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ORIGINAL PAPER

Depth-wise distribution, mobility and naturally occurring glutathione based phytoaccumulation of cadmium and zinc in sewage-irrigated soil profiles

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Abstract Field experiments were conducted to determine the mobility, distribution and naturally occurring glutathione (GSH)-based phytoaccumulation of cadmium and zinc in the sewage-irrigated alluvial soils, Allahabad, India. Frequent sewage-irrigation (up to 100 mL kg^{-1} soil) at 5 days' interval indicated enrichment of soil profiles with Cd and Zn more prominently in surface soils and subsurface soils below to the depth of 0.6 m and augmented cadmium and zinc accumulation in shoot tissues of *Brassica* species up to 10.6 and 31.5 mg kg^{-1}, respectively. Both cadmium and zinc were found significantly correlated with organic matter and cation exchange capacity of the soils indicating their dominant role in the sewage-irrigated soils. *Raphanus sativus* L. and *Brassica napus* L. accumulated significant quantity of cadmium and zinc, and higher concentration of GSH in their shoot tissues synergistically boosted translocation as well as accumulation of metals in plants, especially at plant maturity. However, cadmium showed higher translocation than zinc. Such evidence supports the conclusion that elevated natural GSH concentrations of *Brassica* species during their developed stage of plant growth are involved in metal hyperaccumulation, which ensure their potential for phytoremediation of cadmium and zinc in the sewage-irrigated soils. Thus, the use of the unused part (mostly leaves) of these species as an innovative technology for phytoremediation is suggested.

Keywords Cadmium · Glutathione · Mobility · Phytoaccumulation · Soil profile · Zinc

Introduction

Long-term use of wastewater for cultivation of leafy and other vegetables has resulted in the accumulation of heavy metals in the soil and their transfer to the various crops under cultivation, with levels of contamination exceeding the permissible limits. Sewage-irrigation has received much attention due to enrichment of heavy metals in soils which impacts human health and social problems (Gholamabbas et al. 2010; Nabuloa et al. 2010; Yusuf et al. 2003); however, use of sewage water in agricultural has been an age-old practice and can contribute to a reduction in stress on the utilizable water resource, which will not only reduce disposal problems of sewage water but also contribute towards improvement of soil fertility as it contains appreciable amounts of macro and micronutrients (Mitra and Gupta 1999).

Knowledge of depth-wise distribution of heavy metals like cadmium, chromium, lead, copper and zinc is helpful in understanding the contamination of soil and their downward movement in the soil. Moreover, roots of many plants go beyond the surface layer (0–15 cm) and thus draw part of the heavy metal deposits from sub-surface

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layers. Distribution of heavy metal keeps on continuously changing in soils due to both anthropogenic activities and the natural turn over in rock-soil–plant system below to the depth of 60 m through the soil profile. These metals are associated with various soil components in different ways and these associations influence both their mobility and distribution in the soil profile (Ahumada et al. 1999; Adelekan and Alawode 2011).

Zinc is the most abundant heavy metal found in both industrial and domestic sewage which accumulates in crops. It is also more toxic to plants than to animals or human beings. It, therefore, appears reasonable to assume that the phytotoxicity of Zn provides a safeguard against toxicity to animals or man. Although there is some evidence that Zn competes with Cd in plant uptake (Jarvis et al. 1976), many questions about interactions between Zn and Cd in plants as well as in animal and man remain unanswered.

Vegetables not only provide the daily caloric intake of the diet, but also certain phytochemicals with antioxidant capacity such as vitamins, carotenoids and polyphenols. Recently, there has been increasing interest in the protective biochemical function of these phytochemicals to protect human beings from oxidative stress or from free radicals. Glutathione (GSH) is one of the important endogenous phytochemicals for the defense mechanism of these species. *Brassica* species are grown worldwide, especially for the purpose of dietary vegetables, but very little attention has been paid towards biochemical causes and correlation between metal translocation in plants and hyperaccumulation ability of plants in sewage-irrigated soils. Earlier, the authors had reported phytoremediation potential of *Helianthus annuus* L. in sewage-irrigated Indo-Gangetic alluvial soils (Mani et al. 2012) with very limited information regarding the biochemical causes of phytoremediation.

The vegetable production sites of urban and sub-urban areas around Allahabad city get contaminated with sewage-irrigation through the main non-ferrous industrial units namely Raymonds Synthetic Ltd., Hindustan Cable Ltd., Deys Medicals, Sangam Structurals, Swadeshi Cotton Mills, Bharat Rocklite Plastics, Petroleum Corporation Ltd., Indian Telephone Industry and Baidyanath. Houben and Sonnet (2010) have also reported that mostly non-ferrous industrial units are responsible for significant leaching and phytoavailability of zinc and cadmium in the contaminated soils. Metal mobilization through sewage-irrigation is also causing potential risk for sub-surface and underground soil–water systems; therefore, soil column experiments are being employed to investigate the movement of metals (like Zn and Cd) in soil profiles. However, the rate of Cd and Zn release to soil solution is thus an important factor regulating its supply to plants. The chelating agent, Diethyl Triamine Penta-Acetic Acid (DTPA) is routinely used reagent to estimate available Zn in calcareous soils as it chelates Zn and to an extent simulates both Zn extractions from soil by plant exudates and Zn uptake by plants; it is especially important for Entisols, which contain considerable Zn but have very small amount of Zn in soil solution. The amount of extractable Zn increases with the organic matter content of the soil, and sewage-irrigation contains appreciable amount of both Zn and organic matter. Thus, the studied soil environment provides suitable scope for DTPA extraction in the investigated Entisols, a soil of recent origin.

Keeping in view the above facts, the present study was conducted with the objectives (1) to assess the depth-wise distribution and mobility of Cd and Zn in sewage-irrigated surface and sub-surface soil profiles; and (2) to investigate the role of naturally occurring GSH on translocation and phytoaccumulation of Cd and Zn by growing some common dietary vegetables, especially the *Brassica* species in the sewage-irrigated soils. This would provide knowledge about the biochemical causes of plants hyperaccumulating ability that guide future research for the protection of the environment and aware people from exposure to heavy metals causing potential risk to human health; and the information would be useful for both the vegetable growers as well as to the consumers.

Materials and methods

Experimental site

The experimental sites are located between latitudes 25°20′–25°57′ N and longitudes 81°52′–81°86′ E and belong to the Indian tropical sub-humid region of Indo-Gangetic plain Allahabad, Uttar Pradesh, India. The soils of Gangetic plain are Alluvial Entisols having some recent origin. The mean texture of the experimental soil was silty clay loam (sand 40.98 ± 9.36 %, silt 31.80 ± 5.55 % and clay 23 ± 8.05 %) having pH: 7.8 ± 0.23, electrical conductivity (EC): 1.2 ± 0.03 (dS m^{-1}), organic carbon (OC): 4.4 ± 0.36 (g kg^{-1}) and cation exchange capacity (CEC): 18 ± 2.74 C mol (p^{+}) kg^{-1} soil. The details of site-wise physico-chemical properties are given in Table 1. All the six vegetable production sites namely Phaphamau, Sangam, Rasulabad, North Malaka, Mumfordganj and Naini are located in the agricultural farms receiving sewage-irrigation for more than 25 years having successive declining degree of pollution levels (from A1 to A6) spread around 36 km^2 of the urban and sub-urban area of Allahabad (Fig. 1).

