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Levels of some trace heavy metals and fat content in commercially available milk brands in Addis Ababa, Ethiopia

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ABSTRACT: The present study analyzed six pasteurized milk brands for their trace heavy metal concentrations and fat contents. The concentrations of Zn, Cu, Cr, Cd, Pb, and Ni were determined by flame atomic absorption spectrometry. The acid digestion method was employed and its validity was checked by recovery test and a good percentage recovery was obtained. Zinc in the different milk brands studied was found to be 1.93 - 3.31 µg/g. The trace metals Cu, Cr, Cd, Pb, and Ni were too low to be detected in all the milk samples. A statistical analysis of variance (ANOVA) at 95% confidence level ($p > 0.05$) showed that there is no significant difference in the mean concentrations of zinc among the milk brands. The results were also compared with international maximum permissible limits and the concentrations were within the safe limits for human consumption. The fat content of each milk brand was determined and found to be 2.4 - 3.0%. ANOVA at 95% confidence level ($p > 0.05$) indicated that there is no significant difference observed in the mean fat content among all the milk brands. The fat contents determined were also comparable with the reported values in the literature.

Keywords/Phrases: Ethiopia, Pasteurized milk, Heavy metals, Fat content, Acid digestion, FAAS

INTRODUCTION

Milk is among the highly valuable foods as it supplies the nutritional needs of the body better than any other single foodstuff and can be considered as nearly complete food as it is an appropriate source of proteins, fats, sugars, minerals, and vitamins (Abdulkhaliq et al., 2021; Hussain et al., 2010; Qin et al., 2009). On average milk is composed of 87% water, 4-5% lactose, 3% protein, 3-4% fat, 0.8% minerals, and 0.1% vitamins per hundred grams of a milk sample (Guetouache et al., 2014; Pereira et al., 2014; Tyasi et al., 2015).

Milk is the main constituent of the daily diet consumed by all age groups all over the world as a meal on its own particularly for vulnerable groups such as developing infants, school-age children, and old age people (Farid and Baloch, 2012; Tassew Belete et al., 2014; Ayub, 2007). Although milk is an ideal source of considerable nutritional constituents, contaminants like heavy metals can readily enter the milk and dairy products reaching levels that can pose a risk to human health. They can be contaminated either through water, food, manufacturing, and packing processes or the packing materials (Abdulkhaliq et al., 2021). Toxic heavy metals cause adverse effects on human health by enhancing the production of free radicals in several organs (brain, liver, kidney, and heart) and interfering with cellular mechanisms (Akpanyung, et al., 2014). Their toxicity is largely related to age, sex, routes of exposure, duration of exposure, and frequency of intake (Hameed, et al., 2015; Tunegova, et al., 2016). At higher doses, even essential metals in milk have detrimental effects on living organisms (Salah, et al., 2013).

Different analytical methods have been reported for the determination of heavy metals in milk such as electrothermal atomic absorption spectrophotometry (Kazi, et al., 2009), inductively coupled plasma-optical emission spectrometry (ICP-AES) (Hameed, et al., 2015), and flame atomic absorption spectrophotometry (FAAS) (Maheswara, et al., 2017).

Electrothermal atomic absorption spectrophotometry (ET-AAS), inductively coupled plasma-optical emission spectrometry (ICP-AES), and inductively coupled plasma-mass spectrometry (ICP-MS), are commonly used for multi-element analysis at low detection limits. However, these techniques are less precise, costly, and require highly trained person for the operation and maintenance. Flame atomic absorption spectrometry (FAAS) is more precise.

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It has the ability to detect trace metals that are present at lower concentrations than parts per million using relatively inexpensive equipment that is also easy to maintain and has low running costs. Therefore, FAAS was selected for the determination of trace metals in the present study as it offers simplicity and relatively low cost of equipment for routine analysis with good analytical performance.

Due to the growing pollution by heavy metals that expose humans and grazing animals to health problems, it becomes necessary to determine the level of these metals in milk (Kazi, et al., 2009; Jigam, 2011). In Ethiopia, the levels of these trace heavy metals were determined in bottled mango juices (Dessie Ezez and Mitiku Belew, 2023), irrigation water and onions (Reta Birhanu, et al., 2012), and honey (Weldegebriel Yohannes, et al., 2018) are some among others.

