RISK ASSESSMENT OF HEAVY METAL TOXICITY THROUGH CONTAMINATED VEGETABLES FROM WASTE WATER IRRIGATED AREA OF REWA (M.P.), INDIA

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ABSTRACT

The present study had generated data on heavy metals in water, soil and different kind of vegetables from waste water irrigated sites of Rewa, India and associated risk assessment for consumer's exposure to heavy metals. Present results showed that Pb, Cd, Mn and Cr in waste waters; Cd soils and Pb, Cd and Cr in all tested vegetables were above the national and international permissible limits. People living in the contaminated area are at greater risk for health issues than individuals in the reference area. Children are at somewhat higher risk than adults. Heavy metal concentrations were several fold higher in all the collected samples from waste water irrigated sites compared to clean water irrigated ones. In this study, the soil to vegetable transfer factor (TF) for various heavy metals and for most common vegetables consumed by human being were also calculated and data showed that the TF values differed significantly between soil and vegetable. However, the Daily Intake Rate (DIR) and Hazard Index(HI) of these metals through consumption of vegetables grown in waste water irrigated areas of Rewa was much less than that affect health. The hazardous quotient (HQ) of all studied heavy metals also indicated that all vegetables were safe with no risk to human health except for Pb contamination in Spinach, Cauliflower and Radish had potential for human health risk due to consumption of these vegetables grown in the area having long term uses of untreated waste water for irrigation at Naubasta site.

Keywords - Average Daily Dose, Daily Intake Rate, Hazardous Quotient, Health Risk.(Hazard),Index, Heavy metal, Transfer Factor, waste water.

I. INTRODUCTION

Today, due to constraint in availability of fresh water for irrigation, waste water is being used for irrigation of agricultural fields. Due to the scarcity of water for irrigation purposes compels the use of sources contaminated with industrial effluents. The growing problem of water scarcity has significant negative influence on economic development, human livelihoods, and environmental quality throughout the world. Farmers are mainly interested in general benefits, like increased agriculture production, low cost water source, effective way of effluent disposal, source of nutrients, organic matter etc, but are not well aware of its harmful effects like heavy metal contamination of soils, crops and quality problems related to health. Industrial wastewater is mostly used for the irrigation of crops and vegetables, mainly in periurban ecosystem, due to its easy availability, disposal problems and scarcity of fresh water. Due to lack of facilities these untreated waste water is being used by farmers to satisfy crop water needs. This indiscriminate continuous use of such effluent for crop production could result in the concentrations that may become phytotoxic (Ghafoor *et al.*, 1999). Wastewater may contain various heavy metals depending upon the type of activities it is associated with. Therefore long term use of this waste water, which is mainly used for cultivation of leafy and other vegetables, has resulted in accumulation of heavy metals in the soil and their transfer to the various vegetables under cultivation (Singh *et al.*, 2004; Sharma *et al.*, 2007; Marshall *et al.*, 2007). Uptake and accumulation of elements by plants may follow two different paths i.e. through the roots and foliar surface. Thus toxic metals may be absorbed by vegetables through several processes and finally enter the food chain (Fytianos *et al.*, 2001). These Heavy metals are very harmful because of their non-biodegradable nature, long biological half-lives and their potential to accumulate in different body parts. The intake of heavy metal can lead to altering of humans and animals healthiness state. Food safety issues and potential health risks make this as one of the most serious environmental concerns (Cui *et al*., 2004). Moreover, the carcinogenic effects generated by continuous consumption of fruits and vegetables loaded with heavy metals (International Occupational Safety and Health Information Centre 1999).Transfer factor expressed the bioavailability of a metal at a particular position on a species of plants (vegetables). This is however, dependant on different factors such as the soil pH and the nature of plant itself. As the vegetables are the source of human consumption so the soil-to-plant transfer quotient is the main source of human exposure. A convenient way for quantifying the relative differences of bioavailability of metals to plants is the transfer quotient. So for human Health Risk Index (HRI) transfer quotient should be assessed (Huang and Qiu, 2004). The proposed work aimed to find out the assessing heavy metal concentration in irrigation water, soil and vegetables and also risk of heavy metals through consumption of contaminated vegetables from sites having long term use of waste water for irrigation of agricultural land of Rewa city. However knowledge on the contamination of vegetables with heavy metals from waste water irrigation sites of Rewa was not established in earlier studies. This also help us in evaluating environmental approach for better utilization of only treated waste water for irrigation of agricultural lands. The result of the proposed work elucidated various impact of industrial waste water when used as irrigation of agricultural lands.

II. MATERIALS AND METHODS

General description of the experimental Sites

Rewa is a city in the northern-eastern parts of the state of Madhya Pradesh, India. It is the administrative centre of Rewa District and Rewa Division. The cities lie about 420 km. (261 mi) north east of the state capital Bhopal, Madhya Pradesh and 130 km. (81 mi) south of the city of Allahabad, Uttar Pradesh. It is situated at 24.53^0 North latitude and 81.3^0 East longitudes and covers an area of 6,240 km^2 (2,410 sq mi). It has an elevation of 304 m. (997 ft) above mean sea level. The average rainfall is 980 mm (39 inches) per year. The average temperature is around 25^0C (77^0 F) and the humidity is quite high. Experimental sites of different irrigation sources J.P. Cement Plants Bela (WWI-I), Naubasta (WWI-II) &, Bhiti village (CWI-III) were selected. Cultivated land of these two industrial areas (Bela & Naubasta) received waste water discharge from industries, manufacturing cement while third site of rural area (Bhiti) received clean (ground) water from deep bore well. Thus all sites (WWI-I, WWI-II and CWI-III) of varying irrigation sources were selected and the sampling of water, soils and vegetables of the surrounding areas were carried out in May month, alone accordingly in order to figure out the extent of heavy metals contamination.