There are 57 unlined drains carrying sewage water from the entire city to the rivers Ganga and Yamuna. Out of these 13 join Yamuna, 36 join Ganga and remaining eight

Table 1 Physico-chemical properties of the different levels sewage-irrigated soils prior to the experiment

Soil	pH	EC (dS m^{-1})	Org. C (g kg^{-1})	CEC [C mol (p^+) kg^{-1}]	DTPA extractable Cd (mg kg^{-1})	DTPA extractable Zn (mg kg^{-1})	Sand (%)	Silt (%)	Clay (%)
A1	7.61 ± 0.11	1.25 ± 0.02	4.8 ± 0.13	22.2 ± 1.93	0.256 ± 0.021	15.2 ± 2.42	48.1 ± 5.66	31.4 ± 3.93	16.5 ± 3.53
A2	8.02 ± 0.13	1.22 ± 0.02	4.6 ± 0.12	18.2 ± 1.85	0.213 ± 0.019	11.4 ± 2.26	42.7 ± 5.33	31.2 ± 3.78	23.4 ± 4.22
A3	7.88 ± 0.12	1.17 ± 0.03	4.5 ± 0.12	19.1 ± 1.88	0.092 ± 0.008	8.2 ± 1.36	30 ± 3.34	28.4 ± 3.25	36.4 ± 4.76
A4	7.98 ± 0.12	1.18 ± 0.03	4.3 ± 0.10	18.5 ± 2.03	0.105 ± 0.011	9.4 ± 1.75	49 ± 8.48	25.5 ± 2.57	20.6 ± 3.88
A5	7.56 ± 0.09	1.19 ± 0.02	4.1 ± 0.10	16.2 ± 1.82	0.042 ± 0.007	3.8 ± 0.83	28.5 ± 3.21	41.9 ± 3.44	27.1 ± 4.35
A6	7.50 ± 0.06	1.2 ± 0.02	3.8 ± 0.07	14.1 ± 1.32	0.038 ± 0.005	1.85 ± 0.64	47.6 ± 4.34	32.4 ± 3.59	14.1 ± 1.68
Mean	7.76 ± 0.23	1.20 ± 0.03	4.35 ± 0.36	18.05 ± 2.74	0.12 ± 0.09	8.31 ± 4.90	40.98 ± 9.36	31.80 ± 5.55	23.02 ± 8.05

Data are mean values of three replications (mean ± SD) from each experimental location obtained from surface soils (0−15 cm); the six experimental sites are namely Naini (A1), Mumfordganj (A2), North Malaka (A3), Rasulabad (A4), Sangam (A5) and Phaphamau (A6)

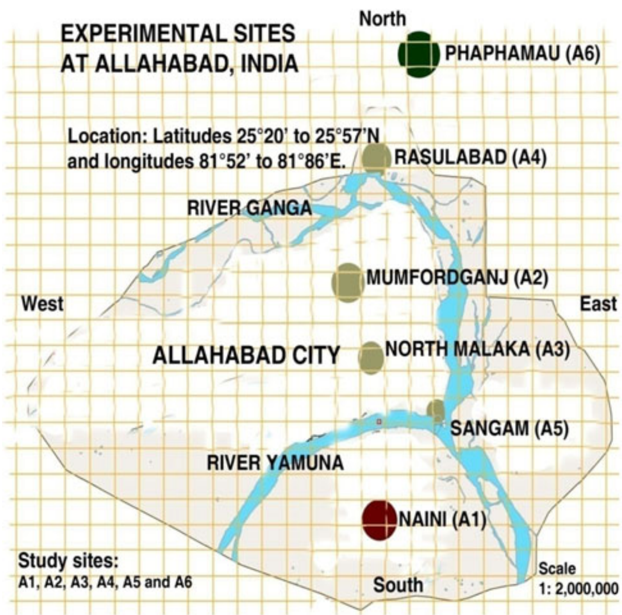


Fig. 1 Map of the experimental sites located at Allahabad, Uttar Pradesh, India

drains only pollute the terrestrial regions of the city. Total amount of wastewater measured in drains at sewage treatment plant (STP) of Allahabad is about 224 million litres per day (mld), out of which only 90 mld is diverted into seven sewer collection systems and the existing treatment capacity is 60 mld only and rest 30 mld wastewater is mixed with the treated effluent from the STP and the mixed (partly treated and partly untreated) water flows down the canal and is disposed off into the river Yamuna. However, a comparison between the total volumes of wastewater produced with total amount measured in open drains indicates that only 10–15 % of the sewage is actually reaching the trunk sewers indicating a serious problem at the investigated sites. The canal water, of which 1/3rd is untreated sewerage, is responsible for contamination of the agricultural products, especially to the dietary vegetables grown in the sewage-irrigated sites. Overall, sewage water management, not only in Allahabad city but also in many urban and sub-urban cities across the globe, is in critical situation.

Naini (A1) is the most polluted site among all the investigated sites having 436.2 hectares (ha) of industrial land use area mainly adjoining the area of Cotton Mills and only 40.7 ha commercial land use area, and the site is located in south of the Allahabad City. One of the investigated sites Mumfordganj (A2) is located just near the Mumfordganj Sewage Pumping Station (having 3 pumps of 6,800 litres per minute or lpm discharge for 27 m head and 2 pumps of 1,350 lpm discharge for 15 m head with a total installed capacity of 272 lpm). However, Phaphamau (A6), the least polluted sub-urban trans-Gangetic site among the investigated areas is located in north of the city having 35 ha commercial land use area and very little industrial land use area. Sangam (A5) is known worldwide as a holy place and the estimates suggest that around 7–10 crore people throng Allahabad city to take a holy bath at the holy Sangam. Mumfordganj, Sangam, Rasulabad (A4) and North Malaka (A3) are situated in the main city of Allahabad.

Six uncontaminated sites (each having 12 micro plots and each of dimensions 1 × 1 m or 1 m^2) were randomly selected in the aforesaid six locations adjoining the contaminated sewage-irrigated soils, to fulfill the objectives. The experiment was replicated thrice and conducted in a total of 72 micro plots (6 sites × 4 vegetable species × 3 replications), covering the total area of 72 m^2, designed in factorial randomized block design (Factorial RBD). The most common dietary vegetable species of the aforesaid regions such as fenugreek (*Trigonella foenumgraecum* L.), cauliflower (*Brassica oleracia* L.), radish (*Raphanus* *sativus* L.) and rapeseed (*Brassica napus* L.) were grown in the investigated sites during 1 August 2006 to 29 October 2006. Thus, vegetables were grown at that time when their early growth stage received higher daytime temperature (mean 37 ± 1.3 °C) and their maturity period received comparatively lower daytime temperature (33 ± 4.1 °C).

Sewage-water obtained from the canal-water (mixture of 2/3rd treated water with STP and 1/3rd untreated water as mentioned earlier) was applied at 0, 20, 40, 60, 80 and 100 mL kg^{-1} soil as per irrigation schedule of the six investigated sites A6, A5, A4, A3, A2 and A1, respectively. To use the partly treated sewage-water efficiently and produce higher biomass of vegetables, localized irrigation with sewage water was carried out at the study sites, which provides the greatest degree of health protection for farm workers and consumers. The plants of *R. sativus* L., *B. napus* L., *T. foenumgraecum* L., and *B. oleracia* L. were first irrigated with sewage water at 15, 20, 15 and 15 days after sowing (DAS), respectively; after that frequent irrigation with sewage water was carried out at 5 days' interval till plant maturity stage. Varying levels of sewage-irrigation were done, accordingly to the degree of pollution of the experimental sites, to reflect the true picture of mobility, distribution and phytoaccumulation of heavy metals (Cd and Zn) in the investigated sites. The irrigation water deficit of vegetable plant species was fulfilled through the municipal tap water system. Thus, nil sewage-irrigated site Phaphamau (A6) required 100 % irrigation through the municipal tap water system while the sewage-irrigated site at 100 mL kg^{-1} of Naini (A1) required only 60 % irrigation through the municipal tap water system. Sewage water quality was characterized as chemical oxygen demand (COD) 239 ± 14.6 mg L^{-1}, biological oxygen demand (BOD) 60 ± 5.4 mg L^{-1}, total dissolved solids (TDS) 231 ± 12.8 mg L^{-1}, EC 2.8 ± 0.4 dS m^{-1}, OC 24 ± 3.7 g L^{-1}, zinc 6.5 mg L^{-1} and cadmium 3.5 mg L^{-1}.

Soil sampling from the experimental sites

Representative soil samples from alluvium soils (Vertisols), which had been receiving sewage effluents for 50 years, were collected from different locations or different types of land use, including industrial, commercial, residential and agricultural to investigate current conditions of cadmium and zinc contamination at a municipal scale (Lee et al. 2006). Soil samples from different depths (0–15, 15–30, 30–45 and 45–60 cm) were collected from six experimental sites to study deep bedding mobility, distribution and enrichment of Cd and Zn in the soil profiles of dietary vegetable production sites in Allahabad, especially to depict the possible risk of Cd-contamination of groundwater.

