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Assessment of Blood Biochemistry and Heavy Metal Contamination in Soil and Forages and their Implications for Livestock

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ABSTRACT

This study explored the influence of soil and forage metal contamination on blood plasma parameters such as calcium, phosphorus, uric acid, total protein, and trace elements (Cd, Cr, Cu, Mn, and Zn) in calves (3, 6, and 9 months) and buffaloes (lactating, non lactating, and pregnant) over three seasonal sampling periods. The soil cadmium (Cd) concentrations ranged from 7.397 to 11.699 mg/kg, with the highest concentration observed at feeding site C during the first period. Forage Cd levels peaked at 0.851 mg/kg in the third sampling period at pasture A. Blood Cd in 3-month-old calves was highest during the first sampling (8.567 mg/kg), which aligns with elevated soil values. Blood calcium in calves varied significantly ($P<0.001$), ranging from 8.52 to 10.16 mg/dl across periods, whereas phosphorus markedly increased from 2.96 to 9.18 mg/dl. Uric acid and total protein also followed a significant upwards trend during the second sampling, suggesting potential metabolic stress or dietary shifts. Zinc in forage peaked at 60.363 mg/kg, with corresponding blood levels showing periodic variation, potentially linked to mineral uptake efficiency. Elevated Cr and Mn in the soil and forage also coincided with increased blood concentrations. These patterns underscore the strong environmental influence on the mineral and biochemical status of animals, with implications for nutrient management and health monitoring.

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INTRODUCTION

The buffalo (*Bubalus bubalis*) was originally an Asian mammal, and most of it is found in Asia. The buffaloes were divided into swamp buffaloes and river buffaloes on the basis of their appearance, wallowing behaviors, and usage. Swamp buffaloes are cultivated in nations ranging from Assam to China and are used for drought, whereas the latter are cultivated in countries ranging from the Indian subcontinent to Mediterranean countries and Egypt and are utilized largely as a source of milk and meat (Cockril, 1974).

Niliravi is a buffalo breed found in Punjab Province, Pakistan. This buffalo breed is known for producing a large supply of milk. Niliravi Buffalo can be found in Punjab districts such as Lahore, Sheikhupura, Faisalabad, Sahiwal, Multan, Bahawal Nagar, and Sargodha (Vijaya, 2007). Buffalo are thought to be the country's primary dairy animal, accounting for approximately 67% of total milk production in Pakistan (GOP, 2005). These buffaloes are especially resistant to adverse weather and have an abnormally extended production period (up to ten years). Among Pakistan's two established buffalo breeds, Nili-Ravi, which originated in the valleys of the Ravi River, dominates Punjab. This breed is now popular throughout Pakistan, especially in Northwest Frontier Province, Sindh, Baluchistan, and Kashmir. Nili-Ravi buffaloe cattle outperform local and crossbred cattle in Muzaffarabad (Kuthu, 2007), indicating their tolerance to a variety of climate conditions.

Many other investigations, such as those conducted by Balakrishnan and Balagopal (1994) and Qureshi (1998), have indicated that stable concentrations of numerous biochemical constituents in the blood are required for the proper functioning of various physiological systems, including the reproductive system. Fluctuations in various biochemical nutrients have been linked to reproductive failure. As a result, the serum biochemical profile could be beneficial in determining these disorders.

Calcium is required for cell development and reproduction, as well as for enzyme activation and the formation of cell walls. It also regulates water transport within cell compartments. Calcium also aids in plant absorption of other minerals, such as nitrogen. Its insufficiency stunts new growth in stems, blooms, and roots, ultimately destroying plant growth (Jing *et al.*, 2024).

Phosphorus (P) is essential for seed germination, photosynthesis, protein synthesis, and, most importantly, feed growth and metabolic activities. It promotes flowering and fruit production in plants. If its availability is limited, plants will generate purple stems and leaves;

maturation and reproduction will be hampered, as will the formation of reproductive components and flowers.

Manganese (Mn) is required for photosynthetic pathways, respiration and nitrogen metabolism in green plants. Owing to its insufficiency, the plant leaves exhibit a green vein network with a light green backdrop. The light green sections turned white, and the leaves fell. The veins have brownish, black, or grayish markings (Khan *et al.*, 2005). Some of these problems stem from the role of manganese (Mn) ions as the most potent activator of glycosyltransferase enzymes in the production of mucopolysaccharides and glycoproteins (Leach 1974).

Zinc (Zn) is essential for the production of numerous plant growth hormones (auxins), as well as for plant development and growth. It is essential for glucose metabolism, protein production, and elongation of internodes (bark development). The visible and clear symptoms include spotted leaves and uneven chlorotic zones. Zinc has a deleterious effect on plant iron levels (Nicholson *et al.*, 1999).

The purpose of this study was to investigate the mineral dynamics and biochemical profiles of grazing buffaloes by analysing blood plasma levels of calcium, inorganic phosphorus, uric acid, and total protein, as well as trace elements such as cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn). To better understand the potential environmental impacts on these variables, soil and fodder samples from grazing sites were analysed simultaneously for the same elements. The goal was to determine whether these minerals are present at acceptable, deficient, or dangerous concentrations in the grazing habitat, as well as to investigate potential relationships between the environmental mineral content and animal health markers. The ultimate goal is to resolve nutritional imbalances via intelligent pasture and mineral supplementation procedures, improving the health and production of ruminant cattle.

MATERIALS AND METHODS

STUDY AREA

The district's administrative subdivision is Khushab Tehsil, and the city is its capital. Khushab, in Punjab, Pakistan, has 102,793 citizens and is located approximately 100 miles (160 kilometres) southwest of Islamabad, the Pakistani government's seat. Khushab has a very dry and hot climate. The topography consists mostly of hills, plateaus, plains, and deserts, with the Jhelum River running to the east.

