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COMPARISON OF PROXIMATE AND HEAVY METAL CONTENTS OF VEGETABLES GROWN WITH FRESH AND WASTEWATER

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Abstract

Due to insufficient clean water resources wastewater is largely used for irrigation of vegetables in many developing countries, particularly in Pakistan. As a result, vegetables in spite of providing nutrients also accumulate toxic metals and causes health risks. In the present study, comparison of heavy metal contents, using two digestion methods, in fresh and wastewater-irrigated vegetables was appraised by ICP-AES and also their proximate composition was accessed. Samples of wastewater-irrigated vegetables; cauliflower, green pepper, spring onion and brinjal edibles and leaves were collected from wastewater-irrigated and fresh water-irrigated area of Faisalabad. In all the samples large variation of elemental concentration was found however, wastewater grown vegetables showed more accumulation of heavy metals than their respective fresh water irrigated vegetables. The amount of Pb (1.87 mg kg^{-1}), Fe (1.09 mg kg^{-1}), Cu (1.01 mg kg^{-1}), Zn (1.03 mg kg^{-1}), Cr (0.97 mg kg^{-1}) showed more accumulation than Mn (0.17 mg kg^{-1}), Cd (0.08 mg kg^{-1}) and Ni (0.45 mg kg^{-1}) both the edible and leaves of investigated vegetables but nearly all metals were found within safe limits. Only Pb (1.87 mg kg^{-1}) concentration in wastewater-irrigated vegetables exceeded the permissible limits defined by World Health Organization (WHO). Of the two digestion methods nitric acid showed more recovery of metals as compared to dry ash procedure. The leaves of vegetables showed more intake capacity of heavy metals. Continuous accumulation of these metals can pose severe threats to health of people.

Introduction

Freshwater is a unique natural resource with intriguing qualities. Worldwide fresh water is inadequate and declining as demand for water has tripled since 1950s (Gleick, 2003) particularly in regions of world such as Africa, Southern Asia and Middle East. Among the entire major water consuming sector, agriculture is the leading sector using freshwater worldwide, accounting for almost 85% of global consumption (Jury & Vaux, 2007). According to Intergovernmental Panel on Climate Change several hundred million people will face serious water shortage due to global warming in the near future.

Municipalities have only two options for wastewater disposal, first in open water bodies and the second to add in ground water and turn a blind eye to use it for agricultural purposes (Rattan *et al.,* 2001). Farmers use wastewater for irrigation with an intention of being a rich source of nutrient and economically feasible, as it saves a lot of fertilizer expenditure (Lone *et al*., 2003; Qadir *et al*., 2008) particularly in agricultural lands located near cities or in the vicinity of an industrial area (Murtaza *et al.,* 2010). Irrigation practices require a large volume of water as 80- 95% of the mass of plants is composed of moisture (Singh *et al*., 2001; Hanif *et al.,* 2006). In spite of containing essential plant nutrients as N, P, K, Ca, Mg and Fe which are important for plant growth, wastewater also contains a variety of inorganic substances from domestic and industrial sources, including potential toxic elements and heavy metals Pb, Cd, Ni, Cr and Hg (Sharma *et al*., 2006) which may be at phytotoxic levels or originate health risks (Pereira *et al*., 2002; Murtaza *et al.,* 2010). Consequently, the usage of wastewater for irrigation ends to soil contamination and heavy metals accumulation both in soil and crops (Bigdeli & Seilsepour, 2008; Zulfqar *et al*., 2012).

Heavy metals no doubt, are important constituents for plants and humans but only in small amount; some micronutrient elements may also be toxic to both animals and humans at high concentrations, for example copper (Cu), chromium (Cr), fluorine (F), molybdenum (Mo), nickel (Ni), selenium (Se) or zinc (Zn). Other trace elements such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) are toxic even at small concentrations (Divrikli *et al.*, 2006). They are persistent and non-biodegradable that are neither removed by normal cropping nor are they easily leached by rainwater as they have strong affinities by soil solid phase (Tandi *et al.*, 2004). The duration of contamination by heavy metals may be for hundreds or thousands of years, even after their addition to soils had been stopped (Mapanda *et al.*, 2005;). Moisture, fibers and ash contents of vegetables and plant species have been considered vital to the human health and also for the soil quality (Hussain *et al*., 2011).

Vegetables accumulate heavy metals in their edible and non-edible parts (Perveen *et al.,* 2012). Leafy parts of vegetables accumulate higher amounts of heavy metals than their fruits (Sawidis *et al.*, 2001). The ability to accumulate these metals depends on several factors as its assimilation capability, levels of sewage sludge amendments applied (Muchuweti *et al*., 2006) and plant metal uptake or transfer factor of metals from soil to plants. Mapanda *et al*., (2005) determined that the concentrations of heavy metals in vegetables per unit dry matter generally follow the order: leaves > fresh fruits > seeds. Human exposure to heavy metals has been linked with increased blood acidity, developmental retardation, various cancers, and interference with essential elements, kidney damage, and even death.

