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Biochemistry of Heavy Metals Uptake in the Grasses Assessing *Cymbopogon Jwarancusa and Arundo Donax* for Livestock Safety in Pollutes Area

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ABSTRACT

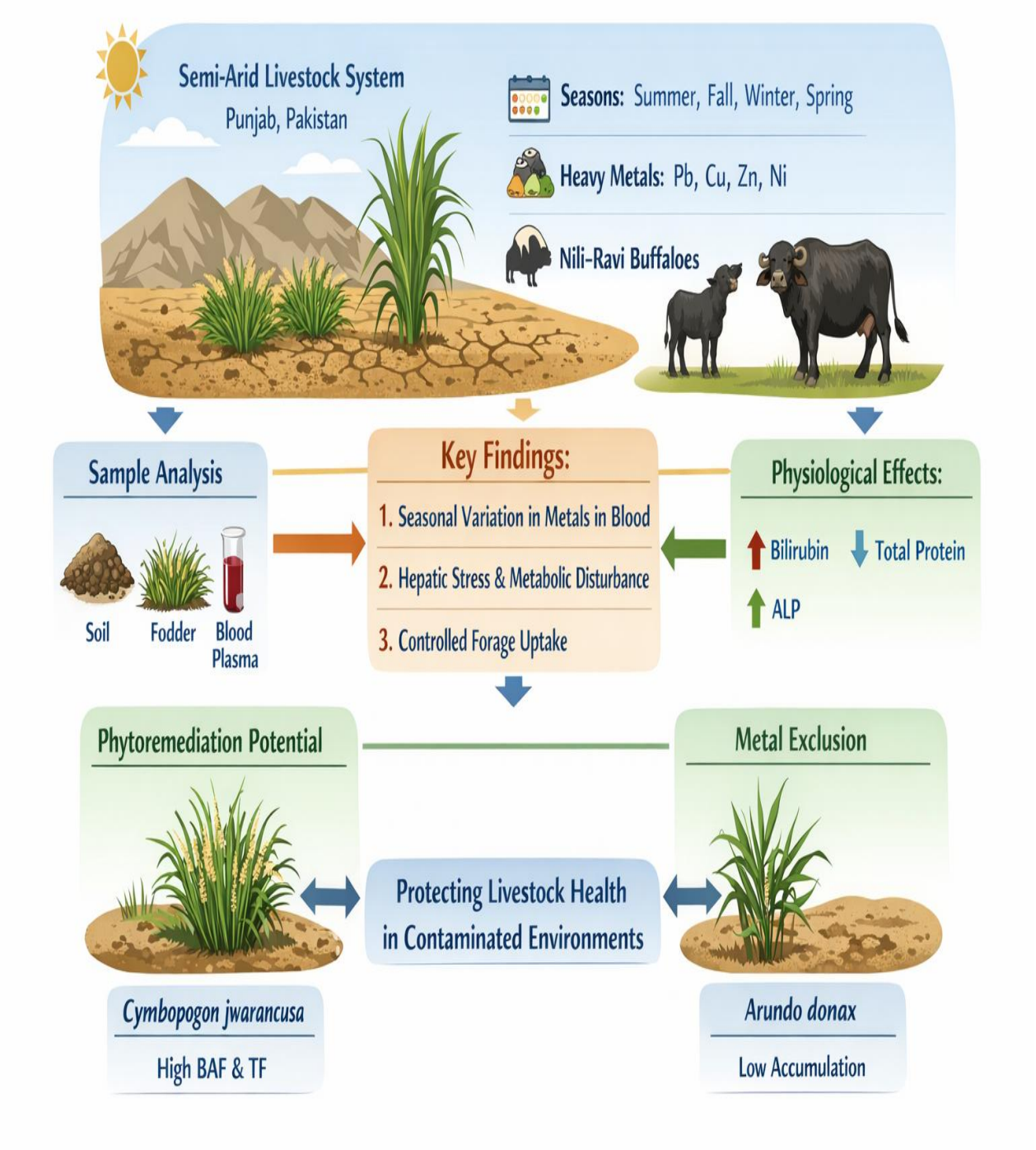
The sustainability of the environment and the health of livestock are increasingly threatened by heavy metal contamination in agro-ecosystems, especially in semi-arid areas. In addition to evaluating the phytoremediation ability of *Cymbopogon jwarancusa* and *Arundo donax*, this study examined the seasonal dynamics of certain heavy metals (Pb, Cu, Zn, and Ni) in soil-forage-animal systems and assessed their physiological effects on Nili-Ravi buffaloes. Samples of soil, fodder, and blood plasma were taken from a semi-arid livestock production system in Punjab, Pakistan, during the summer, fall, winter, and spring seasons. Standard analytical methods were used to measure metal concentrations, and plant absorption and mobility were evaluated by calculating the translocation factor (TF) and bioconcentration factor (BAF). The findings showed that while seasonal changes were noticeable, especially for Pb, metal concentrations in soil stayed within allowable bounds. The rather constant patterns of forage metal levels suggested controlled uptake mechanisms. However, blood plasma showed notable seasonal variations, particularly for Pb and Zn, with larger accumulation in young and nursing buffaloes, indicating enhanced physiological vulnerability. Significant seasonal changes in bilirubin, total protein, and alkaline phosphatase (ALP) were shown by biochemical studies, suggesting possible hepatic stress and metabolic disturbance associated with metal exposure. *C. jwarancusa* demonstrated substantial accumulation potential (BAF ≥ 1) and effective internal translocation (TF ≈ 1) among the plants under study, indicating its potential as a phytoremediator. *A. donax*, on the other hand, demonstrated a metal-exclusion strategy due to its low accumulation and translocation capabilities. Overall, the study emphasizes the necessity of protecting livestock health under changing environmental conditions and highlights the significance of ongoing environmental monitoring. It also identifies *C. jwarancusa* as a good option for phytoremediation in contaminated grazing systems.

Keywords: Heavy metals, Seasonal variation, Phytoremediation, Bioconcentration factor (BAF), Translocation factor (TF), Nili-Ravi buffalo, Livestock health

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Graphical abstract for Metal Accumulation, Nutritional Quality, and Trophic Transfer Potential of Cymbopogon jwarancusa and Arundo donax: Implications for Grazing Animals in Contaminated Environments

Introduction

Pakistan is located between 24-37°N latitude and 62-75°E longitude (in the

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temperate zone), including 8,03,940 square kilometers of land with 6,774 kilometers of coastlines(Ali *et al.*, 2019). *Cymbopogon jwarancusa* is a member of the Poaceae family. The species name is derived from two Sanskrit terms, "Jwar" and "Ankusha," which mean "fever and breaker," respectively. This suggests the highly regarded medicinal quality associated with *Cymbopogon jwarancusa*. Numerous studies conducted in recent years have shown that this plant can treat a variety of issues, including nausea and vomiting, stomach tumors and tumors, mental diseases, impure blood, acne, and skin scars (Rahman *et al.*, 2020).

