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Copper Bioavailability and Accumulation Patterns in the Soil–Plant–Animal Pathway Under Semiarid Conditions

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ABSTRACT

Heavy metal poisoning poses a serious threat to ruminant health. This study aimed to determine Cu accumulation and its transfer in the soil–plant–animal food chain. A study was designed in which three sites irrigated with ground, canal, and sewage water (CW Site I, GW Site II, and SW Site III) in the district of Sargodha were selected. Six types of forages were chosen (*Zea mays*, *Pennisetum glaucum*, *Trifolium alexandrinum*, *Sorghum bicolor*, *Avena sativa*, and *Saccharum officinarum*). The Cu concentrations in these samples were determined by an atomic absorption spectrophotometer (AA-6300, Shimadzu, Japan). In soil, forage, and blood samples, Cu levels ranged from 10.036 to 14.93 mg kg^{-1}, 5.14 to 9.87 mg kg^{-1}, and 1.97 to 5.15 mg L^{-1}, respectively. The highest Cu level was observed at SW Site III, and the lowest was observed at CW Site I. The Cu values in the forage species samples were found to be lower than the permissible limit. On the other hand, a nonsignificant positive association was found between soil and forage at CW Site I, while a strong negative correlation was observed at SW Site III between forage and blood. The maximum levels of the transfer factor (0.8017) and enrichment factor (0.2300) for copper were observed upon wastewater irrigation (SW Site III). A higher value of EDI (0.0190) in *Z. mays* was observed at SW Site III. At all sites, the HQ values for the contaminant Cu were below 1, indicating a relatively low exposure risk. Frequent monitoring of heavy metals is necessary to prevent excessive build-up of copper.

1 | Introduction

Pakistan is an agrarian country where livestock production plays a vital role in meeting the protein requirements of the population and supporting the rural economy. Small ruminants and large livestock species, including buffaloes, are important sources of animal protein, such as meat and milk. However, livestock productivity can be affected by environmental stressors, including increasing temperatures and exposure to environmental contaminants [1]. In many developing regions, wastewater irrigation has become a common practice due to increasing water scarcity. Although wastewater can supply certain nutrients that enhance soil fertility, it may also contain various pollutants, including heavy metals, which can accumulate in agricultural soils and negatively affect crop productivity and environmental quality [2, 3]. These toxic metals can infiltrate soils and water bodies, where they may be absorbed by plants and subsequently enter the food chain through plant consumption by animals and humans [4].

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The permissible limits of copper (Cu) are 100 mg kg^{-1} for soil [5], 40 mg kg^{-1} dry matter for forage/animal feed [6], and 0.7–1.5 mg L^{-1} for ruminant blood [7], and these values were used as reference standards in the present study.

Forage crops play a critical role in livestock production because they serve as the primary feed source for grazing animals. To address issues related to livestock nutrition and environmental contamination, forage plants have attracted increasing attention due to the presence of physiologically active compounds that can influence animal nutrition while also providing potential therapeutic benefits for humans. These compounds are typically low-molecular-weight molecules present in small quantities in plants. Among forage crops, species belonging to the Fabaceae family are known to contain relatively high concentrations of specialized metabolites that contribute to their nutritional and functional value [8].

When livestock consume water and forage contaminated with pollutants, heavy metals can enter their bodies and accumulate in blood, tissues, and animal-derived products such as milk. This accumulation can lead to potential health risks for both animals and humans. Several studies have reported elevated concentrations of heavy metals in milk and other livestock products collected from animals raised in contaminated environments. For example, previous investigations revealed that many milk samples contained lead concentrations exceeding permissible limits, while increased levels of lead and cadmium were also detected in milk produced in contaminated regions [9]. Long-term exposure to heavy metals can weaken immune systems and may lead to neurological disorders, kidney damage, digestive complications, and cardiovascular diseases in both animals and humans [10].

Although numerous studies have reported heavy metal contamination in agricultural soils and crops, most investigations have focused primarily on metal concentrations in individual environmental compartments, such as soil or plant systems, without considering the complete transfer pathway within livestock production systems. Limited research has evaluated the bioavailability and trophic transfer of copper across the integrated soil–forage–animal continuum, particularly in semiarid regions of South Asia where livestock production depends heavily on locally grown forage. In these environments, wastewater irrigation and long-term agricultural inputs can significantly alter soil physicochemical properties and enhance the mobility, bioavailability, and uptake of trace metals by forage crops [11]. However, systematic investigations evaluating copper transfer across the entire soil–forage–animal pathway remain limited, particularly under different irrigation regimes such as canal water, groundwater, and wastewater. Understanding this transfer mechanism is essential for assessing ecological risks and potential impacts on livestock health and food safety in semiarid agricultural systems [12].

Copper (Cu) is an essential micronutrient required for normal plant growth and animal metabolism; however, excessive concentrations can become toxic and pose ecological and health risks. Elevated copper levels in soil may increase its accumulation in forage crops and subsequently in grazing animals, potentially affecting livestock health and food safety. Bioaccumulation of heavy metals can weaken immune systems in ruminants and humans and may also contribute to neurological disorders, kidney failure, digestive problems, and cardiovascular diseases [10, 13]. Forage plants are commonly fed to animals either as fresh pasture or preserved forms such as hay and silage, and therefore play an important role in the transfer of nutrients and contaminants through the food chain. Most fodder crops contain cellulose as their primary fiber source, which is a complex polysaccharide that serves as an important energy source for ruminant animals [14].

Therefore, the novelty of the present study lies in its integrated assessment of copper dynamics within the soil–forage–buffalo system under contrasting irrigation regimes in a semiarid environment. Unlike previous studies that have examined heavy metal contamination in isolated environmental compartments, this research evaluates copper transfer along the entire agricultural pathway and assesses associated ecological risks within livestock production systems. Based on this knowledge gap, it was hypothesized that wastewater irrigation significantly enhances copper accumulation in soil and forage crops, thereby increasing copper levels in buffalo blood and elevating ecological risk under semiarid environmental conditions.

Therefore, this study aimed to evaluate copper bioavailability, accumulation patterns, and potential health risks within the soil–plant–animal pathway under different irrigation regimes. Specifically, the objectives of the present study were to: (i) determine the concentration of copper (Cu) in soil, forage plants, and buffalo blood; (ii) assess copper levels in soils irrigated with different water sources across ecological sites; and (iii) evaluate ecological indices and potential food chain contamination associated with copper transfer in the soil–forage–animal system.

2 | Materials and Methods

2.1 | Location

The study was conducted in a semiarid region characterized by low annual rainfall and high evapotranspiration rates. In the northeastern Pakistani province of Punjab, Sargodha is an urban center and the administrative center of the district of the same name. Sargodha is located at a latitude of 32.08586 and a longitude of 72.67418. It is part of Asia and the northern hemisphere. Sargodha District adumbratively comprises 07 tehsils, namely, Sargodha. The most important airfield in the nation is located in the city, earning Sargodha the moniker “City of Eagles”. The Lower Jhelum Canal colony’s administrative center, Sargodha, was established in 1903, and it became an independent city in 1914.

2.2 | Water Quality

In Sargodha, underground water is the main source of water for drinking, which is extracted through wells and tube wells. However, due to various factors, the quality of groundwater in

TABLE 1 | Area of study sites for sample collection and list of forage crops.

