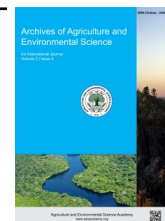This content is available online at AESA

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ORIGINAL RESEARCH ARTICLE

CrossMark

Health risk assessment of heavy metals via dietary intake of vegetables grown in wastewater irrigated areas of Jagjeetpur, Haridwar India

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ABSTRACT

The present study was conducted to appraise the human health risk due to dietary intake of heavy metals contaminated vegetables viz., cabbage (*Brassica oleracea* var. capitata), cauliflower (*Brassica oleracea* var. botrytis) and spinach, (*Spinacia oleracea*) grown in the municipal wastewater irrigated soil in the proximity of sewage treatment plant (STP), Jagjeetpur, Haridwar. The results showed that Cd, Fe and Mn concentrations in *B. oleracea* var. capitata, *B. oleracea* var. botrytis and *S. oleracea* were found beyond the safe limit of the Indian and WHO/FAO standards for heavy metals in the vegetables. The contamination factor of these heavy metals in the soil was recorded in the order of Fe > Mn > Zn > Cu > Cr > Cd after irrigation of municipal wastewater. The higher values of metal pollution index in different vegetables as *B. oleracea* var. capitata (14.82; *B. oleracea* var. botrytis (10.48) and *S. oleracea* (12.59) showed more health risk index for Cd, Fe and Mn in these vegetables cultivated in the wastewater irrigated soil. Therefore, dietary intake of these heavy metals contaminated vegetables may pose a significant threat to the human health. However, cauliflower contained less heavy metal as compared to the cabbage and spinach, but health risk was more due to higher role in the diet. Even though there were low concentrations of heavy metals in the municipal wastewater used for the irrigation, but long term use of the municipal wastewater may cause gradual buildup of heavy metals in the vegetables grown in the municipal wastewater irrigated soil and leads to health risk of consumers due to intake of heavy metals contaminated vegetables.

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INTRODUCTION

Wastewater irrigation is a collective realism in three-fourth of the cities in Asia, Africa and Latin America. Wastewater conveys substantial amounts of trace toxic metals (Pescod 1992; Yadav *et al.*, 2002). Wastewater irrigation is known to have its significant impact to the heavy metal content of soils (Mapanda *et al.*, 2005; Nan *et al.*, 2002). This charging of heavy metals often clues to degradation of soil health and adulteration of food chain mainly through the vegetables grownup on such soils (Rattan *et al.*, 2002). Most municipal farmers in India use wastewater rich in harmful heavy metals alike cadmium (Cd),

chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), manganese (Mn), lead (Pb) and zinc (Zn) and biological negotiators such as pathogenic bacteria, coliform bacteria fungi, protozoans and nematodes etc. and this poses serious health pressures, such as a risk of biomagnifications of heavy metals and conveying intestinal nematodes and bacterial infections especially to consumers and farm hands (Manios *et al.*, 2006; Zhang *et al.*, 2008; Pathak *et al.*, 2011; Kumar *et al.*, 2015, 2017).

Heavy metals are usually not removed even after the treatment of wastewater at sewage treatment plants, and thus cause threat of heavy metal adulteration of the soil and consequently to the food chain (Fytianos *et al.*, 2001). Consumption of heavy

metals through the food chain by human populations has been widely testified throughout the biosphere (Muchuweti *et al.,* 2006). Due to the non-biodegradable and persistent nature, heavy metals are accrued in vital organs in the humanoid body such as the kidneys, bones and liver and are linked with numerous serious health syndromes (Duruibe *et al.,* 2007; Kumar and Thakur, 2017). Individual metals display specific signs of their toxicity. Lead, As, Hg, Zn, Cu and Al poisoning have been concerned with gastrointestinal (GI) disorders, diarrhoea, stomatitis, tremor, hemoglobinuria causing a rust-red colour to stool, ataxia, paralysis, spewing and convulsion, depression, and pneumonia (McCluggage, 1991; Raj and Thakur, 2017).

