# Assessment of cadmium toxicity in buffaloes grazing on forages cultivated in diverse irrigated soils: a comprehensive analysis

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Abstract: This study investigated the potential risks of cadmium (Cd) toxicity in buffaloes grazing on forages that were cultivated in soils irrigated by various sources of wastewater. The absorption of trace metals (TM) by plants and their subsequent entry into the food chain pose a significant danger to grazing animals through the accumulation of contaminated fodder. The mean concentration of Cd in the water ranged from 0.18-1.78 mg/L, in the soil 0.63 to 1.87 mg/kg, in the forage 0.20 to 1.32 mg/kg, and in the blood 0.26 to 1.98 mg/L. Among all three sites, canal water (CW Site I), groundwater (GW Site II), and sewage water (SW Site III), the concentration factor (CF) values were below the threshold of 1 (CF < 1), indicating the nominal environmental concern regarding Cd contents in the soil-plant interface. In addition, a prominent variation was noticed in the transfer factor (TF) of Cd across different sites, with the highest TF observed in Avena sativa L. at SW Site III (0.8) and the lowest in Pennisetum glaucum L. at CW Site I (0.27). Furthermore, the hazard quotient (HQ) exhibited a substantial fluctuation, ranging from 0.39 to 2.6, reflecting varying levels of potential health risks associated with Cd exposure. The outcomes of the current investigation suggested that the prominent increase in Cd levels was recorded at sampling site SW Site III due to continuous wastewater irrigation. Prolonged exposure and increased Cd absorption in buffaloes grazing at these sites could have harmful long-term effects on their health. The correlation analysis between Cd concentrations in water, soil, forage, and blood showed a positive but non-significant relationship for water-soil, soil-forage, and forage-blood interactions. This highlights the need for further research to assess the long-term implications of wastewater irrigation on heavy metal accumulation in livestock.

Keywords: livestock farming; pollution; nutrition; animal fodder; bioaccumulation

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Pakistan's economy mainly depends on agricultural and livestock farming practices, where a large portion of the economy's protein needs are fulfilled by the rearing of animals (Khan et al. 2020). Small ruminants, recognised as the primary source of animal protein, expressed minimal performance with the temperature rise (Hashmi et al. 2021). Wastewater is a source of essential nutrients and plays a prominent role in soil fertility; however, it contains a sufficient amount of trace substances, especially trace metal (TM), that leads to a deterioration of the food web (Khan et al. 2020). TM can easily leach down and penetrate the groundwater bodies, where plant roots can facilitate the absorption into plant tissues. Consequently, these TM can easily be entered into the food web and consumed by humans and animals using edible portions of crops and drinking water (Engwa et al. 2019).

Forage plants are considered crucial for animals because they support both domesticated and wild herbivores as well as ensure the production of meat and milk supply. The significant variation in the nutritional value of forage plants has been recorded in terms of the proportion of their tissues that can be consumed by herbivores, as well as they contain fibre, minerals, and protein. Moreover, the variation in the nutrient composition of forage plants significantly affects the quality of nutrition provided to the end consumers (Lee 2018). When cattle consume water and pastures are contaminated with pollutants, TM can easily be ingested and accumulated in their bodies, including blood vessels, as well as in their products, e.g., milk. The previous study by Norouzirad et al. (2018) investigated cow milk samples containing lead (Pb) and cadmium (Cd) and exceeding the threshold levels. Sewage water is often used as the irrigation source of fodder crops, containing organic and inorganic beneficial nutrients that promote fodder crops. On the other hand, this sewage water also contains excessive amounts of TM, which badly affects the quality of fodder crops and poses severe growth disorders (Uchimiya et al. 2020).

