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Accumulation of Heavy Metals in Vegetable Food under Wastewater Irrigation

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ABSTRACT

Sewage water contains toxic heavy metals which can be translocated and accumulated in plants and subsequently transferred to human body through the food chain, yet it has become the most commonly used water source for irrigating vegetable crops in peri-urban or urban areas of several countries including in Pakistan. Karachi, the metropolitan city of Pakistan, is the largest industrial and financial hub of the country with an estimated 16 Million population of multilingual, multi-cultural and multi-religious peoples. The current study was conducted to examine the accumulation of six heavy metals (Cr, Ni, Cd, Pb, As and Hg) in cabbage, radish, turnip, cauliflower, and carrot crops, irrigated with sewage water (SW) of peri-urban area of the Karachi. Four treatments were designed, the fresh water (FW) was used as the control (T_0), whereas T_1, T_2 T_3 and T_4 contained 25, 50, 75 and 100% of SW respectively. The samples analyzed through atomic absorption spectrophotometer using flame atomic absorption techniques revealed that among the five treatments, accumulation of the six metals was found higher with 100% SW, which was decreased with decrease in SW concentration up to 25% SW. The minimum accumulation of the metal was noted with 100% FW (control). Among the five types of vegetables, cabbage and cauliflower revealed a high tendency of accumulating the metals. Hence, in order to avoid exposure of excess heavy metals to human health through vegetables, the cabbage and cauliflower crops may not be grown in the vicinity of Karachi city where the source of irrigation water is only sewage water.

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Authors' Contribution
ARM and TJ designed the study. AK and MAB performed the field work and collected the data. AAM and AK analyzed the data. SI, FKS and AK drafted the manuscript. AAM and TJ reviewed the manuscript. All the authors read and approved the final manuscript.

Key words
Heavy metals, Karachi, Sewage water, Urbanization, Vegetables

INTRODUCTION

Agriculture is the largest sector of Pakistan's economy with a great diversity of vegetable, horticultural and cash crops (Azam and Shafique, 2017). It is employing 43.3% workforce and contributing 22.8% to grass domestic productivity (GDP) of the country (GOP, 2018). More than 60% of the people in Pakistan rely directly or indirectly on agricultural farming (Khan *et al.*, 2020). Vegetables, amongst the other crops, are the most important and common source of food and business in several countries. In Asia, vegetables are consumed more than the meet (beef or mutton), especially in Pakistan and India, several people are vegetarians and their sole dependency for food is over vegetables. The vegetative parts, fruit and seeds of vegetable plants are rich source of carbohydrates, proteins, vitamins, minerals, antioxidants, dietary fiber and several essential metabolites (Buturi *et al.*, 2021). These crops are cultivated both in rural and urban areas of the world. Currently, the significant increase in sewage water (SW) production due to rapid urbanization and industrialization has left no choice but to use it for agricultural purpose specially in urban areas of a country (Qin *et al.*, 2014). An estimated 2 million km^2 of land is irrigated with SW around the world (Hamilton *et al.*, 2007). The cultivation of vegetable crops using SW has though a positive impact on vegetable crop in term of their yield (Kaur *et al.*, 2012) but the presence of heavy metals in the sewage water has led to the deterioration of food quality and thus it is a serious issue for health of peoples around the globe (Rehman *et al.*, 2018).

Depending upon the source of generation, sewage water is a potential source of many heavy metals including copper (Cu), zinc (Zn), lead (Pb), nickel (Ni), chromium (Cr), magnesium (Mg) and iron (Fe) (Marshall *et al.*, 2006). The contamination of soil and vegetable crops grown in the vicinity of industrial areas of a metropolitan city is reported by (Akram *et al.*, 2014; Bi *et al.*, 2018;

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Cao *et al*., 2010; Chabukdhara *et al*., 2016; Kachenko and Singh, 2006; Khan *et al*., 2010; Proshad *et al*., 2020). The uptake or accumulation of metals in vegetative or reproductive parts of a plant varies with the type of vegetable crop (Cherfi *et al*., 2016; Uzma *et al*., 2016). The leafy vegetables accumulate higher concentration of heavy metals than the non-leafy vegetables (Khan *et al*., 2010).

The irrigation of vegetables crops with sewage water and the accumulation of heavy metals in plant parts is the most serious issue worldwide (Islam *et al*., 2015). The excessive exposure of heavy metals to human body through food chain has profound impacts on its health (Sanaei *et al*., 2021; Zakir *et al*., 2020). For examples, the contribution of Cadmium (Cd), Fe, Pb, Mercury (Hg), Zn and Ni in causing various kinds of cancers has been reported by (Lui *et al*., 2006). According to (Patra *et al*., 2002), the consumption of vegetables with high amount of Pb and Cd can cause heart, kidney, bone and nervous system related problems. Likewise, the consumption of food containing excessive amount of Cu cause iron deficiency and devastation of cellular membranes (Arredondo and Núñez, 2005; Cuypers *et al*., 2012; Tapiero *et al*., 2003). Therefore, it is necessary to investigate the quality of vegetables crops irrigated with sewage water in urban areas of metropolitan cities in the world.