Undeniably, there is no way to stop wastewater use by the farmers, but we can try and make their usage safer by reviewing parts of crops that gather the least heavy metals and thus recommend the farmers and consumers on which parts of the crops is nontoxic to eat (Rattan *et al.,* 2005; Ambika *et al.,* 2010; Kumar and Chopra, 2013 and Kumar *et al.,* 2015, 2016). For farmed soil, irrigation with sewage and application of wastewater will raise crop production because of appreciated sources of vegetable nutrients and organic matter. Another reason for irrigation with wastewater is severe famine of renewed water (Wang and Lin, 2003; Mireles *et al.,* 2004; Singh *et al.,* 2012). However, there are many kinds of heavy metals in wastewater that may be accrued in soil with long-term use. Though, wastewater had a positive effect for short-term submission, heavy metal contaminations with long-term practice of wastewater (Nanjundappa *et al.,* 2002; Oliveira and Pampulha, 2006; Singh *et al.,* 2012).

Vegetables are major constituents of human diet, being sources of key nutrients, antioxidants and metabolites in food items (Kumar *et al.,* 2015). In the current study, the attentions of heavy metals in locally produced vegetables were determined throughout a harvesting period at a peripheral area of Haridwar city of India, where treated and untreated wastewater has been recycled as a source of irrigation water for about 20 years (Kumar *et al.,* 2016). The contamination levels in soil and vegetable crops were evaluated with respect to the prescribed safe limits of different heavy metals set under national and international norms. A number of standard measures were used to assess the health risks associated with the measured levels of heavy metal adulteration at the study sites. Therefore the present study was carried out to assess the health risk assessment of heavy metals via dietary intake of vegetables grown in wastewater irrigated areas of Jagjeetpur, Haridwar India.

MATERIALS AND METHODS

Study sites

The study was conducted around Jagjeetpur sewage treatment plant (JSTP) situated at suburban area in the Haridwar district (29°54'42.53"N latitude 78° 8'19.36"E longitude and 314 M above the sea level). Large-scale vegetables production is carried out in this area and maximum amount of these vegetables is supplied in the market of the Haridwar city. Industrial effluents from various small scale industries positioned in the city are also discharged along with municipal sewage for the treatment at JSTP. These industries include fabric portrait, batteries, dye, plastic recycling and metal surface treatment. A large area around JSTP has no access to sanitary water resources, so farmers use treated and untreated wastewater for irrigation of their crops. During the present study, two major sites were demarcated in Jagjeetpur having different irrigation practices. At the wastewater irrigated (WWI) site, treated wastewater from JSTP has been used for irrigating the fields for about 20 years. Sometimes due to power failure or technical issue the sewage treatment plant does not work and untreated wastewater is used for irrigation. Clean water from bore wells has been used for watering the agricultural fields at the clean water irrigated site (CWI) for a similar period of time.

Soil and water sampling

Soil and water samples were collected at fortnightly interval from September 2016 to March 2017. Soil samples were collected in triplicate by digging out a monolith of $10{\times}10{\times}15$ cm^3 size, from 10 subsites of both clean (CWI) and wastewater irrigated sites (WWI). Soil samples were air-dried, crumpled and passed through 2 mm mesh size sieve and stored at ambient temperature before analysis. Both clean and wastewater samples (100 ml) used for irrigation were composed in triplicate in a pre-acid washed polypropylene bottle and 1 ml of concentrated HNO_3 was added in the water sample to escape the microbial action. These samples were carried back to the laboratory and kept in a refrigerator before digestion.

Plant sampling

All the vegetables *viz.,* cabbage (*Brassica oleracea* var. capitata), cauliflower (*Brassica oleracea* var. botrytis) and spinach, (*Spinacia oleracea*) crops grown in the experimental area were collected either for home feasting or sale. The details of different plants sampled during the experiment are given in Table 1. An area of $5{\times}5m^2$ was indiscriminately marked at 10 sub sites in triplicate and the eatable portion of test vegetables were collected from both CWI and WWI sites. Samples were brought back to the laboratory and wash away with clean tap water to remove the soil elements adhered to the surface of the vegetables. After removing the extra water from the surface of vegetables with blotting paper, samples were cut into pieces, packed into separate bags, and kept in an oven until a constant weight was achieved. The dried samples were grinded and passed through a sieve of 2 mm size and then kept at room temperature for further analysis.

