International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 6; Issue 10; October 2017; Page No. 7065-7074
DOI: http://dx.doi.org/10.24327/ijcar.2017.7074.1075

Research Article

COMPARATIVE STUDY OF LEVELS OF HEAVY METALS IN UNWASHED AND WASHED VEGETABLES AND THEIR IMPACT ON HUMAN HEALTH

Kumar A[1]*., Kumar V[2] and Seema[3]

[1]*Department of Chemistry, Darshan Sah College, Katihar- 854105, Bhupendra Narayan Mandal University, Madhepura, India
[2]Department of Chemistry and Environmental Science, Katihar Engineering College, Katihar 854106, Aryabhatt Knowledge University, Patna, India
[3]Department of Botany and Plant Physiology, College of Horticulture, Noorsarai, Nalanda-803113, Bihar Agricultural University, Sabour, Bhagalpur, and Bihar, India

A R T I C L E I N F O

Article History:

Received 16th July, 2017
Received in revised form 9th August, 2017 Accepted 5th September, 2017
Published online 28th October, 2017

Key words:

Vegetable, Heavy metal, Percentage reduction, Daily intake of metal, Health risk index, Risk Assessment

A B S T R A C T

This study was aimed to compare the level of heavy metals in unwashed and washed vegetables collected from urban and remote areas. The unwashed vegetables showed higher metal concentration than washed vegetables. The mean concentration of Cu and Zn was lower than PFA and FAO/WHO standard, at urban and remote areas, while mean concentration of Pb and Cd was found to be higher than PFA standard in unwashed and washed vegetables at urban areas, whereas lower than PFA standard but exceeded the FAO/WHO standard in both unwashed and washed vegetables at remote areas. Percentage reduction (PR) of heavy metals in vegetables was observed in the order of Pb > Cd > Cu > Zn at urban and Pb > Cd > Zn > Cu at remote areas. The daily intake of Pb, Cd, Cu and Zn were 6.98, 24.98, 1.3 and 0.123% of PDTI respectively. Health risk index (HRI) > 1 was found to be 4.627 (spinach), 4.429 (ladyfinger), 2.403 (cauliflower), 1.954 (radish) and 1.572 (tomato) for unwashed, and 2.031 (spinach) and 1.936 (ladyfinger) for washed vegetables at urban areas posed a severe health risk with respect to Cd. Therefore, proper washing of vegetables before consumption and also appropriate precaution should be taken at the time of marketing of vegetables.

INTRODUCTION

Vegetables are one of the important components of human diet because they provide essential micro and macronutrients, carbohydrates, proteins, vitamins and antioxidants to human body (Kumar *et al*., 2017a; Ramteke *et al*., 2016; Yuan *et al*., 2015). 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 contamination of vegetables with heavy metals due to soil and atmospheric contamination poses a threat to its quality and safety. Aerosols cause the heavy metal contamination in soil through atmospheric deposition which are consequently absorbed and accumulated by plant or get adsorbed on aerial parts of the plant (Udossen *et al*., 2017; Sharma *et al*., 2008b; Al Jassir *et al*., 2005; Temmerman and Hoenig, 2004). Atmospheric deposition also contribute to high levels of toxic metals on the surface of vegetables which 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 (Agrawal, 2003; Dogheim *et al*., 2004). The leafy parts of vegetables were found to be contaminated by heavy metals more frequently than the stem and root parts of plant (Kumar and Seema, 2016; Nwajei, 2009). This is due to higher transpiration rate to maintain the growth and moisture content of these plants (Tani and Barrington, 2005). The major sources of metals are wastewater untreated or partially treated industrial effluents, municipal wastes and vehicles (Kumar *et al*., 2017b; Balkhair and Ashraf, 2016; Rasheed *et al*., 2014; Mahmood and Malik, 2014; Arora *et al*., 2008; Chary *et al*., 2008). A market based study was also conducted by so many researchers to assess the atmospheric deposition of heavy metals in fruit and vegetables (Kumar *et al*., 2017a; Shuaibu *et al*., 2013 Radwan and Salama 2006; Sharma *et al*., 2009). The accumulation of heavy metals in excessive amount was found toxic for plant and human beings. Histidine, citric acid, malic acid and oxalic acid present in the plant form complexes with heavy metals and convert the metals into nontoxic form (Agrawal *et al*., 2007; Hall, 2002).

Corresponding author: **Kumar A**
Department of Chemistry, Darshan Sah College, Katihar- 854105, Bhupendra Narayan Mandal University, Madhepura, India

Consumption of such contaminated vegetables may pose a health risk to human beings. Since non-biodegradable and persistent nature of heavy metals, they are easily accumulated in soft tissues of human beings such kidney liver and bone that result in various problems in the human body (Kumar *et al*., 2015). The degree of toxicity of heavy metals depends on the rate of daily intake of the heavy metals that results in various health problems like retardation in the development of the body, a decrease in blood pH, cancer of many organs and sometimes even death (Gupta *et al*., 2013; Agrawal *et al*., 2007; Varathon, 1997).

Several researchers reported that the washing technique can reduce the levels of heavy metals contamination in vegetables significantly (Udosen *et al*., 2017; Mohite *et al*., 2016; Yusuf and Oluwole, 2009; Al Jassir *et al*., 2005; Ogunyemi *et al*., 2003) and there is a little information on the level of these metals in unwashed and washed vegetables in Bihar, India, however not available in the literature of Katihar district. Therefore, this study was to determine the effect of washing technique on the levels of four heavy metals (Cu, Zn, Pb and Cd) on five common vegetables collected from three traffic density sites at urban and three agricultural fields of remote sites at rural areas of Katihar district and also to assess health risk through their consumption.

MATERIALS AND METHODS

Site Description

Katihar is small but most densely populated district and is situated in the point of north eastern part of the state of Bihar in India (Fig. 1). It is small but has large number of small scale industries. Heavy traffic on narrow roads leading to frequent traffic congestion is of common occurrence in and around the Katihar city. In 2011 it had population of 3,071,029 of which has male and female were 1,600,430 and 1,470,599 respectively. It has an average elevation of 20 meters and is located at 25.53° N 87.58° E.

Vegetable Sampling

Freshly samples of some commonly grown vegetables i.e., tomato (*Lycopersicum esculentum*), radish (*Raphanus sativus* L.), ladyfinger (*Amelmoschus esculentus* L.), cauliflower (*Brassica oleracea* L.), spinach (*Spinacia oleracea* L) were collected randomly from 10 × 8 m area of three agricultural fields of remote sites of rural areas of Katihar district. These are the common vegetables which are grown in the field for own consumption of the farmers and supply to retail and wholesale markets of Katihar city and simultaneously the same vegetables were collected from three selected markets near to traffic density in and around Katihar city (Fig.1), during July 2015 to June 2016 which were located at:

Urban Area

1. Gaidabari- High Traffic Density Area (HTDA) with about 11000 vehicles/day, 18 km from head quarter
2. Kolassi- Medium Traffic Density Area (MTDA) with about 9000 vehicles/day, 10 km from head quarter
3. New Market- Low Traffic Density Area (LTDA) with about 7000 vehicles/day, 5 km from head quarter

Remote Area

1. Site-1, Bijhara Panchayat of Kadwa block, 30 km from head quarter
2. Site-2, Kehunia Panchayat of Pranpur block, 22 km from head quarter
3. Site-3, Sulthanpur Panchayat of Barsoi block, 38 km from head quarter

All the collected vegetables (one intact inflorescence head of *Brassica oleracea* L. and 1 kg each of *Lycopersicum esculentum*, *Raphanus sativus* L., *Amelmoschus esculentus* L, *Spinacia oleracea* L.) were kept in pre-distilled water rinsed polyethylene bags, labeled and brought in the laboratory; here edible part was sliced and chopped into small pieces and then divided into two groups from each site. The first group was thoroughly washed in distilled water while second group was unwashed. All samples were dried at 75^0C for 24 hours, crushed and sieved with 1 mm sieve at room temperature and digested by using the method described by Jamali *et al*. (2009).

