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Determination of Cadmium Concentrations of Vegetables Grown in Soil Irrigated with Wastewater: Evaluation of Health Risk to the Public

Zafar Iqbal Khan[(1)], Anam Nisar[(1)], Ilker Ugulu[(2)], Kafeel Ahmad[(1)], Kinza Wajid[(1)], Humayun Bashir[(1)], Yunus Dogan[(2)#]
[(1)]Department of Botany, University of Sargodha, Sargodha, Pakistan; [(2)]Buca Faculty of Education, Dokuz Eylul University, Izmir, Turkey.

THE MAIN objective of the study was to determine the cadmium (Cd) concentrations in vegetables grown in soil irrigated with canal water and sewage water. The samples were analysed by using atomic absorption spectrophotometer (AAS). The mean Cd concentrations in soil ranged between 1.153-2.294mg/kg and the mean Cd concentrations (mg/kg) in vegetables ranged from 0.789 to 1.575, 0.210 to 0.423, 0.264 to 0.523, 0.203 to 0.404, 0.169 to 0.334, 0.223 to 0.443, 0.723 to 1.443 and 0.344 to 1.450mg/kg for *Raphanus sativus*, *Brassica rapa*, *Zingiber officinale*, *Capsicum baccatum*, *Capsicum frutescens*, *Capsicum annuum*, *Solanum lycopersicum* and *Curcuma longa*, respectively. The recorded health risk index value for Cd concentration was greater than 1 in each vegetable and these values were higher than the determined permissible limit. In all vegetables, health risk index values for Cd were higher during sewage water treatments as compared to the canal water treatments. Finally, the study showed that bioaccumulation of Cd in vegetable samples was high in the study area.

Keywords: Cadmium, Soil, Vegetable, Health risk, Wastewater.

Abbreviations and Acronyms: AAS: Atomic absorption spectrophotometer, BCF: Bioconcentration factor, PLI: Pollution load index, DIM: Daily intake of metal, HRI: Health risk index, Cd: Cadmium; WHO: World Health Organization, FAO: Food and Agricultural Organization.

Introduction

Trace metals are potentially toxic elements due to their physiological effect in many tissues and organs of organisms (Ugulu, 2015). Many of these metals are lethal as they are soluble (Unver et al., 2015; Sahin et al., 2016). Even the less quantity of trace metals in organisms is harmful since their extreme utilization in metabolic processes (Ugulu et al., 2016; Ozyigit et al., 2018). These metals could be collected in the foodstuff that is consumed by humans and other living beings (Baslar et al., 2009; Ugulu et al., 2012; Dogan et al., 2014a).

Sewage water includes a high concentration of trace metals (Chen et al., 2005; Khan et al., 2018a). Excessive usage of sewage water for irrigation can cause contamination of vegetables by metals (Singh & Kumar, 2006). Trace metals released from factories, automobiles or other sources in high amount may get accumulated over the outer part of vegetables (Khan et al., 2018b; Nadeem et al., 2019). Consumption of contaminated vegetables could be lethal to humans and other organisms (Dogan et al., 2010; Durkan et al., 2011).

Vegetables are one of the principal components for the nutrition of humans. They have important dietary components such as proteins, carbohydrates, minerals, vitamins, fibres, and trace elements (Mukerji, 2004; Ugulu et al., 2009). Vegetables are also the latent resource for many important

[#]Corresponding author email: yunus.dogan@deu.edu.tr, +90 532 7699732 0000-0001-9404-6031
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nourishing substances that include protein, iron and calcium which enhance the physical condition of organisms (Arai, 2002; Dogan et al., 2013). The contamination of vegetables and fruits by trace metals is owing to the usage of wastewater of industries and cities, organic and chemical substances used for plant growth, the mass release of radiation energy from factories, automobiles and storage (Khan et al., 2018c; Khan et al., 2019). The food components like vegetables absorb trace metals from land polluted with wastewater (Chojnacka et al., 2005; Ahmad et al., 2018a, b). By observing the possible hazards of heavy metals, their constant life and the regular use of vegetable and fruits by organisms, the matter of concern is to protect humans from health hazards by eating the food items polluted with trace metals (Radwan & Salama, 2006; Dogan et al., 2014b).

