INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
15–925/2016/18–4–689–697
DOI: 10.17957/IJAB/15.0129
http://www.fspublishers.org

Full Length Article

Monitoring of Growth, Yield, Biomass and Heavy Metals Accumulation in Spinach Grown under Different Irrigation Sources

Safina Naz[1*], Muhammad Akbar Anjum[1] and Saeed Akhtar[2]
[1]*Department of Horticulture, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan*
[2]*Department of Food Science and Technology, Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University, Multan*
[*]For correspondence: safina_bzu@yahoo.com; safinanaz@bzu.edu.pk

Abstract

Growing of vegetables by the use of sewage water in peri-urban areas is a common practice which leads to heavy contamination of vegetables with metal ions. An experiment was conducted to compare the effect of canal, tube well and sewage water on growth, yield, biomass production and heavy metals accumulation in spinach. The heavy metals (Pb, Ni, Cu, Cd, Fe and Cr) contents in different water sources, soils and spinach plant parts grown with these irrigation sources were examined using atomic absorption spectrophotometer. The results declared that sewage water resulted in significantly greater growth, yield, biomass production and heavy metals contents compared with canal and tube well water and these metals contents were exceeding above the critical limits. Cu, Cd and Fe contents in sewage water irrigated soils were found above the maximum permissible limits, while Pb, Ni and Cr contents were within safer limits. Significantly greater heavy metals accumulation was recorded in edible parts of spinach grown with sewage water compared with canal and tube well water, which were exceeding the maximum permissible limits (MPLs). Canal water irrigated leaves samples also contained Cd and Fe above the permissible limits while all other metals contents were found within safe limits. The study concludes that regular examine of metals contents may possibly helpful in minimizing accumulation of these metals in the foods. © 2016 Friends Science Publishers

Keywords: Growth; Yield; Heavy metals; Soil; Sewage water; MRLs; Spinach

Introduction

Farmers used sewage water for irrigation with the target of being a valuable source of nutrients and inexpensively reasonable, as it reduces the expenses of fertilizers (Lone *et al*., 2003; Qadir *et al*., 2008) mainly in farming areas situated near cities or in the surroundings of an industries (Murtaza *et al.,* 2010). Irrigating crops with waste water provides higher yields and enhance biomass productivity as it gives large amount of organic and inorganic constituent's indispensable for good growth and development (Mitra and Gupta, 1999). Whereas, waste water irrigation is generally a main cause of buildup of heavy metals in the soil (Sharma *et al*., 2007). Higher contents of heavy metals contamination and micronutrients in soil occurred due to irrigated fields with long term use of waste water (Larchevêque *et al*., 2006; Riaz *et al.,* 2014) which exert harmful influence on the plant growth. Sewage water contains high amount of organic matter, nutrients and some heavy metals which are toxic to plants beyond a certain limit (Mosleh and Almagrabi, 2013). Continuous use of sewage water may contaminate the soil and crops grown (Khan *et al.*, 2008; Gosh *et al.,* 2012). Heavy metals are toxic in nature due to their non-biodegradable and prolonged half-life. They exert severe health concerns because of their ability to store in body parts of humans. This concern is becoming alarming day by day especially for third world countries (Singh *et al*., 2010). Leafy vegetables accrue greater heavy metals contents compared with other vegetables as these vegetables are exposed to environmental deposition and pollution due to their large leaf area (Itanna, 2002).

Higher Pb concentration in plants enhances the production of reactive oxygen species (ROS), which damages lipid membrane that finally destroy the chlorophyll, metabolic and photosynthetic processes of plants and affected the overall growth (Agarwal, 2002). Yongsheng *et al.* (2011) revealed that higher Pb contents inhibits the growth of teat plants by decreasing plant biomass and reduces the quality of plant by changing the quality attributes. Lead is toxic to human organs, tissues and various body processes and the elevated contents of Pb beyond the safe limits caused severe diseases (Abbas *et al*., 2010; Shivhare and Sharma, 2012).

Plants with high Ni contents suffer from nutrient imbalance, disturbed cell membrane functioning and interruption in plasma membrane by destroying composition of lipid and H-ATPase activity (Ros *et al.*, 1992). Copper (Cu) is regarded as an essential element for plants (Thomas *et al.*, 1998), used in the synthesis of ATP and CO_2 absorption and also the important part of electron transport chain and photosynthetic system (Mahmood and Islam, 2006). Higher contents of Cu in soil exert toxicity at cellular level, cause stress, injured the plants, leaf chlorosis and growth retardation (Lewis *et al*., 2001). Excess Cu contents in plants produces oxidative stress (Stadtman and Oliver, 1991) which disturb the metabolic processes and harm the macromolecules (Hegedus *et al*., 2001).

Cadmium causes breakdown of physiological processes (Prasad, 1995), undersized growth of plants and chlorosis. It also affects length of seedling, dry weight, availability of carbon dioxide, photosynthesis, conductance of stoamta, alters the structure of chloroplast and reduce the activity of photosystem II.

Iron is a vital element and play significant biological role for plants such as development of chloroplast, photosynthesis and in biosynthesis of chlorophyll. It's the important part of heme proteins such as catalase, cytochromes, cytochromes, hemoglobin and ferredoxin, superoxide dismutase (Marschner, 2011). Iron toxicity appears in leaf tissues during flooded situations only, which causes microbial reduction of insoluble Fe^{3+} to insoluble Fe^{2+} (Becker and Asch, 2005). Excess Fe^{2+} uptake in plants release free radicals which damage cellular structure irretrievably and destroyed DNA, proteins and membranes (Arora *et al*., 2002; De Dorlodot *et al*., 2005).

The presence of higher Cr contents beyond the maximum permissible limits is harmful to plants as it cruelly destructs the biological factors and enters the food chain by using these plants. Chromium in humans causes respiratory disorders, skin rashes, destabilized resistant systems, alteration of genetic material, stomach upset, cancer of stomach, respiratory and digestive tract (Axelrod and Hamilton, 1981), mucodermal ulceration, allergic contact, damage of kidneys and liver and lung cancer (Avena, 1979). Regarding the consequences of toxic metals and their intake in foods, a study was planned to monitor Pb, Ni, Cu, Cd, Fe and Cr levels in spinach. The aim of the present study was to document the concentration of toxic heavy metals (Pb, Ni, Cu, Cd, Fe and Cr) in leafy vegetable (spinach) grown in Multan, Pakistan and to determine the effect of different irrigation sources in accumulation efficacy of these metals contents.

