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Research Article

Copper Status in Soil, Forage, and Serum and its Association with Blood Parameters in River/Water Buffalo in Babylon Province, Iraq

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Abstract | Many ruminants consume diets that do not meet their nutritional requirements due to the types of forage available and low levels of trace elements in the soil, particularly copper, which directly affects the mineral status of livestock. The current study aimed to evaluate copper levels in soil, forages, and the serum of water buffaloes, and to assess their relationship with various physiological parameters in Babylon Province, Iraq. A total of 180 water buffaloes of both sexes and varying ages were randomly selected from three regions (south, middle, and north) of Babylon Province during the period from January to December 2024. Soil and forage samples, including barley grass, alfalfa grass, and fresh rice straw, were collected from grazing areas, and copper concentrations were measured using atomic absorption spectrometry (AAS). Blood samples were also collected from the jugular vein for hematological and biochemical analysis. The results showed a significant decrease in copper levels in the soil of the middle (6.5 ± 0.35 ppm) and north (5.30 ± 0.29 ppm) regions compared to the reference value of 9.6–76.5 ppm, while the south region remained within the normal range (11.3 ± 0.67 ppm). Copper levels in all forage types were below their respective reference values across all regions. In barley grass (reference: 6–25 ppm), mean copper levels were 1.70 ± 0.18, 2.3 ± 0.08, and 0.6 ± 0.08 ppm in the south, middle, and north, respectively. In alfalfa grass (reference: 11–30 ppm), the values were 1.62 ± 0.07, 1.95 ± 0.05, and 1.0 ± 0.05 ppm, while in fresh rice straw (reference: 0.06–0.1 ppm), the values were 0.05 ± 0.01, 0.01 ± 0.00, and 0.01 ± 0.00 ppm, respectively. Additionally, 91.66% of the water buffaloes showed serum copper concentrations below the reference range of 13.91–15.82 μmol/dL, with a mean value of 5.99 ± 0.42 μmol/dL. Hematological results revealed significant decreases in red blood cells (3.91 ± 4.27 × 10^6/mm^3), hemoglobin (3.77 ± 4.48 g/dL), and hematocrit (19.67 ± 22.14%), compared to their respective reference values. Biochemical analysis also showed reductions in total protein (2.80 ± 4.00 g/dL) and albumin (1.64 ± 1.78 g/dL). The study concludes that there is a widespread copper deficiency in the soils and forages of Babylon Province, which is strongly associated with reduced serum copper levels and significant alterations in hematological and biochemical parameters of water buffaloes.

Keywords | Copper, Analysis, Forage, Soil, River, Water buffalo

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INTRODUCTION

Copper (Cu) is crucial to numerous biological functions; it is a necessary trace element for all living organisms. It is a co-factor of a number of vital enzymes, such as cytochrome C oxidase, tyrosinase, and lysyl oxidase. These enzymes are needed for the production of collagen, myelin, keratin, and hemoglobin, as well as for efficient immunological and antioxidant mechanisms. However, the most prevalent trace element insufficient in ruminants is Cu, which is characterized by decreased production and reproductive capabilities, immune function depression, weakness in the bones, heart failure, and a loss of hair color, especially around the eyes (Balegi, 2023).

Some soils across the world don't give enough of Cu to plants-and subsequently, livestock. Animals fed such low-Cu forages may have a variety of deficiency symptoms (Hussein *et al*., 2025). A typical Cu deficiency occurs in two types of soil. The first are the sandy soils and muck soils recovered from wetlands, sometimes known as "peat," are the second significant type and are more frequently linked to Cu deficiency. Cu may be completely absent from these soils, but more often it is not present to plants, therefore there is insufficient Cu present (Constable *et al*., 2016). The lower Cu levels could be attributed to reasons such as soil pH, biological material content, and agricultural practices. Alkaline soils, common in arid and semi-arid regions, tend to reduce Cu availability due to its immobilization in insoluble forms (Alloway, 2012). The soils of Iraq's southern alluvial plains are of Quaternary origin. These soils are primarily formed from river flood deposits and are characterized by storm-related sedimentation (Jassim and Goff, 2006). The texture of the soil was correlated to the quantity of Cu present i.e., sandy soils had lower Cu levels than loam soils, whereas clay soils had higher Cu levels. This is because Cu is more closely associated with clay minerals than it is with sand alone. The Cu variability is primarily caused by the degree of soil interaction caused by its association with organic matter, which decreases its availability in the soil, and its association with the hydroxide ion, which forms copper hydroxides Hamza (2022).

