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Evaluating the toxicological potential of cadmium in wheat cultivars grown in soils amended with different types of organic fertilizers: Risk assessment for public health

Abid Ejaz¹, Zafar Iqbal Khan¹, Asma Ashfaq¹, Kafeel Ahmad¹, Fatima Ghulam Muhammad¹, Muhammad Nadeem², Farzana Siddique², Naila Riaz³, Faiza Zubair³

¹Department of Botany, University of Sargodha, Sargodha, Pakistan ²Institute of Food Technology and Nutrition, University of Sargodha, Sargodha, Pakistan ³Department of Zoology, University of Sargodha, Sargodha, Pakistan

Abstract

Heavy metal contaminated food crops are one of the major public health concerns these days. The present study evaluates the Cd uptake in some promising wheat varieties subjected to soils amended with different types of organic fertilizers/matter. For this purpose, both pot and field experiments were conducted at the same time in Sargodha, Pakistan. Cadmium uptake in soil and different wheat plant parts were higher in the pot experiment than that in the field experiment. In both experiments, Cd ranged from 0.075 to 0.030 mg/kg in soil, 0.64 to 1.01 mg/kg in root, 0.63 to1.00 mg/kg in shoot and 0.65 to 1.01 mg/kg in grains. Among all soil amendments, farm-yard manure was found to be very effective in mitigating the uptake of Cd in wheat plants. Of all wheat varieties, Gold-16 and Ihsan-16 showed maximum Cd uptake, whereas the minimum Cd uptake was displayed by cv. DHharabi-11. In both experiments, all different indices showed values lower than 1 except the bio-concentration factor. Overall, Cd absorption observed in the present soil-wheat system was lower than the standard Cd absorption values. This study recommends that farmers may use organic soil amendments in this area to increase the fertility of soil. But regular examination practices must be carried out in this area, to limit the exposure to Cd hazards of public being provided Cd contaminated grains.

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Introduction

Wheat is contemplated as a premier staple food crop for humans, and it is also consumed for animal feeding purpose (Mukhtiar et al., 2018). This crop is naturally exposed to a multitude of stressful environments, both biotic and abiotic (Mao, et al., 2023). However, in some parts of the world wheat crop is subjected to heavy metal pollution (Rizvi et al., 2020; Xu et al., 2024) causing the uptake of a variety of heavy metals in grains. For example, like several other metals, cadmium (Cd) accumulation in wheat grains is reported by many studies (Lu et al., 2020; Corguinha et al., 2015; Payandeh et al., 2018;

*CONTACT Zafar Iqbal Khan, 🖳 <u>zafar.khan@uos.edu.pk</u>, 🖃 Department of Botany, University of Sargodha, Sargodha, Pakistan

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© Authors 2025. Published by Society of Eminent Biological Scientists (SEBS), Pakistan IJAaEB is a DOAJ complied Open Access journal. All published articles are distributed under the full terms of the <u>Creative Commons License (CC BY 4.0)</u>. This license allows authors to reuse, distribute and reproduce articles in any medium without any restriction. The original source (IJAaEB) must be properly cited and/or acknowledged. Awan et al., 2019; Yang et al., 2022; Wang et al., 2024), depicting adverse effects of Cd human health. Cadmium is a most hazardous element in environment (Rizwan et al., 2018) and its availability in the environment is due to both geo-genic and anthropogenic activities (Yin et al., 2016; Kubier et al., 2019; Brenko et al., 2024). Agricultural practices such as usage of sewage sludge, mining waste, untreated industrial waste matter and fertilizers add Cd to the environment (Qayyum et al., 2017). As Cd metal is absorbed by the roots from the surrounding soil medium, it directly reduces the root-growth and causes browning of plant roots (Sabella et al., 2022). Other reported symptoms are chlorosis, and reduction in rate of photosynthesis and yield (Rehman et al., 2018; Soni et al., 2024). Cereal grains consumption in human diet is a major route of Cd entrance in human beings, and it induces lung cancer, anemia, bone demineralization and renal damage in humans (Awan et al., 2019; Charkiewicz et al., 2023).