Digestion of Vegetable Samples

1gm of each sample of 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^oC 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 de-ionized water and subjected to atomic absorption spectrophotometer for analysis for heavy metals (Pb, Cd, Cu and Zn). The research work has been done in the P. G. Department of Chemistry, D. S. College, Katihar, Bihar, India and also contacted Shiva Test House, Bailey Road, Patna, Recognized as Environmental Laboratory by Central Govt. under Environment Protection Act 1986, by Dept. of Industry, Forests & Environment, and Govt. of Bihar State Pollution Control Board. The water-removal heavy metals were calculated as the difference between unwashed and washed vegetables concentration.

Percentage Reduction (PR)

Percentage reduction in the concentration of heavy metals between unwashed and washed vegetable samples was calculated by using the following equation:

Percentage reduction=Unwashed vegetable-washed vegetable / Unwashed vegetable x100

Daily Intake of Metals (DIM)

The daily intake of heavy metals by people through consumption of vegetables was calculated by according to equation (Chary *et al*., 2008):

Daily Intake of Metals = $C_{metal} \times D_{food\ intake} \times C_{factor} / B_{average\ body\ weight}$

Where C_{metal}, $D_{food\ intake}$, $B_{average\ weight}$ and C_{factor} represent the heavy metal concentration in plant, daily intake of vegetable, average body weight and conversion factor (Rattan *et al*., 2005) respectively. On the basis of formal interview conducted with about 150 adult (male and female) having an average body weight of 50 kg at study area, the average vegetable intake was calculated 252 g/ person/day.

Health Risk Index (HRI)

The value of HRI depends upon the daily intake of metals (DIM) and reference oral dose (Rf_D), which was computed as described by Jan *et al*. (2010) . Rf_D value for Cu, Pb, Cd, and Zn is 0.04, 0.004, 0.001 and 0.30 (mg/kg bw /day) respectively (US-EPA IRIS. 2006). If HRI is less than 1, people will be safe to eat that kind of vegetables.

HRI = DIM / Rf_D

Statistical Analysis

Descriptive statistics were performed using Lenovo $_{TM}$ Computer Microsoft word and Excel 2007, while Standard deviation and correlation matrix were tested using the Casio Calculator (made in China) fx-*991 MS.* A probability of $p > 0.05$ was considered statically significant

RESULTS

The Concentration of Metals in Vegetables

The maximum, minimum, mean, standard deviation and percentage reduction of concentration of Cu, Zn, Pb and Cd in unwashed and washed vegetables collected from urban and remote areas are presented in Table 1and 2Both unwashed and washed vegetables showed higher mean metal concentrations at the urban areas compare to remote areas. The unwashed vegetables showed higher metal accumulation than washed vegetables at both urban and remote areas. In the unwashed vegetables the highest level of Cu (31.11 µg/g) was found in cauliflower at HTD site (Fig.6), but in washed vegetable the highest level (14.93 µg/g) was recorded in same vegetable at the same site (HTD). The highest Zn concentration in unwashed vegetables was observed (50.13 µg /g) in the ladyfinger at HTD site (Fig.4), whereas in washed vegetables the highest content (30.53 µg /g) was still recorded in ladyfinger at the same site (HTD) and in tomato at the MTD site (Fig.2). Similarly highest Pb concentration (13.81 µg /g) was observed in unwashed spinach at HTD site, whereas highest concentration (3.85 µg /g) was found in the same washed vegetable, but at MTD site (Fig. 5). The highest content of Cd (17.71 µg/g) was found in unwashed ladyfinger

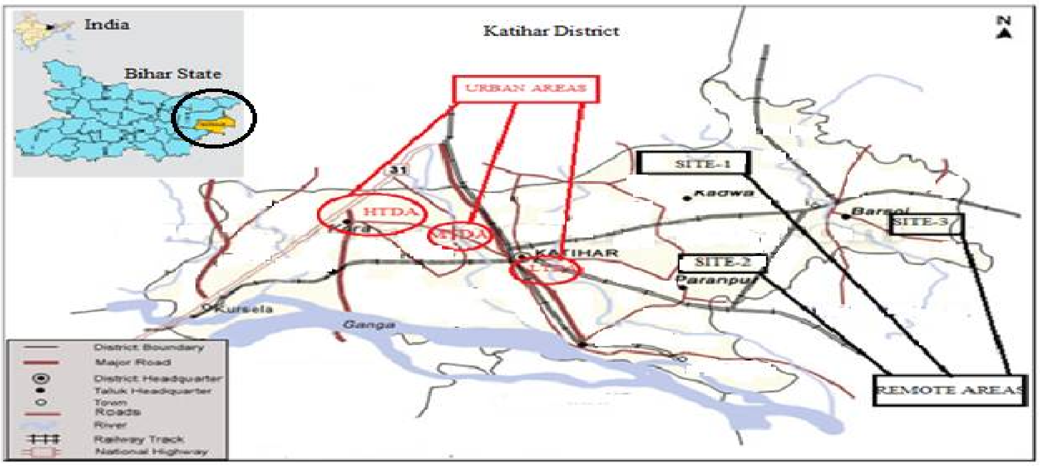

Figure 1 Map of Katihar district showing the relative locations of urban and remote areas

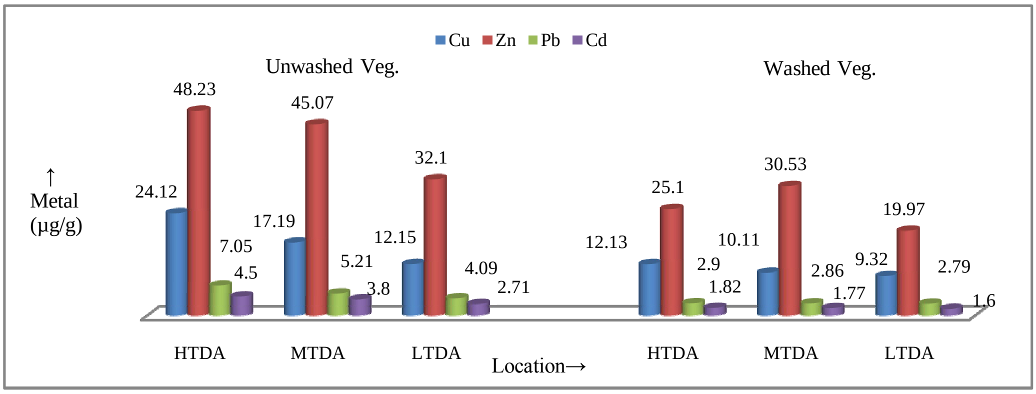

Figure 2 Concentration of heavy metal in unwashed and washed tomato at different locations of urban sites

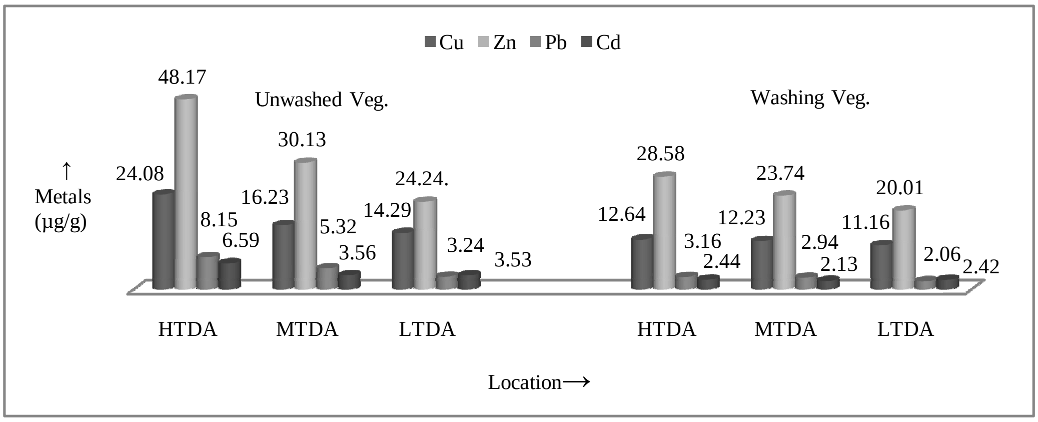

Figure 3 Concentration of heavy metal in unwashed and washed radish at different locations of urban sites

at HTD site, but in washed samples, the highest concentration (6.55µg/g) was observed in same vegetable (ladyfinger) at same HTD site (Fig. 4). The amount of Cu, Zn, Pb and Cd in each vegetable varied among sampling sites in the three traffic density sites and found in the order HTDA > MTDA > LTDA for unwashed vegetables but washed vegetables didn't follow the specific trend at urban areas.

