Toxicity and Daily Intake of Heavy Metals to Adults and Children in Vegetables Irrigated by Different Water Sources

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ABSTRACT

The purpose of the study was to calculate the effects of urban untreated wastewater irrigation on the heavy metals accumulation in vegetable samples consisting turnip (*Brassica rapa*), radish (*Raphanus sativus*), spinach (*Spinacia oleracea*), pumpkin (*Cucurbita moschata*), cabbage (*Brassica oleracea capitata*), onion (*Allium cepa*), smooth luffa (*Luffa aegyptiaca*), coriander (*Coriandrum sativum*), vegetable gourd (*Lagenaria siceraria*) and carrot (*Daucus carota* subsp. sativus). This was accomplished by monitoring waste as well as fresh water-irrigated agricultural fields of Lakki Marwat, Pakistan. Flame atomic absorption spectrometer (FAAS) was used to assess the concentrations of these heavy metals. The concentration of a total of six metals including iron (Fe), zinc (Zn), copper (Cu), nickel (Ni), lead (Pb), and cadmium (Cd) were detected and quantified in vegetable samples. Concentration of Pb was very low, while Fe concentration was higher in all the samples under investigation in the waste water-irrigated vegetables. The highest concentrations of Zn were observed in onion (441.3±2.7 mg.kg^{-1}), and smooth luffa (293.1±0.5 mg.kg^{-1}). Onion (361.95±2.3 mg.kg^{-1}), turnip (292.15±5.6 mg.kg^{-1}) and Spinach (280.25±4.8 mg.kg^{-1}) showed exceedingly higher concentrations of Fe. Daily intakes of metals (DIM) were calculated for both adults as well as children. The DIM values for Ni and Cd exceeded the recommended value. The concentration of Fe and Zn was higher in wastewater-irrigated area samples as compared to fresh water-irrigated vegetables. The data obtained displayed a trend of heavy metal Fe>Zn>Cu>Ni>Cd>Pb. Our findings have demonstrated the accumulation of heavy metals in the vegetables irrigated by the wastewater as confirmed by flame atomic absorption spectroscopy.

KEYWORDS

Daily intake; Heavy metals; Vegetables; Wastewater irrigation

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INTRODUCTION

Water contamination is an issue of major concern in the modern world. This concern arises as water is considered as a symbol of life and hence is of paramount importance in the ecosystem.[1] The last few decades has seen an increase in the utilization of fresh water as well as exposure to contaminated water has become increasingly alarming.[2] Agriculture is the world's largest user of water. Considering water abstraction, agricultural use of water represents near 70% of the global use.[3] The extensive use of available freshwater by industrial, agricultural, and the domestic sectors and the huge production of wastewater are leading to the gradual contribution of contamination of the natural environment.[4] Heavy metal contaminated waste water and as a consequence of industrial or municipal are sources of the problems in current times.[5] Because it is easily accessible, wastewater from industrial and municipalities is commonly used to irrigate a variety of vegetables in the periurban environment.[6] Since wastewater includes large amounts of hazardous heavy metals, irrigation with wastewater is known to greatly increase the amount of heavy metals in the soil.[7] From a health perspective, irrigation with heavy metal-contaminated wastewater is of great concern.[8] These metals transferred to vegetables and pose a threat to human health, due to consumption. Since vegetables are an essential constituent of our daily diet, contamination of vegetables by heavy metals is a worldwide major concern.[9] Inorganic constituents in vegetables play a crucial role in the measurement of their quality in terms of food safety and nutritional value.[10] Specifically heavy metals are highly toxic, due to their non-biodegradable nature, extended physiological half-lives, and capacity to accumulate in many bodily parts.[11] Further, due to their ability to dissolve in water, the majority of heavy metals are thus potentially highly toxic. Also, some heavy metals such as Zn, Fe, and Cu in low doses are generally considered essential nutrients for humans. Nevertheless, these essential micronutrients become exclusively harmful when the concentration rises above the standard limits.[12] In this context, lead, cadmium, and nickel are extremely toxic because a small amount of these metals causes serious deteriorative effects on human health.[13] In addition, by the complex nature, some heavy metals form complexes, and the relative toxicity of these complexes is extremely high as compared to the heavy metals.[14] However, due to the high risks associated with the toxicity of heavy metals, vegetables that have been polluted with them through anthropogenic or natural sources are extremely important. About 90% of the total amount of heavy metals that humans ingest comes from vegetables, with the other 10% coming via contaminated dust and skin contact.[15] Therefore, of the well-known toxicity and metabolic role in the human organism, the precise determination and quantification of daily intake of heavy metals concerning human body weight are of significant importance.[16] Laboratory analytical techniques, such as potentiometry,[17] voltammetry,[18] atomic absorption spectrometry (AAS),[19] and inductively coupled plasma mass spectrometry,[20] have been applied for the metals determination over the years. Comparatively, AAS is one of the most reliable techniques for metal determination, due to its affordable price, excellent sensitivity, simplicity of operation, and elemental analysis capabilities.[21]

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The main goal of the current study is to determine and quantify the potential for the accumulation of heavy metals (Fe, Zn, Cu, Ni, Cd and Pb) in some of the commonly grown vegetables irrigated with various water sources in Lakki Marwat, Pakistan, as well as to compare their potential accumulation rates. Daily intakes of the respective metals through consumption of the vegetables for both adults as well as for children were then calculated.

EXPERIMENTAL

Chemicals and reagents used

High purity chemicals (perchloric acid 70%, nitric acid 68%, and hydrochloric acid 37%) were used and obtained from the local suppliers (Musaji Adam & Sons, Chemicals Suppliers Peshawar, Pakistan) and utilized for experiments as received without further purification. For the experiments, the distilled water used was obtained from the Department of Chemistry at Kohat University of Science & Technology, Kohat, Pakistan.

Sampling and study area

The laboratory analyses were carried out in the Chemistry Department at the Kohat University of Science & Technology in Kohat, Pakistan. Popular crops grown in Lakki Marwat region presented in Figure 1 consisted of turnip, radish, spinach, pumpkin, cabbage, onion, smooth luffa, coriander; vegetable gourd and carrot were investigated. Vegetable samples were taken from areas that were irrigated using untreated municipal effluent water as well as from fresh tube wells irrigated water vegetables of Lakki Marwat Pakistan (as shown in Figure 1). A total of thirty samples of ten different vegetable varieties were collected (triplicate) from both respective water sources. Thus a total of 30 samples were acquired. Selected fresh vegetable were collected from Lakki city of District Lakki Marwat, Khyber Pakhtunkhwa, Pakistan in December 2011 in the morning hours (from 9 am to 11 am). The study only used the edible portions of the vegetable for the laboratory analysis of the metals under investigation.

A location within the vegetable area was chosen for the soil collection. With a shovel, the top layer of soil was removed, and some of the soil was scooped by slanting the shovel beneath the ground. An area of 40 by 50 centimeters was dug. Samples of soil were collected up to a depth of 10 cm. Polyethylene containers were utilized for storing the soil samples.[22] To ensure the homogeneity of soil samples from a location, collections were carried out in triplicate from spots. Soil pH was measured in 1:2.5 soil:water using electronic pH meter.

