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Fractionation of heavy metals and their uptake by vegetables growing in soils irrigated with sewage effluent

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Abstract: Fractionation of heavy metals helps to determine their binding form, toxicity, and availability in terrestrial environments. Wastewater irrigation may lead to the accumulation of these metals in soil and plants. Wastewater irrigated soils and vegetables were collected from 6 sites in the vicinity of Lahore and analyzed for cadmium, nickel, chromium, zinc, manganese, cobalt, and copper. The quality of wastewater used for growing crops was also determined. In soil, sequential extraction was adopted to demarcate 5 metal fractions: exchangeable, acid-soluble, reducible, oxidizable, and residual, which were quantified by inductively coupled plasma optical emission spectrometry. The accumulations of these metals in vegetables (spinach and bitter gourd) were also assessed and it was found that concentrations of all studied toxic metals in edible parts of the vegetables were above the critical level. The total metal contents in soil were in the order of Mn > Co > Zn > Cr > Ni > Cu > Cd. Correlation analysis between metal concentrations in different fractions of soil and vegetables was performed at 95% and 99% confidence levels. Positive and negative correlations were observed; positive values indicated the bioavailability of these metal fractions to vegetables, while negative values showed that metal concentrations in particular fractions were not bioavailable to plants.

Key words: Heavy metals, vegetables, sewage, plant uptake, bioavailable

1. Introduction

With the increasing global population, the gap between the supply and demand for water is widening and is reaching such an alarming level that in some parts of the world it is posing a threat to human existence. Scientists around the globe are working on new ways of conserving water. The use of wastewater for irrigation is one method of water conservation. Wastewater contains a lot of nutrients that may increase the crop yield without using fertilizers [1–4]. Beside these nutrients, wastewater may also contain heavy metals that are added due to the mixing of industrial effluents into municipal wastes and have a negative impact on receiving soils [5,6]. In developed countries where environmental standards are applied, much of the wastewater is treated prior to use for irrigation while in nondeveloping countries standards are set but they are not strictly adhered to. In Pakistan, wastewater in its untreated form is widely used for agriculture, including vegetables. Repeated application of heavy metal contaminated wastewater can result in accumulation of these toxic metals in the soil and plants [7–9].

Transfer of heavy metals from wastewater to soil and subsequently their uptake and accumulation in edible parts of the vegetative tissue represent a direct pathway of incorporation of heavy metals into the human

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food chain. Distribution of heavy metals in soil is continuously altered due to various soil processes and is under the influence of environmental factors. Knowledge of the total metal concentration provides limited information about their potential mobility and bioavailability [10]. Based on primary accumulation mechanism in soils, heavy metals can be classified into 5 categories: (i) adsorbed and exchangeable, (ii) bound to carbonate phase, (iii) bound to reducible phase (Fe–Mn oxides), (iv) bound to organic matter and sulfides, and (v) detrital or lattice metals [11].

Sequential extraction procedures can provide information about the association of heavy metals with geochemical phases of soils, and hence help to reveal the distribution of heavy metals in different fractions and assess the mobility and toxicity of heavy metals in soils [12,13]. Numerous extraction schemes for soil and sediment have been described in the literature [12,14,15]; they explain that water soluble and exchangeable forms are considered readily mobile and available to plants, while metals incorporated into crystal lattices of clays are relatively inactive and not available to plants under normal conditions. The other forms that precipitated as carbonates, occluded in Fe, Mn, and Al oxides or complexed with organic matter, are considered relatively active or firmly bound, depending upon the actual combination of physical and chemical properties of soil [16,17].

The purpose of the present study was to determine the wastewater quality, total and fractional concentrations of cadmium, nickel, chromium, zinc, manganese, cobalt, and copper in soil by Tessier's procedure [12] of sequential extraction. The metal concentrations in spinach and bitter gourd growing in corresponding soil were also analyzed to evaluate the relationship between the uptake of metals by vegetables and different forms of metals in sewage-irrigated soils.

2. Materials and methods

2.1. Chemicals and apparatus

The chemicals used for the study were of analytical grade and solutions were prepared in deionized water. All glassware was cleaned and soaked in 10% (v/v) HNO_3 before use. Inductively coupled plasma optical emission spectrometry (ICP-OES) (PerkinElmer, Optima DV 5300) was used for the analysis of metals in soil and vegetable extracts. Triplicate analysis was carried out and results were expressed as the mean of triplicate values.