Cadmium is widely spread in the environment, specifically in air, water, and soil, and it strongly interacts with animals. The study conducted by Gumasta et al. (2018) explained that animals can consume excessive amounts of Cd from phosphate fertilisers and wastewater, as well as automobile exhaust used in farming ecosystems. It has been noticed that cattle farming near the industrial and urban sites accounts for high levels of Cd in their blood samples. Cd is

a severe environmental hazard in agricultural systems because it is extremely readily soluble in water and rapidly assimilated by plants (Duan et al. 2020). The accumulation of Cd in contaminated water may affect crucial physiological processes and result in either short-term or long-term disorders. It is absorbed in animals' gastrointestinal tract (GI) via several different transporters. High Cd exposure increases digestion, particularly in the duodenum (Ohta and Ohba 2020). At higher dosages, Cd seriously threatens the health of humans, animals, and plants. It is a major contributor to malignant diseases (Haider et al. 2021). The current investigation aims to evaluate the fate and behaviour of Cd in water, soil, cow forage, and cow blood, which exist and grow near the industrial discharge outlets in Sargodha, Pakistan. The research also sought to correlate these findings with health risk assessment with the objective of determining the accumulation and transfer of Cd across the wastewater, soil, forage, and animal blood.

#### MATERIAL AND METHODS

Soil, water and forage collection. Soil and forage sampling was performed from three different water irrigation sources, including canal water, groundwater and sewage water. A total of 18 soil samples with three replicates were collected, each sample containing 1 kg weight. The collected samples were first air-dried and then oven-dried at 70-75 °C for one week to eliminate moisture. The dried samples were crushed and transformed into a fine powder using a crusher. Each sample containing 5 g was then placed in polythene bags for further chemical testing. Tested soil pH and electrical conductivity (EC) were estimated using a pH and EC meter in a 1:2.5 and 1:5 soil-water extract. CaCl<sub>2</sub> extractable Cd contents were estimated using the prescribed method described in our previous study (Bashir et al. 2018). A 20 mL CaCl<sub>2</sub> extractable solution was used to extract Cd from 2 g studied tested soil. The suspension was shaken for 2 h and then filtered via Whatman No. 42 filter paper, and all the extracted samples were kept at room temperature. Soil-available phosphorus and potassium were determined and analysed according to our previous study (Bashir et al. 2018). Soil organic carbon was determined using the Walkaly black method by Bashir et al. (2018).

Similarly, three samples of each forage and three replicates from each location and site were collected and analysed for Cd accumulation (Table 1).

**Blood sampling.** Blood samples from five different buffalo categories were collected from three locations. Each blood sample contained 5 mL and was centrifuged for 2 min at 2 500 rpm to extract plasma. The centrifuged aliquot was placed in plastic bottles and preserved for further analysis (Khan et al. 2021).

**Digestion of soil, water and blood samples.** Soil, water, blood, and forage samples were digested for cadmium estimation. For soil digestion, 0.5 g of airdried studied soil was digested with 10 mL of aqua regia (HCl:HNO<sub>3</sub> = 3:1 v/v) and heated at 180 °C for 2–3 h and then diluted to 50 mL. For water digestion, 5 mL of HNO3 was added to each water sample and heated at 95 °C for 45 min to dissolve total metals before filtration (Bashir et al. 2022). Blood digestion is performed by mixing 2 mL of blood with 5 mL of HNO<sub>3</sub>, pre-digesting for 30 min, heated at 150 °C, then adding 2-3 mL of  $H_2O_2$  to complete the digestion before dilution to 25 mL (Khan et al. 2021). Forage digestion can also be done by digestion of 1 g of dried forage sample with 10 mL of aqua regia at 120 °C, then diluted to 50 mL for Cd determination using AAS.

#### Indices to evaluate heavy metal contamination

**Contamination factor** (CF). The following calculation methods were used to estimate the Cd status in the collected soil samples and selected sites. The following equation was used to determine the exact Cd in soil (Khan et al. 2021).

The CF is calculated as follows:

$$\text{CF} = \frac{C_m \, \text{Sample}}{C_m \, \text{Background}}$$

Where:  $C_{\rm m}$  sample – metal content in the soil;  $C_{\rm m}$  background – metal concentration from a natural source; background value of Cd = 69.

**Transfer factor** (TF). Similarly, the transfer of Cd from soil to plant was estimated using the following equation proposed by Khan et al. (2021).

$$TF = \frac{C_{Plant}}{C_{Soil}}$$

**Enrichment factor** (EF). Meanwhile, the enrichment of Cd in forage was estimated using the following calculation equation described by Khan et al. (2021).

$$EF = \frac{\text{(metal value in forage/metal value in soil) sample}}{\text{soil standard}}$$

**Estimated daily intake** (EDI). The daily intake of trace metals, especially Cd, and its impact on daily life was estimated using the following equation presented by Khan et al. (2021).

$$EDI = \frac{C \times DI \times C. F}{BW}$$

Where: C – metal concentration (mg/kg); DI – daily intake food which is 12.5; C.F – conversion factor which is 0.085; BW – body weight which is 550 (Khan et al. 2021).

