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Uptake and distribution of minerals and heavy metals in commonly grown leafy vegetable species irrigated with sewage water

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Abstract Heavy metal uptake and accumulation behavior in dietary vegetables irrigated with sewage waters is an important issue worldwide. The main objective of this study was to examine and compare the physiological and growth responses of leafy vegetables irrigated with sewage water. A pot experiment was conducted in a wire house with three leafy vegetables, coriander (*Coriandrum sativum*), mint (*Mentha arvensis*), and fenugreek (*Trigonella foenum*), grown under ambient conditions. Plants were irrigated with different concentrations, 0, 50 (T_1), and 100 % (T_2), of sewage water. After harvesting, morphological and physiological parameters of plants were measured. Heavy metal (Cd, Cu, Pb, and Zn) concentrations in the sewage water were found much higher than safer limits. The results revealed that the highest plant biomass and lowest metal contents were observed in control treatments in all studied vegetables. The biomass of all the vegetables were negatively affected when irrigated with sewage water. In T_2, coriander accumulated maximum Cd (μg g^{-1} DW) in shoots (4.97) as compared to other vegetables. The maximum Pb and Cu concentrations were accumulated in mint roots (44 and 3.9, respectively) as compared to coriander and fenugreek. Zinc was accumulated in the sequence of leaves > roots > shoots under polluted water irrigation. The concentrations of potassium increased in leaves, shoots, and roots in all vegetables, while phosphorous concentrations varied with species and plant parts with increasing sewage water concentration. It was found that the leafy vegetables grown with sewage water irrigation may cause severe human health problems.

Keywords Heavy metals · Growth · Vegetables · Phytoremediation

Introduction

Increasing urbanization and industrialization has resulted the increased production of effluents worldwide (Salvatore et al. 2009; Zia et al. 2016). Due to the shortage of freshwater for irrigation, municipal and industrial sewage water is being used for irrigation in various countries including Pakistan (Perveen et al. 2012; Rehman et al. 2015). It contains various

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macronutrients such as potassium (K), calcium (Ca), and phosphorous (P) and micronutrients such as zinc (Zn), boron (B), manganese (Mn), and copper (Cu), which are required for normal plant growth and development (Brar et al. 2000; Zia et al. 2016). In addition, continuous irrigation with municipal sewage water can improve the soil fertility by adding minerals and organic matter contents in the soil (Yadav et al. 2002). However, soils receiving sewage water, applied due to the scarcity of good quality water, may accumulate a considerable amount of toxic heavy metals including cadmium (Cd), lead (Pb), nickel (Ni), and chromium (Cr) (Amin et al. 2013; Liu et al. 2016; Zia et al. 2016). Sometimes irrigation with treated wastewater brings better growth of plants with improved metabolic activities, but the continuous use of such water results in accumulations of metals up to toxic level in soil with reduced growth of the plant and even the barren land (Antil 2012). Due to the undegradable nature and persistence in soil, these heavy metals readily accumulate in the edible parts of the plants grown in these soils (Rizwan et al. 2012; Adrees et al. 2015; Uzma et al. 2016). Uptake and accumulation of these metals could decrease the plant growth and yield (Gill et al. 2015; Rehman et al. 2015). The heavy metals also decreased photosynthesis and gas exchange characteristics in plants (Rizwan et al. 2016a, 2016b).

Dietary uptake of heavy metals can cause severe depletion of some essential mineral elements resulting in various diseases, including immunological disorders and disabilities due to malnutrition and gastrointestinal cancer (Zakir et al. 2009; Amin et al. 2013). For instance, consumption of vegetables contaminated with excessive amount of Pb and Cd can cause heart, kidney, nervous system, and bone-related problems (Patra et al. 2002). Similarly, Cu toxicity caused iron deficiency and the demolition of cellular and sub-cellular membranes (Marschnar 1995).

