

Determination of heavy metals amount in raw milk of Bangladesh

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Abstract

Milk is an indispensable part of the human food regimen that serves several nutritional benefits. Although milk is rich in micronutrients and macronutrients, heavy metal contamination of milk and dairy products is a serious concern. The distribution and deposition of heavy metals and trace elements including Fe, Cu, Zn, Mn, Cd, Cr, Pb, As, etc in different administrative areas of Bangladesh were investigated in this study. All transition metals, regardless of whether they are considered essential or potentially harmful, can have a negative effect on humans and animals if added to the diet in excessive amounts. The samples were prepared for examination by ashing, and after acid digestion, the heavy metals were detected using atomic absorption spectrophotometry. The concentrations obtained in mg/kg were in the range of (0.447 ± 0.043) , (0.314 ± 0.077) , (0.152 ± 0.025) , (0.065 ± 0.022) , (0.008 ± 0.002) , (0.026 ± 0.003) , (0.158 ± 0.027) , (0.007 ± 0.001) for iron, copper, zinc, manganese, lead, cadmium, chromium and arsenic respectively. The study was implemented to assess the concentration of heavy metals and if these heavy metals fell below the World Health Organization's permissible standards (WHO). The investigation disclosed high quantities of iron and other metals that were above WHO guidelines. Iron (Fe) concentrations were higher compared to WHO standards (0.5 mg/kg) in raw milk from all divisions except Chittagong. As heavy metal contamination has been a public health concern in recent years, additional research is needed to ascertain the precise origins of heavy metals in raw milk.

Keywords: Heavy metals, Milk, Atomic Absorption Spectrophotometer.

Introduction

Milk is a liquid meal that is full of nutrients and is formed by the mammary glands of mammals. Young mammals, such as human newborns that are breastfed, acquire the majority of their nutrients from milk before they're able to even metabolize regular meals. [1] Colostrum, or early-lactating milk, contains antibodies that enhance the immune system and reduce the chance of diseases. [2] Milk is an essential component of the human diet that provides numerous health advantages. Besides, it has been considered a complete diet for humans since it includes vital nutrient-rich components. [3] In the human diet, milk is referred to as a nutrient-balanced food. Moreover, it is enriched in micronutrients and macronutrients. However, heavy metal contamination in milk and milk-derived products poses a significant risk. [4] Milk is an important part of a regular diet in Bangladesh and the whole world. These are used as a supplement that is full of nutrients. These are also utilized to treat vulnerable populations like infants, school-aged

teenagers, and the elderly. Additionally, it is utilized in dairy industries and families all over the world, including Bangladesh, to make a diversity of milk products. Milk is packed with calcium, vitamin D, riboflavin, and phosphorus having adequate amounts of vitamin A, vitamin B-12, protein, potassium, and niacin. However, increased pollution has accelerated milk contamination issues and raised questions about the quality of milk. [5] Since neonates and children are the main users of milk and milk-based products, heavy metals including lead and cadmium, which are potential carcinogens, are of critical significance. [6] Deadly heavy metals like arsenic and cyanide can be found in milk and milk products. [7] Essential elements and non-essential elements are the two types of metals found in milk. Lead and cadmium are non-essential compounds, while iron, copper, and zinc are necessary. Dairy milk products like yogurt and cheese have all been improvised milk products that people have created in recent years. Heavy metals can be transferred into powdered milk during production and distribution by a variety of equipment, and they can also contaminate powdered milk through foodstuffs and water as well as production and packaging operations.[8] Heavy metals create serious medical problems, so properly measuring these residues is critical.[9] Metal toxicity is associated with age, sex, method of intake, volume of ingestion, dissolution, metallic elemental composition, retention proportion, length of exposure, periodicity of daily consumption, absorptivity, and excretion processes. [10, 11] Cognitive issues, kidney failure, genetic mutations, cancers, neurodegenerative problems, breathing problems, coronary issues, immunological weakness, and fertility problems may be caused by heavy metals in the body. [9] Lead impairs the central nervous system (CNS), inducing anemia, kidney, liver, heart, and vascular trauma (blood vessel damage), along with damage to the immune system, genital, and digestive tract damage, and contributes to the emergence of cancer and other malignancies.[12, 13, 14] Encephalitis and hepatitis (inflammation of brain and liver) can also be induced by lead. Cadmium builds up in the liver and kidneys, causing anemia and high blood pressure (hypertension). Cadmium is a carcinogen that causes cancers in the lungs and prostate, among other places. Kidneys, bones, lungs, liver, heart, and blood vessels are all affected by cadmium exposure. [12] Because of their toxic effects, bioavailability, and biomagnification in the food chain, pollution from heavy metals has become a very major problem, which has prompted significant concern about anthropogenic pollution's impacts on the environment. The heavy metal contents were measured and compared in this study. Another purpose of this research is to identify whether or not raw milk is safe to ingest.

