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Accumulation of heavy Metals in Soil and Green Leafy Vegetables, Irrigated with Wastewater

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Abstract: *The present study was carried out to assess the level of Cd, Pb, Zn, Cu, Cr, and Ni in soil and edible portion of green leafy vegetables, grown in soil irrigated with wastewater near to katihar town and impact on health of residents of the study area. Results revealed that concentration of Pb, Zn, Cu, and Ni in some agricultural fields and Cd in all fields exceeded the normal soil value but lower than critical soil value. The accumulation of Cd, Pb, Zn, Cu, Cr, and Ni varied from 0.48-1.51, 2.5-25.5, 60.3-134.5, 5.15-18.7, 1.45-6.22 and 3.5-9.2 mg kg-^{1}respectively, in leafy shoot of the vegetable plants, which were higher than root of corresponding test vegetable plant. The soil–plant transfer factor (TF) decreased in the following order- $TF_{Zn} > TF_{Cd} > TF_{Cu} > TF_{Ni} > TF_{Pb} > TF_{Cr}$. Transfer factor more than 0.50 was obtained for Zn, Cd and Pb. The daily intake of heavy metals was found to be lower than the provisional tolerable daily intake (PTDIs) set by the Joint FAO/WHO Expert Committee on food additives and minimal risk levels (MIR) value suggested by AQTSDR. The average value of human health risk index (HRI) for different metals was found in following order Pb (0.726) > Cd (0.327) > Ni (0.1083) > Cu (0.094504) > Zn (0.0652) > Cr (0.00798).The data revealed that HRI value of all individual vegetables were below than 1.0 except Pb (1.689) for mustard. Therefore, it is suggested that regular monitoring of heavy metals in vegetables is essential to prevent excessive build –up of heavy metals in the food chain and further use of wastewater for irrigation should be avoided.*

Keywords*:* *Daily intake of metals, Health risk index, Heavy metals, Soil, Transfer Factor, Vegetables*

I. Introdutction

The soil is a store-house of plant nutrients and reservoir of water for plant nutrition. The main function of the soil is to produce the vegetation that stores the solar energy through photosynthesis. Besides, the production of vegetation by the producers, function of soil is to act as medium for biodegradation of detritus by the decomposers. The micro-organism like bacteria, algae, fungi, protozoa etc. live in or on the soil to decompose the detritus and recycle the nutrients released through decomposition.

Anthropogenic activities add the toxic metal and their compound to land. The use of sewage and wastewater to agricultural land can increase the burden of heavy metals to the soil of adjacent area. Wilson and Pyatt (2007) reported that toxic metal in soil may cause from bedrock itself. Many researchers have shown that sewage water and wastewater is used as potential source of irrigation for raising vegetables and fodder crop around the sewage and wastewater treatment sites which are directly or indirectly consumed by human beings [2, 3, and 4]. Raw sewage effluents are not only rich sources of organic and inorganic nutrients for plant growth but also they elevate the level of heavy metals like Fe, Mn, Hg, Pb, Cr, Ni, Cd and Co in receiving soils. The composition of domestic sewage may vary with type of industrial discharge and their wastes [4, 5]. Most of the heavy metals are essential for normal growth of plant since they are constituents of many enzymes and other proteins, but their elevated concentration in soil of both essential and nonessential heavy metals lead to toxicity symptoms and the inhabitation of growth of most plants [6, 7 ,8, 9]. Once the metals are release to atmosphere they can travel for long distance and are deposited on to soil vegetation and water [10, 11]. Many studies have shown that disposal of these industrial and domestic effluents are major problem is often used for irrigation purpose [12, 13]. If crops irrigated by this wastewater can accumulate certain heavy metals and other dangerous materials which are transferred to food chain can cause serious health hazard to human beings and animals (14, 15]. Prorogated use of wastewater, heavy metals get accumulated in soil and plants and the absorbed minerals settle in edible tissue of the vegetables [16].Thus heavy metals are potentially toxic for plants. Broos et al., [17] and Dan et al., [18] reported that phytotoxicity causes chlorosis, weak plant growth, reduced nutrient uptake, disorders in plant metabolism and reduced ability to N_2 fixation in leguminous plant. After accumulating of certain heavy metals in soil, induce deficiency of other nutrients e.g., Cu, Mn, Fe inhibit plant uptake of Zn, decreased in microbial community [19, 20], decreases organic matter mineralization [21] leaf litter decomposition [22] and N_2 fixation [23]. Thus these toxic metals not only inhibit root growth but can also hamper physiological processes including uptake of nutrients. The absorption of heavy metals from the soil depends on different factor such as pH, organic matter, soil metal availability, cation exchange capacity, plant species plant growth stages and season and presence of other heavy metals in soil [24]. Some metals such as Mn, Cu, Zn, Co, Mo and Fe are essential for human life in appropriate concentration as they catalyze for enzymatic activities in human body, but in excess they become poisonous. They get magnified with rising tropic level and ultimately get accumulated in human beings where they cause chronic and acute ailments and even death [25, 26, and 27]. Some of these heavy metals form complexes with carboxylic (-COOH), amino (-NH_2), imino (>NH) and thiol (-SH) groups present in the proteins and they disturb the activity of the proteins to catalyze the function of enzymes [28]. The new biological complex molecules thus formed lose their function which result in break down or cell damage [28, 29]. Three heavy metals of greatest health concern are Cd, Pb and Hg. There is no biological need of any of them. The increase in environmental pollution caused by toxic metals is of great concern because of their carcinogenic properties, their non-biodegradability and bio-accumulation [30].

In the rural areas of Katihar block vegetables grown on a commercial scale in wastewater irrigated areas, but there is no information on the level of heavy metals in the soil and vegetables produced in these areas. Therefore, our main objective has been to study and asses the accumulation level of heavy metals in soil and vegetables and their impact on human health.

II. Materials And Methods

2.1. Site description

Katihar is located at 25.53° N 87.58° E. It has an average elevation of 20 metres. The present study was carried out in five agricultural fields around Katihar town (in between 3 and 13 km from city center) Bihar India, during August 2014 - March 205 (Table 1). Agricaltural fields such as Sarif Ganj and Sirsa are located near the brick kiln industries and Sarif Ganj, Sirsa and Hajipur are also close to 81 NH. Ganga (southern boundary, 25 kilometers from Katihar Town), Kosi (western boundary, 30 kilometers from Katihar Town) and Mahananda are the main rivers and Kari Kosi (flowing by the side of Katihar town), Kamla etc. are small rivers of the district.