Analytical methods

The irrigation water sample (50 ml) was digested with 10 ml of concentrated HNO_3 at 80^oC until the solution became transparent (APHA, 2012). The solution was sieved through Whatman No. 42 filter paper and the total volume was sustained to 50 ml with distilled water. Vegetables and soil samples were digested in di acid (2:1 ratio of HNO_3 and $HClO_4$) as per the method described in AOAC (1990) for wet washing. 0.5 g of powdered

samples of vegetable, soil and water (10 ml) were taken in digestion tube and then 10 ml nitric acid (HNO_3) and 5 ml perchloric acid ($HClO_4$) were added and the digestions were completed on digestion blocks (Make FOSS) following standard measures as described by Chaturvedi and Sankar (2006). After digestion all samples were sieved through Whatman 42 filter paper and volume was made up to 50 ml. Heavy metals such as Cr, Cd, Cu, Fe, Mn, and Zn in the digested aliquot were determined by atomic absorption spectrophotometer (Perkin-Elemer model 5000).

Data analysis

Enrichment factor (EF): To examine the translocation of heavy metals in the soil and in the edible portion of test plants, and to show the difference in metal concentrations in the plants between the sites, the enrichment factor (EF) was calculated by using the formula given by Buat-Menard and Chesselet (1979):

$$EF = \frac{\frac{\text{Concentration of heavy metals in edible part at WWI site}}{\text{Concentration of heavy metals in soil at WWI}}}{\frac{\text{Concentration of heavy metals in edible part at CWI site}}{\text{Concentration of heavy metals in soil at CWI site}}}$$

Metal pollution index (MPI): To examine the overall heavy metal concentrations in all crops analysed in the wastewater irrigated site, metal pollution index (MPI) was computed. This index was obtained by calculating the geometrical mean of concentrations of all the metals in the vegetables, cereals and milk (Usero et al., 1997).

$$MPI\ (\mu g\,g^{-1}) = (Cf_1 \times Cf_2 \times …………\times Cf_n)^{1/n}$$

Where Cf_n = concentration of metal n in the sample.

Health risk index (HRI): The health risk index was calculated as the ratio of estimated exposure of test crops and oral reference dose (Cui et al., 2004). Oral reference doses were 4×10^{-2}, 0.3 and 1×10^{-3} mg kg^{-1} day^{-1} for Cu, Zn and Cd, respectively (USEPA, 2002) and 0.004, 0.02 and 1.5 mg $kg^{-1}day^{-1}$ for Pb, Ni and Cr, respectively (USEPA, 1997). Estimated exposure is obtained by dividing daily intake of heavy metals by their safe limits. An index more than 1 is considered as not safe for human health (USEPA, 2002).
Daily intake was calculated by the following equation:

$$\text{Daily intake of metal (DIM)} = \frac{C_{metal} \times D_{food\,intake}}{B_{average\,weight}}$$

Where C_{metals} $D_{food\,intake}$ and $B_{average\,weight}$ represent the heavy metal concentrations in plants ($\mu g\,g^{-1}$), daily intake of vegetables and average body weight, respectively. The average daily vegetable intake rate was calculated by conducting a survey where 100 people having average body weight of 60 kg were asked for their daily intake of particular vegetable from the experimental area in each month of sampling (Ge, 1992; Wang et al., 2005).

Statistical analysis

The significance of differences between the concentrations of heavy metals in soil at wastewater (WWI) and clean water irrigated (CWI) sites were shown by using Student's t-test. The data of heavy metal concentrations in the plants at different sites were subjected to two-way analysis of variance (ANOVA) test for assessing the significance of differences in heavy metal concentrations due to different irrigation practices. All the statistical tests were performed using SPSS software (SPSS Ins., version 11).

