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Assessment on level of selected metals and proximate composition of raw cow milk samples from selected sites of Bahir Dar City and it's surrounding

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ABSTRACT

This study systematically assessed the concentrations of selected metals and the proximate composition of milk samples collected from six locations: Agerie Milk Cooperative, Andassa Livestock Research Center, Tekelehymanot Monastery, the College of Agriculture and Environmental Sciences, Bahir Dar Dairy Cooperative, and households in Keble-7, Bahir Dar city. Proximate composition, including moisture, ash, protein, and fat, was determined using the Kjeldahl digestion and Gerber methods, while metal analysis was performed using the Inductively Coupled Plasma Optical Emission Spectroscopy technique. The findings revealed significant variations ($P<0.05$) in both metal concentrations and proximate composition among the milk samples from the different sites. The average concentrations of detected metals were 1550.42 ± 34.76 mg/L for calcium (Ca), 137.26 ± 2.89 mg/L for magnesium (Mg), 23.97 ± 2.02 mg/L for iron (Fe), 1.10 ± 0.11 mg/L for copper (Cu), 40.48 ± 3.03 mg/L for zinc (Zn), 0.32 ± 0.01 mg/L for manganese (Mn), and 0.58 ± 0.05 mg/L for chromium (Cr). Lead (Pb), nickel (Ni), and cadmium (Cd) were not detected in any of the samples. All detected metal levels, except for iron, were below the permissible limits set by the World Health Organization (WHO). The mean proximate composition of the milk samples included moisture at 87.56 ± 1.16%, ash at 0.75 ± 0.04%, protein at 3.24 ± 0.34%, and fat at 4.02 ± 0.38%. These values were consistent with Food and Agriculture Organization (FAO) standards and aligned with findings from previous studies. Based on the findings, milk from these locations is safe for consumption, free from harmful levels of heavy metals, and provides nutritional benefits due to its favorable proximate composition.

1. Introduction

Milk is widely accepted and consumed across all age groups, both in rural and urban areas (Herber *et al.*, 2020). Raw cow milk and its by-products serve as essential food sources, providing vital nutrients in the human diet. The mineral content of milk significantly influences both its biological and technological properties (Amalfitano *et al.*, 2024). Cow milk contains a variety of major, minor, and trace elements, with only a small subset of these being deemed essential for human health (Olowoyo *et al.*, 2024). These elements are introduced into the environment through both natural processes and human activities (Mitra *et al.*, 2022), such as fertilizers, feed supplements, and food additives. These metals can enter the human body through inhalation, ingestion, or absorption (Mitra *et al.*, 2022). Understanding milk composition is vital to addressing nutritional deficiencies by ensuring an adequate supply of essential elements (Cimmino *et al.*, 2023).

The characteristics of cow milk can vary greatly depending on the geographical region and farming practices, which ultimately influence the quality of milk products (Zebib *et al.*, 2023). Therefore, the origin of milk products plays a crucial role in determining their quality. Previous studies have highlighted the importance of evaluating both the proximate and elemental composition of milk as quality indicators (Meneses *et al.*, 2020). Moreover, the assessment of metal levels in cow's milk is crucial not only for evaluating potential health risks to humans but also for assessing environmental quality (Karimi *et al.*, 2023). Several studies have examined the mineral content in milk from different regions; however, the evaluation of milk composition in Bahir Dar City remains unexplored.

While existing research has provided valuable insights into the mineral content and proximate composition of cow milk in various regions, there is a lack of documented data specifically concerning milk samples from Bahir Dar City. This gap in the literature highlights the need for a detailed analysis of both essential and non-essential elements in raw cow's milk from this region. The novelty of this study lies in its

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unique focus on raw cow's milk from Bahir Dar City and its surrounding areas, an under-researched region in terms of milk composition. This research is the first to assess and document the proximate and elemental composition of local milk, providing essential data that can be used to evaluate its nutritional value and safety. By comparing the levels of these elements with internationally accepted standards, this study contributes new insights into the quality and safety of milk in this region.

The objective of this study was to assess the proximate composition (moisture, ash, fat, and protein) and elemental content of raw cow's milk from Bahir Dar City and its surrounding areas. The study aimed to compare the levels of these elements with the permissible limits set by the World Health Organization (WHO) and other relevant published values. Wet Kjeldahl digestion, using nitric and perchloric acid, was employed to break down the organic matter in the milk samples. Wet digestion is preferred over dry digestion due to the reduced loss of volatile elements (Das & Ting, 2017). The digested samples were then analyzed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), a technique recognized for its ability to simultaneously detect elements in biological and environmental samples, with the advantages of rapid analysis and low detection limits (Khan et al., 2022).

2. Method and materials

2.1. Description of the study area

The study was conducted on milk samples obtained from six selected sites namely, Agerie Milk Cooperative (AC), Andassa Livestock Research Center (ALRC), Tekelehymanot Monastery (TM), College of Agriculture Environmental Sciences (CAES), Bahir Dar Dairy Cooperative (Keble-13) and Keble-7, in Bahir Dar and its surrounding (Fig. 1) Amhara region, Ethiopia.

