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Cadmium (Cd) and Copper (Cu) Exposure and Bioaccumulation Arrays in Farm Ruminants: Impact of Forage Ecotypes, Ecological Sites and Body Organs

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Abstract: Copper (Cu) and cadmium (Cd) metal distribution in soil–plant ecosystems and their public health risk impact on ruminants (cows, buffalo, and sheep) are explored in the present investigation. Five different forage crops were selected, and the foraging responses of three types of ruminants (cows, buffalo, and sheep) at three ecological sites were evaluated. The soil of these three ecological sites was metal polluted (due to wastewater irrigation) and was studied to evaluate the metal contamination and pollution load index. For the assessment of Cd and Cu, soil, vegetation, blood, hair, and feces samples were collected and analysed using an atomic absorption spectrophotometer. High consumption of fodder crops (*Sorghum bicolor* Kuntze, *Sesbania bispinosa* (Jacq.) W. Wight, *Cynodon dactylon* (L.) Pers., *Suaeda fruticosa* (L.) Forssk., and *Tribulus terrestris* L.) by cows and buffalo at site-III resulted in an increase in daily Cu and Cd intake. The pollution load index was higher at site-II and site-III, indicating a severe health risk scenario for local inhabitants. Cd and Cu were at their maximum levels in fodder crops. A significant increase in the concentrations of Cd and Cu was found in the blood, hair, and feces of cows and buffalo at site-III. Ecological indicators such as the bioaccumulation factor, the pollution load index, and the enrichment factor were found to be higher in buffalo than cows. The Cd level in forages was highest at the site-III Cd level and in the order of *S. bispinosa* > *S. fruticosa* > *T. terresteris* > *C. dactylon* > *S. bicolor*. Although these levels were lower than the permissible maximum limit, they were generally higher in the forage crops. Exposure of local inhabitants to the consumption of milk and meat from these cattle showed the serious health risks consequences. This situation can be properly managed by general monitoring of soil and vegetation pollution, avoiding metal contamination in the soil and food chain components, and using treated waste water and other alternate water sources for forage irrigation.

Keywords: heavy metals; ecological toxicity; pollution; human health risk; effluents; livestock

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1. Introduction

Unchecked landfilling, industrial effluent disposal, and wastewater used for irrigating crops can lead to the bioaccumulation of heavy metals (HMs); the loss of biodiversity; the deterioration of , water, soil, and atmosphere quality; and pose risks to animal and human health [1,2]. Heavy metal pollution and toxicity severely inhibit the growth, yield, and quality of cereals, forages, and fruit crops via interfering in their physiological,

cellular, and biochemical attributes, such as inhibition of photosynthesis, cell division, energy production, and protein synthesis. These pollutants are serious risks to the ecosystem and to human health [3–5].

Cadmium is a toxic metal that enters the soil–plant system via low-quality urban wastewater irrigation that is most often polluted with industrial discharges with a heavy load of toxic metals [6]. Cd is highly toxic at higher concentrations to plants, animals, and human health and is a major source of carcinogenic disorders [7]. Cadmium is readily soluble in water, easily absorbed by plants, and is a serious environmental pollutant in agroecosystems [8]. In urban or industrial wastewaters, heavy metals gather in sewage sludge and are the biggest source of contamination for the soil rhizosphere. Water bodies receive heavy metals from agricultural drainage because of the constant use of fertilizers that exhibit heavy metals. Heavy metals are considered the most dangerous among numerous soil pollutants due to their persistency and toxicity in the water–soil–plant environment [8–10].

Even though Cu is necessary for agriculture crops, higher amounts pose serious risks to the terrestrial ecosystem as well as to the health of both animals and people. Bioaccumulation of heavy metals might increase the pollution load in the soil rhizosphere, compromise the human and ruminant immune system, and result in some neurological problems, kidney failure, digestive system and heart disease [11]. In the past, several studies have been conducted to quantify the health risks associated with the ingestion of heavy metals, including Cu and Cd, by consuming contaminated food crops [12,13]. For plants, many heavy metals are crucial microelements and are subsequently involved in a varied assortment of enzymatic redox responses. Root nodulation was inhibited, and the quantity of useful nodules significantly decreased [14]. Some of these nutrient elements have a defensive role contrary to the poisonous effects of Cd stress [15]. Heavy metals are non-biodegradable and may be taken up by plants, including agricultural crops. For this reason, for the safety of the environment, communications detailing metal–plant contamination is also significant. Recently, a number of studies have drawn attention to heavy metal accretion in plants [16,17].