Sampling and laboratory analyses

Collection and digestion of water samples

At each site, both waste water and clean water samples collected randomly from different location. The WWI-I, WWI-II and CWI-III samples were not mixed with residential, domestic, sewage or other waste water sources.

All water samples were labelled and brought to the laboratory and kept in a refrigerator at about 4^0C before digestion. As soon as the samples were brought to the laboratory, they were acidified with HNO_3 (Merck), filtered and stored in dark at ambient temperature before analysis. Both waste water and clean water samples were digested according to APHA, (2005); the irrigation water sample, 50 ml. was transferred into beaker and 10 ml. of concentrated nitric acid (HNO_3) was added. The beaker with the content was placed on a hot plate and evaporated down to about 20 ml at 80^0C .The beaker was cool and another 5 ml. concentrated HNO_3 was also added. The beaker was covered with watch class and returned to the hot plate. The heating was continued, and then small portion of HNO_3 was added until the solution appeared transparent. The beaker wall and watch glass were washed with distilled water and the solution was filtered through whatman NO. 42 filter paper and the total volume were maintained to 50 mL with distilled water.

Collection and digestion of soil samples

Waste Water Irrigated soil samples were collected from the cultivated fields near the J.P. Cement Plant (Bela and Naubasta) along a distance of 100m from the Plants. Soil samples taken from each sites were separately labelled and transferred into air tight polythene bags and brought into laboratory. Before its transported to the research laboratory, care was taken, to the extent possible, to ensure that there were no other sources of contamination at the site of investigation such as motor vehicle emission, dumpsite garbage, sewage water, grey water, domestic waste, slurry, or compost to mask the effect of waste water irrigation. Soils were sieved through a 2 mm sieve to remove coarse particles and stored at ambient temperature prior to analysis. Soil samples were digested according to Allen *et al.*, (1986). To 5g of each of the air dried and sieved soil samples was thoroughly grinded, 1.0g of each of the ground soil samples were placed in 100 ml beaker. 15 ml of HNO, H_2SO_4 and HCl mixture (5:1:1) of tri-acid were added and the content heated gently at low heat on hot plate for 2 hrs at 80^0C until a transparent solution was obtained. After cooling, the digested sample was filtered using whatman NO. 42 filter paper. It was then transferred to a 50 mL volumetric flask by adding distilled water.

Collection and digestion of vegetable samples

Vegetable samples were taken in the agricultural fields around the commune where they were known to be affected by waste water and where they were not (control). Samples of seven different kinds of vegetables; leafy vegetables included Spinach (SP) (*Betavulgaris L. CV.* All green), and Cabbage (CA) (*Brassica oleracea L. Var. Capatuta*). Inflorescence vegetable included Cauliflower (CF)(*Brassica oleracea L. Var. botrytis*), Fruit vegetables included Lady's Finger (LF) (*Abelmoschus esculentus L.*), Brinjal (BR)(*Solanum melongena L.*), Toma -to (TO) (*Lycopersicon esculentum L.*) and Root vegetable included Radish (RA) (*Raphanus sativus L.*) were taken from the same experimental sites where waters and soils samples were taken . Vegetable sample were collected randomly by hand using vinyl gloves carefully packed into polyethylene bags and the whole plant body was brought to the laboratory from each experimental site in order to estimate heavy metals. Cleaning (soil removal) of vegetable plant samples was performed by shaking and also by means of a dry pre-cleaned vinyl brush. Only edible parts of different vegetables were randomly taken from each experimental site. Freshly collected mature vegetable samples from each experimental site were brought to the laboratory and washed primarily with running tap water, then in distilled water and finally rinsed carefully in demonized water to remove any attached dust pollen particles (Burton and Patterson, 1979). Vegetable samples were also digested according to Allen *et al.*, (1986) as described above.

Analysis of samples

Concentrations of Fe, Zn, Cu, Pb, Cd, Mn and Cr in the filtrate of digested soil, water and different kind of vegetables samples were estimated by using an Atomic Absorption Spectrophotometer (AAS, Perkin Elmer analyst 400). The instrument was fitted with specific lamp of particular metal. The instrument was calibrated using manually prepared standard solution of respective heavy metals as well as drift blanks. Standard stock solutions of 1000 ppm for all the metals were obtained from Sisco Research Laboratories Pvt. Ltd., India. These solutions were diluted for desired concentrations to calibrate the instrument. Acetylene gas was used as the fuel and air as the support. An oxidising flame was used in all cases.

