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Impact of Environmental Trace Elements on Blood Biomarker in Buffalo and Cow

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ABSTRACT

This research examined the temporal variability of blood albumin and alanine aminotransferase (ALT) levels in buffaloes and calves in relation to trace element exposure (Fe, Pb, Co Se, As, Mo) from soil and forage in six feeding pastures over three seasonal sampling periods. Soil lead (Pb) levels ranged from 26.36 to 31.616 mg/kg, while forage Pb peaked at 13.14 mg/kg in the first sampling period at pasture-E. Blood Fe concentrations in 3-month calves showed a significant decline from 285.27 to 180.92 µg/dl across sampling periods, while ALT levels in buffaloes ranged from 32.8 to 55.4 U/L, with the highest values observed in late sampling periods in 3-month and 9-month calves. Albumin concentrations remained relatively stable but demonstrated physiological-stage-specific variation, being highest in non-lactating buffaloes during the third period (5.12 g/dl). Soil arsenic (As) values were also elevated, reaching up to 29.681 mg/kg, while forage As concentrations exceeded 5 mg/kg in some pastures. Selenium (Se) in soil varied from 0.569 to 1.137 mg/kg, influencing blood mineral balances and enzymatic responses. These trace element fluctuations are closely mirrored in the enzymatic activity and protein levels in animal blood, highlighting environmental exposure as a modulating factor in liver health and metabolic stability.

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INTRODUCTION

The buffalo (*Bubalus bubalis*) is endemic to Asia and may be found primarily in the continent's tropical and subtropical regions. Buffaloes are divided into two categories based on their physical traits, wallowing behaviours, and uses: swamp buffaloes and river buffaloes. Swamp buffalos are generally raised in areas ranging from Assam to China, where they provide draft power. In contrast, river buffaloes are found in countries ranging from the Indian subcontinent to the Mediterranean region and Egypt, and are primarily used for milk and meat production (Martínez-Burnes*et al.*, 2024).

The Nili-Ravi is a well-known buffalo breed that originated in Punjab, Pakistan. Nili-Ravi buffaloes, known for their characteristic black coat, have a significant size disparity between sexes, with mature males reaching an average of 800 kg and females weighing an average of 525 kg. Rehman*et al.* (2021) describes them as having a strong, wedge-shaped form with short, spiralling horns and wall eyes. Nili-Ravi buffaloes are also known for having white markings on their forehead, face, snout, and legs, as well as a white-switched tail.

Buffalo are considered Pakistan's major dairy animal, providing for around 67% of total milk output (GOP, 2005). Buffalo are known for their endurance and amazing tolerance to harsh environmental conditions. They also have an extremely extended production phase, which can last up to a decade. Among Pakistan's two established buffalo breeds, the Nili-Ravi breed, which originated in the Sutlej and Ravi river valleys, dominates in Punjab (Duan*et al.*, 2025; Bashir *et al.*, 2017).

Iron is vital for plant growth because it is involved in several enzymatic activities and acts as a catalyst for chlorophyll synthesis (Rout and Sahoo, 2015). Young, developing plant tissues have a high requirement for iron. When iron levels are low, plants exhibit distinctive symptoms such as pale-colored juvenile leaves with prominent veins. Iron is leachable and accumulates in the soil's lower layers. Its availability to plants depends on pH, with acidic soils having the highest availability. Conversely, in alkaline soils, iron may be abundant, but its availability to plants is severely limited (Riaz*et al.*, 2020; Pandy, 2018).

Selenium is a component of glutathione peroxidase (Murray *et al.*, 2000). Animals grow lame, and mortality is primarily caused by malnutrition as a result of movement impediment. Cobalt is also recognized as vitamin B12 which has chief function in metabolism. Vitamin B12 is interrelated with Iron and Copper in hematopoietic and shortage of Cobalt in ruminant's results in anemia (Yaremchuk and Farionik, 2022).Arsenic enhances tissue culture growth and several

organic arsenicals have been shown to improve pig and poultry health and performance. Its function is similar to that of antibiotics and appears to be primarily focused on the suppression of dangerous intestinal microorganisms (Liao *et al*., 2020).

The purpose of this study is to determine the effect of ambient trace elements such as iron (Fe), lead (Pb), Cobalt (Co), selenium (Se), arsenic (As), and molybdenum (Mo), on liver function and protein metabolism in buffaloes, using blood plasma indicators such as albumin and alanine aminotransferase. The study's goal is to trace the source and channel of mineral exposure and assess the impact on animal health by analysing soil and fodder samples from various grazing sites at the same time. This study focusses on the possibility of chronic toxicity or deficiency associated with certain metals, as well as how they affect enzyme and protein levels in the blood. The findings are intended to help develop strategies for reducing environmental health risks and enhancing nutrient management methods in pasture-based buffalo production systems.