Leaching column experiments

The leaching column experiments were conducted to correlate and signify the data obtained from the field experiments. The column experiments were conducted in Cylindrical Plexiglas columns. The total length of each column was 100 cm with the internal diameter of 5.4 cm. Air-dried samples of column beds were sieved (<1 mm) and autoclaved prior to every experiment. The columns were filled with soil (500 g) to a height of 0.6 m by uniform tapping to achieve uniform bulk density of 1.58 g cm^{-3}, and dry-packed columns were completely saturated with deionized water (pH 6.5–6.6) from the bottom by capillary to facilitate the removal of pore air and guarantee wetting. A Whatman No. 42 filter paper was placed at the bottom of the leaching column. The solution was pounded up to 5 cm above the soil surface on the soil column. The sewage water, which has previously been stored for 3 months, was allowed to equilibrate for 30 days prior to its application in leaching column experiment. Then soil columns were leached with distilled water and stabilized sewage water at 40 and 100 mL kg^{-1} soil placed in each column. Leachates were collected in 10 mL increments and after eight pore volumes (PV) in 50 mL increments, leaching was continued until 20 PVs were collected (Jalali and Khanboluki 2007). The PV of the soil columns was calculated from value for the bulk density and particle density (2.65 g cm^{-3}) of the soil in the column. Pore volume of the columns was 547 mL. The concentrations of Zn and Cd were determined in the leachate using at Central Environment Pollution Control Lab, Indian Farmer Fertilizer Cooperative (IFFCO) Ltd., Phulpur Unit, Ghiya Nagar, Phulpur, Allahabad, 212404, Uttar Pradesh, India.

Methods of soil sampling and analysis

Soil analysis

One gram of soil in 5 mL concentration HNO_3 and 5 mL $HClO_3$ (Perchloric acid 60 %) were added and the contents were heated up to dryness. The hot distilled water was added. The contents filtrated and volume was made up to 50 mL.

Silt and clay were separated by Pipette method and fine sand by decantation. Heavy metals were extracted with tri-acid mixture (con. HNO_3, H_2SO_4 and $HClO_4$ in 5:1:2) and determined with the standard method of Li et al. (1995) using Inductively Coupled Plasma Spectrometry (ICP-AES; Model-LABTEM, Perkin Elmer, Inc.) at IIFCO, Phulpur. DTPA solution [1.97 g (0.05 M) DTPA powder, 13.3 mL (0.1 M) Tri-ethanol amine and 1.47 g (0.01 M) $CaCl_2$ were dissolved in distilled water made up to 1 L after adjusting the pH to 7.3] was prepared (Lindsay and

Norvell 1978) to extract the available heavy metals in soil samples. Five grams of soil was shaken with 20 mL of the above reagent for 2 h. The clean filtrate was used for the estimation of Cd and Zn. Organic carbon was determined by chromic acid digestion method and CEC using neutral ammonium acetate solution. For nitrogen, a known weight of soil (1 g) was taken in a 150 mL conical flask and treated with 10 mL of digestion mixture containing sulphuric acid and selenium dioxide.

Methods for plant analysis

The aforesaid vegetables are grown in the experimental area for own consumption of the farmers and for supply to retail and wholesale markets of Allahabad. Vegetables were collected randomly from the six locations at two stages of their plant growth period first at early growth stage (at 20–30 DAS) and the finally at plant maturity stage (at 60–90 DAS) during the period 1 August 2006 to 29 October 2006. After washing with clean tap water to remove the soil particles, vegetable samples were dried at 60 °C in a forced draught oven to constant weight, ground, passed through a 2-mm sieve and stored in glass jars at room temperature (25 ± 2 °C) for analysis.

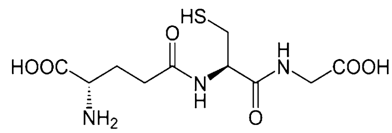
Digestion of plant material One gram of ground plant material was taken in a 100-mL beaker and 15 mL of tri-acid mixture (HNO_3, conc. H_2SO_4 and $HClO_4$ in 5:1:1 ratio) was added. The content was heated on hot plate at low heat (60 °C) for 30 min and the volume was reduced to about 5 mL, until a transparent solution was obtained (Allen et al. 1986). After cooling, 20 mL of distilled water was added to the beaker and the content was filtered through Whatman number 42 into a 100-mL volumetric flask and the volume was made up with distilled water.

Tap water and sewage water analysis

The water quality parameters of tap water was tested as per APHA (2005) and observed normal as per standard drinking water quality guidelines (having pH 8.0, EC 0.9 dS m^{-1}, total alkalinity 160 ppm, hardness 240 ppm, chloride 35 ppm, Zn below 0.02 ppm and Cd not detectable).

Sewage water samples (50 mL) were digested with 10 mL of concentrated HNO_3 at 80 °C until the solution became transparent (APHA 2005). These transparent solutions were filtered through Whitman number 42 filter paper and diluted to 50 mL with distilled water. Concentration of heavy metals was determined using the aforesaid model of spectrophotometer.

Biochemical analysis



GSH

GSH is the tripeptide that contains an unusual peptide linkage between the amine group of cysteine (which is attached by normal peptide linkage to a glycine) and the carboxyl group of the glutamate side-chain. GSH, ethylenediaminetetraacetic acid (EDTA), reduced nicotineamide-adenine dinucleotide phosphate (NADPH) and dithiobisnitrobenzoic acid (DTNB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other chemicals and reagents used were purchased from Himedia, BDH, Merck and Qualigens Ltd. HPLC grade quartz double-distilled water was employed throughout the study.

Samples of dietary vegetables were taken at different two stages of their growth, one at early growth stage and another and final at plant maturity. For early growth stage samples of *R. sativus* L., *B. napus* L., *T. foenumgraecum* L. and *B. oleracia* L. were taken at 20, 30, 20 and 20 DAS, respectively, while final plant samples were taken at 60, 90, 60 and 60 DAS (at plant maturity stage) in the aforesaid plants, respectively. One gram of plant sample in 10 mL of ice-cold 0.9 % (w/v) NaCl containing 5 mM EDTA (final concentration) was homogenized, followed by acid precipitation with 5 mL of ice-cold 30 % (w/v) trichloroacetic acid, followed by centrifugation at 5,000 rpm for 10 min, and the resulting supernatants stored at −80 °C. GSH content was measured using standardized kit from sigma chemical Co., USA. Assays were performed in 96-well microplates and change in absorbance monitored at 410 nm. The method is based on sequential oxidation of GSH by 5, 5′-dithiobis 2-nitrobenzoic acid (DTNB) and reduction by NADPH in the presence of GSH reductase or GR (Griffith 1980) to remove precipitated proteins.

Validation of analytical methods

The analytical methods included the limit of detection (LOD) and quantification (LOQ). Please refer to the supplementary information attached in separate file to see the detection levels obtained for calculating the LOD and LOQ values for the analysis using the 3 (RMSE)/slope method. The statistical analysis including all graphical illustrations except the map of the experimental location was determined and designed using GraphPad Prism 5.04 software in accordance with Motulsky and Christopoulos (2003).

Results and discussion

The profile-wise distribution of heavy metals in sewage-irrigated soils at six aforesaid locations having varying degree of sewage application (at 0, 20, 40, 60, 80 and 100 mL kg^{-1} soil) was studied and significant correlation between metals (Cd and Zn) and physico-chemical properties (especially with OC and CEC) of these soils established. Besides these, leaching of Cd and Zn in soil, TF of heavy metals (Cd and Zn) from soils to plants and the comparative phytoaccumulation potential of the four tested dietary vegetables in the sewage-irrigated soils were investigated, and significant positive correlation between plant GSH content and metals hyperaccumulating potential of *Brassica* species established. The results have been presented under the following sub-headings: influence of sewage-irrigation on physico-chemical properties of soils, DTPA-extractable Cd and Zn in soils, Leaching of cadmium and zinc in soil, Metal translocation factor, Cadmium and zinc phytoaccumulation and Correlation among leaf GSH and metal hyperaccumulation ability of the aforesaid plants.