Table 1: Guideline for safe limits of heavy metals (Source: CPCB-Lucknow)

Samples	Standards	Fe	Zn	Cu	Pb	Cd	Mn	Cr
Water (mg L^{-1})	Indian Standard (Awashthi 2000)	NA	5.0	0.05	0.10	0.01	0.1	0.05
	WHO/FAO (2007)	NA	2.0	0.20	5.0	0.01	0.2	0.10
	European Union Standards (EU2002)	-	-	-	-	-	-	-
	USEPA (2010)	NA	2.00	1.00	.015	.005	.05	0.10
	Kabata-Pendias (2010)	0.80	NA	NA	NA	NA	NA	NA
Soil (mgkg^{-1})	Indian Standard (Awashthi 2000)	NA	300-600	135-270	250-500	3-6	NA	NA
	WHO/FAO (2007)		-	-	-	-	-	-
	European Union Standards (EU2002)	NA	300	140	300	3.0	NA	150
	USEPA (2010)	NL	200	50	300	3.0	80	NA
	Kabata-Pendias (2010)	1000	NA	NA	NA	NA	NA	NA
Plant (mgkg^{-1})	Indian Standard (Awashthi 2000)	NL	50.0	30.0	2.5	1.5	NL	NA
	WHO/FAO (2007)	450	60.0	40.0	5.0	0.2	500	5.0
	European Union Standards (EU2002)	NL	60	40	0.30	0.20	NL	NA
	USEPA (2010)	-	-	-	-	-	-	-

NA = Not Available ; NL = No Limit

Quality Control Analysis

Quality control measures were taken to assess contamination and reliability of data. For this Blank samples (zero metal conc.) were analyzed after seven samples. Concentrations were calculated on a dry weight basis. All analysis was replicated three times. The accuracy and precision of metal analysis were checked against NIST (National institute of standard and Technology)-SRM (Standard Reference Material) 1570 for every heavy metal.

DATA ANALYSIS

Transfer Factor (TF)

Metal concentrations in the extract of soils and vegetables were calculated on the basis of dry weight (mg/kg). TF was calculated as follows (Cui *et al.*, 2005):

$$TF = \frac{C_{(Vegetable)}}{C_{(Soil)}} \quad (1)$$

Where, $C_{(Vegetable)}$ represent the heavy metal concentration (mg/kg) in extract of edible parts of vegetables & $C_{(Soil)}$ represent the heavy metal concentration (mg/kg) in soils from where the vegetable was grown.

Daily Intake Rate (DIR)

For the Daily Intake Rate (DIR), the average metal content in each vegetable was calculated and multiplied by the respective consumption rate. Daily Intake Rate (DIR) was determined by the following equation (Arora *et al.*, 2008; Sajjad *et al.*, 2009):

$$\mathbf{DIR} = \mathbf{C}_{(\text{Metal conc.})} \times \mathbf{C}_{(\text{Factor})} \times \mathbf{D}_{(\text{Vegetable intake})} \qquad \textbf{(2)}$$

Where, $C_{(\text{Metal conc.})}$ = Heavy metal concentration in vegetables (mg/kg); $C_{(\text{Factor})}$ = conversion factor (0.085); $D_{(\text{Vegetable intake})}$ = Daily Intake of Vegetable (kg person^{-1}day^{-1} FW).

The conversion factor of 0.085 is set to convert fresh vegetable weight to dry weight based on Eqn. (Rattan *et al*. 2005; USDA, 2007).

$$IRdw = IRww\left[\frac{100-W}{100}\right] \qquad \textbf{(3)}$$

Where, *IRdw* = dry-weight intake rate; *IRww* = wet-weight intake rate & *W* = percent water content.

Average Daily Dose (ADD: mg kg^{-1} day)

The average daily vegetable intake rate(ADD) 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 (Ge.1992; Wang *et al.*, 2005; Sajjad Khan *et al.*, 2009). Where, the average daily intake for adults and children were set to 0.345Kg and 0.232 kg person^{-1}day^{-1} (expressed as fresh weight), respectively while the average adult and child body weights were set to 60 and 32.7 kg, respectively in this study ;based on Eqn.(EPA 1989d, 2010e).

$$ADD = \frac{C \times IR \times FI \times EF \times ED}{BW \times AT} \qquad \textbf{(4)}$$

Where, C = Contaminant concentration in vegetable (mg kg-1); IR = Ingestion rate per unit time or event (kg day-1); FI = Fraction ingested from contaminated source (unit less); EF = Exposure frequency (days/year); ED = Exposure duration (70 years; lifetime; by convention) is the length of time that a receptor is exposed via a specific exposure pathway; BW = Body weight; AT =Pathway specific period of exposure for no carcinogenic effects (i.e., ED×365days/year), and 70 year lifetime for carcinogenic effects (i.e., 70 years×365 days/year).Upper tolerable daily intake limit (safe limits) for both adults and children through the consumption of vegetables were presented in Table 2.

Table 2. Upper tolerable daily intake limit for both adults and children

Upper tolerable daily intake (mg day^{-1})	
Heavy Metals	**Integrated Risk Information System (US EPA 2009)**
Fe	45E-00
Zn	40E-00
Cu	10E-00
Pb	2.40E-01
Cd	6.40E-02
Mn	11E-00
Cr	1.05 E-02

Hazardous Quotient (HQ)

Hazardous Quotient (HQ) for the locals (consumers) through the consumption of contaminated vegetables was assessed by the ratio of Daily Intake Rate (DIR) to the oral reference dose (R_fD_o) for each metal (USEPA 2013).