**Hazard of quotient** (HQ). The following equation was proposed to evaluate. The proportion of risk associated with an impact on the level above which no adverse effects are anticipated.

$$HQ = \frac{EDI}{RfD}$$

Where: EDI – estimated daily intake (mg/kg/day) of metals; RfD – measured reference dose of metals (mg/kg/day).

**Statistical analysis.** All the observed data of the studied experiment were stated as the mean data. The ANOVA was estimated using the computer-based software Statistix 8.1 (USA). The significant difference among all the studied treatments was differentiated using the least significant difference (*LSD*) at the 5% probability level.

## **RESULTS**

**Physico-chemical properties of water and soil samples.** The outcomes of the studied soil and water samples represent the prominent variation in EC values among all three studied sites EC. The EC values ranged from 266.6 to 5 870  $\mu$ S/cm at (CW Site I, GW Site II, and SW Site III) varied from the mean concentration of Ca<sup>2+</sup> + Mg<sup>2+</sup> ranged from 2.53–13.42 mg/L, while the value of sodium fluctuated between 0.54–

Table 1. List of the studied forage crops grown on the studied sites and locations

Local name	Scientific name	Family	
Maize	Zea mays L.	Poaceae	
Bajra (pearl millet)	Pennisetum glaucum L.	Poaceae	
Berseem	Trifolium alexandrinum L.	Fabaceae	
Jawar	Sorghum bicolor L.	Poaceae	
Joddar/oat	Avena sativa L.	Poaceae	
Sugar cane	Saccharum officinarum L.	Poaceae	

Table 2. Physico-chemical properties of water and soil samples collected from three different sites

		Water					
Site	EC	$Ca^{2+} + Mg^{2+}$	sodium	bicarbonate	chloride	CAD	
	$(\mu S/cm)$		(ppm)			SAR	
CW Site I	810.3 ± 0.88	$6.24 \pm 0.005$	2.44 ± 0.008	7.11 ± 0.005	1.63 ± 0.008	$1.24 \pm 0.008$	
GW Site II	$266.6 \pm 0.88$	$2.53 \pm 0.08$	$0.54 \pm 0.005$	$2.34 \pm 0.005$	$0.15 \pm 0.011$	$0.21 \pm 0.005$	
SW Site III	$5\ 870.0\pm0.5$	$13.42 \pm 0.01$	$45.68 \pm 0.005$	$18.56 \pm 0.05$	$30.5 \pm 0.005$	$16.6 \pm 0.008$	
	Soil						

	3011					
		EC	SOC	A-P	A-K	Saturation
	pH	$(\mu S/cm)$ (%)	(%)	(mg/kg)		(%)
CW Site I	$7.60 \pm 0.05$	$1.95 \pm 0.05$	$0.42 \pm 0.05$	$8.61 \pm 0.08$	$156.0 \pm 0.57$	$42.0 \pm 0.57$
GW Site II	$8.13 \pm 0.08$	$1.05 \pm 0.08$	$0.72 \pm 0.011$	$5.12 \pm 0.017$	$153.0 \pm 0.57$	$43.0 \pm 1.52$
SW Site III	$8.26 \pm 0.08$	$3.84 \pm 0.05$	$0.97 \pm 0.08$	$35.5 \pm 0.08$	$295.0 \pm 0.57$	$43.0 \pm 0.57$

EC – electrical conductivity; SAR – sodium absorption ratio; SOC – soil organic carbon; A-P – available phosphorus; A-K – available potassium. The study sites were categorised as follows: CW – canal water; GW – groundwater; SW – sewage water