The hazardous nature of Cd metal in human food chain compels the scientists to reduce the Cd availability by applying various organic or inorganic amendments (Woldetsadik et al., 2016; He et al., 2017; Li et al., 2019). These amendments have capacity to bind with pollutants, and available nutrients are easily accessible to plants (Hamid et al., 2019). Inorganic amendment readily imparts the nutrients in a soluble form to plants, which enhance crop production rate, but these nutrients may seep down for causing soil acidification or ground-water contamination (Garcia et al., 2018; Kubier et al., 2019; Zulfiqar et al., 2023). In another study, various organic soil amendments like sewage sludge, farm-yard manure, press mud and poultry waste were used to preserve the soil fertility and productivity (Oueriemmi et al., 2021). Organic amendments enhance the water penetration, retention, hydraulic conductivity and buffering competence of soil (Kandra et al., 2024). The presence of organic waste in soil stimulates the microbial activity and nutrient recycling that in turn positively affect plant vegetation and yield (Mujdeci et al., 2017). Despite all these beneficial aspects, the addition of organic waste instigates undesirable changes in soil depending upon soil type and waste composition (Kandra et al., 2024).

It has been observed that fertilizers particularly organic ones can restrict the uptake of heavy metals including that of cadmium, e.g., in a study it was reported that different types of organic fertilizers used in the soil contaminated with Cd reduced the availability of Cd in soil (Wang et al., 2022) as well as its uptake in plants (Zhu et al., 2020). Therefore, the present study aimed to analyze Cd content in promising wheat varieties grown in soils amended with different doses of different types of organic fertilizers.

Materials and Methods

The present research was performed in Chak 102 NB of Sargodha district, Punjab, Pakistan. Annual rainfall was 180-200 mm, average summer temp. 35-49 °C and average winter temp. 5-23 °C.

Experimental design

The present research was arranged in a complete randomized block design (CRD) having 10 wheatvarieties, 9 soil amendment treatments and 5 replicates in each of pot or field trials. For pot experiment, each pot consisted of 8 kg soil, while each field plot with 1.83 × 1.83 m was used in the field trial and 100 or 200 g/kg of fertilizer was added to each plot. Mixing of the fertilizers or different types of organic matters was done manually. Twelve grains of each wheat cultivar were sown in each pot, whereas 600 grains were sown in each field plot. Watering of the pots or field was done carefully to avoid the leaching of Cd or nutrients released from the fertilizers

Wheat cultivars and organic fertilizer treatments

Following soil amendment treatments were used in the present research: T1 = Municipal solid waste (100 g), T2 = Municipal solid waste (200 g), T3 = Poultry waste (100 g), T4 = Poultry waste (200 g), T5 = Press Mud (100 g), T6 = Press Mud (200 g), T7 = Farm-yard manure (100 g), T8 = Farm-yard manure (200 g), and T9 = Control (Without organic matter).

The wheat varieties in the experiment were: Millat-11 (V1), AARI-11 (V2), Galaxy-13 (V3), Gold-16 (V4), Johar-16 (V5), Ujalla-16 (V6), Dharabi-11 (V7), Ihsan-16 (V8), 11CO23 (V9), and Chakwal-50 (V10). The seed of all varieties was obtained from the Ayub Agricultural Research Institute, Faisalabad, Pakistan.

Sample collection and preparation

At the time of harvesting, five replicates of each wheat variety with its soil samples were taken from both amended soil treatments and control treatment. Different parts such as root and shoot were separated from the plant body, while grains were taken out from the spikes. All selected wheat parts were treated with dilute HCl and distilled water to remove contaminated dust particles. The collected samples were dried in sunlight and then placed them in an oven for 6 days at 80 °C to get a constant weight. The dried samples were crushed into a fine powder and stored them in labeled bags.

Five replicates of each of shoot and grain of each wheat variety along with soil were taken from all treatments. All sample contaminations were removed by washing with dilute HCl and distilled water. The

samples were firstly air-dried and then placed in an oven for 80 °C for 5-6 days.

To 1.0 g powdered sample, 5 mL of a mixture of H_2O_2 , HNO_3 , H_2SO_4 and HCl (3:1:2:1) were added and kept overnight. This mixture was heated appropriately on a hot plate till colorless solution was obtained. This digested solution was filtered through Whatman # 42 and final volume 50 mL was made.

Cadmium analysis

The digested samples were subjected to atomic absorption spectrophotometer (Model: 969, Unicam Limited, Cambridge, UK). Analysis of variance and LSD analysis were worked out on the collected data.