The total water resources of Pakistan are not sufficient to meet up its requirements especially of irrigation. Pakistan is an agricultural country and more than 60 different types of vegetable species are cultivated as summer and winter crops in Pakistan (Tahir *et al*., 2011). Total region of Punjab under crop growing is 57% of total cultivation in Pakistan. In Pakistan total geographic area is about 20.63 million hectares of which 12.49 million hectares is under cultivation (Anon., 2008-09) while vegetables are grown in the area of about 253800 hectares during 2007-08 (Perveen *et al*., 2010). In Faisalabad district about 1623 liters/sec of wastewater is used for irrigation and more than 2000 hectares are irrigated by wastewater. The present study was intended to determine heavy metal accumulation and proximate analysis in edible part of vegetables and their leaves, to determine and compare the heavy metal concentrations in different parts of vegetables i.e., edible and leaves irrigated with fresh water and wastewater and also to access the recovery of different metals by wet and dry digestion processes. For this investigative study status of eight heavy metals (Pb, Cd, Cr, Ni, Cu, Zn, Mn and Fe) in four vegetables (Cauliflower, Green pepper, Spring onion and Brinjal) were determined.

Materials and Methods

Collection of samples: The fully grown vegetable's (cauliflower, green pepper, spring onion and brinjal) edible portion and leaves of fresh water irrigated were randomly collected from University of Agriculture, Faisalabad and the same vegetables irrigated with wastewater were randomly collected from industrial areas of Faisalabad, Pakistan. The each variety collected was further authenticated from Department of Botany, GC University Faisalabad, Pakistan.

Pre-treatment: Samples of edible part of vegetables and their leaves were thoroughly washed with distilled water to remove any dust particles and then air dried for an hour and put in an electric oven at 70ºC for a day to remove all of its moisture. Dried samples were ground and sieved to obtain fine powder. All the samples were placed in polythene bags until used for further analysis.

Proximate analysis: The proximate analysis of vegetable's edible part and their leaves were analyzed using standard methods. Moisture content was determined using reported method (Anwar *et al*., 2011). Ash content was evaluated according to ISO (Anonymous, 1977) method. Crude fiber content was determined according to the ISO (Anonymous, 1981) method. Kjeldahl's method was used to estimate the protein contents in the investigated vegetables as described in AOAC (1985).

Digestion of samples: Two different digestion processes i.e., wet digestion/ nitric acid method (Farooq *et al*., 2008; Hseu 2004) and dry ash method (Hseu, 2004) were used to prepare the samples for determination of heavy metal concentration in the fresh and wastewater irrigated vegetables.

Analysis of samples: Metal ion concentrations (Pb, Cd, Cr, Ni, Cu, Zn, Mn and Fe) were measured by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).

Statistical analysis: The recorded data were subjected to two-way analysis of variance (ANOVA) to assess the influence of different variables on the concentrations of heavy metals in the fresh and wastewater irrigated vegetables (Steel & Torrie, 1986).

Results and Discussion

Proximate analysis: Moisture content of leaves, edibles of wastewater irrigated vegetables ranged between 79.1-83.6%, 83.6-95.1% respectively, whereas in fresh water grown leaves, edibles samples it was found 76.2-88.9%, 85.6-95.1% respectively (Tables 1 and 2). The results are comparable (78.53-86.53%) to the moisture content reported in leaves of different vegetables by Effiong *et al.,* (2009). Edible part of vegetable showed high mean moisture content to those of leaves of fresh and wastewater grown vegetables. Hanif, *et al.,* (2006) also found moisture in edible parts of different vegetables in the range of 77-95%.

The ash content of wastewater leaves was found to be in range of 0.4-2.05% (Table 1) and its edible parts contained 0.25-1.4% (Table 2). In fresh water, leaves of vegetables ranged 0.5-1.85% (Table 1) and in edibles it ranged 0.25-1.65% (Table 2). Edible parts showed less ash contents as compared to leafy parts of same vegetables. Hanif *et al*., (2006) found ash content (0.4-1.2%) in leafy and non leafy vegetables which support the results of leafy parts but the results of non leafy parts are slightly higher (Hanif, *et al*., 2006). All vegetables showed lower values of ash to those found by Effiong, *et al.,* (2009) for pumpkin and okra. Variation may occur due to environmental conditions of collection area and different varieties.

