Uptake, Accumulation and Translocation of Heavy Metals in Cauliflower Grown in Integrated Industrial Effluent Irrigated Soil in District Haridwar (Uttarakhand)

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Abstract The combined effluents from various industries (integrated industrial effluent; IIE) in some locations are used as irrigation water to grow cauliflower because it is readily available and low cost. Contents of cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) in soil, roots, leaves and inflorescence of *B. oleracea* cultivated with the IIE irrigated water at Bhagtanpur Village, Ikkarkala Village and Shubrasha Village were higher than the control site (Bongla Village), where irrigation was with well water. The bioaccumulation factor (BCF) and translocation factors (TF) of metals in *B. oleracea* were in Bhagtanpur village due to higher concentration of metals in the effluent. Use of IIE for irrigation of *B. oleracea* increased concentrations of heavy metals in plant parts which may pose a potential threat to human health due to consumption, and to deterioration of soil fertility due to the long-term irrigation practices.

Keywords *Brassica oleracea* var. *botrytis* · Bioaccumulation factor · Enrichment factor · Integrated industrial effluent · Translocation efficiency

1 Introduction

As urban areas in developing countries increase, and people seek better living standards, larger volumes of freshwater are diverted to domestic, commercial, and industrial sectors, which produce greater volumes of wastewater [1–3]. Increasing demand of water for agricultural irrigation has increased use of treated, or untreated, wastewater [4–7]. The State Infrastructure and Industrial Development Corporation of Uttarakhand Limited occupies an area of about 823.13 ha having nearly 700 independent industrial units which are involved in cosmetics, plastic, apparel, agro-food, pharmaceutical products, electronic products, packaging, synthetic fabrics, electroplating, and commercial automotive activities [8, 9]. The areas contain heterogeneous

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industries with varying pollution loads and concentrations but the industries lack individual effluent treatment systems. The industries use a large quantity of fresh water and generate huge quantities of effluent. The industrial effluents are partially treated in a common effluent treatment plant and most of the partially treated effluent is disposed through various channels near the treatment plant. Unprocessed wastewater flows through channels into rivers where it is diverted by subsistence farmers to small vegetable plots grown for nearby municipal markets [10–12].

There are public health risks of using adulterated streams for irrigation [10]. Disposal of wastewater is a major problem for industries, due to generation of high volume of effluent, and limited space, for land-based treatment and disposal [11, 13–15]. Wastewater contains nutrients that can be used for cultivation of agricultural crops [15–17]. Irrigation with waste water contributes to heavy metal contents of soil and crops [15].

Trace elements play roles in chemical, biological, metabolic, and enzymatic responses in living cells of plants and humans [16]. Large deposits of heavy metals in agricultural soils through wastewater irrigation may result in soil contamination and affect food quality and safety [17–19]. Vegetables plants take up heavy metals and accumulate them in tissues in quantities high enough to cause clinical problems to animals and human beings that consume them [15, 20].

Heavy metal pollution in agriculture can come from atmospheric fall-out, pesticides, and contamination by chemical fertilizers and irrigation water [20]. Heavy metals rank high among toxins of leafy vegetables [21]. Uptake of trace elements by plants varies, and depends on soil pH, soil organic matter content and other factors [22, 23]. Plant uptake is a major route of food chain exposure to trace elements from the soil.

Effluents from industries contain measurable amounts of nitrogen (N), phosphorous (P), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), lead (Pb), nickel (Ni), and cadmium (Cd), and their disposal causes contamination of soil and water [21, 22, 24, 25]. Pollutants from industry include suspended solids, compounds colored by lignin, dissolved inorganic salts, chlorinated lignins, and phenolic derivatives. Discharge of untreated effluent can create pollution resulting in deterioration of water quality and toxicity to aquatic life [26–28].

Generally, farmers and consumers do not have information about vegetable physiology and morphology; they only consider undamaged, dark green, big, leaves as characteristics of good quality [29, 30]. External appearance of vegetables can not guarantee safety from contamination especially when farming activities are carried out using industrial effluents for irrigation [31–33]. Growers frequently use industrial effluents due to scarcity of clean irrigation water and to save chemical fertilizer cost as the effluents contain nutrients and toxins [15, 32, 34]. Irrigation with effluents can cause deterioration in soil fertility. Cauliflower (*Brassica oleracea* L. var. *botrytis* L.) has been reported to accumulate heavy metals [34–36], with particular affinity for Cu, Cd, Cr, Pb and Zn.

It may be possible to remediate contaminated agricultural soils using vegetables because they grow rapidly, take up high contents of minerals and metals, and translocate them in their aerial plant parts [32, 37–39], which may make them not safe to eat.