However, there were no studies carried out the on the levels of trace heavy metals and fat contents of pasteurized milk samples marketed in Ethiopia. The objectives of the study were: (i) to determine the trace heavy metals Cd, Pb, Zn, Cr, Ni, and Cu in the milk samples using flame atomic absorption spectrometry, (ii) to compare the results of this research with internationally set limits, (iii) to develop and validate the working procedure for acid digestion method for the extraction of selected trace metals from the milk samples, and (iv) to determine the fat content of the different milk samples.

Experimental

Chemicals and reagents

The reagents that were used for the analysis of the selected metals were all analytical grade. HNO_3 (69–72%, Spectrosol, BDH, England) and $HClO_4$ (70%, BDH Laboratory Supplies AnalaR®, Poole, England) were used for the digestion of the milk sample. Standard stock solutions containing 1000 mg/L in 2% HNO_3 (BDH Chemicals Ltd Spectrosol®, Poole, England) were used to prepare intermediate standard solutions of concentration 10 mg/L, where working calibration standard solutions were prepared from it for the determination of metals in the spiked and non-spiked samples. Distilled and deionized water was used throughout the study.

Reagents that were used for the determination of fat content in the six different milk brands were HCl (1.18 sp. Gr, Sigma-Aldrich, Germany), ethyl alcohol (absolute anhydrous, Fisher Scientific, UK), diethyl ether (peroxide free, Carlo Erba, France) and petroleum ether (boiling point 40-60 ^oC, Sigma-Aldrich, Germany).

Equipment

An atomic absorption spectrophotometer (ZEE nit 700P, Germany) equipped with a deuterium arc background corrector with air-acetylene flame at selected wavelengths was used for the determination of the metals. A refrigerator was used to keep the collected and digested samples until analysis. A 250 mL-round bottom flasks fitted with a reflux condenser were used together with the Kjeldahl digestion block apparatus to digest the milk samples, spiked samples, and blank solutions.

Sample collection

For this study, six different brands of commercially available pasteurized milk in Addis Ababa, Ethiopia were purchased from supermarkets located in different areas of the city. All of them were packed in plastic bags. Since the milk brands have a short shelf life, only three plastic bags (500 mL) of each brand were purchased and stored in a refrigerator at a time until analysis. Three plastic bags of the same brand for each of the six different milk brands (a total of 18 plastic bags) were used to determine heavy metals and fat content. The purchased samples were used immediately for fat content determination as the fat is sensitive to biochemical reactions.

Trace metal determination

Three plastic bags (500 mL each) of the same brand were used for each of the six different samples of pasteurized milk. 100 mL of the sample from each of the three bags of the same brand was transferred to a plastic bottle (totally 300 mL) for homogenization to get a representative sample. After mixing thoroughly, 1.00 g of milk was measured and transferred to a round bottom flask for the digestion to which 2.5 mL of 69-72% concentrated HNO_3 and 1 mL of 70% $HClO_4$ were added to the sample and digested in a Kjeldahl digestion block fitted with reflux condensers for 2 h at 240 ^oC. After cooling the content for 15 min at room temperature (25 ^oC) without removing the condensers, 1 mL of 69-72% concentrated HNO_3 and 1 mL of 70% $HClO_4$ were added and the content was digested for an additional 1 h at the same temperature

until clear and color less solution was obtained. After cooling the digested solution for 15 min, the flasks were detached from the reflux condenser and 5 mL of deionized water was added into it and transferred to a 25 mL volumetric flask using filter paper (Whatman No. 41), made up to the mark with deionized water, and kept in refrigerator for the analysis of their trace metal contents using FAAS. Each milk brand was digested in triplicates. Blank samples were prepared in the same way as that of the sample.