Site	Location	Irrigation source
CW Site I (Location I)	78 NB	Ground Water
GW Site II (Location II)	90 NB	Canal Water
SW Site III (Location III)	45 NB(A)	Sewage Water
Local names	**Scientific names**	**Family**
Maize	*Zea mays*	Poaceae
Bajra (Pearl millet)	*Pennisetum glaucum*	Poaceae
Berseem	*T. alexandrnum*	Fabaceae
Jawar	*Sorghum bicolor*	Poaceae
Jodari	*Avena sativa*	Poaceae
Sugarcane	*Saccharum officinarum*	Poaceae

Sargodha has deteriorated over the years. One of the main factors contributing to poor water quality in Sargodha is the high level of salinity in the groundwater. The high amount of pollution is another element affecting Sargodha's inadequate water conditions. Uncontrolled waste from municipal and business disposal has caused heavy metal, bacterial, and other hazardous material pollution of waterways.

2.3 | Sample Collection

2.3.1 | Water Sampling

Water samples were systematically collected from three distinct types of sources: canal water, groundwater, and sewage water, with six samples obtained from each source at three different locations. For each collection, 5 mL of water was carefully transferred into prelabeled plastic bottles to ensure accurate identification of the source and sampling site. The samples were stored at room temperature until further laboratory analysis. This standardized sampling procedure ensured consistency, proper labeling, and preservation of the water samples for subsequent experimental evaluation.

2.3.2 | Soil and Forage Sampling

For soil and forage sampling, three irrigated soil sites were selected (Table 1). Soil samples were collected from a depth of 0–15 cm using a stainless-steel auger. Each site was sampled in triplicate to ensure statistical independence. Likewise, forage samples were randomly collected from the same plots used for soil sampling. Samples were washed, oven-dried, ground, and digested for Cu analysis.

Each soil sample weighed 1 kg, and it came from each location. The forage yielded an overall of 18 soil samples. Each sample, as well as its three copies, was properly gathered. The soil samples were initially air-dried before being baked in an oven at 70°C–75°C for 5 days to remove the moisture content. The operation of a crusher produced a fine powder. Each sample weighed 1 g and was placed in bags of polythene for testing.

2.3.3 | Ethical Approval

All the study protocols were approved by the Institutional Animal Ethics Committee, University of Sargodha (Approval no. 25-A18 IEC UOS). All the experiments performed complied with the rules of the National Research Council, and all methods were performed in accordance with relevant guidelines and regulations.

2.3.4 | Blood Sampling

Six blood samples from each site (canal water, groundwater, and sewage water) were collected, from which five buffalo categories were selected. Blood samples were collected from healthy buffalo categorized by age and physiological status. Approximately 5 mL of blood was drawn via jugular venipuncture using sterile syringes. Blood samples were transported in ice boxes and stored at 4°C before analysis.

Following collection, samples were centrifuged for two minutes at 2500 rpm to extract plasma. The sample was taken in 5 mL in labeled plastic bottles, and for further analysis purposes, the sample was saved at room temperature.

2.4 | Sample Digestion

2.4.1 | Apparatus and Chemicals

Beaker, flask, H_2O_2 50%, H_2SO_4 2 mL, tripods, stirrer, filter, measuring cylinder, analytical balance, plastic bottles, vinyl gloves, oven, paper, dropper, hotplate, refined water, measuring jars and storage jars, labeling tape.

2.4.2 | Water Sample Digestion

Using 3 mL of H_2SO_4 and 5 mL of H_2O_2, 5 mL of water was added to a glass container and processed for 30 min inside the chamber that was used. When the emissions stopped evaporating, the water-based samples were removed from the chamber. Until the disappearance of the solution color, 2 mL of H_2O_2 was added.

Fifty milliliters of water that was distilled twice were poured into the tank after cooling. The subsequent solution was stored in bottles made of polyethylene.

2.4.3 | Soil Sample Digestion

The organic matter was digested using the wet digestion technique. For 30 min, 1 g of soil in a glass container was processed in a digestion chamber using 4 mL of H_2SO_4 and 8 mL of H_2O_2. When the evaporation stopped, 2 mL of H_2O_2 was subsequently poured. The procedure was repeated until the sample disappeared all its color. The material that was digested was filtered using Whatman No. 42 filter paper. By adding doubly distilled water to the mixture and storing the sample in plastic bottles with tags, the final amount of the samples was increased to 50 mL.

2.4.4 | Forage and Blood Sample Digestion

Approximately 2 mL of serum from the blood and 1 g of forage sample were placed in two different flasks, and the samples were digested for 15–30 min with 2 mL of H_2SO_4 and 4 mL of H_2O_2. After the flask's drying halted, samples were withdrawn, and 2 mL of H_2O_2 was introduced once more to the mixture until the sample color vanished. By adding double-distilled water, filtering the sample via Whatman No. 42 paper filters, and then storing it in marked plastic containers, the sample's ultimate quantity was increased to 50 mL.

2.4.5 | Atomic Absorption Spectrophotometer Principle

Copper concentrations were determined using atomic absorption spectrophotometry (AAS). AAS is a method for determining how much of a specific metal element is present in a sample. The primary cause of this is that single emission and absorption lines from metals are sharp, distinct, and narrow. AAS is an analytical technique that relies on the radiation that is absorbed by atoms when a salt of a metal mixture is aspirated (drawn) over a blaze. Atomic absorption can be used for both quantitative and qualitative evaluation to determine the amount of an element.

To ensure the reliability and precision of the analytical results, quality assurance and quality control (QA/QC) procedures were strictly followed throughout the analysis. These procedures included the use of reagent blanks to detect any contamination during sample preparation, certified reference materials to verify the accuracy of the analytical method, and duplicate analyses to evaluate the precision and reproducibility of the measurements. In addition, instrument calibration was performed after every 10 samples to maintain analytical stability and accuracy. The recovery rate for copper ranged between 92% and 105%, indicating acceptable analytical performance and ensuring the reliability of the obtained results.

For risk assessment analysis, the background soil value of copper (12.9 mg kg^{-1}) was adopted from regional geochemical baseline data. Furthermore, the average body weight of buffalo was assumed to be 550 kg, based on regional livestock statistics, to facilitate accurate estimation of exposure and risk parameters.

3 | Indices to Evaluate Heavy Metal Contamination

3.1 | Contamination Factor

Based on the contaminant quantities in the sample's contents and their background concentration, the contamination factor (C_f) can be utilized to measure the pollution status of contaminants within the soil [15].

The C_f is calculated as follows:

$$C_f = \frac{C_m \text{ sample}}{C_m \text{ background}}$$

where C_m sample indicates the metal content in the soil, C_m background is the metal concentration from a natural source, and the background value of Cu = 12.9.

3.2 | Transfer Factor

The transfer factor (TF) indicates how metals infiltrate soils, including runoff from roads and industries from their substrate [16].

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

where C_{Plant} is the average metal content (fresh value) in the plant and C_{Soil} is average metal content in soil (dry weight).

3.3 | Enrichment Factor

To determine the quantity of contaminants collected in soil from agriculture and forage, a unit of measurement known as the soil enrichment factor (EF) has developed [17]

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

Standard concentration of metals in soil (3.5 mg kg^{-1}) and forage crops (12.2 mg kg^{-1}) [18, 19].

3.4 | Estimated Daily Intake

An estimation of the daily intake of a chemical over the course of an individual's life that is not expected to pose significant harm to health. The following formula is used to express the estimated daily intake (EDI) in mg kg^{-1} day^{-1} [20].

$$\text{EDI} = \frac{C \times \text{DI} \times \text{CF}}{\text{BW}}$$

where C is the metal conc. in mg kg^{-1}, CF is the conversion factor, which is 0.085 [21], DI is the daily intake of food, which is 12.5 [22], and BW is the reference body weight, which is 550 [21].