Materials and Methods

The current study was conducted in areas continuously irrigated with canal (Dera Muhammadi), tube well (Horticultural experimental field area, BZU, Multan) and sewage water (Tawakal Town) separately.

Procurement of Seeds and Sowing

Seeds of spinach variety green queen were procured from four brother's seed company, Multan. Spinach seeds were sown on ridges 45 cm spaced apart with plant to plant of 5cm on 11th October 2011 for 1st year crop and 11th October, 2012 for 2nd year crop. First irrigation was applied immediately after sowing and subsequent irrigations were applied 7–10 days interval according to crop and experiment requirements. Two weeding were carried out manually to remove weeds. Seed @ 32 kg ha^{-1} was broadcasted in the plots. Fertilizers were applied @ 120-100-40 kg ha^{-1} in the form of urea, DAP and SOP. Plants were sprayed with Acetamaprid @ 200 mL ha^{-1} to control aphids and chlorpyriphos @ 500 mL ha^{-1} to control cutworms. Data were collected on various growth and yield parameters.

Preparation of Water Samples for Heavy Metals Extraction

Water samples from three irrigation sources were collected and pretreated with conc. HNO_3 and 50 mL of water samples were separately digested with 10 mL conc. HNO_3 at 80^{o}C. Soil samples were collected at a depth of 0-30 cm from soils irrigated with three water sources and then air-dried, sieved and stored.

Digestion of Soil Samples

Ten grams of dried soil sample was taken in an Erlenmeyer flask (500 mL) and 20 mL of DTPA extracting agent was added into it. The mouth of flask was tightly closed with a stopper and the flask was shaken for 2 h at a speed of 120 rpm on an automated shaker.

Digestion of Plant Samples

The mature plant parts (leaves and roots) of spinach were harvested randomly, brought to laboratory and washed with distilled water to remove dust particles. Spinach leaf and root samples were cut into pieces and kept in an oven at 70°C for 48 h. Then 0.5 g dried samples (three replicates for leaves and roots of spinach) from each source of irrigation were digested in 15 mL of HNO_3 and 5 mL $HClO_4$ mixture (3:1) on a hot plate (ARE, Velp Italy) at 80 ºC until transparent solutions were obtained.

Extraction of Heavy Metals

The digested water samples of all three irrigation sources, soil and spinach plant parts were filtered through Whatman No. 42 filter paper and filtrate was stored in plastic bottles. The heavy metals (Pb, Ni, Cu, Cd, Fe and Cr) contents in the filtrate were detected through an atomic absorption spectrophotometer (Thermo Scientific 3000 series, US.) (Singh, 2013).

Statistical analysis

The recorded data were subjected to an analysis of variance (ANOVA) as described by Steel *et al.* (1997).

Least significance difference test was applied to assess significant differences between the means at 5% level of probability. Statistical analysis software (SAS) was used to prepare graphs.

Results

Effect of Different Sources of Irrigation Water on Growth, Yield and Biomass Production of Spinach

Different irrigation sources had significant effect on growth, yield and biomass production (on fresh and dry weight basis) of spinach. Significantly greater growth attributes like leaf number, relative chlorophyll contents, leaf area, fresh and dry weight of weight per plant at 1st, 2nd and 3rd cuttings of spinach was recorded in the plants irrigated with sewage water, followed by those irrigated with canal water. Whereas, growth attributes were significantly reduced when tube well water was used as irrigation source (Table 1, 2 and 3). Sewage water resulted in significantly greater yield and yield components and biomass production of spinach, which were greater than canal and tube well water. However, tube well water resulted in the minimum yield and biomass production of spinach (Table 4, 5 and 6). However, the effect of years on all the growth, yield and biomass parameters was found statistically non-significant. The interactive effect of irrigation sources and years was also found statistically non-significant for all growth and yield parameters.

Heavy Metals Contents of Water from Three Irrigation Sources

Heavy metals contents in samples of canal, tube well and sewage water used for irrigating vegetables are presented in Table 7, which indicated significant differences in heavy metals contents as affected by different sources of irrigation (sewage, canal and tube well water). Significantly greater Pb (2.480 mg L^{-1}) and Cr (0.460 mg L^{-1}) contents were found in sewage water samples, followed by canal water. While, lower Pb (0.026 mg L^{-1}) and Cr (0.002 mg L^{-1}) contents were recorded in tube well water, which was significantly lesser than two other sources of irrigation. Sewage water contained significantly higher levels of nickel (1.963 mg L^{-1}), copper (3.267 mg L^{-1}), cadmium (1.267 mg L^{-1}), iron (8.767 mg L^{-1}) and chromium (0.460 mg L^{-1}) compared with other two sources of irrigation water. However, canal and tube well water were statistically at par with each other.

Heavy Metals Status of Field Soils Irrigated with Different Water Sources

The results presented in Table 8 revealed significant differences in three soils irrigated with different sources of water for heavy metals accumulation. There was significantly greater accumulation of DTPA-extractable Pb, Ni, Cu, Cd, Fe and Cr in soil samples irrigated with sewage water, followed by those irrigated with canal water. While tube well water irrigated soil samples contained significantly lower contents.

Table 1: Effect of different sources of irrigation water on growth of spinach at 1st cutting during two years of study

Source of irrigation	Year 2011-2012	Year 2012-2013	Mean
Number of leaves per plant			
Canal	6.56a	6.66a	6.61b
Tube well	6.26a	6.20a	6.23c
Sewage	7.60a	7.53a	7.56a
Mean	*6.81a*	*6.80a*	
Relative leaf chlorophyll content			
Canal	33.48a	32.26a	32.87b
Tube well	30.96a	28.96a	29.96c
Sewage	39.00a	37.66a	38.33a
Mean	*34.48a*	*32.96a*	
Leaf area (cm^2)			
Canal	128.33a	124.43a	126.38b
Tube well	122.37a	119.27a	120.82b
Sewage	135.70a	133.67a	134.68a
Mean	*129.02a*	*125.79a*	
Fresh weight of leaves per plant (g)			
Canal	44.65a	45.33a	44.99b
Tube well	42.61a	42.16a	42.38c
Sewage	51.68a	51.22a	51.45a
Mean	*46.31a*	*46.24a*	
Dry weight of leaves per plant			
Canal	3.57a	3.62a	3.59b
Tube well	3.40a	3.37a	3.39c
Sewage	4.13a	4.09a	4.11a
Mean	*3.70a*	*3.69a*	

Mean values sharing the same letter (s) in a group are not significantly different at $p \geq 0.05$

Table 2: Effect of different sources of irrigation water on growth of spinach at 2nd cutting during two years of study