Fat content determination

The procedure in reference [FSSAI, 2015] was adapted to determine the fat content of each milk brand. Accurately weighed 5.00 g of the homogenized milk sample from each brand was taken from the plastic bottle and transferred to a 100 mL beaker. 5 mL conc. HCl (sp. gr. = 1.18) was mixed with the milk sample and heated on a Bunsen burner by stirring continuously with a glass rod until a dark brown solution appeared. After cooling at room temperature, the contents were transferred to a graduated plastic tube. The addition of 5 mL ethyl alcohol and 13 mL of petroleum ether and diethyl ether each into a plastic tube followed by vigorous shaking for 1 min gave two separated layers. The colorless upper layer of the mixture which is a mixture of ethers and fat was decanted into a conical flask and the solvents were evaporated in the water bath. The fat was dried in an oven at 102 ^{o}C until constant mass was obtained. The oven drying removes the last traces of water, alcohol, and solvents and finally, the percentage of fat was determined.

Digestion of milk samples spiked with standard metal solutions

The validity of the modified procedure for milk samples was checked by spiking of a known amount of zinc from 100 mg/L intermediate solution (prepared from 1000 mg/L stock standard solution) to flasks containing one gram of milk samples. For zinc, 40% of its concentration was added to the triplicates by measuring 13.24 µL from the 100 mg/L intermediate solution. Both the spiked and unspiked triplicate samples were digested simultaneously based on the modified developed procedure. The digests were transferred to a 25 mL volumetric flask and diluted to its mark with deionized water. Finally, the solutions were analyzed for the metal concentration with FAAS and the percentage of recovery was calculated.

Method detection limit

The method detection limit of the metal was obtained by using the equation:

$MDL = 3 \times \sigma_{blank}$

where, σ is the standard deviation of the blank. Based on the above equation, the method detection limit of Zn metal was found to be 0.12 mg/L.

RESULTS AND DISCUSSION

Calibration of the instrument

The correlation coefficient (R^2) of the calibration curves of each metal was determined by plotting working standard concentration (mg/L) versus their corresponding absorbance. A calibration curve was used to express the relationship between the response of the measuring technique and the standard concentration of the target analyst. The working standard solutions and correlation coefficient obtained from the calibration curves of the analyzed metals are summarized in Table 1.

Table 1. Concentrations of working standard solutions, and the correlation coefficients of the calibration curves for the respective metals.

Metal analyzed	Conc. of standard working solutions (mg/L)	Correlation coefficient (R^2)	Regression equation
Zn	0, 0.15, 0.5, 0.75, 1	0.995	y = 0.086x+0.000
Cu	0, 0.25, 0.5, 1, 2	0.996	y = 0.022x-0.000
Cr	0, 0.5, 1, 1.5, 2	0.998	y = 0.012x-3e-05
Cd	0, 0.25, 0.5, 0.75, 1	0.998	y = 0.044x-0.001
Ni	0, 0.5, 1.5, 3, 6	0.999	y = 0.006x-0.000
Pb	0, 0.4, 0.8, 1.2, 1.6	0.998	y = 0.003x+6e-05

Digestion procedure for metal determination

For milk samples, the digestion condition was adjusted by modifying the procedure used by Admasu and his co-workers (Engdawork Admasu, et al., 2008). The modified procedure mainly focused on minimizing the time of digestion (3.0 h) and volume of $HClO_4$ (2 mL) and HNO_3 (3.5 mL) at 240 ^oC.

Recovery test

The percentage recovery of the metals was determined and the recovery percentage for the milk samples is given in Table 2.

Table 2. Recovery test for zinc metal for analyzed milk samples.

Metal	Sample ID	Sample (mg/L)	[a]X±SD	Amount added (mg/L)	Spiked (mg/L)	[b]X±SD	% Recovery
Zn	Ete 1	0.0839	0.0988±0.0220	0.0500	0.133	0.148±0.0236	98.9
	Ete 2	0.124			0.175		
	Ete 3	0.0884			0.136		

[a]Mean unspiked. [b]Mean spiked.

Determination of the concentration of heavy metals in the milk samples

The concentrations of heavy metals (Cu, Cr, Cd, Pb, and Ni) in the different milk samples studied were too low to be detected in all the milk samples. This is possibly due to the feeds and the water that the cows use being free from the environmental contamination of the heavy metals from different common anthropogenic sources such as consumer wastes, vehicles, industrial emissions, etc. In this study, zinc is present in all the milk samples with concentrations ranging from 1.93 to 3.31 µg/g as shown in Figure 1. There is no significant difference at a 95% confidence level ($p > 0.05$) in Zn level was observed in the mean concentrations among the six milk brands.