2.2. Sampling technique and sample size

Six sampling sites: Agerie Milk Cooperative (AC), Andassa Livestock Research Center (ALRC), Tekelehymanot Monastery (TM), College of Agriculture Environmental Sciences (CAES), Bahir Dar Dairy Cooperative (K-13) and Keble-7(K-7) were selected purposively on the basis of milk production potential and number of producers as well as consumers who utilize milk through buying. About 500 mL of milk from the bulk milk container of each sampling site was collected using a polyethylene bottle in the morning milking. To ensure proper sterilization of polyethylene bottles and prevent contamination, we used a more reliable procedure. The bottles were initially cleaned with a detergent solution followed by thorough rinsing with deionized water. They were then soaked in a 10% nitric acid (HNO_3) solution for 24 hours to effectively remove any potential contaminants. Afterward, the bottles were rinsed multiple times with sterile deionized water and autoclaved at 121 °C for 15 minutes to ensure complete sterility before being dried and prepared for sample collection (Hee *et al.*, 2022). Then, these samples were immediately kept in a refrigerator (4 °C) until wet digestion was carried out.

2.3. Equipment and apparatus

The laboratory work utilized the following equipment: an electronic balance (Nimbus, ADAM Equipment, USA), an ICP-OES spectrometer (PerkinElmer, Optima 7300V HF Version), desiccators (VAKUUM), a pH meter (AD8000, Romania), an oven (US Binder), a furnace (DAIHAN Scientific), a deionizer (Evoqua Water Technologies), a Kjeldahl digester (VELP Scientifica), a butyrometer (DINKELBERG), a refrigerator (Lec Refrigeration PLC, England), a centrifuge (1020D, Centurion Scientific Ltd, UK), and a water bath (China Hh. S Series).

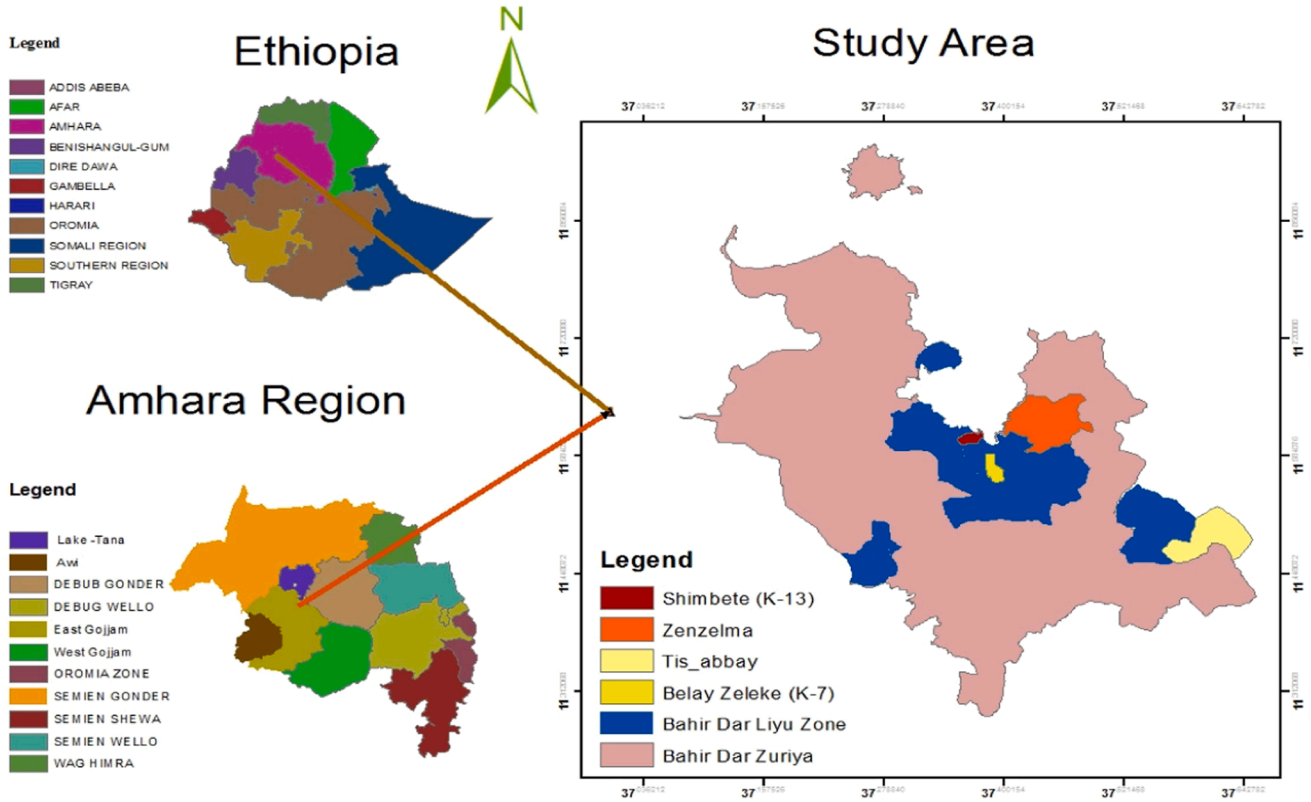

Fig. 1. Map of the study area.