Source of irrigation	Year 2011-2012	Year 2012-2013	Mean
Number of leaves per plant			
Canal	7.43a	7.60a	7.51b
Tube well	7.20a	7.16a	7.18c
Sewage	8.20a	8.43a	8.31a
Mean	*7.61a*	*7.73a*	
Relative leaf chlorophyll content			
Canal	35.66a	35.30a	35.48b
Tube well	33.50a	32.36a	32.93c
Sewage	38.46a	37.83a	38.15a
Mean	*35.87a*	*35.16a*	
Leaf area (cm^2)			
Canal	134.47a	134.33a	134.40b
Tube well	126.23a	125.10a	125.67c
Sewage	150.20a	152.73a	151.47a
Mean	*136.97a*	*137.39a*	
Fresh weight of leaves per plant (g)			
Canal	49.80a	50.92a	50.36b
Tube well	48.24a	48.01a	48.12c
Sewage	54.94a	56.50a	55.72a
Mean	*50.99a*	*51.81a*	
Dry weight of leaves per plant			
Canal	3.98a	4.07a	4.02b
Tube well	3.85a	3.84a	3.85c
Sewage	4.39a	4.52a	4.45a
Mean	*4.07a*	*4.14a*	

Mean values sharing the same letter (s) in a group are not significantly different at $p \geq 0.05$

Table 3: Effect of different sources of irrigation water on growth of spinach at 3rd cutting during two years of study

Source of irrigation	Year 2011-2012	Year 2012-2013	Mean
Number of leaves per plant			
Canal	7.40a	7.46a	7.43b
Tube well	7.20a	7.13a	7.16c
Sewage	8.93a	9.03a	8.98a
Mean	*7.84a*	*7.87a*	
Relative leaf chlorophyll content			
Canal	39.60a	36.20a	36.60b
Tube well	35.43a	34.00a	34.71c
Sewage	39.60a	39.83a	39.71a
Mean	*37.34a*	*36.67a*	
Leaf area (cm^2)			
Canal	134.47a	135.20a	134.83b
Tube well	126.30a	126.00a	126.15c
Sewage	153.53a	150.47a	152.00a
Mean	*138.10a*	*137.22a*	
Fresh weight of leaves per plant (g)			
Canal	48.10a	48.53a	48.31b
Tube well	46.80a	46.36a	46.58c
Sewage	58.06a	58.71a	58.39a
Mean	*50.98a*	*51.20a*	
Dry weight of leaves per plant (g)			
Canal	3.84a	3.88a	3.86b
Tube well	3.74a	3.70a	3.72c
Sewage	4.64a	4.69a	4.67a
Mean	*4.07a*	*4.09a*	

Mean values sharing the same letter (s) in a group are not significantly different at $p \geq 0.05$

Table 4: Effect of different sources of irrigation water on total yield of spinach during two years of study

Source of irrigation	Year 2011-2012	Year 2012-2013	Mean
Total number of leaves per plant			
Canal	21.40a	21.73a	21.56b
Tube well	20.66a	20.50a	20.58c
Sewage	24.73a	25.00a	24.86a
Mean	*22.26a*	*22.41a*	
Yield per hectare (t/ha)			
Canal	66.81a	66.35a	66.58b
Tube well	63.90a	63.38a	63.64c
Sewage	77.43a	78.02a	77.73a
Mean	*69.38a*	*69.25a*	

Mean values sharing the same letter (s) in a group are not significantly different at $p \geq 0.05$

Table 5: Effect of different sources of irrigation water on total biomass production (on fresh weight basis) yield of spinach during two years of study

Source of irrigation	Year 2011-2012	Year 2012-2013	Mean
Total fresh weight of leaves per plant at 1st , 2nd and 3rd (g)			
Canal	142.56a	144.79a	143.67b
Tube well	137.65a	136.54a	137.10c
Sewage	164.69a	166.45a	165.57a
Mean	*148.30a*	*149.26a*	
Fresh weight of roots per plant (g)			
Canal	14.50a	13.06a	13.78b
Tube well	10.43a	11.20a	10.82c
Sewage	18.60a	17.03a	17.82a
Mean	*14.51a*	*13.77a*	
Total plant biomass on fresh weight basis (g)			
Canal	157.06a	157.85a	157.46b
Tube well	148.09a	147.74a	147.92c
Sewage	183.29a	183.48a	183.38a
Mean	*162.81a*	*163.03a*	

Mean values sharing the same letter (s) in a group are not significantly different at $p \geq 0.05$

Table 6: Effect of different sources of irrigation water on total biomass production (on dry weight basis) yield of spinach during two years of study

Source of irrigation	Year 2011-2012	Year 2012-2013	Mean
Total dry weight of leaves per plant at 1st, 2nd and 3rd cutting (g)			
Canal	11.40a	11.58a	11.49b
Tube well	11.01a	10.92a	10.96c
Sewage	13.17a	13.31a	13.24a
Mean	*11.86a*	*11.94a*	
Dry weight of roots per plant (g)			
Canal	2.90a	2.61a	2.76b
Tube well	2.09a	2.24a	2.16c
Sewage	3.72a	3.40a	3.56a
Mean	*2.90a*	*2.75a*	
Total plant biomass on dry weight basis (g)			
Canal	14.30a	14.19a	14.25b
Tube well	13.09a	13.16a	13.13c
Sewage	16.89a	16.72a	16.80a
Mean	*14.76a*	*14.69a*	

Mean values sharing the same letter (s) in a group are not significantly different at $p \geq 0.05$

Heavy Metals Contents in Spinach Plant Parts

Lead (Pb) contents: Irrigation water sources used significantly influenced the Pb contents in roots and leaves of spinach during 2011–2012 and 2012–2013. The levels of Pb in leaves (5.72 mg kg^{-1}) and roots (5.09 mg kg^{-1}) of sewage water grown plants were significantly higher ($p \geq 0.05$) than those of canal water grown plants which had comparatively lower contents. The lowest Pb contents were observed in leaves (0.24 mg kg^{-1}) and roots (0.11 mg kg^{-1}) of the plants irrigated with tube well water (Fig. 1a). The trend of Pb accumulation was observed during 2012–2013 (Fig. 1b).

Nickel (Ni) contents: Fig. 1c and d showed significant differences for Ni contents among various sources of irrigation water. The maximum Ni contents were observed in leaves (13.54 mg kg^{-1}) and roots (13.49 mg kg^{-1}) of sewage water irrigated plants, followed by canal water irrigated ones. The minimum contents were found in the plants of tube well water irrigated spinach leaves (4.02 mg kg^{-1}) and roots (3.73 mg kg^{-1}) during two years of study.

