetables may cause accumulation of heavy metals in the kidney and liver and can cause disturbance of biochemical process and cause cardiovascular, kidney, liver, nervous and bone diseases (Jena *et al.,* 2012; Yuan *et al.,* 2015., Khan *et al.,* 2009).

In the rural areas of Katihar block vegetables grown are on a commercial scale in sewage water, seepage water and municipal wastewater irrigated areas, but there is very little information on the level of heavy metals in the soil and vegetables produced in these areas. Thus the aim of this research work was to monitor and assess the concentration of heavy metals in water, soil and some selected vegetables grown at SWI and SWI zone of Katihar city, Bihar, India and also to estimate human health risk through their consumption.

2. Materials and Methods

2.1. Site Description

Katihar is an agricultural district that covers 3056 square km areas and located at 25.53°N and 87.58°E. Kathiar is too small but has large number of small scale industries. The wastewater and disposal of sewage water are drained to the agricultural land where these are used for growing crops and vegetables. In the present study we have selected two types of agricultural fields at the experimental sites. Groundwater from deep bore well is used for irrigation at groundwater irrigation zone designated as GWI, whereas wastewater from discharge sewage water for irrigation at other site designated SWI. Agricultural fields cover five panchyats such as Bhawara, Garbheli are located near the brick kiln industries and Dalan East, Sirnia East, Deheria are also close to 81 NH and small rivers as shown in Fig. 1.

2.2. Water and Soil Sampling

Sewage water and groundwater used for irrigation purpose were collected in a 100 mL pre acid-washed polypropylene bottle. 1 mL concentrated HNO_3 was added in each bottle to avoid microbial activity (Chary *et al.,* 2008). Soil samples were collected from 5 agricultural fields by digging a monolith of 10cm x 10cm x 10cm size by using plastic scooper. Each field was first sub divided into five parts, then all the collected soil samples were mixed together to form composite soil sample from each field. Non-soil particles were removed from each sample, dried at 40°C for 48 h and ground into fine powder and then sieved through 2 mm nylon sieve. After this each sample was transferred into air tight polyethylene bag and brought into laboratory for analysis as described by (Lei *et al.,* 2008).

2.3. Vegetable Sampling

The vegetables selected for heavy metal analysis were potato (*Solanum tuberosum* L.), coriander (*Coriandrum sativum*), spinach (*Spinacia oleraceae* L.), cauliflower (*Brassica oleracea* L.), carrot (*Dascus carota* L.) and cabbage (*Barssica oleracea* L.). These are the common vegetables which are grown in the experimental field for own consumption of the farmers and supply to retail and wholesale markets of Katihar town. The test vegetables were collected randomly from 10 ×5 m area of five different fields, each from SWI and GWI zones at 20 day interval for one year from February 2015 to March 2016. All the collected vegetables (one intact inflorescence head of cauliflower and 1kg each of potato, carrot, spinach and coriander) were washed with tap water to remove the soil particles and then inedible parts were removed. The edible part was sliced into pieces and dried separately on sheet of filter paper, then dried in oven at 75°C for 24 h and then crushed and sieved at room temperature and digested by using the method described by Jamail *et al.,* (2009).

2.4. Digestion of Samples

1gm of each sample of soil and vegetable was placed in 100 mL beaker separately and digested with 15 mL of tri-acid mixture i.e. HNO_3, $HClO_4$ and H_2SO_4 at 5:1:1: ratio at 80°C on an oven plate till the solution becomes transparent (Allen *et al.,* 1986). The solution thus obtained was filtered and each filtrate was made to 50 mL by mixing deionised water and subjected to atomic absorption spectrophotometer for analysis for heavy metals (Pb, Cd, Cu, Cr, Mn and Ni).

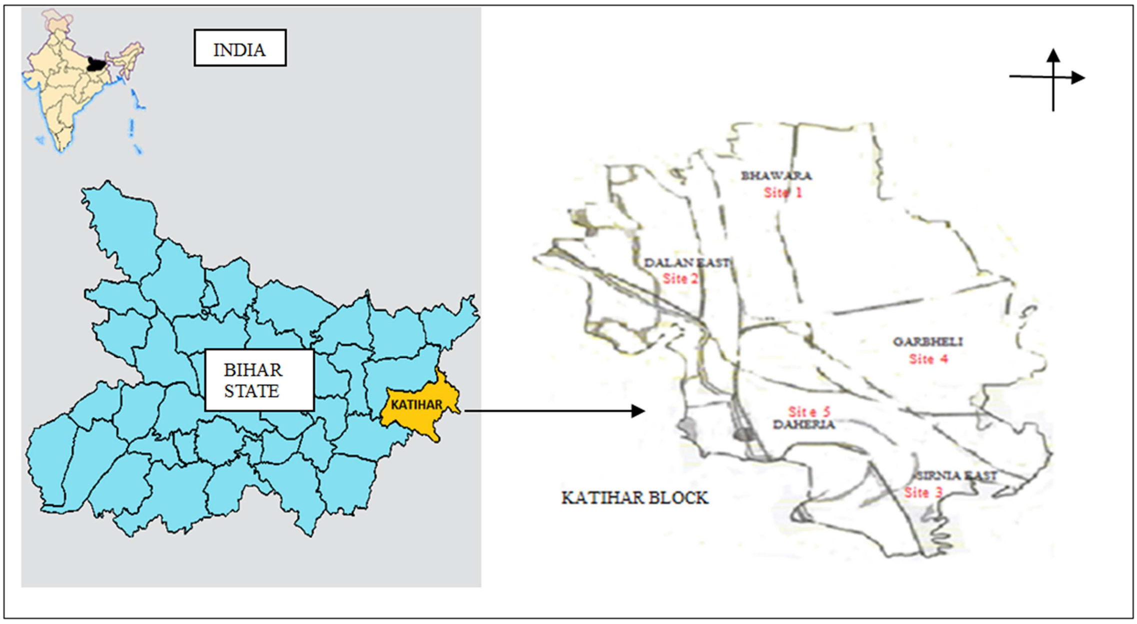

Figure 1. *Geographical location of study area.*

Table 1. *Description of vegetables samples collected from SWI and GWI zone.*

S.N	Common name	Vernacular name	Botanical name	Part used	Family
1.	Potato	Aalo	*Solanum tuberosum* L.	Tuber	Solanaceae
2.	Cabbage	Band Gobi	*Brassica oleracea* L.	Leaves	Brassicaceae
3.	Cauliflower	Phool Gobi	*Brassica oleracea* L.	Fruity flowers	Brassicaceae
4.	Spinach	Palak	*Spinacia oleraceae* L.	Leaves	Amaranthaceae
5.	Carrot	Gajr	*Dascusc arota* L.	Underground stem	Apiaceae
6.	Coriander	Dhania	*Coriandrum sativum*	Leaves	Apiaceae

2.5. Data Analysis

2.5.1. Pollution Load Index (PLI)

The degree of soil pollution for each metal was computed as pollution load index by using following modified equation, (Liu *et al.,* 2005).

$$\text{Pollution load index (PLI)} = \frac{C\ Soil}{C\ Reference} \quad (1)$$

Where, C_{Soil} and $C_{Reference}$ represent the metal concentration in the sewage water irrigated and reference soil respectively.