2.4. Reagents and chemicals

All chemicals and reagents used in this study were of analytical grade and utilized without further purification. These included nitric acid (65.0%), perchloric acid (60.0%), hydrochloric acid (37.0%), potassium sulfate (99.0%), and boric acid (99.5%) from Blulux Laboratories (P) Ltd.; sulfuric acid (98.08%) from UNI-CHEM; sodium hydroxide (extra pure) from Lab Tech Chemicals; amyl alcohol (99.0%) from ASTRRA; and standard solutions for Ca, Fe, Mn, Mg, Zn, Cu, Ni, Pb, Cr, and Cd.

2.5. Sample preparation for metal analysis

2.5.1. Optimization of sample digestion

The optimized wet digestion procedure was selected depending upon the clarity of digests, minimal digestion time and reagent volume, simplicity, and low temperature to obtain a clear and colorless solution. In this study, nitric acid and perchloric acid were used by optimizing (Appendix 1) the volume ratio, time, and temperature with some modification (Moges, 2014).

In this optimization, the volume ratio of reagents HNO_3:$HClO_4$ (4:2), temperature of 160 °C and time of 80′ were the optimum conditions for digestion. In this case, 1.0 mL of each liquid milk sample was transferred into a digestion vessel, and then optimized volumes of 4 mL of 70% nitric acid and 2 mL perchloric acid were added, and the mixture was heated at optimized kjeldhal digestion program up to 160 °C until a clear colorless solution appeared. After heating, the sample was cooled to room temperature to avoid foaming and splashing, and the digestion vessels were opened carefully in a fume hood. Then the digested sample was transferred into a 50 mL flask and then diluted with deionized water up to the mark.

2.5.2. Metal determination in raw cow's milk

The level of the studied metals in the samples was determined using ICP-OES Instrument. As shown in Table 1, six sets of working standard solutions with known concentrations were made for each metal by diluting the intermediate standard solution (10 mg/L) with deionized water. Means of triplicate ICP-OES results for each analyzed metal were used to plot the calibration curves. The concentration of analyte plays a vital role in the sensitivity, precision, accuracy, and linearity of the method.

2.5.3. Proximate composition (moisture, ash, protein and fat) determination in raw cow's milk

Moisture was determined according to the AOAC, 2000 method; briefly, a weight of a dried petridish was recorded. Then, 5 g of sample was weighed using the weighed petridish. The pertidish with the milk sample was put in an oven and heated for 3 hr at 105 °C. Then, the sample was placed in desiccators until the sample was weighed. Finally, the moisture content was calculated by difference. Percentage moisture was calculated as described below in Eq. (1):

$$\%moisture = \frac{wt\ of\ H_2O\ in\ sample}{wt\ of\ wet\ sample} \times 100\% \quad (1)$$

Ash content of the analyzed milk samples was determined following the procedure by (Zebib *et al.*, 2023). 5.0 g of milk sample was taken in a pre-weighed crucible. The crucible containing the milk sample was placed in a furnace and heated for 4 hrs at 550 °C. Total ash content was calculated using Eq. (2) (Azeze and Tera, 2015).

$$\text{Ash } \% = \frac{\text{Residue weight}}{\text{Weight of the sample}} \times 100\% \quad (2)$$

Total protein content of the milk samples was determined by the Kjeldahl method (Ilirjana and Gentiana, 2013). In this method, total protein content was determined following three stages; digestion, distillation, and titration stages. To a 5.0 g milk sample in a Kjeldahl flask, 15.0 g of potassium sulfate, 1 mL of (0.5 molar) copper sulfate solution, and 25 mL of concentrated sulfuric acid were added sequentially. The mixture was then mixed gently. The digestion was carried out in a digestion block until a clear solution appeared.

Then the samples were allowed to cool at room temperature for distillation, the digestion flasks were placed in the distillation unit, and 30 mL of distilled water was added, and ammonium sulfate was then converted into ammonia gas by heating with 75 mL of 50% sodium hydroxide solution.

Then, ammonia was distilled into 50 mL of 40% boric acid solution to trap the volatile ammonia by forming ammonium borate using bromocresol green indicator until blue color appeared.

Finally, the sample was titrated with a 0.1 N hydrochloric acid solution from a burette until a faint pink color solution was formed, and the burette readings were taken. A blank test was carried out using the above procedure except that water was used instead of a milk sample. The percentage of nitrogen and protein in the milk samples was calculated.

$$\%N = \frac{(V_s - V_b)HClconsumed \times N \times HCl \times 1.4007}{Sample\ weight} \times 100\% \quad (3)$$

$$\%Cprotein = \%N \times F \quad (4)$$

Where: % N - percentage nitrogen by weight, Vs - volume of HCl used for titration of sample, Vb - volume of HCl used for titration of the blank, N - normality of HCl, % CP - percentage of crude protein and F- conversion factor.

For dairy products like milk, F = 100/15.68 = 6.38 (crude protein contains 15.68 nitrogen).

Fat content was determined by the Gerber method. 10 mL of sulfuric acid was added to the Gerber Butyrometer. Then 11 mL of a well- mixed milk sample were transferred to Gerber test bottle, and 1 mL of isoamyl alcohol was added into the butyrometer having the sulfuric acid and the milk sample, then closed with rubber. After closing the butyrometer using a butyrometer stopper, the content was shaken and inverted several times until all the milk samples were digested by the acid. Then the butyrometer was placed in a water bath at 65 °C for five minutes.