2.2. Wastewater sampling

The wastewater samples were collected from a site where it is directly used for growing vegetables. The grab method was used for sample collection and samples were preserved accordingly [18] and placed in 1-L polyethylene containers. Temperature and pH were measured in the field while other properties were measured in the laboratory.

2.3. Soil sampling

The study was conducted at 6 randomly selected sites irrigated continuously with wastewater from a drain passing through Lahore, Pakistan. All along its route, wastewater generated by human settlements and industrial units is discharged into this drain without any prior treatment. This practice has polluted the drain's water and also affected the aquatic life of the drain and that of the River Ravi, of which it is a tributary. On both sides of the drain, there are agricultural fields where farmers divert the effluent for irrigating vegetables and other crops. Top (0–15 cm) soil samples were collected using a stainless steel soil auger. All the samples were then air dried, mixed, ground with a wooden implement to homogenize them, and sieved through a 2-mm sieve and then stored in labeled polyethylene bags for further analysis.

2.4. Plant sampling

The samples of vegetables (spinach and bitter gourd) and soil were collected during 2010–2011 from the same sites. The plant material was washed with deionized water, then dried, and ground to powder for metal analysis.

2.5. Wastewater analysis

The wastewater was analyzed by using standard methods, described by the American Public Health Association [18]. The sodium adsorption ratio was calculated as described [19].

2.6. Soil analysis

Tessier's sequential extraction procedure [12] was adopted. It consists of the following steps: exchangeable and extractable fraction (1.00 g of soil treated with 1 M $MgCl_2$ for 1 h at 25 °C with agitation), acid soluble fraction (1 M NaOAc of pH 5 for 5 h at 25 °C with continuous agitation), reducible fraction (20 mL 0.04 M $NH_2OH.HCl$ in 25% (v/v) acetic acid for 5 h at 96 °C with occasional agitation), oxidizable fraction (0.02 M HNO_3 and 5 mL of 30% H_2O_2, agitation for 5 h at 85 °C), and residual fraction (HF and $HCLO_4$ added stepwise and residue was dissolved in 12 N HCl).

After each extraction the suspension was centrifuged in polyethylene tubes at 4000 rpm for 30 min [H-1500FR]. The solution was separated, and residue was washed twice after each extraction with deionized water. For total and residual metal analysis the soil was digested by HF and perchloric acid and then solubilized in 12 N HCl.

2.7. Vegetable digestion

The vegetable samples were oven-dried at 60 °C and then ground in an agate mortar. One gram of ground sample was taken in a conical flask and kept overnight after adding 5 mL of conc. HNO_3 and 5 mL of $HClO_4$. The next day, again 5 mL of conc. HNO_3 was added and the resulting mixture was digested on a hot plate until the material was clear. After digestion the material was cooled and reconstituted with deionized water [20]. The concentrations of metal in digests were determined by ICP-OES.

3. Results and discussion

Table 1 represents the quality of wastewater used for growing vegetables in the studied area. The pH and temperature were 7.81 and 26.8 °C and according to National Environmental Quality Standards (NEQS) as described by the Pakistan Environment Protection Act (PEPA) [21] the permissible limits for pH and temperature are 6–10 and 40 °C respectively. The pH and temperature of the analyzed wastewater were within NEQS limits and pH was also within the Food and Agriculture Organization (FAO) guidelines [22] for the quality of irrigation water.

The chemical oxygen demand (COD) and 5-day biochemical oxygen demand (BOD_5) were 476.4 mg/L and 179.0 mg/L, respectively, and were above the NEQS limits, indicating the high organic pollution load of the wastewater. The NEQS limit for sulfide is 1 mg/L while the concentration was 8.12 mg/L, and so a high concentration of sulfide causes an offensive odor in water.

Table 1. Quality of wastewater used for growing vegetables (spinach, bitter gourd).

Parameter	Units	Results	National Environment Quality Standard	FAO Standard
pH	–	7.81	6–10	6.5–8.4
Temperature	°C	26.8	40	n.l.
Chemical oxygen demand	mg/L	476.4	150	n.l.
Biochemical oxygen demand	mg/L	179.0	80	n.l.
Total dissolved solids	mg/L	917.0	3500	n.l.
Total suspended solids	mg/L	92.8	200	n.l.
Sulfide	mg/L	8.12	1.0	n.l.
Sodium adsorption ratio	–	5.44	n.l.	> 9.0
Cadmium	mg/L	0.01	0.1	0.01
Nickel	mg/L	0.42	1.0	0.2
Chromium	mg/L	0.21	1.0	0.1
Zinc	mg/L	0.78	5.0	2.0
Manganese	mg/L	0.17	1.5	0.2
Cobalt	mg/L	0.02	n.l.	0.05
Copper	mg/L	0.37	1.0	0.2

n.l.: not listed

The sodium adsorption ratio (SAR) is a useful index for determining the sodium hazard in water used for irrigation. The critical value for SAR is 9.0 and above this value there is a danger of sodium hazard. The average value of SAR was 5.44, which lies within normal range of the FAO standards.

