

Health risk assessment of potentially harmful elements and dietary minerals from vegetables irrigated with untreated wastewater, Pakistan

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Abstract In the developing world, vegetables are commonly grown in suburban areas irrigated with untreated wastewater containing potentially harmful elements (PHEs). In Pakistan, there is no published work on the bioaccessibility aspect of PHEs and dietary minerals (DMs) in sewage-irrigated soil or the vegetables grown on such soils in Pakistan. Several industrial districts of Pakistan were selected for assessment of the risk associated with the ingestion of vegetables grown over sewage-irrigated soils. Both the total and bioaccessible fraction of PHEs (Cd, Co, Cr, Ni, and Pb) and DMs (Fe, Cu, Mn, Zn, Ca, Mg, and I) in soils and vegetable samples were measured. The concentrations of these PHEs and DMs in sewage-

irrigated and control soils were below published upper threshold limits. However, compared to control soils, sewage irrigation over the years decreased soil pH (7.7 vs 8.1) and enhanced dissolved organic carbon (1.8 vs 0.8 %), which could enhance the phyto-availability of PHEs and DMs to crops. Of the PHEs and DMs, the highest transfer factor (soil to plant) was noted for Cd and Ca, respectively. Concentrations of PHEs in most of the sewage-irrigated vegetables were below the published upper threshold limits, except for Cd in the fruiting portion of eggplant and bell pepper (0.06–0.08 mg/kg Cd, dry weight) at three locations in Gujarat and Kasur districts. The bioaccessible fraction of PHEs can reduce the context of dietary intake measurements compared to total concentrations, but differences between both measurements were not significant for Cd. Since the soils of the

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sampled districts are not overly contaminated compared to control sites, vegetables grown over sewage-irrigated soils would provide an opportunity to harvest mineral-rich vegetables potentially providing consumers 62, 60, 12, 104, and 63 % higher dietary intake of Cu, Mn, Zn, Ca, and Mg, respectively. Based on Fe and vanadium correlations in vegetables, it is inferred that a significant proportion of total dietary Fe intake could be contributed by soil particles adhered to the consumable portion of vegetables. Faecal sterol ratios were used to identify and distinguish the source of faecal contamination in soils from Gujranwala, Gujarat, and Lahore districts, confirming the presence of human-derived sewage biomarkers at different stages of environmental alteration. A strong correlation of some metals with soil organic matter concentration was observed, but none with sewage biomarkers.

Keywords Sewage irrigation · Potentially harmful elements · Dietary minerals · Micronutrients · Transfer factor · Bioaccessibility · Dietary intake · Faecal sterols

Introduction

The majority of cities in the developing world generate sewage that is either disposed of untreated into rivers and streams or used by growers in suburban areas to irrigate and fertilise crops (Kunhikrishnan et al. 2012). There is often no separate drainage system for industrial and domestic effluents, rather drains carry a blend of both. In Pakistan, the untreated sewage is directly used for irrigating about 32,500 ha (Murtaza et al. 2010; Ensink et al. 2004). Vegetables irrigated with this untreated sewage are typically common crops that attain higher prices in close proximity to urban markets. Approximately, 26 % of national vegetable production is irrigated with wastewater (Ensink et al. 2004). The wastewater used for irrigation is valued by farmers, mainly because of its nutrient content and reliability of supply. In some cases, sewage is auctioned by the municipalities to the highest bidder, often a group of rich farmers, who then rent out their fields to poor landless farmers. Under these conditions, the use of sewage is considered an ideal solution by the authorities responsible for

sewage disposal and the farmers who get its reliable supply with a high nutrient (N, P, K, Ca, Mg, S) and micronutrient content. Ensink et al. (2004) estimated that the average gross margin of US\$ 173 ha⁻¹ for a farmer irrigating with sewage in Pakistan was substantially higher than US\$ 43 ha⁻¹ for farmers using canal water, due to the higher cropping intensities and higher market values of crops.

The potential for contamination of urban agricultural soils from usage of untreated wastewater for vegetables and fodder production is a matter of concern. In particular, with respect to the speciation (e.g. toxicity, mobility) and bio-accessibility of potentially harmful elements (PHE), such knowledge of geochemical data is essential to provide an estimation of risk to health and enable effective land use planning or remediation strategies. In order to identify and remove unacceptable risks to human health and the environment, there is a clear need for a practical methodology that measures the fraction of the contaminants in the soil that can enter the systemic circulation of the human body and cause toxic effects (Ryan et al. 2004). For PHEs in soil, the most important exposure route is through ingestion, and therefore measurement of oral bioaccessibility of a substance, defined as the fraction that is soluble in the gastrointestinal environment and is available for absorption, is important for robust human health risk assessments (Denys et al. 2012; Ruby et al. 1999).

There are only a few studies reported in the literature that focus on the use of published methods for the bioaccessibility measurement of PHEs together with dietary minerals (DMs) essential for human health in the consumable portions of vegetables, rather than total metal contents (Hu et al. 2013; Hur et al. 2011; Khouzam et al. 2011; Moreda-Piñeiro et al. 2011; Intawongse and Dean 2006, 2008). Pelfrène et al. (2015) reported the first bioaccessible concentrations for Cd in vegetables using the Unified BARGE Method (UBM). In Pakistan, Waseem et al. (2014) and Murtaza et al. (2010) reported PHEs and DMs in wastewater- and sewage-irrigated soils and paired vegetable crops. However, there is no published work on the bioavailability or bioaccessibility of PHEs and DMs in sewage-irrigated or contaminated soils and the vegetables grown thereon in Pakistan. Previous studies focused on total metal(s) content in the consumable portion of vegetables and compared those with the published upper threshold limits (Najam et al.

2015; Khan et al. 2015; Ismail et al. 2014; Randhawa et al. 2014; Mahmood and Malik 2014; Amin et al. 2013; Khan et al. 2013; Rehman et al. 2013; Ehsan et al. 2011; Mushtaq and Khan 2010). Since not all of the metal content of ingested vegetables is bioavailable, conclusions made solely on the basis of total metal concentration might be misleading (Denys et al. 2012; Ryan et al. 2004; Ruby et al. 1999).

This study selected several major cities from the upper Punjab in Pakistan for the first comparison of risk via ingestion of vegetables grown on sewage-irrigated soils in suburban areas compared to typical vegetables for from soils not treated with sewage which represent a typical basket of food produce, as represented by control sites at research farms. This is the first study from Pakistan with both the total and bioaccessible measurement of PHEs and DMs in sewage-irrigated soils and vegetables to provide an estimate of the contribution from sewage application to agricultural soils of PHEs and DMs, with subsequent soil-to-vegetable transfer estimates that may be used for human consumption. In addition, the measurement of faecal sterol biomarker compounds (Bull et al. 2002) provided confirmation of the presence of raw urban or human-derived sewage in the agricultural soils.

Materials and Methods

Location of studies

Study sites were selected from major cities (Faisalabad, Lahore, Gujranwala, Kasur, and Gujarat) of the upper Punjab in Pakistan in which suburban agriculture utilised untreated sewage irrigation to grow vegetable and fodder crops. All of the sampled locations were mapped using GIS coordinates and are shown in Fig. 1. All of these cities have industrial units that discharge untreated effluents directly to the sewage drains. The quality of the sewage from these drains varies seasonally and has been reviewed elsewhere (Waseem et al. 2014; Murtaza et al. 2010). For comparison, chemical analyses of vegetable produce grown over canal-irrigated soils (control-1) were also collected from vegetable research farms of the Ayub Agriculture Research Institute (AARI) in Faisalabad. These farms were located in the middle of this industrial

city, with an established history of irrigation with ‘clean’ canal water and occasionally with tube well water. To compare the sewage-irrigated soils with a broader range of country wide soils, a data set from a previous study (Zia et al. 2015) was used (control-2), from which 84 soils were analysed from wheat-growing regions across Pakistan which are irrigated with rain, underground tube well, or canal water.

No records exist to quantify the volume of wastewater irrigation applied to each vegetable crop. The frequency of wastewater irrigation depends upon the season of crop, crop water requirements and number of cuttings of vegetable crop, e.g. spinach, coriander, and fenugreeks. As per the information collected by the authors during sample collection, farmers use wastewater for irrigation purposes every 7 days during the summer months (25 April to 15 September) and every 15 days during the winter months (16 September to 24 April). Many farmers also grow off-season ‘premium’-priced vegetables to improve productivity and income from land resources. For these out-of-season vegetables, farmers frequently use wastewater to overcome the stresses of high temperature during summer months, at approximately 3–4 times in winter months.