Water scarcity is very common in several countries, including Pakistan. Therefore, alternative sources of water, such as treated wastewater, rainwater, and water from retaining ponds constructed in areas at risk of water scarcity, should be used for field crop irrigation [2]. The consumption of crops cultivated on soil that received wastewater during the crop growth cycle might be loaded with large amounts of trace metals, which will ultimately pose a large risk to the public and to ruminants [18]. Previously, some authors documented heavy metal contamination in different fodder crops, translocated to feeding cattle's blood and milk and their possible entry into the food chain [12,15,19,20]. These studies neglected HM entry into the food chain and instead concentrated on the presence of metals in the plant–soil relationship. The present study, for the first time, examines the bioavailability of cadmium (Cd) and copper (Cu) in the four most cultivated pasture crops (*Sorghum bicolor* Kuntze, *Sesbania bispinosa* (Jacq.) W. Wight, *Cynodon dactylon* (L.) Pers., *Suaeda fruticosa* (L.) Forssk., and *Tribulus terrestris* L.) in Punjab and their bioaccumulation in soil, translocation, and pollution load into these pasture crops and subsequent health risks to grazing ruminants (sheep, cows, and buffalo). Different ecological sites were chosen to evaluate the biomonitoring of selected pastures to study environmental hazards and assessment of the potential risks of heavy metal pollution to human health.

2. Materials and Methods

2.1. Experimental Area, Season Expedition and Sample Collections

The present study was conducted in Punjab province (District Toba Tek Singh), Pakistan. Toba-Tek-Singh geographically (Supplementary Figure S1) situated at 30°33′ to 31°2′ N and 72°08′ to 72°48′ E. Dry climate extends from April to October, while May, June, and July are the most sizzling months. The mid-year season temperature is in the range of 42 °C and 29 °C, while cooler months include December, January, and February. Annual precipitation is mostly around 158 mm. The soil of Toba-Tek-Singh is mostly alluvial plain.

2.2. Soil and Forages Sample Collection

The study sites were selected at random and 1 km away from roads. The soil samples were collected (3 replicates) at a soil depth of 0–20 cm [21]. Soil and plant samples were taken and sealed in labeled paper bags. Three different ecological sites were selected to collect the soil samples, and the samples were named soil from the *S. bicolor* plot, soil from the S. *bispinosa* plot, soil from the *C. dactylon* plot, soil from the *P. fruticosa* plot, and soil from the *T. terresteris* plot. The forage chosen was jawar, normally referred to as "*Sorghum bicolor*", Jantar, referred to as *Sesbania bispinosa,* common grass frequently known as *Cynadaon dactylon*, *Psuedo fruticosa,* usually called wild plant, and *Tribulus tristeris*, frequently known as bhakra. Forage and soil samples were dried in an oven at 72 °C for 48 h to achieve a steady dry weight. The same forage plots were used as grazing units for selected animals. The oven-dried soil samples were subjected to digestion and analysis.

2.3. Animal Maintenance and Sample Collection

After securing the necessary ethical approvals, adult livestock (sheeps, cows, and buffalo) were used for the assessment of Cd and Cu bioassimilation. The weight of the buffalo, cows, and sheep ranged from 250–420 kg, 300–350 kg, and 40–60 kg, respectively. Healthy, disease free, and adult animals were used for the feeding trial. Blood, hair, and feces samples were taken from the farm ruminants (sheep, buffalo, and cows). For the purpose of gathering animal samples, a total of 10 animals (from each animal type) were chosen (at each site). The healthy animals were selected for blood plasma sampling and to determine the heavy metals (Cd and Cu). A specimen of blood (15 mL) was collected from the jugular vein of the selected animals with the help of heparin needles to avoid blood coagulation [12]. Plasma was centrifuged at 2500 rpm for 2 min to make a plasma parcel. The serum was taken to the laboratory in a chilled box and maintained frozen at −20 °C before investigation [21]. The animal's body was used to gather hair samples. Hair samples were cleansed of all externally deposited pollutants by first being rinsed in acetone and then in distilled water [3,14,20]. We used the method described by Ghazzal et al. [22] to collect feces samples from each type of animal. First, for digesting purposes, all samples were air-dried, kept in an oven to five days, and then stored in plastic bags with labels.

2.4. Soil Sample Digestion

Soil samples were dried in an oven for 72 h and ground to fine powder. The powder soil sample (2 g) was added to concentrated H_2SO_4 (20 mL) in a flask for digestion. The procedure was repeated twice with the end product a colorless solution. Following the addition of distilled water to achieve the final volume of 60 mL, the samples were stored in glass tubes for further analysis [12].

2.5. Digestion of Fodder Crops Samples

Forage samples (*Sorghum bicolor* Kuntze, *Sesbania bispinosa* (Jacq.) W. Wight, *Cynodon dactylon* (L.) Pers., *Suaeda fruticosa* (L.) Forssk., and *Tribulus terrestris* L.) grown on field plots irrigated with wastewater were harvested. These samples were dried in an oven for 72 h to remove moisture and ground using a blender. A 1 g feed sample was decomposed in H_2SO_4 and H_2O_2 (4:2) at 250 °C for 3–4 h until the solution became colorless and thick white smoke rose in the flask. The mixture was incubated, washed with distilled water, passed through filter paper, and diluted to 50 mL [12].