In this direction, the main objective of the present research was to determine the concentrations of cadmium (Cd), one of the most toxic metals, in vegetables grown at the areas which are usually irrigated with canal water and wastewater. Cadmium toxicity due to the canal and wastewater irrigation is a major concern in the study area. Many current studies support that the Cd concentration in wastewaters is higher than the permissible limits. In the studies conducted in and around the study area of the present research, Khan et al. (2018b) and Ahmad et al. (2018a, b) reported that the Cd accumulation in municipal and sewage water samples was higher than the accumulation of other toxic metals. Based on the fact that the high Cd accumulation in the wastewaters in the region, the focus was to determine the Cd concentration in soil and vegetables. Moreover, it was evaluated whether the consumption of these vegetables was suitable for humans.

Materials and Methods

Study area

The present research was performed in Sargodha City, Punjab, Pakistan. This city has the tolerable winter season and warm temperature from May to July. The temperature differs from 12°C to 50°C in summer. The areas irrigated with sewage and canal water in Sargodha City was selected for this research. Two sites were selected for study, i.e. site I (CWI), where canal water is used for irrigation and site II (SWI), where primary treated industrial sewage water is used (Fig. 1).

Sample collection

In this study, *Raphanus sativus* L. (roots), *Brassica rapa* L. (roots), *Zingiber officinale* Roscoe (roots), *Capsicum baccatum* L. (fruit), *Capsicum frutescens* L. (fruit), *Capsicum annuum* L. (fruit), *Solanum lycopersicum* L. (fruit) and *Curcuma longa* L. (rhizome) were chosen as vegetable samples. In the sample collection season, sold in the open markets in the region and the most consumed plants in the study area were chosen as vegetable samples. Large volumes of canal water and sewage water samples were collected from the sources and one liter of the water samples were applied in experimental pots for irrigation purpose. Pots were irrigated twice a week. All samples were collected at maturity as sold in the open markets. An equal number of plant samples were collected per species (n= 5) (Audu & Lawal, 2005). The soil that was used to grow the vegetables were randomly collected from both sites. From each site, five samples of soil were taken. A metal spade was used to collect the soil at a depth of 15-20cm below the surface. The samples were placed in clean polythene bags before taking them into the laboratory for further analysis (Khan et al., 2010).

Wet digestion process

The procedure for sample arrangement and preparation involves the wet digestion process. This process involves following steps: Firstly, complete digestion of samples is done by using acid and hydrogen peroxide. After digestion, the mixture is diluted with distilled water. The step after dilution is filtration of samples with filter paper. 100ml flask, 10ml pipette, small size beakers, stirrers, Whatman filter paper, acid and hydrogen peroxide are required for wet digestion process. The wet digestion breaks down organic components of the sample and the organic content found in plant tissue is disintegrated into CO_2 by strong oxidizing agents such as H_2O_2. After the wet digestion process, a colourless, transparent and clear solution is obtained (Allen et al., 1986).

Firstly, soil is weighed, and 1g sample is added in flask. The sample is placed on hot plate after the addition of 20ml nitric acid. On hot plate sample is heated till it boils, and the volume reduced to 2 or 3ml. After removing the sample from hot plate, the sample is allowed to cool down. Then 10ml H_2O_2 which is strong oxidizing agent is added to the solution. All steps are repeated till the colourless, clear and transparent solution is obtained (Alloway & Ayres, 1997).

Fig.1. The map of the study area.

The wet digestion process was done for the digestion of the collected food crops. The food crops were converted to powdered form and then dried completely in oven for 24hr. After that the samples were added to the digestion flask. Then hydrochloric acid and nitric acid were added in following proportion, i.e. 1:3 and the solution was measured in measuring beaker and then 20ml of this strong acid solution was added to the dried powdered sample grains. Then the solution was transferred to the digestion flask and after that shifted to the hotplate to heat and boil the solution. When the sample was reduced to 2 or 3ml and the solution was removed from the hotplate to cool it. After that 10ml mixture solution of acid was added to the cooled solution of sample and then again placed on hotplate till the clear and colourless solution was obtained (Allen et al., 1986). The next step after complete digestion was dilution of the samples that were prepared with distilled water. The volume of the solution was made 50ml for each sample. After the samples were diluted, the samples were filtered through Whatman filter paper and were labelled and were made airtight in plastic bottles.