Influence of sewage-irrigation on physico-chemical properties of soils

Data presented in the Table 2 clearly show that soil pH (7.8–6.8), EC (1.2–0.6 dS m^{-1}), OC (4.4–2.9 g kg^{-1}) and

Table 2 Impact of sewage-irrigation on soil physico-chemical environment after the experiment

Depth (in cm)	Experimental sites						
	A1	A2	A3	A4	A5	A6	Mean
pH							
0–15	7.6 ± 0.07	8.0 ± 0.08	7.9 ± 0.06	8.0 ± 0.08	7.6 ± 0.07	7.5 ± 0.05	7.8 ± 0.23
15–30	7.0 ± 0.05	7.1 ± 0.07	6.9 ± 0.05	6.7 ± 0.06	6.9 ± 0.05	7.0 ± 0.05	6.9 ± 0.14
30–45	6.9 ± 0.06	6.8 ± 0.05	6.8 ± 0.06	6.9 ± 0.07	6.7 ± 0.06	7.4 ± 0.05	6.9 ± 0.25
45–60	6.8 ± 0.05	6.6 ± 0.04	6.7 ± 0.05	6.8 ± 0.06	6.8 ± 0.04	6.8 ± 0.04	6.8 ± 0.08
Mean	7.1 ± 0.36	7.1 ± 0.63	7.1 ± 0.55	7.1 ± 0.60	7.0 ± 0.39	7.2 ± 0.33	
Electrical conductivity (dS m^{-1})							
0–15	1.25 ± 0.03	1.22 ± 0.02	1.17 ± 0.02	1.18 ± 0.02	1.18 ± 0.02	1.20 ± 0.02	1.2 ± 0.03
15–30	1.10 ± 0.04	1.00 ± 0.05	1.12 ± 0.04	1.14 ± 0.03	1.21 ± 0.03	1.15 ± 0.03	1.1 ± 0.07
30–45	0.85 ± 0.04	0.70 ± 0.03	0.74 ± 0.03	0.77 ± 0.02	0.70 ± 0.03	0.76 ± 0.03	0.8 ± 0.06
45–60	0.70 ± 0.05	0.63 ± 0.06	0.57 ± 0.05	0.62 ± 0.06	0.65 ± 0.05	0.45 ± 0.05	0.6 ± 0.09
Mean	0.98 ± 0.25	0.89 ± 0.27	0.90 ± 0.29	0.93 ± 0.28	0.94 ± 0.30	0.89 ± 0.35	
Clay (%)							
0–15	16.5 ± 1.22	23.4 ± 1.63	36.4 ± 3.24	20.6 ± 2.69	27.1 ± 3.26	14.1 ± 2.07	23.0 ± 8.05
15–30	24.7 ± 1.67	31.2 ± 2.97	49.1 ± 6.38	22.3 ± 3.13	30.1 ± 4.19	18.2 ± 3.08	29.3 ± 10.9
30–45	27.1 ± 3.53	36.2 ± 3.87	56.2 ± 7.89	26.1 ± 3.55	31.3 ± 4.42	22.3 ± 3.29	33.3 ± 12.2
45–60	29.4 ± 4.69	40.1 ± 4.24	64.1 ± 8.05	37.2 ± 6.33	50.0 ± 6.03	27.2 ± 3.90	41.3 ± 13.8
Mean	24.4 ± 5.62	32.7 ± 7.21	51.4 ± 11.76	26.6 ± 7.46	34.7 ± 10.40	20.4 ± 5.61	
Organic carbon (g kg^{-1})							
0–15	4.8 ± 0.13	4.6 ± 0.09	4.5 ± 0.16	4.3 ± 0.08	4.1 ± 0.11	3.8 ± 0.08	4.4 ± 0.36
15–30	3.6 ± 0.09	3.4 ± 0.08	4.1 ± 0.23	3.8 ± 0.09	3.8 ± 0.26	3.2 ± 0.13	3.7 ± 0.32
30–45	2.9 ± 0.13	2.7 ± 0.16	3.8 ± 0.27	3.4 ± 0.24	3.6 ± 0.25	2.8 ± 0.34	3.2 ± 0.46
45–65	2.5 ± 0.15	2.3 ± 0.19	3.5 ± 0.35	3.2 ± 0.28	3.3 ± 0.29	2.5 ± 0.26	2.9 ± 0.51
Mean	3.5 ± 1.01	3.3 ± 1.01	4.0 ± 0.43	3.7 ± 0.49	3.6 ± 0.34	3.1 ± 0.56	
Cation exchange capacity (C mol (p^{+}) kg^{-1})							
0–15	22.2 ± 1.23	18.2 ± 1.07	19.1 ± 0.91	18.5 ± 1.24	16.2 ± 0.89	14.1 ± 0.97	18.1 ± 2.74
15–30	21.1 ± 1.18	17.4 ± 0.93	18.0 ± 1.02	17.2 ± 1.10	15.1 ± 0.95	13.1 ± 0.83	17.0 ± 2.71
30–45	20.2 ± 1.12	16.1 ± 0.79	17.3 ± 0.87	16.1 ± 1.05	14.0 ± 0.73	12.6 ± 0.76	16.0 ± 2.64
45–65	18.1 ± 0.95	15.1 ± 0.77	16.2 ± 0.83	15.3 ± 0.93	13.3 ± 0.65	11.1 ± 0.68	14.9 ± 2.41
Mean	20.4 ± 1.74	16.7 ± 1.37	17.7 ± 1.22	16.8 ± 1.39	14.7 ± 1.27	12.7 ± 1.25	

Data are mean values of three replications (mean ± SD) collected from soil profiles of six locations A1–A6 (as mentioned under Table 1)

CEC [18.1–14.9 C mol (p^+) kg^{-1}] successively decreased with the increasing depth of soil profile; contrary to this, clay content increased with the increasing depth of soil profiles. Soil pH (7.5–8.02), EC (1.17–1.25 dS m^{-1}), clay (14.1–36.4 %), OC (3.8–4.8 g kg^{-1}) and CEC [14.1–22.2 C mol (p^+) kg^{-1}] in surface soils had wide variation from one to the other site due to varying levels of sewage-irrigation commonly practiced by the vegetable producers. Distribution of OC and CEC in the sewage-irrigated soils shows almost definite pattern as their content synergistically enriched with increasing levels of sewage-irrigation from 0 to 100 mL kg^{-1} soil. This is due to the application of sewage water possessing higher content of OC (24 ± 3.7 g L^{-1}) than the investigated soils (Esteban et al. 2000). EC was observed maximum in the soil profiles of Naini (A1) to the extent of 0.70 dS m^{-1} in the lowest horizon (of 45–60 cm depth) to 1.25 dS m^{-1} in the surface horizon (of 0–15 cm depth), which was followed by the soil profiles of Mumfordganj (A2) to the extent of 0.63 dS m^{-1} in the lowest horizon (of 45–60 cm depth) to 1.22 dS m^{-1} in the surface horizon (of 0–15 cm depth). It is evident from the data that frequent sewage-irrigation contributes to the build-up of soil fertility to some extent and can mitigate the scarcity of water in the dry and tropical regions.

DTPA-extractable Cd and Zn in soils

The data graphically presented in Fig. 2a indicated that extractable Cd in surface soil was minimum (0.038 mg kg^{-1}) at Phaphamau (A6) which had not been receiving sewage-irrigation as compared with those receiving sewage-irrigation in different amounts. The extractable Cd in sewage-irrigated surface soils at various depths varied from 0.038 to 0.256 mg kg^{-1}, which decreases with increase in depth of soil profiles. Sewage-irrigation at 100 mL kg^{-1} soil augments six- to ninefold enrichment of cadmium in the sewage-irrigated soil profiles of the aforesaid six experimental sites.

The extractable Zn differed significantly with the sites receiving six varying levels of sewage-irrigation and with the varying depths of four horizons under the soil profiles.