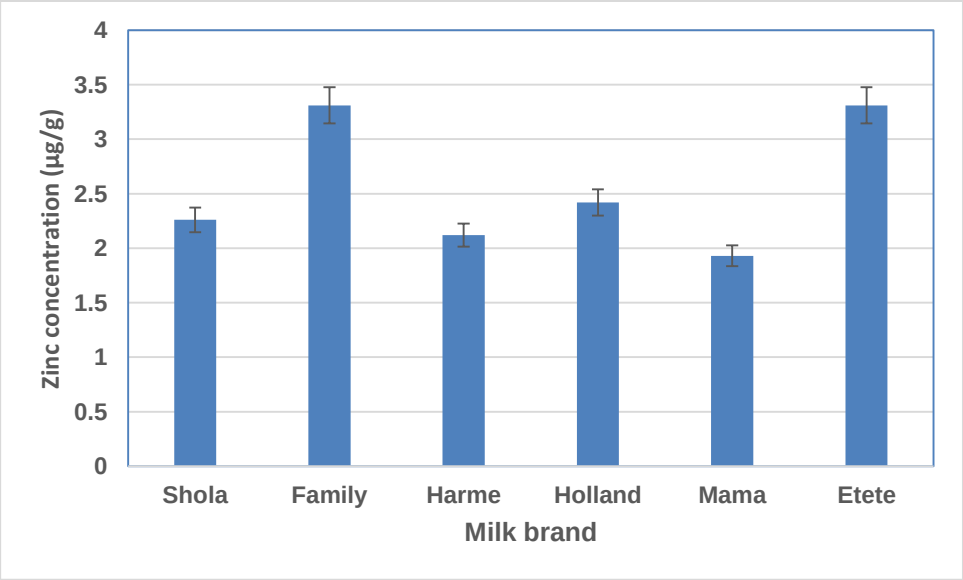

Figure 1. Mean concentration of zinc (µg/g) in the milk brands.

Comparison of the concentration of metals in the different milk brands with values reported in the literature

Table 3 shows the comparison of the obtained results with the literature from different countries. Different countries have wide variations in the concentrations of metals in milk in the published data as recorded in Table 3. The

trace heavy metals investigated in the present study were found too low to be detected in the pasteurized milk samples except zinc. The concentrations of zinc in the different milk samples in this study are generally more or less comparable with most of the reported literature values. The *concentration* of zinc metal in pasteurized *milk* is within the *permissible limits* that may be attributed to the presence of zinc in water sources or the animal feed given to cows.

Table 3. Average metal content of cow's raw and pasteurized milk in different countries (μg/g).

Countries	Zn	Cu	Cd	Cr	Ni	Pb	Ref.
Egypt (raw)	3.15	0.142	0.086	0.03	0.004	0.066	(Tunegova et al., 2016)
Egypt (raw)	3.59	0.170	0.025	0.03	0.036	0.030	(Abou-Arab et al., 2008)
Algeria (raw)	3.48	0.010	0.017	0.086	c NR	c NR	(Bousbia et al., 2019)
Egypt (raw)	c NR	0.0656	0.070	0.1044	c NR	0.1076	(Mahmoud et al., 2023)
USA (raw)	2.34	0.019	0.010	0.03	[c]NR	0.014	(Kinsara and Farid, 2006)
India (raw)	2.89	0.039	0.001	0.04	[c]NR	0.002	(Kinsara and Farid, 2006)
Libya (raw)	1.67	0.160	0.001	c NR	0.062	0.070	(Mohamod et al., 2020)
Ethiopia (raw)	2.90	0.195	0.0572	0.369	0.210	2.31	(Abreham Tadese et al., 2024)
Germany (raw)	3.39	0.037	0.001	[c]NR	[c]NR	0.002	(Kinsara and Farid, 2006)
Spain (raw)	1.42	0.051	[c]NR	0.03	[c]NR	0.009	
Japan (raw)	3.00	0.100	0.00	[c]NR	[c]NR	0.050	(Kinsara and Farid, 2006)
Ethiopia (raw)	5.59	0.109	[c]NR	0.87	[c]NR	[c]NR	(Tassew Belete et al., 2014)
Egypt (pasteurized)	3.11±0.66	0.151 ±0.08	0.020 ±0.02	0.032 ±0.02	0.030 ±0.02	0.021 ±0.02	(Abou-Arab et al., 2008)
Ethiopia (pasteurized)	Etete = 3.31±0.67 Family = 3.31±0.41 Harme = 2.12±0.69 Holland = 2.42±0.73 Mama = 1.93±0.38 Shola = 2.26±0.22	[d]BDL	[d]BDL	[d]BDL	[d]BDL	[d]BDL	Present study

[c]Not reported. [d]Below detection limit.