Soil and plant sampling

Soil samples were collected from 0 to 15 cm depth at all sites along with paired vegetables (edible portions) grown at these locations. This includes leaves for some vegetables, including green fodder for animal feed. The sampled vegetation included: eggplant (*Solanum melongena* L.), onion (*Allium cepa* L.), pumpkin (*Cucurbita maxima* L.), red chilli (*Capsicum annum* L.), bell pepper (*Capsicum annum* Group), radish (*Raphanus sativus* L.), bottle gourd (*Lagenaria siceraria* L.), cabbage (*Brassica oleracea* L.), turnip (*Brassica rapa* L.), bitter melon (*Momordica charantia* L.), okra (*Abelmoschus esculentus* (L.) Moench.), spinach (*Spinacia oleracea* L.), sugarbeet (*Beta vulgaris* L.), and pumpkin (*Cucurbita moschata* L.). All samples were returned to the laboratory and washed with clean tap water followed by three times washing with deionised water to remove soil particles adhered to the surface of the vegetables. After removing excess water from the vegetables with blotting paper, un-

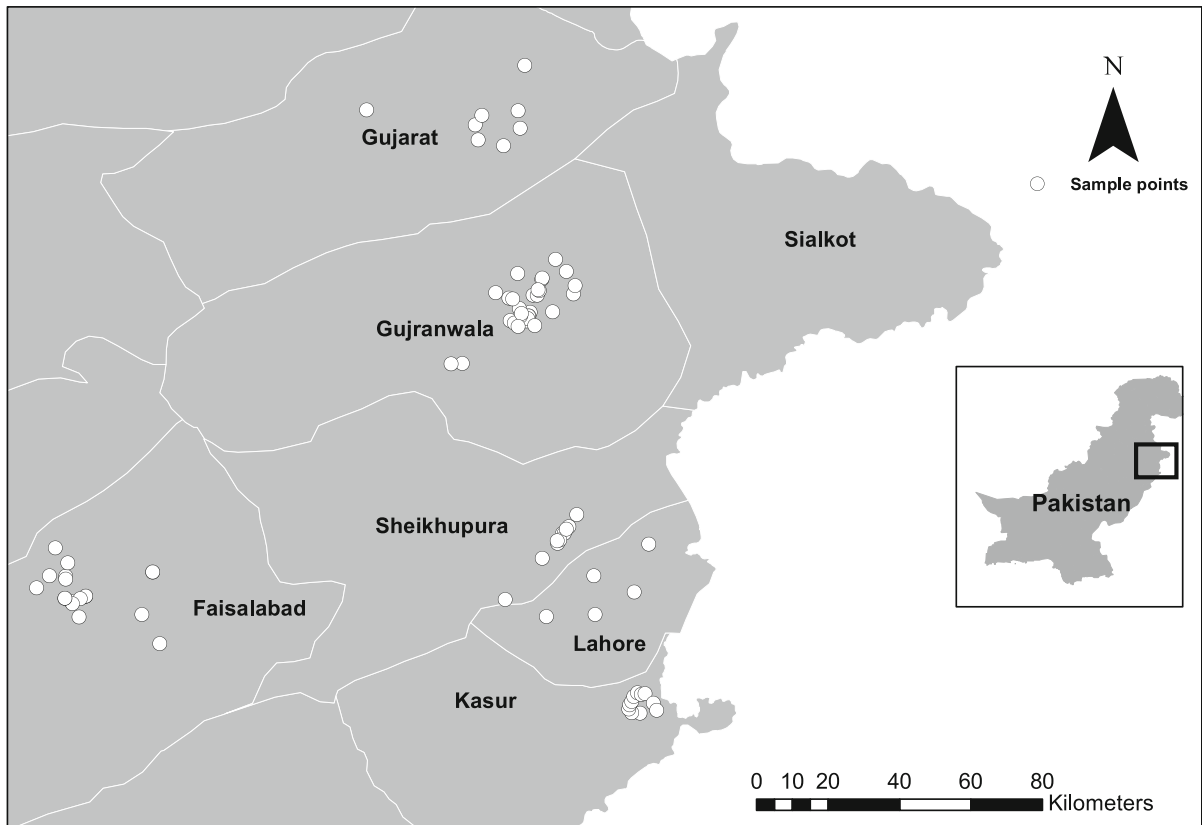


Fig. 1 Sampled locations for the soils and coupled vegetation samples

peeled samples were cut into pieces, packed into separate bags, and dried at 40 °C in an oven.

Analyses

Soil samples were air-dried, crushed, and sieved to 2 mm and 250 μm for bioaccessibility and soil pH measurement. Soil samples were further ground to $<32 \mu\text{m}$ in an agate ball mill for elemental analysis by inductively coupled plasma mass spectrometry (ICP-MS). Plant samples were passed through a food blender prior to milling, as appropriate. For a general suite of elemental analyses, soil samples (0.25 g) were digested in a mixed acid solution (2.5 ml HF/2 ml HNO_3 /1 ml HClO_4 /2.5 ml H_2O_2) on a programmable hot block (Watts et al. 2008); 0.5 g of plant samples was digested in 10 ml HNO_3 /1 ml H_2O_2 mixed solution in a closed-vessel microwave heating system (MARS Xpress). Subsequent total elemental analysis was carried out by ICP-MS (Agilent 7500cx) using (1) collision cell mode (He gas) for Li, Be, B, Na, Mg, Al,

P, S, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Y, Zr, Nb, Mo, Ag, Cd, Sn, Sb, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Tl, Pb, Bi, Th and U; and (2) H_2 -reaction cell mode for Si (in plant material) and Se (Joy et al. 2015a, b). Iodine analyses for soil samples (0.25 g) were extracted using 5 % tetramethyl ammonium hydroxide (TMAH), as described in Watts and Mitchell (2009) and Watts et al. (2010), at 70 °C in a drying oven. Vegetable samples (0.5 g) were extracted using 5 % TMAH in a closed microwave vessel system to ensure complete extraction and breakdown of cellulose material, as described in Watts et al. (2015). All iodine measurements were made by ICP-MS (Agilent 7500cx) in no-gas mode, using tellurium as an internal standard and all standards and samples in a final matrix of 0.5 % TMAH.

To measure the bioaccessible fraction (BAF) of PHEs and DMs in soils and vegetable samples, methodology validated by the Bio-Accessibility

Research Group of Europe (BARGE), known as the Unified BARGE Method (UBM) was utilised. The methodology simulates conditions in the gastrointestinal tract to assess the human bioaccessibility of PHEs and DMs by ingestion. The UBM has been validated for As, Cd, and Pb against an *in vivo* model and employed as a standard method in the UK and by BARGE research collaborators across Europe. Details of the ingredient chemicals and concentrations used for the extraction during oral and gastrointestinal phase can be seen elsewhere (Hamilton et al. 2015).

Certified reference materials (CRMs) were used within each extraction batch to monitor the performance of the total and bioaccessible extraction and subsequent analysis by ICP-MS, soils/sediments (MESS-3, JR-2, BCR-2, BGS 102) and plants (NIST 1573a tomato leaves, NIST 1570a spinach leaves, and NCS ZC 73013 spinach leaves). All measurements were within $\pm 15\%$ of target concentrations, ranging from 1 to 5 repetitions of each CRM (Supplementary Info: Tables 1a, 1b, and 1c).

For soils, the threshold values used for comparison reported in this work have been extracted from several reports from the European Commission (2009), Awashthi (2000), Kabata-Pendias and Mukherjee (2007), Canadian Environmental Quality Guidelines by Canadian Council of Ministers of the Environment (2015) and State Environmental Protection Administration, China (1995). The threshold range (lower and upper limits shown as blue and red dotted lines in figures, respectively) reported for vegetables in this work has been extracted from several reports from the European Commission (2006), FAO/WHO (2011) and State Environmental Protection Administration, China (1995). For Cd, FAO/WHO and the Commission of European Economic Community set a maximum level (ML) in vegetables based on produce group, on a wet weight basis (FAO/WHO 2011). The ML for leafy vegetables is 0.2 mg/kg, stem and root vegetables 0.1 mg/kg, and for fruiting vegetables (excluding leafy vegetables) 0.05 mg/kg. The ML for lead (Pb) in vegetables (excluding brassica and leafy vegetables) is 0.1 mg/kg, whereas the limit for brassica and leafy vegetables is 0.3 mg/kg.

Five metrics were used to measure the presence of sewage biomarkers: (1) sterol concentrations to define the presence of sewage in soil, (2) 5β -stigmastanol to define sewage from animals, (3) coprostanol–cholesterol ratio to define human sewage, (4) coprostanol–

5α -cholestanol + coprostanol ratio also to define human sewage, and (5) epi-coprostanol–coprostanol ratio to define ageing and environmental alteration of sewage. The method used is described in Vane et al. (2010). The GC–MS (gas chromatography–mass spectrometry) limit of quantification for individual compounds ranged from 0.01 to 0.04 $\mu\text{g/g}$ (dry weight). An urban river sediment was used as an in-house quality control material (Supplementary Info: Table 2).

Daily intake of PHEs and DMs

An average daily intake of PHEs and DMs from vegetable crops was previously reported by Murtaza et al. (2010) and Qadir et al. (2000). The daily intake (DI) was calculated using the following Eq. 1.

$$DI = (C_{\text{metal}} \times C_{\text{factor}} \times D_{\text{food intake}}) \quad (1)$$

where the C_{metal} represents the metal concentrations in crop species (mg/kg), C_{factor} is the conversion factor, and $D_{\text{food intake}}$ is the daily intake of food crops (kg). Conversion factor (CF) of 0.087 was used to convert fresh vegetables weight to dry weight. The average daily consumption of food crops was 0.25 kg per person per day (Qadir et al. (2000). Average adult (19–50 years) body weight in the study area was assumed to be 70 kg. Daily intake values in the manuscript are reported as mg.

Soil contamination of vegetables (dust loading)

Foliar uptake from dust could be a pathway for edible parts of vegetables to become contaminated with metals in urban agriculture near to industrial areas (Schreck et al. 2012). According to the findings of De Temmerman et al. (2012), in celeriac leaves exposed to particulate matter, approximately 25 % of Cd accumulated in dust deposits could be removed by thoroughly washing the leaves. Similar findings were reported by Xiong et al. (2014a, b) for leafy vegetables where Zn concentration in the exposed vegetables was 5–6 times higher than that in control vegetables. Indeed, even after thorough washing, cabbage and spinach leaves were still significantly contaminated by foliar uptake-surface adsorption of particulate matter such as Cd and Zn. Xiong et al. (2014a) concluded that this is highly influenced by the plant morphology and physiology (cuticular waxes,

stomata numbers) and the physicochemical properties of the metal species and particles (size, composition, solubility). Stomata openings are particularly enriched with pollutants, suggesting that this is a preferential uptake pathway. When exposed to airborne Pb particles, lettuce, radish, and parsley showed significant translocation of the metal towards roots.