Despite being a hydrophyte, Arundo donax may grow in a wide range of conditions, including moist and depleted soils and those with a water table at or near the surface. Soil inclinations are also prevalent, occurring on coarse sands, gravelly soils, notable dirts, and waterway drags from freshwater to semi-saline soils in trenches or bitter estuaries, as well as along the sides of streams, lakes, and streams (Corno *et al.*, 2014).

Some plants are capable of utilizing and tolerating the impacts of heavy metals. The plants have some robust activity to empower their mechanism and support on the land, the most noticeable of which are Pb, Cd, Zn, Cu and As. These species are classified into two types: pseudometallophytes, which can live on both metal-filled and metal-free soils, and metallophytes, which have an incredible amount of metal storage in their stems and parts and live better in metal-rich soils (Rizwan *et al.*, 2017).

The current study also discloses the use of plants, specifically pseudo metallophytes. Pseudo-metallophytes are those plants that are used to transport metals from one part of the plant to another. When these plants are present, they cause the area to become more filled with metals and salts, so this type of plant is grown in places where soil has a deficiency of metals and salts due to the absence of vegetation over a long period of time (Reeves *et al.*, 2018). Plants have a significant chance of collecting metals from the earth and transporting them to other sections of the plant because when metals are transferred to the upper portions of the plant, the plant will exhibit more metal in those parts (Yan *et al.*, 2020).

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Plants produce less biomass or food per plant than others, and some of these 300+ plants are Ni hyperaccumulators (Ghori et al., 2019). Other plants, such as gigantic reed, exhibit some of these traits. It is found in a region that is known and famous in the globe for having more metals in the soil, and its yield is 32 and 37 t/h (Scordia and Cosentino., 2019). It is easy to carry out the growth of hyperaccumulator in such places (Cosentino *et al.*, 2017).

Buffaloes, like other animals, play a significant role in transforming agricultural residues or trash into milk and meat. If these waste products are not used, they accumulate in our environment and damage our ecosystem, posing a current threat to biodiversity (Khan et al., 2015). There are numerous buffalo breeds in Pakistan, but the Nili-Ravi (Bubalus bubalis) is the most productive, producing 1800-2500 liters of milk per lactation with a high fat content of 6.5%. No breed in the world holds such promise (Ali *et al.*, 2019).

Blood biochemical markers include glucose levels, protein and lipid profiles, and enzymatic profiles such as liver and kidney function, among others. The haematological profile includes a complete blood count (CBC) (Tchounwou et al., 2012). Different pastures are used around the world to supplement ruminants' nutritional diets. If soil and forages are lacking in trace minerals, this will create deficits in ruminants that rely on them (Suttle, 2010).

In a semi-arid area of Punjab, Pakistan, the goal of the current study was to thoroughly assess the dynamics of heavy metal pollution within a soil–forage–animal system. The specific goals were to: (i) measure the seasonal variation of specific heavy metals (Pb, Cu, Zn, and Ni) in the soil, forage, and blood plasma of Nili-Ravi buffaloes; (ii) evaluate the transfer and accumulation of these metals across trophic levels; (iii) assess the phytoremediation potential of *Cymbopogon jwarancusa* and *Arundo donax* using bioconcentration factor (BAF) and translocation factor (TF); and (iv) examine the effects of metal exposure on important biochemical parameters, such as bilirubin, alkaline phosphatase, and total protein, in order to identify potential health risks in livestock.

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Materials and Methods

Study Area

The investigation was carried out at the Livestock Experiment Station (Chak No. 61/Mb, Khushab, Punjab, Pakistan). The area depicts a semi-arid zone where animals are frequently exposed to environmental pollutants via soil and feed.

Plant Material Collection and Preparation

Cymbopogon jwarancusa and *Arundo donax* were taken from specific locations in Jauharabad, Pakistan. Healthy aerial parts were picked, rinsed with distilled water to eliminate pollutants, and air-dried to room temperature. The dried materials were crushed into fine powder and kept in airtight containers for future analysis.

Soil Sample and Preparation

Soil samples were taken from ten selected sites at depths ranging from 12 to 15 cm. The samples were air dried, oven dried at 60 °C, and wet digested with sulfuric acid and hydrogen peroxide. The digested samples were filtered and diluted with distilled water before metal analysis.

Forage Sampling and Preparation

Forage samples were obtained from the same places as soil samples. Samples were rinsed with distilled water and dilute HCl to remove external contamination, then dried at 50°C and crushed into powder. Approximately 1 g of each sample was digested with sulfuric acid and hydrogen peroxide, then filtered and kept for analysis.

Blood Sample Collection

A total of 30 Nili-Ravi buffaloes were selected and divided into three groups: lactating, dry, and young. Throughout four seasons (summer, autumn, winter, and spring), blood samples were drawn from the jugular vein with sterilized syringes. Plasma was extracted by centrifugation and kept at -20 °C for analysis.

Heavy Metal Analysis

Heavy metals (Pb, Cd, Cu, Zn, Ni, Cr) in soil, forage, and blood plasma were determined using standard analytical procedures following wet digestion. Metal concentrations were expressed in mg/kg (soil and forage) and mg/L (blood plasma).

Biochemical Analysis

Blood plasma was tested for biochemical parameters such as total protein, bilirubin,

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and alkaline phosphatase (ALP) using standard spectrophotometric techniques. Total protein was calculated using the Biuret method, while bilirubin and ALP were tested using commercially available diagnostic methods.

Assessment of Metal Uptake and Transfer

Bioconcentration Factor (BAF)

BAF was calculated to evaluate the accumulation of metals from soil to plant:

BAF = Metal concentration in plant / Metal concentration in soil

Translocation Factor (TF)

TF was calculated to assess the movement of metals within the plant:

TF = Metal concentration in shoot / Metal concentration in root

Statistical Analysis

Data were evaluated using analysis of variance (ANOVA) to determine the impact of seasonal fluctuations on metal concentrations and biochemical parameters. Mean values were presented as mean ± standard error (SE), with significance set at $p < 0.05$.