Table 3: Oral reference doses (R_fD_O) mg kg^{-1} day^{-1} for heavy metals

R_fD_O(mg kg^{-1} day^{-1})		
Heavy Metal	**Integrated Risk Information System (US EPA 2013)**	**FAO/WHO (Codex Alimentarious Commission, (2013)**
Fe		7.00 E-01
Zn	-	3.00E-01
Cu	-	4.00E-02
Pb	1.00E-03	4.00E-03
Cd	-	1.00E-03
Mn	-	1.4E-02
Cr	1.5E-00	1.5E-00

If the value of HQ is less than 1, then the exposed local population (consumers) is said to be safe, if HQ is equal to or higher than 1, is considered as not safe for human health, therefore potential health risk occurred, and related interventions and protective measurements should be taken (US-EPA, 2013). An estimate of risk to human health (HQ) through consumption of vegetables grown in metal contaminated soil was calculated by the following equation:

$$HQ = \frac{DIR}{R_fD_O} \quad (5)$$

Where, R_fD_o is the oral reference dose. R_fD_o is an estimate of a daily oral exposure for the human population, which does not cause deleterious effects during a lifetime (US-EPA, 2009). Table 3 showed the values of oral reference doses (R_fD_O) for some heavy metals by IRIS, 2013; DEFRA, 2005 and FAO/WHO, 2013.

Hazard Index (HI)

To estimate the risk to human health through more than one HM, the hazard index (HI) has been developed (US EPA, 1989). The hazard index is the sum of the hazard quotients for all HMs, which was calculated by the Eqn. (Guerra *et al.* 2010):

$$HI = \sum HQ = HQ_{Fe} + HQ_{Zn} + HQ_{Cu} + HQ_{Pb} + HQ_{Cd} + HQ_{Mn} + HQ_{Cr} \quad (6)$$

It assumes that the magnitude of the adverse effect will be proportional to the sum of multiple metal exposures. It also assumes similar working mechanisms that linearly affect the target organ.

Statistical analysis

Statistical analysis of data was done by SPSS 17. For water, soil, vegetable and site, two-way ANOVA was used. Pearson's Correlations between the vegetable and the soil were also worked out. Statistical significance of means was computed using Pair Samples t-test, with a significance level of $P < 0.001$ (Steel and Torrie, 1980).

III. RESULTS AND DISCUSSION

Heavy metal concentrations in irrigation water

Heavy metals in waste water are associated with J.P. Cement Plant (Bela and Naubasta), releasing Fe, Zn, Cu, Pb, Cd, Mn and Cr into water, which were accessed for irrigation. From the results, the concentration of heavy metals in waste water samples obtained from the two source sites (WWI-I and WWI-II) were higher than those of the samples of clean water obtained from clean water irrigation (CWI-III) site (Table 4). At WWI sites, the concentration of Zn and Cu were however below the permissible limit set by Indian Standard (Awashthi, 2000), FAO/WHO (2007), EU Standard (EU 2002) And USEPA (2010), but the concentration of Pb by USEPA (2010), Cd by Indian Standard (Awashthi, 2000) and FAO (1985), Mn by FAO (1985) and Cr by Indian Standard (Awashthi, 2000) were higher than permissible limits (see Table 1 Guideline). However, because no permissible limits were available for Fe, level of Fe suggested by Kabata Pendias (2010) was used for Fe and it was found that Fe concentration in all sites were below the permissible level (Table 1 & 4). While at CWI-III site only concentration of Fe, Zn, Cu and Mn were found (present) in negligible or minor concentration whereas Pb, Cd and Cr were not detected (totally absent) in clean water (Table 4). In comparison of the concentration of heavy metals in waste water of J.P. cement Plant Rewa with Cement Plant of NIGERCEM, Nigeria showed that Cd (0.04 mg L^{-1}) and Cr (0.149 mg L^{-1}) were lower, but for Fe (0.298 mg L^{-1}) and Zn (0.069 mg L^{-1}) were higher during the present study.

Table 4: Mean heavy metals concentration in irrigated water and soil

SAMPLE	SITES	Mean Heavy Metal Concentrations						
		Fe	Zn	Cu	Pb	Cd	Mn	Cr
FOR IRRIGATED WATER (mg L^{-1} DW)	WWI-I	0.689±0.445	0.248±0.005	0.041±0.013	0.067±0.013	0.031±0.006	0.375±0.024	0.077±0.010
	WWI-II	0.371±0.093	0.109±0.0513	0.025±0.004	0.105±0.081	0.027±0.006	0.230±0.030	0.036±0.009
	CWI-III	0.005±0.0032	0.003±0.0008	0.002±0.0008	ND	ND	0.001±0.0002	ND
FOR IRRIGATED SOIL (mg kg^{-1}DW)	WWI-I	174.38±3.39	163.42±5.77	29.19±3.03	20.08±1.74	3.03±0.053	66.08±2.41	21.05±0.779
	WWI-II	185.22±6.12	156.95±11.06	40.70±4.95	14.47±0.799	2.24±0.353	57.88±3.03	24.84±2.02
	CWI-III	61.01±8.25	43.75±6.71	16.54±2.87	7.4±1.04	0.65±0.023	44.52±9.38	5.76±2.27

Mean±SD Values ; ND= Not Detected; Student t-test was done for mean heavy metal concentrations between WWI and CWI sites ; Level of significance: $p<0.001$

Heavy metal concentrations in Soil

Statistical test of significant using the analysis of variance (ANOVA - Two Way), showed significant difference ($p<0.001$) between the concentration of the heavy metals in soil obtained from the sample sites (WWI-I and WWI-II) and those of the control (CWI-III), indicating that the waste water irrigation has increased the heavy metal concentrations in soil, whereas there were no significance differences ($p>0.001$) between the metals concentration in soil obtained from the two sample sites of WWI-I and WWI-II (Table 4). Similar results were also found in the previous studies carried by Liu *et al.*, (2006) in Zhengzhou city, china. In all the sites heavy metal concentrations were below the safe limit of Indian Standard (Awashthi, 2000), FAO/WHO (2007), EU Standard (EU 2002), Kabata-Pendias (2010) And USEPA (2010) with the exception of Cd concentration (WWI-

I site), which showed exceed the permissible limit set by EU Standard (2002) and USEPA (2010) (Table 1 & 4). Since there were no other sources of contamination in the area, the source of Cd in soil may be attributed to dust particles from cement plant (Bela). The lower concentrations of heavy metals (except for Cd) than the safe limits of WWI-I and WWI-II sites may be due to the continuous removal of heavy metals by the vegetables and cereals grown in this area and also due to leaching of heavy metals into the deeper layer of soil.