The concentrations of nickel, chromium, manganese, and copper were above the FAO standard limits, and the concentrations of cadmium, zinc, and cobalt fell within FAO standards. Considering NEQS standards, the metals' concentrations were within the limits. The heavy metal concentrations in untreated wastewater were comparable with the results reported by Rai and Tripathi [23], who investigated metals in industrial wastewater at Lotha village. Gupta et al. [24] also indicated that the concentrations of heavy metals in wastewater varied depending upon the type of industry the effluents came from.

Table 2 represents the total concentration (mg kg^{-1} dry soil) of metals in soil of the sites; they were between 6.30 and 12.70 for Cd, between 36.50 and 51.00 for Ni, between 52.80 and 76.0 for Cr, between 50.0

Table 2. Total concentration (mg kg^{-1}) of metals in sewage-irrigated soils.

Vegetable	Site	Metals (mg kg^{-1})						
		Cd	Ni	Cr	Zn	Mn	Co	Cu
Spinach	S 1	10.9 ± 1.2	41.2 ± 3.2	55.5 ± 3.5	79.5 ± 7.8	216.0 ± 25	83.4 ± 6.5	12.7 ± 1.3
	S 2	12.7 ± 1.3	40.4 ± 2.3	69.2 ± 6.5	50.0 ± 6.3	278.5 ± 32	110 ± 8.6	22.0 ± 2.3
	S 3	9.3 ± 1.3	39.1 ± 3.2	74.3 ± 7.8	132.5 ± 14	376.0 ± 42	117 ± 7.6	36.5 ± 1.5
	S 4	11.7 ± 2.1	48.5 ± 4.3	62.8 ± 6.9	87.3 ± 8.5	460.0 ± 36	88.5 ± 6.8	29.0 ± 2.5
	S 5	8.0 ± 0.89	37.5 ± 2.5	69.7 ± 8.6	105.0 ± 11	251.3 ± 17	103 ± 11	48.5 ± 4.3
	S 6	6.3 ± 0.85	51.0 ± 5.3	76.0 ± 9.2	76.5 ± 7.8	389 ± 54	113 ± 8.5	35.5 ± 3.5
Bitter gourd	S 1	12.3 ± 2.1	43.5 ± 5.2	52.8 ± 7.1	96.0 ± 12	360 ± 33	85.5 ± 12	31.5 ± 4.3
	S 2	10.4 ± 0.87	36.5 ± 3.2	58.5 ± 6.5	76.5 ± 6.3	264 ± 15	96.8 ± 6.7	37.0 ± 3.2
	S 3	7.6 ± 0.68	38.9 ± 2.6	63.5 ± 7.8	108.5 ± 8.7	405 ± 42	108 ± 12	30.5 ± 3.8
	S 4	6.9 ± 0.84	41.5 ± 5.4	69.0 ± 5.6	87.6 ± 6.7	415 ± 38	78.3 ± 9.8	24.6 ± 5.1
	S 5	8.18 ± 1.0	41.9 ± 4.2	71.3 ± 7.5	97.0 ± 10	265 ± 18	98.2 ± 8.6	35.6 ± 4.5
	S 6	7.82 ± 0.94	42.1 ± 5.3	62.0 ± 6.7	81.5 ± 7.3	349 ± 24	101 ± 9.4	38.2 ± 3.6

Table 3. Concentration (mg/kg) of heavy metals in different fractions of soils receiving sewage efflent.