TABLE 2 | Analysis of variance of data for Cu concentrations in samples of water, soil, forage, and serum collected from the study sites.

Water			Soil		
Sources of variance	**Degree of freedom**	**Mean square**	**Sources of variance**	**Degree of freedom**	**Mean square value**
Treatment	2	2.80110***	Treatment	2	27.9180***
Water	2	0.03674***	Soil	5	4.3085***
Treatment*Water	4	0.07934***	Treatment*Soil	10	0.4391***
Error	18	0.00016	Error	36	0.0001
Total	26		Total	53	
Crops			**Buffaloes blood**		
Sources of variance	**Degree of freedom**	**Mean square value**	**Sources of variance**	**Degree of freedom**	**Mean square value**
Treatment	2	3.96499***	Sites	2	8.89638***
Crop	5	0.03442***	Blood	4	1.59440***
Treatment*Crop	10	0.05507***	Sites*Blood	8	0.47403***
Error	36	0.00023	Error	30	0.00023
Total	53		Total	44	

***$p < 0.001$.

3.5 | Hazard of Quotient

It is the proportion of risk associated with an impact on the level above which no adverse effects are anticipated.

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

where EDI is the estimated daily intake (mg kg^{-1} day^{-1}) of metals and RfD is the measured reference dose of metals (mg kg^{-1} day^{-1}). The oral reference dose of Cd is 0.01 [22].

3.6 | Mean Concentration of Cu in Water

The statistical analysis of variance revealed significant variations at the level of ($p < 0.001$) in Cu content across the Treatment, Water, and Treatment*Water (Table 2). The findings demonstrated fluctuating levels of copper across three different sites. The mean concentration of Cu in the water samples ranged from 0.63 to 1.92 mg L^{-1}. The peak mean copper concentration of 1.92 mg L^{-1} was observed at Location I in SW Site III, whereas Location III recorded the minimum mean value of copper of 0.63 mg L^{-1} in water in CW Site I.

3.7 | Concentration of Cu in Soil

The findings from ANOVA revealed significant effects on the Cu concentration in the soil (Table 2). The results revealed varying levels of Cu across three different sites (CW Site I, GW Site II, and SW Site III). The mean concentration of Cu was analyzed in soil samples, which ranged between 10.036 and 14.93 mg kg^{-1}. According to Table 3, *Pennisetum glaucum* exhibited the highest or maximum concentration of Cu metal at SW Site III, with a concentration of 14.93 mg kg^{-1}. At CW Site I, the minimum mean concentration of Cu was observed in *Saccharum officinarum* (10.036 mg kg^{-1}).

3.8 | Mean Cu Concentration in Forage Crops

The outcomes from the ANOVA showed that they were significantly affected ($p < 0.001$) by the Cu concentration in forage crops. The mean concentration of Cu was measured in the forage samples, displaying a variability between 5.14 and 9.87 mg kg^{-1}. At SW Site III, *Zea mays* displayed a maximum mean value of Cu of 9.87 mg kg^{-1}, whereas *Z. mays* recorded a minimum mean value of Cu of 5.14 mg kg^{-1} in forage at CW Site I (Table 3).

3.9 | Mean Concentration of Cu in Blood Serum of Buffaloes

The ANOVA between treatment and blood had a significant impact at the level of ($p < 0.001$) on Cu content (Table 2). The blood serum samples from buffaloes were analyzed for the mean concentration of Cu, resulting in a wide range of values from 1.97 to 5.15 mg L^{-1}. At SW Site III, the blood serum samples taken from pregnant buffalo revealed a mean Cu concentration of 5.15 mg L^{-1}, indicating the maximum value observed Conversely, at CW Site I, the buffalo dry displayed the minimum mean value of 1,97 mg L^{-1}.

Figure 1 represents the fluctuations in mean Cu concentration in water, soil, forage, and serum samples collected from the study sites.

TABLE 3 | Mean concentration of cu in the water, soil, forage, and blood samples at sampling sites.

Water			
Locations	**(CW Site I)**	**(GW Site II)**	**(SW Site III)**
Location I	0.76 ± 0.020	1.43 ± 0.025	1.92 ± 0.028
Location II	0.82 ± 0.020	1.67 ± 0.021	1.82 ± 0.028
Location III	0.63 ± 0.020	1.72 ± 0.025	1.58 ± 0.028
Soil			
Soil of forages	**(CW Site I)**	**(GW Site II)**	**(SW Site III)**
Avena sativa	10.97 ± 0.145	11.57 ± 0.165	12.87 ± 0.188
Trifolium alexandrinum	10.73 ± 0.145	10.81 ± 0.163	12.67 ± 0.188
Zea mays	10.23 ± 0.155	10.94 ± 0.145	12.31 ± 0.185
Pennisetum glaucum	11.88 ± 0.155	12.02 ± 0.165	14.93 ± 0.195
Saccharum officinarum	10.036 ± 0.15	10.93 ± 0.165	12.18 ± 0.195
Forage			
Forage crops	**(CW Site I)**	**(GW Site II)**	**(SW Site III)**
A. sativa	7.34 ± 0.118	7.55 ± 0.188	8.95 ± 0.188
T. alexandrinum	7.56 ± 0.118	7.79 ± 0.188	8.82 ± 0.188
Z. mays	5.14 ± 0.118	7.66 ± 0.188	9.87 ± 0.185
P. glaucum	6.25 ± 0.128	7.43 ± 0.188	8.02 ± 0.188
S. officinarum	6.82 ± 0.128	7.30 ± 0.32	8.76 ± 0.162
Sorghum bicolor	6.92 ± 0.118	7.03 ± 0.181	8.87 ± 0.168
Buffalo blood categories			
Buffalo categories	**(CW Site I)**	**(GW Site II)**	**(SW Site III)**
Buffalo calf	2.17 ± 0.042	2.33 ± 0.065	3.22 ± 0.085
Buffalo lactating	2.03 ± 0.045	2.24 ± 0.065	3.27 ± 0.085
Buffalo non-lactating	2.46 ± 0.045	2.66 ± 0.065	3.53 ± 0.085
Buffalo pregnant	2.34 ± 0.049	3.023 ± 0.065	5.15 ± 0.087
Buffalo dry	1.97 ± 0.042	2.46 ± 0.067	3.16 ± 0.085

3.10 | C_f of Cu

The C_f was determined to evaluate the level of Cu contamination in the soil, with values ranging from 0.7780 to 1.1573. According to Table 4, *P. glaucum* exhibited the highest or maximum C_f value of Cu metal at SW Site III, with a concentration of 1.1573. On the other hand, at CW Site I, *S. officinarum* displayed a minimum value of 0.7780. At SW Site III, the presence of higher Cu contents can be attributed to contamination from sewage water.

3.11 | TF of Cu From Soil to Forage

The TF was calculated to assess the migration of Cu contamination from soil to plants, revealing a range of values from 0.5027 to 0.8017. At CW Site I, *Z. mays* had the lowest concentration (0.5027). The highest TF value of Cu concentration (0.8017) was observed in *Z. mays* at SW Site III.

3.12 | EF of Copper in Forage Crops

The EF was determined to be the degree of Cu enrichment in the soil, resulting in a range of values from 0.1442 to 0.2300. At SW Site III, *Z. mays* exhibited the upper limit of EF with a value of 0.2300, while *Z. mays* showed the lowest range with a value of 0.1442 for the EF of Cu at CW Site I.