Results

Lead (Pb) in Soil

The sampling seasons had significant ($P<0.05$) effect on the mean Pb contents in the soil samples Table 1 and 3. The mean Pb levels at various sampling seasons differed from 15.37 to 16.94 mg/kg. The mean values of Pb contents in the various sampling seasons were 15.94 mg/kg(summer), 15.37 mg/kg(autumn), 16.94 mg/kg(winter) and 16.88 mg/kg(spring). The order of the noticed mean Pb contents in all soil samples were determined as summer>winter>spring>autumn. The mean Pb contents in the soil samples showed irregular pattern of increase and decrease at different sampling seasons. The maximum permissible limit of Pb in the soil is 100 mg/kg suggested by FAO/WHO (2001). The value of Pb observed in the present study is lower than this permissible limit.

Lead (Pb) Forage

The sampling seasons had no significant ($P<0.05$) effect on the mean Pb concentrations in the forage samples Table 1 and 3. The mean Pb levels at various sampling seasons differed from 5.98 to 6.24 mg/kg. The mean values of Pb in the

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various sampling seasons were 6.24 mg/kg(summer), 5.98 mg/kg(autumn), 6.09 mg/kg(winter) and 6.13 mg/kg(spring). The order of the mean Pb contents in all forage samples were determined as summer>spring>winter>autumn.

Lead (Pb) Blood Plasma

The Pb concentration in blood plasma samples significantly ($p<0.001$) influenced with respect to seasons in lactating and young buffaloes but it was non-significantly ($p>0.05$) affected by the sampling seasons in dry buffaloes as shown in Table 1 and 3. The mean values of Pb in blood plasma of lactating buffaloes were 0.457 mg/L(summer), 0.389 mg/L(autumn), 0.592 mg/L(winter) and 0.584 mg/L(spring). The mean Pb concentration in blood plasma of dry buffaloes were 0.524 mg/L(summer), 0.566 mg/L(autumn), 0.505 mg/L(winter) and 0.522 mg/L(spring). The average Pb contents in blood plasma of young buffaloes were 0.851 mg/L(summer), 0.838 mg/L(autumn), 0.595 mg/L(winter) and 0.699 mg/L(spring) (Figures 1a & 1b).

Copper (Cu) in soil

There was no significant seasonal effect ($p>0.05$) on the average Cu concentrations in soil samples. The mean Cu values across sampling seasons ranged from 3.54 to 4.08 mg/kg table 1 and 5. The soil samples obtained during the winter sampling season had greater mean Cu contents, but the Cu concentrations were lower during the spring survey season. Cu contents averaged 3.65 mg/kg throughout the summer, 3.84 mg/kg in the autumn, 4.08 mg/kg in the winter, and 3.54 mg/kg in the spring. The observed mean Cu values in all soil samples were arranged in the following order: winter, autumn, summer, and spring.

Copper (Cu) in Forages

There was no significant seasonal effect ($p<0.05$) on mean Cu contents in pasture samples. The mean Cu values across sampling seasons ranged from 6.75 to 7.06 mg/kg as shown in 1 and 5. Forage samples obtained during the summer sampling season had greater mean Cu contents, while Cu concentrations were lower during the winter sampling season. Cu contents averaged 7.06 mg/kg throughout the summer, 7.02 mg/kg in the autumn, 6.75 mg/kg in the winter, and 6.81 mg/kg in the spring (Figures 1a & 1b).

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Copper (Cu) in Blood Plasma

In all three buffalo types (lactating, dry, and young), the mean Cu content in blood plasma did not differ significantly ($p>0.05$) by season as shown in table 1 and 5. Cu levels in nursing buffalo blood plasma averaged 0.193 mg/L throughout the summer, 0.236 mg/L in the fall, 0.213 mg/L in the winter, and 0.230 mg/L in the spring. Cu concentrations in dried buffalo blood plasma averaged 0.210 mg/L throughout the summer, 0.209 mg/L in the autumn, 0.211 mg/L in the winter, and 0.193 mg/L in the spring. The average copper concentration in adolescent buffalo blood plasma was 0.233 mg/L in the summer, 0.240 mg/L in the autumn, 0.239 mg/L in the winter, and 0.224 mg/L in the spring.

Zinc (Zn) in Soil

All sampling seasons had no significant effect ($P>0.05$) on the mean Zn concentration of soil samples. The mean Zn levels across sampling seasons ranged from 5.04 to 5.42 mg/kg table 1 and 4. Higher mean Zn values were observed in soil samples during the summer sampling season, while lower Zn concentrations were discovered during the fall monitoring season. Zn content averaged 5.42 mg/kg throughout the summer, 5.04 mg/kg in the autumn, 5.18 mg/kg in the winter, and 5.09 mg/kg in the spring.

Zinc (Zn) in Forages

All sampling seasons had no significant effect ($P>0.05$) on the mean Zn concentration of fodder samples Table 1 and 4. The mean Zn levels across sampling seasons ranged from 16.27 to 14.71 mg/kg. The highest mean Zn value was seen in feed samples during the summer sampling season, whereas the lowest Zn content was discovered during the fall survey season. The average Zn concentration in the various sampling seasons was 16.27 mg/kg (summer), 14.71 mg/kg (autumn), 15.06 mg/kg (winter), and 15.23 mg/kg (spring). The discovered mean Zn concentrations in all pasture samples were determined in the following order: summer, spring, winter, and fall.

Zinc (Zn) in Blood Plasma

The ANOVA results and average Zn values in blood plasma during various sample seasons are shown in Tables 1 and 4. The analysis of variance revealed that mean Zn

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levels in blood plasma of breastfeeding and young buffaloes were significantly ($p<0.01$) influenced by seasons, while dry buffaloes showed the reverse effect. Lactating buffaloes' blood plasma Zn levels averaged 0.594 mg/L in the summer, 0.616 mg/L in the autumn, 1.018 mg/L in the winter, and 0.772 mg/L in the spring. The average zinc concentration in dried buffalo blood plasma was 0.768 mg/L in the summer, 0.806 mg/L in the autumn, 0.901 mg/L in the winter, and 0.851 mg/L in the spring. The average Zn levels in the blood plasma of juvenile buffaloes were 0.652 mg/L (summer), 0.691 mg/L (fall), 1.01 mg/L (winter), and 0.695 mg/L (spring).