Within remote areas as shown in Table 2 in unwashed vegetables the maximum level of Cu was recorded in cauliflower (4.18 µg/g) at site-1 whereas in washed vegetable highest content (4.14 µg/g) was still recorded in same vegetable but at site-3. The maximum Zn concentration (25.58µg/g) and (25.42µg/g) was observed at sites- 1 and 2 in unwashed tomato and radish respectively, whereas highest

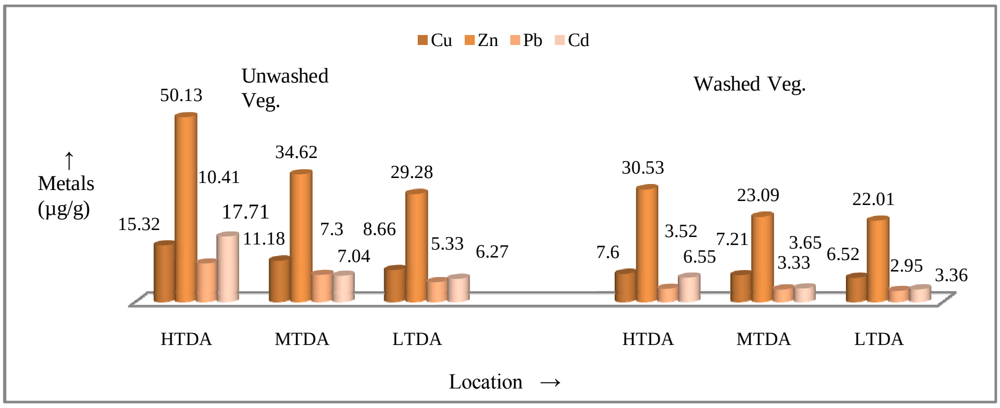

Figure 4 Concentration of heavy metal in unwashed and washed ladyfinger different locations of urban sites

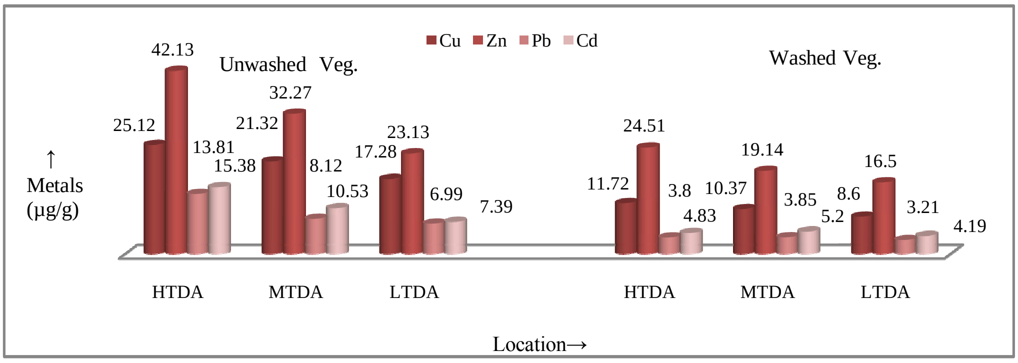

Figure 5 Concentration of heavy metal in unwashed and washed spinach different locations of urban sites

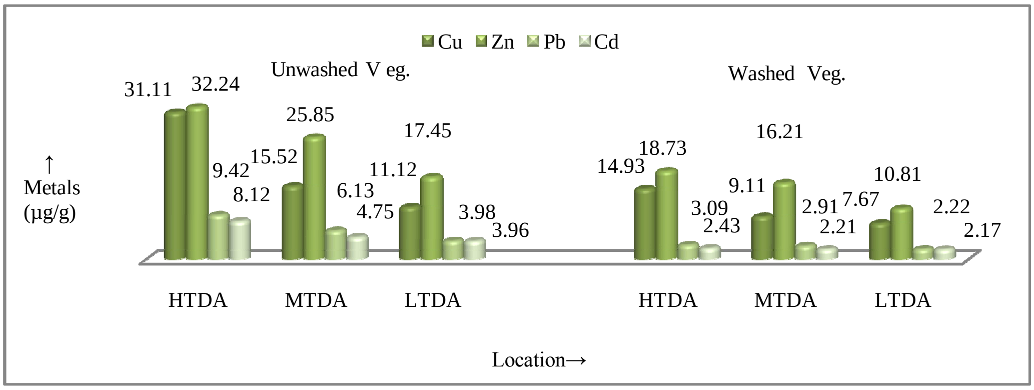

Figure 6 Concentration of heavy metals in unwashed and washed cauliflower different locations of urban sites

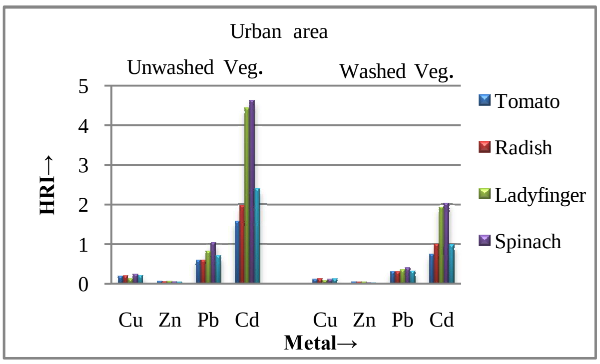

Figure7 HRI of metal by consumption of vegetables at urban areas

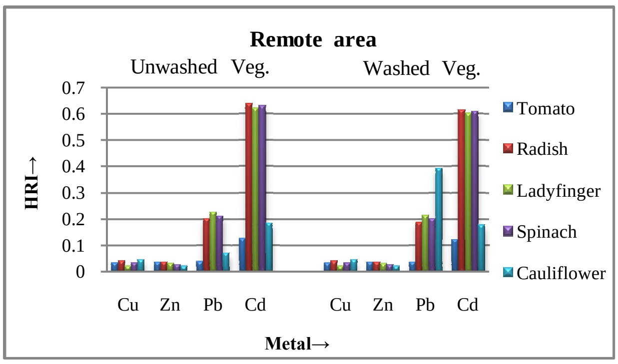

Figure 8 HRI of metal by consumption of vegetables at remote areas

content (25.31 µg/ g) was found in washed tomato at site-2. The highest content of Pb (2.14 µg/g) was observed in unwashed ladyfinger at site -2 and 2.00 µg/g in same washed vegetable but at site -3.

Table 1 Statistical data of metal concentration (µg/g) in unwashed and washed vegetables at urban areas

	Unwashed vegetable at urban areas				Washed vegetable at remote areas			
Metal	Cu	Zn	Pb	Cd	Cu	Zn	Pb	Cd
Tomato								
Min	12.5	32.1	4.09	2.71	9.32	19.97	2.79	1.60
Max	24.12	48.23	7.05	4.50	12.13	30.53	2.90	1.82
Mean	17.82	41.80	5.45	3.67	10.52	25.20	2.85	1.73
σ_{n-1}	5.856	8.548	1.495	0.902	1.449	5.281	0.055	0.115
% Reduction	-------	------	-------	------	40.96	39.71	47.71	52.86
Radish								
Min	14.29	24.24	3.24	3.53	11.6	20.10	2.060	2.420
Max	28.08	48.17	8.15	6.59	12.64	28.58	3.16	2.44
Mean	18.20	34.18	5.57	4.56	12.01	24.11	2.72	2.33
σ_{n-1}	5.18	12.47	2.464	1.758	0.534	4.297	0.582	0.439
% Reduction	-------	-------	-------	-------	34.01	29.46	51.67	48.90
Ladyfinger								
Min	8.660	29.28	5.33 0	6.27	6.520	22.01	2.950	3.360
Max	15.32	50.13	10.41	17.71	7.600	30.53	3.520	6.550
Mean	11.72	38.01	7.68	10.34	7.11	25.21	3.270	4.52
σ_{n-1}	3.362	10.63	2.561	6.394	0.547	6.024	0.290	1.764
% Reduction	-------	-------	------	------	39.33	33.67	57.42	56.29
Spinach								
Min	17.28	23.13	6.99	7.390	8.600	16.50	3.21	4.190
Max	25.12	42.13	13.81	15.38	11.72	24.5`1	3.85	5.200
Mean	21.24	32.51	9.64	10.10	10.33	20.05	3.62	4.70
σ_{n-1}	3.920	4.807	3.655	4.025	1.565	4.082	0.356	0.511
% Reduction	------	------	-------	-------	51.36	38.33	62.45	53.07
Cauliflower								
Min	11.12	17.45	3.98	3.96	7.67	10.81	2.220	2.170
Max	31.11	32.24	9.42	8.12	14.93	18.73	3.09 0	2.43
Mean	19.25	25.18	6.51	5.61	10.57	15.25	2.940	2.270
σ_{n-1}	10.504	7.417	2.739	2.209	3.844	4.046	0.459	0.140
% Reduction	------	-------	------	------	45.09	39.44	54.84	58.54
Safe limit								
PFA	30	50	2.5	1.5	30	50	2.5	1.5
FAO/WHO	40	60	0.3	0.2	40	60	0.3	0.2