45.68 mg/L. The range of the mean bicarbonate concentration was  $2.34{\text -}18.56$  mg/L, whereas the chloride concentration varied from 0.15 to 30.5 mg/L at SW Site III; the Na<sup>2+</sup> absorption ratio shows a higher concentration of 16.6 mg/L. Similarly, the collected soil samples were analysed for pH, EC, P and K availability. The studied soil samples had a loamy texture. The maximum soil pH was noticed by 8.26 at SW Site III. In addition, the mean value of EC of the studied soil ranged from  $1.05{\text -}3.84~\mu\text{S/cm}$  for all three selected sites. Furthermore, the value of organic carbon ranged from  $0.24{\text -}0.56\%$ . Moreover, the available P and K ranged from  $5.12{\text -}35.5$  and  $153{\text -}295.0$  mg/kg. The saturation percentage of the samples varied between  $42{\text -}43\%$  (Table 2).

**Cd** concentration in water. The findings expressed that the concentration of Cd in water samples varied from 0.18 to 1.73 mg/L. At CW Site I and Location I,

Cd's maximum mean value was 0.45 mg/L, whereas the lowest Cd was detected as 0.18 mg/L in water at Location III. GW site II and Location III predominantly exhibited the highest mean Cd value by 1.13 mg/L. Meanwhile, the lowest mean Cd value of 0.62 mg/L was recorded at Location I in the water. The peak mean Cd concentration of 1.73 mg/L was observed at Location II in SW Site III, whereas Location III registered the lowest mean Cd value of 1.35 mg/L in the water (Figure 1).

**Cd concentration in soil.** The estimated results showed that the mean concentration of Cd in soil was detected at 0.71 mg/kg at CW Site I. In comparison, its maximum concentration was observed at 0.92 mg/kg in *Saccharum officinarum* L. However, the minimum concentration of Cd was noticed in *Zea mays* L. by 0.63 mg/kg. In addition, at GW Site II, the highest mean Cd concentration was detected at

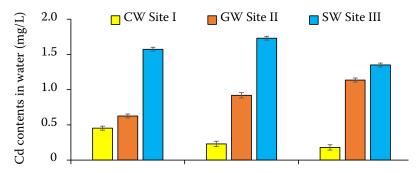


Figure 1. Fluctuations in mean cadmium (Cd) concentration in water samples collected from the study sites. The study sites were categorised as follows: CW – canal water; GW – groundwater; SW – sewage water. All values were the average of three replicates, and the error bar represents the standard deviation

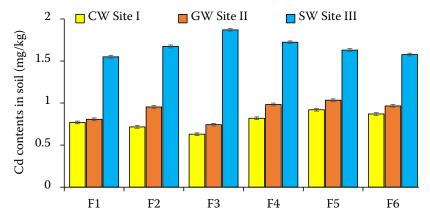


Figure 2. Fluctuations in mean cadmium (Cd) concentration in soil samples collected from the study sites. The studied forage crops were categorised as follows: F1 – Zea mays L.; F2 – Pennisetum glaucum L.; F3 – Trifolium alexandrinum L.; F4 – Sorghum bicolor L.; F5 – Avena sativa L.; F6 – Saccharum officinarum L. All values were the average of three replicates, and the error bar represents the standard deviation. The study sites were categorised as follows: CW – canal water; GW – groundwater; SW – sewage water

1.03 mg/kg in *Saccharum officinarum* L. In contrast, *Zea mays* L. exhibited the lowest mean Cd concentration in soil by 0.74 mg/kg. The maximum level of Cd by 1.87 mg/kg was found in *Zea mays* L. soil, and the minimum level of mean Cd by 1.55 mg/kg was detected in *A. sativa* at SW Site III (Figure 2).

**Cd concentration in forage crops.** The results revealed that the mean concentration of Cd varied between 0.2–1.32 mg/kg in the collected forage samples. At CW Site I, the highest Cd value was recorded in *Trifolium alexandrinum* L. by 0.36 mg/kg, whereas the minimum uptake of Cd by *Z. mays* was estimated by 0.20 mg/kg in forage. At GW Site II, the highest mean value of Cd (0.55 mg/kg) was observed in *Trifolium alexandrinum* L. The lowest mean value, 0.23 mg/kg, was detected in *Z. mays*. At SW Site III,

*P. glaucum* displayed the maximum mean value of Cd by 1.32 mg/kg. Meanwhile, *Trifolium alexandrinum* L. showed the minimum accumulation of Cd at 0.82 mg/kg (Figure 3).