Nickel (Ni) in Soil

Tables 1 and 2 show the ANOVA findings as well as the mean Ni concentrations in soil samples collected during different sampling seasons. According to the analysis of variance, the average Ni values in soil samples were not substantially changed ($P>0.05$) by the sampling seasons. The mean Ni levels at various sampling seasons were between 0.61 and 0.66 mg/kg. The highest mean Ni value in the soil samples was seen during the autumn sampling season, whereas the lowest Ni content was discovered during the summer sampling season. The mean Ni concentrations varied by sample season: 0.61 mg/kg (summer), 0.66 mg/kg (fall), 0.65 mg/kg (winter), and 0.64 mg/kg (spring).

Nickel (Ni) in Forage

Tables 1 and 2 show the ANOVA findings as well as the mean Ni concentrations in fodder samples collected throughout different sampling seasons. The analysis of variance revealed a substantial ($P<0.05$) effect of sampling season on average Ni values in fodder samples. The mean Ni levels during several sampling seasons ranged between 2.01 and 2.58 mg/kg. The highest mean Ni value was seen in feed samples during the spring sampling season, whereas the lowest Ni content was discovered during the fall monitoring season. The mean Ni concentration in the various sampling seasons was 2.33 mg/kg (summer), 2.01 mg/kg (autumn), 2.34 mg/kg (winter), and 2.59 mg/kg (spring) (Figures 1a & 1b).

Nickel (Ni) in Blood Plasma

Tables 1 and 2 show the ANOVA results and mean Ni levels in blood plasma

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samples during different sampling seasons. Lactating and young buffaloes had significantly lower mean Ni levels ($p<0.001$) compared to dry buffaloes ($p>0.05$) . Lactating buffaloes' blood plasma Ni concentrations averaged 0.056 mg/L in the summer, 0.048 mg/L in the autumn, 0.046 mg/L in the winter, and 0.047 mg/L in the spring. Ni concentrations in dry buffalo blood plasma averaged 0.043 mg/L in the summer, 0.046 mg/L in the autumn, 0.047 mg/L in the winter, and 0.045 mg/L in the spring. Young buffalo blood plasma had an average Ni concentration of 0.041 mg/L in the summer, 0.035 mg/L in the autumn, 0.038 mg/L in the winter, and 0.034 mg/L in the spring (Figures 1a & 1b).

Table 1: ***Descriptive analysis for Pb, Ni, Zn, Cu concentration in soil, forage and blood plasma influenced by different season***

Metal	Soil	Forage	Lactating Buffaloes	Dry Buffaloes	Young Buffaloes
Pb	16.290 ± 0.215	6.119 ± 0.096	0.506 ± 0.015	0.530 ± 0.015	0.747 ± 0.017
Ni	0.641 ± 0.011	2.320 ± 0.068	0.049 ± 0.003	0.048 ± 0.002	0.037 ± 0.002
Zn	5.188 ± 0.102	15.324 ± 0.235	0.751 ± 0.027	0.832 ± 0.029	0.761 ± 0.022
Cu	3.783 ± 0.093	6.916 ± 0.131	0.218 ± 0.006	0.206 ± 0.006	0.235 ± 0.005

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Table 2: *Analysis of variance for Ni concentrations in soil, forage and blood plasma influenced by different seasons*

Source of Variation (SOV)	Degrees of Freedom (df)	Mean Squares				
		Soil	Forage	Blood plasma		
				Lactating Buffaloes	Dry Buffaloes	Young Buffaloes
Sampling Season	3	0.014^{ns}	1.695*	0.001^{ns}	0.001**	0.001^{ns}
Error	36	0.004	0.291	0.001	0.000	0.001

Table 3: *Analysis of variance for Pb concentrations in soil, forage and blood plasma influenced by different seasons*

Source of Variation (SOV)	Degrees of Freedom (df)	Mean Squares				
		Soil	Forage	Blood plasma		
				Lactating Buffaloes	Dry Buffaloes	Young Buffaloes
Sampling Season	3	17.278*	0.350^{ns}	0.298*	0.020^{ns}	0.444***
Error	36	1.513	0.596	0.015	0.013	0.016

P <0.001= ***, P <0.05 = *, P >0.05=ns

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Table 4: ***Analysis of variance for Zn concentrations in soil, forage and blood plasma influenced by different seasons***

Source of Variation (SOV)	Degrees of Freedom (df)	Mean Squares				
		Soil	Forage	Blood plasma		
				Lactating Buffaloes	Dry Buffaloes	Young Buffaloes
Sampling Season	3	0.839 ns	13.482 ns	1.147***	0.099 ns	0.805***
Error	36	0.459	2.767	0.030	0.059	0.024

P<0.001= ***, P >0.05=ns

Table 5: ***Analysis of variance for Cu concentrations in soil, forage and blood plasma influenced by different seasons***

Source of Variation (SOV)	Degrees of Freedom (df)	Mean Squares				
		Soil	Forage	Blood plasma		
				Lactating Buffaloes	Dry Buffaloes	Young Buffaloes
Sampling Season	3	1.675 ns	0.713 ns	0.011 ns	0.002 ns	0.002 ns
Error	36	0.370	1.233	0.001	0.001	0.001

P >0.05=ns

Table 6: ***Heavy metal uptake and accumulation parameters (Mean ± SE) in Cymbopogon jwarancusa***

Metal	L1 Uptake (%)	L2 Uptake (%)	BAF (L1)	BAF (L2)	TF (L1)	TF (L2)
Cr	47.33 ± 3.18	33.67±2.03	1.17±0.03	1.07 ±0.03	1.07 ± 0.03	0.97 ± 0.03
Cu	74.33 ±	46.33±4.41	1.33±0.0	1.20	1.13 ±	1.00 ±

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	6.69			3	±0.00	0.03		0.00	
Cd	15.33	±	10.33±1.20	0.77±0.0	0.87	0.97	±	1.07	±
	2.03			3	±0.03	0.03		0.03	
Pb	36.00	±	20.00±2.08	1.07±0.0	1.17	1.00	±	1.00	±
	2.64			3	±0.03	0.00		0.00	
Ni	46.00	±	35.67±1.45	1.17±0.0	1.07	1.07	±	0.97	±
	1.73			3	±0.03	0.03		0.03	
Zn	23.00	±	18.00±1.15	0.97±0.0	1.07	1.00	±	1.00	±
	1.73			3	±0.03	0.00		0.00	

The findings revealed that *C. jwarancusa* exhibited significant heterogeneity in metal absorption, bioconcentration factor (BAF), and translocation factor (TF) across the heavy metals tested.