Sample preparation

For the removal of surface contaminants and dust particulates, all samples of the respective vegetables field sites were collected. The vegetable were washed three times in distilled water (in the laboratory). After weighing and air-drying for a day, all of the samples were subsequently oven-dried for five hours at 105 °C in a hot air oven. Dried samples were ground into powder using a pestle and mortar and then stored in vial in refrigerator at 4 °C for further analysis.

Digestion of vegetable samples

Dried powdered (ash) of vegetable samples (0.5 g) were placed in crucibles after being precisely weighed using an electric balance (AY120 SHIMADZU JAPAN). A mixture of perchloric acid and nitric acid (1:4) was used for the digestion of vegetable sample. For digestion, 6 ml of acid mixture was added to 0.5 g of ash. After 2 hours of dissolution, the sample was boiled in a laminar flow container until the solution became clear and decreased from 2.5 to 2.0 ml. The heated sample was cooled down. Each sample solution was diluted with distilled water to a final volume of 25 ml, and the contents were subsequently passed through Whatman filter paper No. 42.

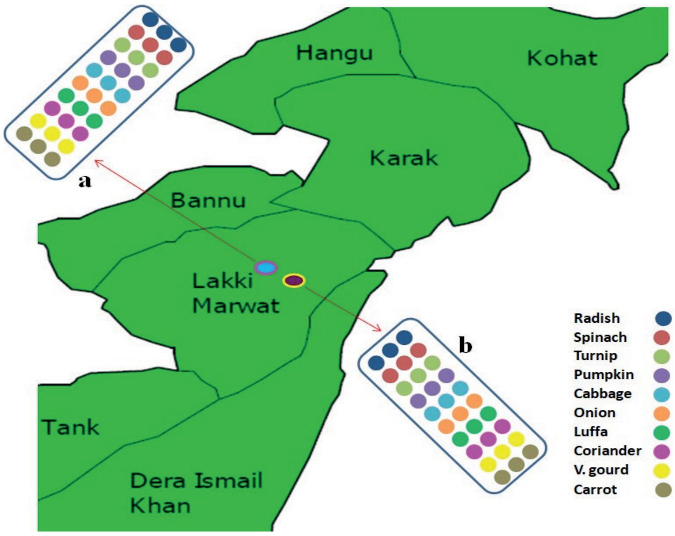

Figure 1. Map of vegetable field sampling site in of Lakki Marwat city. (a) waste water irrigated and, (b) fresh tube well water irrigated of Lakki Marwat city

Digestion of soil samples

The samples were sieved using a 2-mm plastic sieve to remove large particles, gravel-sized pieces, roots of plants, and various other waste after being oven-dried at 45 °C for three days, and stored in sealed plastic containers prior to laboratory analysis. Approximately 2 g of soil sample was accurately weighed into a crucible. These samples were digested with 12 ml of aqua regia (1:3 HNO_3:HCl) under reflux in a conical flask for 2 hr. Reflux continued till the initial sample volume was reduced to 3 ml. The samples were then cooled to ambient temperature. Distilled water was then added to reconstitute the respective samples to 50 ml of final volume. Subsequently, the contents were filtered using Whatman filter paper No. 42. The same process was used to prepare the blank solution but without the soil sample.

Collection and preparation of water samples

Samples were stored in premium polyethylene bottles (Massive Attack Limited, Peshawar Pakistan) with tight caps. The bottles were rinsed overnight in 10% (v/v) nitric acid after being cleaned with distilled water before use. The Whatmann filter No 4 was used to filter the samples. In order to prevent the precipitation of metals and biological growth, a few drops of nitric acid were added to samples to get the pH of the samples to pH 2.[23] Samples were stored in iceboxes at 4 °C for further analysis.

The open digestion method for preliminary digestion of metals was used to reduce the interference of organic matter and to convert metals that are associated with organic matter into a form that could be used for atomic absorption. About 95 ml of water sample that had already been preserved was used for that purpose, and 5 ml of HNO_3 (concentrated) was added to it in a beaker. The beaker's contents were boiled in a water bath until 95 ml remained. Whatman No. 42 filter paper was used to filter the solution. Using a volumetric flask, the filtrate mixture was diluted with distilled water to 100 ml. Similar steps were taken to prepare the blank solution using distilled water.[7]

Standards solutions

Multi-element standard solutions of heavy metals (1000 mg.L^{-1}), namely Fe, Zn, Cu, Ni, Cd, and Pb were procured from Perkin Elmer. By diluting the standards, solutions of various concentrations were created for each metal, preparing of 0.3, 0.6, 1.2, 2.4, 4.8, and 9.6 ppm as calibration standards. Accuracy, reliability, and repeatability of the data, curves for calibration were established and the analytical tests were executed in triplicates.

Determination of concentration of heavy metals in samples

Different concentrations of 0.3, 0.6, 1.2, 2.4, 4.8, and 9.6 ppm, the samples for heavy metals were examined using an atomic absorption spectrophotometer (FAAS, PERKINS ELMER 400). After standardization and the necessary set-up, the samples were sequentially aspirated into the AAS. The concentration of each metal in the blank was subtracted from the analyzed value. Each sample's reading was determined in triplicate.

Daily intake of metal based on vegetable consumption

The amount of metal (mg) absorbed by a person each day is determined by their DIM. DIM of metals is the amount of metal taken per kg of body weight.

The following equation was used to calculate DIM:

$$DIM = \frac{[M] \times K \times I}{W} \tag{1}$$

where: [M] = Concentrations of heavy metal in plants (mg.kg^{-1}); K = Represent conversion factor (same for all metals); I = Daily vegetable intake; and, W= Average weight of body.

The conversion factor[24] for green fresh vegetable weight to dry vegetable weight is 0.085. According to the literature reported, the average daily vegetable consumption for children and adults was estimated to be 0.232 and 0.345 kg/person/day, respectively.[7] The average body weight for an adult is regarded to be 55.9 kg, while that of a child is believed to be 32.7 kg.[7,25]

RESULTS AND DISCUSSION

The purposes of the research were to determine, quantify, and compare the accumulation of heavy metals in commonly grown vegetables in Lakki Marwat District, Khyber Pakhtunkhwa, Pakistan, irrigated with different water sources (Figure 1). To evaluate the usefulness, daily intakes of metals calculated for both adults and children based on the findings of this study are presented in Table 1.