Table 1
The wavelengths, calibration equations, and correlation coefficients of the elements.

Analyzed Metal	Wave length (nm)	Concentration of standards (mg/L)	Equation	Correlation Coefficient (R^2)
Ca	317.933	0.05, 0.25, 0.50, 1.00, 1.50, 2.00	y = 28,717.83x+5303.24	0.9979
Mg	285.213	0.05, 0.25, 0.50, 1.00, 1.50, 2.00	y = 240356x + 9890.83	0.9997
Fe	238.204	0.005, 0.05, 0.50,1.00, 2.00, 4.00	y = 3393.2x + 392.26	0.9987
Mn	257.610	0.005, 0.05, 0.500,1.00, 2.00, 4.00	y=138,905.7x + 9498.86	0.9975
Cu	327.393	0.005, 0.05, 0.500,1.00, 2.00, 4.00	y=131,916.35x+2607.92	0.9980
Zn	206.200	0.005, 0.05, 0.500,1.00, 2.00, 4.00	y = 8129.80x + 105.22	0.9991
Ni	231.604	0.005, 0.05, 0.500,1.00, 2.00, 4.00	y = 770.92x + 107.31	0.9990
Cr	267.716	0.005, 0.05, 0.500,1.00, 2.00, 4.00	y = 2499.92x+ 202.69	0.9993
Pb	220.353	0.005, 0.05, 0.500,1.00, 2.00, 4.00	y = 112.70x+ 12.11	0.9993
Cd	228.802	0.005, 0.05, 0.500,1.00, 2.00, 4.00	y = 1992.26 x+ 164.28	0.9997

The samples were centrifuged for five minutes at 1100 rpm. Finally, the samples were taken back to the water bath adjusted at 65 °C for 5 minutes, and the fat percentage was recorded from the butyrometer reading (Fekata *et al.*, 2022).

2.6. Statistical data analysis

The collected data were subjected to analysis of variance (ANOVA) by using SAS 9.3 and SPSS version 22.0. The least significance difference (LSD) test at a 5% probability level was used for mean comparison.

3. Results and discussion

3.1. Method verification

The applicability of the ICP-OES method for metal determination in the collected milk samples was verified using the concentration dynamic range, limit of detection (LoD), and recovery of spiked standards (Swartz and Krull, 2005).

3.1.1. Constructing calibration curve

A series of standard solutions were prepared for each analyzed element from the respected stock solution through dilution (Table 1). Triplicate measurements were made for all the metal standards, including the blank solution, and mean values were plotted against the concentration. A correlation coefficient greater than or equal to 0.990 confirmed a strong correlation of the signal intensity with the concentration in the entire concentration range.

3.1.2. Recovery test

For testing the accuracy of the method for the determination of metals, a milk sample from Kebele-13 was chosen to which known amounts of the ten studied metals were added. A known amount of each standard solution was added to the sample and digested with the same procedure of wet digestion for milk samples. They were analyzed with the same procedure followed for the analysis of milk samples. As presented in (Table 2) the recovery percentages of the analyzed metals ranged between 86.4% and 108.4%. According to (Muhib et al., 2016) the acceptable recovery percentage for most metals is between 85% and 103%. Therefore, the result was found to be the acceptable ranges. The percentage of recovery calculated according to Eq. (4).

$$\%recovery = \frac{Concentration\ in\ spiked\ sample - Concentration\ in\ sample}{amount\ spiked} \times 100\% \quad (5)$$

3.1.3. Method and instrument detection limits

As presented in (Table 3), the limit of detection (LoD = 3*SD/m) and limit of quantification (LoQ =10*SD/m) were estimated, respectively as three and ten times the standard deviation (SD) of consecutive measurements of the reagent blanks divided by the slope of the calibration curve, which were prepared with the same procedure as the real milk samples (Magnusson, 2014). In order to determine the method detection limits the reagent blank which was digested with the same procedure as the milk samples were analyzed for Ca, Mg, Fe, Cr, Mn, Ni, Cu, Zn, Cd and Pb by using ICP-OES.

Table 2
Summary of recovery analysis of milk sample.

Metal	Amount spiked (mg/L)	Concentration in sample (ppm)	Concentration spiked sample (mg/L)	Recovery (%)
Ca	0.450	1.786	2.210	94.2
Mg	0.450	0.175	0.612	97.1
Cu	0.500	0.051	0.593	108.4
Zn	0.500	0.316	0.769	90.6
Mn	0.450	0.009	0.414	90.0
Fe	0.500	0.181	0.613	86.4
Ni	0.200	ND	0.198	99.0
Pb	0.500	ND	0.469	93.8
Cr	0.500	0.022	0.511	97.8
Cd	0.500	ND	0.505	101.0