Figure 2 represents the C_f, TF, and EF of copper in soil to forage crops at the sampling site.

3.13 | Assessment of Estimated Daily Copper Intake by Buffaloes

Table 5 illustrates the range of EDI values for Cu, which include values from 0.0099 to 0.0190. At CW Site I, the lowest EDI was in *Z. mays* (0.0099), while at SW Site III, *Z. mays* demonstrated a maximum EDI value (0.0190).

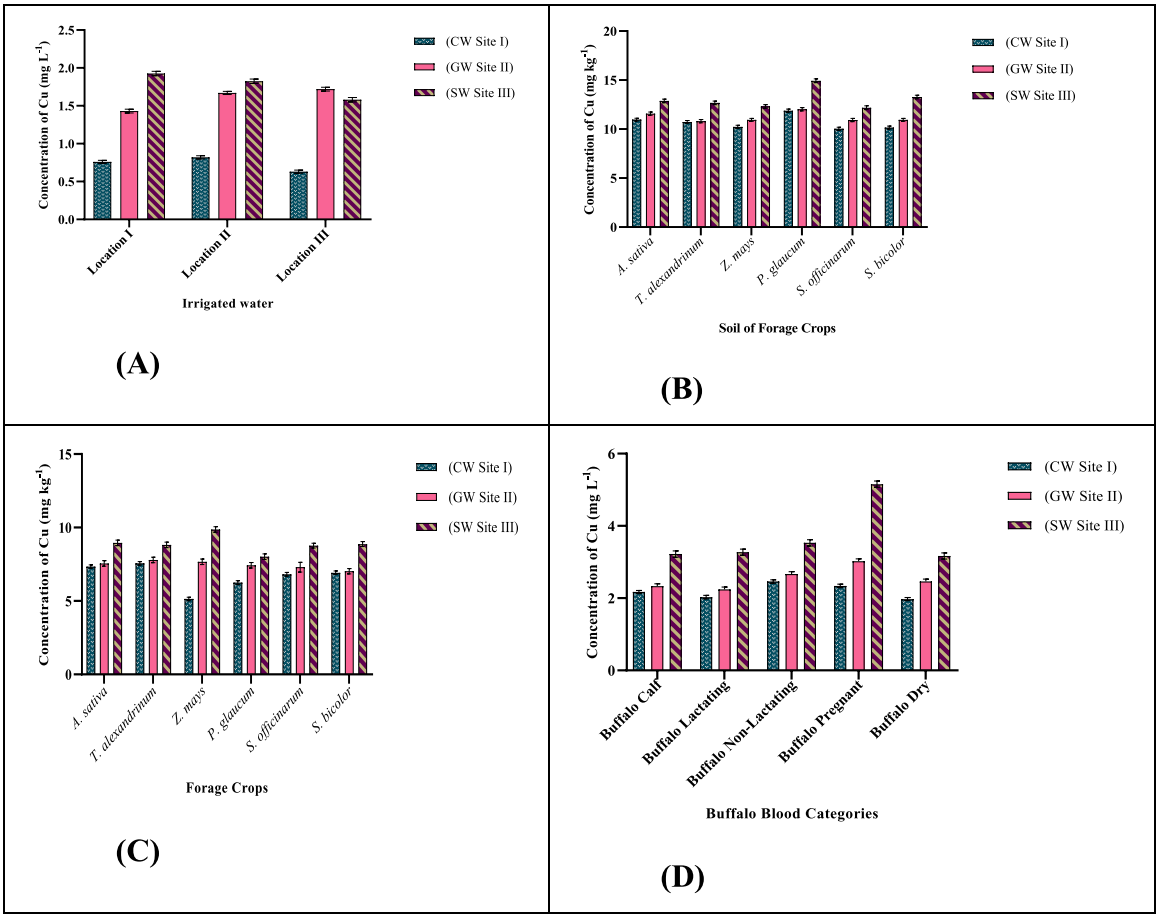


FIGURE 1 | Fluctuations in mean Cu concentration in (A) water, (B) soil, (C) forage, and (D) serum samples collected from the study sites.

3.14 | Hazard Quotient of Cu in Forage Crops

Across the samples, the hazard quotient (HQ) values of Cu fluctuated considerably from 0.248 to 0.4766. At SW Site III, the highest peak level of HQ (0.4766) was found in *Z. mays* for Cu, while *Z. mays* indicated the lowest HQ value of Cu (0.248) at CW Site I. At all sites, the HQ values for the contaminant Cu were all below 1, indicating a relatively low exposure risk.

Figure 3 represents the HQ, estimated daily intake of copper by buffaloes through contaminated forage.

3.15 | Correlation of Cu

Pearson correlation analysis was applied to examine the relationship connecting water and soil, soil and forage, and forage and blood. Forage and blood have been demonstrated to have a substantial negative association, according to the correlation investigation performed at CW Site I, whereas soil and forage showed a nonsignificant positive association. Forage–blood and soil–forage showed a nonsignificant negative connection in the information analysis at GW Site II (Table 6). The relationship between soil and water showed a weakly positive association. Forage–blood and forage–soil were shown to have strong negative correlations at SW Site III, although the relationship across water and soil was nonsignificant there. The results indicated higher Cu concentrations in wastewater-irrigated soils than in canal-irrigated soils. However, copper concentrations in forage and buffalo blood remained within permissible limits recommended by international standards. Interestingly, a negative correlation between forage and blood Cu levels was observed. This may be attributed to physiological homeostatic regulatory mechanisms in ruminants, where excess copper absorption is modulated through hepatic storage and metallothionein binding [23]. Figure 4 represents the correlation across the content of Cu in water and soil, Figure 5 represents the correlation across the content of Cu in soil and forage, and Figure 6 represents the correlation across the content of Cu in forage and blood.

4 | Discussion

Copper in agricultural soils may originate from several natural and anthropogenic sources, including fertilizer and pesticide application, atmospheric deposition, irrigation with contaminated water, and biomass burning. In particular, studies have reported that the burning of vegetation and organic residues can release heavy metals such as Cu from plant biomass and ash, which subsequently accumulate in soil and may be transferred to crops such as rice. This mechanism has been documented in recent research on upland rice cultivation systems, where fire used for land clearing released heavy metals from vegetation and

TABLE 4 | Assessment of contamination factor, transfer factor, and enrichment factor of copper in forage crops from different sites.