Pollution load index (PLI)

PLI represents the soil pollution level (Liu et al., 2005) $PLI = (M)^{Soil}/(M)^{Reference soil}$

Bio-concentration factor (BCF)

BCF represents the metal transfer to plant system (here in this case wheat grains) (Cui et al., 2004) BCF = (M) $^{Grain}/(M)^{Soil}$

Daily intake of metal (DIM) and health risk index (HRI)

According to Chary et al. (2008), DIM and HRI were determined using this equation:

DIM= (M) Grain×Cfood intake/ BW

HRI = Daily intake of metal/oral reference dose

(M) Grain represents Cd level in grains, $C_{food intake}$ represents daily wheat grain intake (0.345) and BW represents human body weight (60 kg).

Results

In both pot and field experiments, Cd content showed significant variations within soil fertilizer treatments and wheat samples, but non-significant effect of metal was observed within the waste treatment (Table 1-2).

Source of	Degrees of	Mean squares		
variation	freedom	Pot	Field	
Treatment (Tr)	8	0.000318**	0.001224**	
Ctrl. vs Tr	1	0.000990**	0.002890**	
Waste (W)	3	0.000119 ^{ns}	0.000062 ^{ns}	
Dose (D)	1	0.000970**	0.002102**	
W x D	3	0.000075 ^{ns}	0.001537**	
Error	36	0.000046	0.000123	
Total	44			

Table 1: Analysis of variance of Cd content in soil samples and different soil amendments

** = Significant at P < 0.01; ns = non-significant (P < 0.05).

Table 2: Analysis of variance of Cd content in wheat samples from both pot and field experiments

Source of variation	Degree of freedom	Pot experiment				
Source of variation	Degree of freedom	Root	Shoot	Grain		
Variety (V)	9	0.147**	0.153**	0.013ns		
Treatment (Tr)	8	3.376**	2.890**	3.598**		
Ctrl. vs Tr	1	6.036**	5.524**	6.715**		
Waste (W)	3	0.011ns	0.012ns	0.016ns		
Dose (D)	1	20.901**	17.537**	21.982**		
W x D	3	0.012ns	0.008ns	0.013ns		
V x Tr	72	0.038**	0.036**	0.009ns		
Field Experiment						
V	9	0.083**	0.129**	0.072**		
Tr	8	1.245**	1.259**	1.164**		
Ctrl. vs Tr	1	3.940**	4.189**	3.999**		
W	3	0.057**	0.029**	0.066**		
D	1	5.720**	5.788**	5.006**		
W x D	3	0.042**	0.003ns	0.037**		
V x Tr	72	0.018**	0.020**	0.026**		

** = Significant at P < 0.01; ns = non-significant (P < 0.05).

Soil analysis for Cd

The soil samples had Cd concentration within the range of 0.030-0.075 mg/kg in both experiments. Maximum Cd concentration was observed in T8 treatment of the pot experiment, while its minimum value was observed in the T9 treatment of the field experiment (**Table 3**).

Pot ex	periment				
	Soil	Root	Shoot	Grain	
T1	0.059±0.003	0.79±0.01	0.78±0.01	0.78±0.01	
T2	0.062±0.003	1.24±0.02	1.20±0.03	1.23±0.02	
Т3	0.060±0.004	0.79±0.01	0.79±0.01	0.77±0.01	
T4	0.070±0.003	1.23±0.02	1.21±0.02	1.27±0.01	
T5	0.056±0.005	0.77±0.01	0.79±0.01	0.79±0.01	
Т6	0.072±0.002	1.26±0.02	1.23±0.02	1.27±0.02	
T7	0.063±0.001	0.77±0.01	0.78±0.01	0.81±0.02	
Т8	0.075±0.001	1.21±0.03	1.18±0.02	1.26±0.02	
Т9	0.050±0.003	0.64±0.01	0.64±0.01	0.63±0.01	
Field e	xperiment				
T1	0.044±0.003	0.66±0.01	0.61±0.01	0.67±0.02	
T2	0.073±0.008	0.84±0.02	0.84±0.01	0.84±0.02	
Т3	0.045±0.004	0.65±0.01	0.63±0.01	0.63±0.01	
T4	0.065±0.003	0.89±0.02	0.88±0.02	0.86±0.02	
T5	0.067±0.006	0.59±0.01	0.59±0.01	0.58±0.01	
T6	0.045±0.006	0.85±0.02	0.83±0.02	0.84±0.02	
T7	0.037±0.004	0.59±0.01	0.60±0.01	0.59±0.01	
Т8	0.068±0.005	0.85±0.02	0.85±0.02	0.83±0.02	
Т9	0.030±0.003	0.44±0.01	0.42±0.01	0.43±0.01	

Table 3: Cd (mg/kg) a	nalysis of fertilizer	treatments of the p	ot and field e	experiments
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Cadmium content in wheat samples