The population of Pakistan has increased up to 6.5 billion and is expected to reach 2.34 billion by 2025 (Ayub *et al*., 2020). This rapid increase in population growth has increased the construction of urban areas, hence the production of SW has been increased every day. In peri-urban or urban areas of Pakistan, the sewage water is most commonly used (32,500 Ha) for growing vegetable crops (Ensink *et al*., 2004; Khan *et al*., 2017). Karachi is the main metropolitan city of Pakistan with an estimated 16 Million population (Chandir *et al*., 2020) of multi-cultural and multi-religious peoples. Majority of the inhabitant relay upon vegetables crops for their daily diet. Several peoples of Hindu community living in the city are also vegetarians, who consume fresh vegetables and pulses as their only source of food. Karachi is an industrial area, harboring textile mills and food industries. Irrigating vegetable crops with sewage water, containing industrial waste municipal water, in the vicinity of Karachi is the most common practice. There is no way to stop irrigating vegetable crops with sewage water in this area due to the lack of sufficient fresh water. However, investigating the quality of SW and vegetables grown on SW to ensure that the toxic metals are under the acceptable limits as per recommendations of the World Health Organization (WHO) is an inevitable goal of a research industry (Ambika and Ambika, 2010; Kumar and Chopra, 2014; Rattan *et al*., 2005).

Karachi is the largest metropolitan city of with Pakistan with a high population density. A very little data is published, reporting the contamination of vegetable crops with heavy metals in metropolitan cities of Pakistan (Jamali *et al*., 2009; Jan *et al*., 2010a, b; Khan *et al*., 2010). This study reports the accumulation of heavy metals in vegetable crops grown under sewage water in the vicinity of the Karachi city.

MATERIALS AND METHODS

Study area and experimental design

The current study was conducted at experimental fields of Pakistan Agricultural Research Council (PARC), University of Karachi, Karachi. The source of the sewage water was the drainage system of Karachi University. The study comprised five treatments for each of the five vegetable crops and each treatment was replicated thrice. The field was divided into seventy-five (75) sub-plots each measuring 3×2 m^2. The treatments included, T_0 (control, 100% FW), T_1 (25% SW and 75% FW), T_2 (50% SW and 50% FW), T_3 (75% SW and 25% FW) and T_4 (100% SW). The vegetable crops tested were carrot, turnip, radish, cabbage and cauliflower. The experiments were completely randomized block design (RCBD).

Seed sowing and fertilizer applications

The seeds of all the five vegetables were sown from 25th to 27th September 2018. Seed sowing process was carried out following the recommended procedures that included sowing to live with a plant distance of 0.9 cm. DAP (8.5 Kg) was applied after preparation of seed bed before sowing and Urea (12 Kg) was split into two equal dozes of 6.0 Kg, one applied after 20 days of sowing and the other was applied along with SOP 5.5 Kg after 45 days of sowing at the time of fruiting initiation.

Sample collection

Soil and water sampling and processing

Five samples of soil were collected randomly in the plastic bags from the field at the depth of 0-45 cm. Water (fresh and municipal sewage) samples were collected in triplicates from the experimental field. The samples were brought to the PARC laboratory for physicochemical analysis at the Institute of Food Quality and safety Research, Karachi University, Karachi. The soil samples were air-died and passed through 2 mm sieve before using for the analysis. The physio-chemical characteristics of the soil and water are present in Table I.

Plant sampling and processing

The edible parts of vegetables i.e. leave of cabbage; roots of carrot, turnip and radish; and flowers of cauliflower were collected as samples in labeled polythene bags from

Table I. Physicochemical properties of soil and sewage water collected from the experimental site.

Sr. No	Parameters	Soil (µg g^{-1})		Sewage Water (µg ml^{-1})		References
		Current	Safe limit	Current	Safe limit	
1	EC (dS m^{-1})	0.43 ± 0.03	2-4	2.7 ± 0.09	3	Anwar *et al*. (2016)
2	pH	8.1 ± 0.01	≤8.5	7.9 ± 0.01	6.0-8.5	Anwar *et al*. (2016)
3	Cr	5.31 ± 0.14	100	2.73 ± 0.027	0.55	Chiroma *et al*. (2014)
4	Ni	31.53 ± 1.3	50	13.27 ± 0.82	1.40	Chiroma *et al*. (2014)
5	Cd	4.18 ± 0.02	3	0.14 ± 0.0007	0.01	Chiroma *et al*. (2014)
6	Pb	12.83 ± 0.06	100	1.73 ± 0.0015	0.065	Chiroma *et al*. (2014)
7	Hg	2.47 ± 0.011	--*	0.02 ± 0.0004	--	--
8	As	7.18 ± 0.009	20	0.31 ± 0.0008	0.10	Chiroma *et al*. (2014)

*Not available.

the field and were immediately brought to the laboratory. The samples were cut into small pieces before these were oven dried for 2 h. Each oven dried sample was ground to powder using piston and mortar. The powdered samples were shifted to new labeled polythene bags for further use.

For predigest process, approximately 0.5 g of each powdered sample was taken in the digestion tube and 20 ml of nitric acid (HNO_3) followed by 3 ml of perchloric acid ($HClO_4$) was added to each tube. The tubes were allowed for predigest process overnight. On next day, the samples were placed on heating block at 180°C for 2 h or till the white fume was appeared. The samples were then allowed to cool before these were transferred to 100 ml volumetric flasks. The volume was raised up-to the mark before the samples were used for analysis, the working concentrations for each of the metal was prepared. Atomic Absorption Spectrophotometer (FS-220) along with Graphite Furnace (GTA-110) was used for the metal analysis. The details of analytical conditions for analysis of heavy metals are present in Supplementary Table I.