Tehsil Khushab includes certain low-lying areas that flood during the rainy and monsoon seasons. According to Punjab Development Statistics 2008, the populations of different domestic animals, such as buffaloes, sheep, and goats, are 345, 168, 167, and 529 thousand, respectively. Because the district of Khushab generates comparatively few agricultural products, cattle/sheep/goat fattening farms, dairy farms, and poultry farms look to be better sources of money and milk for local farmers (Table 1).

SAMPLE COLLECTION

SOIL

For the collection of soil samples, six feeding locations were randomly selected at 1-acre intervals around the area to gather soil samples. The chosen locations were dug up to 12--15 cm deep with a steel auger to partially envelop all of the layers (Sanchez, 1976). The sampling was repeated three times with a two-month gap, yielding 30 soil samples (05 repetitions from each site). The collected samples were air dried and stored in labelled sealed paper bags in an oven at 60°C for 15 days.

FORAGE

The forage samples were collected from the same site as the soil samples via sterilized equipment. The Nilli ravi buffalo grazed on forage. The fodder samples collected included those from Barsem (*Trifolium Alexander*), Sarso (*Brassica compestrs*), and Oat (*Avanasarivxa*). Three samplings were carried out after a two-month hiatus. During each sampling interval, 30 forage samples (five replicates from each pasture) were collected. To remove dust particles and other contaminants, the plants were washed via distilled water. These samples were air-dried before being baked in an oven at 50°C for 15 days to remove all moisture.

BLOOD PLASMA COLLECTION

Blood samples were obtained from buffaloes categorized by age and physiological status, such as pregnancy, lactation, and non lactation.Thirty buffaloes were selected, ten from each class. Similarly, thirty calves were chosen from three classes (three months, six months, and nine months), with ten from each class. Blood samples were obtained from the buffalos' jugular veins while they were standing via a sterile needle. To prevent clotting, the collected blood was promptly transferred to heparinized Na-citrate voiles. Following blood collection, a centrifugation process was used to separate the serum from the collected plasma. The serum was then packaged in small labelled voiles and frozen at 20°C.

SAMPLE PREPARATION

REAGENTS

The work was carried out with distilled water obtained from the Biological Laboratory (University of Sargodha). The analytical reagents used included 10% hydrochloric acid, 40% sulfuric acid, and 35% hydrogen peroxide. Pyrex-branded glassware was used throughout the research. All glassware and plastic items were treated with 10% HCl for 24 hours before being rinsed with distilled water and then double distilled water.

SAMPLE PREPARATION

SOIL AND FORAGE

To study the samples at different levels after 15 days, the collected samples of soil were removed from the incubator and digested via the "wet digestion method" (Vukadinović and Bertić 1988). In a flask, one 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 approximately 30 min. Once the vapours had finished evaporating, the sample was removed from the digestion chamber. Two milliliters of H_2O_2 were added to the digestion chamber, and the mixture was heated again. The process was repeated until the material was colourless. The digested contents were removed from the digestion chamber, filtered through Whatman #42 filter paper, diluted with double distilled water to 50 ml, and stored in labelled plastic bottles.

One gram of oven-dried forage was placed in a flask and digested with 2 ml of H_2SO_4 and 4 mL of H_2O_2 in a digestion chamber for 30 min. When the fumes from the flask had finished evaporating, the sample was removed from the digestion chamber, and the process was repeated.

BLOOD PLASMA

One millilitre of each sample of serum was placed in a flask and digested with 1 ml of H_2SO_4 and 2 ml of hydrogen peroxide (H_2O_2) before being placed in the digestion chamber. Next, the digested material was carefully removed from the digestion chamber, diluted with double distilled water, filtered through filter paper to a volume of 50 ml, and packaged in marked plastic bottles. Each buffalo and calf provided a 0.05 ml serum sample for biochemical analysis of urea, glucose, calcium, inorganic phosphorus, protein, creatinine, and uric acid.

STANDARD PREPARATION

Prior to analysis with an atomic absorption spectrophotometer and a flame photometer, standard preparation is required to calibrate the instrument and obtain precise values. During this project, standards for Cr, Ni, Zn, Cu, Cd, and Mn were developed.

INSTRUMENTATION FOR BIOCHEMICAL TESTS

DETERMINATION OF GLUCOSE

Serum glucose was measured spectrophotometrically via the method published by Braham and Trinder (1972). Each buffalo had a 10 ml blood sample taken. Ten millilitres of blood were placed in a plane tube and centrifuged for 5–10 min to produce serum. Fresh serum was used to measure the glucose level in the serum. Serum glucose levels were tested via a premade kit (Randox, UK) and the Barham method (Barhan and Trindoer, 1972).

DETERMINATION OF URIC ACID

The MaxDiscovery™ Uric Acid Assay Kit is a straightforward and automated approach for testing uric acid levels in serum samples from any mammal. This kit utilizes a coupled reaction scheme: uric acid is initially converted by the uricase enzyme into allatoin, carbon dioxide, and hydrogen peroxide. In the second stage, hydrogen peroxide combines with a chromogenic dye via peroxidase to produce a noticeably colored (red) dye product.

The amount of dye product, as assessed by sample absorbance at 520 nm, is proportional to the concentration of uric acid in the solution. The kit includes a control solution with a uric acid standard (20 mg/dL) that is used to calibrate the test. Uric acid is a key byproduct of purine metabolism. Purine breakdown produces uric acid, which is expelled by the kidneys. The detection of serum uric acid is a useful marker for a variety of diseases. Elevated uric acid levels are significantly linked to gout, renal failure, and other diseases, including myeloproliferative disorders (Yunsheng*et al.*, 2009).

DETERMINATION OF TOTAL PROTEIN

The biuret technique was used to determine total serum protein levels. The test is based on the biuret reaction, which occurs when blood protein and peptides react with an alkaline copper sulfate solution to generate a violet-colored complex. Other nonprotein nitrogen molecules do not react. The color intensity of the complex formed during the Biuret reaction is related to the number of peptide bonds. The optical density (OD) of each serum sample was calculated against a reagent with a UV spectrophotometer (Labomed Inc., USA) at a wavelength of 540 nm.