Determination of fat content of the different milk samples

Measurement of milk fat is considered one of the main requirements for determining the quality of milk and economic relevance of milk. Accurate determination of fat in certain foods is difficult due to the binding of the fat by the matrix (Engdawork Admasu, et al., 2008). Most methods used to determine fat in these difficult matrices include a pretreatment step to denature or destroy the physical structure of the matrix and allow greater accessibility to the fat.

The fat contents of the milk samples were determined using the procedure by Admasu and coworkers (Engdawork Admasu, et al., 2008). The fat contents of the milk samples were quantified by determining the percent dried weight relative to the original milk weight.

The percentage of the fat content of each milk sample is indicated on the corresponding plastic bag as 2.7, 2.7, 2.7, 2.8, 2.7, and 2.8% for Mama, Family, Holland, Etete, Shola, and Harme, respectively. The fat content of each milk sample determined in this study is in the order of: Mama (3.0%) > Family (2.8%) = Shola (2.8%) = Harme (2.8%) > Holland (2.6%) > Etete (2.4%). The fat content of the milk samples is shown in Figure 2. As can be seen, the obtained results are closer to the already determined value in the factory except Etete whose determined value is slightly lower than the labeled value. The fat content of Mama was higher than the fat content from the other samples. The observed high-fat content from the Mama milk sample could be attributed to the different manufacturing (or skimming) practices and the breed nature of the cows. There was also no significant difference at a 95% confidence level ($p > 0.05$) in the fat content among the milk brands.

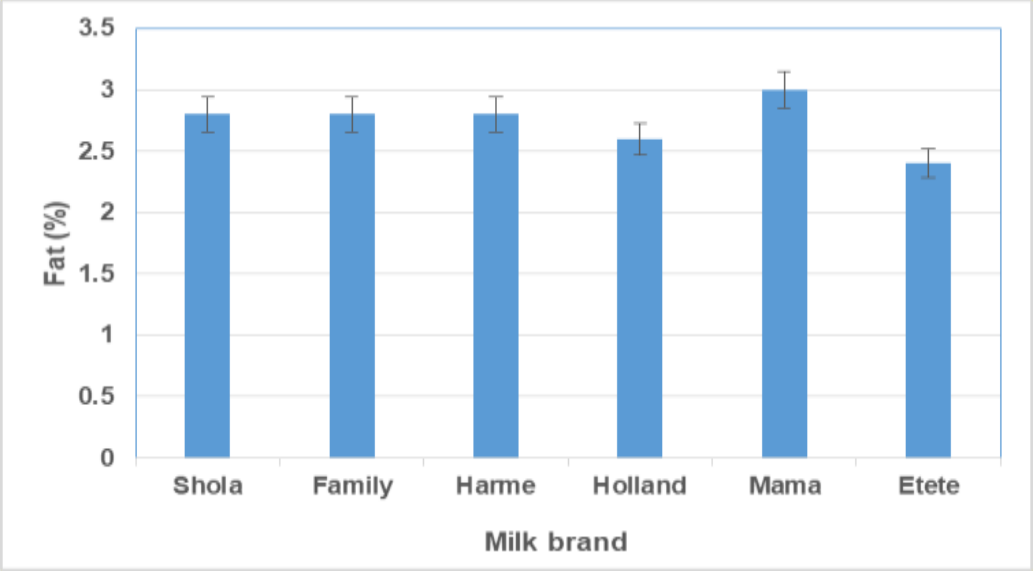

Figure 2. Fat content (%) in the milk brands.