For analysis of metals, first the instrument calibration and working dilutions were prepared from 1000 ppm stock solutions of cadmium (cd), lead (Pb), arsenic (As), mercury (Hg), chromium (Cr) and nickel (Ni) as per Abbas *et al.* (2010). Both the systems attached with atomic absorption spectrophotometer viz: GTA (Graphite Tube Atomizer) for analysis of Cd and Pb and VGA (Vapor generation Assay) for Hg and As were utilized by following the instructions of instrument manual and method 9.01 of (William, 2000) of metals determination.

Statistical analysis

The data obtained from the atomic absorption spectrophotometer was processed for ANOVA analysis using factorial design (factors were varied at 5 levels; treatment was performed at 5 levels including control) using SPSS software version 17, Inc. USA). The differences among the mean were determined through the Duncan's Multiple Range Test (DMRT) at ≤ 0.05 level of significance.

RESULTS

Status of heavy metals in sewage water and soil

The soil used in current study was sandy silt in the texture with an average 47.59%, 38.24% and 14.17% of sand, silt, and clay particles, respectively. The soil was non-saline with an average electrical conductivity of 0.43 dSm^{-1}. The data present in Table II shows that all the metals contents of the soils were under the safe limits, except the Cd concentration (4.18 µg g^{-1}) which exceeded the acceptable limit of (3.0 µg g^{-1}).

The sewage water used for irrigation of the vegetables revealed 2.7 dSm^{-1} EC and 7.9 pH. Regarding the concentration of heavy metal in sewage water, Cr (2.73 µg ml^{-1}), Ni (13.27 µg ml^{-1}), Cd (0.14 µg ml^{-1}), Pb (1.73 µg ml^{-1}), Hg (0.02 µg ml^{-1}) and As (0.31 µg ml^{-1}) crossed safe limits (Table II).

Table II. Inter-metal Pearson correlation of heavy metals found in vegetable tissues.

Variables	Cr	Ni	Cd	Pb	Hg	As
Cr	1					
Ni	0.66	1				
Cd	0.23	0.50	1			
Pb	0.17	0.30	0.41	1		
Hg	0.60	0.76	0.39	0.36	1	
As	-0.44	-0.42	-0.01	0.47	-0.20	1

Values in bold are different from 0 with a significance level P<0.05

Status of chromium (Cr) in vegetables

The accumulation of Cr concentration in plant tissues of five different vegetables crops is given in Table

III. It shows that the maximum mean concentration of Cr was found in cabbage (11.57 mg kg^{-1}) and cauliflower (8.90 mg kg^{-1}). In radish, as compared to 0.99 mg kg^{-1} concentration of Cr accumulated with 0% SW (T_0, control), the significantly highest Cr concentration of 1.60 mg kg^{-1} was found in treatment 4 (T_4), which was followed by 1.56 mg kg^{-1} in T_3 and 1.22 mg kg^{-1} in T_2. The minimum concentration of Cr (after control) in radish tissue was found in T_1 (1.08 mg kg^{-1}). The highest significant concentration of Cr found in cabbage tissue was 11.57 mg kg^{-1} in T4, which was followed by 9.93 mg kg^{-1} in T_3 and 7.62 mg kg^{-1} in T_2. The minimum concentration of Cr in cabbage tissue found was 4.68 mg kg^{-1} in T0 which statistically did not differ from the concentration found in T1 (5.50 mg kg^{-1}). In turnip, the maximum concentration of Cr found was 2.02 mg kg^{-1} in T4, which further decreased from T3 (1.95 mg kg^{-1}) to T2 (1.88 mg kg^{-1}) and T1 (1.74 mg kg^{-1}). The minimum concentration of Cr in turnip found was 1.74 mg kg^{-1} in T0, which statistically did not differ from any of the sewage water treatments from T1 to T4. In cauliflower, the significantly highest accumulation of Cr concentration found was 8.90 mg kg^{-1} in T4 which was followed by 6.75 mg kg^{-1} in T3. The minimum concentration of Cr in cauliflower found was 4.23 mg kg^{-1} in control which did not statistically differ from the concentration found in T1 (4.70 mg kg-1) and T2 (5.26 mg kg-1). In carrot, the significantly highest accumulation of Cr found was 3.48 mg kg^{-1} in T4 which statistically did not vary from concentration found in T3 (3.24 mg kg^{-1}) and T2 (2.78 mg kg^{-1}). Similarly, the significantly lowest concentration of Cr was though observed in T0, but it was significantly not differed from the results obtained in T1 (1.88 mg kg^{-1}) and in T2 (2.78 mg kg^{-1}).