Copper (Cu) contents: Fig. 1e and f demonstrated significant differences for Cu contents among sewage, canal and tube well water irrigated plants during 2011–2012 and 2012–2013. Significantly higher ($p \geq 0.05$) Cu contents were observed in leaves (18.57 mg kg^{-1}) and roots (17.54 mg kg^{-1}) of spinach irrigated with sewage water compared with other water sources, whereas tube well water resulted in the minimum Cu contents in leaves (5.02 mg kg^{-1}) and roots (4.73 mg kg^{-1}) during both the years (Fig. 1e and f).

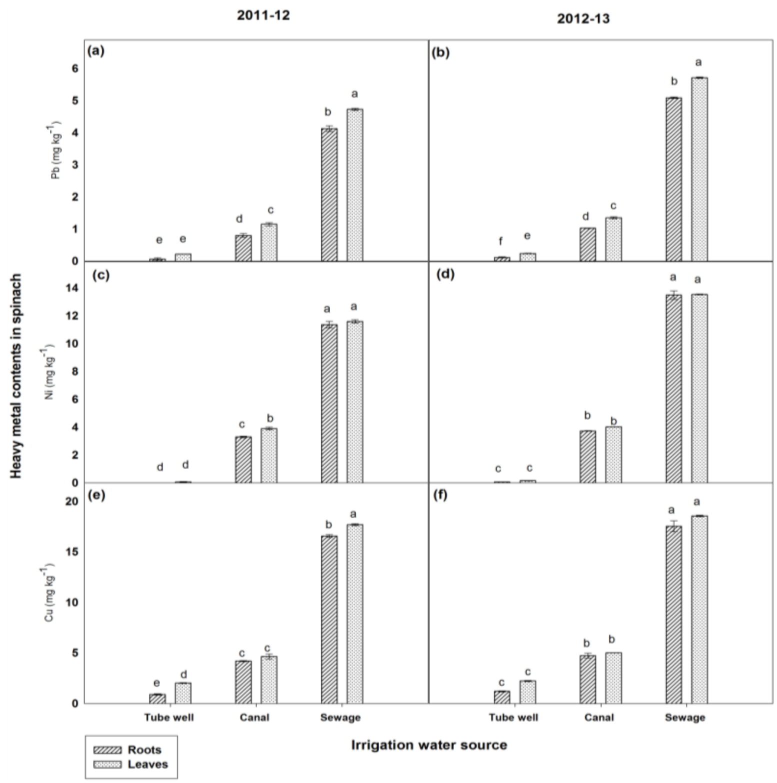

Fig. 1: Effect of different sources of irrigation water on Pb, Ni and Cu accumulation in spinach

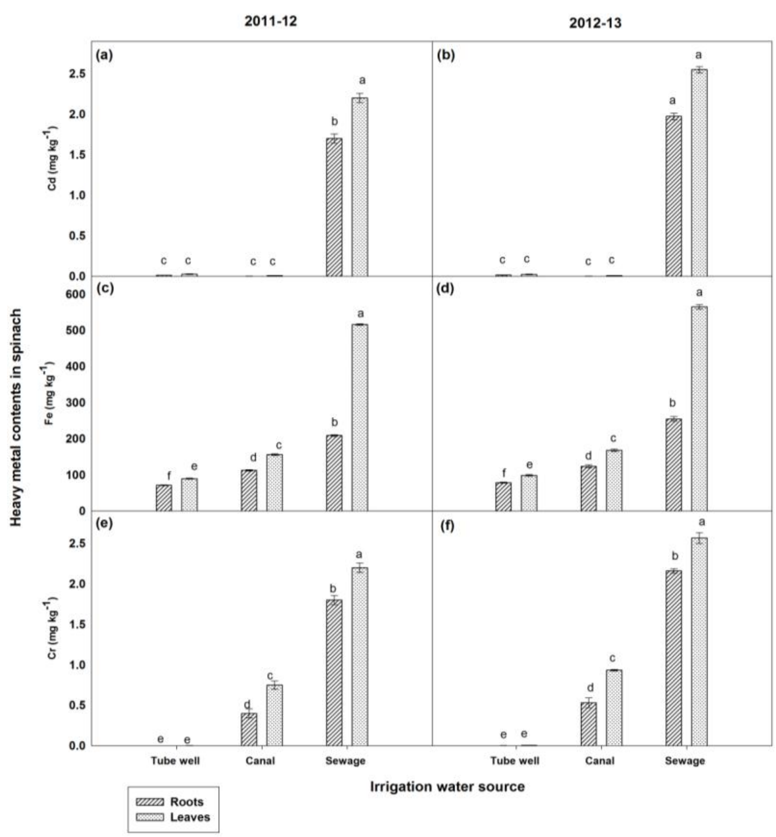

Fig. 2: Effect of different sources of irrigation water on Cd, Fe and Cr accumulation in spinach

Cadmium (Cd) contents: The irrigation sources had significant effect on Cd contents in different plant parts of spinach. Significantly higher ($p \geq 0.05$) Cd contents were examined in leaves (2.20 mg kg^{-1}) and roots (1.70 mg kg^{-1}) of spinach when irrigated with sewage water than those irrigated with other sources of water (Fig. 2a). Similar Cd accumulation was recorded during 2012–2013 (Fig. 2b).

Iron (Fe) contents: The Fe contents in leaves and roots of spinach were significantly influenced by various sources of irrigation water during both the years i.e., 2011–2012 and 2012–2013 (Fig. 2c and d). Significantly higher ($p \geq 0.05$) Fe contents were noted in leaves (516.30 mg kg^{-1}) and roots (208.80 mg kg^{-1}) of spinach plants irrigated with sewage water followed by those irrigated with other sources of water, whereas lower contents were recorded in leaves (89.50 mg kg^{-1}) and roots (71.10 mg kg^{-1}) of those irrigated with tube well water (Fig. 2c). Same pattern of Fe accumulation was recorded during 2012–2013 (Fig. 2d).

Chromium (Cr) contents: Fig. 2e and f depicted significant results for Cr contents between roots and leaves of spinach plants grown with sewage, canal and tube well water (Fig. 2e and f). It is indicated that there was significantly greater ($p \geq 0.05$) Cr content in leaves (2.20 mg kg^{-1}) and roots (1.80 mg kg^{-1}) of the plants irrigated with sewage water than those irrigated with canal and tube well water whereas the lower Cr contents were detected in leaves (0.003 mg kg^{-1}) and roots (0.001 mg kg^{-1}) of the plants irrigated with tube well water (Fig 2e). Similar trend was recorded in spinach plant parts during 2012–13 (Fig. 2f).