CONCLUSIONS

The concentrations of heavy metals (Zn, Cu, Cr, Cd, Pb, and Ni) of commercially available six different milk brands which are found in Addis Ababa, Ethiopia were determined using FAAS. FAAS is the most commonly used method for the determination of metals in different matrices including milk. It offers simplicity and relatively low cost of equipment for routine analysis. It is sensitive, highly selective, reliable and precise method for metal determination. Hence FAAS was used to determine the concentration of heavy metals in the present study. The concentration of the essential metal, zinc was found in the range of 1.93-3.31 µg/g in all brands, which is below the permissible limit set by EFSA, whereas, the other heavy metals were found below the detection limit of the instrument. The efficiency of the modified digestion procedure for this study was checked by the recovery test and a good percentage of recovery was obtained for zinc metal (98.9 %). The fat content of each milk brand was also determined and found to be 2.4-3.0%. The ANOVA result showed that there was no significant variation in the mean fat content and zinc levels of all milk brands. If the milk brands under study contain trace heavy metals and exceed the maximum permissible limit, nano-sized metal oxides like titanium dioxide and others can be fitted to the industry to minimize their concentration. This is a preliminary work on the different brands of pasteurized milk commercially available in Addis Ababa, Ethiopia. This study will help to further investigate the level of chemical contaminants in pasteurized milk or milk products in the capital city of the country, Addis Ababa or in the country at large. In addition the study encourages the regulatory bodies in Addis Ababa and the international community at large in controlling the concentration of trace metals found in the milk and milk products in the city.

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REFERENCES

1. Abdulkhaliq, A., Swaileh, K.M., Hussein, R.M., and Matani, M. (2012). Levels of metals (Cd, Pb, Cu and Fe) in cow's milk, dairy products and hen's eggs from the West Bank, Palestine. *Int. Food Res. J.* **19**(3): 1089-1094.
2. Abou-Arab, A.A.K., AbouDonia, M.A., and Enb, A. (2008). Chemical composition, metals content and pesticide residues in raw, pasteurized and UHT milk and their dietary intake. *J. Egypt. Soc. Toxicol.* **39**(1): 111-121.
3. Akpanyung, E.O., Ekanemesang, U.M., Akpakpan, E.I., and Anadose, N.O. (2014). Levels of heavy metals in fish obtained from two fishing sites in AkwaIbom State, Nigeria. *Afr. J. Environ. Sci. Technol.* **8**(7): 416-421.
4. Ayub, M., Ahmed, Q., Abbas, M., Qazi, I.M., and Khattak, I.A. (2007). Composition and

adulteration analysis of milk samples. *Sarhad J. Agric*. **23**(4): 1127-1130.

