Growth and accumulation of heavy metals in turnip (*Brassica rapa*) irrigated with different concentrations of treated municipal wastewater

Tabassum Parveen, Athar Hussain and M. Someshwar Rao

ABSTRACT

The present study has been carried out by irrigating turnip plants with different concentrations of treated municipal wastewater in order to see the effect on heavy metals accumulation and growth of plants. The turnip plants were watered with normal water and the results compared with results obtained by using treated municipal wastewater. The treatments used were: control (tap water) with 0, 25, 50 and 75% wastewater, and 100% wastewater, in three replications. The results indicated a substantial build-up of heavy metals in turnip irrigated with wastewater. The heavy metals content in the dry matter of the plants increased significantly with increase in wastewater concentration. Analysis of plant samples indicated the maximum accumulation of Fe (1,835 mg/kg in roots and 1,247 mg/kg in leaves) followed by Mn, Zn, Ni, Cu and Cd. The findings of this study regarding daily intake of metals suggest that the consumption of plants grown in wastewater is high, compared to consumption of those grown in tap water, but is nearly free of risks.

Key words | heavy metals, irrigation, municipal wastewater, reuse, turnip

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INTRODUCTION

The continuous increase of the urban population and the increasing per capita water consumption has diverted larger amounts of freshwater to domestic, commercial, and industrial sectors which generates a greater volume of wastewater (Kalavrouziotis *et al.* 2009; Qadir *et al.* 2010). Farmers in urban and peri-urban areas of nearly all developing countries who are in need of water for irrigation have often no other choice than using wastewater (Qadir *et al.* 2010). In many developing countries including India, farmers are irrigating their crops with industrial effluents due to the non-availability of an alternative source of irrigation water. It has been reported that sewage was used for irrigation in ancient Greece and Western Europe in the middle of the 16th century. Also, in the United States wastewater was used for irrigation in the 19th century (Chen *et al.* 2005). Several authors have reported that diluted industrial effluents enhance growth and productivity of crop plants (Chandra *et al.* 2009). Agricultural applications of effluent could provide water and nutrients for crop production, and the nutrient enriched sewage effluent could substantially reduce the reliance on chemical fertilizers. The regular use of treated wastewater for agricultural irrigation concurrently can solve water shortage problems and can also reduce the potential environmental contamination (Ginncken & Oron 2000).

Effluent from municipal sewage treatment plants, apart from supplying water to plants, often contains high levels of macro- and micro-nutrients and heavy metals (Kalavrouziotis *et al.* 2008b, 2010), therefore its long term use may actively affect their uptake by plants and thereby influence, positively or negatively, their consecutive accumulation and plant growth (Rattan *et al.* 2005; Kalavrouziotis *et al.* 2008a, 2009). Groundwater quality is often affected by disposal of untreated wastewater in the environment, percolation

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of wastewater during sewer leakage, deprived agricultural practices and atmospheric deposition of heavy metals (Chen *et al.* 2005; Schmidt *et al.* 2013). It has also a potential to adversely affect water quality in an aquifer that may be the source of drinking water in the particular area (Tang *et al.* 2004; Koussis *et al.* 2010).

The trace elements including copper, zinc, manganese, iron, molybdenum and boron are essential micro-nutrients required for plant growth. With the exception of boron, these elements are also considered as heavy metals as they may be toxic to plants at high concentrations (Batarseh *et al.* 2011). Heavy metals may also adversely affect plant growth as they can influence biochemical processes such as metabolism, photosynthesis and stomatal opening. Heavy metals accumulate mainly in the plant root system and to a more limited extent in leaves or in the edible plant parts (Kalavrouziotis *et al.* 2012). Plants have a natural ability to extract elements from soil and to translocate them between roots, shoots and fruits based on the biological processes in which the elements are involved (Batarseh *et al.* 2011). Agricultural products produced in regions of high micro-nutrients and heavy metals, may have adverse effects on human health due to the high level of these metals in the edible plant parts (Kalavrouziotis *et al.* 2008b).

Vegetables are considered to be an important component of human diet as they are rich sources of vitamins, minerals and fibers. However, the intake of vegetables contaminated with excess heavy metals may pose a risk to human health (Sharma *et al.* 2009). Among root crops, turnip has been used as a vegetable for human consumption in Europe since prehistoric times as it is rich in vitamin C, dietary fiber, has antioxidants and is low in calories. It lowers the risk of high blood pressure and diabetes, as well as cancer of the stomach, pancreas, bladder and lung. It is also a good source of calcium, phosphorus and magnesium. Turnip greens are an excellent source of riboflavin and iron. Considering the above-mentioned facts, the present study was undertaken with the following objectives: (i) to study growth of plants irrigated with treated wastewater and tap water; and (ii) to analyze the heavy metal concentration in leaves and roots of turnip, using different concentrations of 38 ML/d wastewater (Sewage Treatment Plant (STP) located at Saharanpur) and tap water.