MATERIALS AND METHODS

AREA OF STUDY

"Livestock experiment station Khushab, Ckak No. 61/MB, Tehsil &District Khushab" was selected for the current investigation.Khushab is a city of Khushab District in the Punjab province of Pakistan.

Khushab, a city in Pakistan's Punjab province, is the administrative capital of Khushab Tehsil. Khushab, with a population of 102,793, is located about 100 miles (160 kilometres) southwest of Islamabad, the country's capital. The city has a hot and arid climate, with a diversified landscape of hills, plateaus, plains, and deserts. The Jhelum River flows eastward, and certain low-lying regions of Tehsil Khushab are prone to flooding during the wet and monsoon seasons (Figure 1).

According to the Punjab Development Statistics 2008, the district has a large number of domestic animals, including 345,000 buffaloes, 168,000 sheep, 167,000 goats, and 529,000 cattle. Given the district's low agricultural output, local farmers are increasingly turning to alternate forms of revenue, such as cattle, sheep, and goat fattening farms, dairy farms, and poultry farms, for a more consistent supply of money and milk.

SAMPLE COLLECTION

SOIL SAMPLES

Six feeding stations were randomly selected at 1-acre intervals throughout the area to gather soil samples. Soil samples were obtained at each location with a steel auger, burrowing to a depth of

12-15 cm to ensure coverage of all soil strata (Sanchez, 1976). This sampling procedure was performed three times with a two-month delay between each sampling event, yielding a total of 30 soil samples (five from each of the six sites). The collected samples were air-dried and then stored in labelled, sealed paper bags in a 60°C oven for 15 days.

FORAGE SAMPLES

Forage samples were obtained from the same locations as soil samples, using sterilised equipment. The forages tested were those consumed by Nili-Ravi buffalo, notably Barseem (*Trifoliumalexandrinum*), Sarson (*Brassica campestris*), and Oat (*Avena sativa*). Three rounds of sampling were carried out, with a two-month delay between each. Each sampling period yielded 30 samples (five duplicates from each pasture). To remove dust and pollutants, the selected samples were washed with distilled water and diluted HCl. The samples were then air-dried and placed in an oven at 50°C for 15 days to remove all moisture.

BLOOD SAMPLES COLLECTION

Blood samples were obtained from buffaloes based on their age and physiological status, including pregnant, lactating, and non-lactating animals. A total of 30 buffaloes were chosen, with 10 coming from each group. Similarly, 30 calves were chosen from three age groups (3, 6, and 9 months), with ten in each group. Blood samples were collected from the buffaloes' jugular veins while they were standing, using a sterile needle. To prevent clotting, the obtained blood was immediately transferred to heparinised sodium citrate vials. Following blood collection, the serum and plasma were separated using centrifugation. The serum was then separated into tiny labelled bottles and refrigerated at -20°C.

ANALYSIS OF SOIL AND FORAGE SAMPLES

After 15 days, soil samples were retrieved from the incubator and analysed at various levels using wet digestion (Vukadinović and Bertić, 1988). In a flask, 1 g of dried soil was digested with 4 ml of H_2SO_4 and 8 mL of H_2O_2 before being placed in the digestion chamber for around 30 minutes. After the vapours had dissipated, the sample was removed from the digesting chamber and 2 ml of H_2O_2 was introduced. The sample was then cooked again, and the process was repeated until the digestate turned colourless. The digested sample was filtered using Whatman filter paper (#42), diluted with double-distilled water to 50 ml, and kept in labelled plastic bottles.

BLOOD PLASMA ANALYSIS

Each sample's serum was placed in a flask and digested with 1 mL of H_2SO_4 and 2 ml of hydrogen peroxide (H_2O_2) in the digestion chamber. Following digestion, the sample was carefully

removed, diluted with double-distilled water, filtered through filter paper, and reduced to a final volume of 50 ml. The digested sample was then placed in labelled plastic bottles. A 0.05 m serum sample from each bull and calf was collected for biochemical examination, which included Albumin,Alanine Aminotransferase (ALT)levels.

DETERMINATION OF ALBUMIN

The serum albumin levels were determined using the bromocresol green (BCG) dye binding technique. The principle is based on BCG's yellow colour changing to blue green as it binds to albumin. Whole serum from healthy buffaloes was used as standard. The OD of the test serum was measured at 20 0C in a working buffered BCG reagent blank (de-ionized water) using a UV spectrophotometer at 640 nm. A standard curve was created by measuring the OD of standard serum with various volumes of BCG, and the concentrations of albumin in the sample sera were estimated.