DETERMINATION OF CALCIUM AND INORGANIC PHOSPHORUS CONTENTS

After coagulation and centrifugation to remove the proteins, the calcium and phosphorus contents of the blood serum were reliably quantified via an atomic absorption spectrophotometer in an air–acetylene flame (Willis 1960).

MINERAL ANALYSIS

After wet digestion, mineral analysis was performed on the soil, pasture, and blood samples via an Atomic Absorption Spectrophotometer (Perkin-Elmer AAS-5000). Atomic absorption spectroscopy is an analytical chemistry technique for determining the concentration of a certain metal element in a sample. The analysis was performed via AASP, in accordance with Lindsay and Norvell's (1978) method for mineral content determination in buffalo plasma (Table 2).

STATISTICAL ANALYSIS

Data for various attributes were statistically analysed via the SPSS (17.0 edition) program, with 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

GLUCOSE, URIC ACID, TOTAL PROTEIN, CALCIUM AND PHOSPHORUS

The tests were performed to assess uric acid, glucose, total protein, calcium and phosphorus in the blood plasma of buffaloes and ultimately in buffaloes at three different stages. The ANOVA results revealed a significant ($P<0.001$) effect of sampling interval on the glucose, uric acid, total protein, calcium, and phosphorus concentrations in the blood plasma of 3-month-old calves (Table 3).

The average glucose concentrations in the blood plasma at 3MC were 40.8, 66.0 and 41.0 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. A relatively high glucose concentration occurred during the 2nd sampling period, and the lowest concentration occurred during the 1st sampling period. The average uric acid contents in the blood plasma at 3MC were 2.76, 5.94 and 2.78 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. A relatively high uric acid concentration occurred during the 2nd sampling period. The mean total protein content in the blood plasma at 3MC was 5.34, 6.64 and 5.06 g/dl during the 1st, 2nd and 3rd sampling periods, respectively (Table 3).

The total protein concentration was highest during the 2nd sampling period and lowest during the 3rd sampling period. The mean calcium content in the blood plasma at 3MC was 10.16, 8.52 and 10.02 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. The calcium concentration was highest during the 1st sampling period and lowest during the 2nd sampling period. The average phosphorus content in the blood plasma at 3MC was 2.96, 9.18 and 3.92 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. The phosphorus concentration

reached a maximum during the 3rd sampling period, and a minimum occurred during the 1st sampling period (Table 3).

ANOVA (analysis of variance) revealed a significant (P<0.001) effect of sample time on glucose, total protein, and phosphorus contents, as well as a substantial (P<0.01) but nonsignificant effect on uric acid and calcium concentrations in the blood plasma of 6-month-old calves. The average glucose concentrations in the blood plasma at 6MC were 47.3, 70.2 and 50.6 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. A relatively high glucose concentration occurred during the 2nd sampling period, and the lowest concentration occurred during the 1st sampling period. The average uric acid content in the blood plasma at 6MC was 3.786, 4.52 and 3.94 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. A relatively high uric acid concentration occurred during the 2nd sampling period, and the lowest concentration was observed during the 1st sampling period.

The mean total protein content in the blood plasma at 6MC was 5.008, 7.8 and 5.22 g/dl during the 1st, 2nd and 3rd sampling periods, respectively. The total protein concentration was highest during the 2nd sampling period and lowest during the 1st sampling period. The mean calcium contents in the blood plasma at 6MC were 8.736, 9.46 and 9.18 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. The calcium concentration was highest during the 2nd sampling period and lowest during the 1st sampling period. The average phosphorus content in the blood plasma at 6MC was 3.57, 9.08 and 4.2 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. The phosphorus concentration reached a maximum during the 3rd sampling period, and a minimum occurred during the 3rd sampling period (Table 4).

The bar graph for analysis of variance revealed a highly significant effect of sampling period on the glucose, total protein, calcium and phosphorus contents and a nonsignificant effect on the uric acid concentration in the blood plasma of 09-month-old calves (Figure 1a).

The average glucose concentrations in the blood plasma at 9MC were 55.56, 87.4 and 55.8 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. A relatively high glucose concentration occurred during the 2nd sampling period, and the lowest concentration occurred during the 1st sampling period. The average uric acid contents in the blood plasma of 9MC were 4.99, 5.50 and 4.94 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. A relatively high uric acid concentration occurred during the 2nd sampling period, and the lowest concentration was observed during the 3rd sampling period. The mean total protein content in the blood plasma of 9MC was 4.502, 7.52 and 5.12 g/dl during the 1st, 2nd and 3rd sampling periods,

respectively. The total protein concentration was highest during the 2nd sampling period and lowest during the 1st sampling period (Figure 1a).

The bar graph for analysis of variance shows a highly significant effect of sampling period on the glucose, uric acid, total protein, calcium, and phosphorus contents. The average glucose concentrations in the blood plasma of lactating buffalo were 62.16, 102.4 and 60.8 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. A relatively high glucose concentration occurred during the 2nd sampling period, and the lowest concentration occurred during the 3rd sampling period. The average uric acid contents in the blood plasma of lactating buffalo were 6.0, 4.38 and 6.08 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. A relatively high uric acid concentration occurred during the 3rd sampling period, and the lowest concentration was observed during the 2nd sampling period. The mean total protein contents in the blood plasma of lactating buffalo were 4.58, 6.86 and 7.76 g/dl during the 1st, 2nd and 3rd sampling periods, respectively.

The mean calcium contents in the blood plasma of lactating buffalo were 7.28, 9.16 and 7.36 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. The calcium concentration was highest during the 2nd sampling period and lowest during the 1st sampling period. The average phosphorus content in the blood plasma of lactating buffalo was 3.4, 8.8 and 3.58 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. The phosphorus concentration reached a maximum during the 2nd sampling period, and a minimum occurred during the 1st sampling period (Figure 1b).