2.6. Digestion of a Blood Sample

The blood that was anticoagulant was centrifuged. Then, 2 mL of blood plasma and 2 mL of H_2SO_4 were mixed, and a combination of sample tests was left overnight for digestion. All the natural tissues were broken down, and a solution of sample was digested by warming at 120 °C. The treated samples were cooled, and the processed digested samples were diluted up to 50 mL using distilled water and set in glass tubes for assessment.

2.7. Atomic Absorption Spectrum

Metal concentrations were determined utilizing the Atomic Absorption Spectrophotometer (AAS) (Shimadzu double beam AA-6300 and Perkin Elmer Analyst 400) [12]. Quality assurance was achieved by measuring natural matrix certified reference material (CRM-1570) and measuring duplicates for each batch of samples to ensure the stability of the outcomes. The samples were handled cautiously to inhibit pollution. Double distilled H_2O was used during the testing, and glassware was methodically washed. An analysis was carried out to validate the investigative steps by homogenizing the examined samples with a varying quantity of standard solutions. The environmental pollution was determined by different pollution indices as reported previously [12,15,16].

2.8. Statistical Analysis

The results were statistically analyzed using ANOVA, and means were the average of the three replicates ($n = 3$). The significant variation was determined among the different treatments using Tukey's HSD test as post hoc at $p < 0.05$. All statistical analyses were performed by the SPSS statistical package (SPSS 17.0, Chicago, IL, USA).

2.9. Pollution Load Index (PLI)

PLI was estimated by the equation given by Liu et al. [13]:

PLI = 'metal content in examined soil sample/metal concentration in reference soil'.

The reference value of Cd and Cu in soil was 1.49 and 8.39 mg/kg [23,24].

2.10. Bioconcentration Factor (BCF)

It was estimated by following the formula as reported by Chen et al. [14]:

BCF = [M] Fodder samples/[M] Soil sample,

2.11. Daily Intake of Metal (DIM)

Metals enter the body of organisms through diverse pathways, through skin contact, during breathing, or by consuming contaminated fodder [15].

The formula was that reported by Bonnet et al. [16]: DIM = C metal × F conversion factor × D food intake/B average weight of sheep. DIM by ingesting of forages was 1.51 (kg per sheep)m and the normal weight of sheep was 45 kg

A conversion factor of 0.085 was applied to change green plant mass to dry weight [24]. A tolerable daily intake limit of Cd and Cu is 0.21 and 3.01 (mg $kg^{-1}day^{-1}$).

2.12. Health Risk Index (HRI)

The oral reference dose value for Cd and Cu is 0.001 and 0.04 (mg $kg^{-1}day^{-1}$) reported by the World Health Organization [25].

Health risk index = Daily intake of Metal/RfD [13].

2.13. Enrichment Factor (EF)

It was determined by the formula of Buat-Menard and Chesselet [17].

EF = [M] Fodder E/[M] Soil E/[M] Fodder S/[M] Soil S. Average absorption of Cd and Cu in forages is 0.2 and 73.3 mg/kg, and in soil it is 2.8 and 8.39 mg/kg, respectively [18].

3. Results

3.1. Bioaccumulation of Cd in Soil and Forage Crops: Ecological Risk Assessment

The soil of *T. terrestris* at site-III contained the highest concentration of Cd, which was followed by the soil of *P. fruticosa* at site-III (Figure 1A). In the S. bicolor soil at site-I, the lowest Cd level was found. All forages had soil with a Cd content ranging from 2.09 mg/kg to 4.23 mg/kg. The levels of Cd in the soil at Site I were in the following order: *C. dactylon* > *T. terresteris* > *S. bicolor* > *S. bispinosa* > *S. fruticosa*. The soil at site-II had the following Cd levels: *C. dactylon* > *T. terresteris* > *S. bispinosa* > *S. fruticosa* > *S. bicolor*. *T. terresteris* > *S. fruticosa* was the order of the soil's Cd levels at site-III.