Metals	Site	Spinach						Bitter gourd					
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**
Cd	**F 1**	1.18 ± 0.65	0.84 ± 0.53	0.98 ± 0.65	0.88 ± 0.23	0.7 ± 0.23	0.7 ± 0.54	1.74 ± 0.15	1.40 ± 0.81	1.68 ± 0.36	1.12 ± 0.24	1.32 ± 0.20	0.81 ± 0.06
	F 2	2.53 ± 0.86	2.48 ± 0.68	2.37 ± 0.74	1.57 ± 0.56	1.29 ± 0.89	1.26 ± 0.56	3.62 ± 0.56	2.06 ± 0.59	3.01 ± 1.03	2.13 ± 0.14	2.39 ± 0.56	1.28 ± 0.18
	F 3	0.46 ± 0.14	0.49 ± 0.23	0.53 ± 0.25	0.32 ± 0.23	0.45 ± 0.56	0.36 ± 0.45	0.46 ± 0.15	0.61 ± 0.23	0.59 ± 0.28	0.46 ± 0.23	0.52 ± 0.08	0.34 ± 0. 09
	F 4	0.82 ± 0.32	0.84 ± 0.12	1.02 ± 0.32	1.03 ± 0.54	0.92 ± 0.33	0.62 ± 0.41	1.08 ± 0.58	0.89 ± 0.08	1.32 ± 0.45	1.01 ± 0.15	1.12 ± 0.31	0.48 ± 0 .05
	F 5	4.95 ± 1.03	5.46 ± 1.12	4.37 ± 1.30	5.61 ± 1.21	3.98 ± 1.42	3.02 ± 0.74	4.23 ± 1.12	4.30 ± 1.24	3.42 ± 1.25	5.23 ± 1.15	4.7 ± 0.58	6.85 ± 0.58
Ni	**F 1**	2.36 ± 0.24	2.03 ± 0.47	1.8 ± 0.56	1.98 ± 0.35	2.5 ± 0.36	2.78 ± 0.32	2.08 ± 0.45	1.74 ± 0.15	1.63 ± 0.27	1.96 ± 0.09	1.87 ± 0.43	2.06 ± 0.51
	F 2	2.98 ± 0.24	2.3 ± 0.68	3.6 ± 1.02	2.66 ± 0.86	2.32 ± 0.23	3.18 ± 0.74	3.00 ± 0.56	2.25 ± 0.25	3.16 ± 0.12	2.67 ± 0.18	2.41 ± 0.48	2.82 ± 0.69
	F 3	9.43 ± 2.13	8.17 ± 2.12	8.1 ± 2.11	10.2 ± 1.21	8.78 ± 0.87	9.21 ± 1.21	11.26 ± 1.14	7.96 ± 1.27	9.29 ± 2.13	9.55 ± 1.20	8.61 ± 1.12	8.74 ± 2.01
	F 4	6.25 ± 0.78	5.93 ± 1.04	6.68 ± 1.56	6.35 ± 0.86	5.23 ± 0.85	8.93 ± 1.41	7.31 ± 0.95	6.23 ± 0.98	5.78 ± 0.98	7.00 ± 0.59	5.86 ± 0.86	5.80 ± 1.21
	F 5	24.1 ± 3.56	23.6 ± 3.42	23.2 ± 5.43	30.3 ± 4.21	20.55 ± 3.25	32.3 ± 6.28	20.80 ± 2.01	15.5 ± 3.53	16.50 ± 2.52	17.56 ± 2.10	18.65 ± 2.1	20.42 ± 2.26
Cr	**F 1**	1.10 ± 0.24	1.23 ± 0.24	2.16 ± 0.58	1.85 ± 0.56	1.63 ± 0.36	1.89 ± 0.42	1.13 ± 0.25	1.35 ± 0.25	1.60 ± 0.32	1.40 ± 0.11	1.34 ± 0.22	1.68 ± 0.59
	F 2	2.23 ± 0.76	2.36 ± 0.21	5.15 ± 1.2	2.3 ± 0.58	1.15 ± 0.25	2.85 ± 0.72	2.24 ± 0.55	2.76 ± 0.52	3.36 ± 0.31	3.06 ± 0.24	4.21 ± 1.18	3.62 ± 1.02
	F 3	3.53 ± 0.86	5.51 ± 1.20	4.84 ± 1.21	4.21 ± 0.89	3.98 ± 0.57	8.1 ± 1.32	3.28 ± 1.23	4.26 ± 0.26	4.10 ± 0.12	4.23 ± 0.08	5.13 ± 1.32	4.58 ± 1.44
	F 4	14.8 ± 2.13	21.51 ± 2.14	18.5 ± 3.45	17.8 ± 2.15	15.21± 1.78	17.8 ± 3.25	14.40 ± 2.58	16.1 ± 1.27	16.8 ± 2.13	15.6 ± 1.25	14.8 ± 3.85	17.6 ± 6.50
	F 5	31.5 ± 5.67	42.6 ± 3.45	42.1 ± 4.85	38.0 ± 3.40	41.15 ± 5.76	38.5 ± 7.45	31.30 ± 0.40	33.1 ± 2.24	38.0 ± 12.3	38.9 ± 2.35	41.2 ± 3.63	37.8 ± 3.55
Zn	**F 1**	0.64 ± 0.12	0.42 ± 0.25	1.08 ± 0.52	0.86 ± 0.21	0.93 ± 0.18	0.74 ± 0.09	0.85 ± 1.24	0.78 ± 0.53	1.01 ± 0.52	0.82 ± 0.24	0.98 ± 0.18	0.80 ± 0.59
	F 2	4.37 ± 1.20	4.1 ± 0.85	12.8 ± 2.13	6.09 ± 1.42	8.98 ± 1.27	6.13 ± 2.00	8.70 ± 0.45	6.50 ± 1.10	10.13 ± 2.16	8.21 ± 1.26	7.86 ± 1.02	8.06 ± 1.02