Trace elements which are normally poorly available to plants can be used to estimate the likely proportion of soil contamination contributing to apparent crop micronutrient concentrations as described in Joy et al. (2015a, b). Extraneous contamination of plant samples with Fe, as an element of interest in this study, was estimated from the vanadium (V) concentration where coupled soil samples were taken. For example, the proportion of Fe in plant samples originating from contaminant soil or dust (C_{Fe}) was estimated from Eq. 2:

$$\% C_{Fe} = \frac{(R \times Fe_{soil})}{Fe_{veg}} \times 100 \quad (2)$$

where $\% C_{Fe}$ is the contribution of contamination from the dust in the collected samples; R is vanadium_{veg}/vanadium_{soil} (%) and Fe_{soil} and Fe_{veg} are corresponding concentrations in the soil and vegetation (mg/kg).

Results and discussion

Soil loading with potentially harmful elements (PHEs) and dietary minerals (DMs)

Potentially harmful elements (PHEs): Cd, Co, Cr, Ni, Pb, and V

Median, minimum, and maximum concentrations of the PHEs in soils collected from suburban areas of the five districts are shown in Fig. 2a. For Cd, Co, and Ni, the highest median values were observed for Gujranwala and Gujarat districts. For Cr, the highest median values were observed for Kasur where the leather tannery industry is prevalent with a sewage treatment plant that does not have capacity to treat Cr. All of the five industrial districts have no sewage treatment plants except for the primary treatment for 10 % of the sewage that tends to settle suspended solids. Control soils 1 and 2 (urban and arable) depicted higher Co and V median concentrations (13.5 and 12.9 mg/kg Co; 88

and 91.0 mg/kg V) compared to the sewage-irrigated soils from all five industrial cities. This could be due to the use of rock-phosphate-based fertilisers on the control soils for the past three to four decades (Akhtar et al. 2012). Farmers rarely apply chemical fertilisers to sewage-irrigated crops. The phosphate rock contains 23–457 mg/kg of V and 4.4–13.1 mg/kg of Co (Javied et al. 2009; Khiari et al. 2004). The Co concentrations are too low to cause any health or environmental effect, because average toxic effects on plants are unlikely to occur below the soil Co concentrations of 30 mg/kg (Kapustka et al. 2006).

Avci and Deveci (2013) also found similar or somewhat higher concentrations of Cd, and Co in control soils compared to sewage-irrigated soils in Turkey and attributed this to the influence of local geochemistry and regional soil characteristics. When compared to previously reported mean concentrations in soils measured throughout the world (He et al. 2005), Co, Cr, Pb, and Zn concentrations in all soil samples (including the controls) fell within the given range (Co 10–40 mg/kg; Cr 20–200 mg/kg; Pb 10–150 mg/kg; and Zn 100–300 mg/kg, or were similar. The concentrations of all the PHEs in sewage-irrigated and control soils are below the published threshold upper limits, see Table 1, except for a few samples at certain locations (Fig. 2a: Cd for a sample in Lahore; Cr for a sample in Gujarat and for two samples in Kasur; and Ni for a sample in Gujranwala). There is considerable lack of uniformity in maximum allowable concentrations from different countries, thus accounting for the wide range of values for all PHEs shown in Fig. 2a (and DMs shown in Fig. 2b). Generally, the lower concentrations of PHEs are associated with light sandy soils with a pH range of 5–6 (Avci and Deveci 2013). The permissible value for soil Pb is 100 mg/kg in most countries, whereas in the UK, it is 300 mg/kg. In the Netherlands, the permissible value for soil Cd is 0.8 mg/kg, but in other European countries it is 3 mg/kg (Kabata-Pendias and Mukherjee 2007).

In a previous study around Faisalabad (Murtaza et al. 2008), mean concentrations in sewage-irrigated soils were 0.08–0.10 mg/kg, 0.06–0.08 mg/kg, and 177–217 mg/kg for Cd, Co, and Mn, respectively. Despite the fact that Cd concentrations in soils were within permissible limits, higher concentrations of Cd in plant samples were observed due to bioaccumulation (Ensink et al. 2004). In another study around

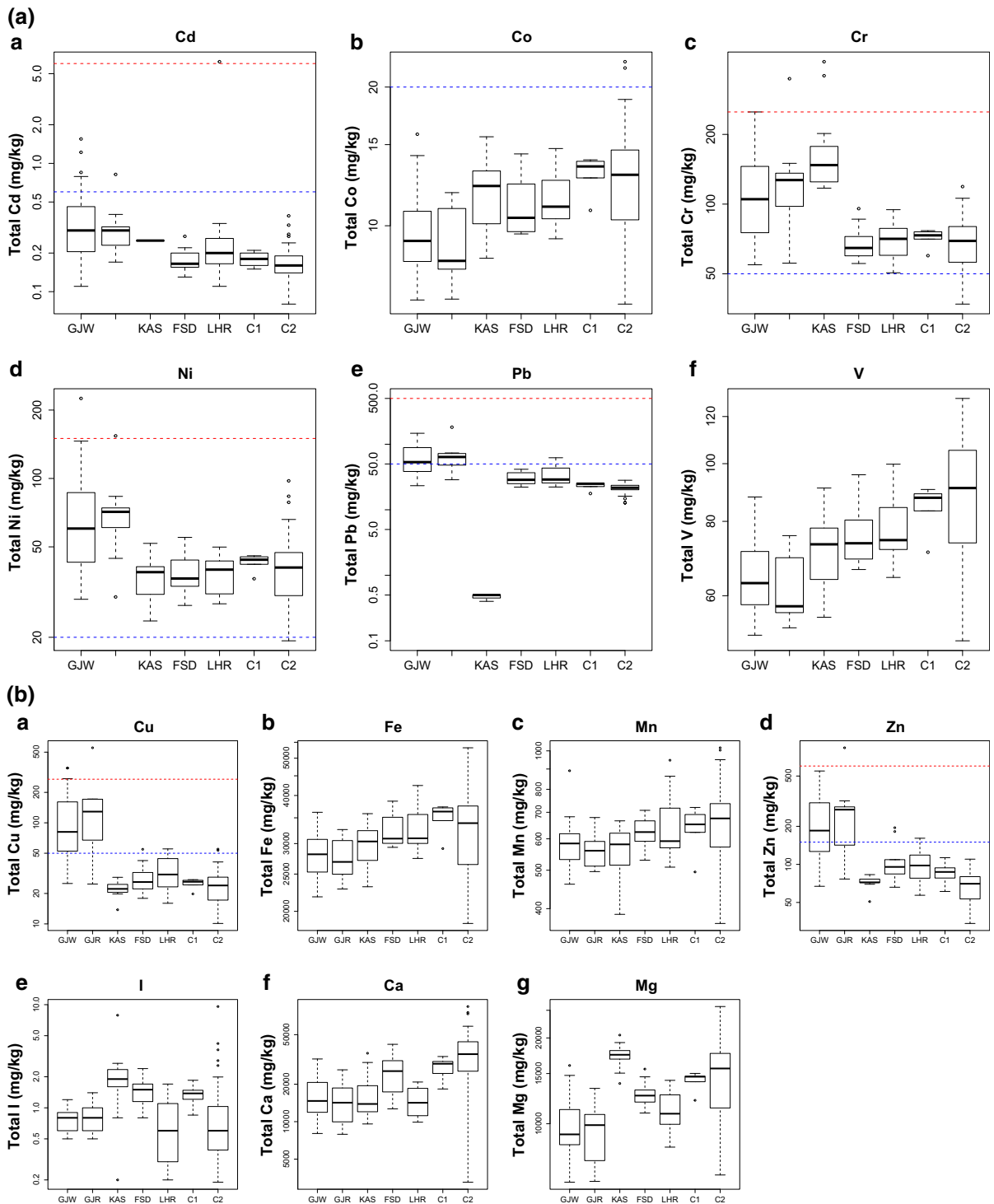


Fig. 2 **a** Concentration of PHEs (mg/kg) in soils across the sampled locations. **b** Concentration of DMS in soils across the sampled locations

Table 1 Published regulatory limits for PHEs and DMs (mg/kg, dry weight) in soils

	Cd	Co	Cr	Ni	Pb	Cu	Mn	Zn
European Commission (EC-DG) (2009)	1.5	n/a	100	70	100	100	n/a	200
Awashthi (2000)—India	3–6	n/a	n/a	75–150	250–500	135–270	n/a	300–600
Kabata-Pendias and Mukherjee (2007)	1–5	20–50	50–200	20–60	20–300	60–150	1500–3000	1.0–300
CCME (2015)—Canada	1.4	40	64	50	70	63	n/a	200
SEPA (1995)—China	0.6	n/a	250	60	350	200	n/a	300

n/a data not available

Lahore (Mahmood and Malik 2014), wastewater-irrigated versus clean-water-irrigated soils had mean concentrations of Cr—21.0 versus 14.0 mg/kg; Co—8.0 versus 3.65 mg/kg; Ni—28.8 versus 14.5 mg/kg; Pb—15.4 versus 7.4 mg/kg; and Cd—3.2 versus 1.2 mg/kg.

It appears from our analysis that compared to control soils irrigated with clean water, sewage increased the concentration of Cd, Cr, Ni, and Pb in soils for the districts of Gujranwala, Gujarat, and Kasur (for Cr only), but not enough to exceed the published threshold upper limits (Table 1). For the districts of Kasur, Faisalabad, and Lahore, sewage irrigation did not influence the build-up of PHEs in soils when compared with control soils. Although lower in pH compared to control soils, sewage-irrigated soils are still alkaline in reaction (pHs > 7.0) which would likely retard the release of Cr, Ni, and Pb from chelates, precipitates, complexes and ion pairs, clay particles, and some other metal interactions (Kunhikrishnan et al. 2012).