Interactions with trace elements might alter mineral metabolism, nutritional factors such as high amounts of iron, sulfur, and molybdenum can decrease copper absorption and bioavailability. Variability in copper status is also influenced by environmental factors, such as seasonal variations in fodder quality and the mineral makeup of the soil. Copper metabolism can be changed by diseases like liver damage or a parasitic relationship which might conceal severe imbalances (Dias Betini *et al*., 2025).

The complex relationship between soils, vegetation, and animals affects how grazing animals are fed. Due to variations in pasture's composition, development stage, and availability, as well as variations in the soil's moisture content, seasonal fluctuation can have a significant impact on the amount of copper consumed through diet (Okalebo *et al*., 2002). Tests on pasture and soil are said to be the first methods used to identify inadequacies in animals. An explanation of the predisposing pasture conditions may be evaluated if pasture samples are collected alongside animal samples. Regular plant analyses that follow may also be able to forecast the varying occurrence of mineral problems (Khan *et al*., 2007).

The current study was designed to examine the influence of Cu levels in soil and forages on the hematological and serum biochemical parameters of water buffaloes in Babylon Province, Iraq.

MATERIALS AND METHODS

Study design

The study involved laboratory analysis and observational surveys to assess copper levels in soil, forages, and serum. A random sampling method was used, and comparisons were made among the three regions of Babylon Province. In the southern region, the soil type was predominantly clay; in the middle region, it was loamy with storm-related sediment formations; and in the northern region, the soil was primarily sandy. Water buffaloes were raised under free-grazing conditions. The local climate is characterized by hot, dry summers and cold, rainy winters.

Animals

In the current study, 180 water buffaloes of both sexes and varying ages were included. These animals were raised in three regions of Babylon Province, Iraq: the southern region (Al-Qasim), the middle region (Abi Gharaq), and the northern region (Al-Musayyib). The study was conducted over a one-year period, from January to December 2024. The buffaloes were fed on three main types of forage: barley grass, alfalfa grass, and fresh rice straw. Each animal underwent a case history evaluation and a thorough clinical examination, which included assessments of behavior, general appearance, skin condition, gait, and mucous membrane color. Additionally, a systemic examination was performed to evaluate vital signs such as respiratory rate, pulse rate, and rectal temperature, following the protocol described by Constable *et al*. (2016).

Parasitological screening

To screen for gastrointestinal parasites, fecal samples from all water buffaloes were examined using flotation, sedimentation, and direct smear techniques, as described by Zajac *et al*. (2021). Buffaloes found to be infected with parasites were isolated and excluded from the study. Initially, the study involved 220 water buffaloes; however, 18% of them tested positive for parasitic infections and were excluded. Therefore, a total of 180 clinically healthy buffaloes were included in the final analysis of copper levels.

Sample collection

Ten milliliters of disposable plastic syringes of the 22 G size were used to draw blood from the jugular veins of the water buffaloes (Shareef and Luaibi, 2020; Al-Wasmee *et al*., 2024). The sample was then separated into 2 parts: 7.5 ml was placed in gel glass test tubes for biochemical analysis, and 2.5 ml was placed in a tube containing an EDTA anticoagulant for blood analyses (Al-Hadithy and Badawi, 2015). Before analysis, blood samples were refrigerated and processed within 24 hours of collection to ensure accuracy of hematological testing. In contrast, serum samples were separated and stored at -20°C for up to one week prior to biochemical analysis, following the procedures outlined by Coles (1986).

After proper restraint of the animals, approximately 10 g of feces was collected directly from the rectum using disposable rubber gloves. The fecal samples were placed in clean plastic containers and immediately transported to the laboratory for parasitological examination (Zajac *et al*., 2021).

Soil samples (50 g) were collected from three distinct regions in Babylon Province where the buffaloes lived or grazed. To avoid surface contamination, the top 5–10 cm of soil was removed before sampling. A sampling tool was used to dig up to a depth of 30 cm, and soil was collected into clean plastic bags from 10 separate locations within a 100-meter radius of each site. These samples were then combined into a single composite sample for each region and allowed to air dry. The dried soil was crushed using a sterile mortar and grinder, then sieved using a 2 mm mesh before being placed in plastic bags for analysis (Razvanchy and Fayyadh, 2023).

Forage samples were also collected from the same three regions. Two types of forage were sampled: Green forage and straw. Barley grass and alfalfa, representing the green forage, were harvested using large scissors at a height of 3–4 inches, collecting only the aerial parts. Samples were taken from various field locations and at different plant growth stages. A 50 × 50 cm quadrat was used to define the sampling area, and all forage within that frame was collected and combined into a single sample for each forage type. The samples were gently cleaned using a damp cotton cloth to remove surface dust, then placed in sealed containers and transported to the laboratory for copper analysis (Simpson, 2019).