2.2. Soil sampling and analysis

Soil samples were collected from five agricultural fields' viz., Sarif Ganj (F1), Sirsa (F2), Sirnia East (F3), Daheria (F4) and Hajipur (F5) near Katihar town (Fig. 1). Each field was first subdivided into five parts (four corner and one centre) and then all the collected soil samples were mixed together to form composite soil sample from each field. Similarly all the five samples were collected and transferred into air tight polyethylene bags and then brought into the laboratory.

The soil samples were dried at 40 ^{0}C for 48 h and ground into fine powder to pass 2 mm nylon sieve. After this 12 gm of powder soil sample was taken in conical flask and then 12 ml 1M HNO_3 and distilled water was added to it, and then made to stand for 24 h. After this, distilled water was added to it, to make the mixture 150 gm by weight. The whole mixture was subject to centrifugation and then filtered by What man filter paper No.1. Finally the filtrate was subjected to Atomic Absorption Spectroscopy for analysis of heavy metals.

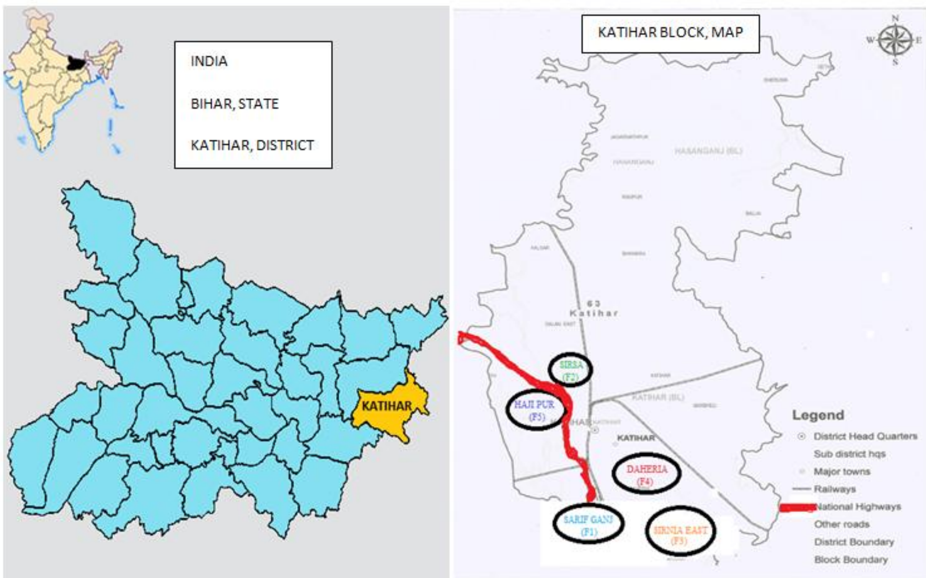


Fig. 1 Geographical locations of study area of 5 agricultural fields (F1, F2, F3, F4 & F5) in Katihar Block

2.3. Plant sampling and analysis

The vegetables selected for heavy metal analysis were mustard (*Brassica compestrits),* coriander (*Coriandrum sativum),* spinach (*Spinacia oleraceae* L), bathua (*Chenopodium album* L) and cabbage (*Barssica oleracea* L) were collected from agricultural fields irrigated with wastewater. These are common vegetables which are repeatedly consumed by the peoples of this area. All the collected vegetables were washed with tap water to remove the soil particles and then root and leafy shoot stalks of each sample were separated and both of these portions were sliced into pieces and dried separately on sheet of paper to eliminate excess of moisture. Air dried samples were directly dried on oven at 75 ^{0}C for 24 h and then crushed and sieved at room temperature.One gm of each plant sample was mixed with 5 ml of concentrated HNO_3 and made to stand for 24 h. After this 12 ml of HNO_3 and $HClO_4$ in the ratio 3:1 was mixed and digested on hot plate till white radish brown fumes of $HClO_4$ comes out .When plant sample was dissolved completely then digested mixture was evaporated until about 5 ml was left in the flask and then subjected for heavy metals analysis by using Atomic Absorption Spectrophotometer.

2.4. Determination of Transfer Factor (TF)

The transfer factor was calculated by dividing the concentration of heavy metal in vegetables (mg kg^{-1}) by the total heavy metal concentration in the soil (mg kg^{-1}) by using following formula [31],

$$\text{Transfer Factor (TF)} = \frac{C\ plant}{C\ soil} \qquad (1)$$

Where, C_{plant} = metal concentration in plant mg kg^{-1} fresh weigh, C_{soil} = metal concentration in soil mg kg^{-1} dry weigh.

2.5. Estimation of daily intake of heavy metals (DIM)

The daily intake of heavy metals by people through consumption of vegetables was calculated by the using following formula [32],

$$\text{DIM } (\mu g\ day^{-1}) = \frac{Cmetal\ \times D\ food\ intake\ \times C\ factor}{B\ average\ weight} \qquad (2)$$

C_{metal} = Heavy metal concentration in plant (mg kg^{-1}), $D_{food\ intake}$ = Daily intake of vegetable (gm day^{-1} person^{-1}), $B_{average\ weight}$ = Average body weight (kg), C_{factor} = Conversion factor (0.085) [33].

The average body weight was considered to be 58 kg by conducting survey of 100 adult (male and female) people from study areas in each period of sampling [34]. The average daily vegetable in take for adult was considered to be 245 gm day^{-1} person $^{-1}$ which was determined by formal interview conducted with people of study areas.