Metal concentrations in waste, fresh tube well water, and soils studied

Table 1 presents the concentration of metal in waste and freshwater used for irrigation. It is found that the physicochemical properties of soil change with the application of wastewater, which ultimately causes the accumulation of toxic heavy metals in vegetables. The physical features of soil samples are represented by the mean concentrations of heavy metals in Table 1. The research region, which included soil that had been irrigated with both fresh water and waste, showed a wide range of heavy metal accumulation (Table 1). The findings shows that the range of Fe contents in soil samples were very high, ranging from 2267 to 2699 mg.kg^{-1}. This is twofold higher than the study carried out by Olawoyin and colleagues 2012 [26]. The soil irrigated with wastewater sources showed the highest concentration of Ni (69.3 mg.kg^{-1}) as compared to freshwater soil concentrations of 25.3 mg.kg^{-1}. The concentration of Cd was between 3.75 and 2.12 mg.kg^{-1}. Table 1 showed the concentrations of Pb ranging from 2.62 to 2.32 mg.kg^{-1}. The concentration of Zn ranged from 100.43 to 263.31 mg.kg^{-1}. which is higher when compared with the literature (58.37 to 37.0 mg.kg^{-1}) [26]. Similarly, the concentration of Cu ranged from 29.93 to 25.01 mg.kg^{-1}. The calculated pH of water and soil samples was 7.6±0.34 and 7.2±0.16 respectively.

Metal concentrations in vegetables grown by waste and fresh water irrigated soils studied

Table 1 displays the concentrations of heavy metals accumulated in edible vegetable parts in the Lakki Marwat District of Pakistan. In all of the vegetable samples, Pb content was the lowest and iron concentration was the highest among all of the heavy metals. The highest concentration of metals was observed in leafy vegetables such as spinach and coriander, as shown in Table 1. It was observed that vegetables watered with wastewater had higher concentrations of all heavy metals than vegetables irrigated with freshwater (Table 1). Vegetables irrigated with wastewater sources show a very high concentration of heavy metals compared to those of freshwater irrigated, as shown in Table 1. Except for onion, radish, and carrot,

Table 1. Heavy metal content (mg.kg^{-1}) (dry weight basis) in vegetables grown in waste and fresh water irrigated soil (± sd, *n* = 3)

Plants	Fe		Zn		Cu		Ni		Pb		Cd	
	Waste	Fresh	Waste	Fresh	Waste	Fresh	Waste	Fresh	Waste	Fresh	Waste	Fresh
Onion	361.95 **±2.3**	73.6 **±0.1**	441.3 **±2.7**	47.9 **±0.**	22.05 **±1.2**	20.6 **±1.15**	9.8 **±0.15**	9.6 **±0.05**	1.42 **±0.02**	0.32 **±0.12**	1.21 **±0.11**	0.23 **±0.1**
Carrot	202.7 **±0.55**	134.5 **±0.85**	91.1 **±.065**	63.85 **±0.2**	38.8 **±1.75**	22.25 **±1.2**	5.4 **±0.2**	4.8 **±0.1**	1.75 **±0.15**	0.45 **±0.05**	1.92 **±0.12**	0.35 **±0.05**
Radish	189.65 **±1.05**	98.9 **±0.6**	73.8 **±.075**	62.65 **±1.5**	15.35 **±0.8**	6.7 **±0.35**	7.55 **±0.05**	7.2 **±0.2**	1.45 **±0.1**	0.65 **±0.03**	1.86 **±0.18**	0.28 **±0.03**
Vegetable Guord	195.9 **±1.2**	165.5 **±5.05**	140.2 **±1.8**	58 **±0.35**	40.4 **±2.1**	39.6 **±2.05**	10.1 **±0.15**	7.25 **±0.15**	0.65 **±0.2**	0.45 **±0.10**	0.95 **±0.2**	0.45 **±0.10**
Smooth Luffa	121.8 **±0.8**	109.5 **±0.8**	293.1 **±0.5**	48.35 **±0.4**	55.05 **±2.5**	24.1 **±1.25**	6.4 **±0.15**	5.15 **±0.15**	0.46 **±0.15**	0.29 **±0.02**	0.95 **±0.2**	ND
Pumpkin	220.5 **±0.6**	117.1 **±0.5**	172 **±1.05**	59 **±1.6**	32 **±1.65**	26.6 **±1.4**	4.75 **±0.2**	3.8 **±0.2**	0.42 **±0.05**	0.23 **±0.06**	0.25 **±0.05**	ND
Spinach	280.25 ±4.8	241 ±6.35	135 ±3.35	88 ±4.9	16.3 ±0.85	13.2 ±0.7	7.7 ±0.2	6.65 ±0.05	0.55 ±0.12	0.25 ±0.05	0.45 ±0.10	0.25 ±0.05
Coriander	215.1 ±0.6	205.05 ±6.6	78.15 ±1.2	75.55 ±3.8	22.85 ±1.2	20.25 ±1.05	7.35 ±0.25	7.0 ±0.15	0.48 ±0.13	0.36 ±0.10	0.45 ±0.10	0.6 ±0.10
Cabbage	144 ±0.45	111.7 ±0.85	142.2 ±0.5	123.4 ±1.6	37.95 ±1.95	9.6 ±0.5	10.4 ±0.2	6.6 ±0.05	0.90 ±0.3	0.80 ±0.15	1.20 ±0.2	0.85 ±0.15
Turnip	292.15 ±5.6	132.4 ±1.1	172.9 ±0.9	59.3± 1.25	20.70 ±0.8	19.2 ±1.00	6.85 ±0.11	6.1 ±0.05	2.56 ±0.11	0.43 ±0.03	2.42 ±0.10	1.05 ±0.35
Soil	2699.7	2267.4	263.31	100.43	29.93	25.01	69.3	25.3	1.75	1.12	1.62	1.32
Water	3.090	1.812	0.360	0.072	0.161	0.142	0.047	ND	0.014	ND	0.058	0.021