Dietary minerals (DMs): Cu, Fe, Mn, Zn, Ca, Mg, and I

Median, minimum, and maximum concentrations of the DMs in soils collected from suburban areas of the five districts are shown in Fig. 2b. For Cu and Zn, the highest median concentrations of Cu: 81 and 129 mg/kg; and Zn: 185 and 271 mg/kg were observed for Gujranwala and Gujarat districts, respectively. For I, the highest median concentrations of 1.5 and 1.9 mg/kg were observed for sewage-irrigated soils from Faisalabad and Kasur, respectively. Control soils exhibited higher median concentrations for Fe, Mn, Ca, and Mg compared to sewage-irrigated soils. Higher concentrations of Mn and Mg in control soils

could be because of the application of rock-phosphate-based fertilisers. The rock-phosphate-based fertilisers being used in arable agriculture across Pakistan are reported to contain 178–5716 mg/kg of Mn and 7410–12,240 mg/kg of Mg (Javied et al. 2009). The concentrations of Cu, Fe, Mn, and Zn are higher than mean concentration reported by Khan et al. (2013) from sewage-irrigated soils in Gujranwala and Sialkot districts; and Mahmood and Malik (2014) from sewage-irrigated soils around Lahore. Khan et al. (2013) found mean concentrations of Cu, Fe, and Zn as 13.7, 1551, and 45.2 mg/kg, respectively, whereas Mahmood and Malik (2014) compared wastewater-irrigated versus clean-water-irrigated soils and reported mean concentrations of Cu—28.7 versus 15.3 mg/kg; Zn—50.8 versus 34.1 mg/kg; and Mn—39.0 versus 21.2 mg/kg.

The significant difference in pH between control and wastewater-irrigated soil were also observed on calcareous soils in Turkey and can be attributed to the impact of long-term influence of wastewater chemistry, causing an increase in hydrogen ion levels in the soil (Avci and Deveci 2013). The concentrations of Cu and Zn in soils are below the published thresholds range (upper limits—red dotted lines) except for the samples from Gujranwala and Gujarat where the values are above the published thresholds (lower limits—blue dotted line), see Table 1. Threshold limits for Fe, Mn, Ca, Mg, and I are not reported in the literature.

From the analysis of the seven DMs studied, sewage influenced the concentration of Zn and Cu in soils of Gujranwala and Gujarat and I in soils of Faisalabad and Kasur. At the same time, Gujranwala and Gujarat districts had also shown high concentrations of Cd, Cr, Ni, and Pb which pose a risk to the harvest of Zn- and Cu-rich vegetables from sewage-

irrigated soils of these two districts. Opposed to this, for the districts of Kasur, Faisalabad, and Lahore, there are no differences in Cu and Zn concentrations in sewage-irrigated soils in comparison with control soils. Therefore, sewage-irrigated soils of these three districts provide an equal opportunity to grow vegetables without any risk. This is also supported from PHEs (Cd, Cr, Ni, and Pb) concentrations that are almost similar to control soils in these three districts, except for Cr in Kasur district. The lower pH in sewage-irrigated soils might enhance the availability of several elements, including Zn and Cu for ready uptake by the vegetables and can also increase the leaching of Ca and Mg from the soil surface. The median value for pH of sewage-irrigated soils in this work is 7.7 against a median pH of 8.1 for control soils. The low pH seems directly responsible for low concentrations of Fe, Mn, Ca, and Mg in sewage-irrigated soils compared to its high concentration in control soils where high pH prevails. The median value for soil organic matter (%LOI) of sewage-irrigated soils is 1.8 compared to 0.8 for control soils. No significant increase in soil iodine was measured for the sewage-irrigated soils at 0.87 mg/kg, compared to 0.62 mg/kg for the control soils.

Uptake of potentially harmful elements (PHEs) and dietary minerals (DMs) by vegetables

Potentially harmful elements (PHEs): Cd, Co, Cr, Ni, Pb, and V

Median, minimum, and maximum concentrations of the PHEs in the coupled vegetable samples collected from suburban areas of the five districts are shown in Fig. 3a. For Co, Cr, Ni, and V, highest median concentrations among the five districts were observed for Gujranwala and Gujrat districts, but the concentrations were still lower than control vegetables (except for V). For Cd and Pb, the highest median concentrations were found for Gujarat, followed by (in decreasing order) Kasur, Gujranwala, Lahore, and Faisalabad districts, whereas the control vegetables had the lowest comparative concentrations for both of the PHEs. Interesting findings are for the Co, Cr, and Ni where control vegetables depicted higher median concentrations compared to the vegetables from all of the five industrial cities. This might be because of the contribution through urban dust

since control vegetables farms are situated in city centre although irrigated with clean water. Another possible reason could be the use of rock-phosphate based fertilisers for the last 30–40 years over these farms. In addition to soil chemistry, biological factors such as plant species and genotype can also influence the total uptake of an element into plant tissues.

It appears from the analysis that sewage irrigation has influenced the concentration of Cd in vegetables grown over all of the districts although the highest median concentrations are noted for Gujarat, and Kasur; Pb in vegetables grown over the districts of Gujarat, Gujranwala, and Kasur; and V in vegetables grown over the districts of Gujranwala and Gujarat. However, only PHE concentration of Cd exceeded the maximum limit (Table 2) in this work. Keeping in view the threshold limits, sewage-irrigated vegetables pose a greater risk only because of higher Cd when compared to vegetable produce on control soils. In preceding sections, sewage-irrigated soils of Gujarat and Kasur districts were described with higher concentrations of Cd compared to sewage-irrigated soils of Faisalabad and Lahore districts as well as controls. This also is transpired in the higher Cd uptake in vegetables grown over these districts. It appears that because of highly dissolved organic matter and frequent irrigation of sewage to vegetables, in comparison with control sites, Cd and possibly Pb was dissolved in soil solution is readily taken up by crops, being bioavailable in the immediate root medium. Del Castillo et al. (1993) also reported that all of the Cd in soils treated with cattle manure slurry was bound in relatively fast dissociating organic metal(loid) complexes. Moreover, higher concentration of inorganic anions like sulphate (SO_4^{2-}) and chloride (Cl^-) in sewage induce the formation of metal(loid)–inorganic complexes (e.g. Cd–Cl complex) which are even more phytoavailable (Weggler et al. 2004).

Summarily, the concentrations of all of the PHEs in vegetables are below the published thresholds range, except for Cd (median Cd 0.06–0.08 mg/kg, fresh weight basis) in the fruiting portion of eggplant and bell pepper at three locations each from Gujrat and Kasur districts. The upper limit of 0.05 mg/kg Cd on fresh weight basis for fruiting vegetables is used in this work as per guidelines set by the European Union Commission Regulation (2006); and FAO/WHO (2011). This could be due to the highest transfer

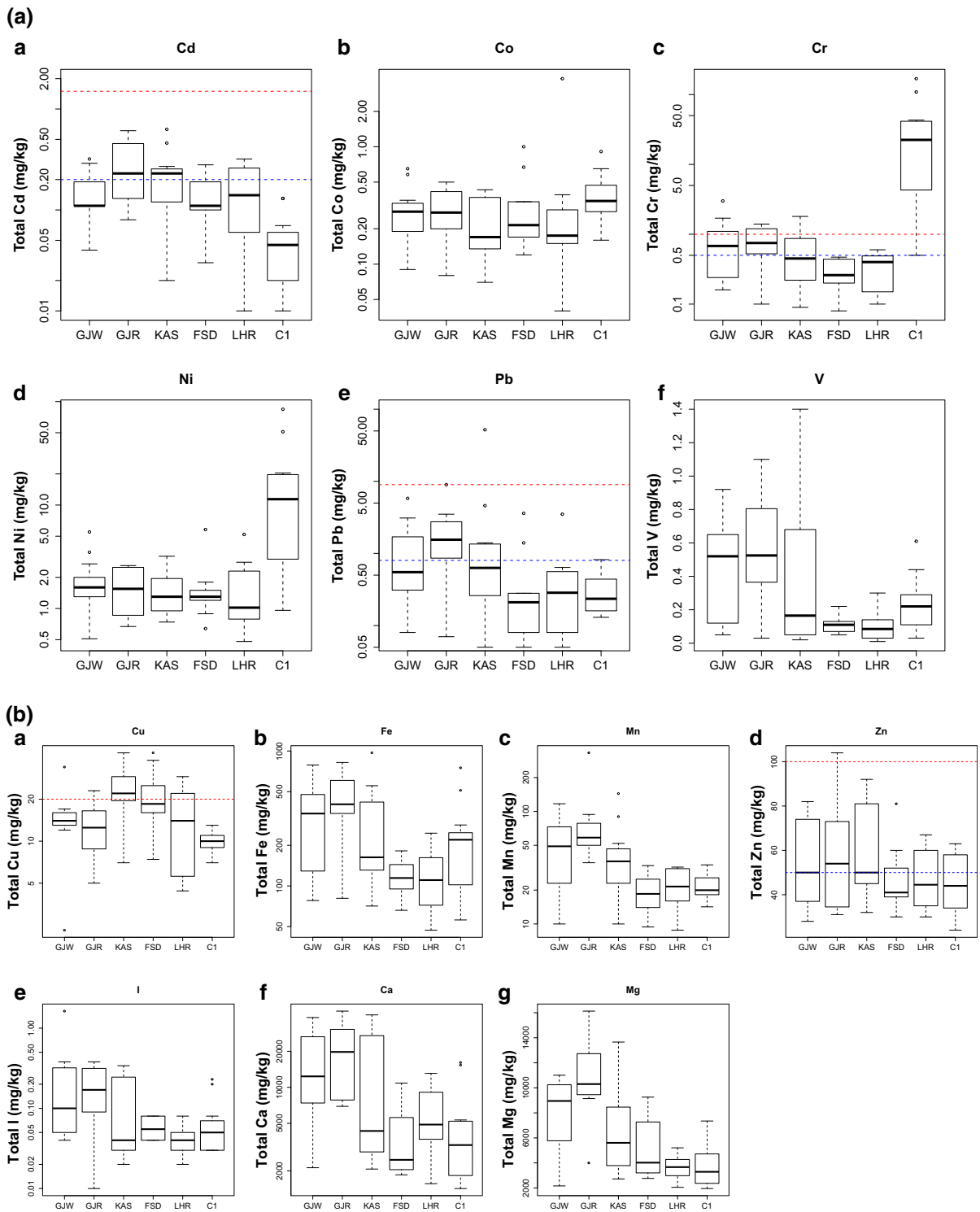


Fig. 3 **a** Concentration of PHEs in vegetables across the sampled locations. **b** Concentration of DMs (mg/kg) in vegetables across the sampled locations. **c, d** TF values for PHEs and DMs for the sampled vegetables