In several countries, and in India, vegetables are irrigated with different types of wastewater, generally from a single source of industrial effluent. The general results are that use of wastewater could expose the consumer to hazards. The State Infrastructure and Industrial Development Corporation of Uttarakhand Limited produced IIE supplies the whole district of Haridwar at low cost. Cauliflower is grown in a large area in the region and is irrigated using IIE, but little is known of the effects on plant development or whether the crop is fit for consumption. The study was conducted to evaluate uptake, accumulation and translocation of heavy metals in cauliflower grown in soil irrigated with IIE and to determine if the plants were safe for human consumption.

2 Materials and Methods

2.1 Study Area

Sampling was collected from Bhagtanpur Village (29°53′36.39″N, 78°4′27.64″E), Ikkarkala Village (29°52′38.85″N, 78°4′36.91″E), and Shubrasha Village (29°52′17.36″N, 78°4′26.57″E) where irrigation was with the IIE. Cauliflower is cultivated in these locations using IIE effluents. In Bongla Village, the control site (29°51′57.81″N, 78°4′41.00″E), bore well water was used for irrigation. The soils of treatment and control sites are sandy loam and slightly alkaline, pH 7.8. Cultivation practices at all sites were similar. Irrigation was twice weekly at all sites.

2.2 Collection of Samples and Analysis

The IIE samples were collected in plastic bottles, immediately acidified with HNO_3, and transported to the laboratory. Samples were filtered through Whatman No. 42 filter paper and stored in a refrigerator at 4 °C. Soil samples from each site were composited separately for analysis. Samples of harvest ready cauliflower were collected from different sites. At each sampling inflorescence, leaves, roots, and soils surrounding roots were collected from 3 or 4 plots of treatment and control sites. Plant samples from all sites were washed with double distilled water and plants separated by hand into different parts which were dried at room temperature for 24 h. Tissues were separately chopped and placed in a hot air oven at 60 °C for 48 h to dry. Samples were ground into powder using a mortar and pestle and stored in plastic bags for further use.

2.3 Analysis of Heavy Metals

For analysis of heavy metals, plant, soil and water samples were digested [17]. A 0.5 g of powdered samples of plant and soil, and a 10 mL sample of water, were placed in a digestion tube and 10 mL of nitric acid (HNO_3) and 5 mL of perchloric acid ($HClO_4$) added and digestions completed on digestion blocks (FOSS, Mumbai, India) following standard procedures [21]. After digestion samples were filtered through Whatman No. 42 filter paper and volume made up to 50 mL. The Cd, Cu, Cr, Fe, Mn and Zn contents in the digested aliquot were determined with an atomic absorption spectrophotometer (model 5000, Perkin-Elmer, GenTech Scientific Inc., Arcade, NY).

2.4 Estimation of Enrichment Factor, Contamination Factor and Bio-concentration Factor

Enrichment factor (EF) of different heavy metals was calculated following the formula of Kim and Kim [27], and used to assess the degree of contamination of heavy metals in the soil. The contamination factor (Cf) of heavy metals in the soil was determined by the method of Håkanson [22]. The bio-concentration factor (BCF) was used to describe the transfer of trace elements from soil to plant tissues. The BCF is calculated as the ratio among the concentration of heavy metals in the plant tissue and in the corresponding soil all based on dry weight for each vegetable [35]. The translocation index was used to determine the ability of plants to translocate heavy metals from roots to harvestable aerial plant parts.

2.5 Statistical Analysis

The data was statistically analyzed using multi-way analysis of variance (ANOVA) to determine the significant difference in the characteristics of IIE effluent irrigated soil and cauliflower crops at different sampling sites using MS Excel 2013.

3 Results and Discussion

The physico-chemical and microbiological components of the IIE and well water varied (Tables 1 and 2). The IIE was had a high pH due to concentrations of alkalis used in the manufacturing processes. The BOD, COD, Ca, TKN, Cd, Fe, Mn, MPN and SPC of the IIE were above recommended limits of the Indian Irrigation Standards [17]. Higher BOD and COD of the IIE were due to existence of high oxidizable

Table 1 Physico-chemical, heavy metals and microbiological characteristics of State Infrastructure and Industrial Development Corporation of Uttarakhand limited effluent and bore well water