DETERMINATION OF ALANINE AMINOTRANSFERASE (ALT)

The serum enzyme alanine transaminase (ALT) was measured by using commercially avalible colorimetric kits (AMP Medizintechnic GmbH, Austria Cat # BR0415, BR061 & BR0202, respectively). The principles for the leakage enzymes (ALT) measurements was to monitored the concentration of L-lactate or L-Malate with α-ketoglutarate and of p-nitrophenol formed with water at wavelength of 340 and 405 nm with a spectrophotometer.

HEAVY METAL ANALYSIS

Following wet digestion, mineral analysis was performed on soil, fodder, and serum samples using sophisticated spectroscopic techniques. The Perkin-Elmer AAS-5000 Atomic Absorption Spectrophotometer (Perkin-Elmer, 2004) and U-2900/2910 Double Beam Spectrophotometer were used to conduct the analysis. Atomic absorption spectroscopy (AAS) is a technique commonly used in analytical chemistry to determine the concentration of specific metal elements in a sample. In this study, AAS was used to determine the concentrations of important and harmful heavy metals in buffalo plasma, including lactation, non-lactating, and calves. The minerals such as Cobalt (Co) Lead (Pb), Selenium (Se), Molybdenum (Mo), Iron (Fe), and Arsenic (As) were being investigated.

STATISTICAL ANALYSIS

Data for various attributes were statistically analysed using the SPSS (17.0 edition) program, with a one-way analysis of variance. Steel and Torrie (1980) recommended that statistical significance between means be tested at the 0.05, 0.01, and 0.001 levels of probability.

RESULTS

BIOCHEMICAL ANALYSIS

BLOOD PLASMA OF 3 MONTHS CALVES (3MC)

The analysis of variance showed a significant ($P < 0.001$) effect of sample periods on uric acid and Alanine Aminotransferase (ALT) concentrations in the blood plasma of 3-month-old calves (3MC), but not on albumin concentration. The average albumin levels in 3MC's blood plasma were 3.94, 4.0, and 3.96 g/dl throughout the first, second, and third sample times, with the highest concentration recorded during the second sampling period. In contrast, the average ALT concentrations were 53.2, 28.8, and 55.4 U/L during the first, second, and third sampling periods, respectively, with the highest concentration detected during the third and lowest during the second (Table 1).

BLOOD PLASMA OF 6 MONTHS CALVES (6MC)

The findings demonstrated a significant effect in the blood plasma of 6-month-old calves (6MC). Significant effects ($P < 0.01$) were reported on albumin and alanine aminotransferase (ALT) concentrations, while uric acid concentrations was not significantly affected. The average albumin values in 6MC were 5.39, 3.9, and 4.04 g/dl throughout the first, second, and third sampling periods, with greater contents found during the first sampling period and lower contents during the second sample period. Similarly, the average ALT concentrations were 48.6, 34.8, and 50.2 U/L throughout the first, second, and third sampling times, with the highest concentration occurring during the third sampling period and the lowest during the second sample period (Table 2).

BLOOD PLASMA OF 9 MONTHS CALVES (9MC)

The analysis of variance showed that sampling times had a significant ($P < 0.01$) effect on albumin and alanine aminotransferase (ALT) concentrations in the blood plasma of 9-month-old calves (9MC), but not on uric acid concentrations. The average albumin levels in 9MC were 5.494, 4.14, and 4.98 g/dl during the first, second, and third sampling periods, respectively, with greater levels detected during the first and third sampling periods and lower levels during the second sample period. Similarly, the average ALT concentrations were 43.1, 36.4, and 48.2 U/L throughout the first, second, and third sampling times, with the highest concentration occurring during the third sampling period and the lowest during the second sample period (Table 3).

BLOOD PLASMA OF LACTATING BUFFALOES

The analysis of variance revealed a highly significant ($P < 0.001$) influence of sample times on

uric acid contents, a substantial ($P < 0.01$) effect on ALT concentration, and a non-significant effect on albumin concentration in the blood plasma of nursing buffalos. The average albumin values were 3.97, 4.14, and 4.18 g/dl for the first, second, and third sample periods, with the third sampling period having higher contents and the first sampling period having lower contents. The average ALT concentrations were 32.75, 32.8, and 40.4 U/L during the first, second, and third sampling periods, respectively, with the highest concentration occurring during the third and lowest during the first (Table 4).

BLOOD PLASMA OF NON-LACTATING BUFFALOES

The analysis of variance demonstrated a significant ($P < 0.01$) effect of sampling times on albumin and alanine aminotransferase (ALT) concentrations, but not on uric acid concentrations in the blood plasma of non-lactating buffaloes. The average albumin readings were 4.89, 3.84, and 5.12 g/dl for the first, second, and third sampling periods, with the third sample period having higher contents and the second sampling period having lower contents. The average ALT concentrations were 40.114, 32.8, and 34.4 U/L throughout the first, second, and third sampling times, respectively, with the highest concentration occurring during the first sampling period and the lowest during the second sampling period (Table 5).