Cd concentration in blood serum (mg/L) of buffaloes. The studied blood sample results exhibited a significant variation in buffaloes among all the selected sites and locations. Cd contents were varied and ranged between 0.26–1.98 mg/L. At CW Site I, the analysis of buffalo non-lactating blood 1.98 mg/L contains the minimum mean value of Cd by 0.26 mg/L. In contrast, buffalo calf displayed the maximum mean value of Cd by 0.54 mg/L. At SW Site III, the blood samples taken from buffalo lactating displayed a mean Cd concentration of 1.05 mg/L, indicating the lowest value observed among all the

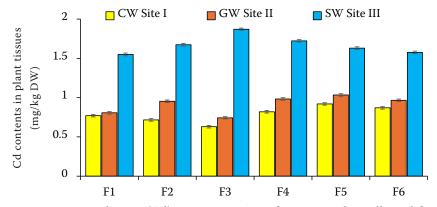


Figure 3. Fluctuations in mean cadmium (Cd) concentration in forage samples collected from the study sites. The studied forage crops were categorised as follows: F1 –  $Zea\ mays\ L$ .; F2 –  $Pennisetum\ glaucum\ L$ .; F3 –  $Trifolium\ alexandrinum\ L$ .; F4 –  $Sorgum\ bicolor\ L$ .; F5 –  $Avena\ sativa\ L$ .; F6 –  $Saccharum\ officinarum\ L$ . All values were the average of three replicates, and the error bar represents the standard deviation. The study sites were categorised as follows: CW – canal water; GW – groundwater; SW – sewage water DW – dry weight

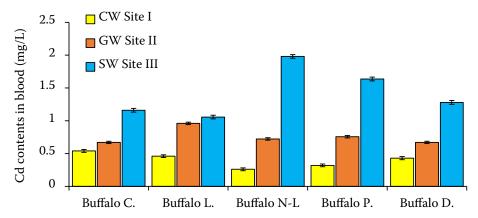


Figure 4. Fluctuations in mean cadmium (Cd) concentration in forage samples collected from the study sites. The studied buffalos were categorised as follows: buffalo C. – buffalo calf; buffalo L. – lactating buffalo; buffalo C. – buffalo calf; buffalo C. – lactating buffalo; buffalo C. – buffalo dry. All values were the average of three replicates, and the error bar represents the standard deviation. The study sites were categorised as follows: CW – canal water; CW – groundwater; CW – sewage water

studied samples. Conversely, the mean value for buffalo non-lactating exhibited the highest average value of Cd by 1.98 mg/L (Figure 4).

**Contamination factor of Cd in soil of forage crops.** The findings of this surveyed study indicated that the Cd contamination factor ranged between 0.0091 and 0.0271 mg/kg. At CW Site I, *Z. mays* showed a minimum CF value of 0.0091 mg/kg for Cd, while *S. officinarum* displayed a maximum value of 0.0133 mg/kg. At GW Site II, the CF values for Cd in both soil samples of forage crops (*Zea mays* L. and *Saccharum officinarum L.*) exhibited 1.98 mg/kg; the same variations were observed at CW Site I.

In addition, the maximum value was recorded by 0.0149 mg/kg for *Saccharum officinarum* L. At the same time, *Z. mays* revealed the minimum value of Cd by 0.0107 mg/kg. The concentration of Cd differed significantly between 0.0224–0.027 mg/kg at SW Site III. *A. sativa* demonstrated the lowest CF value of 0.0224 mg/kg, whereas *Zea mays* L. recorded the highest value of 0.0271 mg/kg.