The analysis of variance revealed a highly significant effect of sampling period on the glucose, total protein, calcium and phosphorus contents and a nonsignificant effect on the uric acid concentration in the blood plasma of nonlactating buffalo. The average glucose concentration in the blood plasma of nonlactating buffalo was 64.79, 104.4 and 63.4 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. A relatively high glucose concentration occurred during the 2nd sampling period, and the lowest concentration occurred during the 3rd sampling period. The average uric acid content in the blood plasma of nonlactating buffalo was 6.69, 6.16 and 6.0 mg/dl during the 1st, 2nd and 3rd sampling periods, respectively. A relatively high uric acid concentration occurred during the 1st sampling period, and the lowest concentration was observed during the 3rd sampling period. The mean total protein contents in the blood plasma of nonlactating buffaloes were 5.94, 7.42 and 5.36 g/dl during the 1st, 2nd and

3rd sampling periods, respectively. The total protein concentration was highest during the 2nd sampling period and lowest during the 3rd sampling period (Figure 1c).

Analysis of variance revealed the least significant effect on uric acid and calcium concentrations and non significant effects on glucose, total protein and phosphorus in the blood plasma of pregnant buffalo (Table 5).

MINERAL ANALYSIS

The analysis of variance revealed that the Zn concentration had a significant influence, whereas the Cr, Mn, Ni, Cu, and Cd concentrations did not (Table 6).

The average Cr concentrations in the soil at feeding sites A were 0.077, 0.071 and 0.08 mg/kg during the 1st, 2nd and 3rd sampling periods, respectively. The Cr concentration was highest during the 3rd sampling period and lowest during the 2nd sampling period. The average Mn contents in the soil at feeding sites A were 8.567, 8.286 and 9.118 mg/kg during the 1st, 2nd and 3rd sampling periods, respectively. The highest Mn concentration occurred during the 3rd sampling period, and the lowest concentration was observed during the 1st sampling period. The average Ni content in the soil at feeding site A was 1.724, 1.24 and 1.324 mg/kg during the 1st, 2nd and 3rd sampling periods, respectively. The concentration of nickel (Ni) was the highest during the 1st sampling period, and the lowest concentration was detected during the 2nd sampling period.

The average Cu values in the soil at feeding site A were 7.32, 6.97 and 6.741 mg/kg during the 1st, 2nd and 3rd sampling periods, respectively. The Cu contents were greater during the 1st period of sampling and decreased during the 2nd period of sampling. The average Zn concentrations in the soil at feeding site A were 6.635, 5.35 and 7.433 mg/kg during the 1st, 2nd and 3rd sampling periods, respectively. The highest Zn concentration occurred during the 3rd sampling period, and the lowest concentration occurred during the 2nd sampling period (Table 6).

The average Cd concentrations in the soil at feeding site A were 8.567, 7.897, and 7.397 mg/kg for the first, second, and third sampling periods, respectively. The Cd levels were greater during the first sampling period and decreased during the third sampling period (Table 6).

The analysis of variance revealed that the Mn concentration had a significant ($P<0.01$) effect on the soil at feeding site B, whereas the Cr, Ni, Cu, Zn, and Cd concentrations did not (Table 7).

The analysis of variance revealed a highly significant ($P<0.001$) influence on the Mn concentration; a less significant ($P<0.05$) effect on the Ni concentration; and no significant effect

on the Cr, Cu, Zn, or Cd concentrations in the soil at feeding site C (Table 8).

FORAGE ANALYSIS

The ANOVA results revealed a substantial ($P<0.001$) influence on the Mn concentration; a significant ($P<0.01$) effect on the Cu and Zn concentrations; and no significant effect on the Cr, Ni, or Cd concentrations in the fodder at pasture A (Table 9).

The results revealed a significant ($P<0.001$) influence on the Mn concentration; significant ($P<0.01$) effects on the Ni, Cu, Zn, and Cd concentrations; and a nonsignificant effect on the Cr concentration in the fodder when the plants were fed pasture B (Table 10).

The results revealed a highly significant ($P<0.001$) influence on the Mn and Zn concentrations, a less significant ($P<0.05$) effect on the Cr and Cu concentrations, and a nonsignificant effect on the Ni and Cd concentrations in the fodder when the plants were fed pasture C (see Table 11).

BLOOD PLASMA ANALYSIS IN CALVES

The data were analysed for the heavy metals in the blood plasma of buffaloes at the 3-, 6- and 9-month stages of the study. The results revealed that the Cr, Mn, Cu, and Cd concentrations had a significant ($P<0.01$) effect on the blood plasma of 03-month-old calves, whereas the Ni and Zn concentrations did not (Table 12).

The results from the ANOVA revealed that there was a highly significant ($P<0.001$) effect on the Cr concentration; a significant ($P<0.01$) effect on the Mn concentration; a least significant ($P<0.05$) effect on the Zn concentration; and a nonsignificant effect on the Ni, Cu, and Cd concentrations in the blood plasma of 6-month-old calves (Table 13).

The analysis of variance revealed a highly significant ($P<0.001$) influence on the Zn concentration; a less significant ($P<0.05$) effect on the Mn concentration; and no significant effects on the Cr, Ni, Cu, and Cd concentrations in the blood plasma of 9-month-old calves (Table 14).

DISCUSSION

SOIL

At all six feeding sites, the chromium (Cr) level above the Gong *et al.* (2024) critical limit was 0.02 mg/kg. The reported soil Cr in our analysis was greater than that reported in earlier investigations (Chen *et al.*, 2024). Slag and solid waste generated during the chromate manufacturing process, when improperly disposed of in landfills, can be dormant sources of chromium exposure (Barceloux 1999). The mean Cr content in this study was lower than Wu *et*

al.'s (2010) critical limit (44.72 mg/kg). In the present study, the mean Cr concentration in the soil decreased compared with that reported by Shallari*et al.* (1998). These results appeared to be similar to those of Kelly *et al.* (1996).