Cu had the highest uptake, with values of 74.33 ± 6.69% (L1) and 46.33 ± 4.41% (L2), followed by Cr (47.33 ± 3.18%; 33.67 ± 2.03%) and Ni (46.00 ± 1.73%; 35.67 ± 1.45%). Pb showed moderate uptake, whereas Cd and Zn had lower uptake levels. Most metals had BAF values of ≥1, indicating high accumulation potential, especially Cu (1.33 ± 0.03; 1.20 ± 0.00), Cr (1.17 ± 0.03; 1.07 ± 0.03), and Ni (1.17 ± 0.03; 1.07 ± 0.03). Cd (0.77 ± 0.03; 0.87 ± 0.03) and Zn (0.97 ± 0.03; 1.07 ± 0.03) had lower accumulation rates (Table 6).

TF values were typically around or equal to one, indicating that metals were transported efficiently inside. Cu and Cd had somewhat higher TF (1.13 ± 0.03) and 1.07 ± 0.03, respectively, while other metals showed balanced translocation between roots and shoots Table 6.

The results showed that *A. donax* has moderate to low metal absorption and accumulation capability for all heavy metals tested. Cu had the highest absorption, with values of 39.67 ± 1.45% (L1) and 48.00 ± 0.58% (L2), followed by Cd (33.67 ± 0.88%; 37.33 ± 0.88%) and Cr (36.00 ± 1.73%; 30.33 ± 1.20%). Pb, Zn, and Ni all showed lower uptake, with Ni having the lowest values. The BAF values for all metals were consistently less than one, indicating low accumulation ability. Cu (0.47 ± 0.01; 0.54 ± 0.01) and Ni (0.22 ± 0.01; 0.53 ± 0.01) had the greatest BAF values, with other metals showing lower values. TF values were likewise less than one,

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indicating that metals were constrained in their translocation within the plant. Cu (0.69 ± 0.01) and Pb (0.56 ± 0.01) had higher TF than other metals in L2, indicating moderate mobility.

A. donax had low bioconcentration (BAF < 1) and limited translocation (TF < 1), suggesting a reduced phytoremediation capability Table 7.

Table 7: ***Heavy metal uptake and accumulation parameters (Mean ± SE) in Arundo donax***

Metal	L1 Uptake (%)	L2 Uptake (%)	BAF (L1)	BAF (L2)	TF (L1)	TF (L2)
Cr	36.00 ± 1.73	30.33±1.20	0.38 ±0.01	0.43 ±0.01	0.24±0.01	0.28 ± 0.01
Cu	39.67 ± 1.45	48.00±0.58	0.47 ±0.01	0.54 ±0.01	0.35±0.01	0.69 ± 0.01
Cd	33.67 ± 0.88	37.33±0.88	0.36 ±0.01	0.39 ±0.01	0.22±0.01	0.25 ± 0.01
Pb	22.67 ± 0.88	26.67±0.88	0.28 ±0.01	0.32 ±0.01	0.39±0.01	0.56 ± 0.01
Ni	18.67 ± 0.88	17.67±0.88	0.22 ±0.01	0.53 ±0.01	0.16±0.01	0.19 ± 0.01
Zn	26.67 ± 0.88	12.67±0.88	0.26 ±0.01	0.35 ±0.01	0.29±0.01	0.33 ± 0.01

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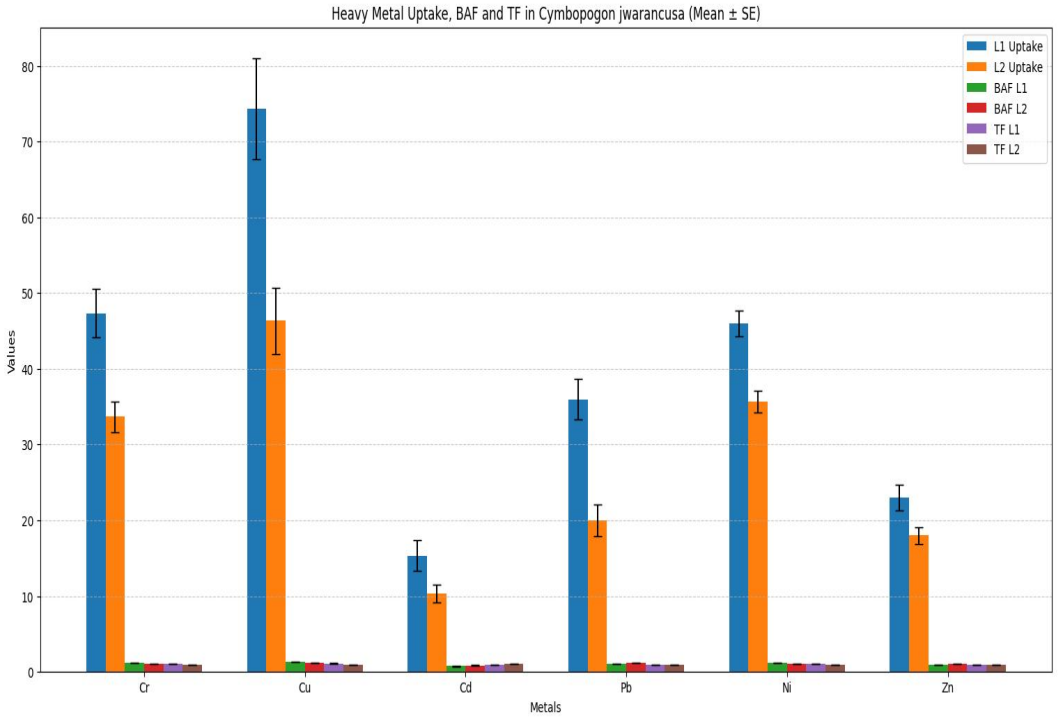


Figure (1a-1b): Heavy metal uptake and accumulation parameters (Mean ± SE) in a) Cambropogan jwarancusa b) Arundo donax

Biochemical Profile

Bilirubin Total

According to the analysis of variance, the bilirubin concentration in blood plasma significantly ($p<0.001$) influenced with respect to seasons in lactating and dry buffaloes but it was non-significantly ($p>0.05$) affected by the sampling seasons in young buffaloes (Table 8 and 9). The mean bilirubin values in blood plasma of lactating buffaloes were 0.573 mg/dl (summer), 0.550 mg/dl (autumn), 0.710 mg/dl (winter) and 0.623 mg/dl (spring). The mean bilirubin concentrations in blood plasma of dry buffaloes were 0.563 mg/dl (summer), 0.503 mg/dl (autumn), 0.622 mg/dl (winter) and 0.583mg/dl (spring). The average bilirubin contents in blood plasma of young buffaloes were 0.560 mg/dl (summer), 0.537 mg/dl (autumn), 0.597 mg/dl (winter) and 0.543 mg/dl(spring) (Figure 2a).