Status of nickel (Ni) in vegetables

The accumulation of Ni concentration in plant tissues of five different vegetables crops is given in Table III. In Radish, as compared to 3.38 mg kg^{-1} concentration of Ni in T0, the significantly highest Ni concentration of 5.72 mg kg^{-1} was observed in T4, which was followed by 5.01 mg kg^{-1} in T3 and 4.62 mg kg^{-1} in T2. The minimum concentration of Ni (after control) found was 3.64 mg kg^{-1} in T1. The highest significant concentration of Ni found in cabbage tissue was 11.48 mg kg^{-1} in T4, which was followed by 9.31 mg kg^{-1} in T3 and 8.93 mg kg^{-1} in T2. The minimum concentration of Ni in cabbage found was 5.43 mg kg^{-1} in T0 which statistically did not differ from the concentration found in T1 (6.83 mg kg^{-1}). In turnip, the maximum concentration of Ni found was 3.23 mg kg^{-1} in T4, which further decreased from T3 (2.81 mg kg^{-1}) to T2 (2.29 mg kg^{-1}) and T1 (1.74 mg kg^{-1}). The minimum concentration of Ni found in turnip was 1.93 mg kg^{-1} in T0, which statistically did not differ from the results obtained in T2 and T1. In cauliflower, the significantly highest accumulation of Ni concentration found was 12.16 mg kg^{-1} in T4 which was followed by 11.64 mg kg^{-1} in T3. The minimum concentration of Ni accumulated in cauliflower tissue was 8.10 mg kg^{-1} in T0 which did not statistically differ from the concentration found in T1 (9.38 mg kg^{-1}), T2 (9.93 mg kg^{-1}) and in T3 (11.64 mg kg^{-1}). Similarly, in carrot the significantly highest accumulation of Ni found was 10.77 mg kg^{-1} in T4, which statistically did not vary from the concentrations observed in T3 (8.75 mg kg^{-1}), T2 (8.60 mg kg^{-1}) and in T1 (7.17 mg kg^{-1}). The lowest significant Ni concentration of 6.10 mg kg^{-1} was observed in T0, which was statistically non-significant to the results obtained with T1, T2 and T3.

Status of cadmium (Cd) in vegetables

The accumulation of Cd concentration in plant tissues of five different vegetables crops is given in Table III. It shows that the maximum mean concentration of Cd found in all the five kinds of vegetables was 0.23 mg kg^{-1}. In radish, as compared to 0.06 mg kg^{-1} concentration of Cd found in T0, the significantly highest concentration of 0.13 mg kg^{-1} was found in T4. While in rest of the treatments from T1 to T3, the concentration of Cd did not significantly differ from the concentration observed in T0 (Table III). Similarly, the maximum concentration of Cd (0.15 mg kg^{-1}) in cabbage tissue was found in T4, which was significantly higher than the concentration found in T0 (0.09 mg kg^{-1}). The concentrations observed in T1, T2 and T3 was 0.09 mg kg^{-1}, 0.10 mg kg^{-1} and 0.13 mg kg^{-1}, respectively. In turnip, the concentration of Cd among all the treatments as well as between the control did not differ significantly. The maximum concentration of Cd found in turnip was 0.15 mg kg-1 in T4, which was followed by 0.15 mg kg^{-1} in T3, 0.15 mg kg^{-1} in T2 and 0.14 mg kg^{-1} in T1. The minimum Cd concentration in turnip was found in T0 (0.14 mg kg^{-1}). In cauliflower, the significantly highest accumulation of Cd concentration found was 0.25 mg kg^{-1} in T4, which was followed by 0.20 mg kg^{-1} in T3. The minimum concentration of Cd accumulated in cauliflower tissue was 0.15 mg kg^{-1} in T0, which did not statistically differ from the concentration found in T1 (0.20 mg kg^{-1}), T2 (0.17 mg kg^{-1}) and in T3 (0.20 mg kg^{-1}). In carrot, the significantly highest accumulation of Cd found was 0.28 mg kg^{-1} in T4, which was followed by 0.21 mg kg^{-1} in T3 and 0.14 mg kg^{-1} in T2. The significantly lowest concentration of Cd was found in T1 (0.12mg kg^{-1}) which was non-significantly different from the results obtained in T0 (0.11 mg kg^{-1}) and in T2 (0.14 mg kg^{-1}).

Table III. Chromium (Cr), nickel (Ni), cadmium (Cd), lead (Pb), mercury (Hg) and arsenic (As) concentrations (mg kg^{-1} in dry weight) in five vegetables.