Allocation of heavy metals depends upon their available concentration as well as plant species. Many species are manic accumulators of heavy metals in their edible parts that may be harmful for plant growth and health of a consumer (Chary et al. 2008; Khan et al. 2008). Fresh vegetables are extensively used for edible purposes and considered as a major component of human diet. These plants are rich in proteins, carbohydrates, vitamins, and minerals (Khan et al. 2008). On the other hand, vegetables irrigated with sewage water are an important source of dietary uptake of heavy metals over an extensive range of concentrations (Uzma et al. 2016; Zia et al. 2016). The heavy metals mainly enter through roots and may translocate to the edible parts of the vegetables (Jabeen et al. 2016; Younis et al. 2016). The excess of heavy metals decreased the essential nutrient uptake by vegetables and as a result decreased the quality of the vegetables (Khan et al. 2016). However, metal uptake and their effects on vegetables considerably varied with vegetable species (Cherfi et al. 2016; Uzma et al. 2016).

It has been widely reported that leafy vegetables grown on metal-contaminated soils or receiving metal-contaminated water may accumulate higher concentrations of heavy metals as compared to other vegetables (Khan et al. 2010, 2016). It was hypothesized that leafy vegetables may accumulate toxic heavy metals in edible parts receiving sewage water for irrigation and could transfer to humans via the food chain. Thus, a wire house experiment was conducted to investigate the effect of sewage water on growth, mineral uptake, and heavy metal accumulation in different parts of three leafy vegetables, i.e., coriander (*Coriandrum sativum* L.), mint (*Mentha arvensis* L.), and fenugreek (*Trigonella foenum* L.). These vegetables were selected as they are commonly grown in Pakistan and worldwide, but the impact of sewage water in these leafy vegetables has rarely been studied.

Materials and methods

Experiment

A pot experiment was carried out in the wire house of Government College University, Faisalabad, located at latitude 31° 26 N and longitude 73° 06 E with altitude of 184.4 m. Sewage water samples were collected from main drain at Tandliawala near the Faisalabad City and subjected to physico-chemical and heavy metal analysis (APHA 1988). Certified seeds of coriander and fenugreek and cuttings of mint were obtained from Ayub Agriculture Research Institute, Faisalabad, Pakistan. Seeds were sown in plastic pots containing 5-kg sandy loam soil in wire house under ambient conditions with a photoperiod of 10-h light/14-h dark, 27 ± 5 °C, and relative humidity of about 65 %. The experiment was performed in a completely randomized design (CRD).

All the pots were irrigated with tap water for 10 days, and then, the pots were treated with 50 % (T_1) and

100 % (T_2) sewage water, whereas tap water was used as a control. Soil moisture content was maintained at about 70 % of soil water-holding capacity throughout the experiment either with tap water or with different sewage water concentrations. There were five replications of each treatment, and weeds were removed on a daily basis.

Measurements

After 20 days of treatments, plants were harvested and separated into leaves, stems, and roots. The plants were harvested at this stage as there were toxic symptoms on the leaves of the plants. Plant heights were measured at the end of the experiment. Plant fresh weights were taken soon after harvest, and then, samples were dried in oven at 65 °C until constant weight and dry weights were measured. Dried plant samples were grounded into powder, sieved at 1 mm, and 0.5 g of the plant samples was digested in 10 mL of strong acid solution (HNO_3/$HClO_4$, 3:1, *v*/*v*). The Pb, Cd, Zn, Cu, and K concentrations in digestions were determined using atomic absorption spectrophotometer (PerkinElmer Analyst-200, USA), following the standard conditions and using the highly sterilized glass apparatus and highly purified deionized water. Calibrated standard was prepared from the commercially available stock solution (AppliChem®) in the form of an aqueous solution. Phosphorous was determined spectrophotometrically (Jackson 1962).

Statistical analysis

Collected data were analyzed statistically by using the analysis of variance (ANOVA) and least significant difference method (LSD) on Statistix 8.1 for Windows program. The level of statistical significance was set at $P < 0.05$.

Results and discussion

Physico-chemical analysis of sewage water (Table 1) indicated that the electrical conductivity (EC; mS cm^{-1}) values recorded were higher (3.93) than the suitable limit (3) for most of the crops (EPA 1991). The increased EC value of underground water was also reported in other studies (Iqbal et al. 2013). In addition, the composition of sewage waters is also changing throughout the year due to several other factors (Antil and Narwal 2008). The higher EC values indicated the increase in soil salinity, which may be harmful for plant growth (Rehman et al. 2016). Deshmukh et al. (2015) reported that sewage water irrigation decreased the EC of the soil as compared to the tube well water-irrigated soil. This showed that changes in EC depend upon the composition of the sewage water used for irrigation.