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Table 8: ***Analysis of variance for bilirubin total concentrations in blood plasma of buffaloes influenced by different seasons***

Source of Variation (SOV)	Degrees of Freedom (df)	Mean Squares		
		Lactating Buffaloes	Dry Buffaloes	Young Buffaloes
Sampling Season	3	0.050*	0.024***	0.006^{ns}
Error	36	0.007	0.003	0.004

P <0.001= ***, P <0.05=*, P >0.05=ns

Table 9: ***Descriptive analysis for bilirubin total concentrations in blood plasma of buffaloes influenced by different seasons***

Parameters	Blood plasma		
	Lactating Buffaloes	Dry Buffaloes	Young Buffaloes
Mean	0.614	0.568	0.558
Std. Error±	0.0142	0.0131	0.0094

Alkaline Phosphatase (ALP)

The concentration of ALP in blood plasma samples significantly ($p<0.001$) influenced by sampling seasons in all categories of buffaloes (Table 10). The mean ALP values in blood plasma of lactating buffaloes were 375.4 U/L(summer), 218.3 U/L(autumn), 393.6 U/L(winter) and 392.8 U/L(spring). The mean concentrations of ALP in blood plasma of dry buffaloes were 210 U/L(summer), 189.4 U/L(autumn), 257.2 U/L(winter) and 256.4 U/L(spring). The average ALP contents in blood plasma of young buffaloes were 372.2 U/L(summer), 204.63 U/L(autumn), 372.03 U/L(winter) and 368.4 U/L(spring) as in Table 11 (Figure 2b).

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Table 10: ***Analysis of variance for alkaline phosphatase concentrations in blood plasma of buffaloes influenced by different seasons***

Source of Variation (SOV)	Degrees of Freedom (df)	Mean Squares		
		Lactating Buffaloes	Dry Buffaloes	Young Buffaloes
Sampling Season	3	72036.576***	11586.958***	69134.517***
Error	36	369.989	964.632	938.972

P <0.001= ***

Table 11: ***Descriptive analysis for alkaline phosphatase concentrations in blood plasma of buffaloes influenced by different seasons***

Parameters	Blood plasma		
	Lactating Buffaloes	Dry Buffaloes	Young Buffaloes
Mean	345.023	228.250	329.325
Std. Error±	7.357	4.306	7.986

Total Proteins (TP)

The total protein concentration in blood plasma significantly (p<0.001) influenced with respect to seasons in all categories of the buffaloes (Table 12). The mean TP values in blood plasma of lactating buffaloes were 2.11 g/dl(summer), 3.65 g/dl(autumn), 2.42 g/dl(winter) and 2.01 g/dl(spring). The mean TP concentrations in blood plasma of dry buffaloes were 3.4 g/dl(summer), 3.36 g/dl(autumn), 4.53 g/dl(winter) and 4.5 g/dl(spring). The average contents of TP in blood plasma of young buffaloes were 3.65 g/dl(summer), 2.02 g/dl(autumn), 3.43 g/dl(winter) and 3.38 g/dl(spring) as in Table 13 (Figure 2c).

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Table 12: ***Analysis of variance for total protein concentrations in blood plasma of buffaloes influenced by different seasons***

Source of Variation (SOV)	Degrees of Freedom (df)	Mean Squares		
		Lactating Buffaloes	Dry Buffaloes	Young Buffaloes
Sampling Season	3	5.707***	4.272***	5.556***
Error	36	0.038	0.050	0.045

P <0.001= ***

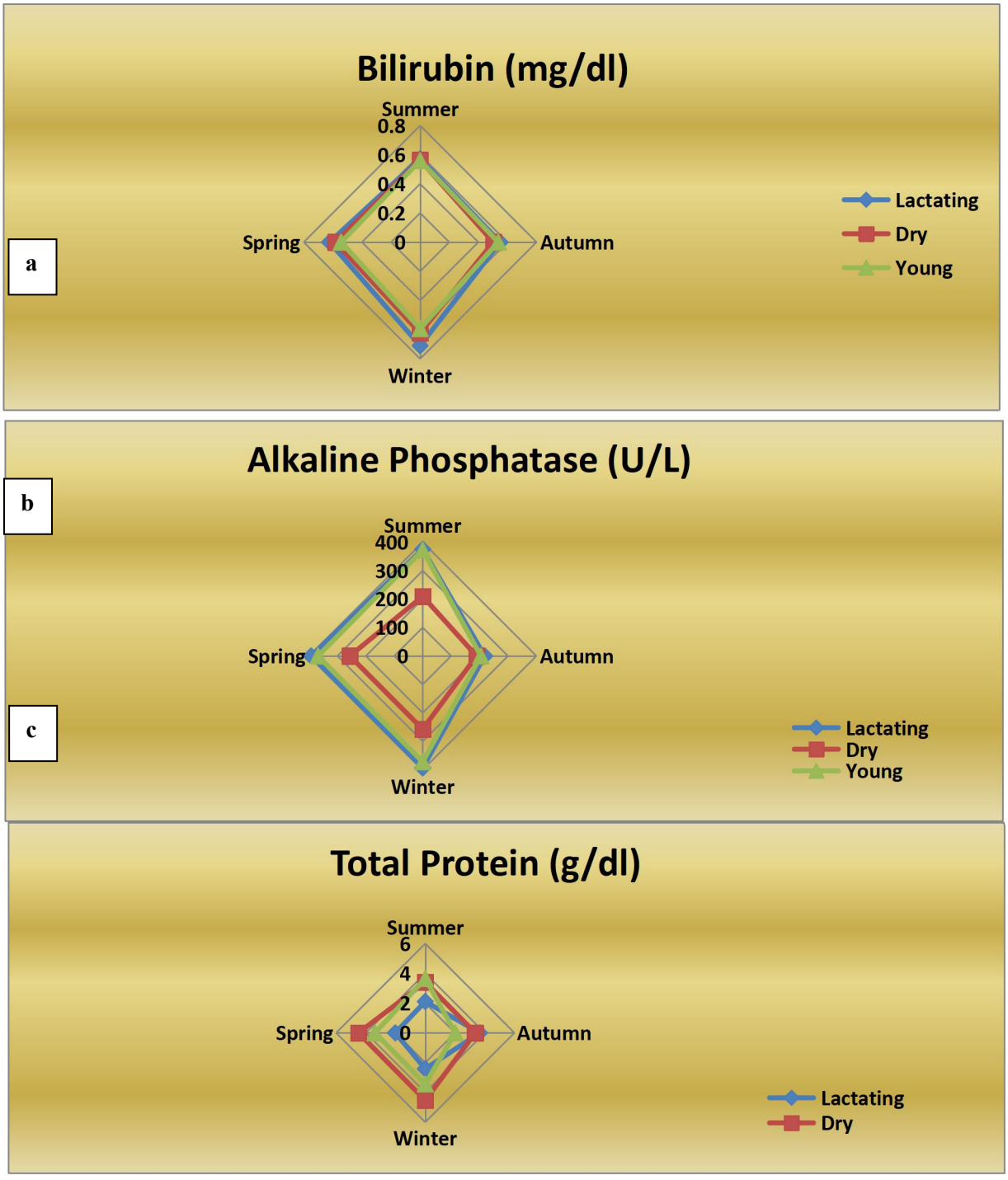
Table 13: ***Descriptive analysis for total proteins concentrations in blood plasma of buffaloes influenced by different seasons***