The crude fiber content in leaves of wastewater grown vegetables was found to be in the range of 7.6-12.8% (Table 1) and in edible part it was found to be in range of 4.6-11% (Table 2). In fresh water is ranged from 7.8-13.2% in leaves whereas in edible portion it was found 4.7-10% as shown (Tables 1 and 2). Results of edible and leafy parts of vegetables demonstrated that leafy vegetables contained more fiber compared to non leafy vegetables. Effiong *et al*., (2009) also reported that leafy parts contained more fiber than non leafy parts. Hanif, *et al*., (2006) evaluated less mean fiber contents in different varieties of vegetables. There is great variation in fiber content comparison of fresh and wastewater grown vegetables and their leaves. In some vegetables wastewater irrigated showed more fiber in others fresh water irrigated showed more fiber.

The crude protein content in leaves, edible portions of wastewater grown vegetables were in the range of 26.2-32.8%, 21.8-37.5%, respectively (Tables 1 and 2). In leaves of fresh water irrigated vegetables crude protein was found to be in the range of 20.6-37.1% (Table 1) and in edible portion ranged 24-30.6% (Table 2). The results of crude protein contents in selected vegetables are in similar to observed by Effiong, *et al*., (2009) for different vegetables but higher than observed by Hanif, *et al*., (2006). Wastewater grown vegetables edible and leafy parts contained more protein contents than fresh water grown edibles and leaves except in cauliflower leaf and edible and onion edible. This may be due to high nitrogen contents in wastewater in comparison to fresh water.

Table 1. Proximate composition of different vegetables leaves grown with wastewater and fresh water.

Vegetables	Moisture		Ash		Fibre		Protein	
	Wastewater	Fresh water	Wastewater	Fresh water	Wastewater	Fresh water	Wastewater	Fresh water
	%							
Cauliflower	83.6 + 2.05	88.9 + 2.07	1.22 + 0.035	1.85 + 0.034	12.8 + 0.3	13.2 + 0.3	26.2 + 0.6	30.6 + 0.9
Green pepper	79.9 + 1.9	79.9 + 1.9	1.5 + 0.04	1.65 + 0.04	7.6 + 0.1	7.8 + 0.1	32.8 + 0.9	37.1 + 1.1
Spring onion	79.1 + 1.9	81.6 + 2.0	0.4 + 0.01	0.5 + 0.01	9.2 + 0.2	11.6 + 0.2	26.2 + 0.7	20.6 + 0.6
Brinjal	79.7 + 2.3	76.2 + 1.9	2.05 + 0.06	1.85 + 0.05	11.1 + 0.2	11.2 + 0.3	32.8 + 0.9	30.6 + 0.7

Values are mean ± SD of three samples of leaves, edibles of each vegetable irrigated with fresh and wastewater

Table 2. Proximate composition of edible parts of different vegetables grown with wastewater and fresh water.

Vegetables	Moisture		Ash		Fibre		Protein	
	Wastewater	Fresh water	Wastewater	Fresh water	Wastewater	Fresh water	Wastewater	Fresh water
	%							
Cauliflower	84.7 + 2.5	88.1 + 2.1	1.25 + 0.03	1.65 + 0.06	7.0 + 0.1	7.6 + 0.1	26.3 + 0.7	28.4 + 0.7
Green pepper	83.6 + 2.5	85.6 + 2.1	0.5 + 0.012	0.4 + 0.01	5.2 + 0.1	5.3 + 0.1	37.5 + 0.9	30.6 + 0.7
Spring onion	95.1 + 2.4	95.1 + 2.85	0.25 + 0.037	0.25 + 0.07	4.6 + 0.1	4.7 + 0.1	21.8 + 0.5	24.0 + 0.8
Brinjal	87.3 + 2.2	89.8 + 2.25	1.4 + 0.03	0.75 + 0.02	11.0 + 0.4	10.0 + 0.3	32.8 + 0.8	29.6 + 0.8

Values are mean ± SD of three samples of leaves, edibles of each vegetable irrigated with fresh and wastewater