Among spinach plant parts, leaves contained greater heavy metals (Pb, Ni, Cu, Cd, Fe and Cr) contents than roots irrespective of irrigation water source during both the years of study.

Discussion

Sewage water application resulted in significantly growth in terms of leaf number, relative leaf chlorophyll content, leaf area, fresh and dry weight of leaves at 1^{st}, 2^{nd} and 3^{rd} cutting of spinach, while the minimum growth was observed in leaves of the plants irrigated with tube well water. Higher relative chlorophyll contents in leaves of the plants irrigated with sewage water might be associated with greater availability of macro and microelements due to sewage water application. These nutrients when taken up by the plant improve growth and enhanced leaf chlorophyll content (Singh and Agrawal, 2009; Khan *et al*., 2011; Balkhair *et al.,* 2013). Higher relative chlorophyll content might also be due to higher nitrogen from sewage water. The organic matter is up taken by plants enhance chlorophyll contents (Kaushik *et al*., 2005). Waste water is a potential source of nutrients and organic matter that enhances the leaf area (Chandra *et al*., 2009) and ultimately photosynthetic activity (Jarvis *et al.,* 1976). Photosynthetic activity is increased with the increase in leaf area, which depends on availability of more nutrients and organic matter (Kaushik *et al.*, 2005).

Table 7: Heavy metals contents of water from different irrigation sources

Water source	Heavy metals (mg L^{-1})					
	Pb	Ni	Cu	Cd	Fe	Cr
Canal	0.289b	0.015b	0.256b	0.013b	1.593b	0.116b
Tube well	0.026c	0.002b	0.073b	0.001b	1.283b	0.002c
Sewage	2.480a	1.963a	3.267a	1.267a	8.767a	0.460a
Permissible limits*	0.10**	0.20*	0.20**	0.01*	5.00**	0.10**

*Maximum recommended concentration in irrigation water (Ayers and Westcot, 1985) **permissible limits as described by WWF (2007) for Pakistan

Table 8: Heavy metals status of field soils irrigated with different water sources

Water source	Heavy metals (mg L^{-1})					
	Pb	Ni	Cu	Cd	Fe	Cr
Canal irrigated	0.713b	0.127b	1.380b	0.034b	19.533b	0.050b
Tubewell irrigated	0.370c	0.042c	0.362c	0.004c	14.467c	0.002c
Sewage irrigated	5.467a	3.803a	7.930a	0.537a	26.767a	0.463a
Critical limits	13.00**	8.10**	0.500*	0.31**	5.00*	8.00**

* = Limits described by Soltanpour (1985); **= limits described by Maclean *et al*. (1987)

Increased supply of nutrients to plants resulted in greater leaf area, more photosynthetic activity and improved CO_2 fixation (Singh and Bhati, 2004). It has been reported that waste water proved better in enhancing leaf number and increasing leaf area of pepper (Alizadeh *et al.*, 2001). Thus, better growth of the vegetables was associated with greater availability of nutrient elements continuously for longer periods from sewage water.

Waste water used for irrigating the lands supplies reasonable quantities of nutrients like N and P, which enhance the fertility status of soil, improve growth and yield attributes and reduces the usage of commercial fertilizers and ultimately enhances the total monetary benefits to the farmers. Higher yield of leafy vegetables such as spinach and cabbage were also reported when the plants were grown with sewage water (Murtaza *et al*., 2003). Earlier Ghafoor *et al.* (2004) and Ahmad *et al*. (2006) also reported that sewage water irrigated spinach plants produced higher yield as it contains a sufficient amount of organic constituents. They further concluded that spinach grows well when irrigated with sewage water and spinach production was increased by 23% when the use of sewage water was enhanced by 1%. Upper layers of waste water irrigated soils contain high organic matter contents, which improve plant growth, lessen the use of chemical fertilizers and enhanced the productivity of poorly fertile soils (Kiziloglu *et al*., 2008).

The concentrations recorded for the heavy metals (Pb, Ni, Cu, Cd, Fe and Cr) in sewage water were greater than the permissible levels prescribed by Ayers and Westcot (1985) and WWF (2007) and were considered unsafe for irrigating vegetables. The Pb, Cu, Cd and Cr contents in canal water were observed slightly higher than the permissible levels, whereas Ni and Fe contents were within the safe range. Tube well water contained all the heavy metals much below the critical levels and safe for irrigating crops. Latif *et al.* (2008) and Gulfraz *et al.* (2002) also observed heavy metals concentrations in sewage water used by farmers for irrigation in Rawalpindi area above the critical limits. Research carried out at Faisalabad (Murtaza *et al*., 2008) and in Haroonabad (Ensink *et al.*, 2004; 2007) indicated higher concentrations of heavy metals in effluents used for irrigation in those areas. Higher levels of Cr, Pb and Cd contents than recommended levels were investigated in waste water of various cities of Pakistan (Khan *et al*., 2008; Murtaza *et al*., 2008; Hussain *et al.*, 2010).

There was a considerable accumulation of DTPA-extractable Pb, Ni, Cu, Cd, Fe and Cr in soil samples irrigated with sewage water as compared to canal and tube well water. The Pb, Ni and Cr contents in all the soil samples irrigated with sewage, canal and tube well water were lower than the critical levels ascribed by Maclean *et al*. (1987). The soils irrigated with sewage water contained higher levels of Cu, Cd and Fe than the recommended levels given by Soltanpour (1985) and Maclean *et al*. (1987). The soils irrigated with canal water had higher Cu and Fe contents than recommended levels. The tube well water irrigated soils accumulated all the heavy metals much below the recommended levels except Fe, which was almost three times of the critical level. Continuous use of waste water for irrigation leads to build up of heavy metals contents in soils (Sharma *et al*., 2007). All the heavy metals contents in tube well water irrigated samples were observed within the critical levels except Fe. Mahmood and Malik (2014) found that bio-available concentration of heavy metals was higher in the soil irrigated with waste water as compared to those irrigated with the ground water. In the present study, the soil irrigated with sewage water had lower soil pH compared to those of irrigated with canal and tube well water. Low soil pH due to sewage water possibly increased heavy metals availability in soil. Lower soil pH in waste water irrigated soils compared to clean water irrigated ones has already been reported by Mohammad and Mazahreh (2003). Sewage water irrigated soils contained Ni, Cu, Cd, Cr and Fe three times greater than those irrigated with tube well water (Kharche *et al.,* 2011). Sewage water irrigated soils contained significant accumulation of Pb, Cu, Ni Cr, Cd and Fe compared with tube well water (Al-Omron *et al*., 2012). Randhawa *et al.* (2014) also reported that sewage and industrial effluents irrigated soils contained higher heavy metals contents especially Cd. Higher heavy metals contents in soils might possibly associated with the fact that these soils were continuously irrigated with industrial and sewage water, which raised heavy metals levels in such soils and also due to exposure of such soil to air pollution, which is caused due to smoke of vehicles rich in these metals. Higher ammonium ions in sewage water led to increase nitrification rate, which released free H^+ ions in the soil, hence reduced the soil pH (Vazquezmontiel *et al*., 1996).