2.5.2. Bioaccumulation Factor (BAF)

The bioaccumulation factor was calculated by dividing the concentration of heavy metal in vegetables (mg /kg) by the total heavy metal concentration in the soil (mg /kg) by using following formula,(Cui *et al.,* 2004),

$$\text{Bioaccumulation Factor (BAF)} = \frac{C\ plant}{C\ soil} \quad (2)$$

Where, C_{plant} and C_{soil} represent the metal concentration in plant fresh weight and in soil dry weight respectively.

2.5.3. Daily Intake of Metals (DIM)

The daily intake of heavy metals by people through consumption of vegetables was calculated by using following formula, (Chary *et al.,* 2008).

$$\text{DIM } (\mu g\ day^{-1}) = \frac{C\ metal\ x\ D\ food\ intake\ x\ C\ factor}{B\ averagebodyweight} \quad (3)$$

Where, C_{metal} = Heavy metal concentration in plant (mg / kg), $D_{food\ intake}$ = Daily intake of vegetable (gm/ day /person), $B_{average\ weight}$ = Average body weight (kg), C_{factor} = Conversion factor (0.085). The average body weight was considered to be 52 kg by conducting survey of 100 adult (male and female) people from study areas in each period of sampling. The average daily vegetable in take for adult was considered to be 262 gm/day/person which were determined by formal interview conducted with people of study areas.

2.5.4. Health Risk Index (HRI)

The value of HRI depends upon the daily intake of metals and reference dose, which was computed as described by Jan *et al.,* (2010).

$$\text{HRI} = \frac{DIM}{RfD} \quad (4)$$

Where, DIM = Daily intake of heavy metals (μg /day), Rf_D-Reference dose, Rf_D value for Cd, Cr,, Ni, Cu, Pb and

Mn (mg/kg bw /day) 0.001, 1.5, 0.02, 0.04, 0.004 and 0.033 respectively (US-EPA IRIS., 2006). If the value of HRI is less than 1 then the exposed population said to be safe (US-EPA IRIS., 2006).

2.5.5. Statistical Analysis

All statistical analysis was performed on Lenovo™ computer using the Microsoft EXCEL and Word 2007 format. Similarly, the significance of differences between the concentrations of heavy metals in soil and vegetables were calculated by using Casio calculator (made in China) *fx-991 MS*. A probability of p>0.05 was considered statically significant.

3. Results

3.1. Concentration of Heavy Metals in Water and Soil Samples

The mean concentration of heavy metals such as Cu, Ni, Mn, Pb, Cr and Cd was found to be 18.87, 8.01, 1.45, 0.49, 0.34 and 0.24 mg/L and 0.0241, 0.0151, 0.0120, 0.0211, 0.0060 and 0.0032 mg/L in sewage water and ground water of study area respectively (Table 3). The results revealed that heavy metal concentrations in irrigated water at SWI zone were higher while at GWI zone were below than Indian Standards (Awasthi 2000). The level of Cu, Ni, Mn, Cd, Cr and Pb in sewage water was found to be 782.9, 530.5, 120.8, 75.0, 56.67 and 23.22 times of ground water. The results also revealed that concentration of heavy metals in soil irrigated with sewage water was below than normal soil value as recommended by Brown (1979) and safe limits of European Union (EU) except Cd. The level of Cd content varied from 1.31-5.82 mg/kg with mean value 3.467mg/kg. The maximum value was recorded at Dalan East (3.37 mg/kg), in Sarinia East (5.82mg/kg) and in Deheria (4.41 mg/kg). The percentage pollution load index (PLI) of each metal in soil at SWI zone compared with normal soil value was in the order of Cd (990%), Cu (83.67%), Ni (58.42%), Pb (46.37%), Cr (36.43%), Mn (8.40%) while at GWI zone was Cd (120%), Cu (9.4%), Ni (8.04%), Cr (4.94%), Pb (2.1%), Mn (0.35%) (Table 4)

Table 2. *Normal and critical value of heavy metal (mg/kg) accumulated by soil and plant.*

Biotopes	Cd	Pb	Mn	Cu	Cr	Ni
NSV (Brown, 1979)	0.35	35	1000	30	70	50
UCSV (K-Pendias*et al.,* (1984)	8.0	400	300 0	125	100	100
NPV (Alloway, 1968)	0.1- 2.4	0.1-10	NA	5-15	0.2 -10	1-2.7
CPV (Pendias*et al.,* (1992)	5 - 30	20- 300	300-500	20-100	5 - 30	10-100
EU (Plant)	0.20	0.43	500	20	1.0	NA
IS (Plant)	1.5	2.5	NA	30	20	67
FAO/WHO/EU (Plant)	0.10	0.30	0.20	0.1	0.10	0.20

NSV- Normal soil value as proposed by Browen (1979), UCSV-Upper critical soil value as proposed by Kabita-Pendias and Pendias (1984), NPV- Normal plant value as proposed by Aloway (1968), CPV- critical plant value as proposed by Kabita-Pendias and Pendias (1992),
IS-Indian Standards (Awasthi 2000), EU- European Union (2002)

3.2. Concentration of Heavy Metals in Vegetable Samples

In the present research work it has been found that accumulation of heavy metals in test vegetables grown in soil at SWI zone moderately enhanced with Cr, Cu, Ni and Mn while strongly enriched with Cd and Pb The mean concentration of Cd in vegetables collected from SWI zone was recorded 1.01- 4.85 mg/kg which was significantly higher than Indian standards (1.5 mg/kg) except potato (1.01 mg/kg) and carrot (1.16 mg/kg). The maximum accumulation of Cd (mg/kg) was in the order of spinach (6.35) > cabbage (6.25) > cauliflower (6.12) > coriander (5.89) > carrot (1.99) > potato (1.81) as shown Fig. 2. The mean concentration of Cd in vegetable grown at GWI zone was found in the range of 0.15-0.22 mg/kg which was below the Indian standards (Table 5). Similar trend was observed by M. S Fazeeli, (1998) and P. J. Doyle, (1998). The concentration of Pb in vegetables grown at SWI zone and GWI zone was in the range of 1.82-25.19 mg/kg and 0.05-0.45 mg/kg respectively. Among the edible parts, the maximum concentration of Pb was recorded in spinach (at Dalan east) followed by cabbage (at Sarinia east), cauliflower (at Deheria), coriander (at Dalan east), carrot and potato (at Deheria) at SWI locations (Fig. 3), which exceeded the Indian safe limits. The level of Pb content in vegetables grown at GWI zone was lower than EU standards (0.43 mg/kg) except cauliflower at Sarnia east and spinach at Deheria. The accumulation of Cu in the test vegetables grown at SWI zone was found to be in the range of 1.68-11.2 mg/kg which was 10 fold greater than vegetables collected from GWI zone (0.35-1.21 mg/kg). The concentration of Cu in vegetables at SWI zone was in the range of normal plant value (5-15 mg/kg) but below the critical plant value, whereas in ground water irrigated vegetables was below than normal plant value. The results also suggest that mean concentration of Cu was found significantly high in non leafy vegetables such as potato (8.72 mg/kg) and carrot (8.45 mg/kg) as shown Fig. 4. Alam *et al.,* (2003) reported that mean Cu accumulation in leafy and non leafy vegetables was 15.5 and 8.51 mg kg^{-1} respectively, which is more than the present observations except potato.