MATERIALS AND METHOD

Pots preparation

Turnip was grown in pots using a statistical approach of simple randomized block design method. In order to carry out the study 45 pots were prepared and monitored during September–November 2008. The composition of water and wastewater used for watering these pots was varied. Pots were further classified into five sets according to the nature of the water used for watering. Pots of size 25 cm diameter filled with 5 kg soil containing 2% of organic manure were used for each experiment. Sufficient quantity of tap water was added to each pot to provide the necessary moisture for germination. In the month of September, seeds of turnip (*Brassica rapa*) were sown manually at equivalent distances in each pot. Seeds germinated within 10 days, after germination thinning was carried out to maintain a single plant in each pot. Turnip plants were harvested at 40, 55 and 70 days after sowing (DAS). Turnip was grown under five different conditions as (i) control (tap water), (ii) 25% wastewater+ 75% control, (iii) 50% wastewater +50% control, (iv) 75% wastewater +25% control, and (v) 100% wastewater. Each set contained nine pots. Three replicates of plant samples were carefully uprooted at 40 DAS, with minimum damage to the root. Out of the remaining six, three pots were harvested after 55 days and the remaining three pots at 70 DAS. Three replicates of plant samples from each treatment were collected and gently washed with running tap water to remove soil particles attached to the plant surfaces and finally rinsed with deionized water. Afterwards leaves and roots were separated and oven dried at 70 °C to a constant weight and dried samples were digested as per the procedure given in the Hach Manual (1999). Heavy metal analysis of the plant samples was carried out by atomic absorption spectrophotometer (Model GBC Avanta M).

Water and soil analysis

Wastewater samples were collected from an Upflow Anaerobic Sludge Blanket (UASB) based 38 ML/d STP located at Saharanpur (U.P.) India. Physico-chemical parameters and heavy metals (Cd, Ni, Fe, Cu, Mn and Zn) of treated effluent and tap water were analyzed using *Standard Methods* (American Public Health Association (APHA) 1998). Samples for heavy metal analysis were collected in plastic bottles containing 2 mL HNO_3. For coliform analysis, samples were collected in sterilized bottles. The bulk of samples were collected in plastic bottles and transported in an ice-box. Procedures listed in *Standard Methods* (APHA 1998) were followed for sample collection, preservation, transportation and analysis. Samples collected for heavy metal analysis were immediately acidified at the sampling point to pH <2.0 by adding HNO_3 to prevent the precipitation of metals. Acidified samples (350 ml) were digested with HNO_3 and filtered. The obtained filtrate was used for heavy metals analysis and the same was aspirated into an Atomic Absorption Spectrophotometer (Model GBC Avanta M) for the analysis of Cd, Ni, Fe, Cu, Mn and Zn. The soil used in the experiment was a top soil collected from an uncontaminated field. The soil samples were air dried, ground and passed through a 2 mm sieve and analyzed for pH, soil texture, total N, P, K and heavy metals (Cd, Ni, Fe, Cu, Mn, and Zn). All the soil samples were analyzed as per internationally accepted methods.

Statistical analysis

The data were analyzed statistically as per the method prescribed by Panse & Sukhtame (1985). The 'F' test was applied to assess the significance of data at 5% level of probability ($P \leq 0.05$). The measures were expressed in terms of mean values and standard errors of three replicates. Critical difference (CD) was also calculated to compare the mean values of various treatments.

Daily intake of metals (DIM)

The DIM was determined by the following equation.

$$\mathrm{DIM} = C_{\mathrm{metal}} \times C_{\mathrm{factor}} \times D_{\mathrm{food\ intake}} / B_{\mathrm{average\ weight}}$$

where C_{metal}, C_{factor}, $D_{\mathrm{food\ intake}}$ and $B_{\mathrm{average\ weight}}$ represent the heavy metal concentrations in plants (mg/kg), conversion factor, daily intake of vegetables and average body weight, respectively. The conversion factor 0.085 is used to convert fresh green vegetable weight to dry weight. The average daily vegetable intakes for adults and children were considered to be 0.345 and 0.232 kg person^{-1} day^{-1} respectively, while the average adult and child body weights were considered to be 55.9 and 32.7 kg, respectively (Rattan *et al.* 2005; Arora *et al.* 2008; Khan *et al.* 2008).

RESULTS AND DISCUSSION

Quality of tap water, treated effluent and soil samples

Analysis of various parameters of wastewater and tap water was carried out in the laboratory and the results are summarized in Table 1. The data from Table 1 indicate that all the parameters are within the limits prescribed by the Food and Agriculture Organization (Ayers & Westcot 1994), World Health Organization (WHO 2006), and Pescod (1992). The concentration of almost all the elements was higher in wastewater than the tap water. It was further observed that wastewater also contained total coliform and fecal coliform in the range of 20×10^3–23×10^4 while it was absent in tap water. The values are much higher than the permissible limits (i.e. 1,000 MPN/100 mL) specified by WHO for unrestricted irrigation (WHO 2006). It may be pointed out that the concentration of heavy metals present in wastewater and tap water (Cd, Ni, Fe, Cu, Mn and Zn) were within the permissible limits as per standards prescribed for land disposal (Pescod 1992).