Manganese (Mn) concentrations in soil above the essential level of 5 mg/kg established by McDowell *et al.* (1983) at all six feeding sites in the Khushab district.Prabowo*et al.* (1990) reported greater soil Mn concentrations in Indonesia, although these concentrations were lower than those determined by Espinoza *et al.* (1991). Mn availability in soil is determined by pH, organic matter content, moisture, and soil aeration levels. The manganese availability increases when the soil pH decreases. Manganese deficiency may occur in soils with high organic matter contents. It has an antagonistic effect on organic compounds. The mean Mn content in our study was lower than the USEPA threshold standard (80 ppm) set in 2000. The mean Mn content in our study was lower than the USEPA threshold standard (80 ppm) set in 2000.

The nickel (Ni) concentrations reported during the current study were lower than Adriano's (1986) threshold value of 0.85 mg/kg for all six feeding sites. A study of soils in Scotland (Berrow and Reaves, 1986) reported a geometric mean nickel concentration of 27 mg per kg. McGrath and Loveland (1992) performed an additional analysis of soils in England and Wales and reported a geometric mean amount of 20 mg kg, which is greater than the current findings. As a result, no hazardous conditions were discovered during the testing.

The present nickel (Ni) values in this study were lower than the USEPA ((2000)) essential level of 50 ppm. In the present study, the Ni concentrations were lower than the mean levels reported by Sharma *et al.* (2007) but comparable to those predicted by Fabis (1987). Ni levels in the soil were below the danger threshold. As a result, it is unlikely to cause any harm.

The copper (Cu) concentrations at all six calf feeding sites were slightly higher than McDowell *et al.*'s (1983) critical threshold of 1 mg/kg. The values obtained in this study were lower than those reported by Jerez *et al.* (1984) in Florida. The copper (Cu) concentration decreased below Wu *et al.*'s (2010) critical limit of 54 mg/kg. The copper levels in both soil surfaces in the current investigation were lower than those reported by Mapanda*et al.* (2005), but Ogebe and McDowell (1998) obtained similar results.

The soil zinc (Zn) concentrations were found to be above the threshold level of 1.5 mg/kg at all six calf feeding sites in the Khushab district (McDowell *et al.*, 1983). According to Dabkowsca-Naskret*et al.* (2004), the low level of available Zn in neutral soil is due to Zn being bound to iron oxide (FeO), making it unavailable to plants; however, in our investigation, a

sufficient amount of Zn was detected, exceeding the normal requirements for plant growth. The current zinc concentration in soil falls below the critical limit of 300--600 µg/g established by Indian standards (Awashthi, 2000).

The cadmium (Cd) concentrations measured in the soil samples during the current investigation were lower than the threshold value of 3 mg/kg reported by McDowell *et al.*(1985) at all six calf feeding sites. According to Ross (1994), soil Cd concentrations ranging from 3--8 mg/kg are considered harmful to organisms. According to these criteria, the soil Cd level in our findings was lower than the toxic limit, as previously reported by Gatashehet *al.* (2025). However, our findings were lower than the Cd content reported in soil by Aksoy *et al.* (1999) in Turkey and Abbas *et al.* (2023) in riparian vegetation in Faisalabad, Pakistan.The decreased concentrations of cadmium found during our investigation could be attributed to harvested crops and leaching, which occurs predominantly during the summer months. A recent study reported higher mean Cd concentrations than the threshold limit of 3 µg/g suggested by Indian standards (Awashthi, 2000).

FORAGE

The results of a forage study at different sites revealed that, compared with the crucial threshold of 3 mg/kg published by McDowell *et al.* (1985), all six pastures had decreased chromium (Cr) concentrations. The current experiment yielded higher results in the winter than in the summer. The mean Cr concentration of forage shoots subjected to various water treatments was lower than that reported by Ahmad *et al.* (2009). According to Anonymous (1980), chromium has been demonstrated to be significantly damaging to livestock health when levels are above the tolerance limit.

The results revealed that manganese (Mn) concentrations were lower than the threshold value of 40 mg/kg (McDowell, 1985) in all six pastures. Our current investigated levels were below the maximum permissible limit (NRC 1984). The lower concentration of Co in the soil could explain the higher level of Mn in the fodder, as these elements have an antagonistic effect on one another in the soil (McKenzie, 1975).

The mean nickel (Ni) forage values in all six pastures were somewhat lower than Adriano's (1986) critical limit of 4.3 mg/kg. According to Painter (1953), Ni forage concentrations between 40 and 60 are poisonous to plants, but the values obtained in our experiment were lower, indicating that there is no possible threat to the usage of these materials by ruminants. The reported Ni values were lower than those suggested for normal plants

(Tokalioglu&Kartal, 2005).

The findings revealed that all six pastures for the calves had mean copper (Cu) contents below the crucial level of 10 mg/kg proposed by McDowell *et al.* (1983). These concentrations were similar to those reported for Indonesia (Prabowo*et al.*, 1990) but lower than those reported in Guatemala by Tejada*et al.* (1987). The Cu content in the current study exceeded that reported in Florida by McDowell *et al.* (1982) and McDowell (1985) and that reported by Khan *et al.* (2007) in different studies conducted in Sargodha, Pakistan.

The zinc (Zn) concentration in fodder was 30 mg/kg in all six pastures. Furthermore, the all-mean Zn values measured during the current study were lower than expected. Dietary Zn consumption can increase the concentration of Zn in feed. According to the findings of this study, Zn supplementation is required for cattle grazing, as well as maintaining the relative ratios of Ca, Cu, and Cd concentrations in soil and forages, because Zn is involved in the absorption and utilization process.