Vegetables	Treatment	Cr	Ni	Cd	Pb	Hg	As
Radish	T0	$0.99^{b} \pm 0.16^{*}$	$3.38^{c} \pm 0.74$	$0.06^{b} \pm 0.02$	$0.16^{b} \pm 0.03$	$0.02^{a} \pm 0.00$	$0.05^{c} \pm 0.01$
	T1	$1.08^{b} \pm 0.16$	$3.64^{bc} \pm 0.42$	$0.06^{b} \pm 0.02$	$0.17^{b} \pm 0.04$	$0.02^{a} \pm 0.00$	$0.07^{bc} \pm 0.02$
	T2	$1.22^{ab} \pm 0.18$	$4.62^{abc} \pm 1.11$	$0.08^{b} \pm 0.03$	$0.19^{b} \pm 0.05$	$0.02^{a} \pm 0.00$	$0.09^{ab} \pm 0.01$
	T3	$1.56^{a} \pm 0.36$	$5.01^{ab} \pm 0.24$	$0.09^{b} \pm 0.02$	$0.25^{b} \pm 0.06$	$0.02^{a} \pm 0.00$	$0.09^{ab} \pm 0.02$
	T4	$1.60^{a} \pm 0.19$	$5.72^{a} \pm 0.78$	$0.13^{a} \pm 0.02$	$0.35^{a} \pm 0.06$	$0.02^{a} \pm 0.00$	$0.11^{a} \pm 0.01$
Cabbage	T0	$4.68^{c} \pm 1.23$	$5.43^{c} \pm 0.75$	$0.09^{b} \pm 0.02$	$0.13^{d} \pm 0.02$	$0.02^{b} \pm 0.01$	$0.03^{b} \pm 0.00$
	T1	$5.50^{c} \pm 0.86$	$6.83^{bc} \pm 1.43$	$0.09^{b} \pm 0.02$	$0.16^{cd} \pm 0.03$	$0.02^{b} \pm 0.01$	$0.04^{b} \pm 0.00$
	T2	$7.62^{bc} \pm 1.54$	$8.93^{ab} \pm 1.92$	$0.10^{b} \pm 0.01$	$0.19^{bc} \pm 0.02$	$0.03^{ab} \pm 0.01$	$0.04^{ab} \pm 0.00$
	T3	$9.93^{ab} \pm 1.85$	$9.31^{ab} \pm 2.33$	$0.13^{ab} \pm 0.04$	$0.22^{ab} \pm 0.02$	$0.03^{ab} \pm 0.01$	$0.04^{ab} \pm 0.01$
	T4	$11.57^{a} \pm 2.38$	$11.48^{a} \pm 1.85$	$0.15^{a} \pm 0.03$	$0.25^{a} \pm 0.04$	$0.04^{a} \pm 0.01$	$0.05^{a} \pm 0.01$
Turnip	T0	$1.72^{a} \pm 0.10$	$1.81^{c} \pm 0.12$	$0.12^{a} \pm 0.02$	$0.17^{b} \pm 0.04$	$0.01^{a} \pm 0.00$	$0.06^{c} \pm 0.01$
	T1	$1.74^{a} \pm 0.12$	$1.93^{c} \pm 0.21$	$0.14^{a} \pm 0.02$	$0.17^{b} \pm 0.02$	$0.02^{a} \pm 0.01$	$0.06^{c} \pm 0.01$
	T2	$1.88^{a} \pm 0.26$	$2.29^{bc} \pm 0.36$	$0.15^{a} \pm 0.02$	$0.19^{b} \pm 0.06$	$0.02^{a} \pm 0.00$	$0.08^{bc} \pm 0.01$
	T3	$1.95^{a} \pm 0.19$	2.81 ± 0.35	$0.15^{a} \pm 0.01$	$0.22^{ab} \pm 0.04$	$0.02^{a} \pm 0.00$	$0.09^{ab} \pm 0.01$
	T4	$2.02^{a} \pm 0.10$	$3.23^{ab} \pm 0.40$	$0.15^{a} \pm 0.02$	$0.28^{a} \pm 0.06$	$0.02^{a} \pm 0.01$	$0.10^{a} \pm 0.01$
Cauliflower	T0	$4.23^{c} \pm 0.58$	$8.10^{b} \pm 1.24$	$0.15^{b} \pm 0.02$	$0.19^{d} \pm 0.01$	$0.02^{b} \pm 0.01$	$0.03^{b} \pm 0.01$
	T1	$4.70^{bc} \pm 1.02$	$9.38^{ab} \pm 2.26$	$0.16^{b} \pm 0.03$	$0.20^{cd} \pm 0.03$	$0.02^{b} \pm 0.01$	$0.04^{ab} \pm 0.00$
	T2	$5.26^{bc} \pm 1.11$	$9.93^{ab} \pm 1.19$	$0.17^{b} \pm 0.01$	$0.23^{bc} \pm 0.02$	$0.03^{ab} \pm 0.01$	$0.04^{ab} \pm 0.01$
	T3	$6.75^{b} \pm 0.58$	$11.64^{ab} \pm 2.13$	$0.20^{ab} \pm 0.03$	$0.26^{ab} \pm 0.03$	$0.03^{ab} \pm 0.01$	$0.05^{a} \pm 0.01$
	T4	$8.90^{a} \pm 1.73$	$12.16^{a} \pm 2.84$	$0.23^{a} \pm 0.04$	$0.28^{a} \pm 0.02$	$0.04^{a} \pm 0.01$	$0.05^{a} \pm 0.01$
Carrot	T0	$1.36^{c} \pm 0.20$	$6.15^{b} \pm 1.13$	$0.11^{c} \pm 0.04$	$0.15^{c} \pm 0.03$	$0.02^{a} \pm 0.00$	$0.04^{b} \pm 0.00$
	T1	$1.88^{bc} \pm 0.35$	$7.17^{ab} \pm 1.2$	$0.12^{c} \pm 0.03$	$0.17^{c} \pm 0.02$	$0.03^{a} \pm 0.01$	$0.04^{b} \pm 0.01$
	T2	$2.78^{abc} \pm 0.87$	$8.60^{ab} \pm 1.99$	$0.14^{bc} \pm 0.04$	$0.18^{bc} \pm 0.03$	$0.03^{a} \pm 0.01$	$0.05^{b} \pm 0.01$
	T3	$3.24^{ab} \pm 1.12$	$8.75^{ab} \pm 2.10$	$0.21^{ab} \pm 0.03$	$0.22^{b} \pm 0.03$	$0.03^{a} \pm 0.00$	$0.06^{a} \pm 0.01$
	T4	$3.48^{a} \pm 1.05$	$10.77^{a} \pm 1.85$	$0.28^{a} \pm 0.07$	$0.29^{a} \pm 0.02$	$0.03^{a} \pm 0.01$	$0.07^{a} \pm 0.01$
MAC**		5***	15***	2***	5***	0.02****	4.3***

Means followed by the same letter in each column are not significantly different from each other at $p \leq 0.05$. *Standard deviation, **Maximum allowable concentration in vegetable tissue, *** Souri *et al.* (2019), **** Huang *et al.* (2014). T_0, 100% freshwater (FW); T_1, 25% sewage water (SW)+75% FW; T_2, 50% SW+50% FW; T_3, 75% SW+25% FW; T_4, 100% SW.