where the trend was Fe>Zn>Cu>Ni>Pb>Cd, while the estimated heavy metal content was in the sequence Fe>Zn>Cu>Ni>Cd>Pb for all other vegetables. The ranges of Fe contents in vegetable samples irrigated with wastewater and those irrigated with fresh water were 121.8 to 361.95 $mg.kg^{-1}$ and 73.6 to 241.01 $mg.kg^{-1}$, respectively (Figure 2a). The Fe contents of wastewater-irrigated vegetables were in close agreement at 116 to 378 $mg.kg^{-1}$ with those previously studied by Monu Arora and colleagues (2008).[27] Due to the chlorophyll structure of leafy vegetables, they are reported as the primary sink for Fe deposition.[28] A study carried out by Sahrawat in 2000 revealed that Fe > 300 $mg.kg^{-1}$ dry weight is the critical limit and toxic to the plants.[29] However, the elevated levels of Fe found in all vegetables examined, together with the smallest percentage loss from plants, can be linked to its comparative abundance in the earth's crust as well as its role in plants' chlorophyll synthesis.[30] A similar trend was observed by authors like Audu and Lawal (2006)[30] and Onianwa and Egunyomi (1983)[31]. Wastewater-irrigated onions and turnips showed significantly higher iron accumulation than onions and turnips irrigated by fresh water. However, the concentration of Fe in spinach and coriander leaves was higher in both water sources as presented in Figure 2a&b. Zinc is considered a less toxic metal and also a necessary element in human food to maintain different body functions.[32] In our study, the range of Zn concentrations in vegetables irrigated with wastewater and freshwater was 73.8 to 441.3 $mg.kg^{-1}$ and 47.9 to 123.4 $mg.kg^{-1}$, respectively (Figure 2b). The concentration of Zn ions was much higher when compared with the studies of Yang and colleagues (2011)[33], who reported a range of 23.9–91.1 $mg.kg^{-1}$, and Monu Arora and colleagues (2010)[27], who reported a range of 22.5–46.4 $mg.kg^{-1}$. Wastewater-irrigated onion and smooth luffa showed a significantly higher accumulation of Zn as recommended limits are 0.3 $mg.kg^{-1}$ (FAO/WHO 1993) and 0.2 $mg.kg^{-1}$ (WHO/EU). For men, the recommended value for zinc is 15 mg/day, and similarly for women 12 mg/day.[34] The bioavailability of Zn in the diet is significantly affected by its composition. The results obtained from a study carried out by Spears and colleagues in 2004, comparing organic and inorganic sources of zinc have shown varying levels of bioavailability.[35] The interactions of Zn have been investigated with other nutrients like fiber, proteins, phytates, and some minerals.[36] Elevated concentrations of Zn in vegetables may however cause renal damage, vomiting, and cramps.[37] The copper concentration in the investigated vegetable was in the range of 15.35 to 55.05 $mg.kg^{-1}$ and 6.7 to 39.6 $mg.kg^{-1}$ in wastewater-irrigated and freshwater-irrigated vegetables, respectively (Figure 2c). The Cu contents were much higher 0.2-1.7 $mg.kg^{-1}$ than Sridhara Chary and colleagues (2008).[38] Waste water-irrigated cabbage and smooth luffa showed the highest accumulation of Cu compared to freshwater-irrigated. Wastewater-irrigated vegetables show close agreement in copper contents with those of the EU and WHO. Due to possible contributions of water metallic burden from external sources such as home garbage and the use of heavy-duty trucks to transport sand from the river, the vegetable sample irrigated with waste water may have a high level of Cu.[39]

Nickel is a poisonous heavy metal. The concentration of Ni was from 4.75 to 10.4 $mg.kg^{-1}$ and 3.8 to 9.6 $mg.kg^{-1}$ in wastewater and freshwater treatment vegetables, respectively (Figure 2d). Cabbage, vegetable guord, and onion irrigated on wastewater treatment show higher accumulation of Ni (Table 1), which is in agreement with 8.00 $mg.kg^{-1}$ Amin and colleagues (2013)[7]. The concentration of Ni in our vegetable samples exceeded the limit (0.2 $mg.kg^{-1}$) set by WHO and EU (1995).

The level of Pb found in different water sources for vegetables was in the range of 0.42 to 2.56 $mg.kg^{-1}$ in the wastewater and 0.23 to 0.80 $mg.kg^{-1}$ in the freshwater-growing vegetables (Figure 3a). Wastewater-irrigated turnips, radish, carrots, and onions show significantly higher accumulations of Pb not only than freshwater irrigated vegetables but also far higher than the FAO/WHO (1993)

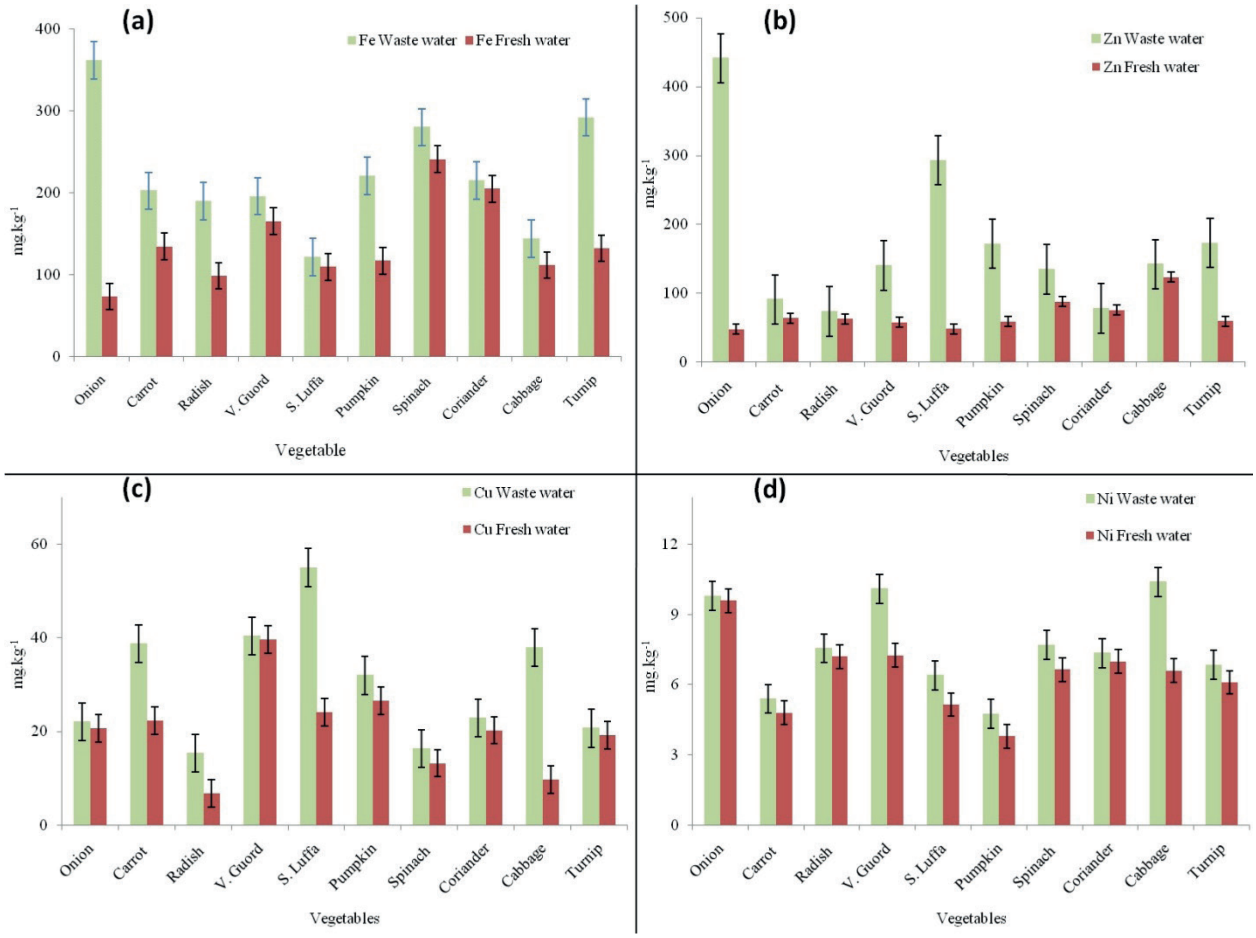

Figure 2. Comparison of (a) Fe, (b) Zn, (c) Cu and (d) Ni in vegetables irrigated with fresh and wastewater