RESULTS AND DISCUSSION

Levels of heavy metals in water samples

The attentions (μgml^{-1}) of heavy metals in the irrigation water at WWI site ranged between 0.03–0.08 for Cr, 0.00–0.02 for Cd, 0.02–0.07 for Cu, 0.47–1.03 for Fe, 0.15–0.28 for Mn and 0.05–0.18 for Zn during September 2016 to March 2017, whereas at CWI site, heavy metal concentrations in irrigation water were very low or below the noticeable limits (Figure 1). Heavy metals in the sewage water are related with small-scale industries such as colouring, electroplating, metal surface treatments, fabric printing, battery and paints, releasing Cd, Cu, Fe, Mn, Zn and other heavy metals into water channels, which are accessed for irrigation. As compared to the present concentration of heavy metals in the wastewater, Kumar et al. (2016) reported Fe (1.72) and Zn (0.38) in the sewage effluent were found very low or below prescribed limit.

Levels of heavy metals in the soil

Eminent levels of heavy metals in irrigation water led to suggestively higher concentrations of heavy metals in the soil at WWI site as compared to those acquired from clean water irrigated site (Table 2). The heavy metal absorptions were, still below the safe limits of Indian standards (Awashthi, 2000) and EU standards (European Union, 2002) at WWI site (Table 2). The lower availability of heavy metals than the safe limits at WWI site may be due to the constant abstraction of heavy metals by the vegetables grown up in this area and also due to percolating of heavy metals into the deeper layer of the soil. The increases in the heavy metal attentions in the soil were 226% for Cr, 274% for Cd, 263% for Cu, 583% for Fe, 480% for Mn and 322% for Zn at WWI site as compared to CWI site in the present study (Table 2).The contamination factor of heavy metals in the soil was recorded in the order of Fe > Mn > Zn > Cu>Cr > Cd after disposal of wastewater. Additionally, the increase in the contents of Fe, Mn, Zn, Cu, Cr and Cd are likely due to the presence of more concentration of these metals in the wastewater.

Content of heavy metals in the plants

Heavy metal concentrations showed variations among different vegetables collected from CWI and WWI irrigated sites (Figure 2). The results showed that the contents of Cu (r = +0.91) and

Mn (*r* = +0.74) in the edible parts of cabbage were found to be positively correlated and contents of Cd (*r* = -0.62), Cr (*r* = -0.68), Fe (*r* = -0.34) and Zn (*r* = -0.98)were found to be negatively correlated, Cauliflower showed slightly negative correlation with Cr and Cd (*r* = -0.06 and -0.06) and showed moderately correlation with Cu (*r* = +0.67) and Spinach showed positively correlated with all the metals except Zn (*r* = -0.22) with the content of Cd, Cr, Cu, Fe, Mn and Zn recorded in the soil (Table 4). The metals in plants were translocated capably from the soil through root system. The higher content of these metals in the soil is likely due to the presence of more contents of the metals in the sewage effluent, used for the irrigation of cabbage in the present study. Kumar *et al.* (2015) also reported the significant correlation of different heavy metals in the different vegetables *viz.*, carrot (*Daucus carota*), radish (*Raphanus sativus*), beet root (*Beta vulgaris*) and sweet potato (*Ipomoea batatas*) grown in municipal wastewater.

Results of two-way ANOVA test showed that variations in the heavy metal absorptions were significant due to site, plant and site plant interaction (*P<0.05/P<0.01/P<0.001*). The dissimilarities in heavy metal attentions in vegetables of the same site may be ascribed to the differences in their morphology and physiology for heavy metal uptake, accumulation and retention (Carlton-Smith and Davis, 1983; Kumar *et al.*, 2009). Several-fold higher absorptions of all the heavy metals were observed in all the vegetables at WWI site as compared to CWI site. The use of adulterated irrigation water at WWI site increased the uptake and accretion of the heavy metals in the plants. This is consistent with reports of higher availability of heavy metals in vegetables from sewage water irrigated areas as compared to the sewage treatment plant STP water irrigated areas of Sarai village of Haridwar (Kumar *et al.*, 2015).