Toxic metals including Cd, Cu, and Pb were detected in sewage water, and their levels were higher than the

Table 1 Physico-chemical properties of wastewater used for irrigation

Parameters	Unit	Wastewater	Recommended values (WHO 2007)
pH		7.3 ± 0.1	6.0–8.5
EC	mS cm^{-1}	3.93 ± 0.3	3
Carbonate	meq L^{-1}	3 ± 0.2	
Bicarbonate	meq L^{-1}	5.2 ± 0.1	
Heavy metals/ ions	mg L^{-1}	–	
Cd	mg L^{-1}	0.93 ± 0.02	<0.01
Cu	mg L^{-1}	1.51 ± 0.3	<0.2
Pb	mg L^{-1}	7.832 ± 0.2	<5.0
Zn	mg L^{-1}	3.945 ± 0.03	≤2.0
Fe	mg L^{-1}	0.819 ± 0.2	≤5.0
K	mg L^{-1}	135 ± 12	
P	mg L^{-1}	14.98 ± 1.4	

Table 2 Physico-chemical properties of the soil used for the pot experiment

Parameters Texture	Unit	Soil Sandy loam	Safe limits
pH		7.3 ± 0.01	≤8.5
EC	mS cm^{-1}	1.91 ± 0.1	2–4
Heavy metals/ions	mg kg^{-1}	–	
Cd	mg kg^{-1}	0.25 ± 0.2	3
Cu	mg kg^{-1}	19 ± 2.1	140
Pb	mg kg^{-1}	26.23 ± 1.8	200
Zn	mg kg^{-1}	3.25 ± 0.5	300
Fe	mg kg^{-1}	2.45 ± 0.7	–
K	mg kg^{-1}	1500 ± 200	–
P	mg kg^{-1}	41.1 ± 3.4	

permissible limits (WHO 2007). Cadmium (0.9 mg L^{-1}) was much higher than the recommended values (0.01 mg L^{-1}). Similarly, Cu (1.51 mg L^{-1}), Pb (7.8 mg L^{-1}), Zn (3.9 mg L^{-1}), and Fe (0.8 mg L^{-1}) were also found higher than acceptable limits set by WHO (Table 1). Long-term application of polluted water results in buildup of heavy metals in the soils (Al-Omron et al. 2012). The increased availability of these metals in soil ultimately results in the increased uptake in different parts of growing vegetables (Ullah et al. 2012). Studies have shown the increased accumulation of toxic metals in different plant parts when grown in sewage and industrial water or sludge-amended soils (Khan et al. 2003; Singh and Agrawal 2007). Brar et al. (2000) also reported the higher accumulation of metals in leaves and tubers of potato grown on sewage-irrigated soils as compared to groundwater-irrigated plants. The pH of soil was 7.3, which was within the safe limit with reference to standard limits of WHO (up to 8.5). Soil EC (1.91 mS cm^{-1}) was also in accordance to the standard permissible limit (WHO 2007). Soil heavy metals were well behind their toxic values, representing that soil was safe for agricultural use (Table 2).

Plant height, shoot, and root fresh and dry weights of the three vegetables were considerably affected under sewage water irrigation (Fig. 1). Plant height of mint and coriander significantly decreased with increasing sewage water levels in the growth medium, while there was no significant difference in plant height of fenugreek as compared to the control (Fig. 1e). Mint showed a maximum reduction in fresh and dry biomass as compared to control treatment followed by coriander and fenugreek, respectively (Fig. 1a–d). As compared to the control, dry weights of mint shoot and root were reduced up to 19 and 13 % under T_1 and 25 and 8 % under T_2 treatments, respectively. Similarly, shoot dry biomass of coriander was reduced up to 12 and 16 % and root biomass was reduced about 14 and 15 % under T_1 and T_2 as compared to control, respectively. Marwari and Khan (2012)