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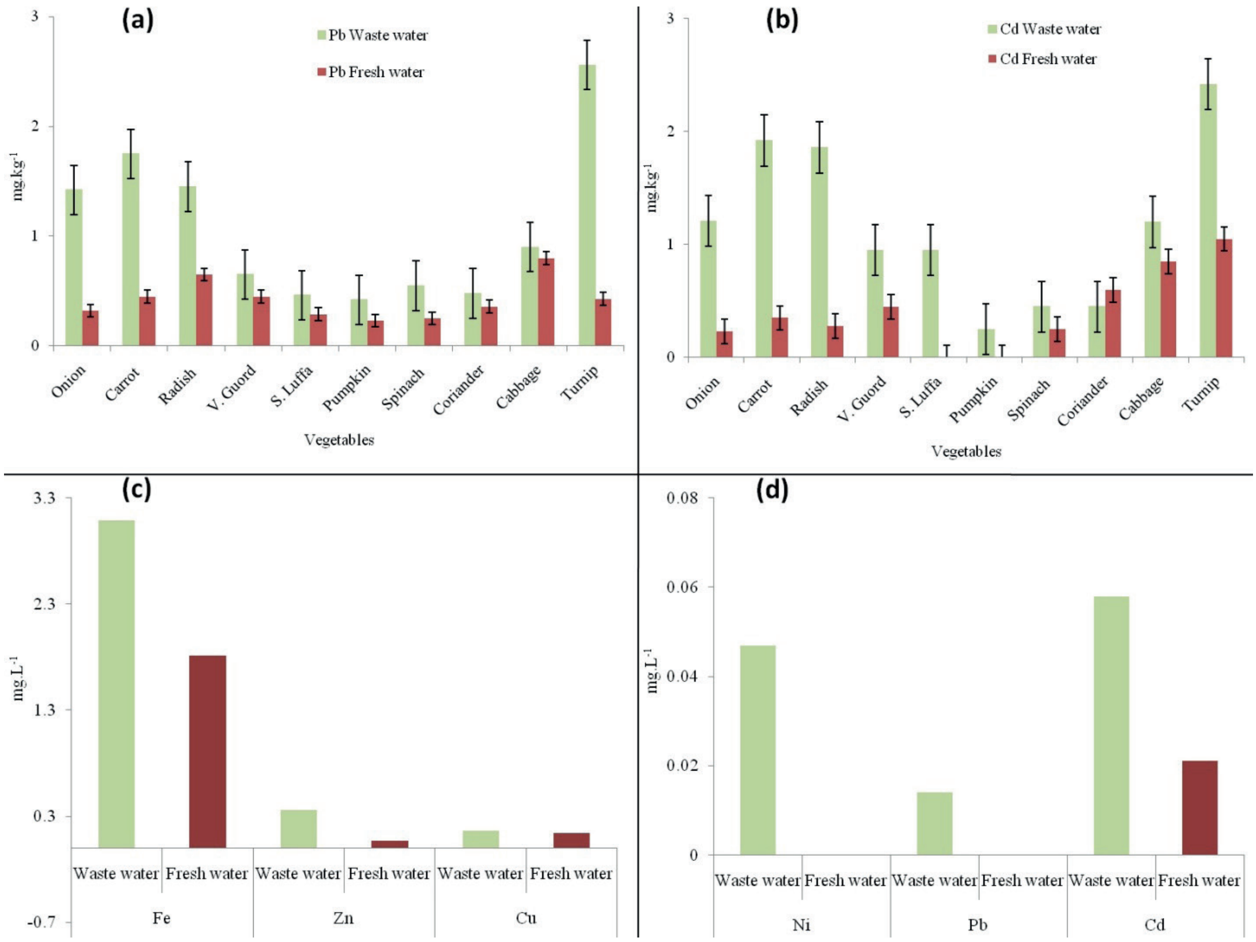

Figure 3. Comparison of (a) Pb and (b) Cd in vegetables irrigated with fresh and waste water, (c) Fe, Zn, Cu in different water sources and (d) Ni, Pb and Cd in different water sources

recommended safe limit (0.3 mg.kg^{-1}) (Table 1). However, the level of Pb was lower 3.09 to 15.74 mg.kg^{-1} as compared to the levels previously studied by Sharmaa and colleagues (2007).[40] The pH of the soil and the amount of organic matter in the soil can also boost Pb uptake.[40]

The cadmium level found in the studied vegetables ranged from 0.25 to 2.42 mg.kg^{-1} and 0.23 to 1.05 mg.kg^{-1} in wastewater-irrigated and freshwater-irrigated vegetables, respectively (Figure 3b). Wastewater turnips, cabbage, vegetable guord, and onion show a higher concentration of Cd than freshwater-irrigated vegetables. The value of fresh water was in close agreement with 0.10 to 1.07 mg.kg^{-1} studied by Yang and colleagues (2011) [33]. The Cd level in vegetables here was much higher than in the previous study in the literature by Li and colleagues (2012).[41]

It is reported in the literature that vegetable samples have been found to contain the highest concentration of iron in vegetables as a result of variations in the uptake of metals by plants through roots and their subsequent transfer within the plant sections. Edible portions of vegetables displayed changes in heavy metal concentrations [27]. The physical and chemical characteristics of the water and soil, as well as the plant's ability to absorb each metal, determine the variations in the metal concentrations of the vegetables under investigation. The use of freshwater-irrigated vegetables might be less hazardous and toxic to human health than those from wastewater-irrigated areas.

Daily intake of heavy metals

Calculating the exposure amount and identifying the routes that pollutants follow to reach the target individual are crucial for identifying the primary risks to human health. A pathways for heavy metal exposure to humans is the food chain.[42] The human body is mostly exposed to pollutants like heavy metals through the food chain. To estimate the average vegetable consumption for both adults and children, the DIM was calculated in µg.kg^{-1} of body weight. (Table 3). The DIM values calculated for wastewater-irrigated vegetables are higher as compared to freshwater-irrigated vegetables. The maximum daily intake rates of Fe via wastewater vegetable consumption were 189.8 and 218.3 µg/day/kg of body weight for adults and children, respectively, from wastewater and 126.4 and 145.3 µg/day/kg of body weight for adults and children from freshwater irrigated vegetables.

Wastewater onions show the highest daily intake of Zn at 231.5 and 266.1 µg/day/kg of body weight for adults and children, respectively. The maximum intakes of iron and zinc were from the utilization of onion, turnips, smooth luffa, vegetable gourd, cabbage, and spinach for both adults and children as wastewater vegetables. The daily intake of onion from wastewater-irrigated vegetables shows a higher concentration of Fe and Zn if taken in large quantities although it is below the recommended acceptable values proposed by the FAO/WHO Joint Expert Committee on Food Additives (1993).[43]

The maximum daily intake of Cu via wastewater vegetable gourd consumption was 21.19 and 24.36 µg/day/kg of body weight for adults and children, respectively, and 20.77 and 23.88 µg/day/kg of body weight for adults and children, respectively, from freshwater irrigated vegetable gourd. However, the Cu DIM was lower than the recommended tolerable levels and is a safe limit, according to WHO (1982).[44]

The daily intake of Ni via wastewater vegetable consumption was 5.46 and 6.27 µg/day/kg of body weight for adults and children, respectively, and 5.03 and 5.79 µg/day/kg of body weight for adults and children, respectively, from freshwater vegetables. The DIM value of Ni in onion and wastewater-irrigated vegetable gourds and cabbage exceeded the recommended acceptable levels proposed by WHO (2000).[45] Thus, the use of onion, vegetable gourd, and cabbage in the wastewater irrigated soil shows a risk if used in a daily routine.