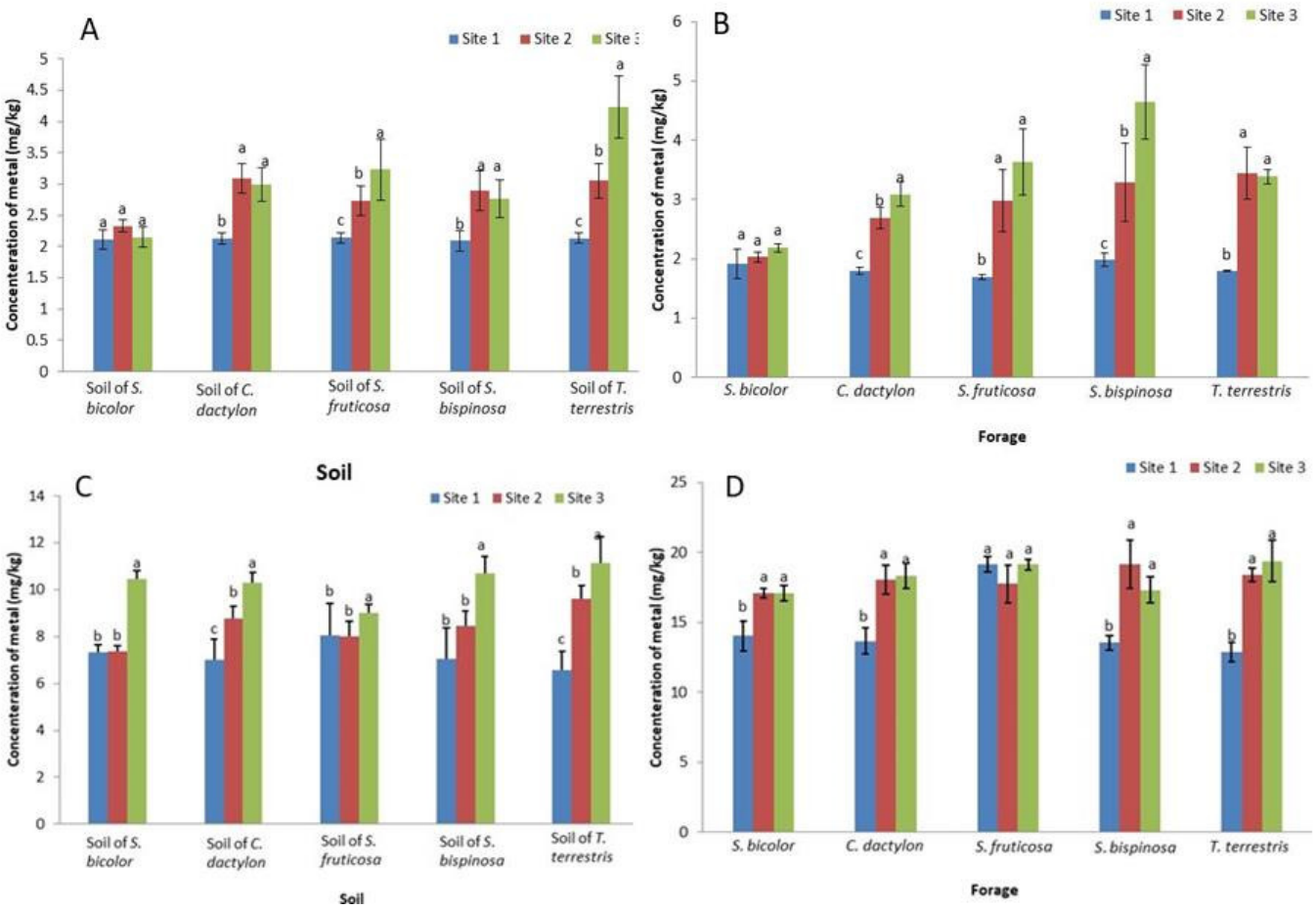

Figure 1. (**A**) The concentration of heavy metal Cd in soil; (**B**) the concentration of heavy metal Cd in the forages; (**C**) the concentration of heavy metal Cu in soil; (**D**) the concentration of heavy metal Cu in the forages. Every bar represents the mean (±S.E.) of three replicates. Means followed by different letters are significantly different ($p < 0.05$) according to Tukey's HSD test.

The total Cd level varied from 1.69 to 4.65 mg/kg depending on the type of feed. *S. bispinosa* at site-III had the highest Cd level in forages (4.65 mg/kg), followed by *S. bispinosa* (3.64 mg/kg) at site-III, and *S. fruticosa* (3.45 mg/kg) at site-II. *S. fruticosa* at site-I had the lowest level (1.69 mg/kg) of the substance (Figure 1B). According to an analysis of variance, Cd had a considerable impact on site and animal but had non-significant impact on site x animal. Cd levels in forage at site-I were found in the following order: *S. bispinosa* > *S. bicolor* > *C. dactylon* > *T. terresteris* > and *S. fruticosa*. The Cd levels in the forage were *T. terresteris* > *S. bispinosa* > *S. fruticosa* > *C. dactylon* > *S. bicolor* at site-II. The order of the forage's Cd levels at site-III was S. bispinosa > *S. fruticosa* > *T. terresteris* > *C. dactylon* > *S. bicolor* (Figure 1B).

The soil of *S. bispinosa* at site-I had a larger bioconcentration factor (BCF) than that of *P. fruticosa*, which had a lower BCF. The BCF was between 0.789 to 1.685 mg/kg (Table 1). *T. terrestris* at site-III had a higher pollution load index, but *S. bispinosa* at site-I had a lower one. The range for the pollutant load index was 0.746 mg/kg to 1.511 mg/kg (Table 1). *S. bispinosa* at site-III had a greater enrichment factor, while *P. fruticosa* at site-I had a lower one. The range of the enrichment factor was 5.883 mg/kg to 12.552 mg/kg (Table 1).

Table 1. Bioconcentration factor (BCF), pollution load index (PLI), and enrichment factor (EF) of Cd metal in soil.