Heavy metal contents

Lead (Pb) contents: In all the samples digested by two methods (Dry ash and HNO_3 digestion method), Pb showed highest concentration in cauliflower irrigated with fresh and wastewater (0.06-1.87 mg kg^{-1}) (Tables 3-10). And lowest concentrations were observed in both parts of green pepper (0.02-0.014 mg kg^{-1}) (Tables 4, 6, 8 and 10) digested by both methods, grown with fresh water. On the other hand, in wastewater grown vegetables, spring onion (1.10-1.31 mg kg^{-1}) showed least accumulation of Pb digested by both methods (Tables 3, 5, 7 and 9). Higher amount of Pb was found in wastewater grown leaves and edible part of all the vegetables (1.10-1.87 mg kg^{-1}) as compared to fresh water grown vegetables digested by both methods (0.014-0.08). Statistically significant differences ($p<0.05$) were obtained between fresh water and wastewater-irrigated samples. The waste water irrigated all the samples have concentrations of Pb exceeding much the safe limits (0.3 mg kg^{-1}) as described by WHO standards and can pose severe health effects. Muchuwti *et al.*, (2006) also evaluated lead contents (6.77 mg kg^{-1}) exceeding the safe limits in wastewater grown vegetables. Continuing irrigation with this water can result in more build up of Pb in all the samples. All the samples grown under fresh water were found to be within the safe limits. Results of analysis for leaves (0.02-1.87 mg kg^{-1}) of samples showed more accumulation of Pb than their respective edible parts (0.014-1.83 mg kg^{-1}). Sawidis *et al.*, (2001) also found that leaves accumulate more concentration of heavy metals than edible parts. Pb showed (Tables 1-8) more recovery in samples digested by dry ash procedure (0.014-1.87 mg kg^{-1}) than their respective samples digested by nitric acid procedure (0.018-1.73 mg kg^{-1}), only a few samples showed equal recovery.

Cadmium (Cd) contents: In all the samples digested by dry ash and HNO_3 digestion method Cd was shown highest in concentration in cauliflower irrigated with both fresh and wastewater (0.039-0.08 mg kg^{-1}) (Tables 3-10). And lowest concentration of Cd was shown in spring onion digested by both methods (0.01-0.039 mg kg^{-1}). Higher amount of Cd was found in wastewater grown leaves and edible part of all the vegetables (0.03-0.08 mg kg^{-1}) as compared to fresh water grown vegetables digested with both methods (0.01-0.05 mg kg^{-1}). The statistical analysis revealed that the difference was non-significant ($p>0.05$) between fresh water and wastewater irrigated leaves and significant ($p<0.05$) between edibles of different vegetables digested by dry ash method. However, nitric acid method showed non-significant ($p>0.05$) difference between fresh and wastewater edibles and significant difference ($p<0.05$) between their leaves. Leafy parts of vegetables showed more Cd contents as determined by Fytianos *et al*., (2001) that roots and leafy vegetables accumulate Cd more effectively and industrial area vegetables contained more Cd than their respective rural area grown vegetables. Cd concentrations in fresh and wastewater grown vegetables were within safe limits (0.1 mg kg^{-1}) as recommended by WHO guidelines. Yususf *et al.*, (2003) found that Cd ranges in leafy and non leafy vegetables were 0.09-0.62 mg kg^{-1}. Al-Jassir *et al*., (2005) determined Cd in different vegetables in the range of 0.046-0.353 mg kg^{-1}. In the present study, both edible part and leafy parts of cauliflower grown with wastewater showed Cd concentration very close to permissible limits (0.1 mg kg^{-1}), and if the wastewater is supplied continually to the same field it may result in exceeded concentration of Cd. In all the selected vegetable samples Cd concentrations vary, it showed vegetables have different capacities for absorbing a metal or may depend on several factors. Cd recovery by both digestion methods did not exhibit much difference. Thus Cd recovered equal in both processes of digestion or slightly more by nitric acid method.

Chromium (Cr) contents: Brinjal showed more accumulation for Cr (0.06-0.97 mg kg^{-1}) and green pepper showed least (0.03-0.24 mg kg^{-1}) Cr concentrations in most of the selected vegetables (Tables 3-10). Results of analysis showed that there was not much variation in Cr recovery in leaves and their respective edible parts. But significant differences ($p<0.05$) were observed between fresh and wastewater irrigated samples digested by dry ash method. However, there were non-significant differences ($p>0.05$) found between fresh and wastewater-irrigated samples digested by nitric acid method. No international guidelines or standards were found for limits of chromium concentration in vegetables. Divirkli *et al*., (2006) examined Cr contents in herbal spices that was high (0.1-9.7 mg kg^{-1}) comparable to the present study (0.03-0.97 mg kg^{-1}). In another study, Sharma *et al.*, (2006) found Cr concentrations within safe limits. In most of the samples, Cr recovery was found more by dry ash digestion method. Hseu (2004) also found recovery of Cr more by dry ash method than nitric acid method in different composts.