2.6. Health risk index (HRI)

To assess the human health risk (HRI) of heavy metals, it is necessary to know their level of human exposure to that metal by knowing the route of exposure of pollutant to human body. The value of HRI depends upon the daily intake of metals, and reference dose which was calculated by using following [35],

$$\text{HRI} = \frac{DIM}{RfD} \qquad (3)$$

Where, DIM = Daily intake of heavy metals (µg day^{-1}), Rf_D -Reference dose, Rf_D value for Cr, Ni, Cu, Pb, Cd, and Zn is 1.5, 0.02, 0.04, 0.004, 0.001 and 0.30 (mg/kg bw /day) respectively [36],

Table 1 Green leafy vegetable samples collected from agricultural fields irrigated with wastewater

S.N	Common name	Vernacular name	Botanical name	Agricultural field	Distance from city
1	Mustard	Tori	*Brassica compestrits*	F1-F5	Sarif Ganj(F1) - 8KM
2	Coriander	Dhania	*Coriandrum sativum*	F1-F5	Sirsa (F2)- 6 KM
3	Spinach	Palak	*Spinacia oleraceae* L	F1-F5	Sirnia Eest (F3)- 8KM
4	White goose foot	Bathua	*Chenopodium album* L	F1-F5	Daheria(F4) - 7KM
5	Cabbage	Bundha govi	*Barssica oleracea* L	F1-F5	Hajipur(F5)- 3KM

F1-Sarif Ganj, F2 –Sirsa, F3-Sirnia East, F4-Daheriya, F5- Hajipur

III. Results And Discussion

Basic analytical data for accumulation of heavy metals in soil, leafy shoots and roots of different type of green leafy vegetables irrigated with wastewater are presented in Table 2, 3 and 4, in term of minimum, maximum, mean, median, sum of the values (Σn) sum of square values (Σn2), population standard deviation (σn), sample standard deviation (σ_{n-1}) and variation. The levels of metals in particular vegetable are shown in Figure 2, 3, 4, 5, 6 and 7.

3.1 Accumulation of heavy metals in soil Samples

Results revealed that concentration of Pb, Zn, Cu and Ni in some agricultural fields and Cd in all fields exceeded the normal soil value as recommended by Bowen 1997 [37], but lower than the critical value as proposed by Kabita-Pendas *et al.,* [38] and maximum levels (ML) of metals in soil as guided by Ewers *et al.,* [39]. The levels of Pb varied from 29.8-37.8 mg kg^{-1} and slightly higher than the normal soil value (35 mg kg^{-1}), at field F2 (35.5 mg kg^{-1}), at field F4 (36.4 mg kg^{-1}) and at field F5 (37.8 mg kg^{-1}) and lower than the critical soil value (Fig. 2). The accumulation of Zn ranged from 78.2-110.3 mg kg^{-1}.The concentration of Zn was also found to be higher at same three fields at F2 (110.3 mg kg^{-1}), at F4 (108.6 mg kg^{-1}) and F5 (102.7 mg kg^{-1}) than the normal soil value (90 mg kg^{-1}) and lower than the critical soil value and maximum levels (ML) (300 mg kg^{-1}) (Fig. 2).The soil Cu and Ni ranged from 17.8- 40.3 mg kg^{-1} and 10.37-51.45 mg kg^{-1} respectively (Table 2). The Higher concentration of Cu was recorded at three soil samples collected from Sirnia East (32.3 mg kg^{-1}), at Sirsa (34.5 mg kg^{-1}) and Daheria (40.3 mg kg^{-1}),whereas accumulation of Ni was slightly more than normal soil value (50 mg kg^{-1}) at only one field of Daheria (51.45 mg kg^{-1}) (Fig. 2). .The concentration of Cr varied from 10.38-35.25 mg kg^{-1}, all of which were lower than the normal soil value (70.0 mg kg^{-1}). The maximum value of Cr was recorded at Sarif Ganj (35.25 mg kg^{-1}) and lowest value was at Sirsa (10.38 mg kg^{-1}).The concentration of Cd was recorded from 1.35-1.82 mg kg^{-1}, which were maximum than the normal soil value (0.35 mg $kg-_1$) at all fields and below than 8 mg kg^{-1} as recommended by Pendas *et al* 1984 and 3 mg kg^{-1} as guided by Ewers et al., 1991. Figure 2 shows that agricultural fields at Sirsa (F2) Daheria (F4) and Hajipur (F5) near Katihar town were moderately enhanced with Cu, Cr and Ni but strongly enriched with Cd, Pb and Zn may be due to anthropogenic contributions. The order of accumulation of metals in soil samples was Zn > Ni > Cu > Pb > Cr > Cd .

Results also showed that concentration of Pb, Cu Cr and Ni in the soil of agricultural field of Hajipur (F5) (37.8 mg $kg-^1$Pb), Sirsa ((34.5 mg $kg-^1$Cu), Sirnia East (19.75 mg $kg-^1$Cr), and Daheria (51.45 mg kg^{-1} Ni), respectively, were high but accumulation in vegetables were low (Fig. 2). This may be due to soil factors such as pH, organic matter and clay contents etc. Various reports [40, 41, and 42] have shown that increased organic matter and increased clay particles [43] can form bond with metal ions and become less available to plants. Besides this concentration of Zn at agricultural fields like Sinia East (F3), Daheria (F4) and Hajipur (F5), was low but accumulation in various vegetables (mustard, coriander and spinach) was high (Fig. 2,3,4). This may be due to easy transportation of Zn from roots into leafy parts of the plant. On the survey of study area, it was found that continuous irrigation of agricultural land with sewage and wastewater may be possible reasons of heavy metal accumulation in the soil and vegetables .The pesticides, agrochemical and chemical fertilizers used in the agricultural field are also known to be major sources of heavy metal pollution in study areas.

3.2 Accumulation of heavy metals in leafy shoot and root of vegetable plants

Copper: The concentration of Cu in the leafy shoot of vegetable plant samples ranged from 5.15-18.7 mg kg^{-1} with mean value 9.6428 mg $kg-^1$. In all vegetable plant samples accumulation of Cu in the leafy part was slightly more than the normal plant value 5 -15 mg kg^{-1}.The maximum accumulation of Cu was found to be 18.7 mg kg^{-1} in the leafy shoot of mustard from Hajipur area at field F5, whereas minimum value (5.15mg kg^{-1}) was found in the leafy shoot of cabbage from Sarif Ganj at field F1.The order of accumulation of Cu in leafy shoot of different vegetable plant samples was mustard > coriander > spinach > bathua > cabbage. Alam *et al.,* [44] reported that mean Cu accumulation in leafy and non leafy vegetables was 15.5 and 8.51 mg kg^{-1}respectively, which is more than the present observations. The accumulation of Cu range in our study (5.15-18.7 mg $kg-^1$) was also lower than accumulation of Cu (22.19-76.50 mg kg^{-1}) in the leafy vegetables reported by Demirezen and Alsoy, 2006 [45].