Heavy metal concentrations in vegetables

Results of two Way ANOVA test indicated that variation in the heavy metal concentrations were significant due to site, plant and site × plant interaction. Heavy metal concentrations varied among different vegetables which may be attributed to differential absorption capacity of test vegetables for different heavy metals (Zurera *et al*., 1989). The variation in heavy metal concentrations in vegetables of same site may be ascribed to the differences in their morphology and physiology for heavy metal uptake, exclusion, accumulation and retention in all vegetables; several fold higher concentrations of all heavy metals at WWI sites as compared to CWI site. Arora *et al*., (2008) have also found higher concentrations of heavy metals in various vegetables grown under waste water irrigation as compared to those at clean water irrigated site. Use of waste water at both WWI sites, increased the uptake and accumulation of the heavy metals in the vegetables. This is consistent with reports of higher concentrations of heavy metals in vegetables from waste water irrigated areas of Ludhiana city of Punjab by Kawatra and Bakhetia (2008).Among all vegetables, Fe, Zn, Cu & Mn concentration did not exceed the permissible limit except for Pb (including control site), Cd & Cr at wastewater irrigated sites whereas at Clean Water Irrigated site, concentration of all metals were below the permissible limit.The results of the present analysis showed that the concentration of Pb was exceedingly and/or slightly above in the different kind of vegetables at all sites including control (CWI) and crossed safe limits and the situation is very much alarming. Among all sites, Pb concentrations in all vegetables from both WWI sites were exceedingly high concentration whereas from CWI site was slightly high concentration (except for Tomato). As there was no industrial unit near the study area of CWI, it seems soil of that area naturally have high concentrations of those elements which may be come from atmospheric deposition by air or other anthropogenic sources. However this crossed concentration of Pb in vegetables was much lower than concentration of Pb reported by Yadav *et al.,* (2013) in WWI area of Naini Allahabad and in WWI area of Varanasi, India reported by Sharma *et. al.,* (2010) but comparatively higher than Pb concentration recorded in Zimbabwe reported by Muchuweti *et al.,* (2006) and in Saudi Arabia reported by Jassir *et al.,* (2005), in vegetables grown in waste water irrigated areas. Accumulation of Pb mainly due to J.P. Cement plants due to transportation, re-suspended road dust and diesel generator sets. Cd concentrations in all vegetables were above the permissible limits at both waste water irrigated sites. The reason for highest Cd accumulation in greens (Spinach) was that they were Cd sensitive and relatively high Cd accumulators. Cd was easily taken up by food crops especially leafy vegetables. Also, comparable to others studies the highest mean concentration of Cd in Spinach from WWI-I site of Rewa was higher than 0.15-0.60mgkg^{-1} reported by Sardans *et al.,* (2005). This was also similar to Demirezen and Ahmet (2006) that highest Cd accumulation in leafy vegetables from WWI site of Turkey and WWI site of Greece, reported by Fytianos *et al.,* (2001). At both WWI sites, Cr concentration in all vegetables above the permissible limits set by Indian Standard (Awashthi, 2000) while below the permissible limits set by WHO/FAO (2007) except for Spinach at WWI-II site.

Pearson's Correlation Coefficient for Transfer factor

Table 5: Correlation Coefficients (r^2) between heavy metal concentrations in vegetables and soil

	VEGETABLES	TF_{Fe}	TF_{Zn}	TF_{Cu}	TF_{Pb}	TF_{Cd}	TF_{Mn}	TF_{Cr}
FOR WWI-I SITE	Spinach(SP)	-0.389*	0.395*	-0.232*	-0.711**	0.837**	-0.502**	0.972**
	Cabbage(CA)	-0.502**	-0.011 NS	0.370*	0.204*	0.947**	-0.226*	0.942**
	Cauliflower(CF)	-0.130*	-0.643**	-0.005 NS	0.409*	0.182*	-0.021 NS	0.850**
	Brinjal(BR)	-0.499*	-0.577**	-0.190*	0.362*	-0.207*	-0.660**	0.842**
	Lady's finger(LF)	-0.217*	-0.208*	-0.144*	-1.35*	-0.001 NS	-0.498*	0.590**
	Tomato(TO)	-0.225*	-0.0522 NS	-0.412*	-0.570**	0.002 NS	0.758**	0.883**
	Radish(RA)	-0.358*	-0.211*	-0.070 NS	0.010 NS	-0.009 NS	0.334*	-0.996**
FOR WWI-II SITE	Spinach(SP)	-0.382*	-0.447*	0.999**	-0.988**	-0.246*	0.996**	0.315*
	Cabbage(CA)	-0.710**	-0.201*	0.946**	0.332*	1.00**	0.936**	-0.760**
	Cauliflower(CF)	-0.529**	0.535**	0.285*	-0.511**	-0.974**	0.972**	0.858**
	Brinjal(BR)	-0.437*	-0.971**	0.971**	-0.837**	-0.710**	0.789**	0.542**
	Lady's Finger(LF)	-0.326*	0.992**	0.169*	-0.989**	0.946**	-0.061 NS	0.356*
	Tomato(TO)	0.793**	-0. 689**	-0. 214*	-0. 888**	0. 683**	-0. 991**	-0. 751**
	Radish(RA)	-0.094 NS	-0.979**	-0.572**	-0.954**	0.893**	-0.239*	-0.629**
FOR CWI-III SITE	Spinach(SP)	0.683**	-0.912**	-0.939**	-0.210*	-0.818**	0.971**	0.539**
	Cabbage(CA)	0.479*	0.845**	-0.569**	-0.236*	-0.972**	-0.318*	0.421*
	Cauliflower(CF)	-1.48**	-0.963**	0.980**	0.451*	-0.689**	0.656**	0.168*
	Brinjal(BR)	0.970**	-0.421*	0.738**	0.516**	-0.044 NS	0.046 NS	-0.139*
	Lady's Finger(LF)	-0.169*	0.437*	-0.371*	0.016 NS	0.283*	0.721**	0.377*
	Tomato(TO)	-0.277*	-0. 999**	0. 923**	0. 844**	0. 283*	0. 989**	-0. 986**
	Radish(RA)	0.429*	0.956**	0.449*	-0.065 NS	-0.689**	0.257*	-0.884**