Nickel (Ni) contents: In some vegetables Ni showed more recovery in dry ash and in others more in nitric acid. More recovery of nickel was found by nitric acid samples than dry ash process. Leaves (0.02-0.45 mg kg^{-1}) (Tables 3, 4, 7 and 8) of nearly all samples contained more metal accumulation than their edible parts (0.018-0.4 mg kg^{-1}) (Tables 5, 6, 9, 10). All the waste and fresh water samples Ni was found within safe limits (67 mg kg^{-1}) though in waste water grown samples (0.03-0.45 mg kg^{-1}) more accumulation of Ni was found than fresh water samples (0.018-0.07 mg kg^{-1}) which were in accordance to those examined by Demirezen & Aksoy (2006) that the concentration of nickel to be high in vegetables grown in urban area (1.8-13.45 mg kg^{-1}) than the same vegetables grown in rural area (0.44-4.02 mg kg^{-1}) of Turkey but nickel was found to be within safe limits in both areas. The statistical analysis revealed that the difference was non-significant ($p>0.05$) between fresh water and wastewater irrigated leaves and edibles of different vegetables.

Table 3. Heavy metal concentrations in leaves of different vegetables grown with wastewater using dry ash method.

Vegetables	Pb	Cd	Cr	Ni	Cu	Zn	Mn	Fe
	mg kg^{-1}							
Cauliflower	1.87 ± 0.04	0.07 ± 0.002	0.53 ± 0.01	0.40 ± 0.01	0.50 ± 0.01	0.91 ± 0.04	0.15 ± 0.003	0.77 ± 0.02
Green pepper	1.43 ± 0.04	0.03 ± 0.002	0.22 ± 0.01	0.05 ± 0.001	0.99 ± 0.03	0.99 ± 0.04	0.05 ± 0.001	0.99 ± 0.04
Spring onion	1.31 ± 0.03	0.03 ± 0.001	0.42 ± 0.01	0.05 ± 0.001	0.13 ± 0.003	0.67 ± 0.02	0.11 ± 0.003	0.72 ± 0.01
Brinjal	1.41 ± 0.03	0.05 ± 0.002	0.97 ± 0.02	0.04 ± 0.001	0.26 ± 0.01	0.63 ± 0.02	0.11 ± 0.003	0.98 ± 0.02
Safe Limit[a]	0.3	0.1	-	67	73	100	500	425

Values are mean ± SD of three samples of leaves of each vegetable, analyzed individually in triplicate. Pb = lead; Cd = cadmium; Cr = chromium, Ni = nickel; Cu = copper; Zn = zinc; Mn = maganese; Fe = ferric; [a] FAO/WHO standard (Codex Alimentarius Comission, 1995, 2001)

Table 4. Heavy metal concentrations in leaves of different vegetables grown with fresh water using dry ash method

Vegetables	Pb	Cd	Cr	Ni	Cu	Zn	Mn	Fe
	mg kg^{-1}							
Cauliflower	0.08 ± 0.002	0.05 ± 0.002	0.09 ± 0.002	0.07 ± 0.001	0.08 ± 0.002	0.77 ± 0.02	0.12 ± 0.008	0.76 ± 0.02
Green pepper	0.02 ± 0.001	0.022 ± 0.001	0.04 ± 0.002	0.03 ± 0.001	0.05 ± 0.001	0.89 ± 0.01	0.02 ± 0.081	0.33 ± 0.01
Spring onion	0.06 ± 0.002	0.02 ± 0.001	0.06 ± 0.002	0.04 ± 0.001	0.05 ± 0.002	0.06 ± 0.001	0.13 ± 0.002	0.61 ± 0.01
Brinjal	0.059 ± 0.001	0.04 ± 0.001	0.08 ± 0.003	0.05 ± 0.001	0.02 ± 0.001	0.54 ± 0.01	0.01 ± 0.001	0.76 ± 0.03
Safe Limit[a]	0.3	0.1	-	67	73	100	500	425

Values are mean ± SD of three samples of leaves of each vegetable, analyzed individually in triplicate. Pb = lead; Cd = cadmium; Cr = chromium, Ni = nickel; Cu = copper; Zn = zinc; Mn = maganese; Fe = ferric; [a] FAO/WHO standard (Codex Alimentarius Comission, 1995, 2001)

Table 5. Heavy metal concentrations in edible part of different vegetables grown with wastewater using dry ash method.

Vegetables	Pb	Cd	Cr	Ni	Cu	Zn	Mn	Fe
	mg kg^{-1}							
Cauliflower	1.83 ± 0.05	0.07 ± 0.001	0.52 ± 0.01	0.31 ±0.01	0.42 ± 0.01	0.88 ± 0.02	0.14 ± 0.004	0.82 ± 0.02
Green pepper	1.41 ± 0.03	0.03 ± 0.001	0.24 ± 0.01	0.04 ± 0.001	0.82 ± 0.02	0.95 ± 0.02	0.05 ± 0.001	0.85 ± 0.01
Spring onion	1.28 ± 0.02	0.03 ± 0.001	0.41 ± 0.01	0.04 ± 0.001	0.09 ± 0.002	0.6 ± 0.03	0.08 ± 0.001	0.59 ± 0.02
Brinjal	1.4 ± 0.3	0.05 ± 0.001	0.95 ± 0.01	0.03 ± 0.001	0.22 ± 0.01	0.64 ± 0.02	0.09 ± 0.001	0.9 ± 0.01
Safe Limit[a]	0.3	0.1	-	67	73	100	500	425