C_f (contamination factor)			
Forage crops	**(CW Site I)**	**(GW Site II)**	**(SW Site III)**
Avena sativa	0.8503 ± 007	0.8968 ± 004	0.9981 ± 007
Trifolium alexandrinum	0.8317 ± 003	0.838 ± 007	0.9824 ± 004
Zea mays	0.7930 ± 009	0.8480 ± 003	0.9542 ± 008
Pennisetum glaucum	0.9209 ± 002	0.9317 ± 006	1.1573 ± 01
Saccharum officinarum	0.7780 ± 007	0.8472 ± 003	0.9441 ± 002
Sorghum bicolor	0.7875 ± 004	0.8478 ± 008	1.0294 ± 003
TF (transfer factor)			
Forage crops	**(CW Site I)**	**(GW Site II)**	**(SW Site III)**
A. sativa	0.6693 ± 004	0.6531 ± 004	0.6953 ± 007
T. alexandrinum	0.7051 ± 004	0.7207 ± 003	0.6962 ± 002
Z. mays	0.5027 ± 009	0.7007 ± 003	0.8017 ± 009
P. glaucum	0.5263 ± 003	0.6184 ± 007	0.5373 ± 004
S. officinarum	0.6795 ± 003	0.6678 ± 003	0.7194 ± 006
S. bicolor	0.6811 ± 003	0.6427 ± 004	0.6679 ± 004
EF (enrichment factor)			
Forage crops	**(CW Site I)**	**(GW Site II)**	**(SW Site III)**
A. sativa	0.1920 ± 003	0.1873 ± 003	0.1994 ± 003
T. alexandrinum	0.2022 ± 004	0.2067 ± 002	0.1997 ± 003
Z. mays	0.1442 ± 004	0.2010 ± 004	0.2300 ± 006
P. glaucum	0.1510 ± 002	0.1774 ± 004	0.1541 ± 002
S. officinarum	0.1949 ± 003	0.1916 ± 003	0.2064 ± 003
S. bicolor	0.1953 ± 003	0.1844 ± 003	0.1916 ± 003

ash that later accumulated in soil and plants. Therefore, biomass burning may contribute to Cu mobilization and its subsequent transfer through the soil–plant system, potentially leading to accumulation in edible plant parts [24].

The range of values for Cu in the present study was found to be from 0.63 to 1.92 mg L^{-1}. A high wastewater concentration (2.17 mg L^{-1}) was found by Ahmad and Goni [25] in the effluent. It is essential to remove copper from aqueous solutions from the perspectives of both environmental preservation and copper resource recycling Hu et al. [26]. According to Abuzaid et al. [27], the copper levels in groundwater were found to range from 0.20 mg L^{-1}, which is lower than those in the present study. Liu et al. [28] evaluated the presence of Cu in water (1.67 mg L^{-1}), which was similar to that found by observation. These variations highlight the importance of considering local water sources when assessing copper exposure risks and potential ecological impacts on aquatic systems, as regional physicochemical conditions and contamination sources can alter metal behavior in water bodies [29].

Usman et al. [30] and Sarwar et al. [31] reported copper concentrations of 29.4–75 mg kg^{-1}, respectively, in soil. This value is higher than the mean concentration reported by the present findings. The higher concentration could be due to various factors, such as differences in soil types, geographical locations, or anthropogenic activities in the study areas. Ahmad et al. [32] found a range of copper values of 2.79–4.13 mg kg^{-1}, which is lower than the mean concentration reported. According to Ahsan et al. [33], when irrigated with city wastewater, peri-urban plants have substantial metal accumulations that cause morphological alterations in the plants. Khan et al. [34] observed a copper concentration of 10.60 mg kg^{-1} in soil. This value falls within the reported mean concentration range. These observations highlight the potential ecological impact of copper accumulation on soil health and plant development.

Higher copper concentrations in forage (69 mg kg^{-1}), as observed by Warman and Termeer [35], might raise concerns about potential environmental and human health implications due to increased exposure to copper. On the other hand, lower copper concentrations (1.60 mg kg^{-1}) in a single crop, as reported by Jung [36], may indicate forage with less copper contamination. According to Napolitano et al. [37], the majority of the trace elements needed by grazing ruminants are obtained from natural forages. Annual fluctuation has an impact on forage

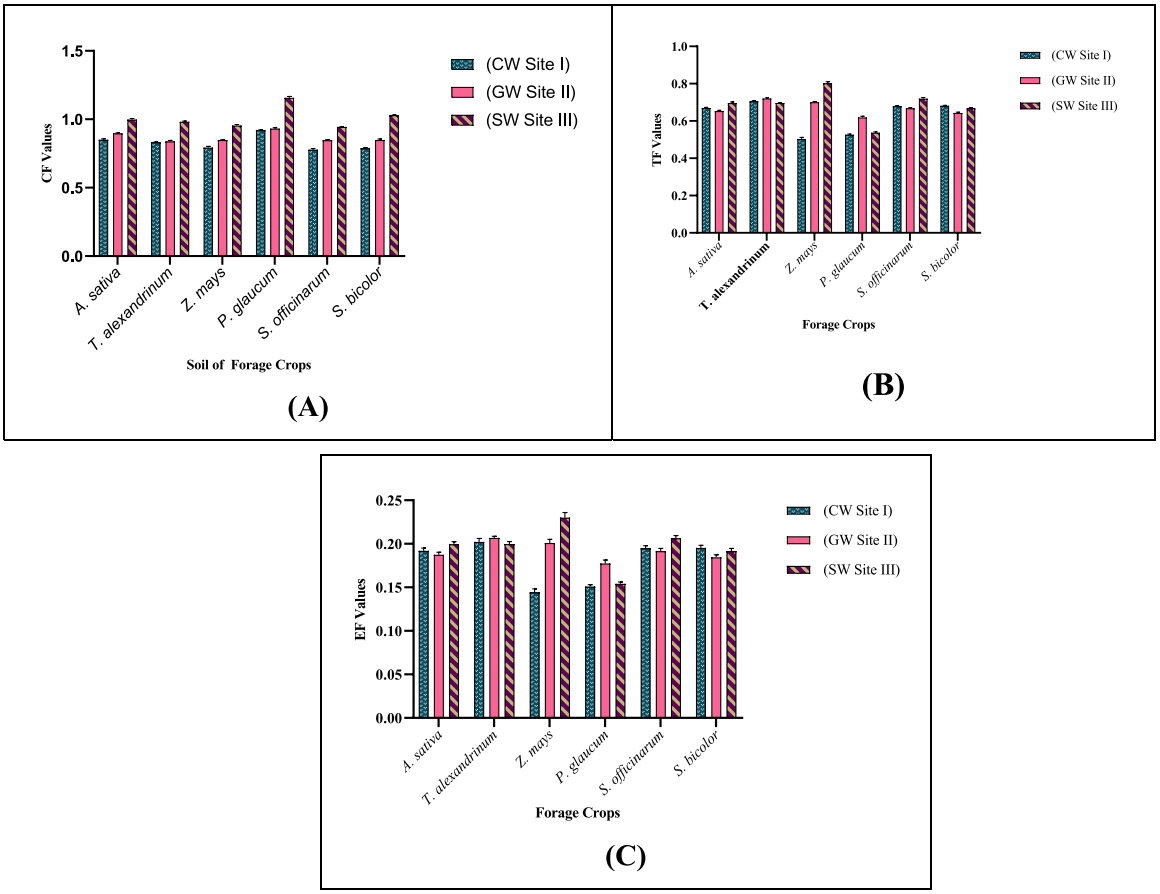


FIGURE 2 | (A) Contamination factor, (B) transfer factor, (C) enrichment factor of copper in soil to forage crops at the sampling site.

TABLE 5 | Comparative analysis of hazard quotient and estimated daily copper intake by buffaloes through contaminated forage from sampling sites.