Table 3. *Heavy metals concentration in sewage water and ground water (mg/L) of study area.*

Sewage water of study area			**Ground water study area**			
Metal	**Range**	**Mean**	**Range**	**Mean**	**C_{SW}/C_{GW}**	**Indian St**
Cr	0.21-0.58	0.34	0.0011-0.0081	0.0060	56.67	0.05
Mn	0.23-2.13	1.45	0.0091-0.0212	0.0120	120.8	0.10
Ni	2.42-9.41	8.01	0.0096-0.0231	0.0151	530.5	1.40
Cu	9.78-20.57	18.87	0.0145-0.0343	0.0241	782.9	0.05
Cd	0.19-0.41	0.24	0.0015-0.0041	0.0032	75.00	0.01
Pb	0.31-0.78	0.49	0.0181-0.0263	0.0211	23.22	0.10.

Table 4. *Heavy metals accumulation (mg/kg) in soil at SWI and GWI zoon of study area.*

Sewage water irrigated soil (SWIS)				**Ground water Irrigated soil (GWIS)**			
Metal	**Range**	**Mean**	**PLI**	**Range**	**Mean**	**PLI**	**C_{SWIS}/C_{GWIS}**
Cr	12.41-35.01	25.50	0.3643	2.01 - 3.87	3.45	0.0494	7.39
Mn	15.35-96.00	84.02	0.0840	1.72- 3.92	3.51	0.0035	23.94
Ni	15.01-44.15	29.21	0.5842	1.98- 4.98	4.02	0.0804	7.27
Cu	12.30-29.15	25.10	0.8367	1.32- 3.01	2.82	0.0940	8.9 0
Cd	1.310-5.820	3.467	99003	0.11- 0.72	0.42	1.200	8.25
Pb	9.140-21.95	16.23	0.4637	0.55 - 1.21	0.75	0.0214	21.64

Table 5. *Accumulation of heavy metals (mg/kg) in the vegetables collected from SWI and GWI zoon.*

	Sewage water irrigated vegetables (SWIV)						**Groundwater Irrigated vegetables (GWIV)**					
Metals	**VG_1**	**VG_2**	**VG_3**	**VG_4**	**VG_5**	**VG_6**	**VG_1**	**VG_2**	**VG_3**	**VG_4**	**VG_5**	**VG_6**
Cd												
Min.	0.21	0.45	0.43	0.58	0.19	0.39	0.03	0.07	0.05	0.06	0.04	0.05
Max.	1.81	6.25	6.12	6.35	1.99	5.89	0.25	0.34	0.31	0.35	0.30	0.32
Median	1.31	5.92	6.12	5.95	1.63	5.89	0.21	0.22	0.31	0.30	0.25	0.32
Mean	1.01	4.52	4.02	4.85	1.16	4.62	0.15	0.21	0.20	0.22	0.16	0.21
Σ_n	5.05	22.6	20.1	24.25	5.80	23.1	0.75	1.05	1.00	1.10	0.80	1.05
σ_{n-1}	0.620	2.348	2.312	2.422	0.717	2.374	0.095	0.028	0.044	0.115	0.11	0.104
Pb												
Min.	1.82	9.31	9.11	9.12	1.95	7.79	0.05	0.17	0.09	0.11	0.09	0.10
Max.	6.52	25.02	24.84	25.19	6.72	23.95	0.31	0.34	0.45	0.45	0.30	0.42
Median	6.62	25.02	20.15	20.22	5.86	19.63	0.19	0.25	0.45	0.22	0.19	0.21
Mean	4.95	19.32	19.25	19.33	5.32	18.11	0.18	0.25	0.21	0.24	0.19	0.22
Σ_n	24.75	96.60	96.25	96.65	26.6	90.55	0.90	1.25	1.05	1.20	0.95	1.10
σ_{n-1}	1.838	5.933	6.049	6.125	1.934	6.097	0.103	0.652	0.148	0.133	0.09	0.123
Mn												
Min.	17.64	61.14	65.17	67.32	19.37	67.84	1.11	2.12	1.41	2.25	1.52	2.13
Max.	55.12	98.72	95.20	99.73	68.36	97.73	2.70	5.15	5.01	5.25	4.01	5.02
Median	54.53	92.18	95.20	90.33	65.76	97.73	1.72	3.01	5.01	5.25	3.01	5.02
Mean	45.23	86.32	85.12	87.34	52.32	87.74	1.75	3.05	2.80	3.21	2.81	3.06
Σ_n	226.15	431.6	425.62	436.7	261.6	438.5	8.75	15.25	14.00	16.05	14.05	15.30
Σ_n^2	11224	38151	36789	38759	152193	9030	16.75	52.46	46.63	57.23	44.23	52.08
σ_{n-1}	15.775	14.962	11.854	12.422	19.574	11.974	0.598	1.219	1.363	1.195	1.09	1.147
Cu												
Min.	4.66	1.68	1.71	4.04	4.62	2.26	0.62	0.42	0.45	0.35	0.63	0.51
Max.	11.2	8.21	9.20	11.02	10.94	9.90	1.21	0.85	0.92	0.83	1.23	0.61
Median	9.28	5.26	6.49	8.29	10.94	7.50	0.96	0.67	0.92	0.45	0.97	0.56
Mean	8.72	5.25	6.01	8.11	8.45	6.72	0.95	0.65	0.67	0.52	0.92	0.56
Σ_n	43.6	26.25	30.05	40.55	42.25	33.6	4.75	3.25	3.35	2.60	4.60	2.80
σ_{n-1}	2.667	2.617	3.084	2.601	2.406	2.976	0.230	0.167	0.174	0.189	0.233	0.038
Ni												
Min.	4.78	5.75	5.48	6.06	4.82	5.04	0.12	0.22	0.18	0.82	0.30	0.35
Max.	10.2	14.15	14.52	15.02	11.25	12.32	2.21	2.78	2.42	3.12	2.32	2.65
Median	8.86	11.95	14.52	12.15	9.02	10.82	1.03	1.52	2.42	2.48	2.32	1.37
Mean	8.251	1.21	10.76	11.51	8.52	10.12	1.12	1.56	1.43	2.01	1.42	1.52
Σ_n	41.25	56.05	53.8	57.55	42.6	50.60	5.60	7.80	7.15	10.05	1.42	7.60
σ_{n-1}	2.176	3.332	3.412	3.372	2.591	3.388	0.787	1.041	0.959	1.054	0.735	0.829
Cr												
Min.	1.01	1.90	1.21	1.41	0.98	1.38	0.16	0. 36	0.48	0.50	0.35	0.38
Max.	3.31	4.62	5.01	5.28	3.94	5.32	0.61	0.84	0.96	0.98	0.79	0.85
Median	2.01	4.01	5.01	4.02	2.10	4.45	0.52	0.74	0.96	0.88	0.74	0.76