Fresh rice straw, commonly stored in large bales, required a separate sampling procedure. Portions were collected and stored in plastic bags before being transported to the laboratory for copper content determination (Drake *et al*., 2002).

Laboratory analysis

Hematological examination

A hematology analyzer (Mindray, China) was used to assess red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hgb) level, hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), following the methods described by Thrall *et al*. (2022) and Alhtheal *et al*. (2024).

Biochemical analysis

Four parts of frozen serum were separated and allowed to thaw at room temperature in order to measure the following parameters. According to the methodology given by Al-Zubaidy *et al*. (2020), atomic absorption spectrophotometer (AAS) was utilized to examine the value of Cu in the first part of serum sample. The ideal conditions of AAS are shown in Table 1. The samples were centrifuged at 3000 rpm for 10 min at 21-28 °C, supernatant was used immediately and the coefficient of variation (CV%) of AAS was <5%. The second part of the analysis involved determining serum ferritin and total iron-binding capacity (TIBC) levels using the Cobas e411 system (Roche, India), following the manufacturer's guidelines (Dahman *et al*., 2022). The CV% for these assays was less than 7%. The third part assessed levels of transferrin, total protein, albumin, and gamma-glutamyl transferase (GGT) using a Fujifilm DRI-CHEM chemical analyzer (Fujifilm, Japan), according to the manufacturer's instructions (Ishioka *et al*., 2011). The CV% was less than 6% for total protein and albumin, and less than 8% for GGT. In the fourth part, serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin were measured using an Abbott Architect clinical chemistry analyzer (Abbott, Japan), following the traditional kinetic method as described by Hubl *et al*. (2002). The CV% for these measurements ranged from 5% to 7%.

Table 1: Ideal Conditions for copper estimation using atomic absorption spectrophotometer (AAS).

Variable	Ideal condition
Lamp current	6 mA
Lighting mode	BGC-D2
Manufacturer	Spectro, USA
Replicates	3
Sample size	100 ml
Slit width	0.5 nm
Wavelength	324.8 nm
Flame type	Air-acetylene
LOD and LOQ	0.01 mg/L and 0.03 mg/L

Soil Cu levels determination

After the soil was allowed to air-dry for 4 days in a well-ventilated shed. Samples were crushed, filtered, and re-stored in a clean, closed container (Grimshaw, 1987). Then digested as described by Jones and Case (1990) by adding one ml of hydrofluoric acid (HF) and one ml of perchloric acid ($HClO_4$) to 0.05 g of soil in a platinum crucible, and the crucible was gently heated by an electro-thermal for 2h at 200 °C without a cover. The mixture was cooled once the white vapors had completely evaporated. Three ml of nitric acid and 0.5 ml of sulfuric acid (H_2SO_4) were then added, and it was heated for 15 min at 200°C before being cooled again. Then, 30 ml of distilled water was added and doubly filtrated with filter paper, then finally it was completed to 50 ml with distilled water in a volumetric flask to be ready for assessment using the AAS in accordance with the recommendations of Al-Paruany *et al.* (2018) and Imeri *et al.*, (2019). Soil analysis was conducted at the Environment, Water, and Renewable Energy Research Center, Authority of Scientific Research, Ministry of Higher Education and Scientific Research.

Forages Cu values determination

The forage samples were digested using the wet digestion method, which oxidizes the sample at a high temperature in an oven: a cup-shaped container used in chemical laboratories to contain laboratory samples when heated to high temperatures, using a combination of acids or chemicals such as hydrogen peroxide (H_2O_2), sulfuric acid, perchloric acid, and nitric acid. AAS calculated the Cu values in compliance with Bankaji *et al.* (2023). The forage analysis was conducted at the Environment, Water, and Renewable Energy Research Center, Authority of Scientific Research, Ministry of Higher Education and Scientific Research.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS), version 27, was used to record and statistically analyze the data in this study. The Chi-square test was applied to assess the significance of qualitative data, while the F-test was used for evaluating quantitative data, which were expressed as mean ± standard error (Daniel and Cross, 2018).