The cadmium (Cd) values found in our study exceeded Kloke's (1994) limits of 0.03 mg/kg in all six pastures. Cicek (2004) reported that the optimum Cd level in plants is approximately 3.0 mg/kg. The Cd levels in our study were below the harmful threshold indicated by Aksoy*et al.* (1999). As a result, the current study revealed that the feed Cd level is safe for animals.

BLOOD PLASMA

The blood plasma glucose levels recorded at various study months were lower than those reported by Majeed*et al.* (1990) in buffaloes. Abnormal hormone-producing organ function can affect blood glucose levels (Coles, 1986).

The origin of hyperpituitarism is unknown, but it may be linked to increased production of adrenocorticotrophic hormones (Coles, 1986). The plasma glucose levels measured in this study are similar to those reported by Anita *et al.* (2010) for normal buffaloes. Singh *et al.* (2010) discovered, however, that plasma glucose is not a metabolic regulator implicated in the onset of ovarian cyclicity. Hypoglycemia in buffaloes inhibits signal transmission along the hypothalamic–hypophyseal–ovarian axis, resulting in anoestrus (Sharma *et al.*, 1998).

The total protein concentration measured in plasma at all sample intervals was lower than the values reported by Fekry *et al.* (1989). The physiological state of buffaloes significantly affects the serum levels of various blood components. Non pregnant, non lactating buffaloes had

the highest serum, glucose, and calcium levels, but the total protein content was lowest in all three categories. All of these variables could be attributed to variations in animal metabolism, milk production requirements, and metabolic changes associated with foetal development.

Buffalos under mild heat stress use amino acids as an energy source more efficiently, resulting in higher urea levels or protein mobilization from muscle mass (Fekry*et al.* 1989). In the present study, the buffaloes presented higher mean blood urea levels, which could be related to differences in protein metabolism or renal BUN excretion in the animals, as BUN is the principal end product of protein synthesis. The results obtained are congruent with those previously reported by Kulkarni and Talvelkar (1993).

Thermal stress in hot surroundings has been shown to promote peripheral vasodilation, allowing body heat to be lost by perspiration and thus reducing blood flow to interior organs. In addition, dehydration can limit blood flow to the kidneys. Heat stress impairs the ability of the kidneys to operate normally. Creatinine excretion is regulated by the glomerular filtration rate, and it is removed more easily than urea is (Guyton and Hall, 1996).

The parathyroid hormone regulates serum calcium levels, acting on bones and kidneys to maintain them steadily in animals. The parathyroid gland detects blood calcium levels in the carotid artery and secretes parathyroid hormone, which activates the renal calcium resorption mechanism, resulting in decreased urine calcium loss (Reece, 2005). In the present study, the buffaloes had increased serum calcium levels, which clearly supported this finding. These findings are similar to those reported by Haque and Verma (1990) in buffaloes.

Phosphorus is essential for protein and enzyme production in the body, as well as for the intermediate metabolism of carbohydrates and creatinine in muscular contraction reactions (Sastry and Rama Rao, 2009). However, Randhawa*et al.* (2006) reported lower values in buffaloes. Animals with low serum glucose and calcium levels clearly had an altered albumin globulin ratio with increased globulin, although the serum urea nitrogen, creatinine, and total protein concentrations remained constant. According to the results presented above, all the biochemical profile values are within the normal range, indicating that no harm was caused to dairy livestock.

BLOOD PLASMA ANALYSIS IN CALVES AT 3 DIFFERENT STAGES

All six buffalo groups had lower chromium (Cr) concentrations in their blood than the average value of 0.34 mg/L reported by Christensen *et al.* (1993). However, Cr supplementation has increased the growth rate of numerous grazing animals (Li *et al.*, 2004). Chromium levels in blood tests indicated a Cr deficit in this specific location. However, mineral supplementation is

essential for fulfilling the cost–benefit ratios of feeding mineral combinations such as Cr to ruminants in this study region.

The findings revealed that the mean plasma manganese (Mn) concentration in buffaloes exceeded the NRC (1996) criterion of 0.05 mg/L at various sample intervals. As a result, no supplements for ruminants were advised. These findings contradict those of Khan *et al.* (2006), who reported lower levels of Mn in blood plasma while working on different animal farms in southwestern Punjab, Pakistan. The elevated blood plasma Mn values could be related to the higher feed Mn levels in the ruminants in the current study.

The nickel (Ni) values measured in the present study were less than the critical limit of 0.25 mg/L reported by Yazar*et al.* (2006) for all three buffalo groups. These different findings could be attributed to differences in geographic area, diet composition, sampling season, etc. (Erdogan, 2002). Ni supplementation for grazing ruminants should be avoided since the cattle on this ranch may ingest more Ni, raising health concerns.

The copper (Cu) values were lower than McDowell's (1997) threshold limit of 0.65 mg/L in all six buffaloes. The values discovered during this investigation were lower than those reported by Jerez *et al.* (1984) in Florida. Khan *et al.* (2008) investigated data on heavy metals in Sargodha, Pakistan, with similar results that were above the critical level. Plasma Cu values that are typically less than 0.65 mg/L are thought to suggest deficiency in animals. As a result, the animals on this ranch should receive a copper supplement.

The results revealed that in all six buffalo groups, the mean zinc level was less than the essential value of 0.80 mg/l reported by McDowell (1997). The current investigation revealed a lower mean zinc concentration than Mills *et al.* (1967). Zinc concentrations in blood plasma vary among buffaloes on the basis of infection and dietary limitations. The zinc serum concentration reduces heat stress and ketosis in ruminants and increases the incidence of mastitis, particularly in elderly cows (Soliman*et al.*, 2024).