BLOOD PLASMA OF PREGNANT BUFFALOES

The analysis of variance demonstrated a significant ($P < 0.01$) effect of sampling times on albumin and alanine aminotransferase (ALT) concentrations, but not on uric acid concentrations in the blood plasma of non-lactating buffaloes. The average albumin readings were 4.89, 3.84, and 5.12 g/dl for the first, second, and third sampling periods, with the third sample period having higher contents and the second sampling period having lower contents. The average ALT concentrations were 40.114, 32.8, and 34.4 U/L throughout the first, second, and third sampling times, respectively, with the highest concentration occurring during the first sampling period and the lowest during the second sampling period (Table 6).

HEAVY METAL ANALYSIS IN SOIL

HEAVY METALS IN SOILS AT SITE-A

The bar graph analysis showed a significant (P 0.01) effect of selenium (Se) concentration, a less significant (P 0.05) effect of iron (Fe) and arsenic (As) concentrations, and a non-significant effect of cobalt (Co), nickel (Ni), molybdenum (Mo), and lead (Pb) concentrations in soil at feeding site-A. Iron concentrations were highest during the third sampling period and lowest during the second sampling period. In contrast, the average As values were 25.85, 27.513, and 24.408

mg/kg during the first, second, and third sampling periods, respectively, with higher contents found during the second sampling period and lower contents during the third sampling period. The average Se concentrations were 1.64, 0.796, and 0.747 mg/kg for the first, second, and third sampling times, respectively (Figure 2A).

HEAVY METALS IN SOILS AT SITE-B

The analysis of variance revealed a highly significant ($P < 0.001$) effect of cobalt (Co) concentration, a significant ($P < 0.01$) effect of arsenic (As) concentration, a less significant ($P < 0.05$) effect of selenium (Se) concentration, and a non-significant effect of molybdenum (Mo) and lead (Pb) concentrations in soil at feeding site-B. The average As values were 24.33, 29.47, and 26.52 mg/kg during the first, second, and third sampling periods, respectively, with the second sampling period having higher contents and the first sample period having lower contents. In contrast, the mean Se contents were 0.992, 1.049, and 0.688 mg/kg during the first, second, and third sampling times, with the highest concentration detected during the second sampling period and the lowest during the third sample period (Figure 2B).

HEAVY METALS IN SOILS AT SITE-C

The analysis of variance showed, significant ($P<0.01$) effect of Co concentration, least significant ($P<0.05$) effect of Fe concentration and non significant effect of As, Se, Mo, and Pb concentration in soil at feeding site-C. The average As values in soil at feeding site-F were 26.72, 28.56 and 26.58 mg/kg during 1st, 2nd and 3rd sampling periods respectively. The As contents were higher during 2nd sampling period and lower were noticed during 3rd sampling period. The mean Se contents in soil at feeding site-F were 1.098, 0.912 and 0.694 mg/kg during 1st, 2nd and 3rd sampling periods respectively. The Se concentration was highest during 1st sampling period and lowest was found during 3rd sampling period (Figure 2C).

HEAVY METAL ANALYSIS OF FORAGE

HEAVY METAL ANALYSIS OF FORAGE AT PASTURE-A

The analysis of variance revealed a very significant influence of lead (Pb) concentration, a least significant effect of manganese (Mn) concentration, and no effect of iron (Fe), selenium (Se), or molybdenum (Mo) concentrations in fodder at feeding pasture-D. The average arsenic (As) levels were 5.276, 4.78, and 4.444 mg/kg during the first, second, and third sampling periods, respectively, but this was not considered significant in the original analysis. The As contents were lower during the first sampling period and even lower during the third sampling period. The mean Se content was 0.147, 0.15, and 0.176 mg/kg during the first, second, and third

sampling periods, respectively, with the highest concentration detected during the third and lowest during the first (Table8).

HEAVY METAL ANALYSIS OF FORAGE AT PASTURE-B

The analysis of variance revealed a highly significant ($P < 0.001$) effect of iron (Fe), arsenic (As), selenium (Se), and lead (Pb) concentrations, as well as a significant ($P < 0.01$) effect of cobalt (Co) concentration and a non-significant effect of molybdenum (Mo) concentration in forage at feeding pasture B. Despite the non-significant effect, the mean Mo concentrations were 12.62, 11.568, and 13.418 mg/kg during the first, second, and third sampling periods, respectively, with the highest concentration found during the third and lowest during the second. In contrast, the mean Pb concentrations were 13.14, 7.895, and 7.94 mg/kg during the first, second, and third sampling periods, respectively, with the highest concentration occurring during the first sampling period and the lowest during the second sampling period (Table9).