Values are mean ± SD of three samples of edibles of each vegetable, analyzed individually in triplicate. Pb = lead; Cd = cadmium; Cr = chromium, Ni = nickel; Cu = copper; Zn = zinc; Mn = maganese; Fe = ferric; [a] FAO/WHO standard (Codex Alimentarius Comission, 1995, 2001)

Table 6. Heavy metal concentrations in edible part of different vegetables grown with fresh water using dry ash method.

Vegetables	Pb	Cd	Cr	Ni	Cu	Zn	Mn	Fe
	mg kg^{-1}							
Cauliflower	0.076 ± 0.001	0.04 ± 0.001	0.07 ± 0.001	0.067 ± 0.002	0.07 ± 0.002	0.79 ± 0.02	0.12 ± 0.005	0.71 ± 0.01
Green Pepper	0.014 ± 0.002	0.02 ± 0.001	0.03 ± 0.001	0.023 ± 0.003	0.03 ± 0.001	0.56 ± 0.01	0.006 ± 0.0001	0.28 ± 0.006
Spring onion	0.05 ± 0.002	0.01 ± 0.002	0.06 ± 0.001	0.03 ± 0.001	0.046 ± 0.001	0.06 ± 0.001	0.12 ± 0.003	0.56 ± 0.02
Brinjal	0.05 ± 0.001	0.04 ± 0.002	0.077 ± 0.002	0.02 ± 0.001	0.02 ± 0.001	0.6 ± 0.01	0.01 ± 0.001	0.77 ± 0.03
Safe Limit[a]	0.3	0.1	-	67	73	100	500	425

Values are mean ± SD of three samples of edibles of each vegetable, analyzed individually in triplicate. Pb = lead; Cd = cadmium; Cr = chromium, Ni = nickel; Cu = copper; Zn = zinc; Mn = maganese; Fe = ferric; [a] FAO/WHO standard (Codex Alimentarius Comission, 1995, 2001)

Table 7. Heavy metal concentrations in leaves of different vegetables grown with wastewater using nitric acid method.

Vegetables	Pb	Cd	Cr	Ni	Cu	Zn	Mn	Fe
	mg kg^{-1}							
Cauliflower	1.73 ± 0.04	0.08 ± 0.002	0.43 ± 0.01	0.45 ± 0.01	0.67 ± 0.02	0.89 ± 0.02	0.17 ± 0.005	0.83 ± 0.002
Green pepper	1.31 ± 0.03	0.04 ± 0.001	0.22 ± 0.01	0.05 ± 0.02	1.01 ± 0.03	1.03 ± 0.02	0.07 ± 0.001	0.46 ± 0.01
Spring onion	1.16 ± 0.02	0.039 ± 0.001	0.39 ± 0.01	0.08 ± 0.001	0.11 ± 0.002	0.75 ± 0.01	0.15 ± 0.003	0.77 ± 0.01
Brinjal	1.43 ± 0.02	0.05 ± 0.002	0.89 ± 0.02	0.04 ± 0.001	0.3 ± 0.01	0.59 ± 0.01	0.13 ± 0.003	1.09 ± 0.01
Safe Limit [a]	0.3	0.1	-	67	73	100	500	425

Values are mean ± SD of three samples of leaves of each vegetable, analyzed individually in triplicate. Pb = lead; Cd = cadmium; Cr = chromium, Ni = nickel; Cu = copper; Zn = zinc; Mn = maganese; Fe = ferric; [a] FAO/WHO standard (Codex Alimentarius Comission, 1995, 2001)

Table 8. Heavy metal concentrations in leaves of different vegetables grown with fresh water using nitric acid method.