	F 3	2.30 ± 0.96	1.88 ± 0.68	2.64 ± 0.36	3.14 ± 0.47	5.82 ± 0.95	2.66 ± 0.74	2.82 ± 1.54	3.14 ± 0.68	5.85 ± 0.56	3.34 ± 0.84	5.21 ± 0.93	4.97 ± 1.44
	F 4	8.47 ± 1.32	6.5 ± 1.02	17.1 ± 2.12	12.1 ± 1.25	14.08 ± 1.24	9.50 ± 1.45	11.10 ± 4.68	9.85 ± 1.25	12.32 ± 3.21	11.86 ± 1.14	11.21 ± 1.08	9.86 ± 6.50
	F 5	60.7 ± 6.57	35.3 ± 3.12	68.4 ± 5.36	94.8 ± 5.46	78.3 ± 6.84	54.8 ± 4.86	72.11 ± 1.12	61.1 ± 6.16	78.5 ± 13.52	85.5 ± 14.23	81.2 ± 12.85	76.5 ± 3.55
Mn	**F 1**	7.58 ± 2.71	10.9 ± 1.63	8.98 ± 1.23	15.3 ± 2.53	9.7 ± 2.13	17.5 ± 2.44	9.86 ± 1.18	8.11 ± 1.36	12.53 ± 2.23	13.63 ± 2.13	8.56 ± 1.32	14.36 ± 0.13
	F 2	13.1 ± 2.86	13.9 ± 1 .14	18.8 ± 2.88	27.8 ± 3.45	18.5 ± 2.10	20.3 ± 3.13	17.50 ± 6.51	14.1 ± 2.53	24.10 ± 3.52	25.13 ± 2.41	14.51 ± 2.31	20.28 ± 1.47
	F 3	47.5 ± 4.35	65.5 ± 5.60	71.2 ± 5.48	102.4 ± 6.87	62.63 ± 6.41	88.9 ± 6.75	89.21 ± 2.14	49.52 ± 5.29	95.63 ± 5.23	92.1 ± 15.41	48.56 ± 5.30	87.90 ± 1.01
	F 4	13.5 ± 2.14	14.25 ± 1.12	14.6 ± 1.23	18.6 ± 2.53	15.45 ± 2.14	16.8 ± 2.16	12.72 ± 19.65	14.52 ± 3.23	15.50 ± 2.13	16.86 ± 2.12	14.81 ± 2.28	15.55 ± 1.28
	F 5	120 ± 21.0	160 ± 14.65	223 ± 16.5	253 ± 45.2	148.0 ± 18.31	232 ± 46	215 ± 0.23	155 ± 21.02	235 ± 26.5	265 ± 36.25	122.8 ± 16.65	206.0 ± 14.41
Co	**F 1**	1.83 ± 0.57	2.85 ± 0.59	1.98 ± 0.56	1.96 ± .36	3.05 ± 0.48	2.95 ± 0.25	1.80 ± 3.45	2.35 ± 0.58	3.15 ± 0.29	1.96 ± 0.51	2.15 ± 0.86	3.65 ± 1.31
	F 2	19.2 ± 2.63	25.2 ± 2.13	25.4 ± 4.55	18.2 ± 2.45	23.56 ± 3.22	25.0 ± 4.55	18.67 ± 0.58	16.86 ± 3.02	23.56 ± 2.05	17.20 ± 3.23	18.23 ± 2.22	24.2 ± 3.23
	F 3	4.12 ± 1.14	4.16 ± 1.17	5.21 ± 1.21	3.89 ± 0.56	5.11 ± 0.69	5.82 ± 0.76	4.15 ± 0.51	5.05 ± 1.13	4.96 ± 1.16	5.65 ± 1.41	5.48 ± 1.00	5.69 ± 13.13
	F 4	1.08 ± 0.24	2.13 ± 0.87	1.93 ± 0.62	1.68 ± 0.35	1.85 ± 0.08	2.41 ± 0.31	1.75 ± 0.51	2.11 ± 0.25	1.68 ± 0.27	2.03 ± 0.53	1.98 ± 0.65	1.28 ± 2.82
	F 5	54.4 ± 6.98	77.5 ± 9.35	78.5 ± 6.85	58.7 ± 7.36	73.41 ± 4.3	80.1 ± 6.23	51.5 ± 5.51	49.2 ± 4.24	72.1 ± 15.25	48.3 ± 7.12	52.62 ± 4.88	74.2 ± 22.28
Cu	**F 1**	BDL	0.25 ± 0.12	0.28 ± 0.21	BDL	0.49 ± 0.06	0.41± 0.12	0.32 ± 0.12	0.56 ± 0.08	0.44 ± 0.14	0.51 ± 0.21	0.27 ± 0.11	0.29 ± 0.66
	F 2	0.37 ± 0.12	0.7 ± 0.25	0.85 ± 0.24	1.12 ± 0.41	1.51 ± 0.12	0.85 ± 0.19	0.86 ± 0.46	1.03 ± 0.51	1.11 ± 0.12	0.80 ± 0.15	0.75 ± 0.12	0.86 ± 0.09
	F 3	0.28 ± 0.11	0.65 ± 0.18	0.73 ± 0.41	0.89 ± 0.23	0.93 ± 0.011	0.73 ± 0.11	0.61 ± 0.35	0.75 ± 0.08	0.95 ± 0.35	0.74 ± 0.35	0.82 ± 0.26	0.79 ± 0.13
	F 4	2.98 ± 0.79	4.65 ± 0.86	7.86 ± 1.19	7.05 ± 1.11	11.64 ± 1.13	7.14 ± 0.85	8.11 ± 1.75	7.37 ± 1.19	7.86 ± 1.41	4.85 ± 1.23	8.21 ± 1.21	7.82 ± 1.23
	F 5	17.85 ± 6.23	14.26 ± 2.42	24.5 ± 2.50	20.1 ± 3.21	29.76 ±	23.8 ± 1.23	16.5 ± 3.65	15.6 ± 3.25	20.6 ± 3.65	16.5 ± 5.36	22.15 ± 3.25	21.36 ± 3.21