Parameter	Water source		BIS[a] for irrigation water
	Effluent	Bore well	
EC ($dc \cdot cm^{-1}$)	2.12 ± 0.15	0.49 ± 0.08	– [b]
TDS ($mg \cdot L^{-1}$)	814.38 ± 143.66	154.16 ± 12.29	1900
pH	8.44 ± 0.44	7.15 ± 0.28	5.5–9.0
DO ($mg \cdot L^{-1}$)	ND[c]	7.27 ± 0.25	–
BOD ($mg \cdot L^{-1}$)	231.89 ± 21.48	3.13 ± 0.16	100
COD ($mg \cdot L^{-1}$)	716 ± 29.92	4.82 ± 0.69	250
Ca ($mg \cdot L^{-1}$)	485 ± 38.87	21.80 ± 2.82	200
Mg ($mg \cdot L^{-1}$)	145.89 ± 9.42	8.71 ± 0.76	–
Na ($mg \cdot L^{-1}$)	103.64 ± 6.01	6.41 ± 0.66	–
K ($mg \cdot L^{-1}$)	60.15 ± 5.75	4.03 ± 0.95	–
TKN[d] ($mg \cdot L^{-1}$)	136.55 ± 10.93	15.02 ± 1.12	100
PO_4^{3-} ($mg \cdot L^{-1}$)	166.49 ± 9.63	0.05 ± 0.01	–
Cd ($mg \cdot L^{-1}$)	3.14 ± 0.27	0.08 ± 0.01	2.00
Cr ($mg \cdot L^{-1}$)	1.75 ± 0.37	0.05 ± 0.01	2.00
Cu ($mg \cdot L^{-1}$)	1.70 ± 0.05	0.08 ± 0.01	3.00
Fe ($mg \cdot L^{-1}$)	3.04 ± 0.07	0.19 ± 0.04	1.00
Mn ($mg \cdot L^{-1}$)	1.32 ± 0.12	0.12 ± 0.03	1.00
Zn ($mg \cdot L^{-1}$)	0.70 ± 0.09	0.32 ± 0.02	15
SPC[f] ($SPC \cdot mL^{-1}$)	86×10^6	NA[e]	10,000
MPN[g] (MPN/100 mL)	27×10^6	NA	5,000

[a]BIS = Bureau of Indian Standards
[b]– = Not given in standard
[c]ND = Not detectable
[d]TKN = Total Kjeldahl Nitrogen
[e]NA = Not available
[f]PC = Standard plate count
[g]MPN = Most probable number

organic matter and rapid consumption of dissolved inorganic materials. The higher bacterial load (SPC and MPN) in IIE was due to presence of more dissolved solids and organic matter including TS, TDS, TSS, EC, BOD, COD, Cl^-, Na^+, K^+, Ca^{2+}, Mg^{2+}, TKN, PO_4^{3-} and SO_4^{2-} [35]. The IIE effluent contained Cd, Cr, Cu, Fe, Mn and Zn [25]. The IIE contained high levels of chemicals that can be plant nutrients as well as heavy metals, which may enhance yield of agricultural crops while still causing them to be contaminated. The ANOVA data presented in Tables 2 and 3 indicated that IIE was significantly enriched with Cd, Cr, Cu, Fe, Mn and Zn in comparison to control (Bore well water). There was no significant effect of location on content of metals in IIE at different sites except Fe (Tables 2 and 3).

Table 2 ANOVA for effect of location, metal, water type and their interactions on heavy metal concentrations in water

Source	Cd	Cr	Cu	Fe	Mn	Zn
Location (L)	ns	ns	ns	ns	ns	ns
Metal (M)	**	**	**	**	**	**
Water type (W)	*	*	*	*	*	*
Interaction						
L × M	ns	ns	ns	ns	ns	ns
L × W	ns	ns	ns	*	ns	*
M × W	*	*	*	**	*	*
L × M × W	ns	ns	ns	*	ns	ns

ns, *, ** not significant or significant at $P \leq 0.05$ or $P \leq 0.01$, ANOVA

Table 3 Interaction of location and water type on concentrations of heavy metals in water

	Concentration (mg L^{-1})[a]						
Location ×	Water type	Cd	Cr	Cu	Fe	Mn	Zn
Bhagtanpur	IEE	3.02[c]	1.68[b]	1.62[b]	2.95[b]	1.17[b]	0.68[b]
Ikkarkala		2.99[b]	1.68[b]	1.62[b]	2.52[b]	1.17[b]	0.60[b]
Shubrasha		2.86[b]	1.73[c]	1.62[b]	3.68[c]	1.32[c]	0.66[b]
Bongla (Control)	Well	0.08[a]	0.05[a]	0.08[a]	0.19	0.12[a]	0.32[a]

[a]Bureau of Indian standards for inland disposal of treated water: Cd (2.0), Cr (1.5), Cu (1.0), Fe (15.0), Mn (2.0) and Zn (10) mg L^{-1}. Data in the interaction analyzed with Least Squares Means and means separated with LSD
[b]values in a group, in the column, followed by ns, *, ** and *** are not different or significantly different at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$