σ_{n-1} (Standard deviation), Safe limit: PFA (Prevention of food adulteration set by Awasthi, 2000), FAO/WHO (Codex Alimentarius Commission , 2001)

Table 2 Statistical data of metal concentration (µg /g) in unwashed and washed vegetables at remote area

	Unwashed Vegetable at remote areas				Washed Vegetable at remote areas			
Metal	Cu	Zn	Pb	Cd	Cu	Zn	Pb	Cd
Tomato								
Min	3.02	24.62	0.32	0.28	2.94	24.05	0.32	0.27
Max	3.08	25.58	0.38	0.30	3.01	25.31	0.35	0.29
Mean	3.05	25.05	0.35	0.29	2.97	24.50	0.33	0.28
σ_{n-1}	0.03	0.488	0.03	0.01	0.036	0.702	0.017	0.01
% Reduction	------	------	-------	-------	2.62	2.19	5.71	3.45
Radish								
Min	3.78	25.09	1.83	1.47	3.71	24.43	1.61	1.34
Max	3.85	25.42	1.93	1.52	3.80	24.95	1.80	1.48
Mean	3.82	25.21	1.87	1.49	3.77	24.67	1.80	1.43
σ_{n-1}	0.036	0.182	0.053	0.026	0.051	0.262	0.104	0.078
% Reduction	------	-------	------	-------	1.31	2.14	3.74	4.03
Ladifinger								
Min	1.84	21.45	2.06	1.44	1.82	20.89	1.95	1.39
Max	1.95	21.88	2.14	1.47	1.91	21.12	2.00	1.41
Mean	1.88	21.68	2.10	1.45	1.85	21.02	1.98	1.40
σ_{n-1}	0.060	0.216	0.04	0.017	0.052	0.118	0.026	0.050
% Reduction	------	------	------	------	1.59	3.04	5.71	3.44
Spinach								
Min	3.08	18.17	1.95	1.43	3.05	17.78	1.86	1.36
Max	3.17	18.31	1.98	1.51	3.12	18.11	1.90	1.48
Mean	3.12	18.25	1.97	1.47	3.08	17.95	1.88	1.42
σ_{n-1}	0.046	0.072	0.017	0.04	0.036	0.165	0.02	0.06
% Reduction	------	------	------	------	1.28	1.64	4.57	3.40
Cauliflower								
Min	4.13	13.87	0.63	0.42	4.10	13.75	0.60	0.40
Max	4.18	14.40	0.66	0.45	4.14	14.23	0.63	0.42
Mean	4.15	14.07	0.65	0.43	4.12	13.96	0.61	0.41
σ_{n-1}	0.026	0.288	0.025	0.017	0.02	0.245	0.017	0.01
% Reduction	-------	------	-------	------	0.72	0.78	6.15	4.65
PFA	30	40	2.5	1.5	30	40	2.5	1.5
FAO/WHO	40	60	0.3	0.2	40	60	0.3	0.2

σ_{n-1} (Standard deviation), Safe limit: PFA (Prevention of food adulteration set by Awasthi, 2000), FAO/WHO (Codex Alimentarius Commission , 2001)

Similarly highest level of Cd (1.52 µg/g) and (1.51 µg/g) was found in unwashed radish and spinach at sites- 1 and 2 respectively while highest level of Cd (1.48 µg/g) was recorded in washed spinach at site -1 and in radish at site-3.

Effects of Washing Technique on Metals Levels in the Vegetables

During study period the percentage reduction of water removal concentrations of heavy metals in vegetables at urban and remote areas was calculated and is presented in Table 1 and 2. The mean concentration of Cu by washing ranged between 34.01% in radish to 51.36 % in spinach at the urban areas, while 0.72 % in cauliflower to 2.62 % in tomato at remote areas, whereas amount of Zn was lowered from 29.46 % in radish to 39.71 % in tomato at urban and 0.78 % in cauliflower to 3.04 % in ladyfinger at remote areas.

Table 3 Percentage reduction of heavy metals by washing of tested vegetables at traffic density sites

Traffic densities	Tomato	Radish	ladyfinger	Spinach	Cauliflower	Mean
Cu						
High	49.71	47.51	50.09	53.34	52.01	50.53
Moderate	41.19	24.65	35.42	51.36	41.30	39.43
Low	23.29	21.90	24.71	50.23	31.02	30.23
Mean	38.06	31.35	36.74	51.64	41.44	40.07
Zn						
High	47.83	40.66	39.09	41.82	41.90	42.26
Moderate	32.26	20.21	33.37	40.25	37.29	32.68
Low	37.38	16.50	24.82	28.66	38.05	29.68
Mean	39.29	25.79	32.42	36.91	39.08	34.70
Pb						
High	58.86	21.23	66.19	72.48	67.19	65.19
Moderate	45.10	44.73	54.38	52.59	52.52	49.86
Low	31.78	36.42	44.65	54.10	44.12	42.21
Mean	45.25	47.46	55.07	59.72	54.61	52.42
Cd						
High	59.55	62.97	63.01	68.59	70.07	64.83
Moderate	53.42	40.16	48.15	50.61	53.47	49.16
Low	40.96	31.44	46.41	43.30	45.20	41.21
Mean	51.31	44.86	52.52	54.17	56.24	51.74
Average	43.76	37.36	44.19	50.61	47.84	-------

Table 4 Daily intake of heavy metal (µg/day) in unwashed and washed vegetables at urban area

	Unwashed Vegetable at urban areas					Washed Vegetable at urban areas				
Vegetable	Cu	Zn	Pb	Cd	Σ_{DIM}	Cu	Zn	Pb	Cd	Σ_{DIM}
Tomato	7.624	17.907	2.335	1.572	29.448	4.507	10.933	1.221	0.7411	17.402
Radish	7.797	14.643	2.386	1.950	26.780	5.145	10.329	1.165	0.9982	17.637
Ladyfinger	5.021	16.383	3.290	4.429	29.023	3.046	10.799	1.401	1.936	17.182
Spinach	9.099	13.927	4.129	4.627	31.782	4.382	8.589	1.551	2.031	16.553
Cauliflower	8.247	1 0.787	2.789	2.403	24.226	4.956	6.533	1.259	0.9725	13.721
Σ_{DIM}	37.798	73.547	14.929	14.985	141.259	22.036	47.183	6.597	6.679	82.495
Mean	7.5596	14.710	2.986	2.997	28.252	4.407	9.437	1.319	1.336	16.499
% of PDTI	1.26	0.123	6.98	24.975	-------	0.735	0.079	3.10	11.13	-------

Table 5 Daily intake of heavy metal (µg/day) in unwashed and washed vegetables at remote area