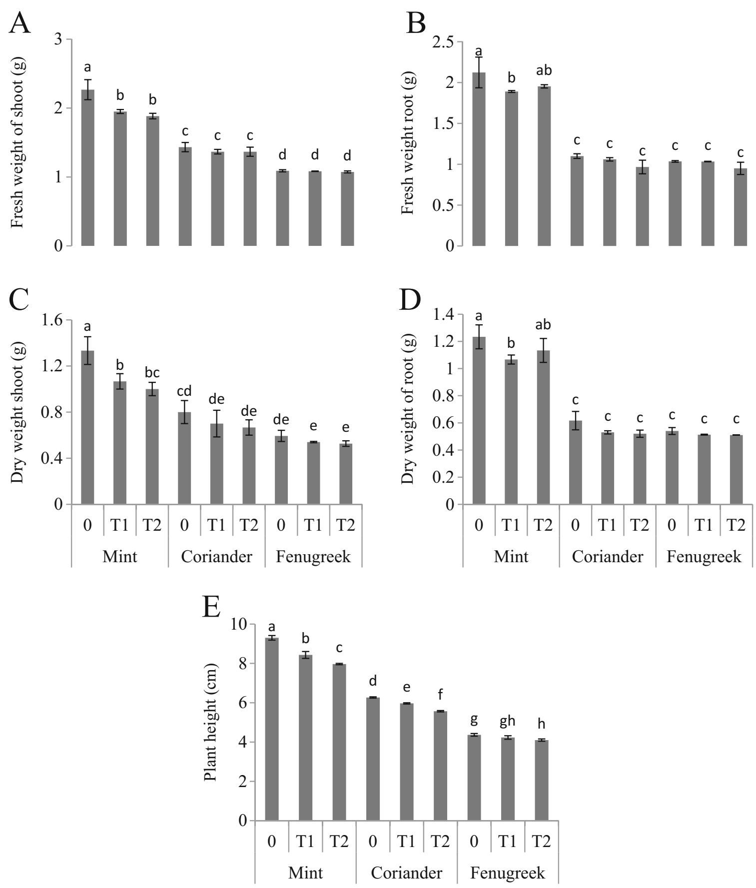
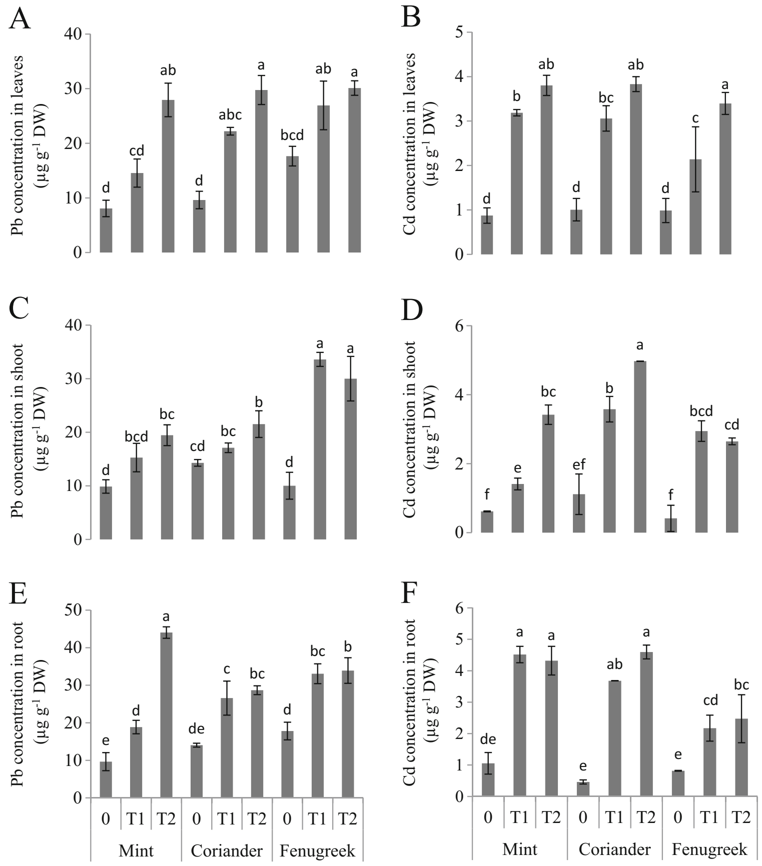