HEAVY METAL ANALYSIS OF FORAGE AT PASTURE-C

The analysis of variance revealed a highly significant ($P < 0.001$) effect of cobalt (Co) concentration, a least significant ($P < 0.05$) effect of iron (Fe), selenium (Se), and lead (Pb), and a non-significant effect of arsenic (As) and molybdenum (Mo) concentrations in forage at feeding pasture-C. The mean Se contents were 0.379, 0.143, and 0.141 mg/kg throughout the first, second, and third sampling times, with the highest concentration detected during the first sampling period and the lowest during the third sampling period. In contrast, the mean Pb concentrations were 8.64, 7.049, and 8.87 mg/kg during the first, second, and third sampling times, respectively, with the highest concentration detected during the third and lowest during the second (Table10).

DISCUSSION

BLOOD PLASMA IN CALVES AND BUFFALOES

The findings show dynamic fluctuations in biochemical parameters such as albumin, alanine aminotransferase (ALT), and uric acid across different age groups and physiological states of calves and buffaloes throughout three sample periods. These alterations are most likely due to developmental, metabolic, and physiological adjustments in response to ageing and reproductive status.

In calves, ALT activity fluctuated significantly across all age groups, with the lowest levels continuously observed during the second sample period. This pattern could reflect transitory hepatic adaptation or decreased metabolic load at this point. Notably, albumin levels

were considerably higher in older calves (6MC and 9MC), indicating that hepatic synthesis activity increases with maturation. Uric acid concentrations were mostly unaltered, except in 3MC, where a considerable variance indicated early metabolic turnover or renal processing abnormalities at younger ages. Our results are similar to previously reported by Carrillo-Muro*et al.* (2024). Albumin was the most prevalent proportion in both young and older calves, ranging from 42.8% in two-month-old calves to 50.6% in one-month-old calves (Nagy *et al.*, 2014).

Lactation and pregnancy proved to have a significant impact on buffalo biochemical profiles. ALT levels were considerably higher in nursing buffaloes during the third sample period, possibly indicating increased hepatic metabolism due to milk production needs. Similarly, lactating individuals' uric acid levels were dramatically impacted, which could be attributed to protein catabolism and oxidative metabolism. In contrast, albumin levels remained rather steady, indicating that protein homeostasis was maintained. Results in our study are similar to previously reported by Hussein *et al.* (2020) and El-Ashker*et al.* (2018).

Albumin and ALT levels in pregnant and non-lactating buffaloes varied significantly over time, with the lowest values occurring during the second sampling period in both groups. This trend could be attributed to a physiological decrease in hepatic activity or nutritional partitioning during mid-gestation, as well as energy conservation in non-lactating animals. Interestingly, uric acid concentrations remained steady in these groups, showing that reproductive state did not significantly affect purine metabolism.The serum Se concentration in ruminants was associated will albumin, glutathione and selenoprotien P (Awadeh*et al.*, 1998).

FORAGE AND SOIL HEAVY METAL CONTAMINATION

Heavy metal studies of soil indicated site-specific contamination patterns. Site-A exhibited high selenium accumulation as well as moderate iron and arsenic levels. Selenium levels rose in the first sampling period and then dropped, indicating episodic intake or leaching. At Site-B, cobalt, arsenic, and selenium levels varied significantly over time, indicating dynamic geochemical or anthropogenic impacts. Site-C had higher cobalt and iron contents, which were highest during the earlier sampling periods, indicating localised contamination likely caused by industrial runoff or agricultural inputs. Hu *et al.* (2021) explained that the less amount of cobalt in soil is not great deficiency to its high availability to plant. Similarly, Reid and Horvath (1980) explain the availability of cobalt from soil to plant. Co has high association with P and cu which make it severe mineral ruminants (McDowell *et al.*, 1984).

Forage samples followed soil contamination trends, with some amplification. A significantly

significant effect was seen for many metals at Pasture-B, including Fe, As, Se, Pb, and Co, raising concerns about longterm ingesting exposure. Notably, lead levels remained continuously high, especially during the first sampling period. The lead level in our study was higher than previously reported by Russi, (2008) andTsoi*et al.* (2016). Pasture-C also accumulated substantial amounts of cobalt and lead, both of which are known to have hepatotoxic effects at sub-lethal concentrations. These findings are consistent with the reported changes in ALT in blood plasma, indicating to probable liver stress due by environmental metal exposure. Results in our study are lower than Nwosu*et al.* (2009) and higher than Dong *et al.* (2012). The current study's mean Co concentration in soil was decreased than that reported by Cetin *et al.* (2022). These results appeared to be similar with those of Zheng*et al.* (2024).