	Unwashed Vegetable at remote areas					Washed Vegetable at remote areas				
Vegetable	Cu	Zn	Pb	Cd	Σ_{DIM}	Cu	Zn	Pb	Cd	Σ_{DIM}
Tomato	1.0307	10.727	0.1499	0.1242	12.3081	1.272	10.496	01414	0.1199	12.029
Radish	1.6364	10.799	0.8011	0.6383	13.8748	1.615	10.569	0.7411	0.6126	13.538
Ladyfinger	0.8054	9.288	0.8996	0.6212	11.6142	0.7925	9.004	0.8482	0.5997	11.244
Spinach	1.337	7.818	0.8439	0.6297	10.6286	1.3195	7.689	0.8084	0.6083	10.422
Cauliflower	1.778	6.0276	0.2785	0.1842	8.2683	1.7650	5.980	0.2613	0.1756	8.1819
Σ_{DIM}	6.8638	44.66	2.973	2.1976	56.694	6.764	43.738	2.7974	2.116	55.415
Mean	1.373	8.932	0.5946	0.4395	11.339	1.353	8.748	0.5595	0.4232	11.082
% of PDTI	0.229	0.078	1.4	3.7	----------	0.225	0.073	1.31	3.52	---------

Table 6 Correlation Matrix for the heavy metal levels in unwashed vegetables at urban and remote areas

	Unwashed vegetables at urban area					Unwashed vegetables at remote area			
Metals	Cu	Zn	Pb	Cd	Metals	Cu	Zn	Pb	Cd
Cu	---------				Cu	--------			
Zn	- 0.4684	----------			Zn	- 0.1367	----------		
Pb	+ 0.1453	- 0.2109	---------		Pb	- 0.4290	+0.1169	---------	
Cd	- 0.2851	- 0.0652	+0.9019	---------	Cd	- 0.3576	+0.1870	+0.9913	--------

Similarly percentage reduction of water removal concentration of Pb was reduced from 47.71 % in tomato to 62.45 % in spinach at urban areas and 4.57 % in spinach to 6.15 % in cauliflower at remote areas, whereas concentration of Cd was lowered from 48.90 % in radish to 59.53 % in cauliflower at urban and 3.4% in spinach to 4.65 % in cauliflower at remote areas. The percentage reduction of water of heavy metals concentration was found in the order: Pb > Cd > Cu > Zn at the three traffic density sites of urban areas and Pb > Cd > Zn > Cu at three agricultural fields of remote areas. The observed data also revealed that water removal concentration of Cu in ladyfinger (39.33%) and in tomato (40.96 %), of Zn in tomato (39.71 %) and in cauliflower (39.44 %) and of Cd concentration in tomato (52.86%) and in spinach (53.07%) was found to be approximately same at urban areas. The percent reduction in the levels of heavy metals by washing of tested vegetables at traffic density sites as shown Table 3 was recorded maximum in spinach (50.61%) followed by cauliflower (47.84 %), ladyfinger (44.19 %), tomato (43.76 %) and radish (37.36 %) and the level of water removal of heavy metals was found in the order: Pb (52.42%) > Cd (51.74 %) > Cu (40.07 %) > Zn (34.27 %) at traffic density sites at urban areas during the time of study period.

Daily Intake of Metals (DIM)

The values of DIM by consumption of unwashed and washed vegetables at both areas were calculated for adults of average age 50 years are given in Table 4 and 5. The results revealed that daily intake of Zn, Cu, Pb and Cd by adults through consumption of unwashed vegetables at urban and remote areas were found to be 73.55, 37.798, 14.93 and 14.99 µg/day, and 47.18, 22.04, 6.597 and 6.697 µg /day respectively. The DIM for consumption of unwashed and washed vegetables was in order of: $DIM_{Zn} > DIM_{Cu} > DIM_{Cd} > DIM_{Pb}$ at urban and $DIM_{Zn} > DIM_{Cu} > DIM_{Pb} > DIM_{Cd}$ at remote areas. The observed data also showed that the values of DIM were high for unwashed and washed vegetables at the urban as compared to the vegetables collected from remote areas.

Human Risk Index (HRI)

The HRI of Cd, Pb, Cu and Zn varied from 1.572 - 4.629, 0.5838-1.0323, 0.126- 0.227 and 0.0359 -0.059; and 0.7411-2.031, 0.2912-0.3880, 0.0762-0.1286, and 0.022-0.0364 respectively in unwashed and washed vegetable at urban areas and their rank appeared as Cd > Pb> Cu > Zn. The same trend but low value of HRI was observed at remote areas in unwashed and washed vegetables compared to the urban area as shown in Figure 7and 8. HRI for individual vegetables were below 1.0 for all tested metals at remote sites. At urban sites the maximum HRI for Cd was greater than 1 was found for unwashed vegetables such as spinach (4.627), ladyfinger (4.429), cauliflower (2.403), radish (1.954) and tomato (1.572), and in washed vegetables only for spinach (2.031) and ladyfinger (1.936) where as for radish and cauliflower was (0.9982) and (0.9725) respectively. HRI for Pb was > 1was also found in unwashed spinach (1.0323) at urban areas. HRI for Cu and Zn was < 1for all unwashed and washed vegetables at urban areas.

DISCUSSION

The variation of concentration of the heavy metals in tested vegetables may be due to nature of soil, air, irrigated water and also the deposition of heavy metals from aerial deposition, during transportation and marketing (Kumar *et al.*, 2017ab). The aerial deposition may be influenced by environmental factors such as temperature, moisture and wind velocity, and nature of vegetables i.e., leafy, root, fruit, exposed surface area, hairy or smoothness of the exposed parts (Sharma *et al*., 2008b; Sharma *et al*., 2009). Anthropogenic activates like vehicular traffic emission, brick kiln activities, addition of metal based agrochemical and pesticides in agricultural field and urban industrial activities of Katihar city may be the cause of variation in the levels of heavy metals in tested vegetables (Kumar *et al*., 2017a ; Kumar and Seema, 2016). .

Among the tested metals, the average concentration of Zn was highest in unwashed and washed vegetables at urban (44.18 and 26.58 µg/g respectively) and at remote (21.12 and 20.75 µg/g respectively) areas in this work, but was found to be lower than PFA (50 µg/g) and FAO/WHO (60 µg/g) standards. The level of Zn in all tested vegetables were lower than those reported by Mohamed *et al*., 2003 (105.20 mg/kg in vegetables collected from an urban and industrial area), by Kumar and Seema, 2016 (116.42 µg/g in vegetables obtained from agricultural fields) and by Kumar *et al*., 2017a (126, and 116.3 µg/g in vegetables collected from market and a production field respectively. The maximum level of Zn (50.13 µg/g /g) in unwashed ladyfinger at HTD site (Fig.4) and (30.53 µg/g) in washed tomato at MTD site (Fig.2) and in ladyfinger at HTD site (Fig.4) at urban areas were in agreement with the value 75 and 47.7 µg/g as reported by Sharma *et al*. (2008a).

The average level of Cu is second highest metal in vegetables at the three traffic density sites of urban areas in this study, could be attributed to different form of anthropogenic activities such as discharge of domestic waste and wind-blown dust as well as decaying vegetation (E. D. Udosen , 2015).The high level of Cu in vegetables collected from HTD sites may also be the fumes of Cu emitted by the automobiles plying the road, as Cu is used as automobile brake pads. The level of Cu in the in unwashed and washed vegetables for both urban and remote areas reported in this study were lower than PFA (30 µg/g) and FAO/WHO (40 µg/g) standards. The high level of Cu in human body could pose serious health threat such as acute renal failure (WHO, 2001), gastrointestinal bleeding, liver and kidney failure (Udosen, 2015; Arayal, 2003) and even death in human. The high concentration of Cu (31.11 µg/g) in unwashed cauliflower at HTD site (Fig.6) was also supported by Kumar *et al*., 2017a (24.11 µg/g in vegetable collected form market sites) and 4.18 µg/g in unwashed cauliflower at site-1of agricultural field at remote areas, was below than 10.07 µg/g reported by Kumar and Seema, (2016) in vegetables collected from agricultural field around the Katihar city.

The average level of Pb in the unwashed and washed vegetables obtained at urban areas (9.77 and 3.30 µg/g respectively) was below than the value of 14.37 mg/kg as reported by Mohamed *et al*.(2003) and at remote areas (1.42 and 1.34 µg/g respectively) reported in this study were close agreement with other values 2.0, 0.65, 1.76, 0.34-0.71mg/kg as reported by respectively. In the traffic density sites high concentration of Pb in vegetables may be car exhaust, dust and gases from various industrial sources. In the present study it could, therefore, be inferred that the source of Pb to the vegetables was from aerial deposition and other foliar absorption. The retention of particulates matter is the main source of contamination of roadsides vegetation. Hana and AL-Bassam, (1983) reported that 25% of the vehicular emitted Pb is coarse grained and deposited close to the road, while the remaining 75% is finer and may be airborne over a long distance.