Fig. 1 Effect of different treatments of sewage water (0 = control, T_1 = 50 %, and T_2 = 100 % sewage water irrigation) on plant height, fresh and dry biomass of shoots and roots of mint, coriander, and fenugreek. The *bars* represent the SD of five replicates. The *different letters* indicate the significant differences among the treatments at a $P < 0.05$

reported the decrease in length, fresh, and dry biomass of tomato when irrigated with 20 and 30 % polluted water. Shoot length, root length, and biomass of *Beta vulgaris* decreased significantly when treated with sewage sludge, and this decrease was more pronounced at higher concentrations of sewage sludge (Singh and Agrawal 2007). More recently, Uzma et al. (2016) reported that wastewater effects on vegetables varied with the type of wastewaters and plant species. Such reduction in plant biomass is attributed to the increased accumulation of toxic metal in the leaves/shoots of the plant, which damage physiological and biochemical characteristics of plants leading to reduced growth (Ali et al. 2014, 2015).

The Cd, Pb, Cu, and Zn concentrations increased with increasing sewage water levels in the growth medium and the response varied with the vegetable species (Figs. 2 and 3). Cadmium accumulation increased with increasing level of sewage water in all parts of vegetables studied (Fig. 2). Amount of Cd was more than the permissible limit suggested by FAO/WHO. Maximum Cd accumulation (μg g^{-1} DW) was found in the shoots (4.97), roots (4.6), and leaves (3.8) of coriander followed by mint roots (4.51), leaves (3.8), and shoots (3.4). Uptake and distribution of Pb (μg g^{-1} DW) vary with the vegetables as well as increasing levels of polluted water (Fig. 2). Fenugreek accumulated more Pb in leaves (30) and shoot (34), whereas mint accumulated the highest amount of Pb in roots (Fig. 2). Trend of Cu partitioning was as follows: root > leaf > shoot in all studied vegetables (Fig. 3). The maximum amount of Cu (μg g^{-1} DW) was accumulated by the mint roots (3.9), followed by coriander leaves (3.8) and fenugreek shoots (3.4) when irrigated with the highest amount of sewage water (T_2). Application of sewage water increased the concentration of Zn in leaves, shoot, and root of all vegetables (Fig. 3). Zinc was accumulated in the sequence of leaves > roots > shoots under sewage water irrigation. The maximum amount of zinc (μg g^{-1} DW) was found in the leaves (400), shoots (172), and roots (203) of coriander when irrigated with 100 % sewage water (T_2).

Fig. 2 Effect of different treatments of sewage water (0 = control, T_1 = 50 %, and T_2 = 100 % sewage water irrigation) on accumulation of Pb and Cd in leaves, shoots, and roots of mint, coriander, and fenugreek. The *bars* represent the SD of five replicates. The *different letters* indicate the significant differences among the treatments at a $P < 0.05$



Potassium content increased with increasing sewage water treatments (Fig. 4). However, increase in K content was less than heavy metals and Zn. Maximum K (mg g^{-1} DW) was recorded in fenugreek leaves (13.3) irrigated with 100 % sewage water, and the minimum (5.3) was in control plants. Phosphorous accumulation did not show any specific trend under polluted water irrigation; in contrast, it decreased in coriander and fenugreek shoots (Fig. 4). However, the amount of P was increased in the leaves and roots of coriander and fenugreek, respectively (Fig. 4). The maximum leave P content (mg g^{-1} DW) was recorded in coriander (49) by the application of 100 % sewage water. Whereas, the maximum amount in a shoot (55) and root (40) was found in fenugreek at control and 100 % sewage water, respectively.

Different vegetables accumulate different metals depending on environmental conditions, type, and available form of metals. Leafy vegetables and root crops are known to accumulate higher amounts of heavy metals than grain crops (Khurana and Singh 2012). Other factors like the composition, duration and rate of sewage irrigation, soil types, soil reaction, and interaction among metals also affect their uptake (Khurana and Singh 2012). The use of sewage water for irrigation may result in the accumulation of toxic metals in the soil up to hazardous levels (Liu et al. 2016). Similar studies regarding the toxic levels of metals in edible plant parts were also reported by other researchers in Pakistan; such as, Ensink et al. (2007) found high concentrations of metals in Faisalabad by irrigating with industrial water. Khan et al. (2013) reported the higher concentrations of Cu, Mn, Ni, and Cd in edible parts of vegetables when irrigated with wastewater in the peri-urban area of Lahore.