The correlation between elevated environmental heavy metal levels (especially Pb, Co, Se, and As) and increased ALT activity in buffaloes and calves points to a possible relationship between environmental pollution and hepatic enzyme induction. ALT is a sensitive sign of liver stress, and its rise in lactating and pregnant buffaloes, as well as older calves, could be a direct result of dietary metal accumulation from forage. Similarly, fluctuations in albumin concentrations, which are exclusively synthesised by the liver, indicate hepatic involvement that may be controlled by contaminant load. The lack of considerable fluctuation in uric acid in most animal taxa, despite elevated As levels in soil and forage, may indicate restricted or regulated excretion under chronic low-level exposure. However, the considerable variability observed in 3-month-old calves suggests age-related sensitivity.Elevated As level in forage previously reported by Andersen *et al.* (2025), Reynaert*et al.* (2025) and Hussain*et al.* (2024).

CONCLUSION

The study indicates that elevated environmental levels of Pb, As, and Fe in soil and forage are reflected in animal blood enzyme and protein profiles, with significant impacts on ALT activity and albumin concentration. ALT levels increased substantially during the third sampling in most animal groups, suggesting cumulative hepatocellular stress likely exacerbated by prolonged exposure to trace metals. The decline in blood Fe over time, along with variable Se and Mo concentrations, points toward a complex interaction between environmental input and physiological regulation. Forage Se ranged from 0.131 to 0.379 mg/kg, and soil Se up to 1.137 mg/kg, indicating potential oxidative stress influences. These findings recommend the integration of environmental monitoring with animal health surveillance to ensure balanced mineral intake, reduce toxicity risks, and enhance metabolic efficiency in ruminants, particularly

under varying seasonal and physiological demands.

DECLARATIONS

ETHICS APPROVAL

The Institutional Ethics and Guideline Committee of the University of Sargodha (Approval No. 128-H33/2019 UOS) 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

Not applicable

AVAILABILITY OF DATA AND MATERIALS

The data are provided within the manuscript or supplementary information files.

COMPETING INTEREST

All authors declare that there are no competing interests.

FUNDING

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AUTHORS' CONTRIBUTIONS

Sajida collected and analyzed the samples; revised the original draft and prepared the graphs,All authors contributed in the manuscript.

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TABLE 1: BIOCHEMICAL PROFILE OF BLOOD PLASMA OF *3*MC (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Uric Acid	2.76±0.18	5.94±0.23	2.78±0.14	***
Albumin	3.94±0.24	4.0±0.08	3.96±0.19	**ns**
Alt	53.2±1.68	28.8±1.39	55.4±1.86	***

***= significant at 0.001level, ns =not significant

TABLE 2: BIOCHEMICAL PROFILE OF BLOOD PLASMA OF 6MC (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Uric Acid	3.786±0.42	4.52±0.23	3.94±0.32	ns
Albumin	5.39±0.20	3.96±0.06	4.04±0.28	**
ALT	48.6±3.52	34.8±1.49	50.2±2.81	**

***= significant at 0.001level,**=significant at 0.01 level, ns =not significant

TABLE 3: BIOCHEMICAL PROFILE OF BLOOD PLASMA OF 9MC (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Uric Acid	4.99±0.14	5.50±0.42	4.94±0.12	ns
Albumin	5.494±0.36	4.14±0.09	3.98±0.18	**
Alt	43.1±1.92	36.4±1.43	48.2±2.15	**

***= significant at 0.001level,**=significant at 0.01 level, ns =not significant

TABLE 4: BIOCHEMICAL PROFILE OF BLOOD PLASMA OF LACTATING BUFFALO (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Uric Acid	6.0±0.187	4.38±0.208	6.08±0.177	***
Albumin	3.97±0.160	4.14±0.143	4.18±0.086	ns
Alt	32.75±1.080	32.8±1.772	40.4±2.561	*

***= significant at 0.001level,**=significant at 0.01 level, ns =not significant

TABLE 5: BIOCHEMICAL PROFILE OF BLOOD PLASMA OF NON-LACTATING BUFFALO (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Uric Acid	6.69±0.221	6.16±0.220	6.0±0.187	ns
Albumin	4.89±0.386	3.84±0.128	5.12±0.168	**
Alt	40.114±0.665	32.80±1.827	34.40±1.435	**

***= significant at 0.001level,**=significant at 0.01 level, ns =not significant

TABLE 6: BIOCHEMICAL PROFILE OF BLOOD PLASMA OF PREGNANT BUFFALO (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Uric Acid	3.74±0.367	5.16±0.372	4.72±0.320	*
Albumin	3.932±0.150	3.62±0.188	5.50±0.130	***
Alt	34.194±2.711	36.8±1.562	37.2±1.428	ns