Table 2: Estimated DIM µg.kg^{-1} body weight/day) (body weight = 60 kg)

Toxic Elements	Cd	Fe	Cu	Ni	Pb	Zn
Recommended values/ **µg**.kg^{-1} body weight/day	1.01	20000	500	5.00	3.50	2000
[References]	48	43	49	45	50	43

Table 3: Daily Intakes of Heavy metals in Vegetables in **µ**g/day/kg of the body weight

Heavy metals	water sources		Onion	Carrot	Radish	Vegetable Gourd	Smooth Luffa	Pumpkin	Spinach	Coriander	Cabbage	Turnip
Fe 20000	WWV[1]	Adults	189.87	106.33	99.49	102.76	63.89	115.67	147.0	112.84	75.54	153.26
		Children	218.3	122.2	114.4	118.1	73.4	132.9	169.0	129.7	86.8	176.1
	FWV[2]	Adults	38.61	70.6	51.9	86.8	57.4	61.4	126.4	107.5	58.5	69.4
		Children	44.4	81.1	59.6	99.8	66.0	70.6	145.3	123.6	67.3	79.8
Zn 2000	WWV[1]	Adults	231.5	47.79	38.71	73.55	153.7	90.23	70.82	40.99	74.59	90.70
		Children	266.1	54.94	44.50	84.55	176.76	103.73	81.41	47.13	85.76	104.27
	FWV[2]	Adults	25.12	33.49	32.86	30.42	25.36	30.95	46.16	39.63	64.73	31.10
		Children	28.88	38.50	37.78	34.97	29.14	35.58	53.07	45.56	74.42	35.76
Cu 500	WWV[1]	Adults	11.56	20.35	8.05	21.19	28.87	16.78	8.55	11.98	19.91	10.86
		Children	13.29	23.40	9.25	24.36	33.20	19.29	9.83	13.78	22.88	12.48
	FWV[2]	Adults	10.80	11.67	3.51	20.77	12.64	13.95	6.92	10.62	5.03	10.07
		Children	12.42	13.42	4.04	23.88	14.53	16.04	7.96	12.21	5.79	11.47
Ni 5.0	WWV[1]	Adults	**5.14**	2.83	3.96	**5.30**	3.35	2.49	4.04	3.85	**5.46**	3.59
		Children	**5.91**	3.25	4.55	**6.09**	3.86	2.86	4.64	4.43	**6.27**	4.13
	FWV[2]	Adults	**5.03**	2.518	3.77	3.80	2.70	1.99	3.49	3.67	3.46	3.2
		Children	**5.79**	2.89	4.34	4.37	3.10	2.29	4.01	4.22	3.98	3.68
Pd 3.5	WWV[1]	Adults	0.744	0.918	0.761	0.341	0.241	0.220	0.289	0.252	0.472	1.343
		Children	0.856	1.055	0.874	0.392	0.277	0.253	0.331	0.289	0.542	1.544
	FWV[2]	Adults	0.167	0.236	0.341	0.236	0.152	0.120	0.131	0.188	0.419	0.255
		Children	0.193	0.271	0.392	0.271	0.175	0.139	0.151	0.217	0.482	0.259
Cd 1.01	WWV[1]	Adults	0.635	1.007	0.976	0.498	0.498	0.131	0.236	0.236	0.629	**1.269**
		Children	0.729	**1.158**	**1.122**	0.573	0.573	0.151	0.271	0.271	0.724	**1.459**
	FWV[2]	Adults	0.212	0.183	0.147	0.236	ND	ND	0.131	0.315	0.446	0.551
		Children	0.139	0.211	0.169	0.271	ND	ND	0.151	0.362	0.513	0.633

[1] WWV = Waste Water Vegetables. [2] FWV=Fresh Water Vegetables

Similarly, the DIM of Pb ranged from 1.343 and 1.544 µg/day/kg of body weight for adults and children, respectively, in wastewater turnips. The DIM value in wastewater soil carrots ranged from 0.918 and 1.055 µg/day/kg of body weight of adults and children, respectively. However, the DIM of Pb in both waste and freshwater vegetables was lower than the recommended levels suggested by WHO (1993).[46]

The DIM of Cd from wastewater vegetable consumption ranged from 1.269 to 1.459 µg/day/kg of body weight for adults and children, respectively. While, in freshwater sample vegetables, the DIM ranged from 0.551 and 0.633 µg/day/kg for adults and children, respectively. The daily intake of Cd in turnips, radishes, and carrots from wastewater-irrigated vegetables exceeds the recommended acceptable levels suggested by the FAO/WHO Joint Expert Committee on Food Additives (2001).[47] So the use of wastewater turnips shows a risk for Cd for both adults and children. Also, wastewater-irrigated radish and carrot show risk of Cd for children. According to the study on DIM, the consumption of vegetables cultivated on waste water-contaminated soils is higher than that of vegetables irrigated with fresh water, but it is almost risk-free, except for Ni and Cd.

When long-term intake of contaminated or waste water-irrigated vegetation occurs, heavy metals have a more detrimental impact. The results of the present and previous studies[7,27,39] showed that wastewater-irrigated growing plants are generally polluted with contaminants such as heavy metals, which are extremely dangerous to human health.

CONCLUSION

The effects of municipal wastewater irrigation on the accumulation of toxic and non-biodegradable heavy metals, like Fe, Zn, Cu, Ni, Pb, and Cd, in the vegetable samples were investigated by monitoring waste as well as fresh water-irrigated agricultural fields in District Lakki Marwat. The results displayed that onions, spinach and turnips irrigated with wastewater are risky with respect to Fe and Zn, if the specific vegetables are consumed in large quantities i.e., more than 0.232 kg/day for children and 0.345 kg/day for adult.

The DIM of Ni and Cd concentrations in the wastewater irrigated soil onions, vegetable gourds, cabbage, and turnips shows potential risk when consumed in excess, as it does not only enter the human body through vegetables, but also through water and other food sources. Cadmium poses a risk factor when taken even in small amounts and shows an elevated level, as set by the Joint FAO/WHO Expert Committee on Food Additives (1993).

The DIM values for Fe, Zn, Cu and Pb detected in all vegetables (Table-3) were low compared to WHO standards and were free of risk. The present study suggests that wastewater-irrigated growing plants are generally polluted with heavy metals contaminants, which are extremely dangerous to human health.