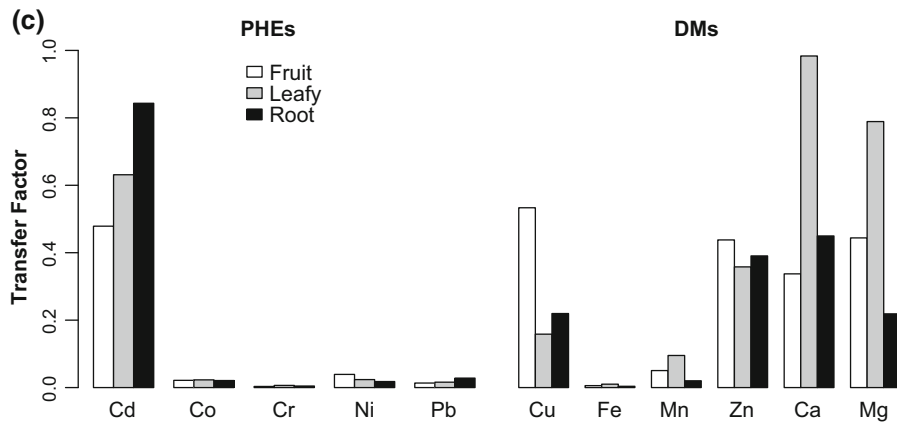


Fig. 3 continued

factor noted for Cd among the studied PHEs in this work. In a study by Qadir et al. (2000), mean Cd concentrations in the fruiting portions of eight diverse vegetables irrigated with sewage were found to be 0.08 mg/kg fresh weight. Ingestion of high-Cd containing crops may contribute substantial Cd to the human diet (Wagner 1993). Of primary concern is its transfer from vegetables to the human body, because vegetable foods contribute about 70 % of Cd intake in humans (Ryan et al. 1982). In a similar study, Murtaza et al. (2008) reported accumulation of PHEs in edible parts of vegetables grown with city effluent around Faisalabad within the following range: Cd 0.06–0.07 mg/kg leafy vegetables and 0.05–0.07 mg/kg in fruiting vegetables; Mn 45–129 mg/kg in leafy vegetables and 7.7–8.3 mg/kg in fruiting vegetables; and Co 0.03–0.05 mg/kg in leafy vegetables and 0.04 mg/kg in fruiting vegetables. Except for the Mn, other metals concentrations were lower than permissible limits (Cd 0.1 mg/kg; Mn 4.9 mg/kg; and Co 0.5 mg/kg) used by the authors.

The transfer factor (TF) quantifies the relative differences in bioavailability of metals to plants and is a function of both soil and plant properties. There is no published threshold set for TF, rather it is just an

indication for comparative risk via consumption of food grown over soil where several contaminants are present. In general, highest TF is reported for Cd while the lowest for Cr and Pb among the PHEs. The TF was calculated by dividing the concentration of an element in a vegetable crop (DW) by the total element concentration in the soil. The range of TF values for the edible portion of vegetable crops from published work of Avci and Deveci (2013) were 0.03–0.7, 0.003–0.1, 0.01–0.2, 0.01–0.2, and 0.01–0.4 for Cd, Co, Cr, Ni, and Pb, respectively. Low TFs reflect the strong sorption of element to the soil colloids (Alloway and Ayres 1997). Higher TFs reflect relatively poor retention in soils or greater efficiency of plants to absorb the element. Of the PHEs, the highest TF was measured for Cd (0.48–0.84), which was highest for root (0.84), followed by leafy (0.63) and fruiting (0.48) vegetables (Fig. 3c). Jan et al. (2010) reported that sewage-irrigated soils around Peshawar district (Pakistan), trace metal TFs for vegetables irrigated with wastewater ranged from 0.59–4.62, 0.003–0.015, 0.005–0.015, 0.56–1.32, 0.944–2.05, 0.05–0.07, 0.51–1.31 for Zn, Cd, Pb, Ni, Cu, Cr and Mn, respectively. In wastewater-irrigated soils around Lahore, Mahmood and Malik (2014)

Table 2 Published regulatory limits for PHEs and DMs (mg/kg) in vegetables

	Cd	Cr	Pb	Cu	Zn
EC/FAO/WHO (fresh weight basis)	0.05–0.2	n/a	0.1–0.3	n/a	n/a
EC/FAO/WHO (dry weight basis)	0.4–1.5	n/a	0.8–2.3	n/a	50
SEPA (2006)—China (dry weight basis)	0.2	0.5–1.0	9	20	100

n/a data not available

reported TF for vegetables in the range of 0.06–0.14, 5.5–6.7, 0.10–0.35, 0.1–0.2, 0.03–0.2, 0.06–0.6, 0.2–1.5, 0.5–1.2 for Cr, Co, Ni, Cu, Pb, Cd, Mn and Zn, respectively. Total concentrations of Cd, Pb, Cr and Co in vegetables grown over wastewater-irrigated soils were higher than maximum limits, while the concentrations of Ni, Cu, Mn and Zn were within published threshold limits, given in Table 2.

Dietary minerals (DMs): Cu, Fe, Mn, Zn, Ca, Mg, and I

Median, minimum, and maximum concentrations of the DMs in the coupled vegetable samples collected from suburban areas of the five districts are shown in Fig. 3b. For Fe, Mn, Zn, I, Ca, and Mg, the highest median concentrations were observed for Gujranwala and Gujarat districts. For Cu, highest median concentrations were observed for Kasur and Faisalabad. Median concentrations for Cu in vegetables from Kasur and Faisalabad were close to the maximum limit of 20 mg/kg, dry weight basis. Among the DMs, the highest TF (soil to plant) was noted for Ca, followed by Mg, Zn, and Cu. Among the vegetable subgroups, the Ca and Mg TF were highest for leafy vegetables, while Zn and Cu for fruiting subgroups (Fig. 3d). In a study by Arora et al. (2008), sewage-irrigated calcareous soils of India had a similar concentration range for DMs: 116–378 mg/kg for Fe, 12–69 mg/kg for Mn, 5.2–16.8 mg/kg for Cu, and 22–46 mg/kg for Zn. All of the vegetables had lower concentrations of Zn and Cu than respective permissible Indian limits of 60 and 40 mg/kg. For Cu, maximum limit in our study was chosen as 20 mg/kg in accordance with State Environmental Protection Administration, China (2006), see Table 2), dry weight basis, as opposed to Indian work where authors chose a limit of 40 mg/kg, dry weight basis although as per Indian standard (Awashthi 2000) the upper limit for Cu in vegetables is 30 mg/kg, dry weight basis.

It appears from the analysis that sewage did not influence concentration of Fe, Mn, Zn, I, Ca, and Mg in Lahore and Faisalabad districts as the concentrations of these DMs are comparable with that of vegetables grown over control soils. Since the vegetables grown over the districts of Faisalabad and Lahore had PHE concentrations similar to those of control soils, therefore sewage irrigation of vegetables in these districts would benefit from higher

bioaccumulation of DMs to the benefit of consumers. The DM of apparent concern in vegetables grown over these two districts is Cu that was approaching the threshold limit of 20 mg/kg Cu, dry weight basis. The threshold limit for Cu (20 mg/kg) seems very conservative since it could contribute 0.44 mg/day Cu thus provides only 22 % of the recommended DI. Keeping this in view, sewage-irrigated vegetables in Lahore and Faisalabad districts are quite safe to consume and comparable to control vegetables while providing the added advantage of supplying higher concentrations of DMs to consumers.

Bioaccessible fraction of potentially harmful elements (PHEs) in soils

Potentially harmful elements (PHEs): Cd, Co, Cr, Ni, and Pb in soils

A selected set of soils was analysed for the bioaccessible fraction (BAF) of PHEs and/or DMs. The analysis revealed that the median, minimum, and maximum bioaccessible fraction of PHEs were as follows (as % of total metal contents, Fig. 4a): Cd 72 (61–85); Pb 55 (42–69); Ni 26 (6–40); Co 16 (8–22); and Cr 14 (1–24). On sewage-irrigated soils, Cd bioaccessibility is the highest of the PHEs followed by Pb, and Ni, while the least bioaccessible were Cr and Co.

The Bioaccessibility Research Group of Europe (BARGE) method, used in this study, has been validated for soils fed to swine (Denys et al. 2012) and mimics the human digestive tract. The method has been employed to assess the bioaccessible fraction of PHEs and DMs from wide range of soils, sediments, dust, vegetables, fruits, grains, and other food. For comparison to our bioaccessibility work findings, we chose the studies of Broadway et al. (2010), Okorie et al. (2011), Barsby et al. (2012), and Hamilton et al. (2015) that reflected the use of the BARGE method for rural agricultural soils collected from across Northern Ireland (Barsby et al. 2012); urban recreational site near Newcastle Upon Tyne (Okorie et al. 2011) and certified reference material, BGS 102, an ironstone soil from Lincolnshire, UK (Hamilton et al. 2015). Compared to the referred studies (Okorie et al. 2011; Barsby et al. 2012; Hamilton et al. 2015), where Cd bioaccessibility was reported as the highest among the five PHEs, focused in this work, followed by Pb.