***= significant at 0.001level,**=significant at 0.01 level, ns =not significant

TABLE 7: MINERAL PROFILE OF SOIL AT FEEDING SITE-A (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Fe	30.71±1.142	30.672±0.789	35.523±1.275	*
Co	7.677±0.774	6.575±0.063	6.853±0.157	ns
As	25.85±1.079	27.513±0.700	24.408±0.403	*
Se	1.64±0.260	0.796±0.062	0.747±0.018	**
Mo	4.104±0.497	4.641±0.095	3.558±0.119	ns
Pb	30.361±0.583	31.616±1.201	31.089±0.414	ns

**=significant at 0.01 level, *=significant at 0.05 level, ns =not significant

TABLE 8: MINERAL PROFILE OF FORAGE AT PASTURE-A (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Fe	38.072±0.652	32.765±0.481	39.685±2.817	ns
Co	0.42±0.032	0.316±0.006	0.303±0.008	*
As	5.276±0.301	4.78±0.018	4.444±0.086	*
Se	0.147±0.0104	0.15±0.010	0.176±0.005	ns
Mo	11.79±2.630	12.657±0.633	12.683±0.436	ns
Pb	12.241±0.678	7.027±0.230	7.916±0.160	***

**=significant at 0.01 level, *=significant at 0.05 level, ns =not significant

TABLE 9: MINERAL PROFILE OF FORAGE AT PASTURE-B (MEAN ± S.E.)

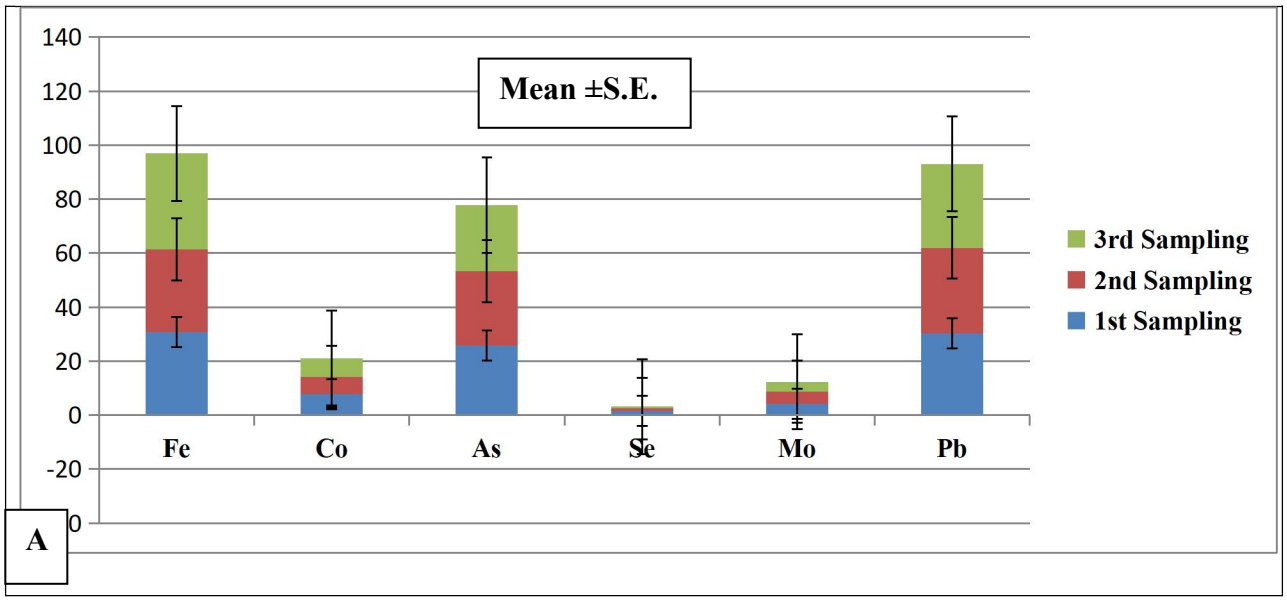
Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Fe	42.59±0.616	36.33±1.094	44.76±0.854	***
Co	0.548±0.049	0.363±0.010	0.342±0.006	**
As	3.768±0.179	4.748±0.041	4.656±0.084	***
Se	0.219±0.014	0.131±0.010	0.149±0.009	***
Mo	12.62±0.761	11.568±0.654	13.418±0.416	ns
Pb	13.14±0.818	7.895±0.482	7.94±0.235	***