Cadmium analysis

The analysis of trace metals requires the formulation of specific standard solution. Before starting the analysis of Cd, the formulation of the standard solution was done. After formulation of the specific standard solution, the Cd analysis was performed by atomic absorption spectrophotometer (AAS-6300 Shimadzu Japan). The instrument operating condition for determination of Cd in soil and vegetables was shown in Table 1.

TABLE 1. Operating conditions for analysis of Cd using AAS.

Metal	Wavelength (nm)	Limit of detection (µg/cm^3)
Cadmium	228.8	1.5

The general method for analysis of metal can be used to prepare the standard solution in which the amount in ppm for stock solution preparation whose stock solution is to be prepared is divided by the volume of solvent such as distilled water and for metal stock solution preparation from metal salt weight of the test metal is multiplied by mole fraction concentration of metal and then divided by solvent concentration.

Quality control

Precision and accuracy of analyses was guaranteed through repetitive samples against National Institute of Standard Technology, Standard Reference Material (SRM 2709). The mean recoveries of SRM of soil was 92% for Cd and the mean recoveries of SRM of vegetables for Cd was 94%.

Among different plants and soil samples, the degree of variation was measured by SPSS (Statistical Package for Social Sciences). To measure the mean concentration values for soil and food crop samples, one-way ANOVA was conducted.

Bioconcentration factor

The concentration of a substance in the tissue of organism is called bioconcentration factor (BCF) (Cui et al., 2004). It is measured by the following formula:

BCF= Metal concentration in vegetable/ metal concentration in soil

Daily intake of metal

Daily intake of metals (DIM) stands for daily intake of metals. For food crops for the normal person normal value of DIM is 0.242kg. Human mean weight is 55.9kg and it was used in the DIM formula by the scientist. It is defined as the amount calculated to the intake of trace metals orally which was obtained by formula given by Sajjad et al. (2009).

DIM= Metal concentration x daily consumption of vegetable (kg per person)/ average body weight of a person

Health risk index

Health risk index (HRI) is calculated relative to DIM value and oral reference dose (R_fD). The formula described by Cui et al. (2004) is used to measure the relative measurement of HRI.

HRI= DIM/ food oral reference dose for the metal

Pollution load index

The presence of metals in soil is assessed by pollution load index (PLI) (Liu et al., 2005). It is calculated by following formula:

PLI= Metal concentration in soil/ metal concentration taken as reference

Results and Discussion

Concentration of metals in soil

The mean Cd concentration in soil samples ranged between 1.153-2.294mg/kg. Higher Cd contents in soil were observed as a result of sewage water irrigation and lower values were found as a result of canal water irrigation. The ANOVA results showed that the irrigation water had significant effect ($P \leq 0.05$) on the Cd concentration in soil (Table 2).

Soil serves as the most central component in agricultural environment. Contents of trace metals and various other minerals in soil determine the accretion of metal in the plant body. In the present study, the level of Cd in soil ranged between 1.153-2.294mg/kg. This Cd level was lower than the maximum permissible limit of Cd (3mg/kg) given by World Health Organization (WHO) (1996). When the recent studies in other cities of Punjab, Pakistan are examined; Ahmad et al. (2018a) conducted a research in Sargodha, the same area with the present study, and detected the Cd values between 1.85-1.98mg/kg. Khan et al. (2018a) performed a study in the areas of Bhakkar District of Punjab and defined the higher Cd values as ranging from 2.42 to 3.20mg/kg.