RESULTS

Table 2 presents the copper concentrations in soil samples collected from the three regions of the study. The reference range for copper in soil is 9.6–76.5 ppm. In the southern region (Al-Qasim), the copper level was within the normal range, with a mean value of 11.3 ± 0.67 ppm. However, copper levels in the middle (Abi Gharaq) and northern (Al-Musayyib) regions showed a significant decrease ($P \leq 0.05$), with mean values of 6.5 ± 0.35 ppm and 5.30 ± 0.29 ppm, respectively.

Table 3 presents the Cu concentrations in the three types of forage analyzed: Barley grass, alfalfa grass, and fresh rice straw. A significant decrease in Cu levels ($P \leq 0.05$) was observed in barley grass across all regions, with mean values of 1.70 ± 0.18 ppm in the south, 2.3 ± 0.08 ppm in the middle, and 0.6 ± 0.08 ppm in the north, all falling below the reference range of 6–25 ppm. Similarly, Cu levels in alfalfa grass were significantly decreased ($P \leq 0.05$) compared to the reference range of 11–30 ppm, with mean values of 1.62 ± 0.07 ppm (south), 1.95 ± 0.05 ppm (middle), and 1.0 ± 0.05 ppm (north). Although the Cu level in fresh rice straw from the southern region approached the normal range of 0.06–0.1 ppm, the mean value remained slightly below normal at 0.05 ± 0.01 ppm. In the middle and northern regions, Cu levels in fresh rice straw showed a significant decline ($P \leq 0.05$), with both regions recording a mean of 0.01 ± 0.00 ppm.

Table 2: Soil copper (Cu) levels in three regions of Babylon Province.

Trace element	Reference level (ppm)[#]	Regions of Babylon province			L.S.D. value
		South (Al-Qasim)	Middle (Abi Gharaq)	North (Al-Musayyib)	
Cu	9.6- 76.5	11.3 ±0.67[a]	6.5 ±0.35[b]	5.30 ±0.29[b]	2.077 *

The means that had different superscript letters in the same row were substantially different, * (P≤0.05), n: 3 samples. [#]The reference value was detected by Manea *et al.* (2019).

Table 3: Effect of Region on Cu levels of forages (barely grass, alfalfa grass and fresh rice straw).

Trace element	Type of forages	Reference level (ppm)[#]	Regions of Babylon province		
			South (Al-Qasim)	Middle (Abi Gharaq)	North (Al-Musayyib)
Cu	Barley grass	6-25	1.70 ±0.18[a]	2.3 ±0.08[a]	0.6 ±0.08[a]
	Alfalfa grass	11-30	1.62 ±0.07[a]	1.95 ±0.05[a]	1.0 ±0.05[a]
	Fresh rice straw	0.06-0.1	0.05 ±0.01[b]	0.01 ±0.00[b]	0.01 ±0.00[b]
L.S.D. value			0.267 *	0.408 *	0.411 *

*(P≤0.05). There was a significant difference (P ≤ 0.05) in the means of the same column with different superscript letters. n: 3 samples. [#]The reference values for copper in barley grass and alfalfa grass were reported by Brown and Hanson (1977), while the reference value for fresh rice straw was provided by Ananta (2023).

Table 4: Comparison of serum copper (Cu) levels among water buffaloes with under normal, normal, and above normal Cu status.

Trace element	Reference level (µmol /L)#	Under normal level, n (%)	Normal level, n (%)	Above normal level, n (%)	X^2	P value	Mean ±SE
Cu	139.1-158.2	165(91.66%)	10(5.55%)	5(2.77%)	413.75	<0.0001*	5.99±0.42

* Highly significant difference at P<0.01; n: 180 sample; #Reference value for copper in water buffalo was mentioned by Alhtheal (2012).

Table 5: Interrelation between different Cu levels and vital signs of the studied buffaloes.

Vital signs	Reference range#	Cu						F value	P value
		Under normal level N=165		Normal level N=10		Above normal level N=5			
		Mean	SE	Mean	SE	Mean	SE		
Respiratory rate (breaths/min)	22-26	37.18	0.21	37.20	0.61	36.20	0.37	0.325	0.72*
Pulse rate (beats/min)	40-60	87.46	0.30	86.00	1.16	85.20	1.71	1.441	0.24*
Temperature (°C)	37.5-39	38.50	0.01	38.51	0.07	38.58	0.09	0.451	0.64*

*No significant difference at P<0.05, n: 180 samples, # Reference values were adopted from the study of Abdulkareem *et al.* (2020).

Table 6: Relationship between the different Cu levels and observed clinical signs of the studied water buffaloes.