**=significant at 0.01 level, *=significant at 0.05 level, ns =not significant

TABLE 10: MINERAL PROFILE OF FORAGE AT PASTURE-B (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Fe	40.27±0.720	33.82±1.417	40.76±2.784	*
Co	0.511±0.035	0.343±0.012	0.341±0.012	***
As	4.73±0.449	4.73±0.055	4.55±0.107	ns
Se	0.379±0.101	0.143±0.009	0.141±0.008	*
Mo	13.95±0.945	12.53±0.588	12.88±0.566	ns
Pb	8.64±0.534	7.049±0.429	8.87±0.265	*

FIGURE 1: STUDY AREA DISTRICT KHUSHAB, PUNJAB, PAKISTAN



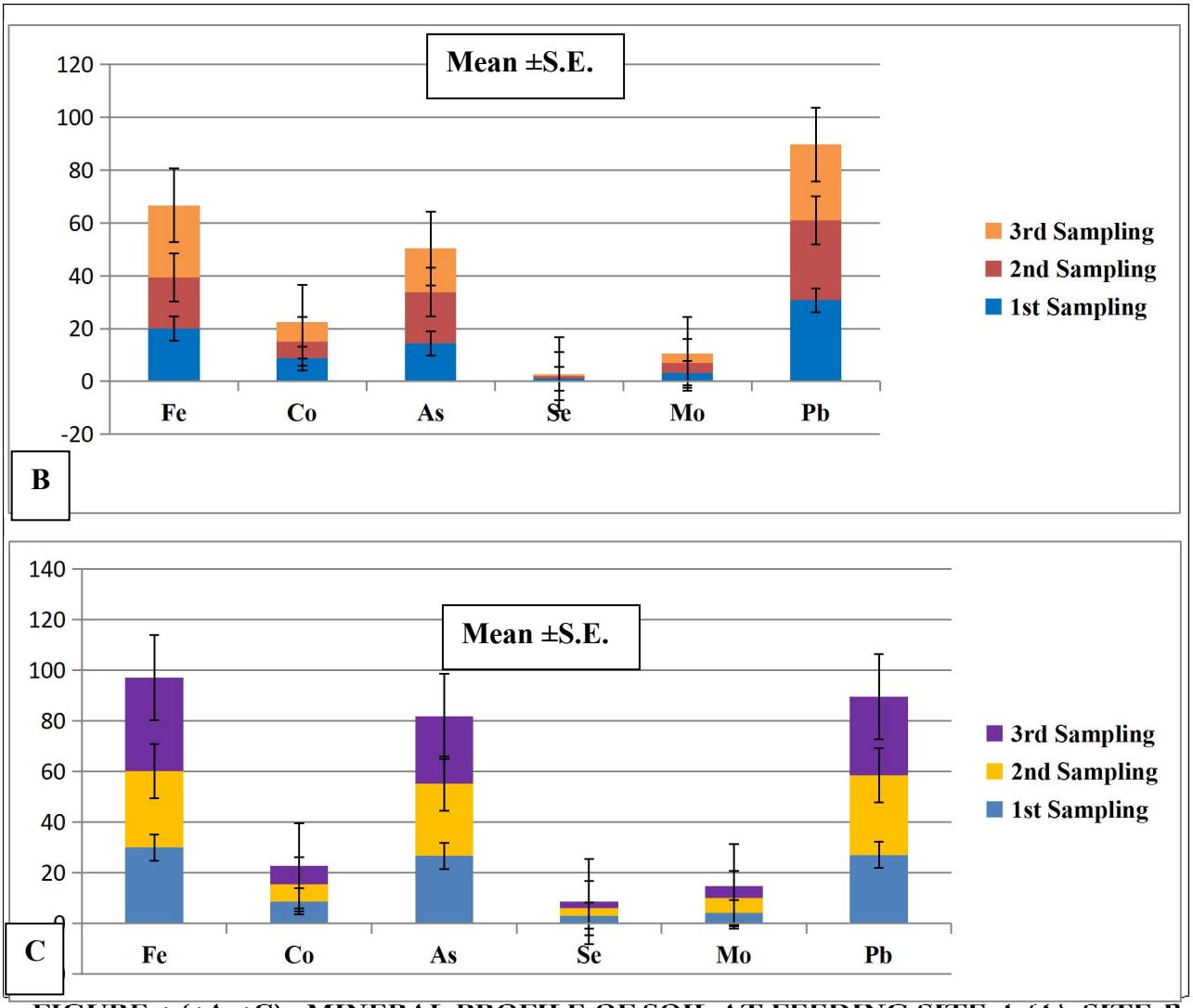


FIGURE 1 (1A-1C): MINERAL PROFILE OF SOIL AT FEEDING SITE-A (A), SITE-B (B), SITE-C (C) (MEAN ± S.E.)

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