The root samples recorded the Cd concentration between 0.44-1.24 mg/kg in both experiments (**Table 3-4**). In the wheat root samples, maximum Cd concentration was found in V4 (1.01 mg/kg) and T2 (1.24 mg/kg) treatment of the pot experiment, while the minimum value was observed in V7 (0.64 mg/kg) and T9 (0.44 mg/kg) treatment of the field experiment (**Figure 1**)

The shoot samples had the Cd content range between 0.42-1.23 mg/kg in both experiments (**Table 3-4**). In the wheat shoot samples, the variety V9 (1.00 mg/kg) and T6 (1.23 mg/kg) treatment showed maximum Cd content in the pot experiment, while V7 (0.63 mg/kg) and T9 (0.42 mg/kg) treatment displayed minimum Cd concentration in the field experiment (**Figure 2**).

The grain samples had Cd concentration as 0.43-1.27 mg/kg in both experiments (**Table 3-4**). Wheat grains showed the maximum level in V8 (1.01 mg/kg) and T6 (11.27 mg/kg) of the pot experiment while V7 (0.65 mg/kg) and T9 (0.43 mg/kg) found the minimum Cd levels (**Figure 3**).

Tab	le 4: Cadmium	content (mg/	kg)) in wh	eat variet	ies grown:	in po	ot and	fielc	experiments
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Varieties	Pot experiment		
	Root	Shoot	Grain
V1	0.82±0.02	0.80±0.01	0.98±0.04
V2	1.02±0.04	0.96±0.04	0.96±0.04
V3	0.97±0.04	0.95±0.04	0.97±0.04
V4	1.01±0.05	0.98±0.04	0.99±0.04
V5	0.98±0.04	0.97±0.04	0.95±0.04
V6	0.93±0.04	0.97±0.04	0.97±0.04
V7	1.00±0.04	0.99±0.04	0.98±0.04
V8	0.98±0.04	0.98±0.04	1.01±0.04
V9	1.00±0.04	1.00±0.04	1.00±0.04
V10	0.97±0.04	0.97±0.04	0.99±0.04
Field experiment			
V1	0.75±0.03	0.74±0.03	0.75±0.03
V2	0.76±0.03	0.75±0.03	0.72±0.03
V3	0.74±0.03	0.75±0.03	0.73±0.03
V4	0.73±0.03	0.73±0.03	0.75±0.02
V5	0.72±0.02	0.72±0.03	0.72±0.03
V6	0.72±0.03	0.72±0.04	0.67±0.02
V7	0.64±0.02	0.63±0.02	0.65±0.03
V8	0.66±0.02	0.65±0.02	0.66±0.02
V9	0.68±0.03	0.65±0.02	0.67±0.02
V10	0.65±0.02	0.62±0.02	0.66±0.02



Figure 1: Box plot for Cd in wheat root of both pot and field experiments

Cadmium-indices assessment

Pollution load index (PLI) was found to be within the range 0.107-0.268 mg/kg Cd in both experiments. Highest value was found in T8 (0.268 mg/kg) treatment of the pot experiment, while the lowest was shown by T9 (0.107 mg/kg) in the field experiment (**Table 5-6**).

BCF fell within the range of 0.866-1.984 mg/kg of Cd. Highest BCF value was observed in V8 (1.603 mg/kg) and T2 (1.984 mg/kg) treatment of the pot experiment, while V7 (1.25 mg/kg) and T5 (0.866 mg/kg) treatment were also the lowest in the field trial (**Table 5-6**).

DIM and HRI ranges for Cd metal were recorded from 0.00019-0.00055 mg/kg/day and 0.187-0.552 mg/kg/day, respectively. In these parameters, highest range values were shown by T4, T6 and T8 treatments in the pot experiment, while the lowest was observed in T9 treatment of the field experiment. The wheat varieties presented the maximum Cd concentration in V8 in the pot experiment, while minimum concentration was noticed in V7 and V10 in the field experiment (**Table 5-6**).