In the root all collected vegetable plant samples the concentration of Cu was recorded 3.35-10.54 mg $k-^1$ (Table 4) with mean value 5.89 mg k^{-1}, which was under the range of normal plant value. The accumulation of Cu in the root of mustard, coriander, spinach, bathua and cabbage was found to be 5.01-10.54 mg kg^{-1}, 3.8 - 8.8 mg kg^{-1}, 3.8-9.1 mg kg^{-1}, 3.38-6.5 mg kg^{-1} and 3.35 -6.11 mg kg^{-1} respectively, which were lower than leafy shoot of corresponding vegetable plants. Within the same environment different vegetables have different accumulation power of Cu. The may be due to differences in ligands at the boundary sites of each vegetable plant. High pH, lime, organic matter and phosphate may reduce uptake of metals [46].

Yang *et al.,[*47] , showed that response of three of vegetable plant to Cu toxicity and reported that Cu levels in both root and shoot increased, but root Cu concentration increased more sharply than shoot with increasing Cu levels in the growth media. Copper mainly accumulated in roots while certain fraction of absorbed Cu was transferred to shoot. The concentration of copper in the shoots was significantly influenced by Cu concentration in soil Cu concentration was also reported by Xiong and Wang [48].

Lead: In the analyzed vegetable plant samples Pb levels ranged 2.5-25.5 mg kg^{-1} in the leafy shoot with mean value 8.092 mg kg^{-1}.The concentration of Pb ranged from 12.5-25.5 mg kg^{-1} in mustard, 3.4-9.1 mg kg^{-1} in coriander, 2.5-8.8 mg kg^{-1} in spinach, 3.7-6.1 mg kg^{-1} in Bathua and 3.5-6.1 mg kg^{-1} in cabbage with mean value of 18.82 mg kg^{-1}, 5.92 mg kg^{-1}, 6.06 mg kg^{-1}, 4.84 mg kg^{-1} and 4.81 mg kg^{-1}, while in roots of the vegetable plants the levels of Pb ranged from 1.2-11.7 mg kg^{-1} with mean value 4.306.The maximum value 11.7 mg kg^{-1} was recorded in mustard from Daheria area at field F4 and minimum value 1.2 mg kg^{-1} was recorded in coriander from Sirnia East at field F3. Maximum Pb limit for human cnsumption has been established for edible part of the crop in the China is 0.2 mg kg^{-1}[49], but its limit by WHO standard is 0.3 mg kg^{-1} [50] and normal plant value 0.1-10 mg kg^{-1} range as recommended by Aloway, [51]. Results showed that in all vegetable plant samples Pb was more than permitted level, so that they are not suitable for consumption.

Table 2 Basic statistics of accumulation of heavy metals in the soil irrigated with wastewater

Vegetable/ Statistical data	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Cr (mg kg^{-1})	Ni (mg kg^{-1})
Min.	1.35	29.8	78.2	17.8	10.38	10.37
Max.	1.82	37.8	110.3	40.3	35.25	51.45
Mean	1.69	34.3	96.46	29.14	21. 28	27.15
Median	1.35	29.8	82.5	20.8	19.75	10.37
Σ_n	8.44	171.51	482.3	145.7	106.42	135.75
Σ_n^2	14.398	5926.7	47428.8	4607.11	2636.59	4802.22
σ_{n-1}	0.194	3.301	15.05	9.51	9.638	16.71
σ_n	0.174	2.953	13.46	8.50	8.62	14.94
Variation	0.038	10.896	226.5	90.44	92.89	289.22

Table 3 Basic statistics of accumulation of heavy metals in the leafy shoots of vegetable irrigated with wastewater

Vegetable/ Statistical data	Cd (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Cr (mg kg^{-1})	Ni (mg kg^{-1})
Mustard						
Min.	0.64	12.5	75.7	8.42	4.12	5.8
Max.	1.51	25.5	125.3	18.7	6.22	9.2
Mean	1.12	18.82	107.08	12.59	5.19	7.52
Median	0.64	20.2	110.9	10.42	5.35	7.3
Σ_n	5.6	94.1	535.41	62.97	25.95	37.6
Σ_n^2	6.942	1872.99	58790.8	878.21	137.66	290.06
σ_{n-1}	0.409	5.050	19.09	4.61	0.864	1.352
σ_n	0.366	4.517	17.076	4.127	0.7723	1.209
Variation	0.167	25.50	364.4	21.289	0.746	1.827
Coriander						
Min.	0.60	3.4	80.5	6.8	2.52	4.7
Max.	1.50	9.1	134.5	16.5	4.63	8.2
Mean	1.032	5.92	110.4	10.74	3.484	6.38
Median	0.60	3.4	110.5	8.2	2.52	6.2
Σ_n	5.16	29.6	552.0	53.7	17.42	31.9
Σ_n^2	6.057	193.66	63006.48	645.67	63.113	210.97
σ_{n-1}	0.428	2.146	22.725	4.151	0.778	1.365
σ	0.382	1.919	20.32	3.713	0.696	1.221
Variation	0.183	4.605	516.42	17.23	0.605	1.863
Spinach						
Min.	0.6	2.5	70.2	6.62	1.85	4.1
Max.	1.48	8.8	120.8	15.7	3.75	7.2
Mean	0.922	6.06	95.54	10.16	2.76	5.48
Median	0.67	2.5	95.6	7.8	1.85	5.1
Σ_n	4.61	30.3	477.7	50.82	13.82	27.4
Σ_n^2	4.875	211.27	47372.65	574.64	40.09	157.16
σ_{n-1}	0.395	2.629	20.816	3.811	0.6879	1.324
σ_n	0.353	2.352	18.618	3.409	0.6153	1.184
Variation	0.156	6.911	433.30	14.523	0.473	1.753
Bathua						
Min.	0.50	3.7	65.5	5.5	2.3	3.6
Max.	1.2	6.1	110.3	12.03	4.7	6.8
Mean	0.768	4.84	89.94	8.66	3.10	5.5
Median	0.59	4.5	91.2	8.70	2.3	4.7
Σ_n	3.84	24.2	449.87	43.33	15.52	27.5
Σ_n^2	3.46	121.6	41978.63	398.67	52.230	158.67
σ_{n-1}	0.313	1.0573	19.6	2.406	1.007	1.362
σ_n	0.279	0.9457	17.507	2.153	0.9007	1.212
Variation	0.097	1.118	384.16	5.789	1.014	1.855