ND Indicates not detected

3.2. Metal analysis in raw cow's milk

The elements concentration in milk varies depending on the cow's age, health condition, lactation period, and the quality of feed (Voronina *et al.*, 2022). The mean of triplicate measurements of calcium content in the milk samples ranged from 1058.67±8.61 (Agerie milk cooperative) to 1941.50±9.26mg L^{-1} (Bahir Dar Dairy Cooperative) with an overall average of 1550.42 mg L^{-1} (Table 4).This result was higher than the earlier findings (Roger et al., 2013) who reported that the calcium content was 1.217±140.67 mg L^{-1}. Moreover, (Malbe *et al.*, 2010) also reported that 1298 mg L^{-1} of calcium from Estonia cow's milk. The statistical analysis of variance (ANOVA) revealed a statistically significant difference ($p < 0.05$) among milk samples from different sites. The average magnesium content of the studied milk samples ranged between 100.30±1.86 in Agerie milk cooperative (AC) and 189.42±0.76 mg L^{-1} in Bahir Dar Dairy Cooperative (Table 4).

The ANOVA analysis result revealed a significant difference ($p < 0.05$) among the magnesium contents across the sampling sites, which might be attributed to differences in their feed, water availability, age, health condition, lactation period and breeding cows (Cadar *et al.*, 2016). The average magnesium content of the study area (137.26 mg L^{-1}) was higher than 103±14.630 and 113 mg L^{-1} reported by (Roger *et al.*, 2013) and (Malbe *et al.*, 2010), respectively. However, the average magnesium content in this study is lower than 214 mg L^{-1} reported by (Birghila *et al.*) from cow's milk in Romania.

As can be seen from (Table 4), the average copper content of the analyzed milk samples ranged between 0.30±0.00 for the milk from AC and 2.88±0.00 mg L^{-1} in the milk from Andassa Livestock Research Center (ALRC). Even though the DMRT analysis showed that there is no significant difference ($p>0.05$) between four sites (AC, TM, CAES, and K-7), the concentration of Cu revealed there were significant differences among six sites. Although the average copper content of the milk samples from the six sampling sites (1.10 mg L^{-1}) was below the WHO recommended value (24.2 mg L^{-1}), it was found to be higher than the results reported by (Roger et al., 2013), (Tunegova *et al.*, 2016), (Malbe *et al.*, 2010) and (Birghila et al.) with respective values of 0.004±0.01, 0.142 ±0.116, 0.191and 0.54 mg L^{-1}.

Similarly, the iron content of triplicate measurements in the analyzed milk samples ranged from 9.08±0.24 mg L^{-1} of the milk from Bahir Dar Dairy Cooperative (K-13) to 66.75±0.71 mg L^{-1} of the milk from ALRC. The ANOVA analysis result also showed significant difference ($p<0.05$) across the sampling sites. The average iron content of the studied milk samples from the six sampling sites (23.97 mg L^{-1}) was found to be higher than the recommended value (0.5 mg L^{-1}) WHO 1973 cited by Enb et al. (2009) and reported results by Roger et al. (2013), Malbe *et al.* (2010), and Tunegova *et al.* (2016) with values of 0.72 ± 0.09, 0.78, 0.68 ± 0.41, 0.72 and 21.73mg L^{-1}, respectively, which still could be attributed to the feed, water, and breeding type.

The zinc content of the studied milk samples varied between 5.92 ± 0.28 mg L^{-1} in K- 7 to 95.78 ± 6.09 mg L^{-1} obtained in College of Agriculture Environmental Sciences (CAES) with the mean value of 40.48 mg L^{-1} (Table 4). According to the analysis of variance, zinc content was significantly different at $p<0.05$ between the study sites. The present study was higher than the findings of (Roger et al., 2013); (Malbe et al., 2010); and (Tunegova et al., 2016) mgL^{-1} who reported with the respective value of 3.34±1.47, 3.24, 3.566, 3.146±1.081 and 0.98 mg L^{-1}. Although high content of Zn was obtained compared with some reported in this study, its value is lower than the recommended

Table 3
Limit of detection, quantification and instrumental detection limit.

Element	Ca	Mg	Fe	Cr	Mn	Ni	Cu	Zn	Cd	Pb
LoD (mg/L)	0.011	0.039	0.020	0.018	0.003	0.045	0.012	0.123	0.023	0.167
LoQ (mg/L)	0.038	0.131	0.066	0.060	0.010	0.150	0.040	0.409	0.078	0.558
IDL (mg/L)	0.010	0.002	0.005	0.007	0.001	0.015	0.01	0.006	0.003	0.042

Table 4
Essential metals concentration (mg/L) of raw cow's milk samples.