NS = Not Significant; *Level of Significance: $p < 0.05$; **Level of Significance: $p < 0.10$

Also comparable to others studies the highest mean concentration was higher than concentration of Cr in Spinach (0.217 mg kg^{-1}) from WWI site of Faisabad, reported by Farooq *et al.*,(2008) but several fold lower than Cr concentration in Spinach (70.79mg kg^{-1}) from WWI site of Bellandur,Bangalore, reported by Ramesh and Murthy (2012). Present findings revealed Cr in Spinach at WWI-II site was really a panic situation and urgent measures are required toward the condition. However value of Cr concentration in all vegetables at all sites (except for Tomato at WWI-II) was well within the permissible limit set by WHO/FAO (2007). This was also similar to Sharma et al., (2006) that studies the Heavy Metals in different vegetable grown in field irrigated with waste water and noted the concentration of Cr to be within safe limits.

Pearson's correlation coefficient of heavy metals in soils and different kind of vegetables are summarized in table 5. TF were computed for the heavy metals to quantify the relative differences in bioavailability of metals to vegetables to identify the efficiency of a vegetables species to accumulate a heavy metal. Computation of Pearson's correlation coefficient of heavy metals between soils and different kind of vegetables showed that for some vegetables; there were positive but not significantly correlation found while for other vegetables it was positively and significantly correlated. Positive correlation suggested that the metal in different kind of vegetables were translocated efficiently from the soil through root system (Agbenin *et al.*, 2009). However most vegetables showed negatively and significantly while other showed negative but not significantly correlation (Table 5). Negative correlation indicated that higher concentration of heavy metals present in soils but in comparison much lower concentration were found to be in vegetables of that soils. This was due to poor retention capabilities of different edible parts of vegetables (Marshall *et al.*, 2010). TF values decreases with increasing respective metal concentration in soils, indicating an inverse relationship between transfer factor and metal concentration such inverse relationship were also reported by Wang *et al.*,(2006) for vegetables.