Enrichment factor

Higher values of enrichment factor (EF) suggest poor retention of metals in soil and/or more translocation in plants. Within the plants, cabbage and spinach (leafy vegetable) showed highest EF values for Cd and Fe (Table 5). Fytianos *et al.* (2001) have

reported higher enrichment factor for Cd through leafy vegetables. Sridhara Chary *et al.* (2008) also reported highest enrichment factor for heavy metals through leafy vegetables. Enrichment factor of heavy metals depends upon bioavailability of metals, which in turn depends upon its concentration in the soil, their chemical forms, difference in uptake capability and growth rate of different plant species (Tinker, 1981). The higher uptake of heavy metals in leafy vegetables may be due to higher transpiration rate to maintain the growth and moisture content of these plants (Tani and Barrington, 2005).

Metal pollution index (MPI)

Metal pollution index (MPI) is suggested to be a reliable and precise method for metal pollution monitoring of wastewater irrigated areas (Usero *et al.*, 1997). Among different vegetables, cabbage showed highest value of MPI followed by spinach. As compared to the cauliflower, cabbage and spinach showed higher metal pollution index (Figure 3). Higher MPI of cabbage, cauliflower and spinach suggests that these vegetables may cause more human health risk due to higher accumulation of heavy metals in the edible portion. To assess the health risk associated with heavy metal contamination of plants grown locally, estimated exposure and risk index were calculated. The results showed that Cd, Fe and Mn contamination in plants had greatest potential to pose health risk to the consumers (Table 6). Health risk index was more than 1 for all the vegetables, only the health risk index for Cu was less than 1 except in cauliflower. Although, cauliflower have lesser concentrations of metals than other two vegetables, but the health risk index was higher. In the present study, only Cu was not found to cause any risk to the local population. Cui *et al.*, (2004) have also reported that local residents of an area near a smelter in Nanning, China have been exposed to Cd and Pb through consumption of vegetables, but no risk was found due to Cu and Zn. Metal transfer factor from soil to plants is a crucial component of human exposure to heavy metals via food chain. Transfer aspect of metals is essential to investigate the human health risk index (Cui *et al.*, 2004).

Table 1. Plant samples collected from the experimental sites.

Edible part of vegetable	Common name	Botanical name	Family
Leaf	Spinach	*Beta vulgaris* L.	Chenopodiaceae
Leaf	Cabbage	*Brasssicaoleracea* L. var. capitata L.	Brassicaceae
Inflorescence	Cauliflower	*Brassica oleracea* L. var. Botrytis L.	Brassicaceae

Table 2. The mean and SE concentrations ($\mu g\,g^{-1}$) of heavy metals in soil of wastewater (WWI) and clean water irrigated (CWI) sites.

| Heavy metals | CWI | | | WWI | | | Safe Limits | |
	Range	Mean	SE	Range	Mean	SE	Indian (Awashthi, 2000)	International (European Union, 2002)
Cr	5.65-6.45	6.07	±0.35	12.26-14.84	13.76[***]	±1.19	--	150
Cd	0.81-2.38	1.41	±0.68	2.68-4.95	3.87[**]	±0.97	3-6	3.0
Cu	5.31-6.57	6.06	±0.57	15.23-16.52	15.95[***]	±0.56	135-270	140
Fe	38.69-42.58	40.90	±1.66	221.58-254.26	238.59[*]	±13.37	75-150	--
Mn	7.59-8.65	8.16	±0.47	35.81-42.38	39.19[***]	±2.70	--	--
Zn	7.28-9.52	8.42	±0.99	25.95-28.62	27.12[***]	±1.16	300-600	300

Student's t-test was done for mean value of heavy metal concentrations between CWI and WWI site.** Level of significance: p ≤ 0.01. *** Level of significance: p ≤ 0.001.

Table 3. The mean and SE concentrations (µg g-1) of heavy metals in plants of wastewater (WWI) and clean water irrigated (CWI) sites.