Ahmad et al. (2018a, b) reported the trace metal values in canal water and sewage water samples collected from Sargodha as 1.69-1.88mg/L for Cd, respectively. They also stated that concentrations of these metals in water samples were higher than the prescribed maximum permissible limits by WWF (2007). However, despite the high Cd content of water in the study area, as stated in the present study, Cd concentrations in soil samples were lower than the maximum permissible limits reported by USEPA (2002). This result may be due to periodic differences in water samples as well as the metal uptake of plant samples.

According to Gupta et al. (2008), the level of Cd in soil elevates due to the application of wastewater. According to some studies in Europe, various household products such as hair conditioners, shampoos, washing powders, fabric, conditioners and cleaners etc. are main causes of Cd pollution in the wastewaters. The higher Cd concentrations found in the washing powders can be explained by the differences in the composition of phosphate ores used in their production. Reducing the amount of phosphate in washing powders or choosing phosphate ores with low Cd concentration could lead to a reduction in Cd in sewage waters from diffuse sources. The concentrated washing powders, usually phosphate-free, have smaller potentially toxic element contents than the traditional powders, and are designed to be used in smaller quantities. As for Cd, apart from the industrial pollution, phosphoric fertilizer is the main source of Cd in agricultural soils (Demirezen & Aksoy, 2006).

Concentration of metals in soil

In the present study, the mean Cd concentrations (mg/kg) in vegetables ranged from 0.789 to 1.575, 0.210 to 0.423, 0.264 to 0.523, 0.203 to 0.404, 0.169 to 0.334, 0.223 to 0.443, 0.723 to 1.443 and 0.344 to 1.450 for *R. sativus*, *B. rapa*, *Z. officinale*, *C. baccatum*, *C. frutescens*, *C. annuum*, *S. lycopersicum* and *C. longa*, respectively (Table 2). Higher Cd values were observed in *R. sativus* as a result of sewage water irrigation and lower Cd values were found in *C. frutescens* irrigated with canal water irrigation (Fig. 2). According to the ANOVA results, the irrigation water has significant effect ($P \leq 0.05$) on the Cd contents in *R. sativus, B. rapa, C. frutescens, C. annuum, S. lycopersicum* and *C. longa* while non-significant effect ($P > 0.05$) on the Cd contents in *Z. officinale* and *C. baccatum.*

In the present study, Cd levels in the vegetable samples were above than the maximum permissible limit of Cd (0.1mg/kg) reported by Chiroma et al. (2014). These levels were also higher than the results of Yusuf et al. (2003) who establish Cd values in leafy vegetables to be 0.09 (µg/g). Cadmium is a non-essential metal and it accumulates mainly in the liver and kidney (Khan et al., 2018d). Various ways of ecological contamination were described for its occurrence in foods and various values were given for leafy vegetables that comprise 0.090mg/kg for fluted pumpkin by Sobukola et al. (2010). The high level of trace metals in wastewater cause excessive accumulation of heavy metals in soil. Excessive irrigation of soil with wastewater cause build-up of trace metals in soil and crop, even if their concentrations were very low in wastewater.

The highest Cd value allowed by the European Economic Commission in leafy vegetables is 0.2mg/kg which was lower than the present findings. The results showed that there was a Cd accumulation in the vegetable from each local government area. Cadmium shows severe toxicity due to its bioaccumulates, and it has a long half-life of about 30 years. Cadmium is easily taken up by plants through ingestion it becomes the part of the food chain. In this direction, Mapanda et al. (2005) stated that the greater the level of trace metals in soil, the greater will be its accumulation possibility in crops.

TABLE 2. Analysis of variance and mean concentration (mg/kg) of Cd in soil and vegetable samples.

Sample	Cadmium content		Mean square	Significance level
	CWI	SWI		
Soil	1.153±0.127	2.294±0.255	2.605	**
R. sativus	0.789±0.014	1.575±0.027	1.236	***
B. rapa	0.210±0.020	0.423±0.041	0.090	*
Z. officinale	0.264±0.051	0.523±0.103	0.134	N.S
C. baccatum	0.203±0.040	0.404±0.079	0.081	N.S
C. frutescens	0.169±0.019	0.334±0.039	0.054	*
C. annuum	0.223±0.005	0.443±0.011	0.097	***
S. lycopersicum	0.723±0.005	1.443±0.011	1.037	***
C. longa	0.344±0.124	1.450±0.027	2.448	***

*, **, ***: Significant at 0.05, 0.01 and 0.001 levels, N.S: Non-significant, CWI: Canal water irrigation, SWI: Sewage water irrigation.