Concentrations of Cd, Cr, Cu, Fe, Mn and Zn in IIE, and bore well, water at sampling sites varied (Tables 1, 2, 3 and 4). Contents of heavy metals in the IIE were higher for Cu, Fe, Mn and Zn than in bore well water. Contents of Cr and Cd were below detection limits in the bore well water. Concentrations of heavy metals in the IIE were above recommended values [35, 36]. The concentrations in the IIE were higher (except Cr) than values reported by Kumar and Thakur [31] for Cd (2.74–2.84 mg·L^{-1}), Cr (1.61–1.65 mg·L^{-1}), Cu (1.42–1.56 mg·L^{-1}), Fe (2.04–2.34 mg·L^{-1}) Mn (1.02–1.08 mg·L^{-1}) and Zn (0.42–0.49 mg·L^{-1}) at sites near the SIDCUL used for irrigation. Values of heavy metals reported here were higher than previous values since the number of industries in operation in SIDCUL has increased and the amount of materials in the effluent has increased. There were increases in Cd, Cr, Cu, Fe, Mn and Zn contents in soil at all sampling sites irrigated with IIE (Tables 5, 6, 7 and 8). The highest Fe content in the IIE irrigated soil was in Shubrasha village followed by Ikkarkala and Bhagtanpur villages (Tables 5, 6, 7, 8 and 9). The increase in Fe content was likely due to Fe content in the water, or soil, at those sites. The ANOVA data presented in Tables 6, 7 and 8 indicated that location have no

Table 4 ANOVA for effect of location, metal, water type, and their interactions on metals found in soil

Source	Cd	Cr	Cu	Fe	Mn	Zn
Location (L)	ns	ns	ns	ns	ns	ns
Metal (M)	**	**	**	**	**	**
Water type (W)	*	*	*	*	*	*
Interaction						
L × M	ns	ns	ns	ns	ns	ns
L × W	**	**	**	*	**	**
M × W	ns	ns	ns	*	ns	ns
L × M × W	ns	ns	ns	*	ns	ns

ns, *, ** not significant or significant at $P \leq 0.05$ or $P \leq 0.01$, ANOVA

Table 5 ANOVA for effect of metal, water type, and their interactions on enrichment of metals in soil

Source	Cd	Cr	Cu	Fe	Mn	Zn
Location (L)	ns	ns	ns	ns	ns	ns
Metal (M)	**	**	**	**	**	**
Water type (W)	*	*	*	*	*	*
Interaction						
L × M × W	*	ns	ns	ns	*	*

ns, *, ** not significant or significant at $P \leq 0.05$ or $P \leq 0.01$, ANOVA

significant effect on Cd, Cr, Cu, Fe, Mn and Zn contents of the soil at all the sampling sites. IIE irrigation have significant effect on the percent increase in the contents of Cd, Cr, Cu, Fe, Mn and Zn in the soil at all the three sampling sites viz., Bhagtanpur, Ikkarkala and Shubrasha villages in comparison to Bongla (Control site) (Table 9). The enrichment factor of Cd, Cr, Cu, Fe, Mn and Zn in the soil was also increased due to the IIE irrigation at all the sampling sites (Tables 6, 7 and 8).

4 Enrichment Factor

The enrichment factor of different heavy metals was varied. The enrichment factor of heavy metals in soils due to IIE irrigation was in the order: Fe > Zn > Mn > Cu > Cd > Cr for Bhagtanpur village; Fe > Mn > Zn > Cu > Cd > Cr for Ikkarkala Village and Fe > Mn > Zn > Cd > Cu > Cr for Shubrasha village. The EF was greatest for Fe and least for Cr in Shubrasha village. The contamination categories established by Sutherland [40] indicated that the IIE irrigated soil was in the significant enrichment category for Fe, Zn and Mn, while Cu, Cd and Cr were in the moderate enrichment

Table 6 Concentration of interaction of metal in soil and location on metal concentration and enrichment factor