Figure 2: Box plot for Cd in wheat shoot of both pot and field experiments

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Trootmonte	Pot expe	eriment		Field ex	Field experiment			
meatments	PLI	BCF	DIM	HRI	PLI	BCF	DIM	HRI
T1	0.211	1.322	0.00034	0.339	0.157	1.523	0.00029	0.291
T2	0.221	1.984	0.00053	0.535	0.261	1.151	0.00037	0.365
Т3	0.214	1.283	0.00033	0.335	0.161	1.401	0.00027	0.274
T4	0.251	1.814	0.00055	0.552	0.232	1.323	0.00037	0.374
T5	0.202	1.411	0.00034	0.343	0.239	0.866	0.00025	0.252
Т6	0.257	1.764	0.00055	0.552	0.161	1.867	0.00037	0.365
Τ7	0.225	1.286	0.00035	0.352	0.132	1.595	0.00026	0.256
Т8	0.268	1.681	0.00055	0.548	0.243	1.221	0.00036	0.361
Т9	0.179	1.262	0.00027	0.274	0.107	1.433	0.00019	0.187

Table 6: Various indices analyzed for Cd metal in wheat varieties grown in the pot and field experiments									
Varieties	Pot exper	iment		Field exp	periment				
	BCF	DIM	HRI	BCF	DIM	HRI			
V1	1.556	0.00043	0.426	1.442	0.00033	0.326			
V2	1.524	0.00042	0.417	1.385	0.00031	0.313			
V3	1.540	0.00042	0.422	1.404	0.00032	0.317			
V4	1.571	0.00043	0.430	1.442	0.00033	0.326			
V5	1.508	0.00041	0.413	1.385	0.00031	0.313			
V6	1.540	0.00042	0.422	1.288	0.00029	0.291			
V7	1.556	0.00043	0.426	1.250	0.00028	0.283			
V8	1.603	0.00044	0.439	1.269	0.00029	0.287			
V9	1.587	0.00043	0.435	1.288	0.00029	0.291			
V10	1.571	0.00043	0.430	1.269	0.00029	0.286			



Panel variable: Treatment

V01 V02 V03 V04 V05 V06 V07 V08 V09 V10

0.8

0.4

Figure 3: Box plot for Cd in wheat grain of both pot and field experiments

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F

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Variety

E

V01 V02 V03 V04 V05 V06 V07 V08 V09 V10

Discussion

Organic fertilizer treatments generally contain high contents of Cd, especially sewage sludge; however, when such materials are mixed with soil, these fertilizers show increased Cd accumulation in crops (Karaca, 2004). In sewage sludge added soil, the values observed in both pot and field trials were considered higher than the limits (3 mg/kg) reported by WHO/FAO (2007). This may have been because of soil pH, organic matter content, soil redox potential, and the rate of metal distribution in soil which influence their adsorption and retention capacity (Rangaraj et al., 2007). The present Cd range was lower than that reported by Khan et al. (2016) elsewhere. The Cd content trend in the fertilizer amended soil was: T8 > T6 > T4 > T7 > T2 > T3 > T1 > T5 > T9 in the pot experiment, while T2 > T8 > T5 > T3 > T6 > T1 > T7 > T9 in the field trial.

The pattern of accumulation of Cd in different plant organs in different wheat varieties was: root > shoot > grain. Cd order in wheat root (mg/kg) of different varieties was: V2 > V4 > V7 > V9 > V5 > V8 > V3 > V10 > V6 > V1 in the pot experiment, and V2 > V1 > V3 > V4 > V5 > V6 > V9 > V8 > V10 > V7 in the field experiment. Moreover, Cd transfer in wheat shoot (mg/kg) was V9 > V7 > V4 > V8 > V5 > V6 > V10 > V2 > V3 > V1 > V3 > V1 > V4 > V5 > V6 > V9 > V7 > V10 in the field trial.

Similarly, Hussain et al. (2010) reported similar variation in Cd content in wheat plants. In comparison to other plant parts, grains had the lowest concentration of Cd, as reported in different earlier published studies (Huang et al., 2008; Zhang et al., 2008; Hirzel et al., 2017; Bai et al., 2024). Karaca (2004) experimented on different organic wastes and found that grain contains less amount of Cd as compared to the shoot and root. In the present research, Cd accumulation in grains was in the following order: V8 > V9 > V4 > V10 > V1 > V7 > V3 > V6 > V2 > V5. The phenomenon of low accumulation of metals including Cd in grains has been explained as the roots generally retain high amounts of metals and only a minimal amount is transferred to the grains (Bai et al., 2024).

It is believed that organic fertilizers resulting from different polluted sources contain substantial amounts of different types of metals (Schmidt, 1997; Naik et al., 2004)). For example, while conducting a study involving the application of different types of organic fertilizers such as farmyard manure (FYM) and press mud to different cultivars of sunflower, Naik et al. (2004) reported higher Cd content in FYM and press mud treatments. Earlier, Schmidt (1997) also reported high levels of different heavy metals (Pb, Cu, Ni, Cd, Zn) in press mud and sewage resources. The modification such as fungus interaction with plant roots (Martino et al., 2004) and structure of plant roots (Welch et al., 1999) helps improve the movement of metals and also their uptakes.