EDI				HQ			
Forage crops	**(CW Site I)**	**(GW Site II)**	**(SW Site III)**	**Forage crops**	**(CW Site I)**	**(GW Site II)**	**(SW Site III)**
Avena sativa	0.0141 ± 0003	0.0145 ± 0003	0.0172 ± 0005	*A. sativa*	0.3546 ± 005	0.3649 ± 003	0.4324 ± 007
Trifolium alexandrinum	0.0146 ± 0004	0.0150 ± 0004	0.0170 ± 0002	*T. alexandrinum*	0.3654 ± 004	0.3763 ± 003	0.4261 ± 003
Zea mays	0.0099 ± 0005	0.0148 ± 0003	0.0190 ± 0007	*Z. mays*	0.248 ± 006	0.3702 ± 003	0.4766 ± 008
Pennisetum glaucum	0.0120 ± 0002	0.0143 ± 0003	0.0155 ± 0003	*P. glaucum*	0.3020 ± 002	0.3589 ± 004	0.3874 ± 003
Saccharum officinarum	0.0131 ± 0003	0.0141 ± 0003	0.0169 ± 0004	*S. officinarum*	0.3293 ± 004	0.3525 ± 003	0.4232 ± 004
Sorghum bicolor	0.0133 ± 0003	0.0135 ± 0003	0.0171 ± 0005	*S. bicolor*	0.3342 ± 003	0.3395 ± 003	0.4283 ± 004

TABLE 6 | Correlation analysis of copper concentrations between water–soil, soil–forage, and forage–blood at different irrigation sites.

Irrigation sites	Water–soil	Soil–forage	Forage–blood
(CW Site I)	-0.036^{ns}	0.066^{ns}	-0.731^{**}
(GW Site II)	0.158^{ns}	-0.015^{ns}	-0.078^{ns}
(SW Site III)	-0.442^{ns}	-0.754^{**}	-0.607^{**}

**Correlation is significant at the 0.01 level (two-tailed).

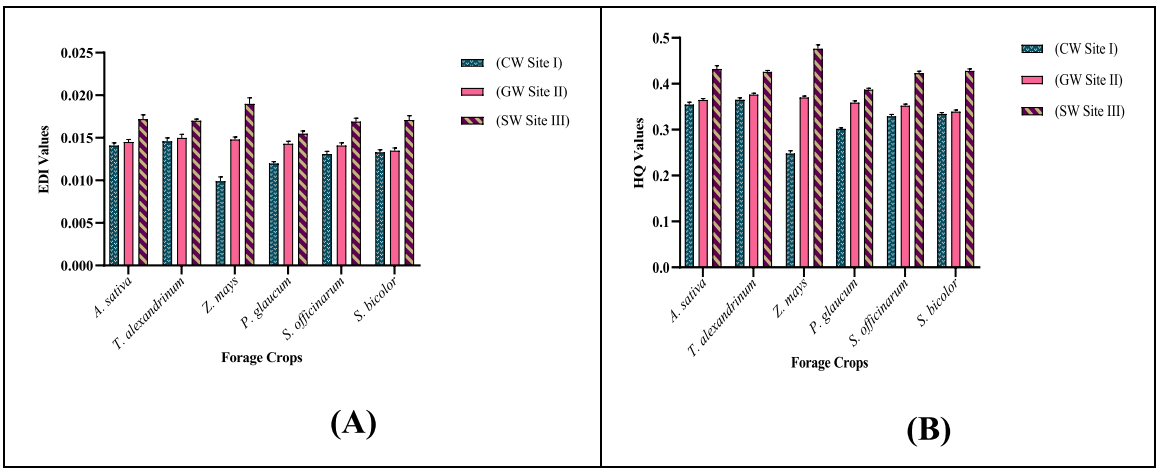


FIGURE 3 | (A) Hazard quotient, (B) estimated daily intake of copper intake by buffaloes through contaminated forage.

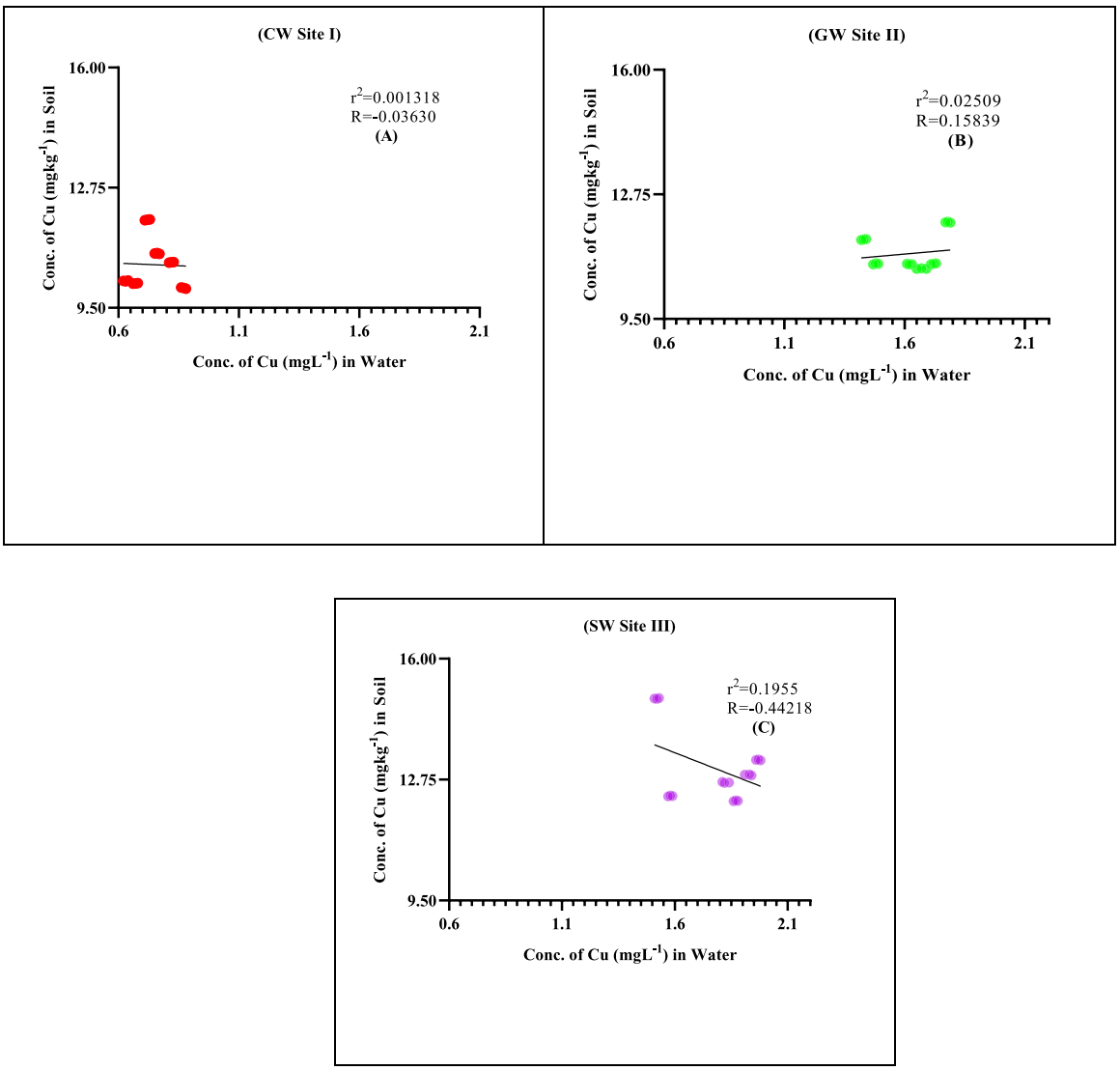


FIGURE 4 | Correlation across the content of Cu in water and soil. (A) Correlation between the level of Cu in the water and soil at CW Site I. (B) Correlation among the level of Cu in water and soil at GW Site II. (C) Correlation between the concentration of Cu in water and soil at SW Site III.