	Site 1	**Site 2**	**Site 3**
BCF			
Soil of *S. bicolor*	0.909	0.871	1.019
Soil of *C. dactylon*	0.845	0.871	1.033
Soil of *S. fruticosa*	0.789	1.092	1.127
Soil of *S. bispinosa*	0.947	1.138	1.685
Soil of *T. terrestris*	0.840	1.131	0.801
PLI			
S. bicolor	0.754	0.832	0.768
C. dactylon	0.761	1.104	1.068
S. fruticosa	0.764	0.975	1.154
S. bispinosa	0.746	1.032	0.98
T. terrestris	0.76	1.089	1.511
EF			
S. bicolor	6.779	6.491	7.589
C. dactylon	6.296	6.486	7.699
S. fruticosa	5.883	8.132	8.396
S. bispinosa	7.058	8.481	12.552
T. terrestris	6.261	8.427	5.971

3.2. Metal Level of Cd in Animal Blood, Hair, and Feces

At site-III, buffalo blood had the highest concentration of Cd, whereas at site-I, sheep blood had the lowest concentration. The range of the Cd concentration in animal blood was 0.99 mg/L to 2.22 mg/L (Table 2). At site-III, sheep hair had a higher concentration of Cd, whereas site-I's buffalo had the lowest concentration. The range of Cd concentrations in animal hair was 1.01 mg/kg to 2.34 mg/kg (Table 2). At site-III and site-I, respectively, cow feces had the highest and lowest concentrations of Cd. Animal waste contained Cd levels ranging from 0.66 mg/kg to 2.19 mg/kg (Table 2).

Table 2. Concentrations of the heavy metal Cd in animal blood, hair, and feces. Each value represents the mean (±S.E.) of three replicates.

Source	Animal	Site 1	Site 2	Site 3
Blood	Cow	1.19 ± 0.27	1.78 ± 0.39	2.13 ± 0.34
	Buffalo	1.29 ± 0.28	1.76 ± 0.29	2.22 ± 0.41
	Sheep	0.99 ± 0.25	1.96 ± 0.35	1.61 ± 0.31
Hair	Cow	1.06 ± 0.19	2.02 ± 0.32	2.25 ± 0.41
	Buffalo	1.01 ± 0.28	1.83 ± 0.31	2.23 ± 0.54
	Sheep	1.14 ± 0.05	1.79 ± 0.35	2.34 ± 0.37
Feces	Cow	0.66 ± 0.14	1.88 ± 0.32	2.19 ± 0.31
	Buffalo	2.03 ± 0.21	1.98 ± 0.35	2.16 ± 0.29
	Sheep	1.55 ± 0.35	2.12 ± 0.33	1.81 ± 0.31

3.3. Human Health Risk Assessment from Dietary Intake

Daily amounts of metal measured for Cd in *S. bispinosa* were higher at site-III in buffalo samples of *T. terrestris* and lower at site-I in sheep samples. Metal consumption per day ranged from 0.0026 mg/kg to 0.0089 mg/kg (Table 3). *S. bispinosa* at site-II in buffalo had a higher health risk index value for Cd, whereas *P. fruticosa* at site-I in sheep had a lower value. The range of the health risk index factor was between 2.4899 and 8.9829 mg/kg (Table 3).

Table 3. Daily intake of metal (DIM) and health risk index (HRI) for Cd.

DIM (Cd)	Cow			Buffalo			Sheep		
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
S. bicolor	0.0032	0.0035	0.0037	0.0037	0.0039	0.0042	0.0028	0.0029	0.0032
C. dactylon	0.0031	0.0046	0.0052	0.0034	0.0052	0.0059	0.0027	0.0039	0.0045
S. fruticosa	0.0029	0.0051	0.0062	0.0032	0.0058	0.0070	0.0025	0.0044	0.0054
S. bispinosa	0.0034	0.0056	0.0079	0.0038	0.0063	0.0089	0.0029	0.0044	0.0069
T. terrestris	0.0030	0.0059	0.0058	0.0035	0.0067	0.0065	0.0026	0.0051	0.0049
HRI (Cd)									
S. bicolor	3.264	3.451	3.723	3.7091	3.9216	4.2307	2.8288	2.9909	3.2266
C. dactylon	3.06	4.573	5.253	3.4773	5.1966	5.9693	2.652	3.9633	4.5526
S. fruticosa	2.875	5.066	6.188	3.2647	5.7568	7.0318	2.4899	4.3905	5.3629
S. bispinosa	3.366	5.593	7.905	3.825	6.3557	8.9829	2.9172	4.8473	6.851
T. terrestris	3.543	5.85	5.763	3.4579	6.6648	6.5489	2.6373	5.083	4.9946

3.4. Bioaccumulation of Copper (Cu) in Soil and Forage Crops: Ecological Risk Assessment

Cu had a significant impact on the site but not on the soil and their interaction (site x soil) according to an analysis of variance (ANOVA). The soil of *T. terresteris* at site-III had the highest concentration of Cu, whereas soil from site-I had the lowest concentration (Figure 1 C). All forages had soil with a range of 6.56 mg/kg to 11.12 mg/kg Cu content. Cu had a non-significant effect on forages but a significant effect on site and site x forage according to an analysis of variance (data not shown). The forage *S. bispinosa* at site-II had the highest concentration of Cu, whereas the forage *T. terrestris* at site-I had the lowest concentration. Cu content in all forages was 12.92 mg/kg to 19.16 mg/kg (Figure. 1D).