The Pb, Ni, Cu and Cr contents in leaves of spinach irrigated with tube well and canal water was found much lower than the maximum permissible limits, while leaves of sewage water irrigated spinach plants had more contents exceeding the maximum permissible limits (MPLs) prescribed by Asaolu (1995) and Ernst (1996). All the studied heavy metals contents in spinach as affected by different irrigation sources were observed in the order: sewage > canal > tube well water irrigated plants. Less Pb content in spinach leaves and roots irrigated with canal and tube well water was possibly due to the presence of less heavy metals contents in canal and tube well water. Whereas, sewage water was found the major source of heavy metals as compared to canal and tube well water. The heavy metals accumulation in various parts of spinach was found in the order: leaves > roots. Schirado *et al.* (1986) also reported higher contents of Pb, Cu, Cd and Fe in spinach leaves grown with waste water compared with those grown with tube well water. Farooq *et al*. (2008) found higher Pb and Cu contents in spinach leaves compared with roots than the maximum permissible limit when irrigated with industrial effluents. Lone *et al*. (2003) recorded higher Pb, Ni, Cu, Cd, Fe and Cr contents in leaves than roots of spinach plants irrigated with sewage water and the minimum in those irrigated with tube well water. Other investigations from Pakistan documented that the waste water grown vegetables accumulated greater heavy metals contents than ground water grown vegetables (Jan *et al*., 2010; Khan *et al*., 2010). Spinach samples collected from waste water irrigated areas of Faisalabad city contained higher Ni contents and were found insecure for eating (Farid, 2003). Ronaq *et al.* (2005) recorded that spinach leaves were risky for eating due to the presence of heavy metals at toxic levels. The results of the present investigation are in accordance with the findings of Liu *et al.* (2005), Khan *et al*. (2008) and Arora *et al.* (2008), who revealed that spinach grown with waste water had higher Cu when compared with fresh water irrigated spinach, thus indicating greater heavy metals absorption capacity of spinach which can affect the human health adversely.

The industrial waste water and municipal sewage water are generated in large quantities daily and are discharged into canals, which carry heavy metals. In suburban this canal water is used for irrigation purposes, it becomes the source of heavy metals. Continuous application of sewage water as irrigation sources changes the physico-chemical properties of soil and leads to build up of heavy metals in soils and food crops mainly vegetables (Jan *et al*., 2010). In the present study, Cd content was higher in the spinach leaves irrigated with sewage water, than those irrigated with canal and tube well water. This difference in the Cd contents was probably due to the various reasons such as sewage water and soil contents of Cd and ability of plant to uptake and accumulates this metal in different parts (Pandey *et al*., 2012). In the present study, spinach leaves had elevated levels of heavy metals, possibly due to the reason that sewage water which was used for irrigation contained heavy metals.

Conclusion

Sewage water irrigation leads to increase growth and yield of vegetables, which attracts the farmers but also accumulated heavy metals ions in soil and leaves of spinach compared with canal and tube well water irrigated ones. Sewage water was major source of heavy metals in soils and vegetables and make them unfit for consumption. By avoiding the sewage water as irrigation source, the concentration of heavy metals can be decreased. The present study will be helpful in providing a base line data for the country to overcome this distressing situation.

Acknowledgement

The authors are grateful to the Higher Education Commission for financial assistance to conduct the research under Indigenous PhD Fellowship Program.

References

Abbas, M., Z. Parveen, M. Iqbal, M. Riazuddin, S. Iqbal, M. Ahmed and R. Bhutto, 2010. Monitoring of toxic metals (cadmium, lead, arsenic and mercury) in vegetables of Sindh, Pakistan. *Kathmandu Uni. J. Sci., Engg. Technol.*, 6: 60–65

Agarwal, S.K., 2002. *Pollution Management*, Vol. IV, pp: 145–163. Heavy metal pollution. A.P.H. Pub. Co., New Dehli, India

Ahmad, B., K. Bakhsh and S. Hassan, 2006. Effect of sewage water on spinach yield. *Int. J. Agric. Biol*., 8: 423–425

Alizadeh, A., M.E. Bazari, S. Velayati, M. Hasheminia and A. Yaghmaie, 2001. Irrigation of corn with wastewater. In: *ICID International Workshop on Wastewater Reuse and Management*, pp. 147–154. Seoul, Korea

Al-Omron, A.M., S.E. El-Maghraby, A.M. El-Eter and H. Al- Mohani, 2012. Long term effect of irrigation with the treated sewage effluent on some soil properties of Al-Hassa Governorate, Saudi Arabia. *J. Saudi Soc. Agric. Sci.*, 11: 15–18

Arora, A., R.K. Sairam and C.G. Srivastava, 2002. Oxidative stress and antioxidative system in plants. *Curr. Sci. Bangalore*, 82: 1227–1238

Arora, M., B. Kiran, S. Rani, A. Rani, B. Kaur and N. Mittal. 2008, Heavy metal accumulation in vegetables irrigated with water from different sources. *Food Chem.,* 111: 811–815

Asaolu, S.S., 1995. Lead content of vegetable and tomatoes at Erekesan Market, Ado-Ekiti. *Pak. J. Sci. Indus. Res.,* 38: 399–401

Axelrod, R. and W.D. Hamilton, 1981. The evolution of cooperation. *Science*, 211: 1390–1396

Avena, J.M. 1979. *Metallic Poisons*, pp: 252–258. Poisoning, Charles C Thomas Springfield, Illinois, USA

Ayers, R.S. and D.W. Westcot, 1985. *Water Quality for Agriculture*, Vol. 29, pp: 37–70. FAO, Rome, Italy