In the present study, the application of sewage water has increased the Cd and Pb contents in all vegetables as compared to the control (Fig. 2). The concentration of Pb found in all the studied vegetables was higher than the permissible limit of 9 mg kg^{-1} set by SEPA (2005). In case of diet, the permissible limit of Cd is 0.3 μg g^{-1} for food samples (WHO and FDA), and it was found in higher amounts in all studied vegetables (Fig. 2). The Cd toxicity decreased the growth, biomass, and

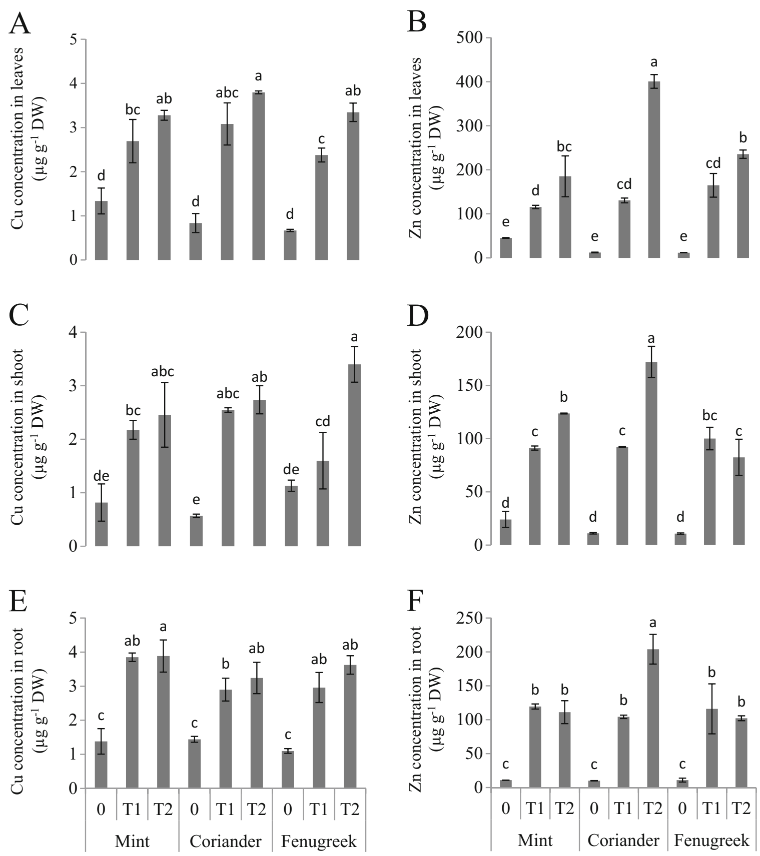


Fig. 3 Concentrations of Cu and Zn in leaves, shoots, and roots of mint, coriander, and fenugreek under sewage water (0 = control, T_1 = 50 %, and T_2 = 100 %) irrigation. The *bars* represent the SD of five replicates. The *different letters* indicate the significant differences among the treatments at a $P < 0.05$

photosynthesis of crops (Rizwan et al. 2016a, 2016b). Torabian and Mahjouri (2002) reported that Cd concentration increased in vegetables, mint, parsley, lettuce, and coriander, irrigated with polluted water. Similarly, higher concentrations of heavy metals have been reported in vegetables irrigated with city effluents (Murtaza et al. 2008). The higher concentrations of Pb, Cu, and Zn were reported in green vegetables, *Allium cepa*, *Allium sativum*, *Solanum lycopersicum*, and *Solanum melongena*, irrigated with wastewater (Amin et al. 2013). Excess heavy metals significantly affected the nutritional quality of the vegetables (Khan et al. 2016; Younis et al. 2016). Ullah et al. (2012) found that Zn, Ni, and Cd had highest translocation in spinach because of their higher mobility. In the present study, the decrease in vegetable biomass with sewage water might be due to the higher uptake of heavy metals by the plants (Figs. 1 and 2).