The texture of the soil was sandy loam. This has great influence on root growth and its ability to absorb water and nutrients in quantities sufficient for optimum growth is most suited for root vegetables. The organic matter content was in the range of 0.72–0.85%, high organic carbon indicates immobilization of many metal ions. The presence of organic matter is important as it is also a source of plant nutrients in addition to its role of providing organic colloids of soil. Therefore, it increases the ion exchange capacity, water holding capacity and soil fertility as it regulates the soil water and air supply, which in turn control the rate at which nutrients are absorbed by the roots. Values of soil pH lie in the range of 7.92–8.10 and the uptake of various plant nutrients is pH dependent. However, the soil contained 215–250 mg/kg P, 60–80 mg/kg K,

Table 1 | Characteristics of treated effluent and tap water

	Year 2008					
Parameters	**Tap water (range)**	**Wastewater 38 ML/d (range)**	**WHO[a]/Pescod[b]/FAO[c] limits of water quality for irrigation**	**Parameters**	**Soil samples (range)**	**SEPA (1995) Limits (mg/kg)**
pH	7.35–7.52	7.24–7.50	6.5–8.4[a]	Soil Texture	Sandy loam	
EC (μmhos/cm at 25 $^{\circ}$C)	608–625	827–858	0.25–3.0[a]	CEC (meq 100 g^{-1} soil)	3.30–3.58	
TDS (mg/L)	360–386	400–738	<2,000[a]	pH	7.92–8.10	
BOD (mg/L)	0–0	20–33	100[b]	Organic Carbon (%)	0.72–0.85	
COD (mg/L)	0–0	49–100	250[b]	EC (μmhos/cm)	290–310	
DO (mg/L)	4.59–4.84	2.4–3.8	–	Total Nitrogen (mg/kg)	145–220	
Calcium as Ca (mg/L)	57–61	76–78	<400[a]	Total Phosphorus (mg/kg)	215–250	
Magnesium as Mg (mg/L)	18.5–25.3	40.5–45.1	<61[a]	Total Potassium (mg/kg)	60–80	
Potassium as K (mg/L)*	3.2–3.9	13.8–14.6	<2.0[a]	Cadmium (mg/kg)*	10–15	0.6
Sodium as Na (mg/L)	31.1–39.5	53.1–60.2	<460[a]	Nickel (mg/kg)*	115–155	60
Total Hardness as CaCO$_3$ (mg/L)	250–283	344–358	–	Iron (mg/kg)	12,410–14,600	–
Total Alkalinity as CaCO$_3$ (mg/L)	252–265	390–400	<610[a]	Copper (mg/kg)	18–30	100
Chloride as Cl^{-} (mg/L)	14.0–16.5	45.8–48.0	<350[a]	Manganese (mg/kg)	300–335	–
Sulfate as SO4^{-2} (mg/L)	15.0–16.2	43.3–45.8	–	Zinc (mg/kg)	140–185	300
Phosphate as PO$_4$-P (mg/L)*	0–0	5.1–5.7	<2.0[a]			
Nitrate Nitrogen as NO$_3$-N (mg/L)	0.026–0.316	0.4–3.2	<10.0[a]			
Ammonical Nitrogen as NH$_4$-N (mg/L)*	0	27.0–50.0	<5.0[a]			
Total Coliform (MPN/100 mL)	< 3	20×10^3–23×10^4	–			
Fecal Coliform (MPN/100 mL)*	< 3	20×10^3–23×10^4	1,000[c]			
Cadmium as Cd (mg/L)	0–0.005	0.006–0.022	0.01[b]			
Nickel as Ni (mg/L)	0.011–0.016	0.012–0.026	0.2[b]			
Iron as Fe (mg/L)	0.019–0.201	0.271–0.772	< 5.0[a]			
Copper as Cu (mg/L)	0.005–0.009	0.022–0.031	0.2[b]			
Manganese as Mn (mg/L)	0.005–0.109	0.072–0.148	< 0.2[a]			
Zinc as Zn (mg/L)	0.006–0.008	0.087–0.115	< 2.0[a]			

*Values crossed the limits of Ayers & Westcot FAO (1994),[a] Pescod (1992),[b] WHO (2006)[c] for irrigation water.

EC: Electric Conductivity, TDS: Total Dissolved Solids, BOD: Biochemical Oxygen Demand, COD: Chemical Oxygen Demand, DO: Dissolved Oxygen, CEC: Cation Exchange Capacity, SEPA: State Environmental Protection Administration.

and 145–220 mg/kg N, which may be an additional source of these macro-nutrients.