**Transfer of Cd from soil to forage.** The result revealed that the TF of Cd varied from 0.27 to 0.8 mg/kg. At CW Site I, *Trifolium alexandrinum* L. demonstrated the highest TF for Cd by 0.50 mg/kg. Meanwhile, *Pennisetum glaucum* L. indicated the lowest Cd at

Table 3. Analysis of variance of data for cadmium (Cd) concentrations in samples of water, soil, forage and serum collected from the study sites

Sources of variance	df	Mean square value	Sources of variance	df	Mean square value		
	Water			Soil			
Treatment	2	3.59290***	treatment	2	4.10114***		
Water	2	0.01618***	soil	5	0.02957***		
Treatment $\times$ water	4	0.17666***	$treatment \times soil$	10	0.04135***		
Error	18	0.00018	error	36	0.00023		
Total	26		total	53			
Crops			buffaloes blood				
	Crops		Buffaloes Blood				
Treatment	2	323.521***	sites	2	5.10477***		
Crop	5	2.843***	blood	4	0.18872***		
Treatment × crop	10	0.988***	sites × blood	8	0.34155***		
Error	36	0.076	error	29	0.00011		
Total	53		total	43			

<sup>\*\*\*</sup>P < 0.001; df – degree of freedom

0.268 mg/kg. At GW Site II, *Avena sativa* L. displayed the maximum Cd contents in TF value by 0.67 mg/kg. Conversely, *Zea mays* L. exhibited the minimum Cd TF value of 0.318 mg/kg. The highest TF value of Cd concentration was estimated at 0.8 mg/kg in *Avena sativa* L. at SW Site III. In contrast, the lowest TF value of Cd was noticed by 0.492 mg/kg among the collected samples.

Enrichment factor of Cd in forage samples. The enrichment factor of Cd varied from 2.04 to 6.08 mg/kg across three different sites (CW Site I, GW Site II, SW Site III). AT CW Site I, the highest EF value of Cd was noted for *Trifolium alexandrinum* L., the highest by 3.82, while *Pennisetum glaucum* L. showed the lowest range with a value of 2.04 mg/kg. According to Table 4, the maximum EF for Cd was observed in *Avena sativa* L. 5.09 mg/kg at GW Site II. On the other hand, *Zea mays* L. found the minimum EF value of 2.42 mg/kg. At SW Site III, *Avena sativa* L. exhibited the upper limit of EF with a value of 6.08. At the same time, *Trifolium alexandrinum* L. displayed the lower limit with a value of 3.74 mg/kg.

Analysis of estimated daily intake and hazard quotient in cadmium. The results presented in Table 5 illustrate the values of Cd-EDI were detected from 0.038-0.025. The highest CD-EDI was observed in Trifolium alexandrinum L. by 0.0069 at CW Site I while, the lowest Cd-EDI in Z. mays 0.004 were recorded. At SW Site III, Pennisetum glaucum L. indicated the maximum estimated daily intake value for Cd by 0.025. In addition, the minimum detection was noted in Trifolium alexandrinum L. by 0.015 among all the sites and locations; the Cd-EDI values showed moderate levels compared to CW Site I and SW Site III. Similarly, Cd's hazard quotient values were detected in a considerable range from 0.39 to 2.6. At CW Site I and GW Site II, Z. mays indicated a minimum HQ value of 0.39-0.46 for Cd, whereas Trifolium alexandrinum L. displayed a maximum value of 0.70-1.07. At SW Site III, the maximum level of HQ 2.6 was found in P. glaucum for Cd, and the minimum level of HQ 1.6 was detected in Trifolium alexandrinum L.

Correlation of cadmium concentrations between water, soil, forage and blood. The association be-

Table 4. Assessment of contamination factor, transfer factor and enrichment factor of cadmium in forage crops from different sites

Forage crop	CW Site I	GW Site II	SW Site III
Contamination factor			
Avena sativa L.	0.0111	0.011	0.0224
Trifolium alexandrinum L.	0.010	0.0138	0.0242
Zea mays L.	0.0091	0.0107	0.0271
Pennisetum glaucum L.	0.0118	0.0142	0.0249
Sorghum bicolor L.	0.0133	0.0149	0.0236
Saccharum officinarum L.	0.0126	0.0140	0.0228
Transfer factor			
Avena sativa L.	0.4025	0.6694	0.8
Trifolium alexandrinum L.	0.5023	0.5804	0.4918
Zea mays L.	0.3174	0.3183	0.6021
Pennisetum glaucum L.	0.2682	0.4271	0.7676
Saccharum officinarum L.	0.2826	0.4193	0.7582
Sorghum bicolor L.	0.3517	0.3724	0.7395
<b>Enrichment factor</b>			
Avena sativa L.	3.0597	5.0876	6.0800
Trifolium alexandrinum L.	3.8176	4.4111	3.7379
Zea mays L.	2.4126	2.4197	4.5762
Pennisetum glaucum L.	2.0390	3.2461	5.8345
Saccharum officinarum L.	2.1478	3.1870	5.7629
Sorghum bicolor L.	2.6731	2.8303	5.6204