Parameters	Blood plasma		
	Lactating Buffaloes	Dry Buffaloes	Young Buffaloes
Mean	2.548	3.948	3.121
Std. Error±	0.066	0.069	0.069

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Fluctuations in bilirubin total values in blood plasma of buffaloes during different seasons

b

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Discussion

Lead (Pb) Dynamics in Soil, Forage, and Blood Plasma

According to the current study, soil Pb concentrations ranged from 15.37 to 16.94 mg/kg, with comparatively higher values seen in the winter and spring. Nevertheless, these concentrations showed mild contamination because they were still below the FAO/WHO allowable level. Khan et al. (2010) and Wei and Yang (2010) showed similar seasonal variations in Pb, attributing this variation to human contributions and climatic variables. The Pb levels found in this study are relatively lower than those seen in highly industrialized areas, indicating limited but ongoing environmental exposure. Changes in soil physical and chemical features including pH, redox potential, and water content could account for the seasonal variance. Pb availability is further increased by decreased microbial immobilization and increased soil moisture throughout the winter (Kabata-Pendias, 2011; Alloway, 2013). The observed variations may also be caused by irrigation techniques and air deposition.

Pb material in pasture showed more consistent absorption throughout seasons. This result is in line with reports by Sharma and Dubey (2005) and Yoon et al. (2006) that Pb shows little mobility in plants because of its significant adsorption to soil particles and root cell walls. Pb uptake appears to be mostly passive and confined at the root level based on the small variation. Mechanistically, Pb is frequently trapped in root vacuoles and binds firmly to carboxyl groups in cell wall pectins, inhibiting its transfer to aerial portions (Clemens, 2006). By reducing Pb toxicity in plants and stabilizing its content in fodder, this immobilization serves as a protective mechanism.

Pb concentrations in blood plasma varied significantly with the season, especially in young and lactating buffaloes. Wintertime levels were higher. Khan et al. (2015) observed similar results, suggesting that the main way that animals are exposed to lead is through feed. Young buffaloes have greater Pb levels, which is consistent with Goyer's (1997) findings that juveniles have immature detoxifying mechanisms and higher absorption efficiency. According to Jarup (2003) and Tchounwou et al. (2012), lead interferes with hemoglobin manufacturing by blocking enzymes such δ-aminolevulinic acid dehydratase (ALAD), which causes

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oxidative stress and poor oxygen transport. Furthermore, Pb damages cellular proteins, lipids, and DNA by producing reactive oxygen species (ROS), which may account for the systemic effects seen in this study.

Copper (Cu) Dynamics in Soil, Forage, and Blood Plasma

The findings revealed a consistent distribution of soil Cu contents with no discernible seasonal fluctuation. These results are in line with those of Kabata-Pendias (2011) and Marschner (2012), who noted that Cu's mobility is restricted by its high binding to clay minerals and soil organic matter. Khan et al. (2010) reported similar findings, indicating that Cu dynamics are less affected by seasonal variations than those of other metals. Cu's strong complexation with organic ligands, which minimizes leaching and preserves constant concentrations, is the fundamental cause of Cu's stability in soil (Alloway, 2013).

Cu contents in fodder also showed seasonal stability, indicating regulated absorption mechanisms. This is consistent with the findings of Yruela (2005) and Broadley et al. (2007), who highlighted the strict regulation of vital micronutrients like Cu in plants. Certain transporter proteins, such as COPT transporters, mediate the uptake of Cu, and excess Cu is detoxified by interacting with phytochelatins and metallothioneins (Clemens, 2006). Cu is kept within ideal physiological bounds because to these systems.

Cu levels in the plasma also did not significantly differ between buffalo groups, suggesting efficient homeostatic control. This is consistent with reports by Underwood and Suttle (1999) and McDowell (2003) that Cu metabolism in animals is strictly regulated by processes of intake, storage, and excretion. Cu is essential to biochemistry as a cofactor for enzymes including ceruloplasmin, superoxide dismutase, and cytochrome c oxidase. Antioxidant defense and oxidative metabolism depend on these enzymes. The animals' ability to retain normal metabolic function in spite of environmental exposure is suggested by the stability of Cu levels.

Zinc (Zn) Dynamics in Soil, Forage, and Blood Plasma

Zn concentrations in soil and fodder did not significantly vary seasonally in the current study, suggesting constant environmental availability and plant uptake. These results are in line with those of Alloway (2013) and Kabata-Pendias (2011),

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who said that the distribution of zinc in soil is generally steady under typical circumstances. In a similar vein, Broadley et al. (2007) noted that because zinc is crucial to metabolic activities, plant uptake is actively controlled.

However, in young and nursing buffaloes, Zn concentrations in blood plasma showed notable seasonal fluctuation. McDowell (2003) and Prasad (2008) observed similar results, emphasizing the impact of physiological status and dietary consumption on zinc levels. Seasonal variations in metabolic demands and fodder quality could be the cause of the observed fluctuation.