CONCLUSION

This study revealed a clear relationship between soil/forage metal concentrations and fluctuations in blood calcium, phosphorus, protein, uric acid, and trace element profiles. The consistent detection of elevated cadmium in both soil (up to 11.699 mg/kg) and forage (up to 0.851 mg/kg) corresponds with high blood Cd levels in calves and buffaloes, indicating a possible bioaccumulation pathway. Blood calcium and phosphorus varied significantly with sampling period and age/physiological state, reflecting not only dietary absorption but also the

antagonistic or synergistic effects of trace metals such as Zn, Cr, and Mn. These results emphasize the importance of routine trace metal monitoring in ruminant environments, particularly in intensively grazed areas, to mitigate the risk of subclinical mineral imbalances and improve herd productivity through informed mineral supplementation strategies.

DECELERATIONS

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.

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AUTHORS' CONTRIBUTIONS

Sajida collected and analyzed the samples; revised the original draft and prepared the graphs,

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TABLE 1: FARM AREA & ITS DISTRIBUTION

Total Area		**971-Acres**
i.	**Direct Cultivable**	**900-Acres**
	Canal Irrigated	900-Acres
Tubewell Irrigated		_ _ _ _
Barani		_ _ _ _
ii.	**Un-Cultivable**	**40-Acres**
iii.	**Road % Buildings etc.**	**31-Acres**

TABLE 2: DETECTION LIMITS OF ATOMIC ABSORPTION SPECTROPHOTOMETER

Elements	Detection limits
Cadmium (Cd)	0.8 (Flame AA)
Chromium (Cr)	3 (Flame AA)
Copper (Cu)	15 (Flame AA)
Cobalt (Co)	9 (Flame AA)
Manganese (Mn)	1.5 (Flame AA)
Zinc (Zn)	1.5 (Flame AA)

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

Mineral	(Mean + S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Glucose	40.8+0.86	66.0+1.51	41.0+1.64	***
Uric Acid	2.76+0.18	5.94+0.23	2.78+0.14	***
Total Protein	5.34+0.067	6.64+0.15	5.06+0.20	***
Calcium	10.16+0.22	8.52+0.18	10.02+0.17	***
Phosphorus	2.96+0.32	9.18+0.22	3.92+0.13	***

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

TABLE 4: BIOCHEMICAL PROFILE OF BLOOD PLASMA OF 6MC (MEAN ± S.E.)

Mineral	(Mean + S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Glucose	47.3+3.26	70.2+2.88	50.6+2.29	***
Uric Acid	3.786+0.42	4.52+0.23	3.94+0.32	ns
Total Protein	5.008+0.25	7.8+0.17	5.22+0.12	***
Calcium	8.736+0.30	9.46+0.26	9.18+0.30	ns
Phosphorus	3.57+0.17	9.08+0.20	4.2+0.12	***

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

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

Mineral	(Mean + S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Glucose	68.324+3.211	69.0+1.760	68.0+2.280	ns
Uric Acid	3.74+0.367	5.16+0.372	4.72+0.320	*
Total Protein	6.41+0.338	6.22+0.321	5.66+0.352	ns
Calcium	8.26+0.284	7.22+0.233	6.66+0.557	*
Phosphorus	3.52+0.227	3.98+0.407	3.38+0.124	ns

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

TABLE 6: MINERAL PROFILE OF SOIL AT FEEDING SITE-A(MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Cr	0.077±0.004	0.071±0.003	0.080±0.007	ns
Mn	8.567±0.382	8.286±0.123	9.118±0.316	ns
Ni	1.724±0.281	1.24±0.023	1.324±0.067	ns
Cu	7.32±0.531	6.97±0.413	6.741±0.128	ns
Zn	6.635±0.306	5.35±0.324	7.433±0.445	**
Cd	8.567±0.382	7.897±0.575	7.397±0.551	ns

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

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

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Cr	0.072±0.007	0.061±0.006	0.067±0.008	ns
Mn	7.55±0.457	7.512±0.290	9.545±0.091	**
Fe	32.15±1.019	29.39±0.676	29.76±1.501	ns
Ni	1.502±0.219	1.528±0.031	1.534±0.086	ns

Cu	5.414±0.882	6.984±0.249	7.15±0.107	**ns**
Zn	6.289±0.711	6.322±0.109	7.146±0.434	**ns**
Cd	7.55±0.457	7.97±0.565	7.997±0.210	**ns**

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

TABLE 8: MINERAL PROFILE OF SOIL AT FEEDING SITE-C(MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Cr	0.058±0.014	0.048±0.005	0.067±0.004	**ns**
Mn	9.25±0.487	6.66±0.286	9.27±0.048	***
Ni	1.77±0.137	1.37±0.119	1.80±0.024	*
Cu	7.073±1.02	6.991±0.495	7.681±0.171	**ns**
Zn	6.332±0.257	7.016±0.661	6.155±0.231	**ns**
Cd	9.249±0.487	7.299±0.616	8.242±0.376	**ns**

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

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

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Cr	6.43±0.468	6.17±0.201	6.35±0.213	**Ns**
Mn	47.74±1.670	37.04±0.583	40.22±1.068	***
Ni	8.523±0.451	7.145±0.476	7.74±0.526	**Ns**
Cu	13.77±1.071	8.352±0.612	8.948±1.066	**
Zn	41.896±1.393	60.363±6.087	58.121±0.488	**
Cd	0.747±0.077	0.838±0.034	0.851±0.028	**Ns**

***= significant at 0.001level, 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	
Cr	6.926±0.435	6.451±0.191	6.898±0.239	**ns**
Mn	51.49±0.632	41.16±1.268	41.79±0.796	***

Ni	9.745±0.858	6.33±0.501	7.671±0.402	**
Cu	14.89±0.867	12.084±1.132	9.58±0.494	**
Zn	44.51±1.192	54.396±2.263	55.312±1.183	**
Cd	0.628±0.040	0.832±0.048	0.841±0.030	**