	Sewage water irrigated vegetables (SWIV)						Groundwater Irrigated vegetables (GWIV)					
Metals	**VG_1**	**VG_2**	**VG_3**	**VG_4**	**VG_5**	**VG_6**	**VG_1**	**VG_2**	**VG_3**	**VG_4**	**VG_5**	**VG_6**
Mean	1.72	3.25	3.27	3.31	2.01	3.29	0.43	0.62	0.71	0.74	0.61	0.64
Σ_n	8.86	26.25	16.35	16.55	10.05	16.45	2.15	3.10	3.55	3.70	3.05	3.20
σ_{n-1}	0.976	1.098	1.456	1.509	1.201	1.632	0.171	0.185	0.197	0.196	0.175	0.18

VG_1-Potato, VG_2-Cabbage, VG_3-Cauliflower, VG_4-Spinach, VG_5-Carrot, VG_6-Coriander, Σn- Sum of the values, σ_{n-1} Sample standard deviation, Min – Minimum, Max-Maximum

The accumulation of Cu in our study (1.68-11.2 mg/kg) was also lower than accumulation of Cu (22.19-76.50 mg/kg) in the leafy vegetables reported by Demirezen and Alsoy, (2006). The results clearly revealed that the level of Mn was in the range of 17.64-99.73 mg/kg and 1.11-5.25 mg/kg for vegetables collected from SWI and GWI zone respectively (Table 5). A considerable increase in the mean concentration of Mn from 2.78- 74.12 mg/kg has been found when vegetables grown at GWI to SWI zone. In the present study Mn content was much higher than safe limit of 0.2 mg/kg recommended by WHO/EU, (1990) but below the critical plant value (300-500 mg/kg). The maximum concentration (mg/kg) of Mn was recorded in spinach (99.73) followed by cabbage (98.72), coriander (97.73), cauliflower (95.2), carrot (68.36) and potato (55.12) as shown in Fig. 5. The concentration of Cr varied from 1.01-5.32 mg/kg and from 0.16 - 0.98 mg/kg for sewage water and ground water irrigated vegetables respectively (Table 5). The level of Cr content in vegetables grown at SWI zone exceeded the permissible limit of EU (1.0 mg/kg). The maximum concentration of Cr was recorded as 5.32 mg/kg in coriander at Dheria and minimum as 1.01 mg/kg in potato at Bhawara locations of SWI zone (Fig. 6). The present finding were lower than concentration reported by Gupta *et al.,* (2008) in west Bengal, India (34.83-96.30 mg/kg) and also lower than 5.35-27.83 mg/ kg as reported by Sharma *et al.,* (2007) in Varanasi. The concentration of Ni was varied form 4.78 to15.02 mg/kg and 0.12 to 3.12 mg/kg in vegetables obtained from SWI and GWI sites respectively (Table 5). All vegetables grown at SWI zone and some locations of GWI zone, concentration of Ni exceeded the normal plant value (1- 2.7 mg/kg). The maximum accumulation of Ni in edible part of spinach at Deheria and minimum was observed at Bhawara location of SWI zone of study area (Fig. 7).

Table 6. *Mean concentration of heavy metals (mg/kg) in different vegetables at SWI zone and percentage accumulation.*

Metal	Potato	Cabbage	Cauliflower	Spinach	Carrot	Coriander	Average	C_{AW}/C_{IS}	% value
Ni	8.25	11.21	10.76	11.51	8.52	10.12	10.06	0.1502	15.02
Cr	1.75	3.25	3.27	3.31	2.01	3.29	2.808	0.1404	14.04
Cu	8.72	5.25	6.01	8.11	8.45	6.72	7.210	0.2403	24.03
Cd	1.01	4.52	4.05	4.85	1.16	4.62	3.368	2.2453	224.53
Pb	4.95	19.32	19.25	19.33	5.32	18.11	14.38	5.752	575.2
Mn	45.23	86.32	85.12	87.34	52.32	87.74	74.01	0.1480	14.80
Total	169.88	129.87	128.46	134.45	77.78	130.06	-------	--------	-------

C_{AW} = Average concentration of metal in plant, C_{IS} = concentration of heavy metal in plant as per Indian Standards

Table 7. *Daily intake of metals established with respect to Rf_D and PTDI.*

Metals	Mean DIM (mg/day)	Rf_D	Rf_D ×70	% of Rf_D	PTDI (µg/day)	% of PTDI
Cd	0.007754	0.001	0.07	11.06	60	12.91
Cr	0.010215	1.5	105	0.01	180	5.675
Ni	0.025850	0.02	1.40	1.185	300	8.62
Cu	0.017524	0.04	2.80	0.625	3000	0.584
Pb	0.036940	0.004	0.28	13.19	214	17.26
Mn	0.190133	0.033	2.31	8.23	8400	2.27