Balkhair, K.S., F.S. El-Nakhlawi, S.M. Ismail and S.G. Al-Solimani, 2013. Treated wastewater use and its effect on water conservation, vegetative yield, yield components and water use efficiency of some vegetable crops grown under two different irrigation systems in Western region, Saudi Arabia. *Eur. Sci. J.*, 9: 395–402

Becker, M. and F. Asch, 2005. Iron toxicity in rice-conditions and management concepts. *J. Plant Nutr. Soil Sci.*, 168: 558–573

Chandra, R., R.N. Bharagava, S. Yadav and D. Mohan, 2009. Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. *J. Hazar. Mat.,* 162: 1514–1521

De Dorlodot, S., S. Lutts and P. Bertin, 2005. Effects of ferrous iron toxicity on the growth and mineral composition of an interspecific rice. *J. Plant Nutr.*, 28: 1–20

Ensink, J.H.J., T. Mahmood, W. Van der Hoek, L. Raschid-Sally and F.P. Amera-singhe, 2004. A nationwide assessment of wastewater use in Pakistan: An obscure activity or a vitally important one? *Water Policy*, 6: 197–206

Ensink, J.H.J., R.W. Simmons and W. Hoek, 2007. *Wastewater use in Pakistan: The cases of Haroonabad and Faisalabad*. The International Development Research Centre, Canada

Ernst, W.H.O., 1996. Bioavailability of heavy metals and decontamination of soils by plants. *Appl. Geochem.*,11: 163–167

Farid, S., 2003. Toxic element concentration in vegetables irrigated with untreated city effluents. *Sci. Technol. Dev.*, 22: 58–60

Farooq, M., F. Anwar and U. Rashid, 2008. Appraisal of heavy metal contents in different vegetables grown in the vicinity of an industrial area. *Pak. J. Bot*., 40: 2099–2106

Ghafoor, A., M. Qadir, M. Sadiq, G. Murtaza and M.S. Brar, 2004. Lead, copper, zinc and iron concentration in soils and vegetables irrigated with city effluent on urban agricultural lands. *J. Ind. Soc. Soil Sci.*, 52: 114–117

Gosh, A.K., M.A. Bhatt and H.P. Agrawal, 2012. Effect of long-term application of treated sewage water on heavy metal accumulation in vegetables grown in Northern India. *Environ. Monit. Assess*, 184: 1025–1036

Gulfraz, M., Y. Mussaddeq., R. Khannum and T. Ahmad, 2002. Quality assessment of effluents from various industries in the vicinity of Rawalpindi and Islamabad. *J. Biol. Sci*., 2: 697–698

Hegedus, A, S. Erdei and G. Horvath, 2001. Comparative studies of H2O2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. *Plant Sci*., 160: 1085–1093

Hussain, A., G. Murtaza, A. Ghafoor, S.M.A. Basra, M. Qadir and M. Sabir, 2010. Cadmium contamination of soils and crops by long term use of raw effluent, ground and canal waters in agricultural lands. *Int. J. Agric. Biol.*, 12: 851–856

Itanna, F., 2002. Metals in leafy vegetables grown in Addis Ababa and toxicological implications. *Ethiop. J. Health Dev.*, 16: 295–302

Jan, F.A., M. Ishaq, S. Khan, I. Ihsanullah, I. Ahmad and M. Shakirullah, 2010. A comparative study of human health risks via consumption of food crops grown on wastewater irrigated soil (Peshawar) and relatively clean water irrigated soil (Lower Dir). *J. Hazard. Mater*, 179: 612–621

Jarvis, S.C., L.H.P. Jones and M.J. Hooper, 1976. Cadmium uptake from solution by plants and its transport from roots to shoot. *Plant Soil*, 44: 179–191

Kaushik, A.R., K.R. Nisha, K. Jagjeeta and C.P. Kaushik, 2005. Impact of long and short term irrigation of a sodic soil with distillery effluent in combination with bioamendments. *Bioresou. Technol*., 96: 1860–1866

Khan, S., Q. Cao, Y.M. Zheng, Y.Z. Huang and Y.G. Zhu, 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environ. Pollut.*, 152: 686–692

Khan, S., S. Rehman, A.Z. Khan, M.A. Khan and M.T. Shah, 2010. Soil and vegetables enrichment with heavy metals from geological sources in Gilgit, Northern Pakistan. *Ecotoxicol. Environ. Safety*, 73: 1820–1827

Khan, M.J., M.T. Jan, N.U. Farhatullah, M.A. Khan, S. Perveen, S. Alam and A.U. Jan, 2011. The effect of using waste water for tomato. *Pak. J. Bot.*, 43: 1033–1044

Kharche, V.K., V.N. Desai and A.L. Pharande, 2011. Effect of sewage irrigation on soil properties, essential nutrients and pollutant element status of soils and plants in a vegetable growing area around Ahmednagar city in Maharashtra. *J. Ind. Soc. Soil Sci.*, 59: 177–184

Kiziloglu F.M., M. Turan, U. Sahin, Y. Kuslu and A. Dursun, 2008. Effects of untreated and treated wastewater irrigation on some chemical properties of cauliflower (*Brassica olerecea* L. var. botrytis) and red cabbage (*Brassica olerecea* L. var. rubra) grown on calcareous soil in Turkey. *Agric. Water Manag.,* 95: 716–724

Larchevêque, M., C. Ballini, N. korboulewesky and N. Montès, 2006. The use of compost in afforestation of Mideterranean areas: Effects on soil properties and young tree seedlings. *Sci. Tot. Environ.*, 369: 220–230

Latif, M.I., M.I. Lone and K.S. Khan, 2008. Heavy metal contamination of different water sources, soil and vegetables in Rawalpindi area. *Soil Environ.*, 27: 29–35

Lewis, S., M.E. Donkin and M.H. Depledge, 2001. Hsp 70 expression in *Enteromorpha intestinalis* (Chlorophyta) exposed to environmental stressors. *Aquatic Toxicol.*, 51: 277–291

Liu, W.H., J.Z. Zhao, Z.Y. Ouyang, L. Soderlund and G.H. Liu, 2005. Impacts of sewage irrigation on heavy metals distribution and contamination. *Environ. Int.*, 31: 805–812

Lone, M.I., S. Saleem, T. Mahmood, K. Saifullah and G. Hussain, 2013. Heavy metal contents of vegetables irrigated by sewage/tube well water. *Int. J. Agric. Biol.*, 15: 533–535

MacLean, K.S., A.R. Robinson and H.H. MacConnell, 1987. The effect of sewage-sludge on the heavy metal content of soils and plant tissue. *Commun. Soil Sci. Plant Anal*., 18: 1303–1316