Clinical signs	Under normal Cu (n=165)	Normal Cu (n=10)	Above normal Cu (n=5)	Total cases (n)	P value
Fatigue	81(49.09)	4(40)	1(20)	86	<0.0001*
Depigmentation of hair	44(26.66)	2(20)	2(40)	48	<0.0001*
Pale mucous membranes	133(80.6)	10(100)	4(80)	147	0.001*
Emaciation	101(61.21)	4(40)	2(40)	107	<0.0001*
Diarrhea	31(18.78)	0(0)	2(40)	33	<0.0001*
Loss of appetite	35(21.21)	2(20)	2(40)	39	<0.0001*
P-value	<0.0001*	<0.0001*	0.559	---	

n: 180 samples. * Highly significant difference at P<0.01.

Table 4 of the current study presents the serum Cu levels in the examined water buffaloes. A highly significant decrease (P < 0.01) in Cu levels was observed in 165 out of 180 buffaloes (91.66%), with values falling below the normal reference range of 13.91–15.82 µmol/dL, and a mean value of 5.99 ± 0.42 µmol/dL. In contrast, only 10 buffaloes (5.55%) had serum Cu levels within the normal range, while 5 buffaloes (2.77%) exhibited Cu levels above the reference value.

Table 5 in the present study shows that there were no statistically significant differences (P < 0.05) between Cu levels and the vital signs of the studied water buffaloes. However, slight increases were observed in both respiratory and pulse rates compared to the reference values. The mean respiratory rate was 36.20 ± 37.18 breaths/min (P = 0.72), and the mean pulse rate was 85.20 ± 87.46 beats/min (P = 0.24). Body temperature remained within the normal range, with a mean value of 38.50 ± 38.58°C.

Table 6 of the current study presents the relationship between different serum copper (Cu) levels and observed clinical signs in the studied water buffaloes. The findings indicate that both Cu deficiency and excess were associated with clinical symptoms; however, Cu deficiency was significantly more prevalent and clinically severe. A strong association was observed between low Cu levels and specific clinical signs (P < 0.0001), including fatigue (49.09%), hair depigmentation (26.66%), emaciation (80.6%), and pale mucous membranes (61.21%). In contrast, buffaloes with elevated Cu levels showed clinical signs primarily of diarrhea and loss of appetite.

Table 7 of the current study showed the influence of copper levels on ferritin, transferrin, and TIBC in the studied water buffaloes. Ferritin levels were below the reference values across all groups, with a mean of 17.46 ± 21.21, but no significant differences were observed between different copper levels (P > 0.05). Similarly, transferrin levels were elevated above the reference range in all groups, with a mean of 14.25 ± 16.82, yet showed no statistically significant differences related to copper status (P > 0.05). Additionally, TIBC levels were higher than normal in all groups, averaging 71.10 ± 117.41, with no significant variation across copper levels (P > 0.05).

Table 7: Effect of Cu levels on ferritin, transferrin, and TIBC levels in studied buffaloes.

Parameter	Reference level#	Cu						F value	P value
		Under normal level (n=165)		Normal level (n=10)		Above normal level (n=5)			
		Mean	SE	mean	SE	Mean	SE		
Ferritin (μg/l)	33-55	21.21	0.77	17.46	1.82	20.74	3.37	0.816	0.444*
Transferrin (g/l)	2-6.6	15.23	0.40	14.25	1.39	16.82	1.06	0.44	0.64*
TBIC (μmol/L)	48-80	117.41	11.49	94.57	2.57	71.10	16.46	0.37	0.70*

*No significant difference at P<0.05; n: 180 samples; # The reference value for ferritin was adopted from Smith (1997), for transferrin from Moser *et al*. (1994), and for total iron-binding capacity (TIBC) from Herdt and Hoff (2011).

Table 8: Effect of Cu levels on total protein, albumin, and total bilirubin levels in studied buffaloes.

Parameter	Reference level#	Cu						F value	P value
		Under normal level (N=165)		Normal level (N=10)		Above normal level (N=5)			
		Mean	SE	Mean	SE	Mean	SE		
Total protein (g/dl)	6.3-8.7	4.00	0.29	3.00	0.49	2.80	0.70	0.60	0.55*
Albumin (g/dl)	3.2-4.1	1.64	0.04	1.90	0.16	1.78	0.24	1.76	0.18*
Total bilirubin (mg/dL)	0.01-0.5	1.38	0.04	1.30	0.12	1.49	0.33	0.24	0.79*

*No significant difference at P<0.05; n: 180 samples; # The reference value for total protein and albumin was adopted from Abdulkareem *et al*. (2020), and for total bilirubin from Constable *et al*. (2016).