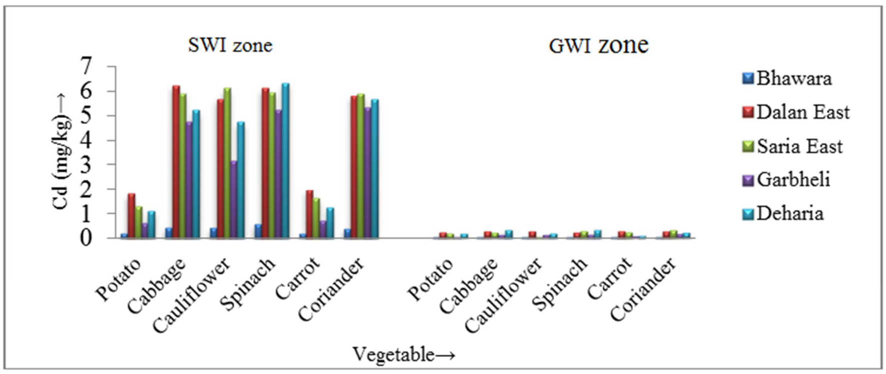

Figure 2. *Concentration of Cd in vegetables grown at SWI and GWI zone.*

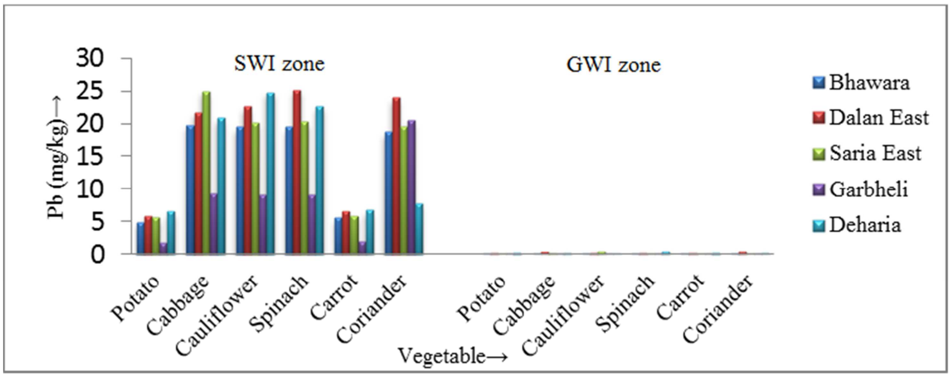

Figure 3. *Concentration of Pb in vegetables grown at SWI and GWI zone.*

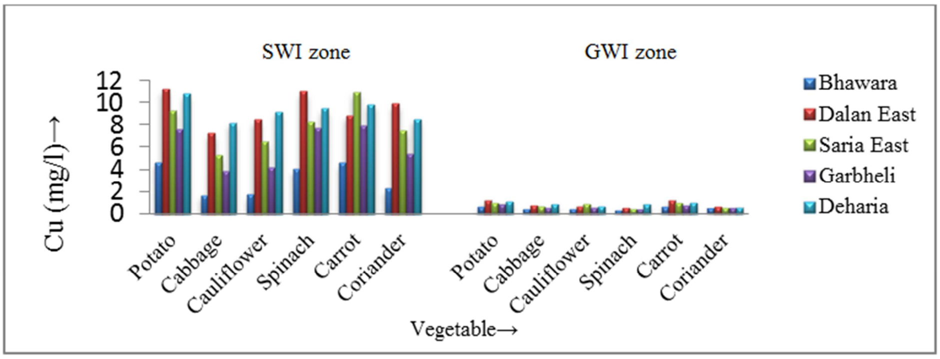

Figure 4. *Concentration of Cu in vegetables grown at SWI and GWI zone.*

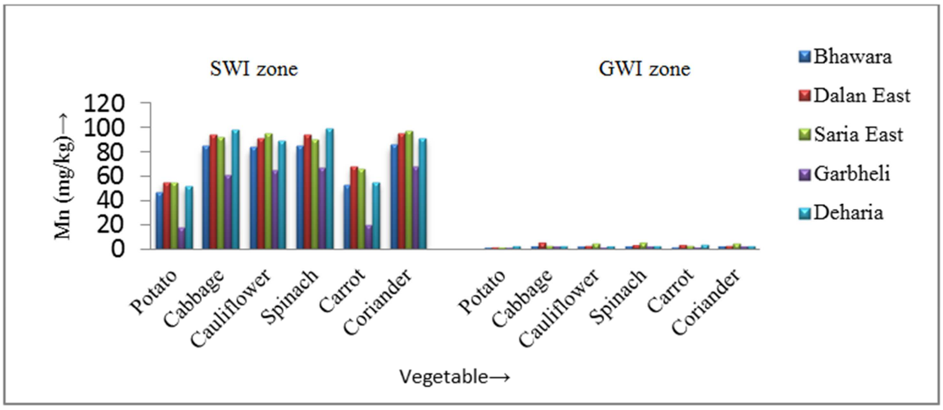

Figure 5. *Concentration of Mn in vegetables grown at SWI and GWI zone.*

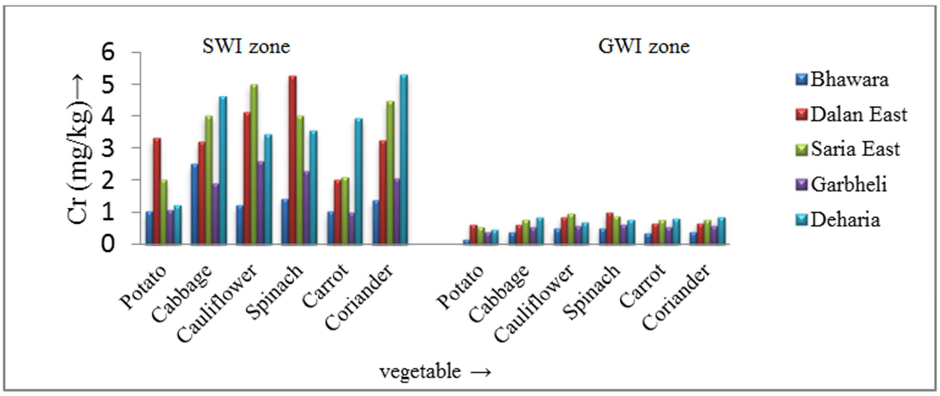

Figure 6. *Concentration of Cr in vegetables grown at SWI and GWI zone.*

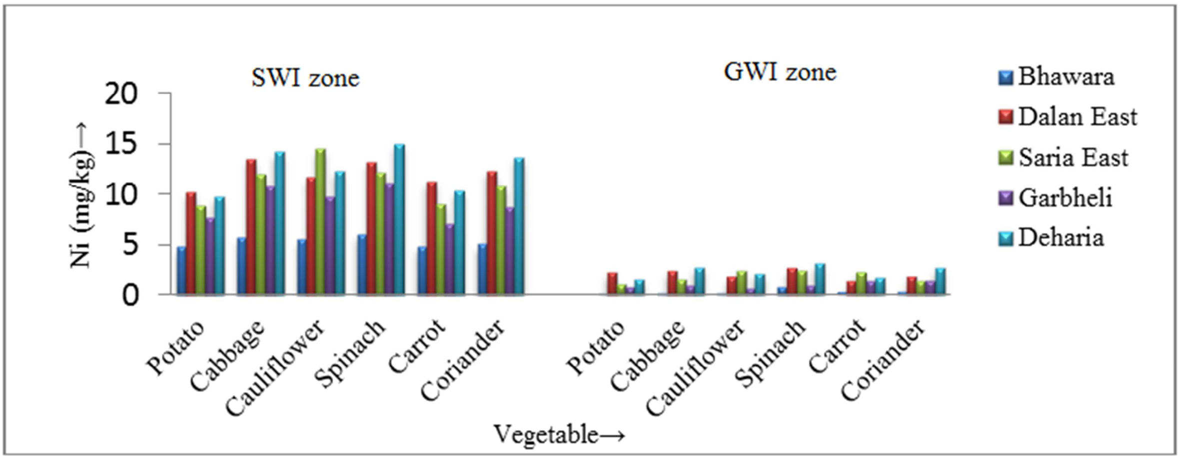

Figure 7. *Concentration of Ni in vegetables grown at SWI and GWI zone.*

3.3. Bioaccumulation Factor of Metals from Soil to Vegetables

The BAF of heavy metals from soil to vegetables in the study area is presented in Fig. 8 which showed that BAF of Cd, Pb, Mn, Cu, Ni, Cr for vegetable grown at SWI zone varied from 0.291-1.399, 0.304-1.191, 0.538-1.039, 0.209-0.347, 0.282-0.394 and 0.0675-0.135 respectively. Cd, Pb and Mn showed BAF greater than 1 in all vegetables expect potato and carrot whereas Cu, Ni and Cr showed lower than 1 for all vegetables grown at SWI zone and was found in the order Cd > Pb > Mn > Ni > Cu > Cr. BAF of metal from soil to vegetables grown at GWI zone for Cd, Pb, Mn, Cu, Ni and Cr ranged from 0.366-0.537, 0.240-0.333, 0.498-0.915, 0.184-0.337, 0.279-0.500 and 0.125-0.215 respectively and was trend of Mn > Cd > Ni > Pb > Cu > Cr. In the GWI zone highest BFA for Mn was found in spinach (0.915) and lowest for Cr in potato (0.125).