The BCF for Cu was found to be higher at site-I for *S. fruticosa* and lower at site- III for *S. bispinosa*. Between 1.620 mg/kg and 2 mg/kg, the BCF factor was present (Table 4). At sites I and III, it was discovered that *T. terrestris* had pollution load indices in Cu that were greater and lower, respectively. The range for the pollutant load index was 0.782 mg/kg to 1.325 mg/kg (Table 4). *P. fruticosa* at site-I had a greater enrichment factor than *S. bispinosa* at site-III. From 0.185 mg/kg to 0.271 mg/kg was the enrichment factor (Table 4).

Table 4. Bioconcentration factor (BCF) and pollution load index (PLI) enrichment factor (EF) of Cu metal in the soil.

	Site 1	**Site 2**	**Site 3**
BCF			
Soil of *S. bicolor*	1.917	2.322	1.636
Soil of *C. dactylon*	1.956	2.059	1.786
Soil of *S. fruticosa*	2.371	2.213	2.118
Soil of *S. bispinosa*	1.929	2.265	1.620
Soil of *T. terrestris*	1.969	1.917	1.745
PLI			
S. bicolor	0.872	0.877	1.247
C. dactylon	0.833	1.04	1.225
S. fruticosa	0.962	0.956	1.076
S. bispinosa	0.838	1.008	1.274
T. terrestris	0.782	1.144	1.325
EF			
S. bicolor	0.219	0.266	0.187
C. dactylon	0.224	0.236	0.204
S. fruticosa	0.271	0.253	0.242
S. bispinosa	0.221	0.259	0.185
T. terrestris	0.225	0.219	0.199

3.5. Cu Metal Animal Blood, Hair, and Feces

Cu had a substantial impact on site and source, but not on animal, animal × site, site × source, or site × animal × source according to an analysis of variance (Tables S1 and S2). At site-III, cow blood had the highest concentration of copper, whereas at site-I, buffalo blood had the lowest concentration. Animal blood contained 2.07 mg/L to 3.86 mg/L of copper (Table 5). Cows in site-II had the highest concentration of copper, while buffalo at site-I had the lowest concentration. Animal hair had 2.05 mg/kg to 3.80 mg/kg of copper. At site-III, cow feces had the highest concentration of Cu, whereas buffalo feces had the lowest concentration. Cu concentrations in animal waste varied from 2.17 mg/kg to 3.97 mg/kg (Table 5).

Table 5. Concentrations of the heavy metal Cu in animal blood, hair, and feces. Each value represents the mean (±S.E.) of three replicates.

Source	**Animal**	**Site 1**	**Site 2**	**Site 3**
Blood	Cow	3.15 ± 0.58	3.45 ± 0.24	3.86 ± 0.34
	Buffalo	2.07 ± 0.28	2.64 ± 0.20	2.88 ± 0.30
	Sheep	2.39 ± 0.29	3.23 ± 0.33	3.13 ± 0.29
Hair	Cow	2.76 ± 0.19	3.80 ± 0.31	3.67 ± 0.23
	Buffalo	2.05 ± 0.23	2.79 ± 0.36	3.27 ± 0.33
	Sheep	2.09 ± 0.21	2.90 ± 0.26	3.04 ± 0.35
Feces	Cow	2.29 ± 0.17	3.52 ± 0.29	3.97 ± 0.30
	Buffalo	2.17 ± 0.19	2.88 ± 0.32	3.28 ± 0.35
	Sheep	2.25 ± 0.20	3.15 ± 0.32	2.89 ± 0.33

3.6. Human Health Risk Assessment (Cu)

Daily metal intakes for Cu *T. terrestris* were higher and lower in buffalo samples at site-III and lower in sheep samples at site-I, respectively. The daily metal intake was between 0.0190 mg/kg to 0.0375 mg/kg (Table 6). *S. bispinosa* at site-II in buffalo had a higher health risk index value for Cu, whereas *T. terrestris* at site-I in sheep had a lower value. From 0.4759 mg/kg to 0.9253 mg/kg, the health risk index factor was measured (Table 6).

Table 6. Daily intake of metal (DIM) and health risk index (HRI) for Cu.