± BDL: below detection limit

and 132.50 for Zn, between 216.0 and 460.0 for Mn, between 78.30 and 117.50 for Co, and between and 12.70 and 48.50 for Cu. The average metal concentration of the soil was found to decrease in the order Mn > Co > Zn > Cr > Ni > Cu > Cd. The total concentration of Cd in soils was above the Indian, European, and Rowell described limits [25–27] as given in Table 3. The concentrations of Ni, Cr, Zn, and Cu were within Indian and European standards, and the Ni and Cu concentrations were above the limits according to Rowell reported standards, except Zn. About 60% of soils contained Zn above the permissible limit of 80 mg kg^{-1}.

The metals' mobility was studied by the fractionation scheme of Tessier's sequential extraction, which divides metal in soil into 5 fractions: exchangeable (F_1), bound to carbonate (F_2), bound to manganese and iron oxide (F_3), bound to organic matter (F_4), and residual (F_5). The results are reported in Table 3. This procedure has been applied and accepted by many scientists [28,29]. Considering species in the first 2 fractions (F1+F2), exchangeable and carbonate bound in soil samples are potentially available to plants and are mobile in the environment; the species in these fractions can be called bio-available species. The observed concentration of bio-available species for Ni, Cr, Zn, Mn, and Cu presented a lower proportion of 3.83%–11.58% and Co and Cd of 24.07% and 35.09%, respectively. It is well known that the carbonate fraction in soil contains a significant concentration of trace metals and is sensitive to change in pH. Cadmium is a nonessential metal with no benefit to humans and is a critical element due to its easy incorporation in the food chain and its general biotoxicity [30]. Other studies also support the findings that uptake of Cd from soil by plants is relatively easy [31,32]. With the exception of Ni and Mn (21.77% and 22.36%, respectively), the metals in Fe–Mn oxide bound species (F3) showed a small percentage of the total metal concentration (Figure 1). Based on Tessier's conclusion in this extraction step, only amorphous iron hydroxide could be attacked by 0.04 mol dm^{-3} $NH_4OH.HCl$ and crystalline hydroxide could not be destroyed. We can therefore deduce that the amount of Ni and Mn associated with amorphous iron hydroxide was higher in the studied wastewater irrigated soils.