5. Bousbia A., Boudalia S., Gueroui1 Y., Ghebache R., Amrouchi M., Belase B., Meguelati S., Belkheir B., Benidir M., and Chelaghmia M. L. (2019). Heavy metals concentrations in raw cow milk produced in the different livestock farming types in Guelma province (Algeria). *J. Anim. plant Sci.* **29**(2): 386-395
6. Engdawork Admasu., Tadesse Wondmu., Girma Akalu., and Chandravanshi, B.S. (2008). Levels of Some Nutrients and Non-Essential Metals in cows' Whole Milk from Addis Ababa, Ethiopia, Aspect of Africa Biodiversity, Proceedings of the Pan Africa Chemistry Network Biodiversity Conference, Nairobi, pp. 91-104.
7. Dessie Ezez., and Mitiku Belew. (2023). Analysis of physicochemical attributes, contamination level of trace metals and assessment of health risk in mango fruits from Southern region Ethiopia. *Toxicol. Rep*. **10**: 124-132.
8. Farid, S., and Baloch, M.K. (2012). Heavy metal ions in milk samples collected from animals feed with city effluent irrigated fodder. *Greener J. Phys. Sci*. **2**(2): 036-043.
9. Guetouache, M., Guessas, B., and Medjekal, S. (2014). Composition and nutritional value of raw milk. *Issues Biol. Sci. Pharm. Res*. **2**(10): 115-122.
10. Hameed, K.G.A., and El-Zamkan, M.A. (2015). Determination of some heavy metals in flavored milk by inductively coupled plasma optical emission spectrometry (ICP-OES) and their public health importance. *World J. Dairy Food Sci*. **10**(2): 193-198.
1. Hussain, Z., Nazir, A., Shafique, U., and Salman, M. (2010). Comparative study for the determination of metals in milk samples using flame-AAS and EDTA complexometric titration. *J. Sci. Res*. **40**(1): 1-6.
12. Jigam, A.A., Dauda, B.E.N., Jimoh, T., Yusuf, H.N., and Umar, Z.T. (2011). Determination of copper, zinc, lead and some biochemical parameters in fresh cow milk from different locations in Niger State, Nigeria. *Afr. J. Food Sci*. **5**(3): 156-160.
13. Kazi, T.G., Jalbani, N., Baig, J.A., Kandhro, G.A., Afridi, H.I., Arain, B.M., Jamali, M.K., and Shah, A.Q. (2009).Assessment of toxic metals in raw and processed milk samples using electrothermal atomic absorption spectrophotometer. *Food Chem. Toxicol*. **47**(9): 2163-2169.
14. Kinsara, A.A., and Farid, S.M. (2006).Concentration of trace elements in human and animal milk in Jeddah, Saudi Arabia. *Med. J. Islamic World Acad. Sci*. **16**(4): 181-188.
15. Food Safety and Standards Authority of India (FSSAI); Ministry of Health and Family Welfare. *Lab. Manual 4; Manual of Methods of Analysis of Foods*; 2015.
16. Maheswara, Rao, V., and Ananda Murthy, H.C. (2017). Determination of concentrations of selected heavy metals in cow's milk: Dodoma Urban District, Tanzania. *Int. J. Eng. Res. Appl*. **7**(4): 76-84.
17. Mohamod, A.E., Antisar, A.B., and Kahled, M.E. (2020). Evaluation of essential and heavy metal levels in pasteurized and long-life cow milk. *Int. J. Adv. Chem*. **8**(1): 6-14.
18. Mahmoud, E., Marwa, E., Khalid, I.S., Noha, M.S., Samir, M.A., Adel, A., and Amira, H.E. (2023). Heavy metal residues in milk and some dairy products with insight into their health risk assessment and the role of *Lactobacillus rhamnosus* in reducing the lead and cadmium load in cheese. *Food Chem. Adv*. **2**: 100261.
19. Pereira, P.C. (2014). Milk nutritional composition and its role in human health. *Nutrition* **30**(6): 619-627.
20. Qin, L.Q., Wang, X., Li, W., Tong, X., and Tong, W. (2009). The minerals and heavy metals in cow's milk from China and Japan. *J. Health Sci*. **55**(2): 300-305.
21. Reta Birhanu., and Chandravanshi, B.S. (2012). Concentration levels of major and trace metals in onion (Allium cepa L.) and irrigation water around Meki Town and Lake Ziway. Ethiopia. *Bull. Chem. Soc. Ethiop.* **26**: 27-42.
22. Salah, F.A.A.E., Esmat, I.A., and Mohamed, A.B. (2013).Heavy metals residues and trace elements in milk powder marketed in Dakahlia Governorate. *Int. Food Res. J.* **20**(4): 1807-1812.
23. Abreham Tadese, Girma Salale, and Girma Regassa (2024). Assessment of essential and potentially toxic metals in raw cow milk from Mukaturi town, Oromia Regional State, Ethiopia. *Sci. Total Environ.* 926: 171987.
24. Tassew Belete, Ahmed Hussen, and Rao, V.M. (2014). Determination of concentrations of selected heavy metals in cow's milk, Borena Zone, Ethiopia. *J. Health Sci.* **4**(5): 105-112.
25. Tunegova, M., Toman, R., and Tancin, V. (2016). Heavy metals - Environmental Contaminants and their Occurrence in different types of Milk. *J. Anim. Sci.* **49**(3): 122-131.
26. Tyasi, T.L., Gxasheka, M., and Tlabela, C.P. (2015). Assessing the effect of nutrition on milk composition of dairy cows. *Int. J. Curr. Sci.* **17**: 56-63.
27. Woldegebriel Yohannes, Ghirma Moges, Chandravanshi, B.S. (2018). Assessment of trace metals and physicochemical parameters of commercially available honey in Ethiopia. *Chem. Int*. **4**(2): 91-101