Metal ×	Location	Concentration ($\mu g \cdot g^{-1}$)	Enrichment factor
Cd[a]	Bhagtanpur	23.95[b]	2.6[c]
	Ikkarkala	25.16[a]	2.8[b]
	Shubrasha	27.26[a]	3.0[a]
	Bongla (control)	9.06[c]	– [c]
Cr	Bhagtanpur	15.71[a]	2.4[a]
	Ikkarkala	16.62[a]	2.6[a]
	Shubrasha	14.43[a]	2.2[a]
	Bongla (control)	6.47[b]	–
Cu	Bhagtanpur	40.58[a]	2.9[a]
	Ikkarkala	40.60[a]	2.9[a]
	Shubrasha	34.93[b]	2.5[a]
	Bongla (control)	13.82[c]	–
Fe	Bhagtanpur	391.87[c]	7.3[a]
	Ikkarkala	415.68[b]	7.8[a]
	Shubrasha	425.65[a]	8.0[a]
	Bongla (control)	53.10[c]	–
Mn	Bhagtanpur	50.03[b]	5.0[b]
	Ikkarkala	53.41[b]	5.3[b]
	Shubrasha	55.61[a]	5.6[a]
	Bongla (control)	9.95[c]	–
Zn	Bhagtanpur	43.15[a]	6.4[a]
	Ikkarkala	34.68[b]	5.2[b]
	Shubrasha	36.05[b]	5.4[b]
	Bongla (control)	6.72[c]	–

Data in the interaction analyzed with Least Squares Means and means separated with LSD

[a]Safe Limit of India (Awashthi, 2000): Cd (3–6 $\mu g \cdot g^{-1}$), Cu (135–200 $\mu g \cdot g^{-1}$), Fe (75–150 $\mu g \cdot g^{-1}$), Zn (300–600 $\mu g \cdot g^{-1}$)

[b]values in a group, in columns, followed by the same letter are not significantly different, $P \leq 0.05$

[c]"–" = Not determined since the values from the control were used as the base line

category (Tables 6). The interaction of location, metals and water type indicated the significant increase in the contents of Cd, Cr, Cu, Fe, Mn and Zn in the IIE irrigated soil in different villages (Tables 7 and 8). There were positive correlations between Cr and Cu, Fe and Cd, Zn and Mn, Cd and Cr, Cd and Zn, Cr and Fe, Mn and Cu in the soil and IIE (Table 9). Therefore, the IIE irrigation increased Cd, Cr, Cu, Fe, Mn and Zn contents in soils in those villages.

Table 7 ANOVA for effect of location, metal, water type, and their interactions on percent increase of metals in soil

Source	Cd	Cr	Cu	Fe	Mn	Zn
Location (L)	ns	ns	ns	ns	ns	ns
Metal (M)	**	**	**	**	**	**
Water type (W)	*	*	*	*	*	*
Interaction						
L × M × W	*	*	*	*	*	*

ns, *, ** not significant or significant at $P \leq 0.05$ or $P \leq 0.01$, ANOVA

Table 8 Percent increase in metals in soil as affected by interaction of metal and location

Metal ×	Location	Percent increase in soil
Cd	Bhagtanpur	264.35b[a]
	Ikkarkala	277.70[a]
	Shubrasha	159.27[c]
Cr	Bhagtanpur	242.81[b]
	Ikkarkala	256.87[a]
	Shubrasha	223.03[c]
Cu	Bhagtanpur	293.63[a]
	Ikkarkala	293.78[a]
	Shubrasha	152.78[b]
Fe	Bhagtanpur	737.98[c]
	Ikkarkala	782.82[b]
	Shubrasha	801.60[a]
Mn	Bhagtanpur	502.81[c]
	Ikkarkala	536.78[b]
	Shubrasha	558.89[a]
Zn	Bhagtanpur	642.11[a]
	Ikkarkala	516.07[b]
	Shubrasha	336.46[c]

Data in the interaction analyzed with Least Squares Means and means separated with LSD
[a]values in a group, in columns, followed by the same letter are not significantly different, $P \leq 0.05$

There are concerns about accumulation of heavy metals present in IIE used for irrigation, and consequently their transference to plants and their eventual entrance into the food chain. The contents of heavy metals in edible and non-edible parts of cauliflower (*B. oleracea*) grown in the IIE and bore well water irrigated soil varied (Tables 10, 11, 12 and 13). The highest concentration of Fe was in cauliflower roots and the lowest concentration of Mn was in the inflorescence. Levels of Fe in

Table 9 Coefficient correlation (r) of heavy metals in soil at locations

	Cd	Cr	Cu	Fe	Mn
Bhagtanpur					
Cr	−0.61				
Cu	−0.14	0.22			
Fe	−0.14	0.18	0.99		
Mn	−0.13	0.85	−0.03	−0.07	
Zn	−0.40	−0.24	0.63	0.65	−0.69
Ikkarkala					
Cr	0.12				
Cu	0.74	0.27			
Fe	−0.17	−1.00	−0.27		
Mn	0.15	−0.95	−0.15	0.93	
Zn	0.14	0.89	−0.04	−0.91	−0.78
Shubrasha					
Cr	0.23				
Cu	0.43	0.84			
Fe	−0.53	−0.07	−0.59		
Mn	−1.00	−0.25	−0.46	0.57	
Zn	−0.05	−0.90	−0.91	0.32	0.08
Bongla (control)					
Cr	0.47				
Cu	0.61	−0.31			
Fe	−0.89	−0.01	−0.84		
Mn	−0.82	0.02	−0.66	0.95	
Zn	−0.72	−0.66	−0.44	0.46	0.22