Cabbage						
Min.	0.48	3.5	60.3	5.15	1.45	3.5
Max.	1.01	6.1	109.3	11.05	3.02	6.8
Mean	0.718	4.82	87.52	8.234	2.202	5.3
Median	0.61	4.6	90.3	8.52	1.45	6.8
Σ_n	3.59	24.1	437.6	41.17	11.01	26.5
Σ_n^2	2.82	120.75	40059.96	358.91	25.565	147.11
σ_{n-1}	0.244	1.0709	20.983	2.2316	0.5747	1.290
σ_n	0.219	0.9579	18.768	1.996	0.514	1.154
Variation	0.059	1.147	440.28	4.98	0.33	1.612

Table 4 Basic statistics of accumulation of heavy metals in the roots of vegetable parts irrigated with wastewater

Vegetable/	Cd	Pb	Zn	Cu	Cr	Ni
Statistical data	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})
Mustard						
Min.	0.56	6.3	40.2	5.01	2.5	3.7
Max.	1.46	11.7	78.7	10.54	3.5	5.2
Mean	1.028	9.3	61.24	7.33	2.92	4.56
Median	0.56	9.7	61.2	6.65	3.1	4.2
Σ_n	5.14	46.5	306.2	36.65	14.6	22.8
Σ_n2	5.943	453.17	19622.8	293.59	43.24	105.66
σ_{n-1}	0.406	2.276	14.757	2.497	0.3898	0.6504
σ_n	0.363	2.036	13..20	2.234	0.3487	0.5817
Variation	0.165	5.18	217.76	6.235	0.152	0.423
Coriander						
Min.	0.58	1.2	41.2	3.8	1.35	2.4
Max	1.47	5.0	70.2	8.8	2.38	4.5
Mean	0.996	3.302	55.82	6.56	1.914	3.39
Median	0.58	1.2	56.1	7.9	1.35	3.8
Σ_n	4.98	16.51	279.1	32.8	9.57	16.96
Σ_n^2	5.676	64.79	16199.97	235.38	18.87	60.073
σ_{n-1}	0.423	1.603	12.456	2.248	0.3718	0.798
σ_n	0.378	1.433	11.14	2.011	0.3325	0.7133
Variation	0.179	2.567	155.15	5.053	0.138	0.637
Spinach						
Min.	0.51	1.9	30.8	3.8	1.01	2.0
Max.	0.92	5.8	54.2	9.1	2.1	2.9
Mean	0.678	3.56	46.72	5.98	1.71	2.43
Median	0.53	1.9	50.1	3.8	1.01	2.12
Σ_n	3.39	17.8	233.6	29.9	8.56	12.13
Σ_n^2	2.464	76.54	11280.22	199.91	15.4426	30.55
σ_{n-1}	0.204	1.815	9.571	2.297	0.4438	0.5308
σ_n	0.182	1.623	8.561	2.055	0.397	0.475
Variation	0.042	3.294	91.60	5.278	0.197	0.281
Bathua						
Min.	0.43	1.6	28.2	3.48	1.35	1.81
Max.	0.90	3.8	55.8	6.5	2.3	3.49
Mean	0.634	2.84	42.94	5.12	1.792	2.83
Median	0.50	2.8	46.7	5.5	1.35	2.41
Σ_n	3.17	14.2	214.7	25.58	8.96	14.15
Σ_n^2	2.215	43.34	9838.27	136.22	16.56	42.14
σ_{n-1}	0.226	0.8677	12.44	1.157	0.355	0.7308
σ_n	0.202	0.7761	11.127	1.0347	0.3175	0.6537
Variation	0.52	0.753	154.75	1.339	0.126	0.0.534
Cabbage						
Min.	0.41	1.5	30.3	3.35	0.9	1.75
Max.	0.87	4.1	62.59	6.11	7.4	3.48
Mean	0.61	2.78	48.98	4.76	2.71	2.68
Median	0.45	2.7	45.1	5.01	0.9	3.48
Σ_n	3.05	13.9	244.89	23.8	13.55	13.41
Σ_n^2	2.049	42.35	12589.5	117.52	64.85	37.738
σ_{n-1}	0.217	0.9628	12.199	1.029	2.652	0.666
σ_n	0.194	0.8611	10.912	0.9204	2.372	0.5954
Variation	0.047	0.927	148.81	1.058	7.033	0.443

Table 5 Normal and critical value of heavy metal (mg kg^{-1}) accumulated by soil and plant

Biotopes	Cd	Pb	Zn	Cu	Cr	Ni
NSV (Brown, 1979)	0.35	35	90	30	70	50
UCSV (K-Pendias *et al.*, (1984)	8	400	400	125	100	100
NPV (Alloway, 1968)	0.1-2.4	0.1-10	20-400^{B}	5-15	0.2-10	1-2.7
CPV (Pendias *et al.*, (1992)	5-30	20-300	100-400	20-100	5-30	10-100
ML (Ewera, 1991)	3	100	300	100	100	50
WHO	0.2	0.3	50	10	1.3	10

NSV- Normal soil value as proposed by Browen (1979), UCSV-Upper critical soil value as proposed by Kabita-Pendias and Pendias (1984), NPV- Normal plant value as proposed by Aloway (1968), CPV- critical plsant value as proposed by Kabita-Pendias and Pendias (1992), Ml –Maximum Allowance value as guided by Ewera (1991). WHO- Hassan et al., 2012

Pb is immobile in soil, there was high lead accumulation in both leafy shoot and root of vegetable plants for study area of Katihar Block accumulate Pb through root uptake and atmospheric fall out. Lead is toxic element and can be harmful to plants. In many plants, Pb accumulation can exceed several hundred times the threshold of maximum level permissible for human consumption [52]. The introduction of lead in to the food chain may affect human health. Therefore, all vegetable plant samples that were analyzed in this study were more contaminated by Pb and they were harmful for the consumers.