According to the WHO (2007) reports Cd limit in plants should be close to 0.02 mg/kg (Nazir et al., 2015), whereas in contrast, the Cd levels detected in our study were higher than the WHO (2007) standards, regardless of treatments or varieties. For instance, if grains accumulate high amounts of metals, these can accumulate in the body parts of animals (Harter et al., 2002) as well as humans (Wieczorek et al., 2023).

Depending on the concentration of each metal in the ground, pollution load index (PLI) always offers an estimate of the status of metal contamination and the required action. As PLI values indicate whether soil quality is suitable for agricultural use or not, so it is to be considered as a good parameter to analyze soil quality for crop production (Liu et al., 2005). The current study showed that PLI of Cd is in line with that already reported elsewhere (Khan et al., 2016). These authors conducted a trial to appraise metal toxicity in the soil as well relations among themselves (Fe, Zn, Pb, Ni, Mo, Cu, As, Se). The PLI values found in this study were higher in the amended soil than those in the control soil. High PLI rates can be attributed to a variety of factors such as agricultural flow, human activity and industrial activities (Islam et al., 2018). The PLI (Cd) index in our study was: T8 > T6 > T4 > T7 > T2 > T3 > T1 > T5 > T9 in the pot experiment, whereas T2 > T8 > T5 > T4 > T3 > T6 > T1 > T7 > T9 in the field trial.

If bioconcentration factor (BCF) is > 1, it indicates that the plant has accumulated metals (Singh et al., 2010). The BCF trend for Cd in the wheat varieties was: V8 > V9 > V4 > V5 > V10 > V1 > V7 > V2 > V3 > V6 in the pot experiment, whereas V1 > V4 > V3 > V2 > V5 > V6 > V9 > V8 > V10 > V7 in the field experiment.

Eating foods which are contaminated and are essential parts within our diet, need to be carefully assessed by the Human Risk Assessment (HRA) and Health Risk Index (HRI) (Ungureanu et al., 2023). Especially, in countries where these wastewater laws are not regulated, the magnitude of this indicator is very important. If the HRI value of a particular food is 1, a significant metal toxicity may be caused to human health. In the current study, the HRI (Cd) index observed was: V8 > V1 > V4 > V7 > V9 > V10 > V2 > V3 > V6 > V5 in the pot experiment, whereas V1 > V4 > V3 > V2 > V5 > V6 > V8 > V9 > V10 > V7 in the field trial. To identify consumer-based health risks, certain approaches can be identified. In this study, a metal method was used on daily diet amount to assess health risks (Balkhair and Ashraf, 2016) and in the

present study, the health risks posed by Cd are different from those reported earlier in a different study (Singh et al., 2010).

Conclusion

The results reported in the present study showed that Cd availability in soil-wheat system under different soil amendments was within the WHO standard limits. Among various treatments and varieties, highest cadmium was taken up by cv. Ihsan-16 grown under farm-yard manure treatment. Soil amendment treatments usually increase the heavy metal mobility in food crops and ultimately in the human food chain. It is a need of the time to recognize those soil amendments that overcome the toxic metal absorption in wheat and enhance the crop productivity. The study recommends that soil amending treatments prove to be a source of organic fertilizers for sustainable wheat production. These amendments decrease the metal contamination in agricultural land, and encounter the food security and solid waste management issues. Further research work is needed to uncover the suitable mechanism and soil parameters which stabilize the toxic metals present in the soil environment.

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No supplementary material is included with this manuscript.

Conflict of interest

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Contribution of authors

Conceptualization: ZIK, KA. Conduct of experiment: AE, AA. Writing - original draft preparation: AE, ZIK, AA, KA, FGM, FS, NR, FZ. Data analysis: AE, AA. Presentation of data: AE, ZIK, MN< FS, NR, FZ. All authors have read and agreed to publish this article in IJAaEB.

Ethical approval

This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials

The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of the experiment, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent

All authors contributed in designing and writing the entire article. All contributors have critically read this manuscript and agreed to publish in IJAaEB.

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It is declared that we the authors used ChatGPT 3.5 free version for paraphrasing 3-4% of text during the preparation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB). After using this tool, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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