Site	Essential metals (mg/L)						
	Ca	Mg	Cu	Fe	Zn	Mn	Ni
AC	1058.67 ± 8.61^{f}	100.30 ± 1.86^{d}	0.30 ± 0.00^{c}	23.23 ± 0.50^{b}	34.05 ± 0.66^{c}	0.17 ± 0.003^{c}	ND
ALRC	1256.17 ± 0.76^{e}	121.57 ± 1.35^{c}	2.88 ± 0.03^{a}	66.75 ± 0.71^{a}	52.85 ± 0.92^{b}	0.20 ± 0.005^{c}	ND
K13	1941.50 ± 9.26^{a}	189.42 ± 0.76^{a}	1.89 ± 0.12^{b}	9.08 ± 0.24^{f}	15.80 ± 0.96^{d}	0.45 ± 0.00^{a}	ND
K7	1830.67 ± 13.88^{c}	134.03 ± 3.20^{bc}	0.55 ± 0.00^{c}	14.37 ± 0.14^{d}	5.92 ± 0.28^{d}	0.47 ± 0.006^{a}	ND
TM	1365.18 ± 3.51^{d}	130.93 ± 1.48^{c}	0.43 ± 0.03^{c}	17.37 ± 0.03^{c}	38.48 ± 1.07^{c}	0.30 ± 0.00^{b}	ND
CAES	1850.33 ± 14.84^{b}	147.33 ± 0.86^{b}	0.60 ± 0.00^{c}	13.03 ± 0.51^{e}	95.78 ± 6.09^{a}	0.35 ± 0.00^{b}	ND
Total mean	1550.42 ± 34.76	137.26 ± 2.89	1.1 ± 0.11	23.97 ± 2.02	40.48 ± 3.03	0.32 ± 0.01	—
WHO cited in Ahmad *et al.* (2017)	NA	NA	24.2	0.5	121	55.5	0.43
LSD(0.05)	15.08	13.87	0.88	0.83	11.91	0.05	—-
P	*	*	*	*	*	*	

NA =not available, ND = not detected, LSD= Least Significance Difference and P = Probability level; * significantly different at $p<0.05$. Means followed by the same letters in a column are not significantly different at $p<0.05$.

value of 121 mg L^{-1}. WHO cited in (Ahmad *et al.*, 2017).

The content of manganese was ranged from 0.17 ± 0.003 mg L^{-1}in AC to (0.47 ± 0.006 mg L^{-1}) in household Keble-7 in Bahir Dar city (K-7) (Table 4). The mean value was 0.32 mg L^{-1}, this value was higher than the earlier findings of (table 4) (Roger *et al.*, 2013) and (Tunegova et al., 2016) mg L^{-1} and who reported with the respective values of 0.02 ± 0.01, 0.29, 0.056 ± 0.038, and 0.08 mg L^{-1} while compared with WHO recommended value level Mn the present study was lower. This may be due to sampling sites having a limited amount of manganese in the soil, water, and feed which are the main sources of Mn in the sampling environment (Karanja *et al.*, 2010). This value was below the recommended value WHO cited in (Ahmad *et al.*, 2017).

The analysis of variance showed that there was significant difference between the manganese content in six study sites; however, there were no significant differences ($p<0.05$) between AC & ALRC, K-13 & K-7 and Teklehayimanot Monastery (TM) and CAES sites. This may be due to soil, water, and feed being free from Mn around the sampling environment (Wu *et al.* (2022).

The result showed that, the concentration of Ni was not detected in any of the six sampling sites. The below detection limit of Ni in the milk sample has been reported by (Belete et al., 2014). These might be due to the absence of the sources that introduce Ni into the environment, like metallurgy and refining industries, coal combustion, diesel and fuel oil, sewage, etc.

The result showed that the average chromium content of the analyzed milk samples ranged from ND in the samples from K-7 and TM to 1.15 ± 0.09 mg L^{-1} in the milk sample collected from CAES. Although the ANOVA analysis result showed a significant difference ($p<0.05$) in chromium contents across all the sampling sites, it also revealed that the chromium content in the milk collected from AG and ALRC has no significant difference. The result of the study also showed that the average chromium content of the six sites (0.58 ± 0.05 mg L^{-1}) is below the recommended value (1.61 mg L^{-1}) WHO cited in (Ahmad *et al.*, 2017). The heavy metals Pb and Cd were found to be below the detection limit of the instrument (Table 5). As (Belete et al., 2014) who report, concentration of Pb, and Cd is BDL in Borena Cow's milk. Cd and Pb are not detected in all of the six sites. This may be because there are no industries or car emissions that pollute the milk for cadmium and lead sources in the study area, or because the cows' feed and water are clean from such contamination (Ali *et al.* (2023).

As indicated in (Table 6) the findings in this study were somewhat comparable with the findings in other studies that were conducted in different countries throughout the world.

Table 5
Levels of non-essential metals (mg/L) in the analyzed raw cow's milk samples.

Site	Non-essential metals (mg /L)		
	Cr	Pb	Cd
AC	0.58 ± 0.25^{b}	ND	ND
ALRC	0.67 ± 0.06^{b}	ND	ND
K13	1.10 ± 0.035^{a}	ND	ND
K7	ND	ND	ND
TM	ND	ND	ND
CAES	1.15 ± 0.09^{a}	ND	ND
Total mean	0.58 ± 0.05	——	——
WHO	1.61	0.02	0.01
LSD(0.05)	0.36		
P	*		

ND = not detected, LSD= Least Significance Difference and, P =Probability level; * significantly different at $p<0.05$.Means followed by the same letters in a column are not significantly different at $p<0.05$.