Plant growth

The growth of a plant is measured with respect to plant fresh weight, root fresh weight, leaf fresh weight, plant height, leaf number and root diameter as shown through Figure 1(a)–(f). It is also seen that growth can be significantly affected by the concentration of wastewater used for irrigation because in 100% wastewater most of the growth parameters were found to be better as compared to those for all four wastewater concentrations. Irrigation with 100% wastewater proved more effective than 0, 25, 50 and 75% wastewater treatments, while plant growth was low in lower concentrations of wastewater and tap water. Weights of the plants roots and leaves increased with the advancement of age up to 70 days. The root diameter also increased with the increase in growth period (Figure 1(e)). Plant height increased marginally (Figure 1(d)) however, the number of leaves did not exhibit any trend. On the contrary an increase in leaf number from 40 to 55 days followed by a decline was observed in some cases which may be due to the senescence in older leaves at the last stage of growth. It may be recalled that the presence of sufficient essential macro-nutrients plus Na which is categorized as a beneficial element for plants (Salisbury & Ross 1992) in addition to trace elements like Cl, Fe, Cu, Mn, Zn, Ni cumulatively have played a key role in enhanced growth. These nutrients were available to plants due to regular watering and the role of these nutrients is very well documented. For example, consistent supply of N in two forms (NH_4^+ and NO_3^-) present in wastewater could have played a key role in plant fresh weight, by maintaining adequate cation–anion ratio, while P stands second after N in plant nutrition and is also important for all forms of life because of its role in energy transfer via ATP. Like nitrogen and phosphorus, presence of potassium also enhanced the growth as sufficient K was present in the wastewater as well as in the soil. It is thought to be essential for the formation and translocation of carbohydrates and is needed in large quantities by most root crops. In the case of low K the growth rate, cell size and water content of the tissue may be reduced as these are essential growth components of turnip. Another macro-nutrient, Ca, present in wastewater has an important role in the structure and permeability of cell membranes and is also essential for cell elongation and division thereby promoting growth. Therefore the presence of Ca (76–78 mg/L) has also provided

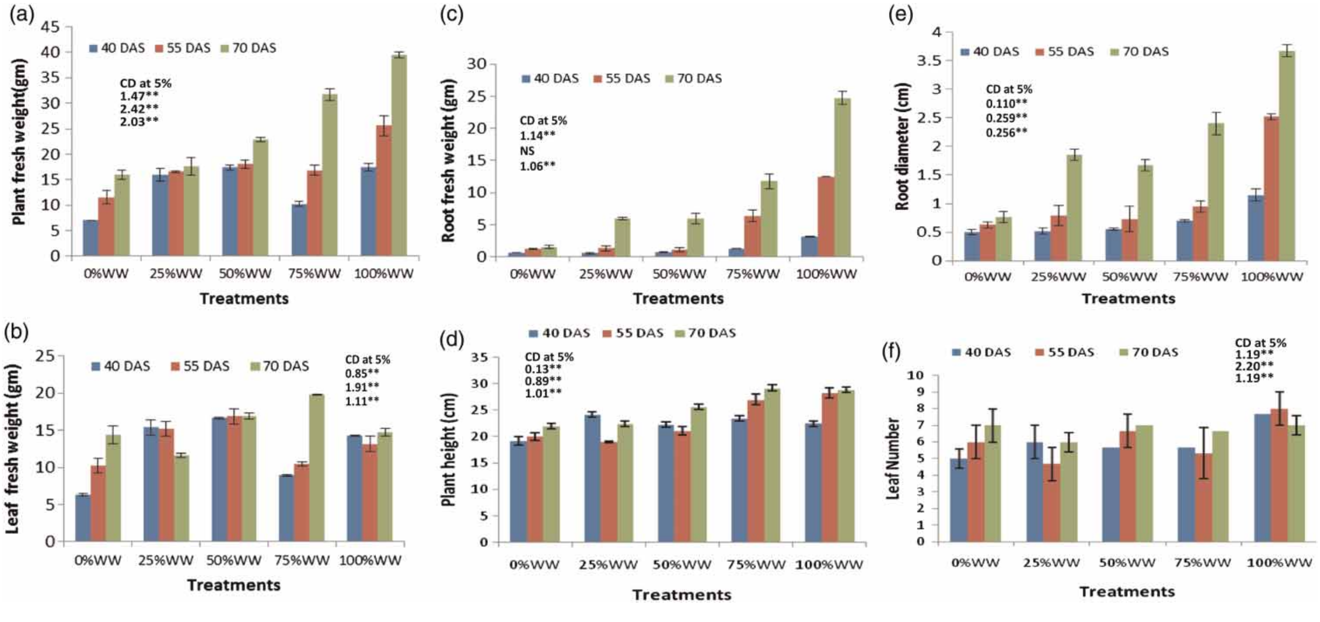

Figure 1 | (a) Plant fresh weight, (b) Leaf fresh weight, (c) Root fresh weight, (d) Plant height, (e) Root diameter, (f) Leaf number at 40, 55 and 70 DAS.

some regulation of cation uptake (Schmit & Le 1981). Magnesium, another essential macro-nutrient (40.5–45.1 mg/L) is an important constituent of the chlorophyll molecule and without it a green plant would fail to carry on photosynthesis (Marschner 2002). Similarly sulfur, absorbed by the plant root almost exclusively as the sulfate ion (SO_4^{-2}), is so important for this crop and it is present in equal or lesser amounts than P in plants such as wheat, corn, beans and potatoes but in larger amounts in alfalfa, cabbage, and turnips.