Table 10 ANOVA for effect of location, metal and tissue on heavy metals found in plant tissue

Source	Cd	Cr	Cu	Fe	Mn	Zn
Location (L)	ns	ns	ns	ns	ns	ns
Metal (M)	ns	ns	ns	ns	ns	ns
Tissue (T)	ns	ns	ns	ns	ns	ns
Interaction						
L × M	ns	ns	ns	ns	ns	ns
L × T	ns	ns	ns	ns	ns	ns
M × T	ns	ns	ns	ns	ns	ns
L × M × T	ns	ns	ns	ns	ns	ns

ns, *, ** not significant or significant at $P \leq 0.05$ or $P \leq 0.01$, ANOVA

Table 11 ANOVA for effect of location, water type and translocation factor of metal found in the entire plant

Source	Cd	Cr	Cu	Fe	Mn	Zn
Location (L)	ns	ns	ns	ns	ns	ns
Metal (M)	ns	ns	ns	ns	ns	ns
Entire plant (E)	ns	ns	ns	ns	ns	ns
Interaction						
L × M	ns	ns	ns	ns	ns	ns
L × E	ns	ns	ns	ns	ns	ns
M × E	ns	ns	ns	ns	ns	ns
L × M × E	ns	ns	ns	ns	ns	ns

ns, *, ** not significant or significant at $P \leq 0.05$ or $P \leq 0.01$, ANOVA

Table 12 ANOVA for effect of metal and organ on bio-concentration factor of metal found in *B. oleracea* plant

Source	Cd	Cr	Cu	Fe	Mn	Zn
Location (L)	ns	ns	ns	ns	ns	ns
Metal (M)	*	*	*	*	*	*
Organ (O)	*	*	*	*	*	*
Interaction						
L × M × O	*	*	*	*	*	*

ns, *, ** not significant or significant at $P \leq 0.05$ or $P \leq 0.01$, ANOVA

Table 13 Bio-concentration factor for heavy metals in *B. oleracea* as affected by interaction of location and organ

Location ×	Organ	Cd	Cr	Cu	Fe	Mn	Zn
Bhagtanpur Village	Root	$0.067b^{a}$	0.157^{b}	0.130^{a}	0.179^{a}	0.124^{a}	0.154^{a}
	Leaves	0.027^{c}	0.064^{c}	0.057^{c}	0.087^{c}	0.034^{b}	0.061^{c}
	Inflorescence	0.077^{a}	0.183^{a}	0.071^{b}	0.114^{b}	0.020^{b}	0.071^{b}
Ikkarkala Village	Root	0.052^{a}	0.156^{a}	0.145^{a}	0.135^{a}	0.112^{a}	0.172^{a}
	Leaves	0.018^{c}	0.055^{c}	0.058^{b}	0.066^{c}	0.026^{c}	0.063^{b}
	Inflorescence	0.043^{b}	0.093^{b}	0.055^{b}	0.094^{b}	0.080^{b}	0.063^{b}
Shubrasha Village	Root	0.051^{a}	0.176^{a}	0.143^{a}	0.134^{a}	0.127^{a}	0.142^{a}
	Leaves	0.018^{c}	0.071^{c}	0.057^{b}	0.044^{c}	0.028^{c}	0.051^{c}
	Inflorescence	0.042^{b}	0.109^{b}	0.064^{b}	0.101^{b}	0.065^{b}	0.075^{b}
Bongla Village (Control)	Root	0.034^{a}	0.102^{a}	0.064^{a}	0.113^{a}	0.102^{a}	0.126^{a}
	Leaves	0.028^{b}	0.077^{b}	0.048^{a}	0.081^{b}	0.054^{b}	0.067^{b}
	Inflorescence	0.026^{b}	0.102^{a}	0.059^{a}	0.074^{c}	0.030^{c}	0.068^{b}

Data in the interaction analyzed with Least Squares Means and means separated with LSD
[a]values in a group, in columns, followed by the same letter are not significantly different, $P \leq 0.05$.