Zinc: The concentration of Zn in the leafy shoot of vegetable plant samples was recorded 60.3-134.5 mg kg^{-1} with mean value 98.096 mg kg^{-1},wheras in the root varied from 28.2 -78.2 mg kg^{-1} with mean value 51.14 mg kg^{-1}.The maximum Zn limit for human health has been established for edible part of crop is 20 mg kg^{-1} by recommended CDPM [53], but WHO permitted the maximum level of Zn in vegetables to be 50 mg kg^{-1} and Bowen (1979) proposed normal plant value to be 20-400 mg kg^{-1}. Results showed that there is not any pollution in mustard leaves collected from Sarif Gnj, in coriander rleaves collected from Sirinia East and Sirsa respectively, in spinach, bathua

and cabbage vegetables collected from Sarif Ganj, Sirsa and Sirinia East. Hyper-accumulation (higher than soil value) of Zn was observed in mustard and Coriander vegetables collected from Sirnia West, Deheria and Hajipur and spinach collected from only Daheria and Hajipur. The easy transportation from the roots into the aerial parts may be possible cause for hyper-accumulation of Zn in the leafy shoot of vegetable plants [54].

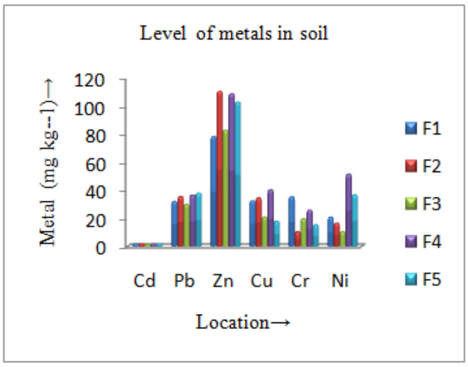


Figure 2 Level of metal in soil

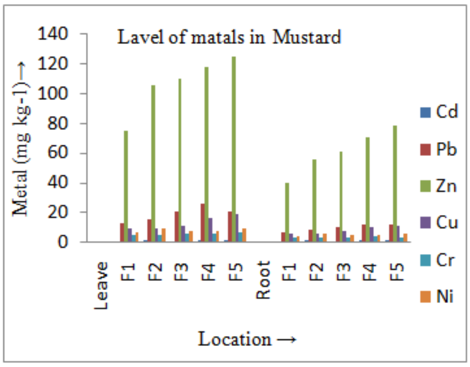


Figure 3 Level of metal in mustard

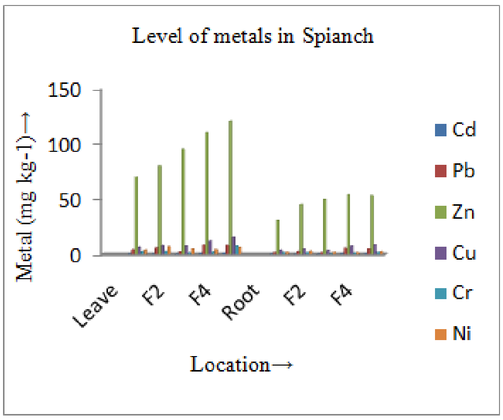


Figure 4 Level of metal in spinach

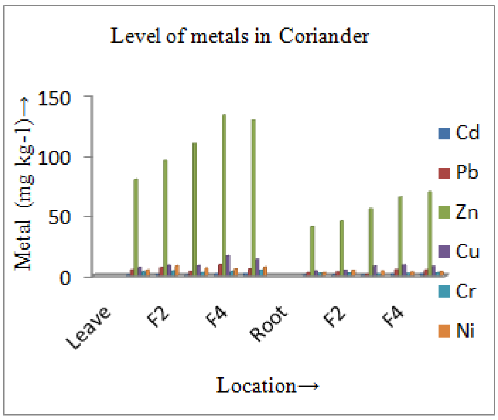


Figure 5 Level of metal in coriander

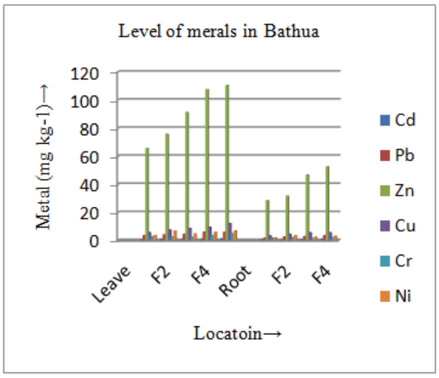


Figure 6 Level of metal in bathua

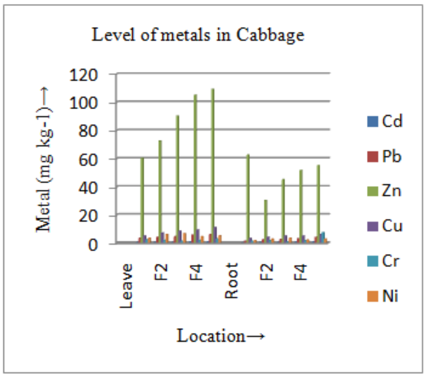


Figure7 Level of metal in cabbage

Zinc has been considered to be an important trace element that play vital role in the physiological and metabolic process of animal and plant organisms. It also plays an important role in protein synthesis. [55]. Zn toxicity isminimal in humans, but higher concentration can be toxic to the organism. Several researchers reported that Zn interferes with Cu metabolism. The symptoms of acute oral Zn dose are tachycardia, dyspeptic nausea, vascular shock, diarrhea, vomiting, and damage of heptic parenchyma [56]. Thus vegetables growing in soil having heavy metals contamination can accumulate high concentration of Zn to cause of serious health risk to consumers.

Nickel: Ni is one of the essential trace elements for human and plant health [57]. The mean level of Ni in the leafy part was 6.03 mg kg^{-1} and in the root part of all vegetable plant samples was 3.178 mg kg^{-1}, which was higher than normal plant value (0.1- 2.7 mg kg^{-1}) but lower than critical value (10-100 mg kg^{-1}) and WHO standard (10 mg kg^{-1}). The maximum concentration of Ni (9.2 mg kg^{-1}) was recorded in mustard from Hajipur agricultural field at F5 and minimum value was recorded from Sarif Gang at field F1. Figure showed that accumulation of Ni in vegetable plant samples was as follow mustard> coriander >spinach >bathua> cabbage.