Among micro-nutrients, Cl^- plays an important role in the opening and closing of stomata which is so vital for gaseous exchange for greater photosynthesis. It is also required in the cell division in leaves and shoots and plants deprived of it tend to have reduced growth (Hopkins & Huner 1995). Its excess, if approaching 300 mg/L, can be harmful but it was found to be within permissible limits in 38 ML/d effluent wastewater. Among other micro-nutrients the sufficiency range of iron in plant tissue is normally between 50 and 250 mg/L and it is a structural component of some important molecules. Also, Cu acts as an electron carrier and part of plastocyanin which has an important role in photosynthesis and dry matter accumulation. Similarly, the involvement of Mn in photosynthesis, particularly in the evolution of O_2, is well known while zinc concentration ranges from 25 to 150 mg/L in plants and its absence or deficiency adversely affects the leaf development as leaf margins are often distorted and it is also essential for certain enzymes (Marschner 2002). On the contrary, Na comes under the category of beneficial elements, which was also present in the wastewater, and it is beneficial for turnips, beets, sugar beets and celery as these crops grow better in its presence and may actually require it (Salisbury & Ross 1992). In this experiment, 14 essential and beneficial elements were thus estimated in wastewater and most of them were in the permissible range as per the FAO limits for irrigation water. The lower concentration of wastewater also improved the growth when compared with tap water as most of these essential nutrients were also present, although in lesser quantity, due to its dilution with tap water. These results were in agreement with the studies conducted by Inam *et al.* (1993) and Aziz *et al.* (1994) on Mathura refinery wastewater, Javid *et al.* (2003) on thermal power plant wastewater, and Jacobs & Ward (2006), Nair *et al.* (2008) and Shahroz (2009) on city wastewater.

Metal accumulation and translocation ratio in plant

The average concentration of heavy metals in leaves and roots of turnip on different days (40, 55 and 70 DAS) is shown through Figure 2(a)–(f). The range of heavy metal concentrations (mg/kg) in all the plants harvested on different days shows that Cd varied from 0.5 to 25.0, Ni 15 to 99, Fe 668 to 1,835, Cu 0 to 21.5, Mn 72 to 233 and Zn 40 to 216 mg/kg, while in leaves Cd varied from 0.53 to 7.8, Ni 12 to 110, Fe 602 to 1,247, Cu 0 to 26.5, Mn 81 to 212 and Zn 46 to 128 mg/kg. Heavy metal concentrations in the root and shoot tissues of plants are also compared with the standards (Kabata-Pendias & Pendias 1984; Alloway 1990). The levels of Cd, Cu, Mn and Zn in the leaves were found to below the toxic limits given by Kabata-Pendias & Pendias (1984) and Alloway (1990) at harvest stage. While in root samples levels of Cu and Mn were found to be below the toxic limits. Heavy metal concentration in leaves was in the order of Fe > Mn > Zn > Ni > Cu > Cd. The trend of heavy metal concentrations in roots was Fe > Mn > Zn > Ni > Cu > Cd. In the present study Fe concentration was higher in plant samples irrigated with wastewater than in the plants irrigated with tap water. The concentration of Fe was greater in the roots than in the leaves and the content decreased with growth of the plant (Figure 2(c)). A similar trend was observed for the other metals, i.e. maximum accumulation in wastewater irrigated plants and minimum in tap water irrigated samples. The differences in the metal contents in these vegetables depend on the physical and chemical nature of the soil and absorption capacity of each metal by the plant, which is altered by various factors like environmental, human interference and the nature of the plant (Arora *et al.* 2008). The concentration trend of Zn was followed by Ni, as it is also readily taken up by most plant species (Havlin *et al.* 1999). Among the six trace elements, comparatively lower concentration of Cu and Cd may be due to their greater dependability on solubility and soil pH. Most of the heavy metals decreased with growth in both organs. This decreasing trend can be ascribed to the exponential increase in growth and, as a result of dilution with growth