The average concentration of Cd observed in unwashed and washed vegetables at three traffic density sites of urban areas (10.46 and 3.69 µg/g respectively) were higher than values of 0.03-0.05 mg/kg reported in dumpsite plants by Udosen *et al*. (2006). However, 1.05 and 1.02 µg/g amount of Cd in unwashed and washed vegetables respectively at three sites of agricultural fields of remote areas were in agreement with the value of 0.6 mg/kg and 0.37mg/kg as reported by Cheng *et al*. (2003); Garba and Jimoh, *(*2015). The Cd level reported at urban areas was higher than permissible limit of 1.5µg/g was stipulated by PFA (Awashthi, 2000) in unwashed and washed vegetables but was within the limit in the unwashed and washed samples at remote areas.

The water-washing removal technique mainly removes particles deposited on the surface of the vegetables and suggested the difference of levels of heavy metals between unwashed and washed vegetables might be due to aerial deposition that adhere on vegetable surface. Similar results were also reported by Udosen *et al*. (2017); Yusuf and Oluwole, (2009); De Nicol *et al*. (2008) and Sharma *et al*. (2008b). It has been found that during washing process 72.48 % of Pb and 68.59 % of Cd was removed by spinach whereas 67.19% of Pb and 70.07 % Cd was removed by cauliflower at HTD sites of urban areas. The results further showed observed trend of the level of water removal of heavy metal at the urban sites ((Pb>Cu>Zn) is also supported by so many researchers De Nicol *et al*.(2008); Caselles *et al*. (2002) and Gratani *et al*. (2000).The results suggested that all the vegetable tested were contaminated maximally by Pb and Cd during transporting and marketing in the contaminated environment of Katihar city. The different levels of the heavy metals in tested vegetables at the urban areas varied with the length of exposure and types of vegetables. The concentration of Pb was higher in spinach, ladyfinger and then cauliflower, while concentration of Cd was higher in cauliflower, spinach and then ladyfinger at traffic density sites (Table 3). The results also suggest that spinach is leafy type, cauliflower having a higher exposed area of inflorescence and ladyfinger having hairy surface so they all have greater capacity to adsorb heavy metals from the atmosphere (Sharma *et al*., 2009).

There are several pathways of exposure to human but amongst them food chain is the most important pathway. Cu is an essential micronutrient which functions as a biocatalyst required for body pigmentation in addition to Fe, maintained and interrelated with the function of Zn and Fe in the body (Radwan and Salama, 2006; Akinyele and Osibanjo, 1982) but in high dose it can cause anemia, liver, kidney damage, stomach and intestinal **i**rritation. The average daily intake of Cu was estimated at 7.559 and 1.372 µg/day through consumption of unwashed vegetables collected from urban and remote areas respectively, which represent 27 and 12.1% for 60 kg adult (WHO, 1993).The contribution for the Cu intake through consumption of unwashed vegetables at urban areas was in the order of spinach > cauliflower > radish tomato > ladyfinger. Cu is easily accumulated in body hence, even low level intake of Cu have damaging effect on human beings and other animal (Bermudez *et al*., 2011).

Zn is the least toxic and an essential element in human diet as it is required for maintaining the functioning of immune system. The average daily intake of Zn was estimated at 14.71 and 8.93 µg/day through consumption of unwashed vegetables collected from urban and remote areas respectively, which were lower than Rf_D established at 0.3 mg/kg/bw/day (US EPA, 2010) and 0.268 mg/kg as reported by Shuaibu *et al*. (2013) and represent 52.1 and 78.77 % of the total intake respectively. The greatest contribution for Zn intake came from tomato (17.91) and ladyfinger (16.38) followed by radish (14.64), spinach (13.93) and cauliflower (10.79) µg/day. Low level of Zn concentration can result from inadequate dietary intake, impaired adsorption, excessive excretion or inherited defect in Zn metabolism (Narin *et al*., 2005) but high level of Zn in vegetables may cause vomiting, renal damage, cramps etc.

Pb does not have a specific function in living beings. Pb affects several organs like liver, kidney, spleen and lung causing a variety of biochemical effect. The average daily intake of Pb was estimated at 2.986 and 0.5946 µg/day through consumption of unwashed vegetables collected from urban and remote areas respectively, which were lower than Rf_D established at 0.004 mg/kg/bw/day (US EPA, 2010). The order of contribution of the Pb intake came from spinach > ladyfinger > cauliflower > radish > tomato, accounting for 10.6 % of the total daily intake. These values are lower than 0.77 mg as reported in by Gupta et a1. *(*2013).

Cd is non essential in foods and natural waters and its accumulation is mainly in the kidney and liver .The average daily intake of Cd was estimated at 2.997 µg/day through consumption of unwashed vegetables collected from urban sites, was higher than Rf_D established at 0.001 mg/kg/bw/day (US EPA, 2010), whereas 0.4395 µg/day collected from remote areas was lower than $Rf_{D.}$ The greatest contribution for Cd intake appeared as spinach > ladyfinger > cauliflower > radish > tomato, accounting for 10.61 % of the total dilly intake. 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 Saplakogclu and Iscan, (1997) have also reported that long term exposure of Cd can cause renal prostate and ovaries cancer.

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, Cu-3000 µg/day and Zn-60000 µg/day. The consumption of unwashed vegetables collected from urban areas to daily intake of Pb, Cd, Cu and Zn were 6.98, 24.98, 1.26, and 0.123 % of PTDI 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.

Many researchers considered the health risk estimation methods among which health risk index is most reliable (Chary *et al*., 2008; Khan *et al*., 2009; Wang *et al*., 2005). The results revealed that HRI value for all individual vegetable collected from urban areas was higher compare to vegetable collected from remote areas and found to be less than 1.0 except Cd. The sequence of average value of HRI for adults followed the increasing order Cd (2.997) > Pb (0.746) > Cu (0.189) > Zn (0.045). The results show in 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 value for Cd in our present finding show that unwashed tested vegetables such *Spinacia oleracea*, *Amelmoschus esculentus*, *Brassica oleracea*, *Raphanus sativus* and *Lycopersicum esculentum*, and *Spinacia oleracea* and *Amelmoschus esculentus* for washed vegetables at urban areas pose severe health risk while other were almost safe for consumption (Ikeda *et al*., 2000; Zhuang *et al*., 2008). The vegetables grown at remote areas were entirely free from any risk. So, the consumption of these vegetables can be considered safe with no risk to human health except with regard of Cd.

Correlation of Heavy Metal Levels in the Vegetables

Table 6 shows the correlation matrix between level of heavy metals in unwashed vegetables at the three traffic density sites of urban and three agricultural fields of remote areas. Results showed significantly positive and negative correlation were observed for metals in vegetables. Cu shows mild negative correlation with Zn and Cd, and weak positive relation with Pb, and Zn shows weak negative relation with Cd and Pb, whereas Pb shows strong positive relation ($r = + 0.9019$) with Cd in vegetables at urban areas . In vegetables collected at remote areas Cu also shows mild negative correlation with Zn, Pb and Cd, and Zn shows mild positive correlation with Pb and Cd ,whereas Pb shows very strong positive correlation with Cd ($r = + 0.9913$). Negative correlation between metals in plant indicates metals accumulated in plant from different sources whereas positive correlation indicated that the element come from the same sources or are influenced by the same anthropogenic sources was also supported by Udosen *et al*. (2017) .

CONCLUSION

The present study showed that level of heavy metals in all unwashed vegetables was higher than washed vegetables at both urban and remote areas. The study also suggested that transportation and marketing of vegetables in pollute environment of urban activities of Katihar city may elevate the level of heavy metals in the test vegetables through atmospheric deposition. The water removal extractions for Cu, Zn, Cd and Pb were more notable at the urban compare to remote areas showing greater atmospheric deposition on surface of vegetables during marketing. The present study further suggested that washing technique can be used as a mechanical tool to assess the heavy metals load in vegetables through aerial deposition. DIM and HRI values for heavy metals in all test vegetables at urban areas were higher than vegetables grown at remote areas. Dietary intake of food results in long-term low level body accumulation of heavy metals and the detrimental impart become visible only after long time exposure. The findings of this study regarding DIM compared with PTDI suggest that the consumption of vegetables collected from urban areas is nearly free from risk, but HRI >I for Cd, indicating potential health risk. Finally it can be concluded that the regular monitor of heavy metals in vegetables is essential to reduce the health risk and vegetables should be washed properly to remove aerial contamination from surface of vegetables and also appropriate precaution should be taken at the time of marketing of vegetables.