cauliflower were above safe limits for Cd (3–6 $\mu g \cdot g^{-1}$), Cu (135–200 $\mu g \cdot g^{-1}$), Fe (75–150 $\mu g \cdot g^{-1}$), Zn (300–600 $\mu g \cdot g^{-1}$) reported by Awashthi (2000). Contents of Cr, Cd, Cu, Fe, Mn and Zn in the soil increased as the number of effluent irrigation increased. The contamination factor was highest for Fe and lowest for Mn at all sampling sites irrigated with IIE. The contamination factor of heavy metals in cauliflower was on the order: Fe > Zn > Cu > Cd > Cr > Mn in Bhagtanpur and Ikkarkala village due to irrigation with IIE (Tables 10, 11, 12 and 13). The concentration of Cd, Cr, Cu, Fe, Mn and Zn were higher in soil irrigated with effluent than in soil irrigated with well water. Fertigation with IIE increased nutrients as well as metals content in soils used for the cultivation of *B. oleracea*. The ANOVA data indicated that location; metal, tissue and their interaction did not show significant effect on the contents of different metals Cd, Cr, Cu, Fe, Mn and Zn on the tissues of *B. oleracea* (Table 10). The contamination factor of metals in *B. oleracea* inflorescence was ranged for Cd (2.99–3.59), Cr (2.70–3.10), Cu (3.59–3.97), Fe (6.19–6.97), Mn (1.92–3.37) and Zn (5.28–5.84) while the average contamination factor of Cd (3.31), Cr (2.88), Cu (3.76), Fe (6.71), Mn (2.56) and Zn (5.50) in the inflorescence of *B. oleracea* was at these sampling sites due to irrigation of *B. oleracea* with IIE. Similarily, location, water type and translocation factor of different metals in the entire plant of *B. oleracea* did not show significant effect on the contents of different metals Cd, Cr, Cu, Fe, Mn and Zn in the entire plant of *B. oleracea* (Table 11). The translocation factor of different metals in the entire plant of *B. oleracea* was ranged for Cd (34.62–40.63%), Cr (35.52–41.05%), Cu (39.73–43.73%), Fe (32.82–48.77%), Mn (22.14–27.65%) and Zn (34.48–39.40%) while the average translocation factor of Cd (36.90%), Cr (38.91%), Cu (41.18%), Fe (43.40%), Mn (24.39%) and Zn (36.81%) in the entire plant of *B. oleracea* was at these sampling sites after irrigation of *B. oleracea* with IIE.

The average contents of Cd (3.33 $\mu g\ g^{-1}$), Cr (5.52 $\mu g\ g^{-1}$), Cu (10.06 $\mu g\ g^{-1}$), Fe (129.92 $\mu g\ g^{-1}$), Mn (10.98 $\mu g\ g^{-1}$) and Zn (10.80 $\mu g\ g^{-1}$) in entire *B. oleracea* plant at IIE irrigated sites as Bhagtanpur, Ikkarkala and Shubrasha villages in comparison to Cd (1.07 $\mu g\ g^{-1}$), Cr (1.82 $\mu g\ g^{-1}$), Cu (1.70 $\mu g\ g^{-1}$), Fe (19.56 $\mu g\ g^{-1}$), Mn (3.63 $\mu g\ g^{-1}$) and Zn (1.76 $\mu g\ g^{-1}$) in entire plant of *B. oleracea* irrigated with bore well water at Bongla (Control site). The content of Cd, Cr, Cu, Fe, Mn and Zn in different tissues and in the entire plant of *B. oleracea* was varied and it was insignificantly different in IIE irrigated *B. oleracea* (Table 10), while content of Cd, Cr, Cu, Fe, Mn and Zn in *B. oleracea* was significantly different at Bhagtanpur, Ikkarkala and Shubrasha villages in comparison to Bongla village (Control). The translocation factor of Cd, Cr, Cu, Fe, Mn and Zn in different parts of *B. oleracea* were also observed varied in IIE irrigated *B. oleracea* (Table 11).