3.3. Determination of proximate composition (ash, moisture, fat &protein) of raw cow's milk

The mean moisture content of the milk samples was 87.56 ± 1.16, with a range of 86.10 ± 0.10 (AC) to 89.78 ± 0.01% (K-7) (Table 7). The findings showed that, with the exception of the samples from AC and TM, the moisture content of the milk samples was higher than the (FAO, 2023) standard of 87.20 (Table 7). The results of the ANOVA analysis showed that the moisture content of the milk samples taken from the six locations varied significantly ($P < 0.05$). (Li et al.,2012) and (Soliman, 2005) reported moisture values of 87.5 ± 0.13 and 86.7 ± 0.13%, respectively, which were consistent with the average moisture content of the milk samples that were examined.

The mineral makeup of the milk is reflected in its ash content. The mean value of the milk analysis results obtained for this study was 0.75 ± 0.04 and varied from 0.70 ± 0.00 (AC) to 0.79 ± 0.02 percent (ALRC and CAES). The findings indicated that all milk samples had an ash concentration that was both within and over the FAO-recommended threshold of 0.70 (Table 7). The analysis of variance revealed a statistically significant difference ($P < 0.05$) between sample sites, despite the

Table 6
Essential and non-essential metals concentration (mg/L) in raw cow's milk from various studies.

Country	Metals										Ref.
	Ca (mg/L)	Mg (mg/L)	Ni (mg/L)	Cu (mg/L)	Fe (mg/L)	Zn (mg/L)	Mn (mg/L)	Cr (mg/L)	Pb (mg/L)	Cd (mg/L)	
Cameroon	1217 ±140.67	103 ±14.63	NR	0.04±0.01	0.72 ±0.094	3.34±1.47	0.02±0.01	NR	NR	NR	Roger et al. (2013)
Estonia	1298	113	NR	0.191	0.778	3.566	NR	NR	NR	N	Malbeet al. (2010)
Romania	NR	214	0.18	0.54	21.73	3.24	0.29	0.04	0.0012	0.004	Birghilaet al. (2008)
Romania	NR	NR	0.00361 ±0.89	0.0756 ±11.1	NR	2.356 ±231	0.0327 ±5.63	0.0182 ±1.32	0.0158 ±5.45	0.00256 ±1.53	Cadaret al. (2016)
Egypt	NR	NR	0.004 ±0.002	0.142 ±0.116	0.682 ±0.406	3.146 ±1.081	0.056 ±0.038	0.034 ±0.014	0.066 ±0.056	0.086± 0.062	Tunegova et al. (2016)
	NR	NR	0.04	0.17	0.72	0.98	0.08	0.04	0.12	0.004	Semaghiul et al. (2008)
Gondar, Ethiopia	NR	NR	NR	0.840–1.532	NR	1.208–5.267	1.614–2.806	0.468–0.828	ND–0.186	ND–0.330	Akeleet al., (2017)
Borena, Ethiopia	NR	NR	ND	0.109 ±0.006	NR	5.592 ± 0.092	0.427 ±0.018	0.868 ±0.026	ND	ND	Belete et al. (2014)
Bahir Dar D	1550.42 ±34.76	137.26 ±2.89	ND	1.1 ± 0.11	23.97 ±2.02	40.48±3.03	0.32 ± 0.01	0.58 ± 0.05	ND	ND	Present study

NR = not reported, ND = not detected

Table 7
Moisture, ash, protein and fat content in raw cow's milk.

Site	Ash (%)	Protein (%)	Fat (%)	Moisture (%)
AC	0.70 ± 0.00^{d}	3.13 ± 0.26^{cd}	3.63 ± 0.06^{e}	87.50 ± 0.10^{c}
ALRC	0.79 ± 0.01^{a}	3.51 ± 0.01^{ab}	4.43 ± 0.06^{b}	86.10 ± 0.10^{f}
K-13	0.76 ± 0.01^{c}	2.98 ± 0.01^{de}	3.83 ± 0.06^{d}	87.27 ± 0.06^{d}
K-7	0.78 ± 0.01^{b}	2.74 ± 0.01^{e}	3.67 ± 0.06^{e}	89.78 ± 0.01^{a}
TE	0.70 ± 0.00^{d}	3.67 ± 0.01^{a}	4.60 ± 0.00^{a}	86.94 ± 0.04^{e}
CAES	0.79 ± 0.02^{a}	3.28 ± 0.01^{bc}	3.97 ± 0.06^{c}	87.76 ± 0.04^{b}
Total mean	0.75 ± 0.04	3.24 ± 0.34	4.02 ± 0.38	87.56 ± 1.16
FAO (2023)	0.70	3.50	3.70	87.20
LSD(0.05)	0.01	0.25	0.09	0.09
P	*	*	*	*

LSD= Least Significance Difference and, P =Probability level; * significantly different at $p<0.05$.Means followed by the same letters in a column are not significantly different at $p<0.05$.

fact that two sampling sites (ALRC and CAES) did not exhibit any significance. Breed, nutrition, parity, individuals, feeding, season, lactation period, environment, farm management, location, and animal health status could all be contributing factors to the variation (Boyazoglu *et al.*, 2005). This study's findings concur with those of the studies by Boyazoglu et al. (2005) and Soliman (2005). However, the results were higher than those of Roger et al. (2013), who said that the milk's ash content was 0.61± 0.09.