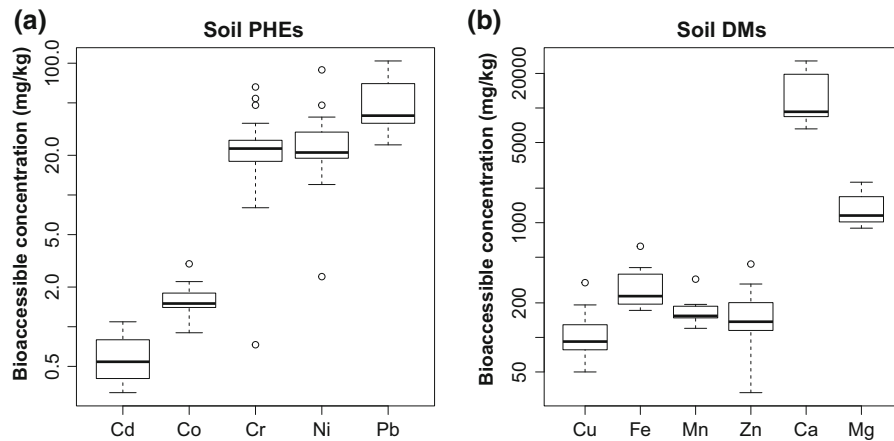


Fig. 4 Bioaccessible fraction of PHEs and DMs in soils

In this study, there were large differences in the minimum and maximum bioaccessibility of the different PHEs (and DMs), which indicate that they are bound to different fractions of the soil (organic matter, carbonate, (hydr)oxides, clay surfaces) and with different strengths. In three of the above referred studies, Ni has been reported to be bioaccessible in the range of 8–25 % depending upon the geochemistry of the studied UK sites. As in this study, Co was reported to be 16–21 % bioaccessible by Barsby et al. (2012) and Hamilton et al. (2015). Chromium bioaccessibility was reported in the range of 1–17 % (Broadway et al. 2010; Okorie et al. 2011; Barsby et al. 2012; Hamilton et al. 2015), which is almost in agreement with this study of 1–24 %.

Dietary minerals (DMs): Cu, Fe, Mn, Zn, Ca, and Mg in soils

Median, minimum, and maximum levels of the bioaccessible fraction (BAF) of DMs in selected soils were as follows (as % of total mineral)—Fig. 4b: Cu 53 (44–70); Fe 0.9 (0.4–2.1); Mn 29 (22–38); Zn 43 (22–61); Ca 77 (60–100); and Mg 13 (10–18). Calcium, Cu, and Zn have a relatively high bioaccessible fraction at 77, 53, and 43 %, respectively. However, compared to Ca, Cu and Zn, the bioaccessibility of Fe is almost negligible from sewage-irrigated soils. In Pakistani soils, the median bioaccessible fraction for Cu was 53 % and comparable to the reported range of 25–57 % (Okorie et al. 2011; Barsby et al. 2012; Hamilton et al. 2015). The median BAF of Fe was 0.9 %, similarly low compared to Hamilton et al. (2015) at 0.5 %. Manganese, Ca, Mg, and Zn had median BAFs of 29, 77, 13,

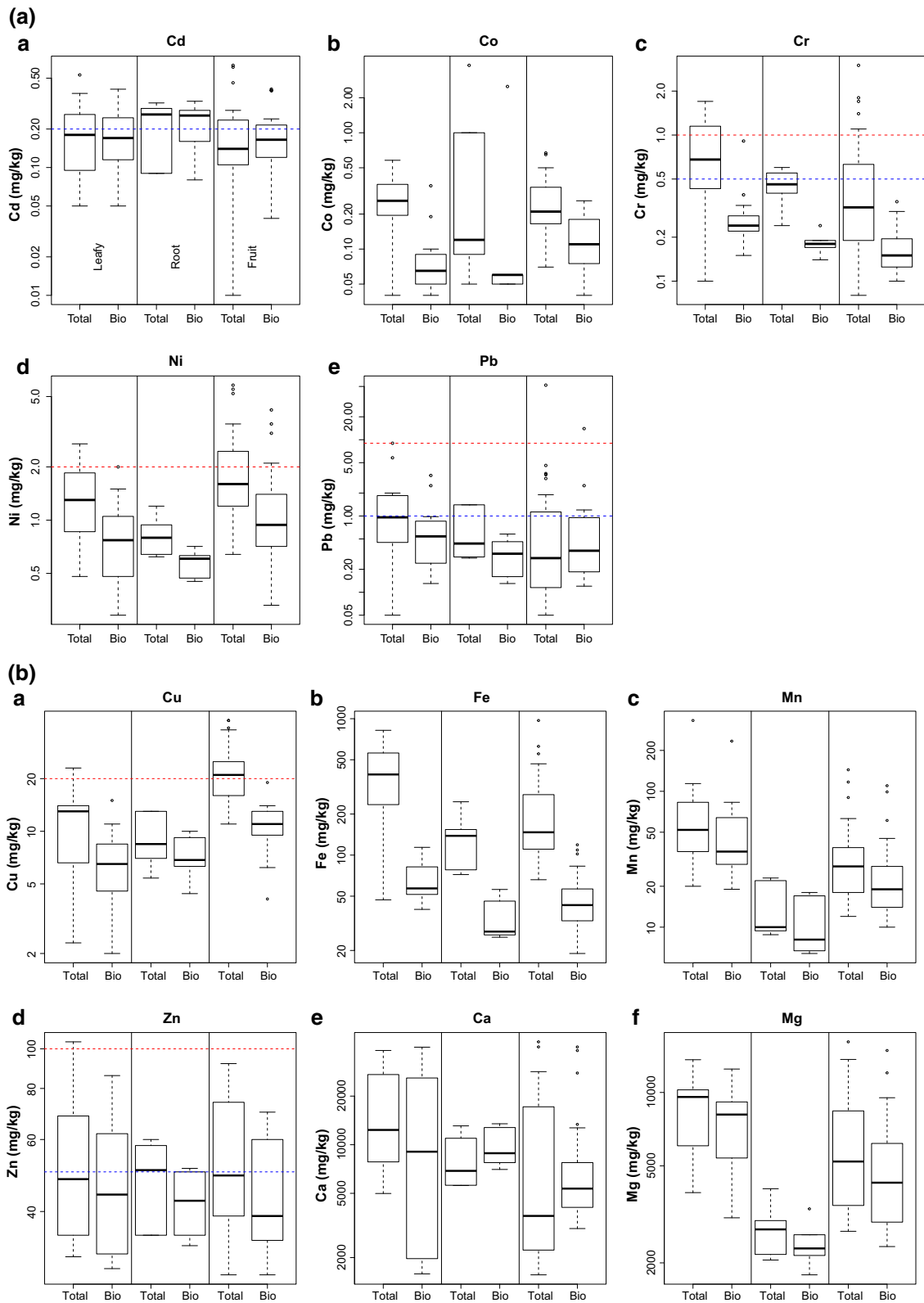
and 43 %, respectively compared to reported values of 41, 95, 25 %, respectively (Hamilton et al. 2015) and 17–39 % for Zn (Okorie et al. 2011; Barsby et al. 2012; Hamilton et al. 2015).

Bioaccessible fraction of potentially harmful elements (PHEs) and dietary minerals (DMs) for vegetables

Potentially harmful elements (PHEs): Cd, Co, Cr, Ni, and Pb in vegetables

The consumable portions of vegetable samples were analysed for the bioaccessible fraction (BAF) of PHEs from all five districts as illustrated in Fig. 5a. Cadmium had the highest median BAF (92 %) followed by Cu (63 %) and Ni (60 %) for vegetables. Leafy, fruiting, and root vegetables did not exhibit any significant difference in BAF for any of the PHEs. Bioaccessible concentrations were lower than total concentrations for all of the PHEs except Cd where no significant difference was observed for leafy, fruit, and root vegetables. Median concentrations of Co, Cr, Ni, and Pb were lower than ML’s set by EU/UK and the Chinese State Environmental Protection Administration (2006), as summarised in Table 2.

In this study, a median BAF of 92 % for Cd in the consumable portion of vegetables was comparable to 79–91 % reported by Pelfrêne et al. (2015) using the BARGE UBM approach for Cd in seven raw vegetables; radish, lettuce, French bean, carrot, potato, leek, and tomato, which upon steam cooking, the BAF of Cd increased by 0.7–23 % in French beans, 0.5–13 % in



◀ **Fig. 5 a** Bioaccessible fraction of PHEs for sampled vegetables. **b** Bioaccessible fraction of DMs for sampled vegetables

carrot, and 0.6–11 % in leeks. This is in contrast to the findings of Fu and Cui (2013), who reported a decrease through cooking of Cd bioaccessibility by 55 % using the physiologically based extraction test (PBET). Median BAF for Co in this study was 49 %, but no comparable data is available in the literature. For Cr, a median BAF of 39 % in this study is within the range of 11–62 % reported for vegetables by Hu et al. (2013) and Intawongse and Dean (2008) who employed an in vitro gastrointestinal and PBET model, respectively. For Ni, and Pb, median BAF were 60 % and 42 %, respectively, in this study, compared to Hu et al. (2013) and Intawongse and Dean (2008) reported values for vegetables of 29–60 and 20–68 % for Ni, and Pb, respectively.

Dietary minerals (DMs): Cu, Fe, Mn, Zn, Ca, and Mg

The consumable portions of vegetable samples were analysed for the bioaccessible fraction (BAF) of DM from all five districts as shown in Fig. 5b. Calcium had the highest median BAF (105 %) followed by Zn (88 %), Mg (86 %), Mn (74 %), and Cu (63 %), while the lowest median BAF (27 %) was noted for Fe. Leafy, fruit, and root vegetables did not exhibit any significant difference in BAF for any of the DMs except for Cu where higher values were noted for root vegetables followed by leafy vegetables. Median bioaccessible concentrations of Cu, Fe, Mn, Zn, Ca, and Mg were lower than the maximum limit set by EU/UK and State Environmental Protection Administration in China (2006), see Table 2. The BAF for Cu (63 %), Fe (27 %), Mn (74 %) and Zn (88 %) in this study are comparable to 30–77, 4–55, 36–58 and 69–94 %, respectively, reported in the literature (Hu et al. 2013; Khouzam et al. 2011; Intawongse and Dean 2008).