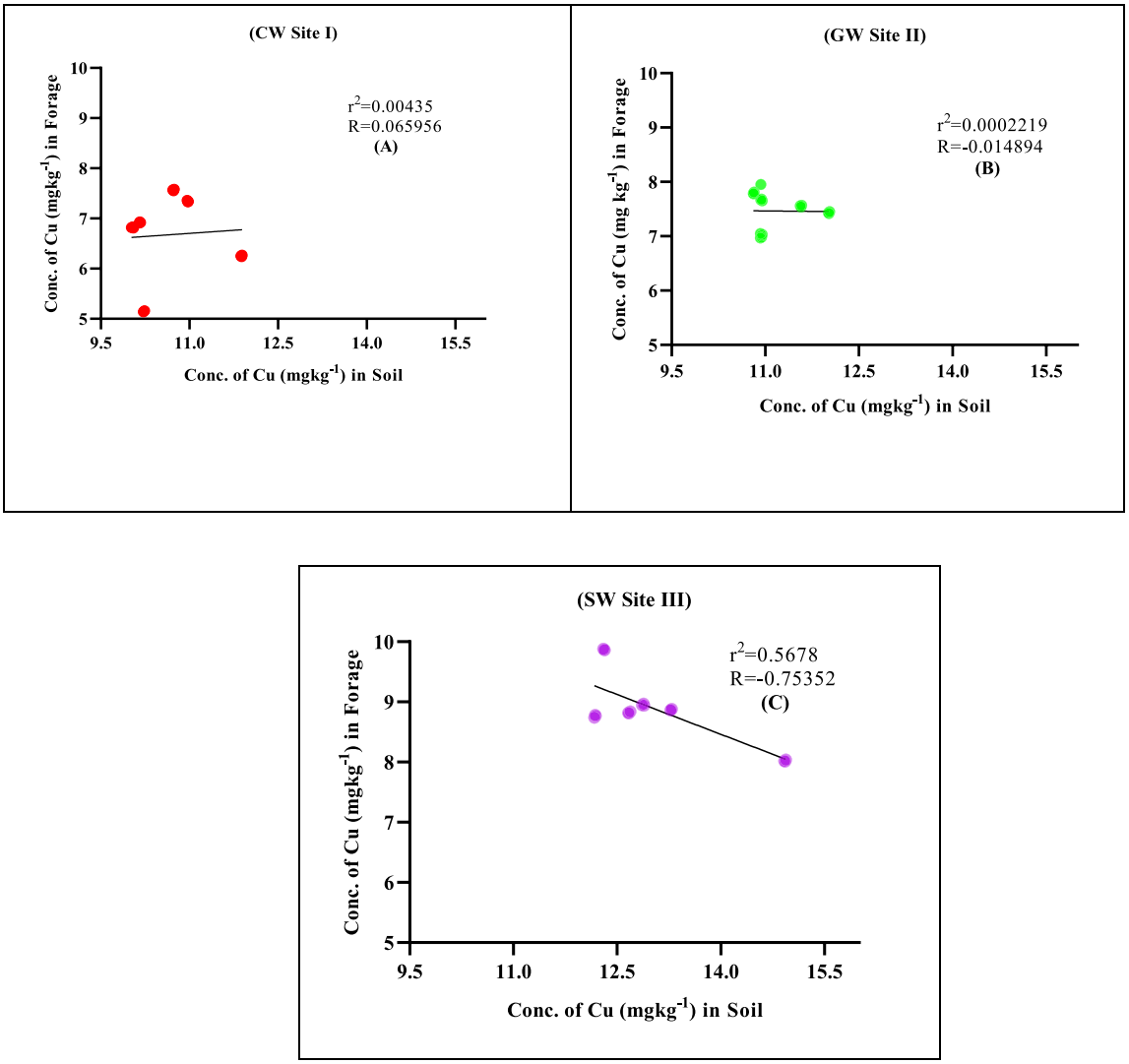


FIGURE 5 | Correlation across the content of Cu in soil and forage. (A) Correlation between the level of Cu in the soil and forage at CW Site I. (B) Correlation among the level of Cu in soil and forage at GW Site II. (C) Correlation between the concentration of Cu in soil and forage at SW Site III.

maturity and is closely connected to the mineralization of forage. Mwesigye and Mwavu [38] found a copper concentration (6.85–6.99 mg kg^{-1}) similar to the mean concentration, which adds weight to the reliability and validity of the average reported value. These observations suggest that copper exposure through forage can have cumulative effects on livestock health, which may vary regionally based on local contamination sources, as supported by research showing that forage quality and metal uptake are influenced by soil composition and irrigation practices [39].

Copper from contaminated forage accumulates in animal blood, serving as a biomarker of environmental exposure. Similar to cobalt, blood, hair, and feces reflect metal uptake in sheep, cows, and buffalo, indicating potential health risks from contaminated diets [23]. In the present work, the mean range of copper concentrations in the serum of different buffaloes was from 1.97 to 5.15 mg L^{-1}. Rasheed et al. [40] reported a copper concentration range of 0.93 mg L^{-1} in lactating buffalo serum. It is the lower end of the reported mean range. Ahmed et al. [41] found a value of 88.45 mg L^{-1} of Cu in serum. This value is significantly higher than the present findings. Shukla et al. [42] observed a copper concentration of 1.83 mg L^{-1}, which shows a similar value range as that registered in the present work.

The reported C_f values for copper (Cu) in soil vary from 0.7780 to 1.1573 degrees of contamination across different studies, reflecting the importance of considering local environmental conditions and sources of contamination.

Maisto et al. [43] found a relatively low C_f range of 0.0917–0.0928, suggesting minimal Cu contamination in the soil compared to the present study. Conversely, Ugwu and Ofomatah [44] reported a moderate C_f value of 1.57, indicating a notable level of Cu contamination in the soil. Similarly, Bampoe et al. [45] reported a moderate C_f range of 0.98–1.06, indicating a slightly higher potential for Cu accumulation compared to the present finding.

Rehman et al. [46] found that separate sites with household wastewater had a lower copper TF in soil and pasture (0.460) compared to the present findings. Hao et al. [47] demonstrated