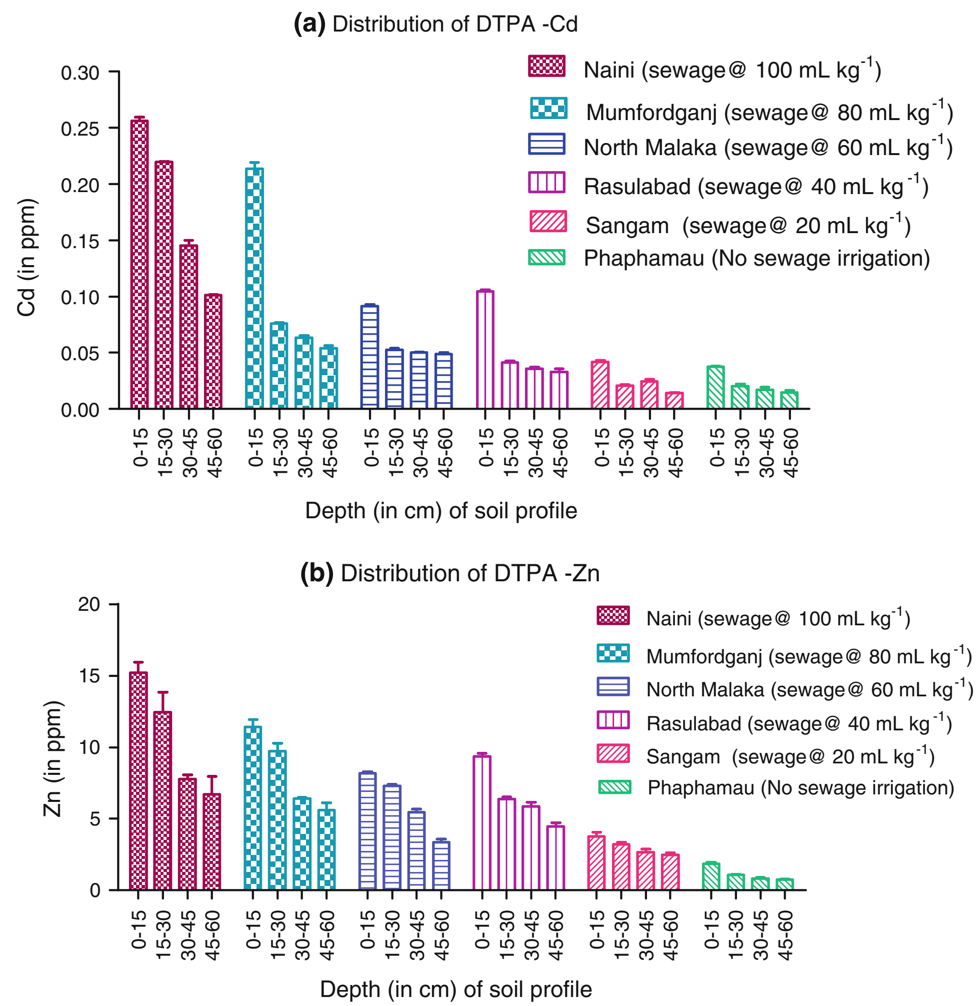


Fig. 2 Distribution of **a** DTPA-Cd and **b** DTPA-Zn in sewage irrigated soil profiles; data are mean values of three replications (mean ± SD); mean distribution of DTPA-Cd in six soil profiles are as follows: Naini (0.18 mg kg^{-1}), Mumfordganj (0.10 mg kg^{-1}), North Malaka (0.06 mg kg^{-1}), Rasulabad (0.05 mg kg^{-1}), Sangam (0.03 mg kg^{-1}) and Phaphamau (0.02 mg kg^{-1}); mean distribution of DTPA-Zn are as follows: Naini (10.6 mg kg^{-1}), Mumfordganj (8.3 mg kg^{-1}), North Malaka (6.1 mg kg^{-1}), Rasulabad (6.6 mg kg^{-1}), Sangam (3.1 mg kg^{-1}) and Phaphamau (1.1 mg kg^{-1})

Figure 2b showed that concentration of extractable Zn ranged from 0.75 to 15.2 mg kg^{-1} and indicated a trend almost similar to Cd in the distribution of metals in the soil profiles. However, sewage-irrigation at 100 mL kg^{-1} soil caused nine- to tenfold enrichment of zinc in the sewage-irrigated soils. In the site receiving municipal tap water for irrigation (A6), Zn content remained more or less constant throughout the depth of soils. In light textured soils (A1, A2, A4 and A6), decline in the extractable Zn was higher than that in heavy textured type of soils (A3 and A5). This is attributed to high infiltration and percolation rates of sewage in light-textured soils than in the heavy-textured soil. Thus, continuous and successive sewage-irrigation results in the build-up of heavy metals in the surface soils due to higher adsorption and low permeability of these soils.

Leaching of Cd and Zn from the soil

The results of the leaching are presented in Fig. 3a, b showing the relationship between concentration of cations and cumulative water passing out the columns. Leaching of Cd with distilled water and sewage-irrigation (at 40 and 100 mL kg^{-1} soil) was observed negligible to low because of low Cd-content in these soils (Fig. 3a). The lower

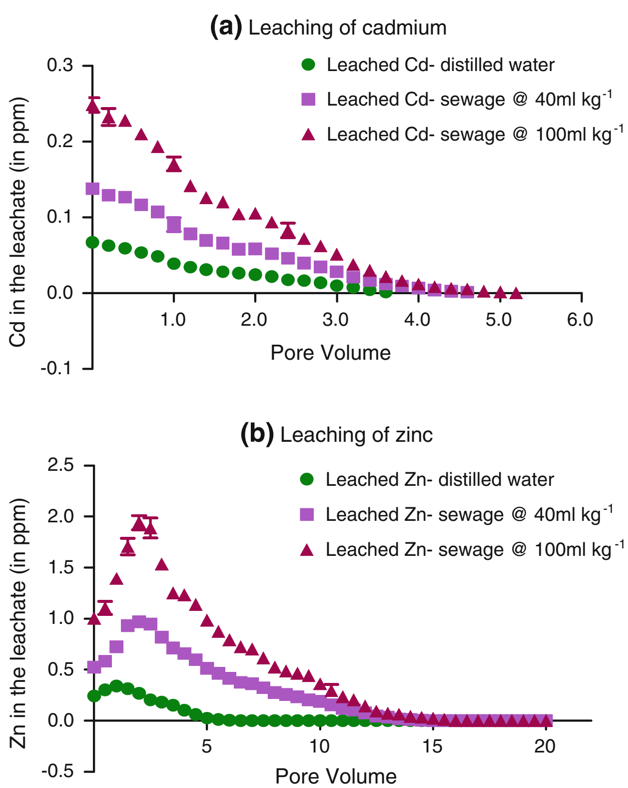


Fig. 3 Curves for **a** leaching of cadmium and **b** leaching of zinc, leached with (1) distilled water, (2) sewage water at 40 mL kg^{-1} soil and (3) sewage water at 100 mL kg^{-1} soil

leaching of cadmium was due to adsorption of Cd onto the soil surfaces. At the initial breakthrough curves of the heavy metals, the adsorption coefficient is large when the heavy metal concentration in the liquid phase is low. With increasing concentration of heavy metals, it drops considerably to a maximum value at the highest breakthrough concentration of the heavy metals. After the peak breakthrough concentration has passed away, adsorption coefficients increase gradually with decreasing concentration of the heavy metals. Sewage-irrigation at 40 and 100 mL kg^{-1} soil enhanced leaching of Cd by 3.4–4.25 fold and 3.8–5.25 fold, respectively, over the distilled water (control).

Leaching of Zn with distilled water in the native soil was negligible and in the presence of different levels of sewage water was moderate. The breakthrough curves for Zn are presented in Fig. 3b. Peak concentration for Zn in the leachates of distilled was observed at one PV followed by a decrease in the subsequent leachate fractions till the flow of five PV. However, peak concentration of Zn in sewage water application at 40 and 100 mL kg^{-1} soil was observed at two PV followed by a decrease in the subsequent leachate fractions till the flow of 15 PV. The maximum concentration of Zn (1.94 mg L^{-1}) in the leachates of sewage application at 100 mL kg^{-1} soil (at Naini) was observed than the other tested concentrations. However, the data are well below from 5.0 mL kg^{-1}, the maximum permitted concentration of Zn in potable water, but the concentration of Zn is likely to surpass the limit in the coming years in future. Sewage-irrigation at 40 and 100 mL kg^{-1} enhanced leaching of Cd by 5.13–5.33-fold and 9.89–10.2-fold, respectively, over the distilled water (control).

The graphical illustrations (Fig. 3a, b) show that total amount of Cd leaching stabilizes in about five PV whereas Zn leaching in about 15 PV for the tested three leaching solutions (distilled water, sewage water at 40 mL kg^{-1} soil and sewage water at 100 mL kg^{-1} soil). This result indicates that addition of sewage water promoted the availability of soluble organic matter which increased the sorption of Cd in soil. Petruzzelli et al. (1992) also showed that addition of sewage sludge extract increased the amount of heavy metals retained by soil. Thus, long-term application of wastewater could elevate the trace element content in soil and accelerate the leaching of Cd and Zn in the soil profile.

Correlation between available metals and physico-chemical properties of soils

The statistical analysis revealed that correlation between EC with extractable Cd ($r = 0.79^*$, $P < 0.05$) and between EC with extractable Zn ($r = 0.79^*$, $P < 0.05$) was significant. However, the correlation between pH with available Zn ($r = 0.51$, $P > 0.05$) was non-significant.