The studied sites were categorised as follows: CW - canal water; GW - groundwater; SW - sewage water

Table 5. Comparative analysis of hazard quotient (HQ) and estimated daily cadmium intake (EDI) by buffaloes through contaminated forage from sampling sites

Forage crop	EDI			HQ		
	CW Site I	GW Site II	SW Site III	CW Site I	GW Site II	SW Site III
Avena sativa L.	0.0056	0.010	0.023	0.59	1.04	2.4
Trifolium alexandrinum L.	0.0069	0.0106	0.015	0.70	1.07	1.6
Zea mays L.	0.0038	0.0045	0.021	0.39	0.46	2.2
Pennisetum glaucum L.	0.0042	0.0081	0.025	0.42	0.81	2.6
Saccharum officinarum L.	0.005	0.0083	0.023	0.50	0.84	2.4
Sorghum bicolor L.	0.0059	0.0069	0.022	0.59	0.69	2.3

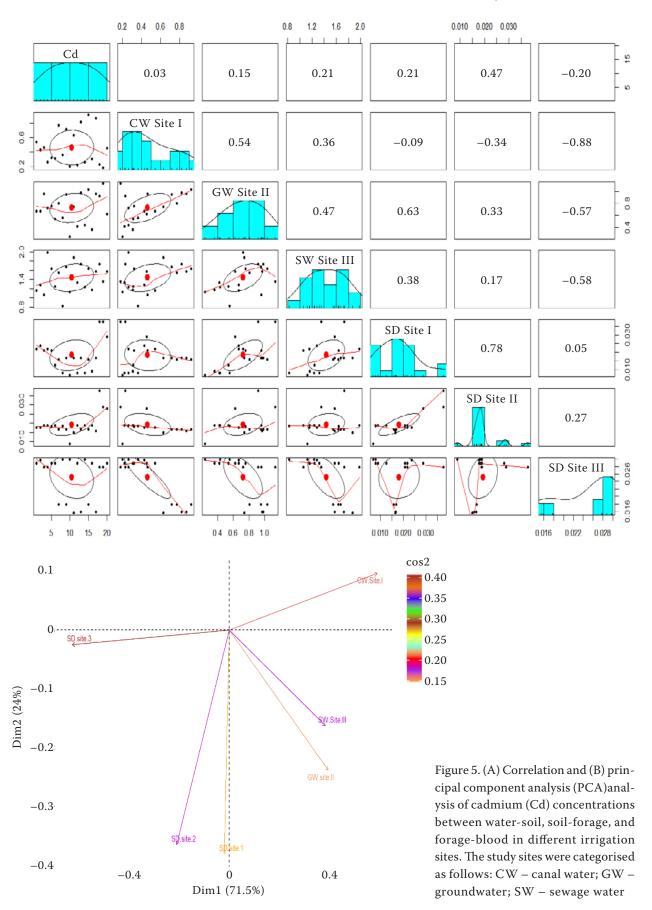
The study sites were categorised as follows: CW - canal water; GW - groundwater; SW - sewage water

tween water and soil, soil and forage, and forage with blood was investigated using the Pearson's correlation assessment technique (Figure 5). A non-significant connection between water and soil was found in Cd, which was analysed from the CW Site I and GW Site II samples. Nevertheless, a negative non-significant result between the water and soil variables was found at SW Site Location-III. The principal component analysis (PCA) separated the Site I, Site II and Site III variations regarding Cd concentration in soil, water, forage and blood samples of animals. Soil variables and treatments in orthogonal space (Figure 5). The PC1 and PC2 explained 71.5% variability in the data set (71.5% and 24% of total variability, respectively). A significant positive correlation was recorded in the PCA analysis between the SW II, GW II, and CW I.