3.4. DIM and HRI of Metals

The degree of toxicity of heavy metals to human health being depends upon their intake. The estimated DIM through food chain is given in Fig. 9. DIM (µg/day) for Cd, Pb, Mn, Cu, Ni and Cr varied from 0.432 - 2.077, 2.119 - 8.269, 19.368 - 37.553, 2.248 - 3.734, 3.533 - 4.929 and 0.861 - 3.737 respectively in the vegetables grown at SWI zone and the values of DIM ranged from 0.0642 - 0.116, 0.077 - 0.107, 0.0695 - 1.375, 0.0287 - 0.407, 0.479 - 0.861 and 0.184-0.317 for Cd, Pb, Mn, Cu, Ni and Cr respectively.

The human health risk index calculated for metals of vegetables for adult was calculated and is presented in Fig. 10. The HRI varied from 0.4323 - 2.0767, 0.5299 - 2.0693, 0.5869 - 1.1379, 0.0562 - 0.946, 0.1766 - 0.240 and 0.00049-0.00094 for Cd, Pb, Mn, Cu, Ni, and Cr respectively and its rank appeared as Pb > Cd > Mn > Ni > Cu > Cr in case of vegetable grown at SWI locations. The trend of HRI for vegetables grown at GWI locations was in the order Cd > Mn > Ni > Pb > Cu > Cr. The spinach, cabbage, cauliflower and coriander collected at SWI zone showed HRI greater than one, while rest vegetables grown at SWI locations and all vegetables collected from GWI locations showed HRI less than one.

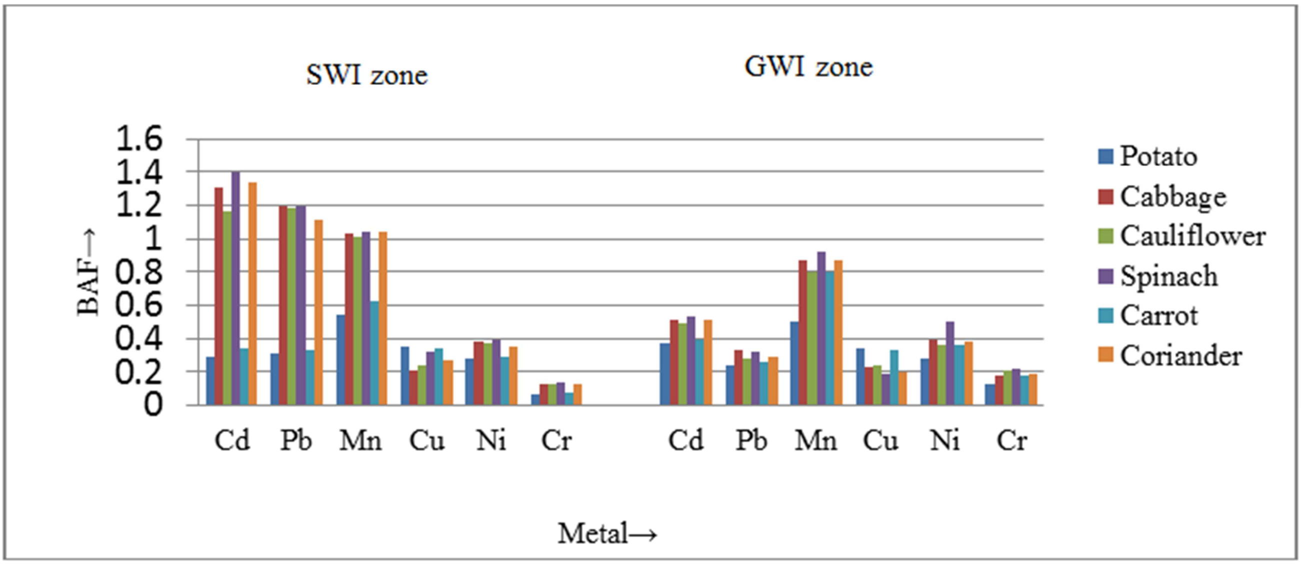

Figure 8. *BAF of heavy metals from soil to vegetables grown at SWI and GWI zone.*

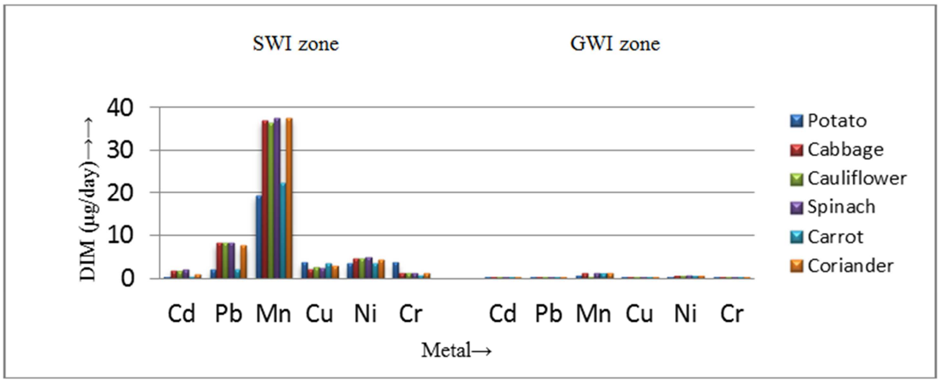

Figure 9. *DIMsthrough consumption of vegetables grown at SWI and GWI zone.*

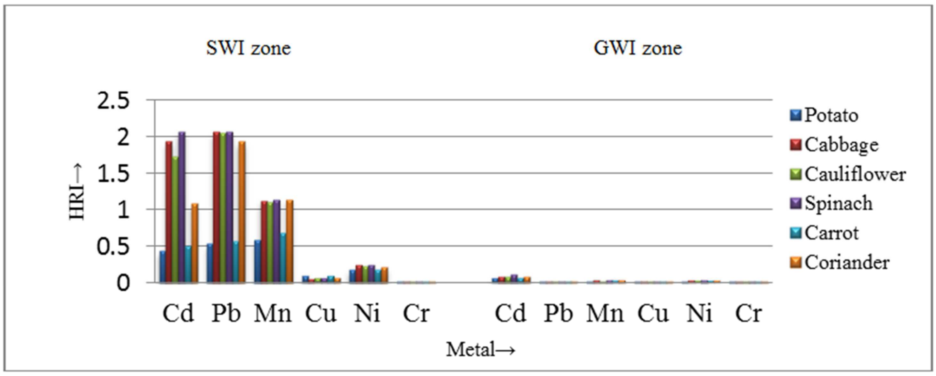

Figure 10. *HRI of heavy metals through consumption of vegetables grown at SWI and GWI zone.*

4. Discussions

Sewage water of the studied areas contains mainly municipal, domestic and hospital wastes of Katihar city that are discharge into drainage around agricultural fields. On the basis of survey of studied area and observed data, it was found that sewage water near the discharge point contaminated appreciably high and gradually decreased as distance increased (Khan *et al.,* 2001; Lone *et al.,* 2013). The high concentration of heavy metals in sewage water was also due to availability of different sources of sewage water.