DIM (Cu)	Cow			Buffalo			Sheep		
	Site 1	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**	**Site 1**	**Site 2**	**Site 3**
S. bicolor	0.0238	0.0290	0.0291	0.0271	0.0330	0.0331	0.0207	0.0252	0.0252
C. dactylon	0.0232	0.0307	0.0312	0.0264	0.0349	0.0355	0.0201	0.0266	0.0271
S. fruticosa	0.0325	0.0302	0.0325	0.0369	0.0342	0.0369	0.0282	0.0262	0.0282
S. bispinosa	0.0231	0.0326	0.0294	0.0262	0.0370	0.0335	0.0199	0.028	0.0255
T. terrestris	0.0219	0.0313	0.0329	0.0249	0.0355	0.0375	0.0190	0.0271	0.0286
HRI (Cu)									
S. bicolor	0.5963	0.7262	0.7272	0.6776	0.8252	0.8263	0.5168	0.6294	0.6302
C. dactylon	0.5809	0.7667	0.7803	0.6602	0.8713	0.8867	0.5035	0.6645	0.6763
S. fruticosa	0.813	0.7544	0.813	0.9239	0.8572	0.9239	0.7046	0.6538	0.7046
S. bispinosa	0.5763	0.8143	0.7361	0.6549	0.9253	0.8365	0.4995	0.7057	0.6379
T. terrestris	0.5491	0.782	0.8245	0.6239	0.8886	0.9369	0.4759	0.6777	0.7146

4. Discussion

Cd is a harmful metal and is toxic to people and other living organisms. It exhibits biological activity in both terrestrial and aquatic environments [22]. Numerous anthropogenic releases of toxic heavy metals into environments are indeed massive and pervasive and cause cadmium release into terrestrial ecosystems. Due to its high mobility in contaminated soils, the deposition of Cd in plants in Cd-polluted soil causes major issues for livestock and public health [3,4,12]. Cd toxicity damages the human body's various organs, but it concentrates primarily in the kidneys and leads to major problems such as kidney stones, pulmonary emphysema, and damage to the renal tubules [23].

Our results are in agreement with the Cd level detected by Khan et al. [5], who reported that among the top toxins, cadmium ranks seventh. The levels of cadmium in unpolluted soils were typically lower than 0.5 mg kg^{-1} but can extend up to 3.0 mg kg^{-1} depending on the soil-parent material. Cd can be easily absorbed by plants growing in Cd-accompanied soils, and its accumulation may cause numerous physiological, biochemical, and organizational disorders. Cd adversely affects seed germination, stand establishment, plant growth, nutrient uptake and assimilation, enzymatic activity, ultrastructural and oxidative damage, alterations in antioxidant defense systems and stress proteins, carbon metabolism, and yield reduction [24,25].

Feeding of farm ruminants (sheep, cows and buffalo) on the contaminated pasture crops (*Sorghum bicolor* Kuntze, *Sesbania bispinosa* (Jacq.) W. Wight, *Cynodon dactylon* (L.) Pers., *Suaeda fruticosa* (L.) Forssk., and *Tribulus terrestris* L.) caused a significant increase in Cd and Cu concentration in feces, hair, and blood plasma of sheep, cows, and buffalo. The Cd content was minimal in sheep blood and maximum in buffalo blood (Table 1). The maximum concentration of Cd was detected in sheep hair and the lowest in buffalo hair (Table 1). The highest and lowest levels of Cd were found in cow feces at site-III and site-I, respectively. According to reports from Spain [26] and Nigeria [27], the Cd level in blood was higher during different sampling campaigns. The higher level of Cd in blood may be associated with the consumption of pastures grown on contaminated soils with Cd. The heavy metal content in hair can be a good indicator of the accumulation of heavy metals in the animal's body. A study by Rashed and Soltan [28] with Fe, Mn, Pb, and Cd in wool

from goats, sheep, and camels showed an association between the metal content in hair and Fe and Mn in wool. The hair of cows in the exposed area showed higher Cd and Pb values than in the untreated area, and the Cd level correlated with the Cd level in blood [29].

The pollution of soils caused by heavy metals has been increased due to excavating, melting, industrial pesticides, discharges of trace metals, metalloids, and industrial effluents [30]. The metals that are present in wastewater are less soluble in water. They tend to gather in soils and then accumulate in plants and cause severe ecological risks [31]. In this context, the transfer of heavy metals from soil to plants is one of the main pathways for human contact through the food chain. In urban or periurban areas, unprocessed wastewater is commonly used for agriculture [32]. The Cd take-up, toxicity, and detoxifying components in the water–soil–plant system have been completely examined previously by several authors [32–34]. From plants and animals feeding on contaminated forages, Cd can easily enter into the food chain. Meanwhile, Cd contamination influences the various organs of the human body, including kidneys, and causes serious harm, including pneumonic emphysema, renal cylindrical harm, and kidney stones [23]. Cadmium in minerals replaces calcium (Ca), due to having indistinguishable charge and comparable ions and substance conduct [34]. In this way, it can undoubtedly move to the human body and be stored in different organs at higher concentrations. Cadmium toxicity seriously harms the liver and bones and can significantly decrease Ca uptake in the body [23,34].