The correlations between organic carbon and extractable Zn ($r = 0.95^{**}$, $P < 0.01$) and between CEC and extractable Zn ($r = 0.94^{**}$, $P < 0.01$) were strongly positive as well as highly significant, whereas correlation between organic carbon and extractable Cd ($r = 0.89^{*}$, $P < 0.05$) and between CEC and extractable Cd ($r = 0.77^{*}$, $P < 0.05$) were significant. Similarly, extractable Zn in soils was strongly correlated with organic carbon ($r = 0.95^{**}$, $P < 0.01$) and CEC ($r = 0.94^{**}$, $P < 0.01$) and non-significantly correlated with EC ($r = 0.60$, $P > 0.05$) and pH ($r = 0.51$, $P > 0.05$) in the soils. However, correlations between soil pH and extractable Cd ($r = -0.33$), between clay and extractable Cd ($r = -0.21$) and between clay and extractable Zn ($r = -0.04$) were slightly negative but non-significant. This study indicates that organic carbon and CEC play dominant role in the mobility of Cd and Zn in the sewage-irrigated soils receiving varying levels of sewage application. Banin et al. (1981) also reported abundance of metal ions (Cd and Zn), organic carbon and CEC in the sewage-applied soils.

Metal translocation factor

The translocation factors (TF) of Cd and Zn from soils to shoot tissues of vegetables were calculated to understand the risk and associated hazards due to different levels of sewage-irrigation and consequent heavy metal accumulation in test vegetables following Cui et al. (2004) as follows:

$$\mathrm{TF} = \frac{\text{Concentration of metal in plant tissue (fresh weight basis)}}{\text{Concentration of metal in corresponding soil or root (dry weight basis)}}$$

The ratio of metals between soil and plant parts (TF) is an important criterion for the contamination assessment of soils possessing higher level of heavy metals. The ratio >1 means higher accumulation of metals in plant parts than in soil (Barman et al. 2000).

The average translocation of metals from soil to plant was found to be in the order of Cd (2.64) > Zn (1.91). It was seen in all tested vegetables that transfer factor of Cd was >1.3, indicating its high mobility from soil to plants (Table 3). Between the two metals, Cd showed maximum values for transfer factor, which ranged from 1.354 (in *B. oleracia* L.) to 6.175 (in *R. sativus* L.) at six investigated sites with varying degree of contamination. Transfer factor for Zn was observed comparatively lower than for Cd, ranging from 0.082 (in *T. foenumgraecum* L.) to 0.477 (in *R. sativus* L.). Different species of vegetables exhibited the following order for their degree of Cd-contamination: *R. sativus* L. > *B. napus* L. > *T. foenumgraecum* L. > *B. oleracia* L.; however, these species exhibited quite different order for Zn-contamination: *R. sativus* L. > *B. napus* L. > *B. oleracia* L. > *T. foenumgraecum* L. In common, both *R. sativus* L. and *B. napus* L. showed higher translocation of Cd and Zn as compare to *T. foenumgraecum* L. and *B. oleracia* L. Therefore, both species are

Table 3 Translocation of heavy metals (Cd and Zn) in the leaves of dietary vegetables in the sewage irrigated soils

Sites	*T. foenumgrecum* L.	*B. oleracia* L.	*R. sativus* L.	*B. nupus* L.
TF of cadmium				
A1	1.668 ± 0.033	1.354 ± 0.108	4.134 ± 0.037	1.668 ± 0.033
A2	1.825 ± 0.067	1.458 ± 0.060	4.565 ± 0.240	1.955 ± 0.084
A3	2.257 ± 0.166	1.387 ± 0.441	5.096 ± 0.100	2.772 ± 0.550
A4	2.680 ± 0.028	1.843 ± 0.032	6.175 ± 1.495	3.502 ± 0.526
A5	2.698 ± 0.087	1.907 ± 0.364	6.968 ± 1.054	4.278 ± 0.540
A6	2.973 ± 0.502	1.772 ± 0.077	5.407 ± 0.245	5.879 ± 0.701
TF of zinc				
A1	0.086 ± 0.012	0.135 ± 0.033	0.205 ± 0.032	0.130 ± 0.049
A2	0.086 ± 0.012	0.149 ± 0.012	0.246 ± 0.102	0.173 ± 0.080
A3	0.082 ± 0.009	0.123 ± 0.013	0.310 ± 0.075	0.144 ± 0.022
A4	0.098 ± 0.016	0.096 ± 0.017	0.215 ± 0.087	0.112 ± 0.032
A5	0.148 ± 0.013	0.196 ± 0.020	0.372 ± 0.070	0.155 ± 0.003
A6	0.244 ± 0.009	0.299 ± 0.15	0.477 ± 0.165	0.299 ± 0.044

Data for translocation of Cd and Zn are mean values of three replications (mean ± SD) collected under four different soil–plant systems at six locations A1–A6 (as mentioned in Table 1) recommended for phytoremediation technology owing to their higher potential of metal-phytoaccumulation. Variation in transfer factors among different vegetables is attributed to differences in the concentration of heavy metals in the sewage-irrigated soils and differences in metal uptake by different vegetables (Cui et al. 2004; Zheng et al. 2007).

The translocation values from soil to plant tissue followed almost the similar or regular pattern indicating enrichment of metals in the contaminated soils and their frequent translocation from soil to plant. The TF values also reveal that some species like *R. sativus* L. and *B. napus* L. show higher biomagnifications of metals. Different TF values under different plant species grown under various levels of sewage contamination suggest that GSH serves to alleviate the toxicity of heavy metals in plants (Singh et al. 2003), which have high affinity for binding metal cations such as Cd and Zn in the rhizosphere of soil–plant system.

Cadmium and zinc phytoaccumulation

Phytoaccumulation of cadmium in different crop species varies from 2.0 to 10.6 (mean 5.7) mg kg^{-1} for *R. sativus* L., 2.2–4.3 (mean 2.9) mg kg^{-1} for *B. napus* L., 1.13–4.3 (mean 2.4) mg kg^{-1} for *T. foenumgraecum* L. and 0.67–3.5 (mean 1.8) mg kg^{-1} for *B. oleracia* L. (Fig. 4a). Cadmium accumulation in different vegetable species grown under sewage-irrigated soils ranged from 0.67 to 10.6 mg kg^{-1} and the variation in build-up of Cd and Zn in soils and vegetable crops are attributed to a larger variation in the initial values of Cd and Zn in soils prior to study and preferential absorption of a particular cation by different crop species under study.

The extractable Cd in soils exhibits highly significant correlation with Cd accumulation in shoots of *R. sativus* L. ($r = 0.98^{***}$, $P < 0.001$), *B. napus* L. ($r = 0.97^{**}$, $P < 0.01$), *T. foenumgraecum* L. ($r = 0.99^{***}$, $P < 0.001$) and significant correlation with *B. oleracia* L. ($r = 0.91^{*}$, $P < 0.05$). The findings suggest that frequent application of sewage water on agricultural land increases the extractable Cd which augments Cd concentrations in different plant species nearly to the level of phytotoxicity. Hence, precaution in biomonitoring of heavy metals status of sewage-irrigated soils is needed for the future application of sewage-irrigation to the plants in agricultural farms.

Different plant species absorbed different concentrations of Cd, and the following order was observed: *R. sativus* L. > *B. napus* L. > *T. foenumgraecum* L. > *B. oleracia* L. A linear relationship (regression analysis) between Cd concentration in plant species and extractable Cd was formulated as given below:

Raphanus sativus L.: Cd = 36.51 + 1.44 extractable Cd ($r = 0.98^{***}$, $P < 0.001$)
Brassica napus L.: Cd = 9.37 + 1.98 extractable Cd ($r = 0.97^{**}$, $P < 0.01$)
Trigonella foenumgraecum L.: Cd = 12.43 + 1.04 extractable Cd ($r = 0.99^{***}$, $P < 0.001$)
Brassica oleracia L.: Cd = 11.42 + 0.611 extractable Cd ($r = 0.91^{*}$, $P < 0.05$)

These regression equations are suitable tools for prediction of concentration of cadmium in different plant species with the help of estimated of extractable Cd in soils. The rate of metal uptake by these plants has also been affected by other factors such as plant species, soil pH, nature of soil and climate. Elevated levels of Cd in crops may have certain health hazards to humans and animals consuming these crops. For instance, Cd exposures result in kidney damage, bone deformities, and cardiovascular problems.