The accumulation of selected heavy metals (mg/kg) in different vegetables was observed in the order of spinach (134.45) > coriander (130.06) > cabbage (129.87) > cauliflower (128.46) > carrot (77.78) > potato (69.88) as shown in Table 6. Spinach (*Spinacia oleracea* L.) is cultivated as a crop in the various parts of world. Fresh leaves are rich source of flavonoid, vital anti-oxidant, vitamins and polyphenolic-antioxidant. Health benefits of spinach are good eye-sight, regulates blood pressure, strengthens muscles and prevents age-locked muscular degeneration, cataracts and maintains proper metabolic activity. The mean concentration of Ni, Cr, Cu, Cd, Pb and Mn was recorded as 11.51, 3.31, 8.11, 4.85, 19.33 and 87.34 mg/kg at SWI zone respectively (Table 6). The levels of Ni, Cd and Pb in spinach at SWI zone were found to be comparable with the values reported in the spinach of the Chhattisgarh, Bangalore and Delhi in India and Bangladesh regions (Ramteke *et al,* 2016; Nasser *et al,* 2011; Singh and Kumar 2006; Ramesh *et al,* 2012). Coriander is an essential annual herb in the family Apiaceae. Fresh leaves of coriander are widely used as an ingredient in Indian food. It is rich in source of vitamin K, protein and carotien antibacterial chemical. It helps to lower cholesterol, blood pressure, blood sugar naturally. The mean concentration of Ni, Cr, Cu, Cd, Pb and Mn in coriander obtained from SWI zone was 10.2, 3.29, 6.72, 4.62, 18.11 and 87.74 mg/kg respectively (Table 6). The level of Pb and Cd content in coriander was found to be higher than value reported by Ramtake *et al.,* (2016) in Chhattisgarh, India. Cabbage (*Brassica oleracea* L.) is leafy vegetable of Brassic family and is round or oval shaped. It is very rich in fiber and is an abundant source of vitamin C and frequently used as treatment for constipation, stomach ulcer, headaches, skin diseases, eczema, jaundice arthritis, eye-disorders and heart diseases. The mean concentration of studied heavy metal like Ni, Cr, Cu, Cd, Pb and Mn in cabbage grown at SWI zone was 11.21, 3.35, 5.25, 4.52, 19.32 and 86.32 mg/kg respectively (Table 6). The content of three metals Cr, Cd and Pb in cabbage was found to be higher than safe limits of 1.0, 0.20 and 0.43 as recommended by EU standard, (2002). Cauliflower is a member of the Cruciferous family of vegetables and contains an impressive array of nutrients including vitamins, minerals, antioxidant and other photochemical. The accumulation of heavy metals in cauliflower was found 10.76, 3.27, 6.01, 4.05, 19.25 and 85.12 for Ni, Cr, Cu, Cd, Pb and Mn mg/kg at SWI zone respectively. The content of Cr, Cd and Pb in cauliflower was found to be higher than safe limits of EU whereas levels of Ni, Cu and Mn were below the EU but in the range of the normal plant value. Carrot (*Daucus carota* L.) is a root vegetable and is the second most popular type of vegetable after potato. It is good source of antioxidant, vitamin A, vitamin C, vitamin K, vitamin B_8, pantothenic acid; it reduces cholesterol, prevents heart attacks and improves vision. The range of Ni, Cr, Cu, Cd, Pb and Mn in carrot was varied from 4.82-11.25, 0.98-3.94, 4.62-10.94, 0.19-1.99, 1.95-6.72 and 19.37-68.36 mg/kg at SWI zone respectively (Table 5). The data revealed that the concentration of Cr, Cd and Pb in carrot was found to be higher than safe limits of EU standard but below the critical plant value as proposed by Pendas *et al.,* (1992). Potato is a stem vegetable and recent studies at agriculture research service suggested that flavonoid, antioxidant, quescetin are present in potato. It has anticancer and cardio protective properties. It is one of the finest sources of starch, minerals, dietary fiber. The range of Ni, Cr, Cu, Cd, Pb and Mn in potato was observed as 4.78-10.2, 1.01-3.31, 4.66-11.2, 0.21-1.81, 1.82-6.52, and 17.67-55.12 mg/kg at SWI zone respectively (Table 5). The concentration of Mn, Cu and Ni in potato was lower whereas the concentration of Cr, Cd and Pb was above the safe limit of EU standards.

Within the same environment, different vegetables have different accumulation power to accumulate heavy metals. The accumulation of heavy metals in coriander, cabbage spinach (leafy vegetables) was observed high due to the large surface area of their leaves, their high transpiration and faster growth rate, which enhances the metal translocation in leafy vegetables sensitizes them to be recipient of dust and rain water splashes (Luo *et al.,* 2011). The concentration of Ni, Cr, Cu, Cd, Pb and Mn was also high in cauliflower, this may be due to higher exposed area of inflorescence and has greater capacity to absorb heavy metals from atmosphere (Sharma *et al.,* 2009).