Plants	WWI						CWI					
	Cr	Cd	Cu	Fe	Mn	Zn	Cr	Cd	Cu	Fe	Mn	Zn
Cabbage	3.85± 0.95	1.86± 0.25	5.35± 1.03	39.67± 2.40	6.4±1. 03	8.06±1 .38	0.56± 0.06	0.08±0 .01	3.62±0 .69	12.44±1 .57	3.54±0 .58	4.15± 0.38
Cauliflower	2.54± 0.54	0.89± 0.07	8.02± 1.16	37.12± 2.40	6.34± 0.96	9.31±1 .43	0.68± 0.13	0.09±0 .02	2.97±0 .53	9.12±0. 79	2.76±0 .37	3.06± 0.39
Spinach	2.99± 0.15	1.21± 0.19	4.98± 0.84	30.79± 2.10	4.25± 1.21	5.60±1 .21	0.41± 0.01	0.05±0 .01	2.61±0 .32	9.69±1. 80	1.67±0 .78	0.80± 0.14

Table 4. Correlation coefficients (r^2) between heavy metals concentrations in the edible parts of the plants and metal concentrations in soil.
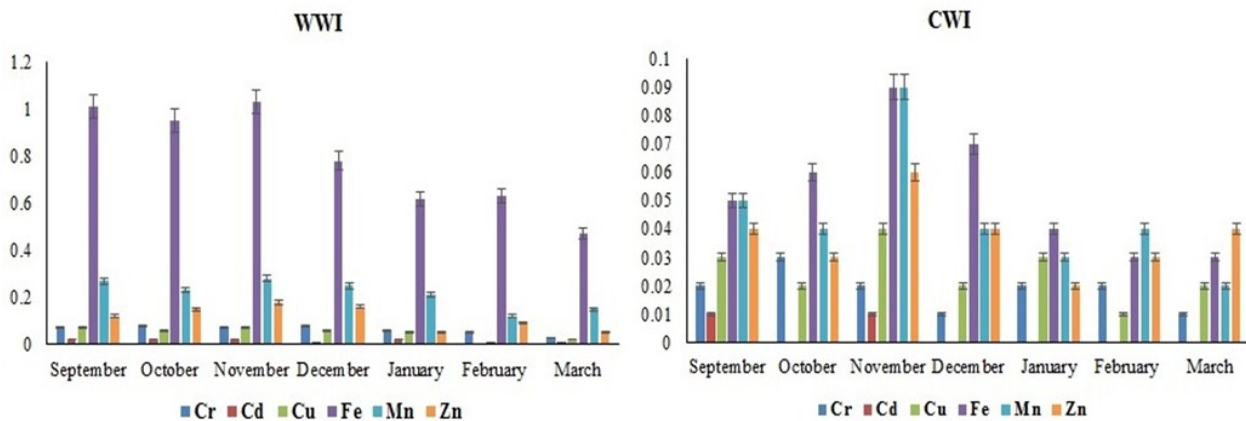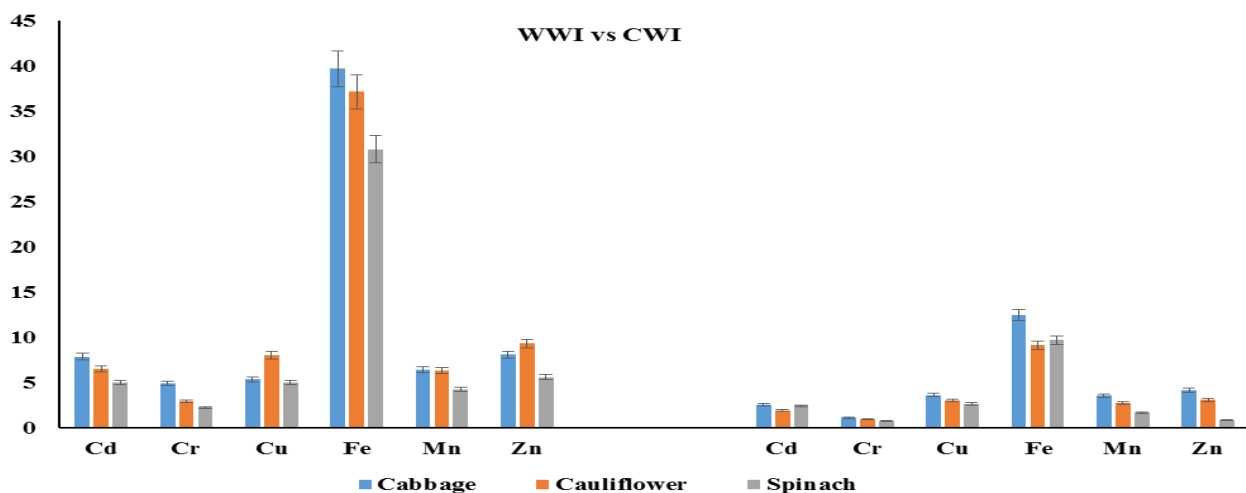
Vegetables	Cr	Cd	Cu	Fe	Mn	Zn
Cabbage	-0.68	-0.62	0.91	-0.34	0.74	-0.98
Cauliflower	-0.06	-0.06	0.67	-0.34	-0.43	0.05
Spinach	0.22	0.46	0.35	0.03	0.26	-0.22