Potential dietary-based human health hazards: daily intake of potentially harmful elements (PHEs) and dietary minerals (DMs) via vegetables

Potentially harmful elements (PHEs): Cd, Co, Cr, Ni, and Pb via vegetables

The daily intake (DI) of PHEs was calculated for leafy, fruiting, and root vegetables (Supplementary info:

Figure 1a) in comparison with the Recommended Dietary Allowances (RDA). The daily dietary intake (DI) level of a nutrient element considered sufficient to meet the requirements of 97.5 % of healthy individuals in 19–70 years adults (Food and Nutrition Board 2004). The DI threshold limit for Cd of 0.07 mg/day, set by WHO was used for comparison (WHO 1996). For Ni there is no RDA rather an upper limit of 1.0 mg/day is suggested by the Food and Nutrition Board (2004). The US Food and Drug Administration set provisional total tolerable intake (PTTI) levels for DI of Pb at 0.025 mg/day (Carrington and Bolger 1992).

For Cd, all vegetable subgroups had significantly lower median DI values (0.003 mg for fruiting vegetables; 0.004 mg for leafy vegetables; and 0.01 mg for root vegetables) compared to the DI threshold limit of 0.07 mg. Root vegetables potentially contributing a higher daily Cd intake, followed by leafy vegetables. For Co, all vegetable subgroups had significantly higher median DI values (0.005 mg for fruiting vegetables; 0.006 mg for leafy vegetables; and 0.003 mg for root vegetables) compared to the DI threshold limit of 0.00012 mg. For Cr, all of the leafy, root and fruiting vegetable subgroups had lower median DI values (0.007 mg for fruiting vegetables; 0.015 mg for leafy vegetables; and 0.01 mg for root vegetables) than the threshold DI limit of 0.035 mg. For Ni, all of the leafy, root and fruiting vegetable subgroups had lower median DI values (0.007 mg for fruiting vegetables; 0.015 mg for leafy vegetables; and 0.01 mg for root vegetables) than the threshold DI limit of 1.0 mg. For Pb, all of the leafy, root and fruiting vegetable subgroups had lower median DI values (0.006 mg for fruiting vegetables; 0.02 mg for leafy vegetables; and 0.01 mg for root vegetables) than the threshold DI limit of 0.03 mg.

Through consumption of sewage-irrigated vegetables, DI of PHEs would be within threshold limits. For Co there is no threshold limit mentioned in the published literature. The possible source of Co might be from urban dust since control vegetables grown in urban centres had still higher concentrations of Co and contributed a higher DI compared to sewage-irrigated vegetables. Median DI calculated in this work for bioaccessible Co is 0.002 mg which can reduce DI of Co by 40 % but is still higher than the recommended maximum DI. Further investigation is warranted to measure the contribution of Co from urban dust, and

phosphatic fertilizers in urban and suburban vegetables. Overall, control vegetables ($n = 14$) grown with clean water in city centre had median Cd, Co, Cr, Ni, and Pb DI values of 0.001, 0.007, 0.49, 0.25, and 0.005 mg, respectively. It is evident from the comparative bioaccessible DI data that daily intake of PHEs is generally on the higher side if the total concentration of PHEs is taken into account instead of bioaccessible concentration. However, in the case of Cd, differences are not considered significant.

Dietary minerals (DMs): Cu, Fe, Mn, Zn, Ca, Mg, and I via vegetables

The daily intake (DI) of DMs was calculated for sewage-irrigated leafy vegetables, fruiting vegetables, and root vegetables (Supplementary info: Figure 1b). The DI was also calculated using BAF for DMs, with a higher median DI for Fe, Mn, Ca, and Mg from leafy vegetables followed by fruiting and root vegetables. For Cu, a higher median DI was calculated for fruiting vegetables, whereas no difference was observed for Zn among any of the three vegetable subgroups. The DI was significantly lower for all of the sewage-irrigated vegetable subgroups; Cu (0.35 mg), Fe (3.4 mg), Mn (0.7 mg), Zn (1.1 mg), Ca (156 mg), and Mg (119 mg), compared to the recommended daily allowance of 2.0, 15.0, 5.0, 11.0, 1000, and 400 mg/day, respectively. In contrast, the DI of DMs from control vegetables for Cu, Fe, Mn, Zn, Ca, and Mg were 0.22, 4.8, 0.4, 0.96, 71, and 73 mg, respectively. In summary, compared to control vegetables, sewage-irrigated vegetables upon consumption would supply 62, 60, 12, 104, and 63 % higher DI values for Cu, Mn, Zn, Ca, and Mg, respectively, but 28 % lower Fe. Arora et al. (2008) also reported similarly low DI related to the consumption of vegetables grown on wastewater-irrigated soils, suggesting a low risk with DIs for Cu, Fe, Zn, and Mn in adults in India 1.2–3.0, 10–50, 5–22, and 2–20 mg, respectively.

Sewage-irrigated vegetables had the highest iodine content in Gujranwala and Gujarat districts, with an overall median of 0.058 mg/kg (dry weight). Assuming moisture content of 87 % and adult vegetable consumption of 0.25 kg/day, both sewage-irrigated and control vegetables could contribute a similar DI of 1 μ g/day. The iodine DI from vegetables is 72 %

lower than from Pakistani wheat flour at 3.5 μ g (Zia et al. 2015).

Approximately 3.5 and 1.1 billion people are at risk of calcium (Ca) and zinc (Zn) deficiency, respectively, due to inadequate dietary supply (Kumssa et al. 2015a, b; Joy et al. 2014). Approximately 90 % of those at risk of Ca and Zn deficiency in 2011 are in Africa and Asia (Kumssa et al. 2015a, b). In this study, a DI of 156 and 1.1 mg/day is calculated for Ca, and Zn, respectively, compared to a recommended DI of 1000 and 11 mg/day, respectively. Therefore, from the districts studied, an average adult DI provides 16 and 10 % of the daily requirement for Ca and Zn from the consumption of vegetables grown on sewage-irrigated soils. Compared to control vegetables grown with clean water, the DI values calculated through consumption of sewage-irrigated vegetables are higher by 104 and 12 %, respectively, for Ca, and Zn.

Possible solutions to Zn deficiency include: supplementation, direct fortification, fertiliser application, and plant breeding (Joy et al. 2015a; Kumssa et al. 2015a, b; Ahmad et al. 2012). At present, zinc fertiliser consumption in Pakistan is less than 10 % of the potential market. The cost per DALY (Disability Adjusted Life Year) saved was estimated at US\$ 624–5893 via granular fertilisers and US\$ 46–347 via foliar fertilisers (Joy et al. 2015a). Foliar applications are likely to be more cost-effective than soil applications due to the fixation of Zn in soil, but may be more difficult to deploy. Factors contributing to Zn deficiency in humans include low consumption of animal products, high phytate intakes that inhibit Zn absorption and low concentrations of Zn in crops grown on Zn deficient soils (Cakmak et al. 1999). Phytate refers to mixed salts of phytic acid (PA), the principal form of phosphorus (P) in cereal grains, and is a potent inhibitor of Zn absorption in the human gut. A PA:Zn molar ratio of >15 is commonly used to classify diets having low levels of bioavailable Zn (Joy et al. 2015a).

The global weighted mean Mg supply is 613 mg per capita per day compared to a weighted estimated average requirement for Mg of 173 mg per capita per day. Estimates of deficiency risk based on supply do not account for potential inhibitors of Mg absorption, including Ca deficiency, phytic acid and oxalate (Kumssa et al. 2015a). On alkaline soils, carbonate formation and excess Ca, potassium (K), and Na reduce Mg phytoavailability. It is also likely that the incidence of Mg deficiency in crop plants is increasing

as a result of intensive crop production without concomitant Mg fertilisation (White and Broadley 2009; Broadley et al. 2012; Joy et al. 2013). Magnesium in sewage-irrigated vegetables in this study had a median concentration of 5470 mg/kg, (dry weight), accounting for an intake of 119 mg/day for Mg, which was lower than the recommended intake of 400 mg/day. Compared to control vegetables grown with clean water, the DI values calculated through consumption of sewage-irrigated vegetables are higher by 63 % for Mg. Since vegetables sourced from sewage-irrigated and highly calcareous soils are still unlikely to deliver greater than 30 % of the estimated average requirements, agronomic solutions to rectify Mg malnutrition via vegetables are limited, in comparison with strategies for dietary diversification. In addition, some vegetables are high in phytic acid, which can inhibit Mg absorption through the formation of stable chelate complexes (Hurrell 2003).

In this study, the median concentration of 156 mg/kg (dry weight) in sewage-irrigated vegetables would provide 3.5 mg/day of Fe, accounting for only 23 % of an adult requirement, compared to 4.8 mg/day and 32 % from control soils. Vegetables grown on control soils had a higher Fe content, probably resulting from soil particles and/or urban dust, details of which are discussed in the next section. It is worth noting that a 0.6 % BAF for Fe sourced from sewage-irrigated vegetables, and this would provide a DI of only 0.02 mg/day of Fe.

Soil contamination of vegetable samples

Trace elements are normally poorly available to plants and can be used to estimate the likely proportion of soil contamination contributing to apparent plant sample element concentrations. Vanadium (V) may be a reliable indicator of extraneous contamination with soil dust. Although mobile forms of V exist in calcareous soils (e.g. vanadate, HVO_4^{2-}), most soil V is bound predominantly within ferric oxides where (reduced) trivalent V^{III} (0.064 nm) isomorphically substitutes for Fe^{III} (0.065 nm; Schwertmann and Pfab 1996). Vanadate is also likely to be retained as a specifically adsorbed anion on Fe oxides under acidic conditions. Thus, as vanadyl and vanadate are unlikely to be systemically absorbed by plants concurrently with Fe^{II} or Fe^{III} , it seems reasonable to assume that a

high correlation between V and Fe in plants indicates contamination with Fe oxides present in soil dust. In this study, there was a positive correlation between V and Fe concentrations in plant samples ($R^2 = 0.98$, $p < 0.001$, Fig. 6). Thus, extraneous contamination of plant samples with Fe was estimated where coupled soil samples were taken ($n = 50$).