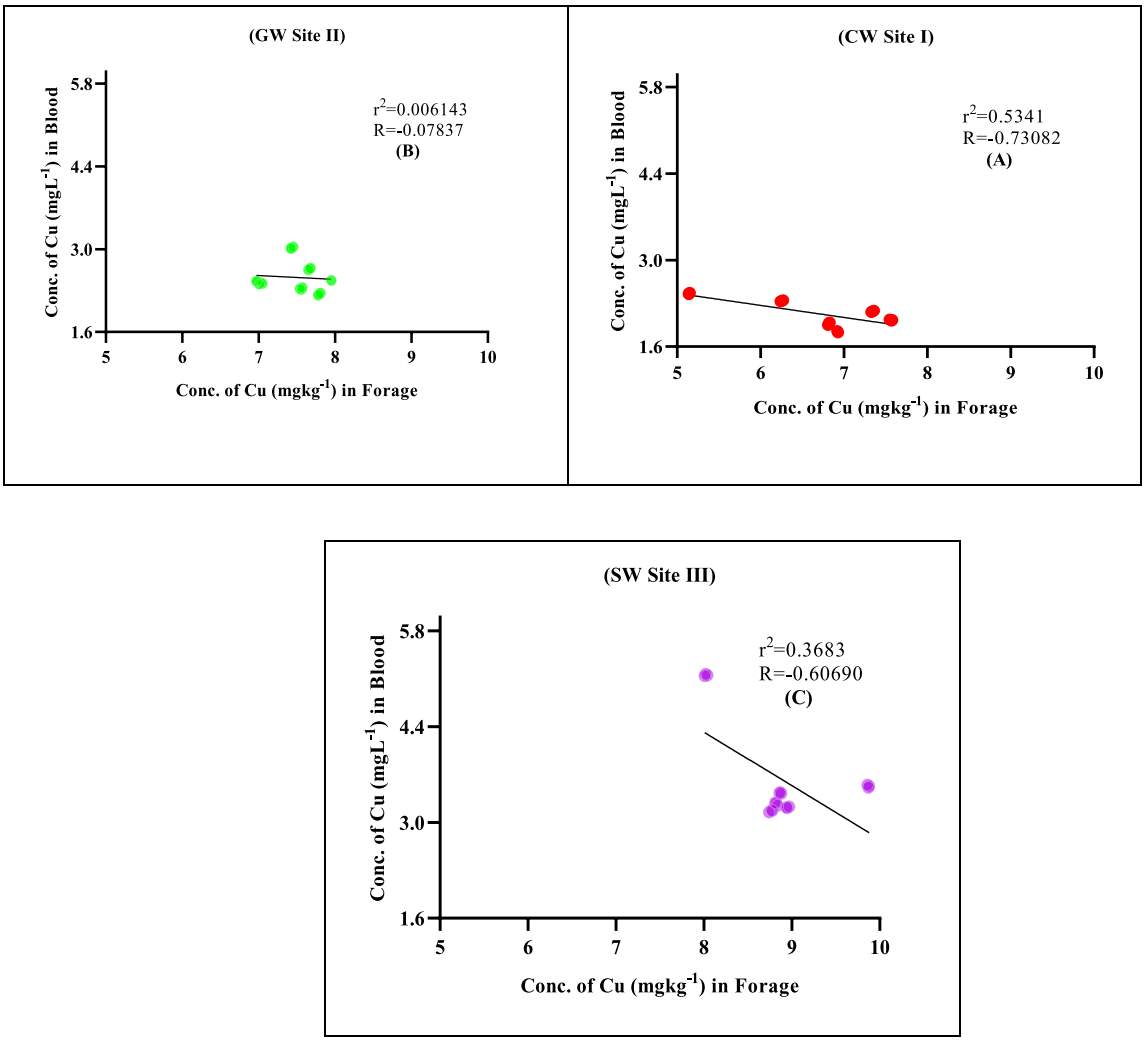


FIGURE 6 | Correlation across the content of Cu in forage and blood. (A) Correlation between the level of Cu in the forage and blood at CW Site I. (B) Correlation among the level of Cu in forage and blood at GW Site II. (C) Correlation between the concentration of Cu in forage and blood at SW Site III.

that cultivation in somewhat polluted soil had higher TF values for four metals than crops grown in substantially contaminated soil. Khan et al. [48] found that copper had a higher TF value (4.04). On the other hand, Forcada et al. [49] looked into the equivalent value of Cu in TF.

The results from the cited studies reveal a wide range of 0.1442–0.2300 EF values for copper (Cu). Barakat et al. [50] reported a notably high EF value of 0.61, pointing to significant Cu bioaccumulation compared to the present study. In contrast, Jalali and Moradi [51] found a more moderate EF range of 0.175–0.182, suggesting a lower but still notable level of Cu bioaccumulation in the studied environments. Abdullah et al. [52] reported that a value of EF 1 denotes no enrichment, a value of EF: 2–5 denotes moderate enrichment, a value of EF: 5–20 denotes considerable enrichment, a value of EF: 20–40 denotes very high enrichment, and a value of EF > 40 denotes extreme enrichment. Król et al. [53] reported a considerably low EF value of 0.011, indicating minimal Cu bioaccumulation, reflecting a less concerning environmental exposure to copper in that specific area.

According to Takarina et al. [54], an EDI value of copper concentration of 0.038–0.14450 was recorded, which is higher than that in the present work. Metal deposition in soils and crops may eventually occur from the use of wastewater for irrigation, whether directly or indirectly Singh and Agrawal [29]. Oppong et al. [55] reported an EDI range of Cu 0.00109–0.00112. This range is lower than the present investigated work. Zemenchik et al. [56] observed an EDI of 0.013; this value is similar to the reported present range.

Usman et al. [57] reported a relatively lower Cu concentration range of 0.001 compared to the present investigation. In contrast, Liu et al. [58] highlighted a significantly higher Cu concentration range of 3.02–3.92, raising concerns about potential copper toxicity. According to Avigliano et al. [59], an HQ value larger than 1 indicates that chronic health hazards may arise in the exposed population, but an HQ value less than 1 suggests that daily ingestion of a particular metal is unlikely to have any carcinogenic health impacts. Meanwhile, Yun et al. [60] reported a moderate Cu concentration range of 0.567–0.68, suggesting a more balanced Cu intake through forage consumption in the studied findings.

Overall, the data emphasize that copper accumulation in water, soil, forage, and animal serum has significant ecological implications, including potential effects on soil fertility, plant growth, and animal health. Regional variations in contamination and metal transfer highlight the necessity of site-specific monitoring and mitigation strategies to reduce long-term environmental and health risks. These findings also underscore the importance of integrating ecological assessments with contamination indices (C_f, TF, EF, EDI) to inform sustainable management practices and protect both ecosystems and human populations [61].

4.1 | Conclusion

To save money, Pakistani farmers used wastewater for irrigation. Wastewater has a high concentration of micronutrients—nutrients needed by plants for growth—as well as heavy metal contamination. The findings showed that although the mean levels of Cu in wastewater were greater, they were still within an acceptable range in the forages and blood of buffaloes that were fed on these kinds of forages that were grown in Tehsil Sargodha's canals and groundwater. Long-term usage of wastewater for irrigation may result in problems, such as high metal accumulation that could eventually reach dangerous levels for people. The study demonstrates that wastewater irrigation increases copper accumulation in soils under semiarid conditions; however, current concentrations in forage and buffalo blood remain within safe limits. Long-term monitoring is recommended to prevent potential bioaccumulation and ecological imbalance.

Author Contributions

Kafeel Ahmad: conceptualization, investigation, writing – original draft, formal analysis. **Menal Hamdani:** investigation, visualization, validation, methodology, formal analysis, resources. **Zafar Iqbal Khan:** funding acquisition, investigation, resources, writing – review and editing. **Asma Ashfaq:** formal analysis, writing – review and editing. **Mudassir Hassan:** conceptualization, visualization, formal analysis, data curation. **Muhammad Nadeem:** investigation, writing – original draft, conceptualization, supervision. **Ilker Ugulu:** supervision, visualization. **Muhammad Adeel Ghafar:** investigation, validation, writing – review and editing, methodology. **Ahsan Habib:** writing – original draft, formal analysis, methodology, conceptualization. **Yusuf Tutar:** supervision, formal analysis, investigation.

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[Correction added on 5th June 2026, after first online publication: funding statement needs to be updated.]

Ethics Statement

The institutional Human Ethics Committee of the University of Sargodha (Approval no. 25-A18 IEC UOS) has allowed all the protocols used in this experiment. All the experimental methods of this study followed all the appropriate guidance and regulations, including the NRC standards. In this study, involving human participants, informed written consent to take part in the research was obtained before the commencement of the study. The authors declare that the manuscript has not been published previously.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated or analyzed during this study are included in this research article.

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