Mahmood, T. and K.R. Islam, 2006. Response of rice seedlings to copper toxicity and acidity. *J. Plant Nutr.*, 29: 943–957

Mahmood, A. and R.N. Malik, 2014. Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arab. J. Chem.*, 7: 91–92

Marschner, H., 2011. *Marschner's Mineral Nutrition of Higher Plants*. Academic press, Toronto

Mitra, A. and S.K. Gupta, 1999. Effect of sewage water irrigation on essential plant nutrients and pollutant elements status in a vegetable growing area around Calcutta. *J. Ind. Soc. Soil Sci.*, 47: 99–105

Mohammad, M.J. and N. Mazahreh, 2003. Changes in soil fertility parameters in response to irrigation of forage crops with secondary treated waste water. *Commun. Soil Sci. Plant Anal.*, 34: 1281–1294

Mosleh, Y.Y.I and O.A.E. Almagrabi, 2013. Heavy metal accumulation in some vegetables irrigated with treated waste water. *Int. J. Green Herbal Chem*., 2: 81–90

Murtaza, G., A. Ghafoor, M. Qadir and M.K. Rashid, 2003. Accumulation and bioavailability of Cd, Co and Mn in soils and vegetables irrigated with city effluent. *Pak. J. Agric. Sci.*, 40: 18–24

Murtaza, G., A. Ghafoor and M. Qadir, 2008. Accumulation and implications of cadmium, cobalt and manganese in soils and vegetables irrigated with city effluent. *J. Sci. Food Agr.*, 88: 100–107

Murtaza, G., A. Ghafoor, M. Qadir, G. Owens, M.A. Aziz and M.H. Zia, 2010. Disposal and use of sewage on agricultural lands in Pakistan: A review. *Pedosphere*, 20: 23–34

Pandey, R., K. Shubhashish and J. Pandey, 2012. Dietary intake of pollutant aerosols via vegetables influenced by atmospheric deposition and wastewater irrigation. *Ecotoxicol. Environ. Safety*, 76: 200–208

Prasad, M.N.V., 1995. Cadmium toxicity and tolerance in vascular plants. *Environ. Exp. Bot.*, 35: 525–545

Qadir, M., D. Wichelns, L. Raschid-Sally, P.G. McCornick, P. Derchsel, A. Bhari and P.S. Minhas, 2008. The challenges of wastewater irrigation in developing countries. *Agric. Water Manage.*, 97: 561–568

Riaz, S., M. Iqbal, I. Hussain, R. Rasheed, M.A. Ashraf, S. Mahmood, M. Younas and M.Z. Iqbal, 2014. Chronic cadmium induced oxidative stress not the dna fragmentation modulates growth in spring wheat (*Triticum aestivum*). *Int. J. Agric. Biol.*, 16: 789–794

Randhawa, M.A., G. Ahmad, F.M. Anjum, A. Asghar and M.W. Sajid, 2014. Heavy metal contents and their daily intake in vegetables under peri-urban farming system of Multan, Multan. *Pak. J. Agric. Sci.*, 51: 1025–1031

Ronaq, R.N., I. Haider, M. Qadir and N. Hussain, 2005. Studies on distribution of heavy and toxic metals (Copper, Lead, Zinc and Cadmium) in different vegetables using Atomic Absorption Spectroscopy. In: *Proc. 5th National Chemical Conference*, pp: 63–64, Islamabad, Pakistan

Ros, R., D.T. Cook, C.A.R.M.E.N. Martinez-Cortina and I. Picazo, 1992. Nickel and cadmium-related changes in growth, plasma membrane lipid composition, ATPase hydrolytic activity and proton-pumping of rice (*Oryza sativa* L. cv. Bahia) shoots. *J. Exp. Bot.*, 43: 1475–1481

Schirado, T., I. Vergara, E.B. Schalscha and P.F. Pratt, 1986. Evidence of movement of heavy metals in soil irrigated with untreated waste waters. *J. Environ. Qual.*, 15: 9–12

Sharma, R.K., M. Agrawal and F. Marshall, 2007. Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. *Ecotoxicol. Environ. Safety*, 66: 258–266

Shivhare, L. and S. Sharma, 2012. Effect of toxic heavy metal contaminated soil on an ornamental plant georgina wild (Dahlia). *J. Environ. Anal. Toxicol*., 2: 1–3

Singh, J., 2013. Determination of DTPA extractable heavy metals from sewage irrigated fields and plants. *J. Integ. Sci. Technol.*, 1: 36–40

Singh, G. and M. Bhati, 2004. Soil and plant mineral composition and productivity of *Acacia nilotica* (L.) under irrigation with municipal effluent in an arid environment. *Environ. Conserv.*, 31: 331–338

Singh, R.P. and M. Agrawal, 2009. Use of sewage sludge as fertiliser supplement for *Abelmoschus esculentus* plants: Physiological, biochemical and growth responses. *Int. J. Environ. Waste Manage.*, 3: 91–106

Singh, S., M. Zacharias, S. Kalpana and S. Mishra, 2010. Heavy metals accumulation and distribution pattern in different vegetable crops. *J. Environ. Chem. Ecotoxicol*., 4: 75–81

Soltanpour, P.N., 1985. Use of ammonium bicarbonate DTPA soil test to evaluate elemental availability and toxicity 1. *Commun. Soil Sci. Plant Anal.*, 16: 323–338

Stadtman, E.R. and C.N. Oliver, 1991. Metal-catalyzed oxidation of proteins. Physiological consequences. *J. Biol. Chem.*, 266: 2005–2008

Steel, R.G.D., J. Torrie and D.A. Dickey, 1997. *Principles and Procedures of Statistics: A Biometrical Approach*, 3rd edition. McGraw Hill Book Co., New York, USA

Thomas, F., C. Malick, E.C. Endreszl and K.S. Davies, 1998. Distinct responses to copper stress in the halophyte, *Mesembryanthemum crystallium*. *Physiol. Plant.*, 102: 360–368

Vazquezmontiel, O., N.J. Horan and D.D Mara, 1996. Management of domestic waste water for reuse in irrigation. *Water Sci. Technol.*, 33: 355–362

WWF, 2007. *Report on National Surface Water Classification Criteria, Irrigation Water Quality Guidelines for Pakistan.* February-2007. Waste Water Forum Pakistan

Yongsheng, W., L. Qihui and T. Qian, 2011. Effect of Pb on growth, accumulation and quality component of tea plant. *Procedia Eng.*, 18: 214–219

(Received 24 November 2015; Accepted 04 December 2015)