Ahmad et al. [35], reported that Cu showed elevated quantities of 2.79–4.13 in contaminated soil irrigated with wastewater. Environmental factors such as low soil pH, including excessive pesticide or insecticide use, can increase the accumulation of copper in the soil. The results showed that the Cu content in the forage samples varied with the season and site. The Cu content in the different forage crops ranged from 12.92–19.16 mg/kg, with the Cu content lower in *T. terrestris* and higher in *S. bispinosa*. In addition, the Cu content in the forage crops was above the permissible limit value for Cu in animal feed [36] from India. However, the Cu content in the forage crops was higher than that of Khan et al. [12]. A significant difference ($p < 0.05$) in the plant Cu level was observed between the plant tissues, such as the roots (26–53 mg kg^{-1}), leaves (23–28 mg kg^{-1}), and stems (14–21 mg kg^{-1}), of *Boehmeria nivea* L. [37], while the bioaccumulation and translocation factors were less than one. The Cu levels in the blood ranged from 2.07–3.86 mg/L (Table 5).

In Rasheed et al. [38], the Cu level was significantly higher in the soil samples (3.54 to 4.08 mg/kg). Meanwhile, a higher concentration of Cu was observed in cow blood plasma. In another study, higher levels of different heavy metals were observed in milk and cheese samples from contaminated areas [39]. It was found that the Cu concentration in cheese samples on the roadside was significantly higher than in uncontaminated cheese samples from the green zone. The higher Cu content is due to the use of copper-contaminated pasture forage, water, and animal feed on contaminated soils. Asthma and heart problems are a common cause of consumption of food commodities by Cu [40]. The proven Cu content was well above the recommended values and can therefore have a negative effect on living organisms. The cow blood serum from 30 different farms in Poland showed significantly higher concentrations of Cu [41]. The concentration of toxic metals was higher in calves from contaminated areas of northern Spain [42].

Low soil and water pH levels have been linked to macro mineral deficiencies and micro mineral abundance in buffalo pastures [43,44]. Toxic metals can build up in food and in grazing buffalo. However, research on this aspect is limited, especially in flooded meadows. An increase in the pH was observed by several workers following bioaccumulation of Cu, Pb, Zn, and Cd [45]. Human activity near sewage treatment plants and household waste can contribute to environmental pollution and terrestrial system toxicity. Earlier studies in flooded grasslands showed that the dietary levels of Cu, Fe, and Mn exceeded the upper limits for grazing ruminants [46], which could be related to the infectivity of various plants. Anthropogenic activities have caused cadmium to be present in soil,

water, and air. The acceptable limit for Cd in cattle diets is 0.05 mg/kg. Cattle with high levels of Cd experience poisoning, appetite loss, and reduced growth [46].

Use of industrial, municipal, and sewage wastewater for irrigation of fields crops is a common practice. The freshwater supplies are in short supply and wastewater is discharged into the drainage canals which reach the fields located near where vegetables and fodder crops are grown [12,31,34]. Lack of freshwater resources leads farmers to use wastewater for irrigation: wastewater that might contain many important nutrients.

Through the consumption of contaminated forage crops, heavy metals were dissolved in the sheep, cow, and buffalo stomachs, taken into the blood, and integrated into the body's circulatory system. The blood circulatory system is a very sensitive system because the blood not only reflects how these metals are transported to different organs, but also serves as a crucial target for their harmful effects when Cd and Cu are present [47–52]. In the current investigation, Cd and Cu were assimilated into the blood, hair, and feces of cows, buffalo, and sheep through their blood, and their quantities were much greater after consuming metal-contaminated forages. It has been found that consumption of contaminated forage crops has a significant effect on the bioassimilation of Cd and Cu in cows, buffalo, and sheep.

5. Conclusions

From the present study, we conclude that Cd and Cu bioaccumulation was below the permissible limits, but forage crops cultivated on metal-contaminated soil (irrigated with wastewater) showed higher Cd and Cu concentrations. The results showed that ruminant samples (blood, hair, and feces) also had higher Cd and Cu concentrations, which varied from one site to another. Cd and Cu entry via dietary intake was the major source of exposure to these environmental pollutants. Cd was highest in buffalo blood, while higher and lowest concentrations of Cd were found in cow feces. However, the bioconcentration factors for Cd and Cu demonstrate low toxicity in the soil–plant–livestock ecosystem.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su141912595/s1, Figure S1: Map of the study district (Toba Tek Singh), Punjab, Pakistan; Table S1: Analysis of variance for Cd metal in soil, in forages and in animal blood, hair and faces; Table S2: Analysis of Variance for Cu metal in soil, in forages, in Animal blood, Hair and Faces; Table S3: TREATMENT DETAILS.

Author Contributions: M.N. (Majida Naeem): data curation, formal analysis, investigation, writing—original draft; Z.I.K.: formal analysis, writing—original draft; M.I.H.**:** data curation, investigation, resources, formal analysis, investigation, writing—original draft, writing—review and editing; M.N. (Majida Naeem), Z.I.K., S.A., and M.N. (Muhammad Nadeem): supervision, writing—original draft, writing—review and editing; M.A.A.: formal analysis, writing—review and editing; O.V.: writing—original draft, writing—review and editing; K.A., H.-H.Y.: resources, supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from formers to conduct the study and to collect the samples. They were briefed about the research plan in detail.

Data Availability Statement: Data and material are available for research purposes and for reference.

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