From a biological standpoint, zinc is an essential part of more than 300 enzymes, such as carbonic anhydrase, RNA polymerase, and DNA polymerase. It is essential for immunological response, antioxidant defense, and protein synthesis. By controlling the synthesis of metallothionein, zinc also stabilizes cell membranes and guards against oxidative damage. Therefore, variations in buffaloes' metabolic activity, immunological response, and stress adaptability may be reflected in seasonal variations in zinc levels.

Nickel (Ni) Dynamics in Soil, Forage, and Blood Plasma

Ni concentrations in soil remained stable across seasons, which is consistent with Kabata-Pendias (2011), who reported minimal variation in Ni levels under non-industrial conditions. However, forage Ni concentrations showed significant seasonal variation, suggesting that plant uptake is influenced by environmental factors. Similar findings were reported by Chen et al. (2009), who observed that Ni availability increases under higher soil moisture and temperature conditions. In blood plasma, Ni concentrations were relatively low, particularly in lactating and young buffaloes, indicating limited accumulation. This aligns with Das et al. (2008), who reported that Ni is poorly absorbed and efficiently excreted in animals.

Biochemically, Ni is required in trace amounts for certain enzymatic functions, such as urease activity in plants, but its role in animals is limited. Excess Ni can interfere with iron metabolism and enzyme activity, leading to oxidative stress. The low levels observed in this study suggest that Ni did not pose a significant toxicological risk under the given conditions.

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Heavy Metal Uptake, Bioconcentration Factor (BAF), and Translocation Factor (TF)

The study showed that *C. jwarancusa* and *A. donax* have significantly different metal uptake. Strong accumulation potential was shown by *C. jwarancusa* increased absorption percentages and BAF values larger than one. Baker et al. (2000) and Reeves and Baker (2000) reported similar results and categorized these plants as accumulators.

Detoxification processes, improved root absorption, and effective metal transport systems could all account for the high accumulation capacity. Phytochelatins and metallothioneins chelate metals that enter plant cells, facilitating their sequestration into vacuoles (Cobbett and Goldsbrough, 2002). Furthermore, antioxidant enzymes like catalase and superoxide dismutase aid in reducing oxidative stress brought on by metal buildup (Hall, 2002).

Arundo donax, on the other hand, displayed reduced BAF and TF levels, suggesting limited absorption and translocation. This behavior is in line with reports from Yoon et al. (2006) and Monni et al. (2001) that certain plants use exclusion methods to reduce metal toxicity. These include immobilization of metals in root tissues, which restricts their transport to aerial regions, and decreased membrane permeability.

Biochemical Responses in Buffaloes

Significant seasonal differences in bilirubin, alkaline phosphatase (ALP), and total protein levels were found by the biochemical study, suggesting physiological reactions to environmental exposure. During the winter, lactating and dry buffaloes had much higher bilirubin levels, which may indicate hepatic stress. Kaneko et al. (2008) observed similar results, connecting increased bilirubin levels to compromised liver function. Bilirubin is a biochemical byproduct of hemoglobin breakdown, and its build-up suggests a problem with hepatic absorption, conjugation, or excretion. By harming hepatocytes and blocking detoxification pathways, heavy metals like lead can affect liver function (Flora et al., 2012).

All buffalo groups' levels of alkaline phosphatase (ALP) varied significantly, with greater values seen in the winter and spring. An established biomarker of liver and

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bone problems is elevated ALP (Radostits et al., 2007). Heavy metal exposure has been linked to similar increases (Satarug et al., 2003).

Heavy metals cause oxidative stress, which damages cells .Significant variations were also seen in total protein levels, which reflect dietary and metabolic health. Coles (1986) and Tchounwou et al. (2012) revealed similar results, connecting heavy metal poisoning with changed protein levels. By interfering with ribosomal function and enzyme activity, heavy metals can cause disruptions to protein synthesis. Furthermore, protein breakdown brought on by oxidative stress might further change the levels of total protein.

Overall, the biochemical changes found in this study imply that buffaloes' liver function, enzyme activity, and metabolic processes can be impacted by even modest levels of heavy metal exposure. By changing exposure levels and physiological reactions, seasonal fluctuation further affects these impacts.

Effects on Hematological Parameters and Livestock Health

Animal physiology has been shown to be impacted by heavy metal exposure, especially in terms of hematological parameters. Reduced red blood cell (RBC) counts and anemia are caused by decreased heme synthesis, which is linked to elevated Pb levels. Similar to how Ni exposure is associated with oxidative stress and changes in immunological responses, Cd buildup can cause renal dysfunction and interfere with enzymatic systems (Khan et al., 2018; Ahmed et al., 2010).

The differences in blood plasma metal concentrations across immature, dry, and nursing buffaloes indicate varying vulnerability, which may be impacted by physiological condition and metabolic activity. For example, increasing metabolic demands in lactating animals may result in higher metal mobilization, which presents extra problems for milk safety and animal health.

Conclusion

This study provides a comprehensive assessment of heavy metal dynamics within a soil-plant-animal continuum and highlights the complex interactions between environmental exposure, plant behavior, and livestock health. Although the concentrations of Pb, Cu, Zn, and Ni in soils and forages remained largely within safe limits, their seasonal variability significantly influenced metal bioavailability and

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subsequent accumulation in buffaloes. The elevated levels of Pb and Zn in blood plasma particularly in young and lactating animals indicate increased vulnerability linked to physiological demands and metabolic activity. The observed alterations in key biochemical parameters, including bilirubin, alkaline phosphatase, and total protein, suggest that even low to moderate metal exposure can induce subclinical physiological stress, potentially affecting liver function and overall metabolic health. These findings emphasize the importance of integrating environmental risk assessment with animal health monitoring in livestock production systems. From a phytoremediation perspective, *C. jwarancusa* demonstrated strong metal accumulation and translocation capabilities, making it a viable candidate for remediation of contaminated soils. In contrast, *A. donax* exhibited limited uptake and mobility, reflecting its role as a metal excluder rather than an accumulator.

Declarations

Ethics approval

The Institutional Ethics and Guideline Committee of the University of Lahore, Lahore Pakistan has allowed all the protocols used in this experiment. All the experimental methods of this study followed all the appropriate guidance and regulations.

Consent for publication

All subjects gave their "informed consent" for the publication of details within the text ("informed consent") to be published in the above Journal and Article. Written "informed consent" was obtained from all authors for the publication of this manuscript.

Availability of data and materials

The data generated are provided within the manuscript and will be available from author at reasonable request

Competing Interest

All authors declare that there are no competing interests.

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