Cadmium: The concentration of Cd in leafly part of vegetable plant samples varied from 0.48-1.51 mg kg^{-1}, whereas in root part varied from 0.41- 1.47 mg kg^{-1}. In all the collected samples concentration of Cd was higher than WHO standard (0.2 mg kg^{-1}) and under limit of normal plant value (0.1-2.4 mg kg^{-1}) but lower than critical plant value. By this way accumulation of Cd in vegetables was in the order of mustard > coriander > spinach > bathua> cabbage. Similar trend was observed by Giordano and Mays [58], Fazeeli, [59], Torabian and Mahjouri [60], Doyle [61]. Mahjouii [60] also reported that accumulation of Cd in plant irrigated with wastewater in south Tehran is in the following order of ranking Mint > coriander > Radish >spinach.

Most of leafy (aerial part) of vegetable shows higher Cd accumulation compare to root (ground part). Our result show agreement with previous studies [59 ,61, 62] it has been found that Cd is a highly mobile metal and can be easily absorbed by the plant through root surface and moves to wood tissue and transfers to upper parts of the plant. Therefore there is direct relation between the levels of presence of Cd in the root zone and its absorption by plant is also supported by Gardinar et al., [63] and Ramos et al., [64].

Chromium: The concentration of Cr in leafy part of the vegetable plant varied from 1.45 mg kg^{-1} at Sirnia East to 6.22 mg kg^{-1} at Hajipur, with mean value 3.35 mg kg^{-1}, while in root from 0.9 mg kg^{-1} at Sirnia East to 3.5 mg kg^{-1} at Deheria, with mean value 2.21 mg kg^{-1}. The maximum value of Cr was recorded in mustard at Hajipur (F5), whereas lowest value was recorded in cabbage at Sirnia East (F3).Cr accumulation in all vegetables was found within the normal plant value (0.2 -10.0 mg kg^{-1}) and lower than critical plant value The present finding were lower than concentration reported by Gupta *et al.*, [65] in west Bengal, India (34.83-96.30 mg kg^{-1} and also lower than 5.35-27.83 mg kh^{-1} as reported by Sharma *et al.*, [66] in Varanasi. The possible reason for low concentration of Cr in plant is due lower chromate uptake which occurs only in hexavalent and readily reduced to immobile trivalent from soil [46].

3.3Transfer Factor of heavy metals from soils to vegetables (TF)

The mobility of metals from soil to plant is a function of bioavaility of metal, which in turn depends upon its concentration in the soil their chemical forms, difference in uptake capacity and growth rate of different plant species [68]. Soil to plant transfer is a parameter used to describe the transfer of trace elements from soil to plant body so that soil to plant transfer is one of the key components of human exposure to metals through food chain. The higher value of metals in leafy parts may be due to higher transpiration rate to maintain the growth and moisture content of the plant [69]. The transfer of the heavy metal from soil to vegetables is presented in Table 6. The transfer values ranges were: Cd 0.425-0.663, Pb 0.141-0.548, Zn -0.907-1.14, Cu 0.282-0.432, Cr 0.092-0.237 and Ni 0.195-0.277, . The soil–plant transfer factor of different heavy metals shows the following order- $TF_{Zn} > TF_{Cd} > TF_{Cu} > TF_{Ni} > TF_{Pb} > TF_{Cr}$ (Fig.8)

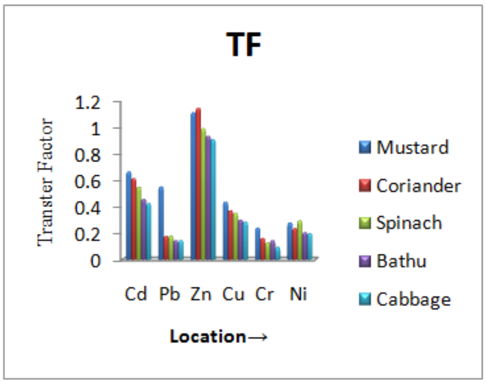
The similar trend was also reported by various researchers. The highest transfer factor value was recorded 1.14 0.663 and 0.548 for Zn, Cd and Pb respectively. The higher TF value of Zn, Cd and Pb in the study area indicates the plant will have a greater chance of metal contamination by anthropogenic activities [69].

The TF for different heavy metals in particular vegetable were in order

Mustard- $TF_{Zn} > TF_{Cd} > TF_{Pb} > TF_{Cu} > TF_{Ni} > TF_{Cr}$
Coriander- $TF_{Zn} > TF_{Cd} > TFCu > TF_{NI} > TFPb > TF_{Cr}$
Spinach - $TF_{Zn} > TF_{Cd} > TFCu > TF_{Ni} > TFPb > TF_{Cr}$
Bathua- $TF_{Zn} > TF_{Cd} > TFCu > TF_{Ni} > TFPb > TF_{Cr}$
Cabbge $TF_{Zn} > TF_{Cd} > TFCu > TF_{Ni} > TFPb > TF_{Cr}$

Table 6 Transfer factor of heavy metals from soils to vegetables irrigated by wastewater

Vegetable	Cd	Pb	Zn	Cu	Cr	Ni
Mustard	0.663	0.548	1.11	0.432	0.237	0.277
Coriander	0.611	0.173	1.14	0.368	0.159	0.235
Spinach	0.545	0.177	0.990	0.349	0.126	0.202
Bathua	0.454	0.141	0.932	0.297	0.142	0.203
Cabbage	0.425	0.142	0.907	0.282	0.092	0.195
Min	0.425	0.141	0.907	0.282	0.092	0.195
Max	0.663	0.548	1.14	0.432	0.237	0.277
Mean	0.540	0.236	1.01	0.346	0.151	0.222



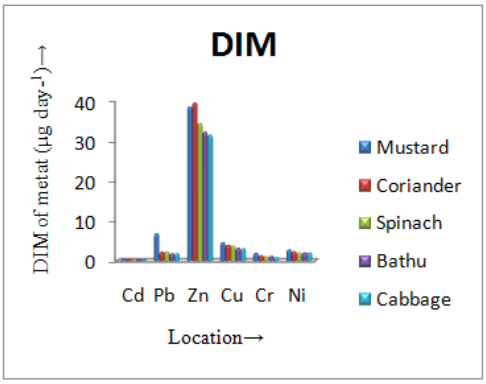


Figure 8, TF of heavy metals from soils to vegetables **Figure 9,** Daily intake of heavy metals