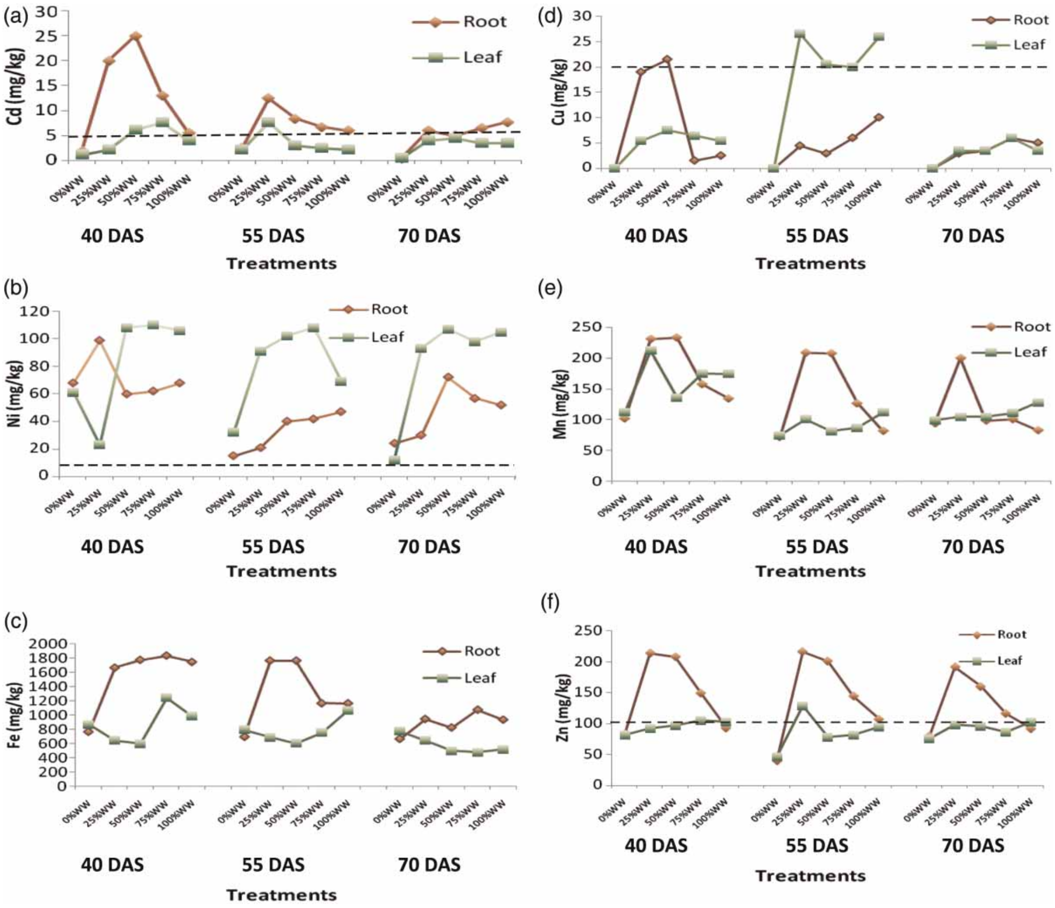

Figure 2 | (a)–(f) Cd, Ni, Fe, Cu, Mn and Zn in leaf and root at different stages of growth, while horizontal lines indicate the limits given by Kabata-Pendias & Pendias (1984) and Alloway (1990).

effect, even higher quantities of elements appear to be less when expressed on a per unit basis (Moorby & Besford 1983).

In most of the experiments heavy metal concentration was greater in the leaves and roots of the plants grown with 25%, 50% and 75% wastewater than the plants grown with 100% wastewater. This was not surprising because metal uptake may differ in relation to external concentration and genotypes (Figure 3). It may also be pointed out that uptake is not linear in relation to the increase in wastewater concentration. This is because metals are bound in the tissues causing saturation which is governed by the rate at which the metal is conducted away. Therefore, the uptake efficiency is greater at low concentration which has been observed in solution culture (Greger *et al.* 1991) as well as in soil (Greger 1997). This may be because of low metal concentration per absorption area giving low competition between the ions at the uptake sites and vice versa. The Cd concentration was found to be maximum in plants irrigated with 50% wastewater at 40 days, and at 55 days it was maximum in plants irrigated with 25% wastewater while at harvest stage it was maximum in plants irrigated with 100% wastewater. Its concentration generally decreased in plant roots with increased growth of the plant. While in leaves its concentration was more or less similar at all the stages of plant growth. Cd concentration was greater in roots then in leaves which was also in agreement with the work of Demirezen & Aksoy (2004). Significantly the level of Cd in roots in the majority of the cases exceeded the toxic levels at 40 DAS, while at harvest stage Cd was surprisingly below the toxic level (Figure 2(a)). It was obviously due to the increased plant fresh weight at this stage which was supposed to be responsible for its dilution. However, the