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

TABLE 11: MINERAL PROFILE OF FORAGE AT PASTURE-C (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Cr	8.046±0.406	7.391±0.069	7.047±0.147	*
Mn	51.882±1.094	43.497±1.39	41.72±1.147	***
Ni	8.288±1.031	6.909±0.62	8.223±0.457	**ns**
Cu	15.05±1.298	10.76±0.803	11.28±0.446	*
Zn	38.499±2.791	56.748±0.743	57.58±0.668	***
Cd	0.765±0.037	0.769±0.042	0.775±0.029	**ns**

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

TABLE 12: MINERAL PROFILE OF 3MONTH CALVES (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Cr	0.512±0.020	0.455±0.011	0.459±0.012	**
Mn	0.0697±0.006	0.079±0.004	0.094±0.006	**
Ni	0.557±0.0322	0.548±0.0316	0.521±0.0224	**Ns**
Cu	1.145±0.175	0.693±0.011	0.736±0.032	**
Zn	0.825±0.208	0.452±0.013	0.432±0.009	**Ns**
Cd	0.165±0.0087	0.126±0.006	0.103±0.007	**

***= significant at 0.001level, **=significant at 0.01 level, *=significant at 0.05 level, ns =not significant

TABLE 13: MINERAL PROFILE OF 6 MONTH CALVES (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Cr	0.605±0.024	0.447±0.012	0.441±0.014	***
Mn	0.066±0.002	0.070±0.007	0.095±0.007	**
Ni	0.451±0.088	0.507±0.014	0.488±0.010	**ns**
Cu	1.121±0.154	0.844±0.029	0.85±0.018	**ns**
Zn	0.951±0.128	0.613±0.018	0.573±0.021	*
Cd	0.167±0.151	0.151±0.008	0.144±0.012	**ns**

***= significant at 0.001level, **=significant at 0.01 level, *=significant at 0.05 level, ns =not significant

TABLE 14: MINERAL PROFILE OF 9 MONTH CALVES (MEAN ± S.E.)

Mineral	(Mean ± S.E.)			Significance Level
	1st Sampling	2nd Sampling	3rd Sampling	
Cr	0.619±0.020	0.625±0.041	0.655±0.047	**ns**
Mn	0.071±0.002	0.071±0.004	0.087±0.005	*
Ni	0.527±0.029	0.468±0.005	0.464±0.016	**ns**
Cu	1.439±0.171	1.183±0.035	1.059±0.089	**ns**
Zn	1.517±0.160	0.709±0.027	0.665±0.022	***
Cd	0.184±0.023	0.148±0.006	0.154±0.009	**ns**

***= significant at 0.001level, **=significant at 0.01 level, *=significant at 0.05 level, ns =not significant

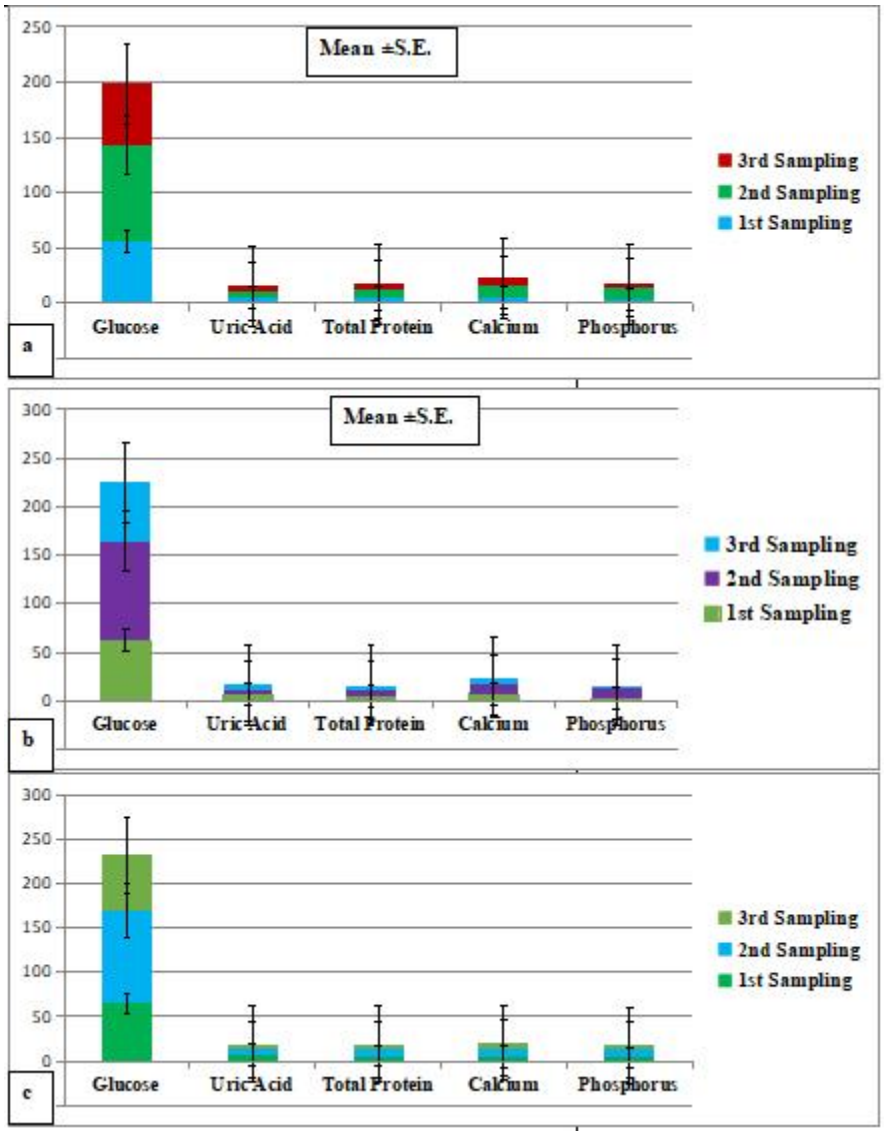

FIGURE 1 (1A-1C):BIOCHEMICAL PROFILE OF BLOOD PLASMA OF 9MC (MEAN ± S.E.)

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