Vegetables	Pb	Cd	Cr	Ni	Cu	Zn	Mn	Fe
	mg kg^{-1}							
Cauliflower	0.07 ± 0.002	0.05 ± 0.001	0.09 ± 0.002	0.07 ± 0.002	0.08 ± 0.002	0.76 ± 0.01	0.13 ± 0.004	0.81 ± 0.01
Green pepper	0.02 ± 0.001	0.03 ± 0.001	0.04 ± 0.001	0.02 ± 0.001	0.08 ± 0.003	0.89 ± 0.02	0.008 ± 0.0003	0.39 ± 0.01
Spring onion	0.02 ± 0.001	0.032 ± 0.002	0.06 ± 0.001	0.05 ± 0.001	0.06 ± 0.002	0.09 ± 0.001	0.14 ± 0.003	0.65 ± 0.01
Brinjal	0.06 ± 0.002	0.04 ± 0.001	0.07 ± 0.001	0.04 ± 0.001	0.05 ± 0.002	0.51 ± 0.01	0.02 ± 0.001	1.00 ± 0.03
Safe Limit[a]	0.3	0.1	-	67	73	100	500	425

Values are mean ± SD of three samples of leaves of each vegetable, analyzed individually in triplicate. Pb = lead; Cd = cadmium; Cr = chromium, Ni = nickel; Cu = copper; Zn = zinc; Mn = maganese; Fe = ferric; [a] FAO/WHO standard (Codex Alimentarius Comission, 1995, 2001)

Table 9. Heavy metal concentrations in edible part of different vegetables grown with wastewater using nitric acid method.

Vegetables	Pb	Cd	Cr	Ni	Cu	Zn	Mn	Fe
	mg kg^{-1}							
Cauliflower	1.69 ± 0.04	0.076 ± 0.001	0.42 ± 0.01	0.4 ± 0.02	0.59 ± 0.02	0.90 ± 0.02	0.15 ± 0.004	0.72 ± 0.001
Green pepper	1.33 ± 0.02	0.03 ± 0.001	0.21 ± 0.01	0.04 ± 0.001	0.89 ± 0.02	0.97 ± 0.01	0.08 ± 0.002	0.41 ± 0.02
Spring onion	1.10 ± 0.01	0.03 ± 0.001	0.38 ± 0.01	0.07 ± 0.003	0.9 ± 0.02	0.69 ± 0.01	0.11 ± 0.002	0.61 ± 0.02
Brinjal	1.40 ± 0.03	0.047 ± 0.001	0.87 ± 0.02	0.03 ± 0.001	0.29 ± 0.01	0.63 ± 0.02	0.10 ± 0.003	1.08 ± 0.01
Safe Limit[a]	0.3	0.1	-	67	73	100	500	425

Values are mean ± SD of three samples of edibles of each vegetable, analyzed individually in triplicate. Pb = lead; Cd = cadmium; Cr = chromium, Ni = nickel; Cu = copper; Zn = zinc; Mn = maganese; Fe = ferric; [a] FAO/WHO standard (Codex Alimentarius Comission, 1995, 2001)

Table 10. Heavy metal concentrations in edible part of different vegetables grown with fresh water using nitric acid method.

Vegetables	Pb	Cd	Cr	Ni	Cu	Zn	Mn	Fe
	mg kg^{-1}							
Cauliflower	0.06 ± 0.001	0.039 ± 0.001	0.08 ± 0.003	0.059 ± 0.002	0.07 ± 0.002	0.81 ± 0.02	0.13 ± 0.003	0.75 ± 0.01
Green pepper	0.018 ± 0.004	0.026 ± 0.003	0.04 ± 0.001	0.018 ± 0.001	0.07 ± 0.001	0.57 ± 0.03	0.007 ± 0.0002	0.28 ± 0.01
Spring onion	0.05 ± 0.001	0.02 ± 0.0006	0.05 ± 0.002	0.045 ± 0.004	0.05 ± 0.001	0.08 ± 0.002	0.13 ± 0.004	0.59 ± 0.01
Brinjal	0.05 ± 0.001	0.03 ± 0.001	0.06 ± 0.002	0.02 ± 0.001	0.04 ± 0.001	0.6 ± 0.01	0.02 ± 0.001	0.98 ± 0.02
Safe Limit[a]	0.3	0.1	-	67	73	100	500	425

Values are mean ± SD of three samples of edibles of each vegetable, analyzed individually in triplicate. Pb = lead; Cd = cadmium; Cr = chromium, Ni = nickel; Cu = copper; Zn = zinc; Mn = maganese; Fe = ferric; [a] FAO/WHO standard (Codex Alimentarius Comission, 1995, 2001)

Copper (Cu) contents: Cu contents were found more in wastewater-irrigated samples (0.09-1.01 mg kg^{-1}) (Tables 3, 5, 7 and 9) than fresh water samples (0.02-0.08 mg kg^{-1}) (Tables 4, 6, 8 and 10), but Cu was found within safe limits (73 mg kg^{-1}) in all the samples as described by WHO standards. There were great variation found in accumulation of Cu by different vegetables but in most of the samples, green pepper (0.03-1.01 mg kg^{-1}) showed more accumulation of Cu. In nitric acid method copper showed more recovery of metal supporting the results of Hseu (2004). Leaves (0.02-1.01 mg kg^{-1}) showed more concentration of copper than edible portion (0.02-0.9 mg kg^{-1}) except spring onion edible part (0.9 mg kg^{-1}) grown with waste water and digested by dry ash procedure showed much higher concentration of Cu than its leaf part (0.11 mg kg^{-1}). In fact, non-significant (p>0.05) difference was found between fresh and wastewater grown all vegetable portions except for nitric acid digested fresh water grown edible parts (0.02-0.9 mg kg^{-1}) of vegetables. Fytianos *et al*., (2001) observed that Cu contents in vegetables grown in urban and rural areas did not vary significantly while Farooq *et al*., (2008) determined Cu contents were below 10 mg kg^{-1}.