The mean protein content of milk samples was 3.24 ± 0.34 (Table 7), with a range of 2.74 ± 0.0.01 (ALRC) to 3.67 ± 0.01 % (K-7). The findings showed that, with the exception of the TM sample, the protein level of the milk samples was below the global standard limits of 3.50 (Table 7). Between the six sampling sites, there was a statistically significant difference ($P < 0.05$). This study's findings concur with those published by Boyazoglu et al. (2005). With an average mean value of 4.02 ± 0.38 percent of fat contents, the analysis findings of the milk obtained for this study ranged from 3.63 ± 0.06 (AC) to 4.60 ± 0.00 percent (TM) (Table 7). The results of the ANOVA analysis showed that the fat contents at the sampling site varied significantly ($P<0.05$). The findings indicated that while the fat content of other milk samples was determined to be above global standards, the fat content at two sampling sites (AC and K-7) was found to be below the FAO recommended value. A statistically significant difference ($P < 0.05$) was discovered between the milk samples, according to the analysis of variance. This study's findings concur with those of other authors (Akele *et al.*, 2017; Soliman, 2005).

The proximate composition (moisture content, ash content, protein content, and fat content) in raw cow's milk is comparable with other countries studies as indicated below in (Table 8).

4. Conclusions

The mean concentrations of essential metals in raw cow's milk were as follows: Ca (1550.42 ± 34.76 mg/L), Mg (137.26 ± 2.89 mg/L), Fe (23.97 ± 2.02 mg/L), Cu (1.10 ± 0.11 mg/L), Zn (40.48 ± 3.03 mg/L), and Mn (0.32 ± 0.01 mg/L). The mean concentration of Cr was 0.58 ± 0.05 mg/L, while Pb and Cd were not detected. The overall mean of proximate composition was 87.56 ± 1.16% moisture, 0.75 ± 0.04% ash, 3.24 ± 0.34% protein, and 4.02 ± 0.38% fat.

The results show that, except for Fe, the metal concentrations were below the WHO permissible limits. Regarding proximate composition, moisture, ash, and fat levels exceeded FAO recommendations, while protein content was below the FAO value. These findings are consistent with most literature. Variations in metal levels and proximate composition across different sites may be attributed to factors such as breed type, forage, feeding practices, milking processes, seasonal changes, lactation periods, and potential adulteration. To ensure milk safety and quality, factors affecting milk composition should be carefully

Table 8
Summary of reported proximate contents in milk samples.

Country	% moisture	% ash	% P*	% fat	Ref.
Pennsylvania	87.00	0.80	3.10	3.70	Ferguson *et al.* (2001)
Africa	NR	0.61	2.83	2.66	Roger *et al.* (2013)
USA	NR	0.70	3.20	3.60	Boyazoglu et al. (2005)
Egypt	86.70	0.71	3.48	4.14	Soliman (2005)
	87.50	0.70	3.30	3.40	Li et al. (2012)
Borena, Ethiopia	NR	0.80	0.80	6.01	Azeze and Tera, (2015)
West Africa	3.93	5.27	8.58	29.1	Olagunju *et al.* (2013)
Nigeria	86.30	ND	ND	ND	Abubakar (2023)
	87.20	0.70	3.50	3.70	FAO (2023)
Bahir Dar, Ethiopia	87.56	0.75	3.24	4.02	Present study

controlled and monitored. Further studies are needed to explore the impact of environmental and management factors on milk quality. The study was limited by regional variability and the lack of control over all potential influencing factors.

CRediT authorship contribution statement

Yetinebersh Shitahun: Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Minbale Endaye:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Adane Kassa:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

Appendix 1
Acid digestion optimization procedure.

Volume of sample	Trial no.	HNO_3:$HClO_4$ vol ratio (mL)	Temperature (°c)	Time in min	Color change
1 mL	1	3:2	180	80	Dark black
	2	3:3			Yellow
	3	4:1			Black
	4	4:2*			Clear colorless
1 mL	6	4:2	100		Yellow
	7		120		Clear yellowish
	8		160*		Clear colorless
	10			40	Yellow
	11			50	Black
1 mL	12	4:2	160	70	Clear yellowish
	13			80*	Clear and colorless

* indicates optimized condition for digestion

Appendix 2
Correlations between essential, non-essential metals and proximate contents of milk.

Parameters	Ash	Protein	Fat	Moisture	Mg	Ca	Cu	Fe	Zn	Mn
Protein	-0.195									
Fat	-0.050	.844**								
Moisture	.132	-0.755**	-0.672**							
Mg	.321	-0.316	-0.088	.053						
Ca	.536*	-0.494*	-0.299	.501*	.816**					
Cu	.487*	.144	.327	-0.534*	.251	-0.001				
Fe	.301	.437	.476*	-0.585*	-0.441	-0.574*	.663**			
Zn	.326	.491*	.313	-0.376	-0.114	-0.047	-0.038	.184		
Mn	.329	-0.576*	-0.305	.626**	.750**	.909**	-0.097	-0.620**	-0.351	
Cr	.379	-0.056	-0.206	-0.335	.437	.298	.330	-0.016	.468	-0.006

**. Correlation is significant at the 0.01 level. *. Correlation is significant at the 0.05 level.

Data availability

The data that has been used is confidential.

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