Recently published work suggested that the presence of soil particles on plant samples influenced plant elemental concentrations, particularly for Fe (Joy et al. 2015a, b; Gibson et al. 2015). In this study, the greatest effect of possible dust contamination was on the Fe concentrations in leafy and fruiting vegetables, with a median contribution from soil of 51 % in leafy vegetables and 34 % in fruiting vegetables. Gibson et al. (2015) concluded that Fe intakes could be underestimated from weighed food records using food composition data. This discrepancy was identified through a positive correlation of food Al and Ti with Fe concentrations (Siyame et al. 2014). Soil contains high concentrations of both Ti and Al, yet their uptake and transport to the edible parts of plants is relatively low resulting in a demonstrably large soil:plant ratio (Chilimba et al. 2011). Hence even minor soil contamination (by mass) of the diet composites will strongly affect Fe, Al, Ti concentrations (Gibson et al. 2015). Other elemental plant concentrations are influenced to a lesser degree by soil contamination.

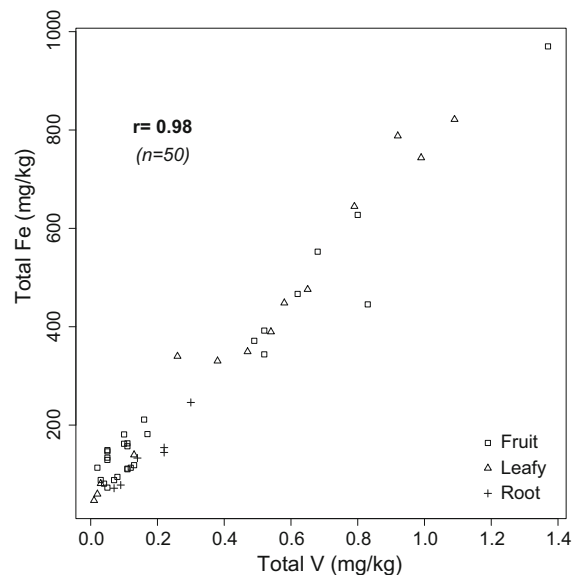


Fig. 6 Correlation between V and Fe concentrations in vegetable samples

The level of care taken to remove soil from samples in this study is likely to exceed that of households during food preparation, and it would therefore be possible that a significant proportion of total dietary Fe intake may come from soil particles adhered to the plant. This can be extended to other DMs and may have consequences in estimating DI of minerals.

Sewage biomarkers

Sterol and stanol concentrations in selected set of soils from Gujranwala, Gujarat, and Lahore districts are summarised in Supplementary Info: Table 2. Sterol concentrations were also determined in human faeces and were found to fall within published criteria. Total sterol concentrations in all three districts are broadly similar, with the highest value observed at Marali Wala, Gujranwala district (3.78 $\mu\text{g/g}$), of which coprostanol is 2.54 $\mu\text{g/g}$. This, combined with a high coprostanol/cholesterol ratio of 8.1 (Fig. 7), suggests a larger and less environmentally altered application of sewage.

With a history of urban sewage application, the sum of coprostanol + epi-coprostanol is generally the most abundant sterols, except at three sites. The presence of coprostanol (0.24 to 2.54, mean 0.74 $\mu\text{g/g}$) and epi-coprostanol (0.05–0.36, mean 0.13 $\mu\text{g/g}$) demonstrates that the soils contain sewage, particularly where coprostanol concentrations are greater than 0.5 $\mu\text{g/g}$ (Readman et al. 2005), as measured in 13 of 18 soils. Grimalt et al. (1990) reported that 5 β -stigmastanol is derived from intestinal microbial reduction in the plant-derived marker sitosterol and was elevated at Barah Sandha, Ghosia Colony, Lahore

district, relative to other sterols and could indicate faecal matter from herbivorous animals, particularly ruminants.

The coprostanol–cholesterol ratio is a proxy indicator of human sewage pollution. In this study human faeces range was 6.7–8.4 (Fig. 7). As the raw faecal matter (ratio of ~ 10) is dispersed in the environment, the ratio will decrease caused by microbial action and/or as more (non-faecal) cholesterol from animals is encountered. Fattore et al. (1996) suggested that coprostanol–cholesterol ratio >1 indicates a human source of sterols, equivalent to 12 of 18 soils in this study. There is a slow conversion of coprostanol to epi-coprostanol in the environment and this ratio will indicate either the degree of sewage treatment or its age in the environment. The cross-plot of the coprostanol–cholesterol ratio with the epi-coprostanol–coprostanol (Fig. 7) indicates both faecal contamination and environmental alteration. All the soils gave an epi-coprostanol–coprostanol ratio of <0.38 (Fig. 7) suggesting that the coprostanol is derived from humans (Venkatesan and Santiago 1989). The coprostanol/(5 α -cholestanol + coprostanol) ratio is another human faecal contamination index (Grimalt et al. 1990). 5 α -Cholestanol is formed naturally in the environment by bacteria and generally does not have a faecal origin. Samples with ratios >0.7 may be considered as contaminated with human faecal matter, as was the case in 15 of 18 soils, the remaining three gave ratios of 0.55–0.68 and cannot readily be categorised as contaminated on the basis of this ratio alone.

Chromium concentrations were compared to coprostanol + epi-coprostanol and soil organic matter (OM) (Fig. 8). There was little or no correlation of Cr with sterols (R^2 0.137), compared to soil OM (R^2

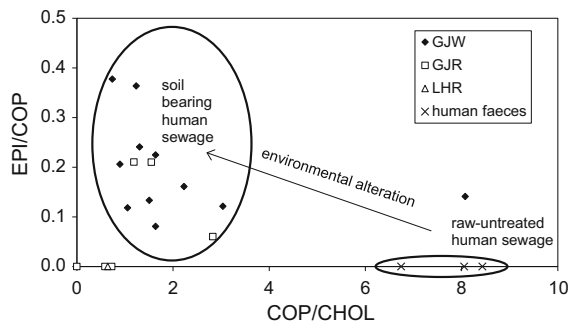


Fig. 7 Coprostanol (COP) ratios with respect to cholesterol (CHOL) and epicoprostanol (EPI). Environmental alteration is indicated

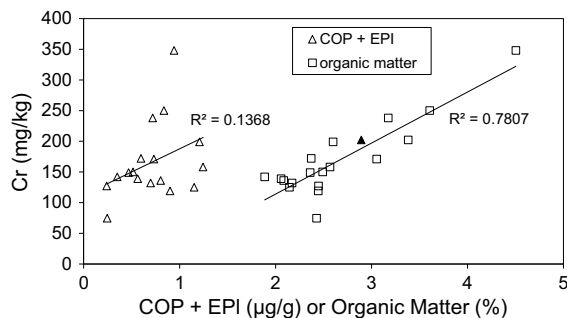


Fig. 8 Correlation of chromium with soil organic matter and coprostanol (COP) + epi-coprostanol (EPI). Outlier omitted at Marali Wala (filled triangle COP + EPI = 2.89 $\mu\text{g/g}$)

0.781), which is likely due to the presence of humic substances in the soil derived from decomposed vegetation. The presence of β -sitosterol, derived from vascular plants, supports this because β -sitosterol is one of the main sterols which undergo reduction by enteric bacteria to yield 5β -stanols (Leeming et al. 1996). Humics are macromolecular compounds consisting of carboxylate and phenolate functional groups and they form chelate complexes with cations which is an important aspect of their biological role in regulating bioavailability, transport and natural attenuation of metal ions (Tipping 2004). However, coprostanols cannot form these chelates with cations and no resulting correlation is observed. This is substantiated in the current study where soil OM showed the stronger correlation for Cr, Cu, Ni, Zn and U ($R^2 = 0.646$ – 0.781), Pb ($R^2 = 0.551$), and Cd, Co, Fe, Mn, Ca, Mg and V ($R^2 < 0.145$). However, coprostanol + epicoprostanol displayed little or no correlation for these metals ($R^2 = 0.094$ – 0.287).

Conclusions

Compared to control soils irrigated with clean water, sewage irrigation did exhibit an increase in concentration of Cd, Cr, Ni, and Pb in soils for the districts of Gujranwala, Gujarat, and Kasur (for Cr only), but within threshold limits. For the Kasur, Faisalabad, and Lahore districts, sewage irrigation did not result in a greater concentration of PHEs in soils, with comparable Fe, Mn, Zn, I, Ca, and Mg concentrations in vegetables grown from control sites in Lahore and Faisalabad districts. This implies that there is no harm in harvesting of vegetables with the use of sewage irrigation. It appears that sewage irrigation over the years decreased soil pH at control sites (7.7 vs 8.1) and increased its dissolved organic matter content, which directly influence the retention and phyto-availability of PHEs and DMs from soils. Concentrations of PHEs in most of the sewage-irrigated vegetables were below the published upper threshold limits, except for Co. On sewage-irrigated soils, the Cd bioaccessibility was the highest of the PHEs. Leafy, fruiting, and root vegetables did not show any differences in the BAF for any of the PHEs or DMs, except for Cu. The BAF for PHEs can reduce comparable DI measurements compared to total concentrations in general, but not significantly for

Cd. Since the sewage-irrigated soils were not considerably higher in PHEs compared to the control sites, sewage-irrigated vegetables could provide an opportunity to harvest mineral-rich vegetables thus providing consumers 62, 60, 12, 104, and 63 % higher DI of Cu, Mn, Zn, Ca, and Mg, respectively. The effect of possible soil dust contamination on the Fe concentrations, i.e. 51 % in leafy vegetables and 34 % in fruiting vegetables, depicts the possibility that a significant proportion of total dietary Fe intake may come from soil particles adhered to the consumable portions of vegetables. The presence of sewage in the wastewater-irrigated soils was confirmed and indicated largely human and faecal sources, with some degree of natural attenuation, but with association of metals to organic matter rather than sewage content.

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