Status of lead (Pb) in vegetables

The accumulation of Pb concentration in plant tissues of five different vegetables crops is given in Table III. In radish, the maximum accumulated concertation observed was 0.35 mg kg^{-1} in T4 which was significantly higher than rest of the treatments and the control (0.16 mg kg^{-1}). The minimum accumulated concentration after control (T0) was found in T1 (0.17 mg kg^{-1}) which was non-significantly different from the concentrations observed in T2 (0.19 mg kg^{-1}) and in T3 (0.25 mg kg^{-1}). The highest significant concentration of Pb found in cabbage tissues was 0.25 mg kg^{-1} in T4, which was followed by 0.22 mg kg^{-1} in T3 and 0.19 mg kg^{-1} in T2. The minimum concentration of Pb in cabbage found was 0.13 mg kg^{-1} in T0 which statistically did not differ from the concentration achieved in T1 (0.16 mg kg^{-1}). In turnip, the maximum concentration of Pb found was 0.28 mg kg^{-1} in T4, which further decreased in T3 up to 0.22 mg kg^{-1}. The minimum concentration of Pb found in turnip was 0.17 mg kg^{-1} in T0, which statistically did not differ from the results found in T1 (0.14 mg kg^{-1}) and in T2 (0.17 mg kg^{-1}). In cauliflower, the significantly highest accumulation of Pb concentration found was 0.28 mg kg^{-1} in T4, which was followed by 0.26 mg kg^{-1} in T3 and 0.23 mg kg^{-1} in T2. The minimum concentration of Pb accumulated in cauliflower was 0.19 mg kg^{-1} in T0, which was statistically non-significant from the concentration found in T1 (0.20 mg kg^{-1}). In carrot, the significantly highest accumulation of Pb found was 0.29 mg kg^{-1} in T4 which statistically did not vary from concentration found in T3 (0.22 mg kg^{-1}) and in T2 (0.18 mg kg^{-1}). Similarly, the significantly lowest concentration of Pb was found in T0, which was significantly non-significant to the results obtained in T1 (0.17 mg kg^{-1}) and in T2 (0.18 mg kg^{-1}).

Status of mercury (Hg) in vegetables

The accumulation of Hg concentration in plant tissues of five different vegetables crops is given in Table III. It shows that the maximum mean concentration of Hg found in five different kind of vegetables was 0.04 mg kg^{-1}. The data presented in Table III shows that the maximum accumulated concentration of As in radish tissues was 0.022 mg kg^{-1} in T4 but, it did not significantly differ from the concentrations found in T3 (0.020 mg kg^{-1}), T2 (0.019 mg kg^{-1}), T1 (0.019 mg kg^{-1}) and in T0 (0.019 mg kg^{-1}). The highest significant concentration of Hg found in cabbage tissue was 0.038 mg kg^{-1} in T4, which was followed by 0.030 mg kg^{-1} in T3 and 0.029 mg kg^{-1} in T2. The minimum concentration of Hg in cabbage found was 0.021 mg kg^{-1} in T0, which was statistically non-significant to the results found in T1 (0.022 mg kg^{-1}), T2 and in T3. In turnip, all treatments revealed non-significant difference with the control in terms of the accumulation of Hg concentration in vegetable tissues. The maximum concentration of Hg found in turnip was 0.023 mg kg^{-1} in T4, which was followed by 0.20 mg kg^{-1} in T3, 0.019 mg kg^{-1} in T2 and 0.016 mg kg^{-1} in T1. The minimum concentration of Hg (0.015 mg kg^{-1}) found in turnip was observed in T0. In cauliflower, the significantly highest accumulated concentration of Hg found was 0.038 mg kg^{-1} in T4, which was followed by 0.030 mg kg^{-1} in T3 and 0.029 mg kg^{-1} in T2. The minimum concentration of Hg found in cauliflower was 0.021 mg kg^{-1} in T0, which was statistically non-significant to the results obtained in T1 (0.022 mg kg^{-1}), T2 and T3. In carrot, accumulation of Hg did not vary significantly among the treatments as well as between the treatments and the control. However, the highest accumulated concentration of 0.033 mg kg^{-1} was found in T4, which was followed by 0.031 mg kg^{-1} in T3, 0.029 mg kg^{-1} in T2 and 0.025 mg kg^{-1} in T1. The minimum accumulated concentration of Hg was found in T0 (0.023 mg kg^{-1}).