FUTURE RECOMMENDATION

To prevent an excessive accumulation of toxic heavy metals in the human food chain, it is recommended that routine monitoring of heavy metals in vegetables and other food items be carried out. To find any risks connected to wastewater irrigation, regular risk assessments should be carried out. It is important to design and apply efficient risk management measures in order to reduce the potential negative effects on crop quality and human health.

LIMITATION OF THE STUDY

Here the study is limited to only ten vegetable and not considered all the vegetable grown on the municipal wastewater. Also here we selected specific area of Lakki Marwat city, not throughout the district or province. We have selected only inorganic hazardous few heavy metals and not the organic carcinogenic compounds.

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AUTHOR CONTRIBUTIONS

Muhammad Ismail, Salma Khan, Saima Gul: conceptualization, methodology, investigation, visualization, formal analysis, and validation;
Nafeesa Zahid, Alaud Din, Muhammad Afzal Kamboh: investigation, writing – original draft, formal analysis, methodology, validation, writing – review and editing;
Muhammad Afzal Kamboh, Sher Bahadar Khan, M.A. Khan, M.I. Khan: project administration, supervision, visualization, data curation, resources, writing – review, and editing.

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DATA AVAILABILITY STATEMENT

From the corresponding author, the data presented in this research are available on request.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Richardson SD, Ternes TA. Water analysis: emerging contaminants and current issues. Anal Chem. 2014;86(6):2813–2848. https://doi.org/10.1021/ac500508t.
2. Hasan Z, Jhung SH. Removal of hazardous organics from water using metal-organic frameworks (MOFs): plausible mechanisms for selective adsorptions. J Hazard Mater. 2015;283:329–339. https://doi.org/10.1016/j.jhazmat.2014.09.046.
3. Makarigakis AK, Jimenez-Cisneros BE. UNESCO's contribution to face global water challenges. Water. 2019;11(2):388. https://doi.org/10.3390/w11020388.
4. Shao L, Chen G. Water footprint assessment for wastewater treatment: method, indicator, and application. Environ Sci Technol. 2013;47(14):7787–7794. https://doi.org/10.1021/es402013t.
5. Ahmad I, Malik SA, Saeed S, Rehman A, Munir TM. Phytoextraction of Heavy Metals by Various Vegetable Crops Cultivated on Different Textured Soils Irrigated with City Wastewater. Soil Syst. 2021;5(2):35. https://doi.org/10.3390/soilsystems5020035.
6. Mateo-Sagasta J, Raschid-Sally L, Thebo A, Global Wastewater and Sludge Production, Treatment and Use. Wastewater. Springer; 2015. pp. 15–38. https://doi.org/10.1007/978-94-017-9545-6_2.
7. Amin N, Hussain A, Alamzeb S, Begum S. Accumulation of heavy metals in edible parts of vegetables irrigated with waste water and their daily intake to adults and children, District Mardan, Pakistan. Food Chem. 2013;136(3-4):1515–1523. https://doi.org/10.1016/j.foodchem.2012.09.058.
8. Qureshi AS, Hussain MI, Ismail S, Khan QM. Evaluating heavy metal accumulation and potential health risks in vegetables irrigated with treated wastewater. Chemosphere. 2016;163:54–61. https://doi.org/10.1016/j.chemosphere.2016.07.073.
9. Hang X, Wang H, Zhou J, Ma C, Du C, Chen X. Risk assessment of potentially toxic element pollution in soils and rice (Oryza sativa) in a typical area of the Yangtze River Delta. Environ Pollut. 2009;157(8-9):2542–2549. https://doi.org/10.1016/j.envpol.2009.03.002.
10. Miller BDD, Welch RM. Food system strategies for preventing micronutrient malnutrition. Food Policy. 2013;42:115–128. https://doi.org/10.1016/j.foodpol.2013.06.008.
11. Ismail M, Akhtar K, Khan MI, Kamal T, Asiri AM, Seo J, Khan SB. Pollution, Toxicity and Carcinogenicity of Organic Dyes and their Catalytic Bio-Remediation. Curr Pharm Des. 2019;25(34):3645–3663. https://doi.org/10.2174/1381612825666191021142026.
12. Kazi TG, Jalbani N, Baig JA, Afridi HI, Kandhro GA, Arain MB, Jamali MK, Shah AQ. Determination of toxic elements in infant formulae by using electrothermal atomic absorption spectrometer. Food Chem Toxicol. 2009;47(7):1425–1429. https://doi.org/10.1016/j.fct.2009.03.025.
13. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology. 2011;283(2-3):65–87. https://doi.org/10.1016/j.tox.2011.03.001.
14. Akcil A, Erust C, Ozdemiroglu S, Fonti V, Beolchini F. A review of approaches and techniques used in aquatic contaminated sediments: metal removal and stabilization by chemical and biotechnological processes. J Clean Prod. 2015;86:24–36. https://doi.org/10.1016/j.jclepro.2014.08.009.
15. Khan A, Khan S, Khan MA, Qamar Z, Waqas M. The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review. Environ Sci Pollut Res Int. 2015;22(18):13772–13799. https://doi.org/10.1007/s11356-015-4881-0.
16. Baldwin DR, Marshall WJ. Heavy metal poisoning and its laboratory investigation. Ann Clin Biochem. 1999;36(3):267–300. https://doi.org/10.1177/000456329903600301.
17. Ayenimo JG, Adeloju SB. Inhibitive potentiometric detection of trace Metals with ultrathin polypyrrole glucose oxidase biosensor. Talanta. 2015;137:62–70. https://doi.org/10.1016/j.talanta.2015.01.006.
18. Baldo MA, Daniele S. Anodic Stripping Voltammetry at Bismuth-Coated and Uncoated Carbon Microdisk Electrodes: Application to Trace Metals Analysis in Food Samples. Anal Lett. 2004;37(5):995–1011. https://doi.org/10.1081/AL-120030293.
19. Quintana AV, Olalla-Herrera M, Ruiz-López MD, Moreno-Montoro M, Navarro-Alarcón M. Study of the effect of different fermenting microorganisms on the Se, Cu, Cr, and Mn contents in fermented goat and cow milks. Food Chem. 2015;188:234–239. https://doi.org/10.1016/j.foodchem.2015.05.008.
20. Şahan Y, Basoglu F, Gücer S. ICP-MS analysis of a series of metals (Namely: Mg, Cr, Co, Ni, Fe, Cu, Zn, Sn, Cd and Pb) in black and green olive samples from Bursa, Turkey. Food Chem. 2007;105(1):395–399. https://doi.org/10.1016/j.foodchem.2006.12.026.
21. Jalbani N, Soylak M. Ligandless ultrasonic-assisted and ionic liquid-based dispersive liquid–liquid microextraction of copper, nickel and lead in different food samples. Food Chem. 2015;167:433–437. https://doi.org/10.1016/j.foodchem.2014.07.015.