Table 9: Effect of Cu level on ALT, AST and GGT levels in studied buffaloes.

Type of parameters	Reference level (U/L)#	Under normal Cu (N=165)		Normal Cu (N=10)		Above normal Cu (N=5)		F value	P value
		Mean	SE	Mean	SE	Mean	SE		
ALT	83-219	245.16	3.05	250.71	11.33	210.20	10.53	2.12	0.12*
AST	46-189	18.36	1.50	309.08	19.65	317.50	28.03	1.33	0.27*
GGT	6.1-17.4	26.47	0.35	26.22	1.51	25.84	1.58	0.06	0.94*

*No significant difference at P<0.05; n: 180 samples; #Reference value for GGT was adopted from Kaneko *et al*. (2008), and for ALT and AST from Abdulkareem *et al*. (2020).

The study results presented in Table 8 showed that total protein levels in all groups were below the reference range, with a mean value of 2.80 ± 4.00 g/dL, but no significant differences were observed among groups based on Cu levels (P > 0.05). Similarly, albumin levels were lower than the reference values across all groups, with a mean of 1.64 ± 1.78 g/dL, without statistically significant influence from Cu levels (P > 0.05). Total bilirubin levels were above the reference range in all groups, averaging 1.38 ± 1.49 mg/dL, but no significant differences were found between different Cu level groups (P > 0.05).

The results of the present study shown in Table 9, demonstrated that the levels of ALT, AST, and GGT were elevated in all Cu level groups compared to their respective reference values. The mean values recorded were 210.20 ± 245.16 U/L for ALT, 317.50 ± 18.36 U/L for AST, and 26.47 ± 25.84 U/L for GGT. However, statistical analysis revealed no significant influence (P > 0.05) of different Cu levels on these liver enzyme values.

Table 10 of the present study shows the influence of Cu levels on hematological parameters in the studied water buffaloes, including RBC count, Hct, Hb, WBC count, MCV, MCHC, and MCH. There were no statistically significant differences (P < 0.05) among Cu level groups for any of the measured parameters.

The RBC count was below the reference range in all groups, with a mean value of 3.91 ± 4.27 $\times 10^6$/mm^3, indicating mild anemia. Similarly, Hct values were decreased in all groups, with a mean of 19.67 ± 22.14%, but this was not significantly associated with Cu levels. Hemoglobin levels were markedly lower than the reference range across all groups, with a mean of 3.77 ± 4.48 g/dL, yet no significant effect of Cu levels was found.

The WBC count remained within the normal range, with a mean value of 9.94 ± 10.03 $\times 10^3$/mm^3, and no significant difference was observed across Cu levels (P > 0.05). Moreover, MCV, MCHC, and MCH were all below reference values, particularly in buffaloes with low Cu levels, with respective means of 30.66 ± 28.48 fL, 24.3 ± 22.22 g/dL, and 15.70 ± 14.94 pg.

Table 10: Effect of Cu level on the hematological parameters in studied buffaloes.

Blood parameters	Reference level#	Cu						F value	P value
		Under normal level (N=165)		Normal value (N=10)		Above normal level (N=5)			
		mean	SE	mean	SE	mean	SE		
RBCs (10^6/mm)	5-10	3.91	0.05	4.27	0.27	3.37	0.44	2.74	0.07*
Hct (%)	24-46	19.67	0.28	20.03	0.86	22.14	0.40	1.20	0.30*
Hb (g/ dl)	5-15	3.77	0.08	3.83	0.30	4.48	0.30	1.28	0.28*
WBCs (10^3 /mm)	4-12	10.41	0.08	10.03	0.38	9.94	0.43	1.03	0.36*
MCV (fL)	40-60	29.56	0.87	28.48	4.65	30.66	3.85	0.067	0.935*
MCHC (g/dl)	30-36	22.22	0.58	24.3	2.18	22.3	2.49	0.364	0.696*
MCH (pg)	14-18	15.70	0.09	15.04	1.48	14.94	1.26	1.27	0.28*

*No significant difference at $P<0.05$; n: 180 sample; #The reference values of blood parameters were adopted from Abdulkareem *et al*. (2020).

DISCUSSION

In the current study, copper (Cu) levels in the soils of Babylon Province were found to be deficient when compared to the standard reference range of 9.6–76.5 ppm. Significant regional variation was observed: the southern region (Al-Qasim) exhibited relatively higher Cu concentrations, though still on the lower end of the reference range, while the middle (Abi Gharaq) and northern (Al-Musayyib) regions showed markedly lower Cu levels.