Status of arsenic (As) in vegetables

The data regarding the accumulation of As concentration in vegetable tissues of five different vegetables crops is given in Table III. It shows that the maximum mean concentration of As accumulated in tissue of five different vegetable crops was 0.05 mg kg^{-1}. In radish, as compared to 0.05 mg kg^{-1} concentration of As in T0, the significantly highest concentration found in T4 was 0.11 mg kg^{-1}, which was followed by 0.09 mg kg^{-1} in T3 and 0.09 mg kg^{-1} in T2. The minimum concentration of As (after control) found was 0.07 mg kg^{-1} in T1. In cabbage, the highest significant concentration of As found was 0.05 mg kg^{-1} in T4, which was followed by 0.03 mg kg^{-1} in T3 and 0.04 mg kg^{-1} in T2. The minimum concentration of As in cabbage found was 0.03 mg kg^{-1} in T0, which statistically did not differ from the concentration found in T1 (0.04 mg kg^{-1}). In turnip, the maximum concentration of As found was 0.10 mg kg^{-1} in T4, which further decreased to 0.09 mg kg^{-1} in T3 and to 0.08 mg kg^{-1} in T2. The minimum concentration of As found in turnip was 0.06 mg kg^{-1} in T0, which was statistically non-significant to the results obtained in T1 (0.06 mg kg^{-1}). In cauliflower, the significantly highest accumulation of As concentration found was 0.05 mg kg^{-1} in T4, which was followed by 0.05 mg kg^{-1} in T3. The minimum concentration of As accumulated in cauliflower was 0.03 mg kg^{-1} in T0, which was found statistically non-significant to the concentrations found in T1 (0.04mg kg^{-1}) and in T2 (0.04 mg kg^{-1}). In carrot, the significantly highest accumulation of As found was 0.05 mg kg^{-1} in T4, which statistically did not vary from the results obtained in T3 (0.06 mg kg^{-1}). The minimum concentration of As observed in T0 was 0.04 mg kg^{-1}, which was statistically non-significant to the results obtained in T1 (0.04 mg kg^{-1}) and T2 (0.05 mg kg^{-1}).

Inter-metal correlation and PCA analysis

To find any association among the heavy metal in five different vegetables, an inter-metal correlation method was applied on the obtained data (Table III). Results revealed that the Cr was found highly positive and significantly correlated with Ni (r = 0.66; P<0.05) and Hg (r = 0.60; P<0.05) but correlated negatively with As (r = 0.44; P<0.05). Ni revealed highly positive correlation with Hg (r = 0.76; P<0.05) and Cd (r = 0.50; P<0.05), however it's correlation with As was found negative (r = 0.42; P<0.05). Among all the metals, As was having significantly positive correlation with only Pb (r = 0.47; P<0.05).

A multivariate statistical method, PCA, was applied on the obtained data to analyze the inter-dependencies within heavy metals and for their qualitative evaluation of clustering behavior (Fig. 1). Four factors having a cumulative variance of 68.32% were obtained. Factor-1 contributed 43.21% to the total variability with a high loading on Ni (r = 0.95), Hg (r = 0.80), Cr (r = 0.71) and Cd (r = 0.49). Hence, Factor-1 supported three primary cluster, i.e. Hg-Cr, Hg-Ni and Pb-Cd. Factor-2 contributed 22.39% to the total variability with high negative loading on As (r = -0.81) and Pb (r = -0.76), supporting the As-Pb cluster.

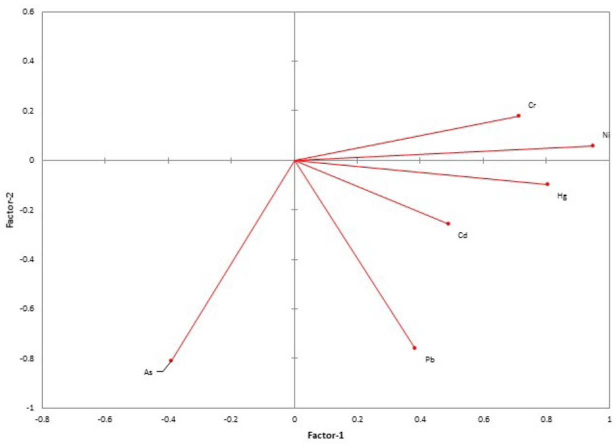

Fig. 1. PCA analysis of the heavy metals found in vegetable tissues.

DISCUSSION

The accumulation of heavy metals in vegetable tissues is a serious threat to human health. Industrial and municipal sewage water is an important source of heavy metals that may accumulated in the agricultural soil and subsequently translocated into the vegetable tissues. Depending upon its source of generation, it may contain different types and concentration of heavy metals (Marshall *et al*., 2006). The contamination of agricultural soil with heavy metals and their subsequent uptake and accumulation within plant tissues depend upon the physiochemical properties of the soil and type of vegetable crops (Karami *et al*., 2011; Zhou *et al*., 2016). The level of contamination of the heavy metals in plants relies on, amongst other, the time of crop harvesting as well as the soil type, humidity, pH and micronutrient contents (Gu *et al*., 2016; Hu *et al*., 2017; Leitzmann, 2003; Właśniewski and Hajduk, 2012).