The average contents of different metals in the various organs as root, leaves and inflorescence of *B. oleracea* were varied and higher at all the IIE irrigated sampling sites (Bhagtanpur, Ikkarkala and Shubrasha village) in comparison to the bore well water irrigated sampling site (Bongla village). The average contents of Cd (1.44 $\mu g\ g^{-1}$), Cr (2.53 $\mu g\ g^{-1}$), Cu (5.38 $\mu g\ g^{-1}$), Fe (60.91 $\mu g\ g^{-1}$), Mn (6.44 $\mu g\ g^{-1}$) and Zn (5.92 $\mu g\ g^{-1}$) in the root, Cd (0.53 $\mu g\ g^{-1}$), Cr (0.98 $\mu g\ g^{-1}$), Cu (2.21 $\mu g\ g^{-1}$), Fe (26.65 $\mu g\ g^{-1}$), Mn (1.57 $\mu g\ g^{-1}$) and Zn (2.21 $\mu g\ g^{-1}$) in the leaves while the contents of Cd (1.36 $\mu g\ g^{-1}$), Cr (2.00 $\mu g\ g^{-1}$), Cu (2.47 $\mu g\ g^{-1}$), Fe (42.35 $\mu g\ g^{-1}$), Mn (2.97 $\mu g\ g^{-1}$) and Zn (2.67 $\mu g\ g^{-1}$) was recorded in the inflorescence of *B. oleracea* at all the IIE irrigated sites. the contents of Cd (0.40 $\mu g\ g^{-1}$), Cr (0.66 $\mu g\ g^{-1}$), Cu (0.88 $\mu g\ g^{-1}$), Fe (8.67 $\mu g\ g^{-1}$), Mn (1.01 $\mu g\ g^{-1}$) and Zn (0.85 $\mu g\ g^{-1}$) in the root, Cd (0.34 $\mu g\ g^{-1}$), Cr (0.50 $\mu g\ g^{-1}$), Cu (0.66 $\mu g\ g^{-1}$), Fe (5.36 $\mu g\ g^{-1}$), Mn (1.33 $\mu g\ g^{-1}$) and Zn (0.45 $\mu g\ g^{-1}$) in the leaves while the contents of Cd (0.33 $\mu g\ g^{-1}$), Cr (0.66 $\mu g\ g^{-1}$), Cu (0.82 $\mu g\ g^{-1}$), Fe (5.53 $\mu g\ g^{-1}$), Mn (1.29 $\mu g\ g^{-1}$) and Zn (0.46 $\mu g\ g^{-1}$) were observed at bore well water irrigated *B. oleracea* site (control).

5 Bio-concentration Factor

The BCFs for transfer of heavy metals from soils to cauliflower varied (Table 12). The BCF for heavy metals due to irrigation with IIE was in the order: Cr > Zn > Cu > Fe > Mn > Cd; for the control, the order was: Fe > Mn > Cr > Zn > Cu > Cd. The BCF values for Mn and Cd in cauliflower plants were comparatively low. The highest BCF values were for Cr, Zn and Fe due to irrigation with IIE (Table 13).

The food chain (soil–plant–human) is recognized as the main route whereby humans are exposed to contaminants in the soil [41]. When the BCF is < 1 or BAF = 1, it indicates the plant absorbs, but does not store heavy metals; when the BCF is >1 the plant accumulates metals. The BCF values of <1 were obtained for Cd, Cr, Cu, Fe, Mn and Zn in cauliflower. The bioavailability of metals was low in the areas of study.

The biological interaction between heavy metals and the plant occurs at the root surface and within cauliflower which ultimately affects uptake and translocation of heavy metals [42]. Accumulation of heavy metals depends on plant age and tissue [43]. Plant species differ in their tolerance and ability to take up and transport Cd within the plant [44]. Differences in heavy metal concentrations in vegetables were due to variations in their ability to absorb and accumulate heavy metals [45, 46]. The soil pH, organic matter content, cation exchange capacity (CEC), redox potential, soil texture, and clay content may affect heavy metal uptake [47]. The BCF indicated that cauliflower contained Cd, Cr, Cu, Fe, Mn and Zn after irrigation with IIE. Regular use of IIE for irrigation of cauliflower may build up levels of metals in soil and affect development and yield of cauliflower.

The translocation index of heavy metals in cauliflower irrigated with IIE, or well water, varied. Concentrations of heavy metals in cauliflower confirmed translocation of metals into different parts of cauliflower (root, leaves and inflorescence) from IIE irrigated soil as is evident from the higher translocation factor (Tf) of Fe followed by Cu and Cr in plants grown in the IIE irrigated soil. Cauliflower efficiently translocates higher contents of Cd, Cr, Cu, Fe, Mn and Zn in to leaves and inflorescence of plants irrigated with IIE. These results agree with Sharma et al. [44, 45] who reported higher heavy metals contents in cauliflower tissues after irrigation with municipal wastewater, and concluded that vegetable crops are capable of taking up, accumulating, and translocating higher concentrations of heavy metals, and can be used for the remediation of contaminated agricultural soils.

6 Conclusions

Those results generally indicated that the plants irrigated with the various wastewaters were not fit for human consumption. Cauliflower accumulated and translocated high contents of heavy metals after irrigation with IIE. The BOD, COD, Ca, TKN, Cd, Fe, Mn, MPN and SPC of IIE were above recommended limits. The accumulation of possibly toxic elements can produce adverse effects on plant morphology, and make them hazardous to eat indicating that use of the IIE for agricultural purposes for consumption of the crop is no better than for the other sources previously tested. Cauliflower takes up heavy metals but the levels do not raise to those which indicate it can be used for bio-remediation.

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