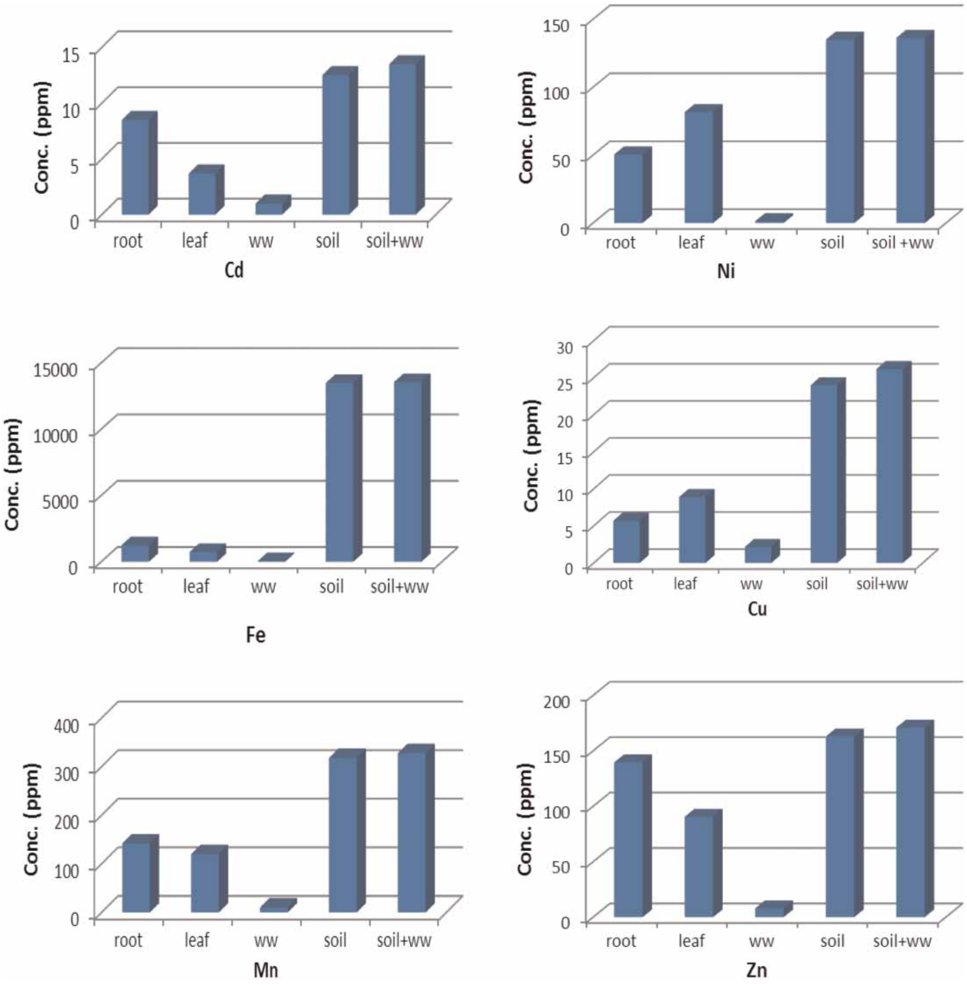

Figure 3 | Distribution of heavy metals (Cd, Ni, Fe, Cu, Mn and Zn) content in turnip leaves and roots, as well as total forms of heavy metals (average values) in soil and wastewater.

total Cd in leaves was below the toxic levels at the last stage of sampling as it was generally retained by the roots instead of being transported towards the shoot. Ni concentration was generally greater in the leaves of the plants. This may be because of its easy mobility in plants as after the initial plant growth phase of 40 days, its concentration was higher and it was then transported towards the growing leaves. Cu was greater in the leaves at 55 DAS while at 70 DAS it was more or less similar in both the plant organs (Figure 2(d)). Mn concentrations in plant samples were found within a range which is lower than the toxic level. Its concentration generally decreased with plant growth. In the present study the range of Mn varied from 72 to 233 mg/kg. In the case of leaves, Mn concentration ranged from 81 to 212 ppm (Figure 2(e)). The concentration of Zn varied from 40 to 216 mg/kg in roots while in leaves Zn concentration varied from 46 to 128 mg/kg. Its concentration was greater in roots than in leaves at all the stages of sampling and the concentration in leaves was almost similar at all the stages of plant growth, while in roots it generally decreased with plant growth (Figure 2(f)). However, Kabata-Pendias & Pendias (1984) reported that toxic or excessive levels of Zn in plants range from 100–400 mg/kg. Comparing with normal and toxic range, all the treatments were found to be within the sufficiency range except for the roots of plants irrigated with 25 and 50% wastewater. Its normal concentration range is from 25 to 150 ppm in plants. The deficiency of Zn is usually associated with concentrations of less than 20 ppm and toxicities with concentration of 400 ppm or more. However, in the present study, the concentration of Zn ranged from 40–216 mg/kg. The translocation ratio was calculated by dividing the heavy metal concentration in the shoot by the concentration in the root. It was highest for Cu followed by Ni, Mn, Fe, Zn and Cd (Figure 4) at 40 and at 55 DAS, while at 70 DAS, it was highest for Ni. The translocation ratio was more than one suggesting greater concentrations of heavy metals in the shoot than in the root portion. Mostly the heavy metals tend to remain in the root portion of horticultural crops (Paivoke 2003), in general the same trend as was observed in the present study.

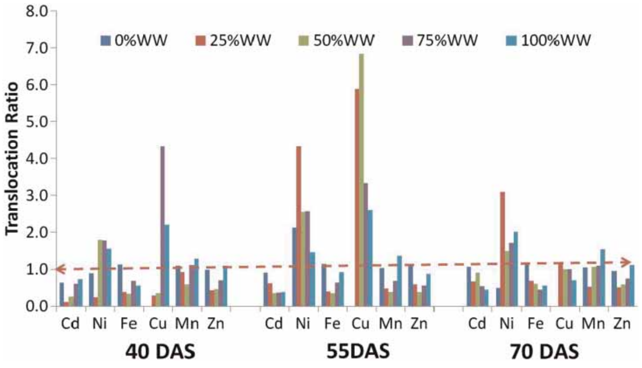

Figure 4 | Translocation ratio (TR) of turnip at different stages of growth.

Daily intake of metals

The daily intake of heavy metals was estimated according to the average vegetable consumption for both adults and children (Tables 2 and 3). The DIM values for heavy metals were higher when based on the consumption of vegetables grown in wastewater than those grown in tap water. The findings of this study regarding DIM suggest that the consumption of plants grown in wastewater and tap water is nearly free of risks, as the dietary intake limits of Cu, Fe, Zn, and Mn in adults can range from 1.2 to 3.0 mg, 10.0 to 50.0 mg, 5.0 to 22.0 mg and 2.0 to 20.0 mg, respectively (WHO 1996). The DIM for adults and children through the consumption of plants irrigated with treated municipal wastewater is summarized in Tables 2 and 3. The highest DIM results in both adults and children were measured for Fe followed by Zn and Mn. The DIM of Fe varied