### **DISCUSSION**

According to the findings of this study, it can be attributed that the Cd concentration range between (0.18–1.73 mg/L) in three different irrigated water was greater than the mean concentration (0.06 mg/L) of Cd for water as described by Idrees et al. (2018). These results suggested that the presence of Cd at this location might be due to the dumping or discharge of Cd-based residues, which might have a strong contribution to increasing the Cd levels in the soil and water as well as its accumulation in forage and transferred to buffalo's milk and blood. Several studies confirmed that Cd detection in water sources near agriculturally productive areas could enhance Cd transfer in the food chain. The previous report by Chaoua et al. (2019) confirmed the detection of Cd by 0.086 mg/L in freshwater bodies near the productive areas, while this detection is lower compared to the findings of the current investigation. The present study findings align with different research by Khan et al. (2018), revealing a similar level of 1.69 mg/L of Cd concentration in water.

The Cd concentration observed in the current study was lower (0.034-0.47 mg/kg) than that recorded by Ahmed et al. (2018). The quantity, quality, and interaction of the applied wastewater with the irrigated soils may be to blame for this erratic tendency. There have been reports of the considerable increase in Cd content caused by wastewater irrigation in different regions of the world (Farahat and Linderholm 2015). The amount of Cd in the current investigation was comparable to the level of exposure (1.0-1.8 mg/kg) suggested by Anderson et al. (2022). The concentration of Cd in soil samples detected in our current study closely resembled the concentration (1.5 mg/kg) proposed by Kaur et al. (2020). Similar findings were suggested by Anderson et al. (2022), who reported a Cd value of (5.23–5.94 mg/kg), which exceeded the range of Cd concentration found in the current study. Madanan et al. (2021) reported higher Cd concentrations in forage (2.8 and 10.3 mg/kg, respectively) compared to the current value. In agricultural soil, the Cd concentration is introduced by the excessive use of phosphatic fertilisers as well as wastewater irrigation. It has been demonstrated that several municipalities worldwide are promoting the establishment of trees planted in urban public areas and the reuse of wastewater, which is also helpful for the improvement of forage cultivation (Amato-Lourenco et al. 2020). According to the findings of the current work, Cd contents were detected in buffalo blood samples ranging from 0.26-1.98 mg/L which were higher than the permissible limit. These results aligned with the previous study reported by Hussain et al. (2022) recorded a high value of Cd (0.99-2.22 mg/L). According to Ghazzal et al. (2020), the concentration



of Cd ranged from 0.20–0.24, reflecting the greatest as compared to the observed value.

The contamination factor of Cd in the present study was found in the range of 0.0091 to 0.0271, which aligns with Khan et al. (2021), which reported the contamination factor values ranged from 2.56-3.0. These values exceed the contamination factor range observed in the present findings. The plant's ability to absorb metal is directly influenced by how metals interact with the soil and plant roots. The transfer factor of Cd in the present study was found in the range of 0.27 to 0.8. Ebong et al. (2023) and Yang et al. (2018) recorded a transfer factor of 0.06-0.139, notably lower than the observed range of the current study. Alaboudi et al. (2018) investigated a transfer factor of Cd, which was in the range of 1.2 mg/kg. It has been established that the effluents discharged from industrial sectors and urban areas are the major contributors to TM pollution in the enrichment factor, increasing distance from the sources of pollution. The estimated daily intake range of the specific substance in the present study was 0.00038-0.0025. Njoga et al. (2021) reported EDI values of 0.009, representing the average intake. These values are notably higher than the EDI range observed in the study. Ainerua et al. (2020) noted similar EDI values of 0.001. The values are aligned with the lower end of the observed EDI range of the current study.

The present study's hazard quotient range was between 0.3863–2.5557. Muhib et al. (2016) and Tschinkel et al. (2020) recorded lower HQ values of 0.030–0.05714, respectively. These values are notably lower than the HQ range observed in the study, suggesting that the hazard potential of the studied is relatively lower than those reported in these studies. Orellana Mendoza et al. (2021) and Wang et al. (2015) investigated higher HQ values of 3.97–2.86, respectively. The animal that absorbs these chemicals daily by free grazing would be in danger if these chemicals were present in the pasture tissue by Kabata-Pendias and Szteke 2015.

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