According to Al-Madhesh *et al*. (2004), copper occurs in soils at variable concentrations, with global averages typically ranging from 10-80 ppm. They also reported that soils in arid and semi-arid regions especially sandy and organic types are often deficient in copper. The results of the current study align with these findings, showing that total soil Cu levels were below both the maximum allowable concentration (MAC) and threshold acceptable values (TAV), which are internationally set at 60–150 ppm and 60–500 ppm, respectively, for agricultural soils (Ahmed and Mohamed, 2016).

Furthermore, our findings exceed those reported by Al-Amar (2025), who recorded extremely low Cu concentrations in the Shatt Al-Hilla River, ranging from 0.0061 ppb to non-detectable levels, with an average of just 0.0042 ppb. Similarly, Al-Khalidi (2004) reported negligible or undetectable levels of Cu in soil samples from Babil Governorate, Iraq.

Festa and Thiele (2011) indicate that the soil is the primary natural source of copper. The concentration of Cu in the soil is mostly determined by the parent material of the soil. (Poggere *et al*., 2023) indicate that copper moves slowly through the soil, it adsorbs onto the mineral and organic components, causing it to accumulate over the soil's surface. According to Al-Dabbas (2024), Cu was absorbed by the aqueous environment from illite and clay minerals, resulting in a decrease in its levels.

Ruminants require 8–14 ppm of copper per kilogram of body weight (Khan *et al*., 2007), while Raisbeck *et al*. (2006) considered 2 ppm to be the minimum acceptable level of copper in forage. The majority of forage samples analyzed in the current study did not meet these nutritional requirements for ruminants. Similarly, Singh (2012) found that copper levels in green forages from Mansa and Ferozepur districts were 0.68 ppm and 0.98 ppm, respectively. In dry forages, the levels were even lower, at 0.24 ppm and 0.39 ppm. Humphries *et al*. (1981) noted that copper bioavailability can be significantly reduced when forage Cu levels exceed 1 g/kg. Moreover, forage Cu concentrations tend to be higher in leaves than in stems and generally decrease with plant maturity (McDowell, 1996). Elevated levels of zinc (Davis *et al*., 1987) and iron (Phillippo *et al*., 1987) can further impair copper absorption, thereby increasing the animal's copper requirements.

One of the major limitations of this study was the unequal group sizes based on serum copper levels, with 165 buffaloes in the deficient group, 10 within the normal range, and only 5 in the elevated group. Although the 180 water buffaloes were randomly selected across various ages and both sexes, this imbalance may have affected the statistical power and the generalizability of comparative analyses among groups.

In the present study, 91.66% of the examined buffaloes exhibited low serum Cu levels, consistent with findings by Suttle (2010) and Smith (2014), who reported reduced serum Cu concentrations in animals due to either dietary Cu deficiency or poor Cu availability in the soil. Additionally, the presence of high levels of antagonistic elements such as molybdenum, zinc, or cadmium may interfere with Cu absorption, even when dietary intake is sufficient. Al-Khalidi (2004) also reported severe copper deficiency in Baghdad's soils, supporting this observation.

The current study showed that body temperatures remained within normal limits. This contradicts the findings of Constable *et al.* (2016), who reported lowered body temperature in animals with poor nutritional status due to reduced metabolic activity, and Al-Shawi (2012), who observed similar results in goats with mineral deficiencies. Conversely, pulse and respiratory rates were elevated, aligning with observations by Abd El-Raof and Ghanem (2006) and Al-Agealy *et al.* (2007). Kusiluka and Kambarage (1996) explained that such elevations occur as compensatory responses to anemia and hypoxia resulting from reduced red blood cell counts.

Clinical signs recorded in this study including fatigue, hair depigmentation, pale mucous membranes, and emaciation are consistent with those reported by Constable *et al.* (2016), Al-Wasmee and Gharby (2021), and Al-Zubaidy *et al.* (2020). Additional signs such as diarrhea and loss of appetite were also observed, in agreement with Suttle (2010).

Although ferritin levels were decreased and both TIBC and transferrin were elevated, these parameters were not significantly associated with Cu levels. Kincaid (2000) noted a correlation between blood Cu and iron status, as Cu deficiency affects the mobilization of iron from endogenous stores. Akhtar *et al.* (2010) highlighted the essential role of Cu in enzyme function, including those involved in iron metabolism.