The present study indicated that the sewage water also added a considerable amount of macronutrients (K and P) and micronutrients (Zn and Cu) in vegetables (Figs. 3 and 4). These results are in accordance with the previous reports indicating that application of sewage water increased mineral nutrient uptake by plants (Yadav et al. 2002). Zia et al. (2016) reported that toxic heavy metals, Cd, Pb, Ni, and Cr, and dietary minerals, Cu, Zn, Ca, and Mg, increased in vegetables irrigated with untreated wastewater. In the present study, Zn contents were increased in all vegetables with the increase of sewage water concentration and found higher than the maximum permissible limit of 100 mg kg^{-1} set by SEPA (2005). Copper is an essential nutrient for plant growth and is required for normal plant growth and development (Adrees et al. 2015). Our results showed that the Cu concentration was also increased under sewage water irrigation; however, it was found within the permissible limit for vegetables, i.e., 10 mg kg^{-1} (SEPA 2005). By the application of sewage water, contents of K and P were increased at 100 % sewage water treatment. It has been reported that

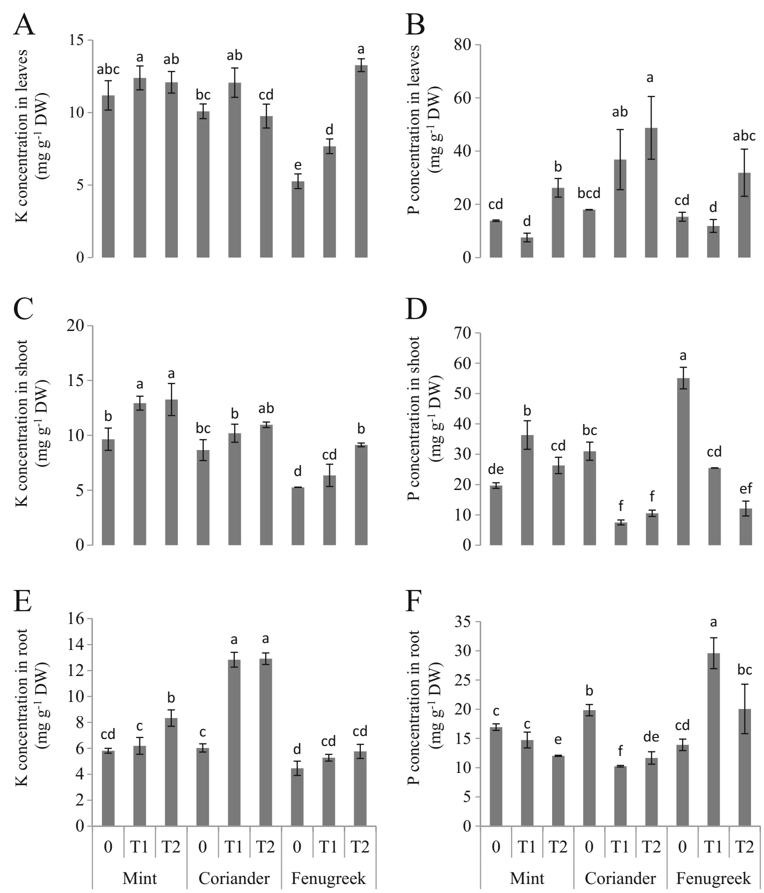


Fig. 4 Concentrations of K and P in leaves, shoots, and roots of mint, coriander, and fenugreek under sewage water (0 = control, T_1 = 50 %, and T_2 = 100 %) irrigation. The *bars* represent the SD of five replicates. The *different letters* indicate the significant differences among the treatments at a $P < 0.05$

polyphosphate compounds released from detergents increased the P contents of the sewage water and subsequently in plants (Singh and Agarwal 2007).

Conclusion

The concentrations of Cd, Pb, and Zn were higher in all studied vegetables than the permissible limit of these metals in vegetables, whereas Cu was far below the tolerable limits. The leafy vegetables like mint, coriander, and fenugreek may not be grown with sewage water application to avoid the risk of food chain contaminations. It can be concluded that application of untreated sewage water may be the source of heavy metals in vegetables under sewage water irrigation. Hence, it is necessary to treat the sewage water before application for irrigation especially under leafy vegetable production. Furthermore, awareness should be given to the concerned farmers regarding the serious consequences of using untreated sewage water for vegetable production.

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