Most of leafy (aerial) part of vegetable shows higher Cd accumulation compared to root (ground) part. Our results show in agreement with previous studies by Sanita and Gabbrielli (1999); M. S Fazeeli (1998) and P. J. Doyle (1998), it has been found that Cd is a highly mobile metal and can be easily absorbed by the plant through root surface and moves to wood tissue and transfers to upper parts of the plant. Therefore, there is direct relation between the levels of presence of Cd in the root zone and its absorption by plant is also supported by Ramos *et al.,* (2002). Cd induces the gastrointestinal problem and severe toxicity on different organs of the body like kidney, liver, testis, ovaries and cardiovascular system. Harwting, (1998) and Saplakogelu and Iscan, (1997) have also reported that long term exposure of Cd can cause renal prostate and ovaries cancer. One of the explanations for high level of Pb in sewage water and some locations of ground water irrigated vegetables could be the uptake of Pb promoted by soil pH and the levels of organic matter (Balkhair *et al.,* 2015). The high level of Pb in all vegetables grown at SWI zone could also be attributed to acid lead batteries; household, paint/pigments industrial waste discharge in the irrigation system at the studied area and also due to agricultural land near to National highway. These results are consistent with previous studies which reported that the concentration of Pb in the washed and unwashed coriander was 0.171 and 0.226 mg /kg (Al Jassir *et al.,* 2005). Thus in the present study vegetables grown in soil at SWI zone may cause health risk of local population. Pb is toxic element because of its global distribution, accumulation tendency in the body and toxicity of Pb is at even low concentration. So many pathological conditions are associated with Pb such as anemia, reduced hemoglobin synthesis, bone metabolism, cardiovascular diseases and in children and adults (Zheng *et al.,* 2007; Nagajyoti *et al.,* 2010). Yang *et al.,* (2002) reported that Cu levels in both root and shoot increased, but in root Cu concentration increased more sharply than shoot with increasing Cu levels in the growth media. Copper mainly accumulated in roots while certain fraction of absorbed Cu was transferred to shoot. The concentration of copper in the shoots was significantly influenced by Cu concentration in soil, was also reported by Xiong and Wang, (2005). Among all the test vegetables leafy vegetable including cauliflower, the accumulation of Mn was significantly higher than in root (carrot) and stem (potato) type. The high concentration of Mn in aerial part of vegetables may be due to the soil type and application of agrochemicals like pesticides and fertilizer (Chary *et al.,* 2008). The possible reason for low concentration of Cr in plant is due to lower chromate uptake which occurs only in hexavalent and readily reduced immobile trivalent from soil (Streit and Stumm, 1993). Cr is an important element for insulin and DNA transcription. However intake below 0.02 mg/kg could reduce cellular responds to insulin (M. Kohlmeier, 2003). The range of Ni content (4.78 -15.02 mg/kg) in vegetable grown at SWI and some locations of GWI zone was significantly higher than 0.98-2.93 mg/kg as reported by Khan *et al.,* (2015) and in good agreement with 1.0-3.25 mg/Kg. reported by Mahmud and Malik, (2014). The high level of Ni in the study area may be due to ultramafic rocks and soil derived from these rocks and also due to extensively used catalyst in different industrial and chemical processes.

To investigate the human health risk index metal bioaccumulation factor from soil to plant is essential tool (Cui *et al.,* 2004). Among studied vegetables cabbage, spinach, cauliflower and coriander showed high metal transfer from soil to plant. The high uptake of metals in these vegetables may be reason to high transpiration rate to sustain the growth and moisture content in leafy vegetables (Tam and Barriangton, 2005). The bioaccumulation factor for Cd, Pb and Ni greater than one in most test vegetables indicates, greater accumulation and these metals have high transfer factor that migrate to the edible part of the vegetables (Luo *et al.,* 2011). This may be possible reason that these metals reflect their high accumulation value in various food crops to such a high ratio, High BAF values were observed for Cd, Pb and Ni metals with sewage water irrigated vegetables and could be one of the possible reasons for health risk in human via their consumption. The BAF value greater than 0.5 suggests that vegetables will have greater chance of metal contamination by anthropogenic activates (Suijd *et al.,* 2009).

DIM depends upon the properties of soil and also species of vegetables; the highest DIM (µg/day) was found for spinach (56.571), for cabbage (55.606), for cauliflower (55.005), for coriander (55.006), for carrot (33.306) and potato (29.922). The results show in agreement with previous studies showing levels of heavy metals in edible part of food crops irrigated continuously with waste water (Khan *et al.,* 2007; Liu *et al.,* 2005). There are various possible exposure pathways of pollutants to the human health but food chain is one of the most important pathways for exposure to pollutant including heavy metals. The daily intake of Pb was estimated to 0.0394 mg/day, which is about 13.2% of the Rf_D, established to 0.28 mg/day for 70 kg adult (WHO. 1993). Similarly DIM values for Cd, Mn, Ni, Cu and Cr were estimated at 0.007754, 0.19013, 0.02585, 0.01752 and 0.010215 mg/day, which represent approximately 11.06%, 8.23%, 1.185%, 0.625% and 0.01% of the Rf_D, established to 0.07, 2.31, 1.4, 2.8 and 105 mg /day for 70 kg adult respectively (Table 7). The exposure of consumers and related health are generally expressed in term of the provisional tolerable intake (PTDI), the FAO/WHO which have a set of PTDI limit for the heavy metal intake based on body weight for an average adult (60 kg) is given Pb-214 µg/day, Cd-60 µg/day, Mn-8400 µg/day, Ni-300 µg/day, Cu-3000 µg/day and Cr-180 µg/day. The consumption of vegetables collected from SWI zone to daily intake of Pb, Cd, Mn, Cu, Ni and Cr were 17.26%, 12.91%, 2.27%, 0.5845%, 8.26% and 5.67% of PTDI respectively. In the studied area vegetables grown at sewage water irrigated soil may be possible reason for health risk for the local residents due to metal contamination, as the estimated daily intake values for Pb (0.03694), Cd (0.007754), Ni (0.02585) and Mn (0.190133) mg/day were found to be higher as compare to Rf_D for Pb, Cd, Ni and Mn is 0.004, 0.001, 0.02, 0.033 mg/kg bw/day respectively. The data also revealed that our estimated daily intake for studied heavy metals is below the PTDI limits, reported by the FAO/WHO. Thus, the consumption of average amount of these vegetables does not pose a health risk for consumer.

In our study Cd, Pb and Mn showed HRI greater than one indicate a possible future human health risk, via intake of vegetables grown in soil irrigated with sewage water. Our results show the agreement with those reported by Khan *et al.,* 2010; Jan *et al.,* 2010 and Mahmood and Mlik (2014). Cd and Pb are considered to be non-essential hazard, even at extremely low concentration. The HRI values of our present finding also show that cabbage cauliflower, spinach and coriander collected from SWI zone pose severe health risk while potato and carrot were almost safe for consumption (Ikeda *et al.,* 2000; Zhuang *et al.,* 2009). The vegetables grown at ground water irrigated soil were entirely free from any risk.

5. Conclusion

The soil irrigated with sewage water in the studied area was extremely contaminated with heavy metals specially Cd, Pb and Mn. They were accumulated in the vegetables and their continuous consumption may disturb the biological and biochemical function in the human body. The heavy metals in the edible parts of vegetables were as follows Cd (575%) > Pb (224.5%) > Cu (24%) > Ni (15%) > Mn (14.8%) > Cr (14%). The BAF for Cd, Pb and Mn greater than one in most of studied vegetables indicates greater accumulation, which may be due to high biomass production. The estimated dietary intake of Cd, Pb and Mn were for human through the consumption of vegetables in the study was higher than Rf_D limit set by the US - EPA, IRIS. The findings of this study regarding DIM compare with PTDI suggest that the consumption of vegetables grown at SWI locations is nearly free from of risk but HRI >I for Cd, Pb and Mn indicating potential health risk. Therefore, continuous monitoring of the water quality, soil and plant of the study area and develop different strategies to prevent the entering of heavy metals in food chain may ultimately minimize the potential health hazards to human beings.

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