22. Anyakora C, Ehianeta T, Umukoro O. Heavy metal levels in soil samples from highly industrialized Lagos environment. Afr J Environ Sci Technol. 2013;7(9):917–924.
23. Assubaie FN. Assessment of the levels of some heavy metals in water in Alahsa Oasis farms, Saudi Arabia, with analysis by atomic absorption spectrophotometry. Arab J Chem. 2015;8(2):240–245. https://doi.org/10.1016/j.arabjc.2011.08.018.
24. Rattan R, Datta S, Chhonkar P, Suribabu K, Singh A. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater—a case study. Agric Ecosyst Environ. 2005;109(3-4):310–322. https://doi.org/10.1016/j.agee.2005.02.025.
25. Chen T, Zheng Y, Chen H, Wu H, Zhou J, Luo J, Zheng G. Arsenic accumulation in soils for different land use types in Beijing. Geogr Res. 2005;24(2):229–235.
26. Olawoyin R, Oyewole SA, Grayson RL. Potential risk effect from elevated levels of soil heavy metals on human health in the Niger delta. Ecotoxicol Environ Saf. 2012;85:120–130. https://doi.org/10.1016/j.ecoenv.2012.08.004.
27. Arora M, Kiran B, Rani S, Rani A, Kaur B, Mittal N. Heavy metal accumulation in vegetables irrigated with water from different sources. Food Chem. 2008;111(4):811–815. https://doi.org/10.1016/j.foodchem.2008.04.049.
28. Ravet K, Touraine B, Boucherez J, Briat JF, Gaymard F, Cellier F. Ferritins control interaction between iron homeostasis and oxidative stress in Arabidopsis. Plant J. 2009;57(3):400–412. https://doi.org/10.1111/j.1365-313X.2008.03698.x.
29. Sahrawat KL. Elemental composition of the rice plant as affected by iron toxicity under field conditions. Commun Soil Sci Plant Anal. 2000;31(17-18):2819–2827. https://doi.org/10.1080/00103620009370630.
30. Audu A, Lawal A. Variation in metal contents of plants in vegetable garden sites in Kano Metropolis. J Appl Sci Environ Manag. 2009;10(2):105–109. https://doi.org/10.4314/jasem.v10i2.43680.
31. Onianwa P, Egunyomi A. Trace metal levels in some Nigerian mosses used as indicators of atmospheric pollution. Environ Pollut B. 1983;5(1):71–81. https://doi.org/10.1016/0143-148X(83)90051-4.
32. Ismail F, Anjum M, Mamon A, Kazi T. Trace metal contents of vegetables and fruits of Hyderabad retail market. Pak J Nutr. 2011;10(4):365–372. https://doi.org/10.3923/pjn.2011.365.372.
33. Yang Q, Xu Y, Liu S, He J, Long F-y. Concentration and potential health risk of heavy metals in market vegetables in Chongqing, China. Ecotoxicol Environ Saf. 2011;74(6):1664–1669. https://doi.org/10.1016/j.ecoenv.2011.05.006.
34. Castillo-Durán C, García H, Venegas P, Torrealba I, Panteon E, Concha N, Perez P. Zinc supplementation increases growth velocity of male children and adolescents with short stature. Acta Paediatr. 1994;83(8):833–837. https://doi.org/10.1111/j.1651-2227.1994.tb13154.x.
35. Spears JW, Schlegel P, Seal MC, Lloyd KE. Bioavailability of zinc from zinc sulfate and different organic zinc sources and their effects on ruminal volatile fatty acid proportions. Livest Prod Sci. 2004;90(2-3):211–217. https://doi.org/10.1016/j.livprodsci.2004.05.001.
36. Olalla M, Fernández J, Cabrera C, Navarro M, Giménez R, López MC, Nutritional Study of Copper and Zinc in Grapes and Commercial Grape Juices from Spain. Nutritional Study of Copper and Zinc in Grapes and Commercial Grape Juices from Spain. J Agric Food Chem. 2004;52(9):2715–2720. https://doi.org/10.1021/jf030796w.
37. Wang B, Feng W-Y, Wang T-C, Jia G, Wang M, Shi J-W, Zhang F, Zhao Y-L, Chai Z-F. Acute toxicity of nano- and micro-scale zinc powder in healthy adult mice. Toxicol Lett. 2006;161(2):115–123. https://doi.org/10.1016/j.toxlet.2005.08.007.
38. Sridhara Chary N, Kamala CT, Samuel Suman Raj D. Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer. Ecotoxicol Environ Saf. 2008;69(3):513–524. https://doi.org/10.1016/j.ecoenv.2007.04.013.
39. Gupta S, Satpati S, Nayek S, Garai D. Effect of wastewater irrigation on vegetables in relation to bioaccumulation of heavy metals and biochemical changes. Environ Monit Assess. 2010;165(1-4):169–177. https://doi.org/10.1007/s10661-009-0936-3.
40. Sharma RK, Agrawal M, Marshall F. Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. Ecotoxicol Environ Saf. 2007;66(2):258–266. https://doi.org/10.1016/j.ecoenv.2005.11.007.
41. Li Q, Chen Y, Fu H, Cui Z, Shi L, Wang L, Liu Z. Health risk of heavy metals in food crops grown on reclaimed tidal flat soil in the Pearl River Estuary, China. J Hazard Mater. 2012;227-228:148–154. https://doi.org/10.1016/j.jhazmat.2012.05.023.
42. Li Y, Wang Y, Gou X, Su Y, Wang G. Risk assessment of heavy metals in soils and vegetables around non-ferrous metals mining and smelting sites, Baiyin, China. J Environ Sci (China). 2006;18(6):1124–1134. https://doi.org/10.1016/S1001-0742(06)60050-8.
43. Bagdatlioglu N, Nergiz C, Ergonul PG. Heavy metal levels in leafy vegetables and some selected fruits. J Verbraucherschutz Lebensmsicherh. 2010;5(3-4):421–428. https://doi.org/10.1007/s00003-010-0594-y.
44. WHO. Evaluation of certain food additives and contaminants. WHO Technical Report Series, Number 683. World Health Organization, Geneva, Switzerland; 1982.
45. Badawy SH, El-Motaium RA, Faruque Hossain MD, Abdel-Lattif HM, Ghorab HM, El-Sayed MA. Health Risk Assessment and Nickel Content in Soils, Rice (*Oryza Sativa* L.) and Wheat (*Triticum Aestivum* L.) Grown in Damietta Governorate, Egypt. Environment and Pollution. 2023;11(2):1. https://doi.org/10.5539/ep.v11n2p1.
46. WHO. Evaluation of Certain Food Additives and Contaminants: Forty-first Report of the Joint FAO/WHO Expert Committee on Food Additives. Vol. 41. World Health Organization; 1993.
47. WHO. Evaluation of certain food additives and contaminants: fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization; 2001.
48. WHO. Safety evaluation of certain food additives and contaminants; 2001; pp 392-392.
49. Fao J. In *Toxicological evaluation of certain food additives and contaminants*, Thirty Seventh Meeting of JECFA; WHO Food Additives Series, Citeseer: 1989; p 219.
50. WHO. Trace elements in human nutrition and health. World Health Organization; 1996.