3.4 Daily intake of heavy metals (DIM)

The food chain is the most important pathway to estimate the level of metals exposure and to observe the health risk of any pollutant. The DMI values of different metals based on the consumption of vegetables grown in soil irrigated with wastewater is present in Table 7. The result revealed that average daily intake of Cd, Pb, Zn, Cu, Cr and Ni by adults in vegetables grown in soil irrigated with wastewater was found to be 0257-0.402, 1.73-6.757, 31.398-39.495, 2.956- 4.520, 0.770-1.863 and 1.902-2.699 µm day^{-1} with mean value 0.327, 2.905, 19.582, 3.618, 1.198 and 2.167 µg day^{-1} respectively. The average DIM was in order of: $DIM_{Zn} > DIM_{Cu} > DIM_{Pb} > DIM_{Ni} > DIM_{Cr} > DIM_{Cd}$ (Fig.9). The results show agreement with previous studies showing levels of heavy metals in edible part of, food crops with continuous wastewater [70,71] the results also show that the present finding were lower compare to 54 µm day^{-1} and 412 µm day^{-1} of Pb in adult as reported by Debeca et al., [72] and Dick et al., [73] also lower than 21.6,858.6, 426.6 and 3.7 mg k day-1 for Cd, Cu, Pb and Zn respectively as reported by Bahemuka and Mubofu [74]. Therefore it can be suggested that the consumption of average amount of these contaminated vegetables do not pose health for consumers as the obtained value are lower than the provisional tolerable daily intake set by Joint FAO/WHO expert Committee on Food Additives

The daily intake of heavy metals through different vegetable plants were in the following order-

Mustard $DIM_{Zn} > DIM_{Pb} > DIM_{Cu} > DIM_{Ni} > DIM_{Cr} > DIM_{Cd}$
Corinder $DIM_{Zn} > DIM_{Cu} > DIM_{Ni} > DIM_{Pb} > DIM_{Cr} > DIM_{Cd}$
Spinach- $DIM_{Zn} > DIM_{Cu} > DIM_{Pb} > DIM_{Ni} > DIM_{Cr} > DIM_{Cd}$
Bathua $DIM_{Zn} > DIM_{Cu} > DIM_{Pb} > DIM_{Ni} > DIM_{Cr} > DIM_{Cd}$
Cabbage- $DIM_{Zn} > DIM_{Cu} > DIM_{Ni} > DIM_{Pb} > DIM_{Cr} > DIM_{Cd}$

Table 7 Daily intake of metals (µg day^{-1}) for heavy metals in vegetables irrigated by wastewater

Vegetable	Cd	Pb	Zn	Cu	Cr	Ni
Mustard	0.402	6.757	38.447	4.520	1.863	2.699
Coriander	0.370	2.125	39.495	3.856	1.251	2.291
Spinach	0.330	2.175	34.303	3.647	0.991	1.967
Bathua	0.275	1.737	32.208	3.109	1.113	1.974
Cabbge	0.257	1.730	31.398	2.956	0.770	1.902
Mean	0.327	2.905	19.582	3.618	1.198	2.167
PIDI	60	214	60 mg day^{-1}	3 mg day^{-1}	------	-------
MRI	30	214	1800	600	300	720

MRL: Minimal Risk Levels stated as µg /day for a 60 kg person
MRL: Sources: ATSDR 2013, FAO/WHO, 2010(Pb) and WHO, 2005 (Ni)

3.5 Human health Risk Index

The human health risk index calculated for heavy metals by consumption of vegetables for adults are given in Table 8. The HRI for Cd, Pb, Zn, Cu, Cr, and Ni ranged from 0.257-0.402, 0432-1.699, 0.1046 0.1316, 0.074-0.113, 0.00066-0.001242 and 0.0951-0.1349 with mean value 0.3268, 0.726, 0652, 0.0945, 0.000798 and 0.1083as shown in Table 8. The data revealed that HRI for all individual vegetables were lower than for 1.0.for Cd, Pb, Zn, Cu, Cr, and Ni except Pb for mustard (1.689). The mean HRI values for consumers showed the following order Pb (0.726) > Cd (0.327) > Ni (0.1083) > Cu (0.094504) > Zn (0.0652) > Cr (0.00798). Our results show the agreement with those reported by Khan et al., [76], Jan et al., [77] and [78] and lower than those reported by Gupta et al., [79] Σ=6.25) and Wang et al., [80]. The HRI of the study area suggest that all vegetables grown in agricultural field irrigated by wastewater are almost safe for consumer except mustard which regard to Pb (HRI= 1.929).

Table 8 Human health risk index for heavy metals in vegetables irrigated by wastewater

Vegetable	Cd	Pb	Zn	Cu	Cr	Ni
Mustard	0.402	1.689	0.1281	0.1130	0.001242	0.1349
Coriander	0.370	0.531	0.1316	0.0964	0.000834	0.1145
Spinach	0.330	0.543	0.1143	0.0911	0.000660	0.0983
Bathua	0.275	0.434	0.1074	0.0777	0.000742	0.0987
Cabbage	0.257	0.432	0.1046	0.0739	0.005153	0.0951
Min	0.257	0.432	0.1046	0.0739	0.000660	0.0951
Max	0.402	1.689	0.1316	0.1130	0.001242	0.1349
Mean	0.327	0.726	0.0652	0.0945	0.0007986	0.1083(Σ=1.213)

IV. Conclusion

From the present research work it may be concluded that heavy metals such as Cd Pb, Zn, Cu, Cr, and Ni in all the vegetable plant samples vary with the vegetable. The level of Pb, Cu, Cr, and Ni in the soil samples of certain agricultural fields at F5, F2, F3, and F4 respectively were high but accumulation in vegetable were low, Result also revealed that the concentration of Zn at F3, F4 and F5 was low but accumulation in mustard coriander and spinach was high. This may be due to differences in ligands at the binding sites of each vegetable and also due to soil factors. The soil-plant transfer factor (TF) of different heavy metal shows following order Zn > Cd > Cu > Ni > Pb > Cr. The high TF of Zn was found to be mustard (1.11) and coriander (1.14). The level of Pb Cu and Ni in leafy part of the vegetables are not still safe for consumption .However daily intake of metals suggests that the consumption of average amounts of these polluted vegetables does not pose a health risk for the residents of the study area. The human health risk index indicated that the vegetables grown in soil irrigated with wastewater are free from any risk, except mustard with regard to Pb for which HRI value is greater than 1 (1.929), Finally, We conclude that regular monitoring of levels of these metals from wastewater, in soil, in vegetables and other food materials is essential to prevent excessive build-up of heavy metals in the food chain.

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