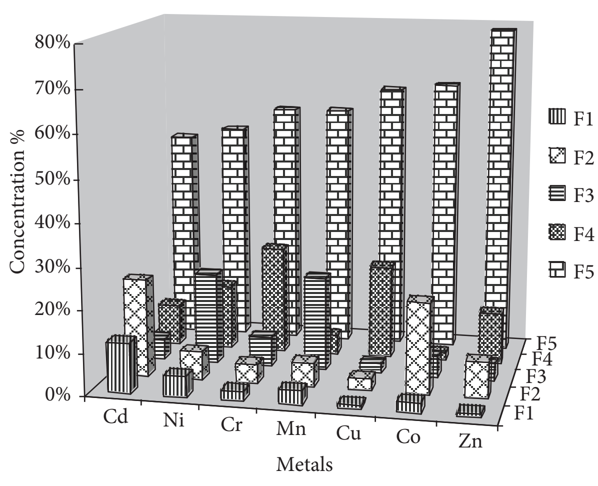

Figure 1. Fractionation patterns of the Cd, Ni, Cr, Mn, Cu, Co, and Zn in the soil samples using Tessier's sequential extraction procedure.

The organic fraction for Mn and Co was small, i.e. 4.55% and 1.85%, respectively, and all other metals showed higher levels (9.95%–25.65%) in this fraction as shown in Figure 1. This indicates that the coordination ability of organic matter is stronger in the investigated soils. The heavy metal speciation procedure showed that all the metals are dominant (>50%) in the residual phase (Figure 1). Metals in the residual fraction are expected to be chemically stable and biologically unavailable. Considering their phytoavailability, the elements under study can be arranged as follows: Zn > Co > Cu > Mn > Cr > Ni > Cd.

3.1. Heavy metal contents in vegetables

The distribution of metals in vegetables varies with the type of metal and crop. The concentrations of metals in spinach and bitter gourd collected from 6 sites are presented in Figures 2a and b. The uptake of Ni, Cr, Zn, and Mn by spinach (leaves) was greater than that by bitter gourd (fruit), and the uptake of Cd, Co, and Cu by spinach was slightly higher than that by bitter gourd. The metal concentrations in the vegetable samples were compared with their typical plant values and all the metal ions and found much more than the safe limits as described in Table 4 [33]. In both vegetables no visual toxicity symptoms were observed due to high level of the metals, but such high concentrations of these metals may be harmful to humans who consume these vegetables.

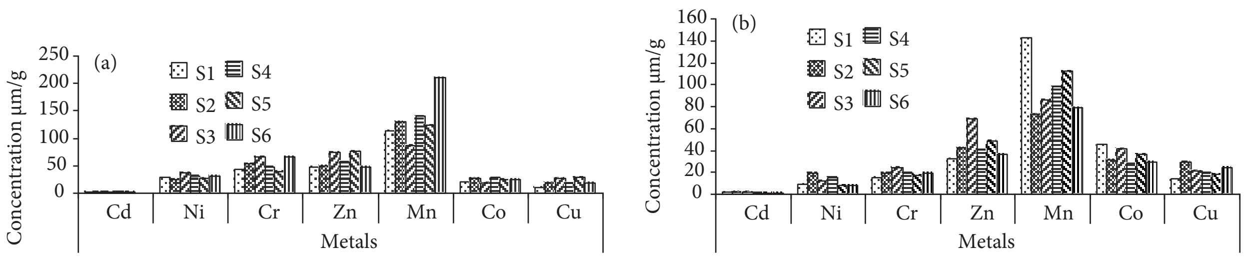

Figure 2. Results of metal analyses (a) in spinach and (b) in bitter gourd.

Table 4. The contamination levels of different metals in vegetables and soils.

Parameter	Vegetable, Fruit WHO Standard (mg/kg on dry wt. basis)	Soil (mg/kg) Indian Standard	European Standard	Rowell
Cadmium	0.02	3–6	3.0	0.5
Nickel	10	75–50	75	20
Chromium	1.3	300–600	150	n.l.
Zinc	50	n.l.	300	80
Manganese	6.61	n.l.	n.l.	n.l.
Cobalt	n.l.	n.l.	n.l.	25*
Copper	10	135–270	140	20

n.l.: not listed
*Baralkiewicz and Siepak (1999)