The Zn content in plants ranged from 9.0 to 31.5 (mean 20.8) mg kg^{-1} for *R. sativus* L., 5.5–20.8 (mean 12.1) mg kg^{-1} for *B. napus* L., 5.5–21.0 (mean 11.4) mg kg^{-1} for *B. oleracia* L. and 4.4–12.77 (mean 7.8) mg kg^{-1} for *T. foenumgraecum* L. (Fig. 4b). Zinc concentration in different crops certainly depended upon different levels of sewage-irrigation (at 0, 20, 40, 60, 80 and 100 mL kg^{-1} soil). Different plant species varied in their zinc concentration in the following sequence: *R. sativus* L. > *B. napus* L. > *B. oleracia* L. > *T. foenumgraecum* L.

The extractable Zn in soils was significantly correlated with Zn concentration in *R. sativus* L. ($r = 88^{*}$, $P < 0.05$), *B. napus* L. ($r = 0.89^{*}$, $P < 0.05$), *T. foenumgraecum* L. ($r = 0.96^{**}$, $P < 0.01$) and *B. oleracia* L. ($r = 0.87^{*}$, $P < 0.05$). These results suggest that successive dosage of sewage-irrigation increases the Zn build-up in crops as compared to ground-water-irrigated soils.

The linear relationships (regression equations) between Zn concentration in crops and extractable Zn in soils are given below:

Raphanus sativus L. Zn = 1.48 + 9.05 DTPA-Zn ($r = 0.88^{*}$, $P < 0.05$)
Brassica napus L. Zn = 1.16 + 2.76 DTPA-Zn ($r = 0.89^{*}$, $P < 0.05$)
Trigonella foenumgraecum L. Zn = 0.60 + 2.83 DTPA-Zn ($r = 0.96^{**}$, $P < 0.01$)
Brassica oleracia L. Zn = 1.08 + 2.65 DTPA-Zn ($r = 0.87^{*}$, $P < 0.05$)

Thus, Zn concentration in various vegetable crops can be computed and predicted with the help of estimated extractable Zn through the aforesaid regression equations. Heavy metal concentration of vegetables grown in the vicinity of sewage-irrigation indicates that crop species

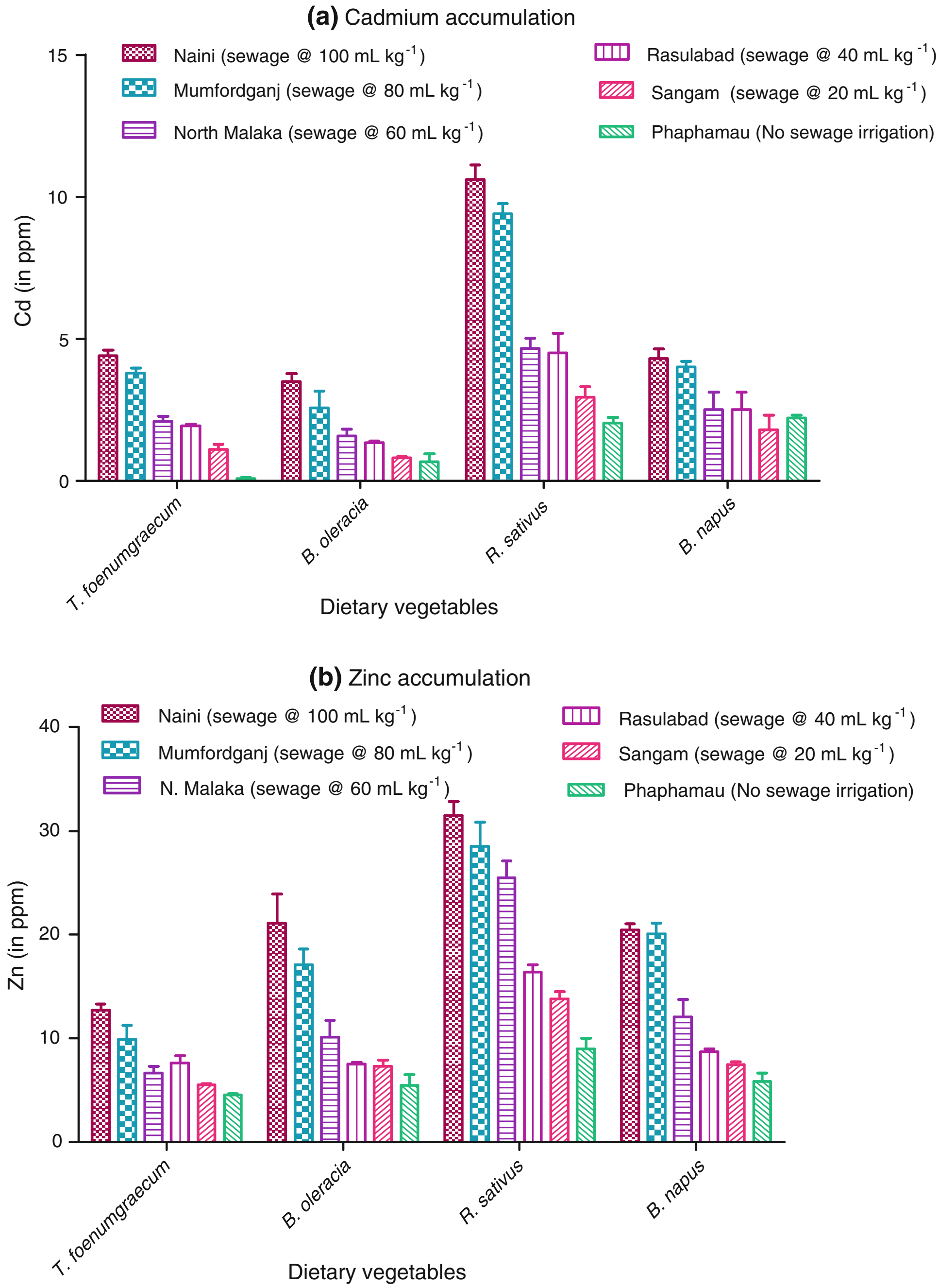


Fig. 4 Phytoaccumulation of **a** cadmium and **b** zinc in dietary vegetables grown in sewage irrigated soils; data are mean values of three replications (mean ± SD); mean accumulation of Cd in shoots of vegetables is as follows: *Raphanus sativus* L. (5.7 mg kg^{-1}), *Brassica napus* L. (2.9 mg kg^{-1}), *Trigonella foenumgraecum* L. (2.4 mg kg^{-1}) and *Brassica oleracia* L. (1.8 mg kg^{-1}). Mean accumulation of Zn in shoots of vegetables is as follows: *Raphanus sativus* L. (20.8 mg kg^{-1}), *Brassica napus* L. (12.1 mg kg^{-1}), *Brassica oleracia* L. (11.4 mg kg^{-1}) and *Trigonella foenumgraecum* L. (7.8 mg kg^{-1})

exhibit differently in metal accumulation in their shoot tissues. The study also reveals that Cd is absorbed more rapidly than Zn by the shoot tissues of plants. By and large, concentrations of both metals in all the crops were below the generally accepted critical levels of phytotoxicity (>50 mg kg^{-1}). *Raphanus sativus* L. (5.7 and 20.8 mg kg^{-1}) and *B. napus* L. (2.9 and 12.1 mg kg^{-1}) were observed good accumulators of cadmium and zinc, respectively; therefore, growing of both these crops, for human consumption, on the sewage-contaminated soils should be minimized or restricted; and to reduce the health risk and the extent of heavy metal contamination in soils, steps for phytoremediation must be undertaken for efficient treatment of sewage, while regular and constant monitoring of heavy metals in the vegetable-growing areas, especially in sewage-irrigated soils, is also equally important.