Human health risk assessment

Estimation of Daily Intake Rate

Table 6: DIR for individual heavy metals caused by the consumption of vegetables

	VEGETABLES	INDIVIDUALS DIR (mg person $^{-1}$ day $^{-1}$)													
		Fe		Zn		Cu		Pb		Cd		Mn		Cr	
		Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children	Adults	Children
WWI-I SITE	SP	3.02 E-02	3.48 E-02	1.5 E-02	1.75 E-02	9.71 E-03	1.16 E-02	2.17 E-03	2.50 E-03	5.29 E-04	6.09 E-04	7.91 E-03	9.09 E-03	2.15 E-03	2.47 E-03
	CA	2.70 E-02	3.17 E-02	1.75 E-02	2.01 E-02	9.04 E-03	1.04 E-02	1.94 E-03	2.23 E-03	6.81 E-04	7.83 E-04	6.24 E-03	7.18 E-03	2.10 E-03	2.42 E-03
	CF	2.59 E-02	2.98 E-02	1.32 E-02	1.52 E-02	8.50 E-03	9.78 E-03	1.72 E-03	1.98 E-03	4.93 E-04	5.66 E-04	5.51 E-03	6.33 E-03	1.29 E-03	1.48 E-03
	BR	2.09 E-02	2.41 E-02	1.36 E-02	1.57 E-02	8.48 E-03	9.75 E-03	1.51 E-03	1.74 E-03	4.61 E-04	5.30 E-04	4.31 E-03	4.95 E-03	1.33 E-03	1.53 E-03
	LF	2.41 E-02	2.77 E-02	1.23 E-02	1.41 E-02	8.01 E-03	9.20 E-03	1.58 E-03	1.82 E-03	4.77 E-04	5.48 E-04	4.57 E-03	5.26 E-03	1.91 E-03	2.20 E-03
	TO	2.65 E-02	3.05 E-02	1.38 E-02	1.59 E-02	9.16 E-03	1.05 E-02	1.70 E-03	1.95 E-03	5.19 E-04	5.54 E-04	6.44 E-03	7.41 E-03	2.04 E-03	2.35 E-03
	RA	2.78 E-02	3.20 E-02	1.60 E-02	1.84 E-02	9.67 E-03	1.11 E-02	1.80 E-03	2.08 E-03	5.29 E-04	6.09 E-04	5.70 E-03	6.55 E-03	1.84 E-03	2.10 E-03
WWI-II SITE	SP	2.14 E-02	2.47 E-02	1.91 E-02	2.19 E-02	7.54 E-03	8.67 E-03	3.93 E-03	4.52 E-03	3.97 E-04	4.52 E-04	8.30 E-03	9.55 E-03	2.75 E-03	3.16 E-03
	CA	2.02 E-02	2.32 E-02	1.77 E-02	2.03 E-02	6.42 E-03	7.38 E-03	3.57 E-03	4.11 E-03	5.77 E-04	6.63 E-04	8.22 E-03	9.44 E-03	2.47 E-03	2.84 E-03
	CF	1.81 E-02	2.08 E-02	1.78 E-02	2.05 E-02	5.60 E-03	6.44 E-03	2.82 E-03	3.20 E-03	3.82 E-04	4.44 E-04	7.41 E-03	8.52 E-03	2.49 E-03	2.86 E-03
	BR	1.84 E-02	2.12 E-02	1.56 E-02	1.80 E-02	7.32 E-03	8.42 E-03	1.52 E-03	1.74 E-03	4.72 E-04	5.42 E-04	6.70 E-03	7.71 E-03	2.19 E-03	2.52 E-03
	LF	1.91 E-02	2.20 E-02	1.71 E-02	1.97 E-02	7.05 E-03	8.10 E-03	2.13 E-03	2.45 E-03	3.20 E-04	3.67 E-04	5.51 E-03	6.34 E-03	2.27 E-03	2.61 E-03
	TO	1.91 E-02	2.20 E-02	1.85 E-02	2.12 E-02	6.14 E-03	7.06 E-03	1.82 E-03	2.09 E-03	3.77 E-04	4.34 E-04	7.21 E-03	8.29 E-03	2.62 E-03	4.48 E-03
	RA	1.96 E-02	2.25 E-02	1.82 E-02	2.10 E-02	7.13 E-03	8.20 E-03	3.54 E-03	4.07 E-03	3.82 E-04	4.40 E-04	7.94 E-03	9.13 E-03	2.03 E-03	2.39 E-03

For this formal interviews conducted in the urban areas of Rewa showed that the average consumption of fresh vegetables including Spinach, Cabbage, Lady's Finger, Cauliflower, Brinjal, Tomato and Radish and also may be other vegetables per person per day. The degree of toxicity of heavy metal to human being depends upon their daily intake (Singh, Sharma, Sridhara Chary, Agrawal and Marshall 2010). DIR as a function of body weight and intake. The DIR estimated for both WWI sites shown in table 6 but did not show for CWI site because it showed negligible values. In both WWI sites of Bela & Naubasta, the estimated Daily Intakes of heavy metals for both adults and children through the consumption of vegetables in this study was less than tolerable daily intake limit set by the US-EPA, IRIS (2013) (Table 2 & 6). Radwan and Salama (2006) & Khan *et al.*, (2008) had also observed no risk due to consumption of vegetables grown under waste water irrigated areas. Singh (2010); Sharma (2010); and Zheng *et al.*, 2007 (except for Cd), Khan *et al.*, (2008); and Guerra *et al.*, (2010) also found lower values than tolerable daily intake limits. On the other hand Sridhara Chary *et al.*, (2007) recorded higher DIR values for heavy metals than tolerable daily intake limits. In present study the highest DIR value in vegetables were for Fe (3.48E-02) for children at WWI-I site while lowest was for Cd (4.15E-04) for adults at WWI-II site. The highest daily intake of Fe was estimated as 0.034 mg/kg per day which represents approximately 4.97% of R_fD_o value of 0.700 mg/kg per day for 0.232 kg for children. This higher DIR of Fe was lower than 0.329 mg/kg per day, reported by Santos *et al.*, 2004 and 0.248 mg/kg per day, reported by Biego *et al.*, 1998. While the lowest DIR of Cd was estimated to 0.000415 mg/kg per day which represent approximately 41.5% of R_fD_o value of 0.001g/kg per day for a 0.345Kg for adults. However The DIR of Fe and Cd were lower than tolerable daily intake (Table 2). This lower DIR of Cd was lower than that reported in literature, which ranged between 0.008 mg/kg and 0.052 mg/kg per day by Santos *et al.*, (2004) & Tripathi *et al.*, (1997).

Estimation of Hazardous Quotient (HQ)

HQ values were calculated on the basis of the oral reference dose. Oral reference doses (R_fD_o) for heavy metals are presented in table 3 (US-EPA, IRIS and FAO/WHO 2013). From the result, in waste water irrigated sites; the HQ values of all heavy metals, in all vegetables were all below the one (1) (except for Pb in Spinach, Cabbage and Radish at WWI-II site) for both adults and children. When HQ exceed one (1), there is concern for potential health effect (Huang *et al.*, 2008). In present study HQ was found to be more than 1 for Pb in Spinach 1.12 E-00 (for adults) and 1.29E-00 (for children); In Cabbage 1.02 E-00 (for adults) and 1.17 E-00 (for children) and In Radish 1.01 E-00 (for adults) and 1.16 E-00 (for children) in WWI-II site. Sridhara Chary *et al.*, (2008) also found HQ in Spinach as high as 5.3 E-00. This high HQ for Pb observed in Spinach, Cabbage and Radish had greatest potential to pose health risk to the consumer. The results indicated that those living around the Cement Plant of Naubasta area of Rewa were probably exposed to some potential health risk through the intake of Pb via consuming locally grown Spinach, Cabbage and Radish but for remains vegetables it was found to be nearly free of risk. Even though there was no apparent risk when each metal was analysed individually, the potential risk could be multiplied when considering all heavy metals. Although HQ was higher for Pb in SP, CA and RA neither population suffered from ingestion of vegetables contaminated with heavy metals. Higher HQ for Pb were also reported by Zheng *et al.*, (2007) in vegetables collected from waste water irrigated area of Huludao Zinc Plant in Huludao city, China; & In vegetables from Pb and Sb smelter in Nanning, China reported by Cui *et al.*, 2004. In the present study, all heavy metals (except for Pb at WWI-II site)were least responsible for causing risk to the local population as the value of HQ was below 1 for all the vegetables from waste water irrigated area of Rewa (M.P.), India.