References

1. Agrawal, M. (2003). Enhancing food chain integrity: quality assurance mechanisms for air pollution impacts on fruit and vegetable system. Final Technical Rreport II submitted to Department of International Development UK, R-7530.
2. Agrawal, S. B., Singh, A., Sharma, R. K. and Agrawal, M. (2007). Bioaccumulation of Heavy metals in Vegetables: A Threat to Human Health. Terrestrial and Aguatic Environment Toxicology, 1(2): 13-23.
3. Akinyele, I. O. and Osibanjo, O. (1982). Levels of trace elements hospital diet. *Food Chemistry*, 8: 247-25.
4. Aktauzzaman, M., Fukhruddin, A. N. M., Chowdhury, M. A.A., Fardous, Z., Alum, M. K. (2013). Accumulation of heavy metals in soil and their transfer to leafy vegetables in the region of Dhaka Aricha Highway, Savar, Bangladesd. *Pakistan Journal of Biological Science,* 16(7): 332-338, DOI: 10.3923/pjbs.2013.332.338.
5. Al-Jassir, M. S., Shaker, A., Khaliq, M. A. (2005). Deposition of heavy metals on green leafy vegetables sold on roadsides of Riyadh city, Saudi-Arabia. Bull. Environ. Contam. *Toxicol*., 75: 1020-1027. doi:10.1007/s00128-005-0851-4.
6. Allen, S. E., Grimshaw, H. M., Rowland, A. P. (1986). Chemical analysis. In: Moore, P. D., Chapman, S. B. (Eds.) Methods in Plant Ecology. Oxford: Blackwell Scientific Publication, London, pp. 285-344.
7. Arayal, M. (2003). Gastrointestinal symptoms Indicators of copper load in apparently healthy adults undergoing controlled copper exposure, *Am. J. Clinic Nutri.*, 77 (3): 646-650.
8. Arora, M., Kiran, B., Rani, S., Rani, A., Kaur, B. and Mittal, N. (2008). Heavy metal accumulation in vegetables irrigated with water from different sources. Food Chemistry, 111: 811-815. doi:10.1016/j.foodchem.2008.04.049.
9. Awasthi, S. K. (2000). Prevention of Food Adulteration Act no. 37 of 1954. Central and State Rules as Amended for 1999, third ed. Ashoka Law House, New Delhi.
10. Balkhair, K.S., and Ashraf, M. A. (2016). Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. *Saudi Journal of Biological Sciences*, 23: S32-S44. http;//dx.doi.org/10.1016/j-sjbs.2015.09.023.
11. Bermudez, G. M., Jasan, R., Pla, R. and Pignata, M. L. (2011). Heavy metal and trace element concentrations in wheat grains: Assessment of potential non-carcinogenic health hazard through their consumption. *J. Hazard. Mater,* 193: 264-271.
12. Caselles, J., Colliga, C. and Zornosa, P (2002). Evaluation of trace elements pollution from vehicle emission in petunia plant. *Water Air Soil Pollut*., 136: 1-9.
13. Chary, N. S., Kamala, C. T. and Raj, D. S. S. (2008). Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. *Ecotoxicol. Environ. Safety,* 69: 513–524, DOI: 10.1016/J.ecoenv.2007.04.013.
14. Cheng-Jung, C. and Su-Lan, L. (2003). Zinc toxicity on National Cortical Neuron: Involment of Glutathione Chelate. J. Neurochem, 85: 443-453.
15. Codex Alimentarius Commission, (FAO/WHO), (2001). Food additives and contaminants. Joint FAO/WHO Food Standards Programme, ALINORM 01/12A: 1-289.
16. Degheim, S. M., El-Asharif, M. M., Gad Alla, S. A., Khorshid, M. A. and Falmuy, S. M. (2004). Pesticides

and heavy metals levels Egyptian leafy vegetables and some aromatic medical plats. *Food Additives and contaminated*, 21(4): 323- 330.

17. De Nicola, F., Maisto, G., Prati, M. V. and Alfani, A. (2008) Leaf accumulation of trace elements and polycyclic aromatic hydrocarbons (PAHS) in Quercus ilex *L. Environ. Pollut.* 153, 376-380.
18. Garba I. and Jimoh, W. (2015). Evaluation of heavy metals and micro-element in irrigated vegetable from Challawa-Yandako and Kano River basin project, Nigeria. *Inter. L. Scieti Res. Engin. Stud.* (IJSRES), 2 (2): 2349-8862.
19. Gratani, L., Crescente, M. F. and Petruzzi, M. (2000). Relation between leaf life-span and photosynthetic activity of Quercus ilex in polluted urban areas (Rome). *Environ. Pollut.*, 110: 19-28.
20. Gupta, S., Jena, V., Jena, S., Davic, N., Matic, N., Rodajevic, D., Solanki, J. S. (2013). Assessment of heavy metal contents of green leafy vegetables. *Croot. J. Food Sci. Technol*., 5(2). 53-60.
21. Hall, J. L. (2002). Cellular mechnisum for heavy metals detoxification and tolerance. *Journal of Experimental Botany*, 53(366): 1-11.
22. Hamid, A., Raza, H., Akhtar, S. and Ahmad, S. R. (2016). Heavy metal contamination in vegetables, soil and water and potential health risk assessment. American- Eurasian *J. Agric. & Environ. Sci*., 16 (4): 786-786, DOI: 10.5897/idosiaejaes. 2016.16.04. 103149.
23. Hana, A. A. K. and Al-Bassam, K.S. (1983). A survey of lead pollution in Bagdad .Water air and soil pollution 19. 3-14 Hartwig, A., 1998. Carcinogenicity of metal compounds: possible role of DNA repair inhibition. *Toxicol. Lett*., 102-103: 235-239.
24. Ikeda, M., Zhang, Z. W., Shimbo, S., Watanable, T., Nakatsuka, H., Moon, C., S. and Higashikawa, K. (2000). Urban population exposure to lead and cadmium in east and south-east Asia. Sci. Total Environ,. 249 (1-3): 373-384.
25. Jamali, M. K., Kazi, T. G., Arain, A. M., Afridi, H. I., Jalbani, N., Kandhro, G. A., Saha, A. Q. and Baig, J. A. (2009). Heavy metals accumulation in different varieties of wheat *(*Triticum aestivum L.) grown in soil amended with domestic sewage sludge. *J. Hazard. Mater*, 164, 1336-1391.
26. Jan, F. A., Ishaq, M., Khan, S., Ihsanullah, I., Ahmad, I. and Shakirullah, M. (2010). A comparative study of human health risks via consumption of food crops grown on wastewater irrigated soil (Peshawar) and relatively clean water irrigated soil (lower Dir*). J. Hazard. Mater*, 179: 612-621.
27. Liu, W., Li, X., Li, H. H., Li., S.R. and Wang, Y.W. (2006). Heavy metal accumulation of edible vegetable cultivated by people ׳s Republic of China. Bullet. Environ. Contam. *Toxicol*., 76: 163-170.
28. Joint FAO/WHO Expert Committee on Food Additives, (1999). Summary and conclusions, In: the 53rd Meeting, Rome, June 1-10.
29. Khan, S., Farooq, R., Shahbaz, S., Khan, M. A. and Sadique, M. (2009). Health risk assessment of heavy metals for population via consumption of vegetables. *World Applied Science Journal*, 6 (12):1602-1606.
30. Khan, S., Rehman, A. Z., Khan, M. A. and Shah, T. (2010). Soil and vegetables enrichment with heavy metals from geological sources in Gilgit, northern Pakistan. *Ecotoxicol. Environ. Safety*, 73: 1820-1827.
31. Kumar, A. and Seema, (2016). Accumulation of heavy metals in soil and green leafy vegetables, irrigated with wastewater. *IOSR-J. Environ. Sci. Toxicol. Food Technol.*, 10 (7), 8-19.
32. Kumar, A., Kumar**,** V. and Kumar, A. (2015). Seasonal variation of toxic metals in groundwater resources of Kishanganj district, Bihar, *India. J. Chem. Pharm. Res.*, 7(4): 187-198.
33. Kumar. A., Seema and Kumar, V. (2017a). Risk Assessment of heavy metals via consumption of contaminated vegetables collected from different agricultural fields and market sites. *Advances in Biochemistry*, 5(3): 47-56 http://www.science publishinggroup.com/j/ab doi: 10.11648/ j.ab.20170503.13.
34. Kumar. A., Seema and Kumar V. (2017b). Human health risk of heavy metals in vegetables grown in contaminated soil irrigated with sewage water. *American Journal of Food science and Nutrition*, 4(4): 23-35, http://www.aascit.org/jouranl/ajfsn.
35. Mahmood, A., Malik, R. N., 2014, Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arabian Journal of Chemistry* 7, 91-99.
36. Mohamed, A., Rashed, M. N. and Mofty, A. (2003). Assessment of essential and toxic elements in some kinds of vegetables. *Ecotoxicology Environ. Safety*, 55(3): 251-260.
37. Mohite, R.D., Basavaiah, N., Singare, P.U., Reddy A. V. R., Singhal, R. K. and Balha, U. 2016. Assessment of heavy metals accumulation in washed and unwashed leafy vegetables Sector-26 Vashi, Navi Mumbai Maharashtra. *J. Chem. Bio. Phy. Sci. Sec. C*, 6 (4): 1130-1139.
38. Narin, I., Tuzen, M., Sariand, H. and Soylak, M. (2005). Heavy metal content of potato and corn chips from Turkey. *Bull. Environ. Contam. Toxicol*., 74: 1072-1077.
39. Nwajei, G. E.(2009). Trace element in soil and vegetables in the vicinity of the shell Petroleum Development Company opening area in Ughelli, delta state of Nigeria. *American Eurasian Journal of Sustainable Agriculture*, 3: 547-578.
40. Ogunyemi, S., Bamgbose, O. O. and Awodoyin, R.O. (2003). Heavy metal contamination of some leafy vegetables growing within Ibadan metropolis, South-Western Nigeria. *Tropical Agricultural Research and Extension,* 6: 71-76.
41. Radwan, M.A. and Salama, A. K. (2006). Market basket survey for some heavy metals in Egyptian fruits and vegetables. *Food Chem. Toxicol.*, 44 (8): 1273-1278.
42. Ramteke, S., Sahu, B. L., Dahariya, N. S., Patel, K. S., Blazhev, B. and Matini, M. (2016). Heavy metal contamination of vegetables. *Journal of Environmental Protection*, 7: 996-1004, http;//dx.doi.org/10.4236/jep.2016.77088.