Manganese (Mn) contents: Ellen *et al*., (1990) found Mn concentrations in different fruits and vegetables in the range of 0.95-5.50 mg kg^{-1} whereas in the present study the tested vegetables showed values below to that range. In present study, all the samples cauliflower (0.12-0.17 mg kg^{-1}) showed higher accumulation of Mn but green pepper (0.006-0.08 mg kg^{-1}) showed least accumulation of Mn (Tables 3-10). The variation in heavy metal concentration in vegetables may be due to variation in their absorption, and accumulation tendency. (Khan *et al*., 2012; Fardous *et al*., 2010; Sinha *et al*., 2006) Wastewater gown samples depicted more Mn concentration but in spring onion samples fresh water samples (0.12-0.14 mg kg^{-1}) showed slightly more concentration of Mn than waste water grown spring onion (0.08-0.15 mg kg^{-1}). Thus, no significant difference (p>0.05) was found between fresh and wastewater grown samples. In the present study, all the vegetable samples, Mn recovery was found more in nitric acid method than dry ash method. Leaves (0.008-0.17 mg kg^{-1}) of the vegetable samples showed more accumulation than their edible parts (0.006-0.15 mg kg^{-1}). In most of the samples nitric acid method gave high recovery for Mn.

Zinc (Zn) contents: Zn was found highly variable in different vegetable samples, more values were found in green pepper and cauliflower samples and low concentrations of Zn were found in brinjal and spring onion. Non-significant differences (p>0.05) were found within fresh and wastewater samples. However brinjal and cauliflower edible parts showed more concentration of Zn than their leafy parts. Demirezen & Aksoy (2006) analyzed different vegetables and found Zn contents (3.56-4.59 mg kg^{-1}) which are also in limits (100 mg kg^{-1}). In the present study, wastewater irrigated sample (0.6-1.03 mg kg^{-1}) were found to contain more Zn concentrations than their fresh water irrigated samples (0.06-0.89 mg kg^{-1}). In most of the samples Zn recovery was found better in nitric acid digestion process but some samples showed better recovery by dry ash procedure.

Iron (Fe) contents: Fe accumulation was found highest in leaves and edible part of brinjal (0.76-1.09 mg kg^{-1}) (Table 3-10). Ismail *et al*., (2005) also found that Fe, Zn and Mn were most commonly detected metals and Fe concentration was found below (0.33-0.54 mg kg^{-1}) than that examined in the present study. But all samples of Fe were found to be within limits defined by WHO (425 mg kg^{-1}). Though wastewater grown (0.41-1.09 mg kg^{-1}) samples showed more accumulation of Fe than their fresh water grown parts (0.28-1.0 mg kg^{-1}). Fe recovery was found better in nitric acid digestion method (0.28-1.09 mg kg^{-1}) as compared to dry ash method (0.28-0.99 mg kg^{-1}). In dry ash digested samples non-significant difference (p>0.05) was found in fresh and wastewater irrigated samples. In nitric acid digested samples, however, it differs non-significantly (p>0.05) in fresh and wastewater edible while fresh and waste leaves was found to differ significantly (p<0.05).

Conclusion

The results from the present study suggested that elemental concentration existed more in wastewater-grown vegetables than fresh water irrigated vegetables. Pb contents were above the toxicity level in wastewater grown vegetables and Cr contents were found near to toxic levels. Other heavy metals were within permissible limits. Elemental concentrations also varied between different vegetable varieties. This may be due to their structures and assimilation capacities. Comparatively, cauliflower showed highest metal accumulation. Mainly leafy parts of all the vegetables depicted more concentration of metals than their fruits. Of the two methods used nitric acid yields more recovery of metals except for Pb and Cr. Mean moisture, ash and fiber contents found more in fresh water vegetables but mean protein contents examined more in wastewater vegetables. Moisture contents were found more in edibles and ash contents in leaves of vegetables. It could be concluded that in wastewater grown vegetables, uptake of metals may increase and nutritional value decrease that severely damage health of people.

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