The most important application of chemical fractionation of metals in soils is to estimate their bioavailability to different plant tissues. Different forms of metals in soils extracted by chemical reagents were usually assessed for their availabilities to plants based on the correlation between metal concentration in the plant tissue and different fraction of metals in soil. If the accumulation of any element by a plant correlates significantly with the extractable fraction of soil, it can be assumed that this fraction of metal is readily available to plants. In this study, correlation analysis was performed between different chemical fractions (F1, F2, F3, F4, and F5) and total concentration of metal in soil and the metal accumulated in the edible parts of spinach and bitter gourd. Correlation coefficients (r) were estimated with 95% and 99% confidence level as described in Table 5. There was a significant negative correlation between Cd_{F5} and $Cd_{Bitter\,gourd}$ and between $Ni_{F5,Total}$ and $Ni_{Bitter\,gourd}$, indicating the nonavailability of the residual fraction and total metal concentration for plants.

A negative correlation was also been observed between Co_{F3} and $Co_{Bitter\,gourd}$. A strong positive correlation was observed between Cd_{F2} and $Cd_{Spinach}$, between Cd_{F1} and $Cd_{Bittergourd}$, between Ni_{F2} and $Ni_{Spinach}$, between Cr_{F2} and $Cr_{Spinach}$, between Cr_{F1} and $Cr_{Bitter\,gourd}$, between $Zn_{F1,F2,F4,FTotal}$ and $Zn_{Spinach}$, between Zn_{F1} and $Zn_{Bittergourd}$, between Mn_{F1} and $Mn_{Spinach}$, and between $Cu_{F2,F3,F4,F5}$ and $Cu_{Spinach}$. The positive correlation indicates the bioavailability of these metal fractions to plants. The highest correlation coefficients between vegetables and soil samples were obtained from F1 (exchangeable) and F2 (acid soluble) fractions of Tessier's sequential extraction procedure.

Table 5. Correlation coefficients (r) between the metal contents in soil fractions (i.e. F1, F2, F3, F4, F5, Total) and the spinach and bitter gourd samples.

Vegetable	Fraction	Cd	Ni	Cr	Zn	Mn	Co	Cu
Spinach	F 1	0.409	–0.246	0.570	**0.796***	**0.873****	0.380	0.710
	F 2	**0.861****	**0.929****	**0.830***	**0.861****	0.295	–0.254	**0.826***
	F 3	0.708	–0.027	0.726	0.693	0.531	–0.359	**0.825***
	F 4	0.074	0.512	0.556	**0.889****	0.539	0.280	**0.911****
	F 5	0.545	0.304	0.361	0.511	0.370	–0.082	**0.737***
	Total	0.685	0.237	0.700	**0.821***	0.341	–0.204	**0.918****
Bitter gourd	F 1	**0.814***	–0.509	**0.759***	**0.792***	–0.274	–0.237	0.528
	F 2	0.641	–0.390	0.240	0.265	–0.150	0.117	0.515
	F 3	0.686	–0.352	0.124	–0.334	0.145	**–0.859****	0.333
	F 4	0.657	0.161	0.710	0.330	–0.670	-0.087	–0.038
	F 5	**–0.823***	**–0.821***	0.348	0.219	0.027	0.050	–0.092
	Total	0.263	**–0.816***	0.266	0.607	0.098	0.145	0.506

*Significant correlation at 95% confidence level
**Significant correlation at 99% confidence level

4. Conclusions

From this study, it can be concluded that:

1. Concentrations of Cd, Ni, Cr, and Cu in wastewater were above the FAO guidelines.
2. Irrigation with wastewater has increased the toxic heavy metal concentration in soil and plants.
3. Total concentrations of metals in the studied soils follow the order Mn > Co > Zn > Cr > Ni > Cu > Cd.
4. All the metals were dominant (>50%) in the residual phase.
5. The highest positive correlation coefficients between vegetable and soil samples were obtained from F1 and F2 extraction steps of Tessier's sequential extraction procedure. There was a negative correlation coefficient between Cd_{F5} and $Cd_{Bitter\,gourd}$ and between $Ni_{F5,Total}$ and $Ni_{Bittergourd}$. This strong negative correlation supports the nonlabile nature of the residual fraction and total metal concentration.

The results of this study showed that potential bioavailability of heavy metals is controlled by their chemical forms and exchangeable and acid soluble forms are the most important forms of heavy metal uptake by vegetables. All the metal ions were beyond the safe limits in the edible parts of the vegetables. The elements under study can be arranged as follows (i.e. from less phytoavailable to more phytoavailable): Zn > Co > Cu > Mn > Cr > Ni > Cd.

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