Table 5. Enrichment factor of heavy metals in collected foodstuffs from the experimental site.

Foodstuff's	Cr	Cd	Cu	Fe	Mn	Zn
Cabbage	3.06	4.50	1.48	3.19	1.81	1.94
Cauliflower	3.48	3.25	2.70	4.07	2.30	3.04
Spinach	2.08	2.95	1.91	3.18	2.54	7

Table 6. Health risk index (HRI) of heavy metals via intake of foodstuffs from wastewater irrigated sites.

Foodstuff's	Cr	Cd	Cu	Fe	Mn	Zn
Cabbage	5.47	10.88	0.59	51.92	17.22	3.08
Cauliflower	1.66	2.30	1.71	28.33	18.71	1.42
Spinach	3.47	2.63	0.94	23.08	18.47	1.63

Figure 1. *Monthly variations in heavy metal (µg g^{-1}) concentrations of water at WWI and CWI sites.*

Figure 2. *Mean concentration of heavy metals (µg g^{-1}) in plant samples collected from WWI and CWI sites.*
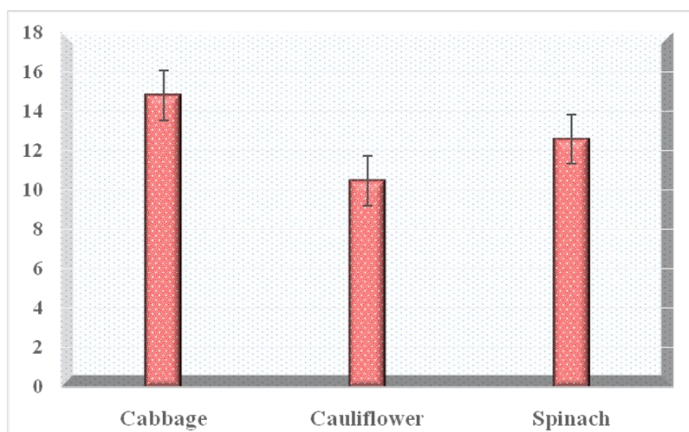
Figure 3. *Metal pollution index in different foodstuffs from wastewater irrigated site.*

Conclusion

The result of the study, show that soils in the vicinity of Jagjeetpur area were found to be significantly contaminated with metals. Variations in the heavy metals concentration between the vegetables reflect the variances in uptake abilities and their further translocation to the comestible portion of the plants. Cadmium, Iron and Manganese concentrations were overhead the national and various international tolerable limits in all the vegetables. The metal pollution index and health risk index of heavy metals also recommend that Cd, Fe and Mn adulteration in most of the plants had latent for human health risk due to consumption of plants grown at waste water irrigated site. Cauliflower is found to be slightest contaminated by heavy metals as its metal pollution index and health risk index were lower likened to other foodstuffs. The health risk index was higher in vegetables due to higher proportion in the diet. Ingesting of foodstuff with higher levels of heavy metals may lead to high level of accretion in the body causing related health complaints. The study proposes that even though there are low concentrations of heavy metals in irrigation water, its long term use produced heavy metal contamination leading to health risk of clients. Thus urgent attention is needed to implement suitable means of intensive care and modifiable industrial and domestic effluent, and providing proper advice and support for the safe and fruitful use of wastewater for irrigation.

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