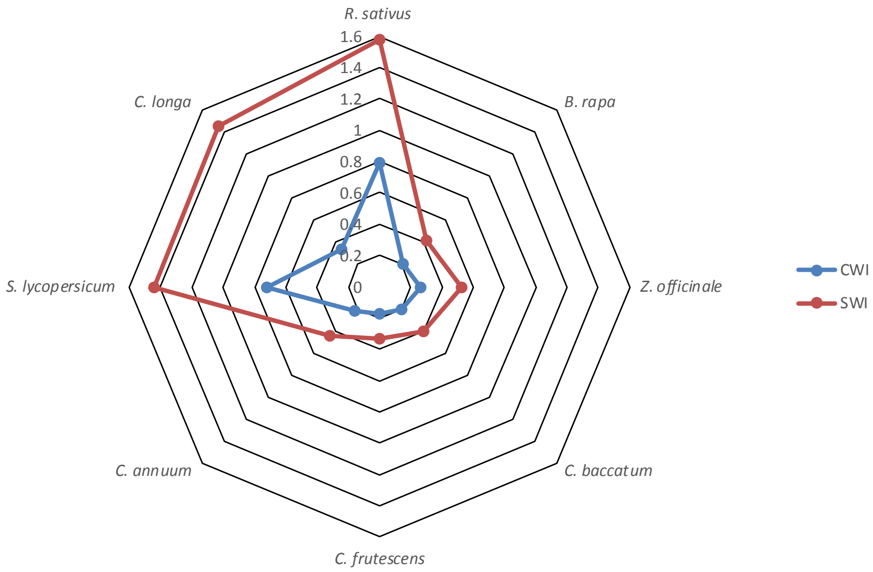

Fig. 2. Fluctuations in Cd concentrations in vegetables treated with canal and sewage water.

Bioconcentration factor

Bioconcentration factor values of Cd in different vegetables were presented in Table 3. In various vegetables, the BCF values differed from 0.684 to 0.687 (*R. sativus*), 0.182 to 0.184 (*B. rapa*), 0.229 to 0.228 (*Z. officinale*), 0.176 to 0.176 (*C. baccatum*), 0.146 to 0.146 (*C. frutescens*), 0.193 to 0.193 (*C. annuum*), 0.627 to 0.629 (*S. lycopersicum*) and 0.298 to 0.632 (*C. longa*). Higher BCF values were recorded for *R. sativus* as a result of sewage water irrigation and lower BCF values were found in *C. frutescens* as a result of canal and sewage water irrigation. BCF values for canal water irrigation and sewage water irrigation were determined at close values for each plant. Similar to this result, close BCF values for canal water irrigation (1.14) and municipal water irrigation (1.08) were determined by Khan et al. (2018a) for *Abelmoschus esculentus* samples collected from Bhakkar District of Punjab Province, Pakistan.

TABLE 3. Bioconcentration factor for vegetable/soil system with reference to Cd.

Plant	BCF	
	CWI	SWI
R. sativus	0.684	0.687
B. rapa	0.182	0.184
Z. officinale	0.229	0.228
C. baccatum	0.176	0.176
C. frutescens	0.146	0.146
C. annuum	0.193	0.193
S. lycopersicum	0.627	0.629
C. longa	0.298	0.632

BCF: Bioconcentration factor, CWI: Canal water irrigation, SWI: Sewage water irrigation.

Cadmium is a very toxic and causes an adverse effect on plants. The high Cd level in soil and plant parts leads to Cd contamination and can lead to the reduction in biomass and yield of vegetables (Qixing et al., 1994). Furthermore, high levels of Cd are severely damaging to public health and could raise cardiovascular and nervous disorders (Tataruch & Kierdorf, 2013). When BCF is ≤1, this signifies that the plant only absorbs but does not accumulate trace metals; when BCF>1, this signifies that plant accumulates metals (Singh et al., 2010). In current findings, BCF values of metals were lower than 1 in all vegetables for Cd. The results showed a lower bioavailability of metals for CWI and SWI.