Table 2 | Daily intake of metals (DIM) for adults and children at 40, 55 and 70 DAS for Cd, Ni and Fe

Treatments	**DIM**		**DIM**		**DIM**	
	Cd		**Ni**		**Fe**	
40 DAS	**Adults**	**Children**	**Adults**	**Children**	**Adults**	**Children**
0%WW	0.002	0.002	0.068	0.078	0.860	0.989
25%WW	0.012	0.013	0.064	0.074	1.217	1.399
50%WW	0.016	0.019	0.088	0.101	1.249	1.436
75%WW	0.011	0.013	0.090	0.104	1.617	1.859
100%WW	0.005	0.006	0.091	0.105	1.438	1.654
55 DAS						
0%WW	0.002	0.003	0.025	0.028	0.782	0.899
25%WW	0.011	0.012	0.059	0.068	1.289	1.482
50%WW	0.006	0.007	0.074	0.086	1.247	1.433
75%WW	0.005	0.006	0.079	0.090	1.010	1.161
100%WW	0.004	0.005	0.061	0.070	1.174	1.350
70 DAS						
0%WW	0.001	0.001	0.019	0.022	0.759	0.872
25%WW	0.005	0.006	0.065	0.074	0.837	0.962
50%WW	0.005	0.006	0.094	0.108	0.700	0.805
75%WW	0.005	0.006	0.081	0.093	0.819	0.942
100%WW	0.006	0.007	0.082	0.095	0.764	0.879

Table 3 | Daily intake of metals (DIM) for adults and children at 40, 55 and 70 DAS for Cu, Mn and Zn

Treatments	**DIM Cu**		**DIM Mn**		**DIM Zn**	
40 DAS	**Adults**	**Children**	**Adults**	**Children**	**Adults**	**Children**
0%WW	0.000	0.000	0.112	0.129	0.086	0.099
25%WW	0.013	0.015	0.232	0.267	0.161	0.185
50%WW	0.015	0.017	0.194	0.223	0.159	0.183
75%WW	0.004	0.005	0.175	0.201	0.133	0.153
100%WW	0.004	0.005	0.162	0.186	0.102	0.118
55 DAS						
0%WW	0.000	0.000	0.077	0.089	0.045	0.052
25%WW	0.016	0.019	0.163	0.187	0.180	0.207
50%WW	0.012	0.014	0.152	0.174	0.146	0.168
75%WW	0.014	0.016	0.112	0.129	0.118	0.136
100%WW	0.019	0.022	0.102	0.117	0.105	0.121
70 DAS						
0%WW	0.000	0.000	0.102	0.117	0.082	0.094
25%WW	0.003	0.004	0.160	0.184	0.151	0.174
50%WW	0.004	0.004	0.107	0.123	0.134	0.154
75%WW	0.006	0.007	0.111	0.128	0.106	0.122
100%WW	0.004	0.005	0.111	0.127	0.102	0.117

DIM: Daily Intake of Metals, DAS: Days After Sowing.

from 0.70 to 1.62, Mn 0.077 to 0.232, Zn 0.045 to 0.180, Ni 0.019 to 0.094, Cu 0 to 0.19 and Cd 0.001 to 0.016, respectively for adults. Meanwhile, the DIM of Fe varied from 0.805 to 1.86, Mn 0.089 to 0.267, Zn 0.052 to 0.207, Ni 0.022 to 0.108, Cu 0 to 0.022 and Cd 0.001 to 0.019, respectively for children. In addition, children were exposed to higher health risks than adults, since the daily intake of metals via consumption of plants was found to be significantly higher for children compared to adults. These results are in agreement with the study conducted by Khan *et al.* (2008) and Arora *et al.* (2008).

CONCLUSIONS

Wastewater irrigation increased the growth of plants because of the presence of more nutrients in wastewater than tap water. The microbiological examination of the wastewater revealed the presence of some pathogenic microorganisms therefore, the growers may be warned to be careful during irrigation operations. It is concluded that the irrigation with treated effluent increased the growth as well as concentration of heavy metals in plant parts. It is believed that translocation of heavy metals from the soil to plant occurred due to the presence of these metals in treated effluent. Plant fresh weight, root fresh weight, leaf fresh weight and root diameter increased with increasing concentrations of wastewater. In leaves, Cd, Cu, Mn and Zn concentrations were below the toxic level at harvest stage. The concentration of heavy metals was at excessive levels at 40 and 55 DAS, while at 70 DAS, metal concentration was comparatively low. Concentration of heavy metals in plants is found to be in the order of Fe > Mn > Zn > Ni > Cu > Cd. The high heavy metal content of the edible plant parts (leaves and roots), and the heavy load of fecal coliform are high health risk factors, which prohibit the use of the treated municipal wastewater for irrigation of vegetables. DIM (daily intake of metals) suggests that the consumption of plants grown in wastewater is high, compared to those grown in tap water, but is nearly free of risks. In the present study, it has been found that excessive levels of heavy metals accumulated in the plant root. Therefore, the root may be treated as a heavy metal accumulator. Under this situation, it is recommended that the roots should be discarded and only the leafy part of the plant, which has a nutritive value and also contains low concentration of heavy metals, may be used for consumption.

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