Variation of glutathione in dietary vegetables at different stages of plant growth

The content of GSH in leaves of the selected four dietary vegetables at their early stage and mature stage of

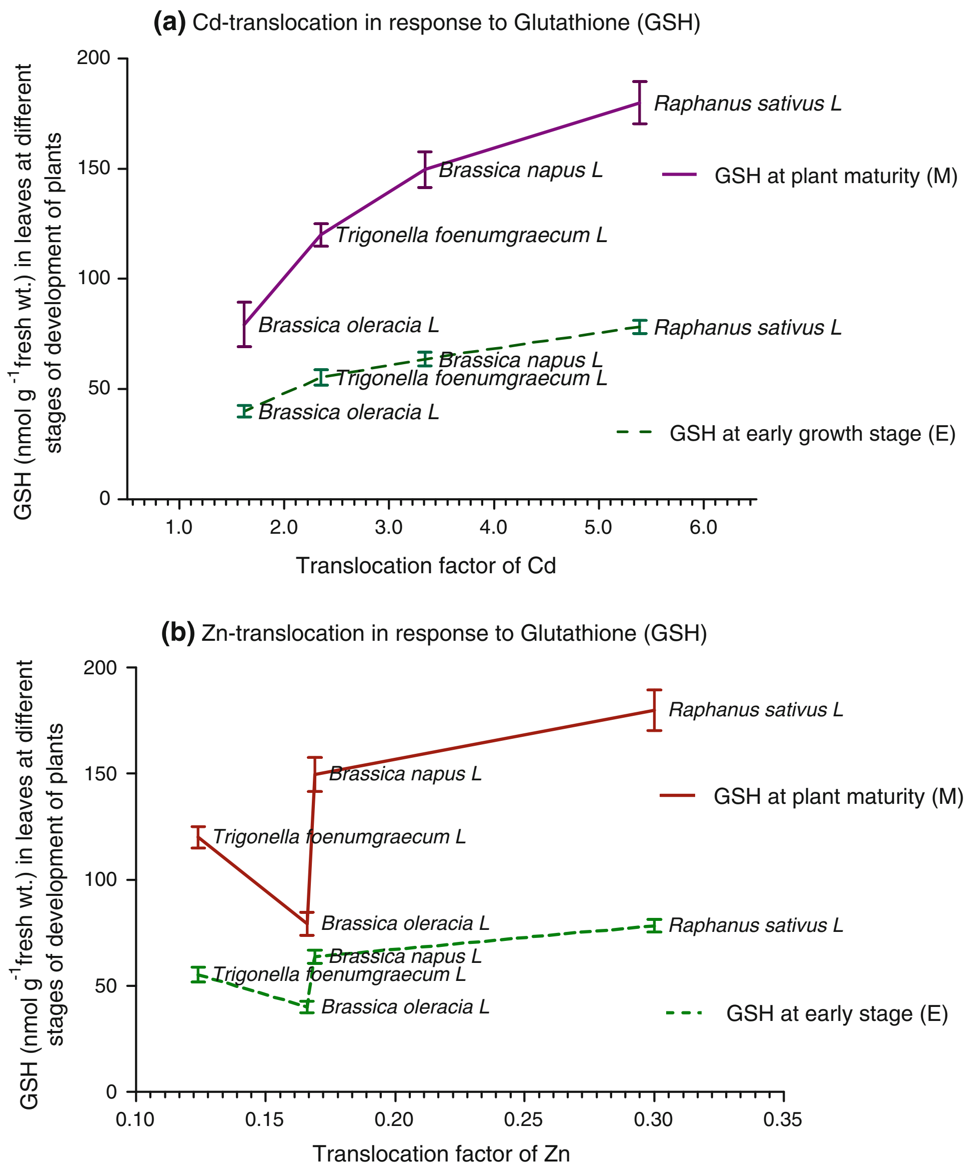


Fig. 5 Translocation of **a** cadmium and **b** zinc from soil to plant leaves in response to naturally occurring glutathione; data are mean values of three replications (mean ± SD); *Raphanus sativus* L. and *Brassica napus* L. possess comparatively higher glutathione (GSH) as well as show higher translocation of Cd and Zn from soil to plant tissues

development and their respective cadmium and zinc TF are summarized in Fig. 5a, b, respectively. The GSH contents in early stage of *R. sativus* L., *B. napus* L., *T. foenumgraecum* L. and *B. oleracia* L. were 78.33, 63.67, 55.33 and 40.0 n mol g^{-1} fresh weight (FW), respectively. In mature aforesaid plants the GSH contents were 180.0, 149.67, 120.0 and 79.33 n mol g^{-1} FW, respectively. Results show that during the maturity of these plants the GSH content increased up to 129.79, 135.08, 116.87 and 98.33 %, respectively. The perusal of Fig. 5a, b also reveals that out of the four tested species, *R. sativus* L. and *B. napus* L. (Brassicaceae family) both species are capable of translocating higher quantity of cadmium and zinc from soil to the leaves of plants due to their higher GSH content naturally occurring in their leaves. These results suggest that the GSH content as well as metal phytoremediation potential of these dietary vegetables increased with the maturity of plants. The increase in GSH content in maturity stage was recorded 1.98- to 2.30-fold higher over the early growth stage of these plants. The above data reveal that GSH is linked with enhancement of plant immune system and plays a major role in the protection of plants against photo-oxidative processes. The study reveals that there was sharp decrease in day temperature to the extent of mean 4 °C from the early growth stage of plant to the plant maturity. The temperature was recorded minimum during the maturity stage of *B. napus* L. which was observed at 90 DAS; whereas, the maturity stage of the remaining three vegetable species was observed at 60 DAS. Low temperature (33 ± 4.1 °C) during the plant maturity as compared with the higher temperature (37 ± 1.3 °C) during the early stage of plant growth is an important factor responsible for enhancement of GSH-like phytochemicals, especially in *Brassica* species of the dietary vegetables commonly grown in the vegetable production sites. The study indicated significant variation in GSH levels with the stage of development of plants. Singh et al. (2010) reported significant variation in some phytochemicals in *T. foenum-gracecum* L. at different stages of development of plants.

Correlation between glutathione in leaves of mature plants and metal hyperaccumulation potential

Analysis of plant species indicated positive correlation ($r = 0.95^*$, $P = 0.046$) between shoot GSH concentrations with Cd translocation ability in the investigated soils (Fig. 5a); however, positive correlation ($r = 0.88$, $P = 0.12$) between shoot GSH concentration with the Zn translocation ability was also observed (Fig. 5b). Such evidence supports the conclusion that elevated GSH concentrations are involved in metal translocation in hyperaccumulators, especially in *Brassica* species, which ensure their phytoremediation potential in the sewage-irrigated soils. The results are in conformity with the findings of Freeman et al. (2004).

The present study study reflects that interactions of soil–plant system and natural biosynthesis of GSH within plants play important role in regulating heavy metal movement from soil to the edible part of the plants. Given the dietary exposure of heavy metals from the contaminated vegetables, urban and sub-urban agriculture has now become a particular research topic, especially in metropolitan cities, which deserves special attention; besides these, high organic matter, CEC, microbial activities and root exudates also lead to create redox conditions and affect metals availability around the rhizosphere in soils. These bioremediation processes for metal uptake and metal detoxification should be the future research criteria and focus must be given on biological indicators, such as species biodiversity, soil invertebrate, plant biochemical assays and soil microbial assays (Aelion et al. 2009), which would improve the assessment of soil quality rather than physico-chemical measurement alone.

Conclusion

Frequent sewage-irrigation on agricultural land increases the DTPA-extractable heavy metals (Cd and Zn) in soils, causes manifold build-up of Cd and Zn in soils and augments cadmium and zinc accumulation in plants. Heavy sewage application (at 100 mL kg^{-1} soil) adversely increases the heavy metal concentration of a soil, leading to increased plant uptake of heavy metals, which is injurious to human and animal health. Thus it is justifiable either to improve the capacity of municipal sewage treatment plants before such sewage water is diverted for irrigation or the community opt for phytoremediation technology. The maximum accumulation of Cd and Zn in *R. sativus* L., followed by *B. napus* L., supports the views that both crops are good accumulators of Cd and Zn from the soils and growing of such *Brassica* species for human dietary consumption in the sewage-irrigated sites should be avoided. The sewage treatment plant (technology) should involve steps like phytoremediation technology to remove heavy metals causing risk to human health.

The presence of phytochemicals in *Brassica* species is greatly influenced by environmental conditions and degree of development. The present study suggests that GSH increases with maturity and enhanced level of natural GSH is synergistically related to the metals and nutrient translocation from soil to plants. It is, therefore, suggested that mature unused parts of these plants possess high potential for extraction of valuable phytochemicals as well as it would be an innovative technology for phytoremediation. It is also recommended that the impact of toxic heavy metals be studied in the individual cities and in semi-urban areas where wastewater irrigation has become a common practice and that crops with least susceptibility to contamination be grown for human consumption. The present findings provide us a clue for the site-wise selection of plant species, especially the *Brassica* species, which contain higher GSH like phyto-chemicals, for detoxifying and phytoaccumulating heavy metals in the soil.

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