In the current study, both total protein and albumin levels were below normal. According to Haas and Brownlie (2001), protein especially albumin is critical for Cu transport in the body, moving Cu from the liver and spleen to the bone marrow. Weiss and Wardrop (2011) also noted that albumin binds to Cu for distribution. On the other hand, total bilirubin levels were elevated. While Peng and Wei (2017) noted an unclear relationship between trace elements and serum bilirubin, elevated bilirubin may indicate liver dysfunction or hemolysis.

Buffaloes with Cu deficiency in this study showed elevated levels of liver enzymes (ALT, AST, and GGT), although not statistically significant. This is in agreement with Humann–Ziehank *et al.* (2001) and Laven *et al.* (2004), who suggested that hepatic enzymes are sensitive indicators of subclinical Cu deficiency due to hepatocellular damage. Li and Hai (2014) proposed that Cu deficiency could alter the synthesis, activity, or elimination of these enzymes during hepatic necrosis.

Our findings revealed reductions in RBC count, hemoglobin, and hematocrit values, aligning with Brooks *et al.* (2022), who reported anemia due to nutritional deficiencies. Constable *et al.* (2016) and Al-Saad *et al.* (2010) also confirmed that Cu deficiency leads to anemia. Coles (1986) identified soil Cu deficiency as a contributing factor. In this study, MCV and MCHC were reduced, suggesting microcytic hypochromic anemia, although MCH remained within normal limits. This is supported by Roland *et al.* (2014), who linked Cu deficiency with microcytic hypochromic anemia, and by Abramowicz *et al.* (2021), who found normocytic normochromic anemia in some cases.

Constable *et al.* (2016) emphasized the role of Cu in hemoglobin formation and in recycling iron from hemolysis, noting that Cu deficiency can lead to anemia and hemosiderin deposition (hemosiderosis). López-Alonso and Miranda (2020) stated that Cu deficiency becomes the primary nutritional cause of anemia in ruminants when parasitism, infections, and iron deficiency are ruled out. Suttle (2010) noted that iron deficiency is rare in ruminants due to efficient recycling and high environmental availability. Even with adequate dietary protein, anemia can develop under Cu deficiency, with hematological recovery only occurring after Cu repletion (Ward *et al*., 1997). Additionally, anemia has been closely linked to reduced serum Cu and ceruloplasmin activity in disease-free herds (Suttle *et al*., 1987). Ceruloplasmin, a ferroxidase, requires Cu to convert Fe^{2+} to Fe^{3+} for hemoglobin synthesis and iron transport. Thus, Cu deficiency causes functional iron deficiency, in which iron is present but cannot be effectively utilized (Suttle, 2010).

In summary, the findings of the current study provide strong evidence that copper deficiency is a significant and reliable nutritional factor contributing to anemia and poor clinical outcomes in grazing water buffaloes, particularly when other confounding causes are absent.

CONCLUSIONS

In this study, both soil and forage samples were found to be generally deficient in copper. Serum copper levels were below the reference range in 91.66% of the examined water buffaloes, indicating widespread copper deficiency. A significant limitation of the study was the absence of liver copper analysis, which is considered the gold standard for assessing copper status in ruminants. Additionally, the study did not evaluate soil pH or the presence of potential antagonistic minerals such as sulfur, iron, zinc, and molybdenum, all of which can interfere with copper absorption and complicate the interpretation of deficiency. While copper deficiency was the primary concern, it is important to note that 2.77% of the animals exhibited serum copper concentrations above the normal range, highlighting the potential for copper toxicity and the need for careful mineral monitoring.

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NOVELTY STATEMENT

The majority of this research has focused on using a spectrophotometer to determine and evaluate the level of copper available in water buffalo serum, soil, and forage samples. Various perspectives exist concerning the most effective goals to use, and comparing labs is difficult due to the inadequate diagnostic instruments.

AUTHOR'S CONTRIBUTION

AKKA-W: Practical work.
SSS: Study design and editing.

Ethical approval

The current study was approved by the Scientific Committee of the College of Veterinary Medicine, University of Baghdad, Department of Internal and Preventive Veterinary Medicine (Approval No. PG: 2399, dated 18/12/2024).

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The authors declare that the present study has no financial issues to disclose.

Generative AI and AI-assisted technology statement

During the preparation stages of this study project, generative artificial intelligence (AI) and AI-assisted technologies were utilized in restricted ways. These tools were used just to improve the text's structure, presentation, grammar, and language clarity. Data, findings, and interpretations were not created or altered by any AI system. The researcher is the sole author of the research design, data analysis, critical evaluation, and findings. The truth, validity, and integrity of the information provided in this project are entirely the responsibility of the author or authors.

Conflict of interest

The authors have declared no conflict of interest.

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