Estimation of Hazard Index (HI)

An Index of Risk called Hazard Index (HI) for residents of ingesting these metals by consuming vegetables grown around waste water irrigated areas were calculated by summation of HQ of all heavy metals for each vegetable. HI values of Heavy metals for all vegetables were between the 1 to 5 (one to five) by US-EPA, IRIS, indicated that there was no risk from the intake of these vegetables. Huang *et al.*, (2008) and Wang *et al.*, *(*2005) were also recorded minimum contribution of heavy metals to aggregated risk via consumption of vegetables in Kunshan and Tianjin,China.

IV. CONCLUSIONS

Scarcity of water forces mankind to use alternative, easily available and cheaper water sources. Farmers are attracted to use waste water, a water stream caring liquid waste from Cement Plant in the city for irrigated purpose. Cement plants developed in the surroundings provides additional pollutant including heavy metals deposited in cement dust. Vegetables grown in such waste water irrigated soils accumulate heavy metals on their surface or inside their tissues. Analyses of different vegetables were observed significantly lower vegetables yield in case of waste water irrigated (WWI) site in comparison to the clean water irrigated (CWI) site of Rewa city. While 94 per cent of total yield was contributed by area under cultivation in clean water irrigated (CWI) site and it was about 75 per cent in case of waste water irrigated (WWI) Site. Therefore studied were also indicated that waste water use had adversely affecting the productivity of vegetables. The present study was carried out around sub urban area of Rewa city, a small sized city of India, where irrigation of vegetables with waste water was a very common practice. Knowledge on the contamination of vegetables with heavy metals from waste water irrigation (WWI) site of Rewa is not yet established. The present study had assess data on heavy metals in water, soil and different kind of vegetables (edible parts) from waste water irrigated sites of Rewa, India and associated risk assessment for consumer's exposure to heavy metals showed that Pb, Cd, Mn and Cr (waste waters); Cd (waste water irrigated soils) and Pb, Cd and Cr conc. in all tested vegetables were above Indian Standard (2000), WHO/FAO(2007), EU(2002) & USEPA(2010) permissible limits. These accumulated heavy metals from Waste Water Irrigated area of Rewa (J.P.Cement Plant of Bela &Naubasta) had affected soil and water for a long time. People living near industrial areas are at greater chances of risk for illness than individuals in the reference area. Children are more affected to health risk than adults. From the result it was noticed that the toxicity levels of Heavy metals were found to be several fold higher in all the collected samples from waste water irrigated sites of Rewa, India, compared to clean water irrigated site of control. The finding of this study regarding DIR, HQ and HI showed that the consumption of vegetables grown in waste water irrigated soils was nearly free of risks (except for HQ values for Pb in Spinach, Cabbage and Radish at WWI-II site), it is therefore indicated that there is a relative absence of health risks associated with the ingestion of contaminated vegetables. But the situation could however change in the future depending on the dietary pattern of the community and the volume of contaminants added to the ecosystems.

RECOMMENDATIONS

- Even though there was low concentration of heavy metals (except for Pb, Cd, Mn and Cr) in irrigation water, its long term use caused heavy metal contamination leading to health risk of consumers. Thus urgent attention is needed to advise and implement appropriate means of maintaining and regulating industrial (plants) and domestic effluent, and providing appropriate advice and support for the safe and productive use of waste water for irrigation.

- Due to untreated discharge of waste water, the soil has been contaminated, which is demonstrated by accumulation of heavy metals in surface soils. This may in some circumstances pose a risk to public health. Policies should be developed that enable authorities to migrate negative environmental impacts of cement plants and empowers communities to future a safer environment whilst maintaining the economic benefits of development.
- Taking the health risks in diet as a result of high level of heavy metals in vegetables, the maximum allowable levels of these metals in vegetables should not exceed levels that reflect good agriculture practices. Farmers should be educated on the problems associated with excessive usage of fertilizers and other chemicals, as well as irrigating the vegetables with waste water and the need to grow vegetables with safe levels of heavy metals. The data generated must be used as baseline wastewater quality framework to serve as a basis for monitoring irrigation water quality in urban areas of Rewa to ensure safety.
- The high HQ of Pb suggested that the consumption of Spinach grown in waste water irrigated site–II (Naubasta Cement Plant) is not free of risks. Responsible agencies should carry out public health education within the consumption area to sensitive the general public on the potential effects of indiscriminate disposal of waste and the potential health hazards associated with the consumption of vegetables cultivated with wastewater. Measures must be taken to reduce heavy metal pollution and nutrient loading of irrigation water and soils to protect the safety of both farmers and consumers.

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