43. Rasheed, H., Jaleel, F. and Nisar, M. F. (2014). Analyzing the status of heavy metals in water in Suburban areas of Bahawalpur City, Pakistan. American-Eurasian *J. Agric. & Environ. Sci.*, 14 (8):732-738, DOI: 10.5897/idosi-aejaes. 2014.14.08. 12380.
44. Rattan, R. K., Datta, S. P., Chhonkar, P. K., Suribabu, K. and Singh, A. K. (2005).Long term impact of irrigation with sewage effluents on heavy metal contenst in soils, crops and groundwater - a case study. Agriculture, *Ecosystem and Environment*, 109: 310-322. doi:10.1016/j.agee.2005.02.025.
45. Saplakogelu, U. and Iscan, M. (1997). DNA single-strand breakage in rat lung, liver and kidney after single and combined treatments of nickel and cadmium. *Mutat Res.*, 394 (1): 133-140.
46. Sharma, R. K., Agrawal, M. and Marshall, F. M. (2008a). Heavy metals (Cu, Cd, Zn and Pb) contamination of vegetables in urban India: a case study in Varanasi. *Environ. Poll.* 154: 254-263.
47. Sharma, R. K., Agrawal, M. and Marshall, F. M. (2008b). Atmospheric deposition of heavy metals (Cd, Zn Pb and Cu,) in Varanasi city, *India. Environ. Monit. Assess.*, 142 (1-3): 269-278, DOI 10.1007/s 10661-007-9924-7.
48. Sharma, R. K., Agrawal, M. and Marshall, F. M. (2009).Heavy metal in vegetables collected from production and market sites of tropical urban of India. *Food and Chemical Toxicology*, 47: 583-591, doi: 10.1016/j.fct.2008.12.016.
49. Shuaibu, I. K., Yahaya, M. and Abdullahi, U. K. (2013). Heavy metal levels in selected green leafy vegetables obtained from Katsina central market, Katsna, North-western Nigeria. *African Journal of Pure and Applied Chemistry*, 7(5): 179- 183, DOI: 10.5897/AJPAC 2013.0499
50. Stalikas, C. D., Mantaloves, A.C. and Pilidis, G. A. (1997). Multi-element concentration in vegetables species grown in two typical agricultural areas of *Greece. Sci. Total Environ*, 206 (1): 17-24.
51. Tani, F. H. and Barrington, S.V. (2005). Zinc and copper uptake by plants under two transpiration ratios Part I. Wheat (Triticum aestivum L.). *Environ. Pollut.*, 138:538-547
52. Temmerman, L. and Hoenig, M. (2004). Vegetables crops for biomonitoring lead and cadmium deposition, *Journal of atmospheric Chemistry*, 49: 129-135.
53. Udosen, E. D., Uwah, E.I. and Jonathan., I (2017). Levels of trace metals in washed and unwashed laves of roadsides Vernonia amygdaline obtained in Abak, AkwaIbom State, Nigeria. *International Journal of Advances in Pharmacy, Biology and Chemistry*, 6 (2): 131-138.
54. Udosen, E.D., 2015, Concepts in Environment Chemistry, Anikzo Global Ventures.
55. Udosen, E.D., Benson, N. U., Essien, J, P. and Ebong, G.A. (2006). Relation between aqua-regia extractable heavy metals in soil and Manihotutilissima within municipal dumpsites. *Inter. J. Soil Sci.*, 1 (1): 27-32.
56. US-EPA IRIS, (2006). United States, Environmental Protection Agency, Integrated Risk Information System. <http://www.epa. gov/iris/substS>.
57. Varathon, P. (1997). Fact Sheet Environmental Health, Number-1, Vol. 1, Ministry of Public Health, Thailand, pp.14.
58. Wang, X., Sato, T., Xing, B., Tao, S. (2005). Health risk of heavy metals to the general public in Tainjan, China, via consumption of vegetables and fish. *Sci. Total Environ.* 350 (1-3): 28-37.
59. WHO, (2001). Food additives and Contaminants, Joint FAO/WHO Standards Programme; Alonorm.
60. Yuan, Li-zhu, Bo Song, Yu-fei Huang, Feng-yan Fu, Lu. Su-fen Lu and Xue-mei Zhong, (2015). Health Risk of Heavy Metals to the General Inhabitants in Guilin, China via Consumption of Vegetables. In the Proceeding of the AASRI International Conference on Industrial Electronics and Applications (IPA 2015), UK, June 27-28 and pp: 445-457.
61. Yusuf, K. A. and Oluwole, S.O. (2009). Heavy metal (Cu, Zn, Pb) contamination of vegetables in urban city: A case study in Lagos. *Research J. of Environment Science*, 3 (3): 292-298. doi: 10.3923/rjes. 2009. 292.298.
62. Zhuang, P., McBride, M. B., Hanping, X., Li, N. and Li, Z. (2008). Health risk from metals via consumption of food crops in the vicinity of Dabaoshan Mine, South China. *Science of the Total Environment*, 407: 1551-1561. doi:10.1016/j.scitotenv.2008.10.061.

How to cite this article:

Kumar A., Kumar V and Seema (2017) 'Comparative Study of Levels of Heavy Metals in Unwashed And Washed Vegetables And Their Impact on Human Health', *International Journal of Current Advanced Research*, 06(10), pp. 7065-7074. DOI: http://dx.doi.org/10.24327/ijcar.2017.7074.1075

****