Daily intake of metal and health risk index

Daily intake of metal values for Cd in vegetables were presented in Table 4. Among two irrigations, DIM values for Cd were between 0.004-0.009 (*R. sativus*), 0.001-0.002 (*B. rapa*), 0.002-0.003 (*Z. officinale*), 0.001-0.002 (*C. baccatum*), 0.001-0.002 (*C. frutescens*), 0.001-0.003 (*C. annuum*), 0.004-0.008 (*S. lycopersicum*) and 0.002-0.008 (*C. longa*). In all vegetables, the DIM values for Cd were higher for sewage water irrigation as compare to canal water irrigation. The recorded HRI value for Cd concentration was greater than 1 in each vegetable that was greater than the determined permissible limit. For the description of the HRI associated with trace metal contamination of plants grown-up, predictable exposure and risk index were calculated. In all vegetables, the HRI for Cd was higher as a result of sewage water treatments as compared to canal water treatments (Table 4).

TABLE 4. Daily intake of metals and health risk index of Cd via intake of vegetables.

Plant	DIM		HRI	
	CWI	SWI	CWI	SWI
R. sativus	0.004	0.009	4	9
B. rapa	0.001	0.002	1	2
Z. officinale	0.002	0.003	2	3
C. baccatum	0.001	0.002	1	2
C. frutescens	0.001	0.002	1	2
C. annuum	0.001	0.003	1	3
S. lycopersicum	0.004	0.008	4	8
C. longa	0.002	0.008	2	8

DIM: Daily intake of metal, HRI: Health risk index, CWI: Canal water irrigation, SWI: Sewage water irrigation.

Health risk index is an effective determination of contamination that is induced in food chain via the consumption of contaminated foods. The value of HRI (>1) is considered as unsafe and could pose serious health risk to public health while the value of HRI (<1) is considered safe for human consumption (USEPA, 2002). In the present study, the HRI values of Cd fall within the acceptable range of HRI value and were measured fit for human consumption. Zhuang et al. (2009) gave similar results as compared to the present findings.

Pollution load index

Pollution load index values for soil samples were 10.77103 and 21.43692 for CWI and SWI treatment, respectively (Table 5). Contamination level in the soil can be determined using PLI. This index gives a simple and comparative means for evaluating the excellence of various combinations of water irrigation. Tomlinson et al. (1980) determined that a value of zero indicates no risk, whereas a value of one and values above one would present progressive deterioration of the site irrigated with this water quality.

TABLE 5. Pollution load index for cadmium in soil.

Site	Reference soil value	Pollution load index
CWI	0.107	10.77103
SWI	0.107	21.43692

CWI: Canal water irrigation, SWI: Sewage water irrigation.

The degree of accumulation of metals in soil can be estimated efficiently using pollution index parameter. It is determined by dividing the concentration of metals in test soil as compared to their reference values. PLI value greater than 1 denotes that soil is contaminated. In the present study, PLI value for Cd was greater than 1 showed that soil is highly contaminated with Cd. Our results were similar to the findings of Singh et al. (2010).

Conclusion

Shortage of freshwater resources diverted the attention of farmers towards the use of wastewater to get the optimum yield of crops. Wastewater contains the useful materials that are necessary for the growth of plants, but it also contains toxic chemical substances. The findings of the current study demonstrated that the mean concentration of Cd in all vegetables was higher than the permissible limit given by FAO/WHO. The bioaccumulation of Cd in vegetable samples was quite high. The value of HRI for Cd was greater than 1. Many studies have revealed that consumption of this vegetables could lead to arthritis, diabetes, anemia, reduced fertility; cardiovascular disease, cancer, cirrhosis, hypoglycemia, kidney disease, headaches, osteoporosis, and strokes. Supporting the findings of this study with new data will be useful for the health of the people living in the region.

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