The soil used in current study was sandy silt in the texture with an average 47.59%, 38.24% and 14.17% of sand, silt, and clay particles, respectively. The average electrical conductivity of the sewage water and soil was 3.0 and 0.43 dSm^{-1}, respectively. Regarding the concentration of heavy metal in sewage water, Cr (2.73 $\mu g\ ml^{-1}$), Ni (13.27 $\mu g\ ml^{-1}$), Cd (0.14 $\mu g\ ml^{-1}$), Pb (1.73 $\mu g\ ml^{-1}$), Hg (0.02 $\mu g\ ml^{-1}$) and As (0.31 $\mu g\ ml^{-1}$) exceeded the international standards (Chiroma *et al*., 2014). However, the metals contents of the soils were under the safe limits, except the Cd content (4.18 $\mu g\ g^{-1}$) which exceeded the acceptable limit of 3.0 $\mu g\ g^{-1}$ (Chiroma *et al*., 2014). This increase in soil Cd contents may be associated with granulometric composition of soils and the properties of soil top layer including soluble and total contents of Cd (Właśniewski and Hajduk, 2012). The soil pH also plays an important role in metals uptake by plant roots. According to Zwolak *et al*. (2019) acidic soil pH increases the absorption of heavy metals by plant roots. The change in soil pH from acidic to basic pH (7.1-8.1) increase the leaching of heavy metals and lowers the bioavailability to plant roots (Bielicka *et al*., 2009). The absorption of heavy metals by roots is also inhibited with the addition of organic matter to soil (Paltseva *et al*., 2018; Zhang *et al*., 2010). In current study the soil pH (8.1) and sewage water pH (7.9) were basic which perhaps have discouraged the accumulation of heavy metals in the soil. Thus, the pH of soil and sewage water in current study are under the safe limits as per international standards that do not favor the uptake of heavy metals by plant roots (Anwar *et al*., 2016; Bielicka *et al*., 2009).

Depending upon the type of vegetable, the leafy vegetables are reported to accumulate higher concentration of heavy metals than the non-leafy vegetables (Gu *et al*., 2016; Hu *et al*., 2017; Khan *et al*., 2010; Właśniewski and Hajduk, 2012). Chromium, lead, mercury and cadmium are considered amongst the top toxic heavy metals. Chromium is commonly found in soil as Cr (III) and Cr (VI) with distinct chemical and toxic properties (Sandeep *et al*., 2019). Cr (VI) is reported to be 10 to 100 times more toxic than Cr (III) (Garnier *et al*., 2006). Cr (VI), being a strong oxidizing agent cause harmful effects on overall microbial population in agricultural soil (Jie *et al*., 2009).

In current study, the mean concentration of Cr contents at 100% SW (T4) in cabbage (11.57 $mg\ kg^{-1}$) and cauliflower (8.90 $mg\ kg^{-1}$) exceeded the allowable concentration of 5 $mg\ kg^{-1}$ in vegetable tissue (Souri *et al*., 2019; WHO, 2007). In radish, turnip and carrot the maximum of Cr concentration found was 3.48 $mg\ kg^{-1}$ at 100% SW (T4). However, it was noticed that the concentration of Cr in all the kinds of vegetables, except in turnip, was significantly decreased with a decrease in swage water concentration from 100% (T4) to 75% (T3), and from 50% (T2) to 25% (T1) and 0% (T0, control). The vegetables grown on 0% SW (T0) accumulated the minimum concentration of Cr that falls under the acceptable limits of 5 $mg\ kg^{-1}$. In cabbage and cauliflower, the minimum concentration that falls under acceptable limits were found on 0% SW (T0) and 25% SW (T1), respectively. Similarly, the maximum mean concentration of Hg found in five different kind of vegetables (0.04 $mg\ kg^{-1}$) exceeded the acceptable concentration of 0.02 $mg\ kg^{-1}$ (Huang *et al*., 2014; WHO, 2007). However, the concentration of Hg in cabbage and cauliflower was significantly decreased with a decrease in swage water concentration from 100% (T4) to 75% (T3), and from 50% (T2) to 25% (T1) and 0% (T0, control).

In contrast, the concentration of Ni, Cd and Pb concentrations among all the five types of vegetables were under acceptable limits as per defined by international health organizations (Souri *et al*., 2019; WHO, 2007). The maximum concentration of Ni, Cd and Pb accumulated with 100% SW (T4) among all the five vegetables were 12.16 mg kg^{-1}, 0.23 mg kg^{-1} and 0.35 mg kg^{-1}, respectively. These concentrations were significantly decreased with a decrease in swage water concentration from 100% (T4) to 75% (T3), and from 50% (T2) to 25% (T1) and 0% (T0, control). The current results are in agreement with the reports published by several scholars from Pakistan on the contamination of irrigated soil and plant tissues with heavy metals under wastewater treatments. For example, Mahmood and Malik (2014) revealed the change of chemical and physical properties of the soil which led to the uptake of heavy metals by plants including vegetables. Similarly, other reports published from Pakistan has showed the higher uptake of heavy metals by vegetable plants under sewage water irrigation than the groundwater irrigation (Jan *et al*., 2010a, b; Khan *et al*., 2013).

CONCLUSION

It can be concluded from the obtained results that among the four treatments, the accumulation of the six metals was higher under 100% SW irrigation. The accumulated concentration was decreased with decrease in SW concentration up to 25% SW. The minimum accumulation of the metals was noted with 100% FW (control). Among the five types of vegetables crops, cabbage and cauliflower accumulate higher contents of Cr than radish, turnip, and carrot. Hence, these results suggest that in order to avoid exposure of heavy metals specially the Cr to human health through plant food, the cabbage and cauliflower crops may not be grown in the vicinity of Karachi city where the source of irrigation water is only sewage water.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20210818080816

Statement of conflict of interest

The authors have declared no conflict of interest.

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