Materials and Methods

Sample collection

For this analysis, 192 samples of cow's milk that was raw were taken from 64 administrative regions of 8 divisions throughout Bangladesh. 39 samples were taken from Dhaka, 33 from Chittagong, 24 from Rajshahi, 24 from Rangpur, 18 from Barishal, 30 from Khulna, 12 from Sylhet, and 12 from Mymensingh. The samples were obtained local marketplaces. The samples were collected in sterile glass vials using conventional collecting procedure that were immediately stored in a refrigerator to control temperature below 4 degrees Celsius. Then the samples were taken in the laboratory immediately and stored at 4°C until biochemical analysis. Within 24 hours, the analysis procedure had begun. To stay away from any metallic sources, the samples were kept in glass bottles. During this study, the levels of specific heavy metals were estimated in the collected milk samples. These metals are Copper (Cu), Cadmium (Cd), Iron (Fe), Manganese (Mn), Zinc (Zn), Chromium (Cr), Lead (Pb) and Arsenic (As).

Preparation of standards of heavy metals

Atomic absorption spectroscopy was used to find out how much heavy metals mentioned above (i.e., Fe, Cu, Mn, Zn, Cd, Pb, Cr and As) were present in the samples. For each heavy metal, we prepared standard solutions at four concentrations and one blank. The spectral ranges for Cd, Cr, Mn, Pb, Zn, Cu, Fe and As are, in order, 228.67, 357.65, 279.43, 217.35, 248.30, 324.57, 213.9, and 193.7 nm. The certified reference materials (CRM) were used to assure quality control (Sigma Aldrich, USA). The mean of four measurements of each standard solution was used. To ensure that the standard solutions were accurate, a blank solution of distilled water was utilized after every 10 samples.

Acid digestion of raw milk

A microwave digestion process was used to remove the components from the samples (Berghof, Germany). Before being rinsed with distilled water, the glass equipment was completely rinsed in a 10% HNO₃ solution. 10 ml of milk was treated with a 1:3 mixture of H₂O₂ and HNO₃ on a heated plate to process it. On a hot plate, the samples were torched until their volume was reduced to 2 ml. 2 ml of this condensed prepared sample solution was produced transparently by adding 20 mL of distilled water. A Flame Atomic Absorption Spectrophotometer was used for evaluating the contents of beaker, which were distilled to the necessary volume.

Data assessment

All of the data was collected and organized into tables. The amount of heavy metals from milk samples were measured in ppm then converted to values in mg/kg. For statistical analysis, the collected data were saved in Microsoft Excel 2010. Percentages, mean, and standard deviation for several variables were used in descriptive analysis. Finally, a one-way ANOVA was performed to assess heavy metal levels in milk sample from several districts of Bangladesh. In our study, the significance level was set at ≤ 0.05 .

Results

Milk is a significant source of nutrition for people of different ages, due to its nutritious value. However heavy metal contamination is becoming more common as a result of ongoing industrialization. Heavy metal contamination necessitates special care since heavy metals in excess of their typical range pose a significant risk to public health. As a result, it was interesting to undertake a study to evaluate the concentration of heavy metals levels in various samples using AAS. This study looked at the concentrations of several metals in samples from various administrative areas. Finally, the findings were compared to WHO's suggested maximum allowable limits.

Table 3.1: Heavy metals concentration (mg/kg) from raw milk samples in eight divisions of Bangladesh, the values are mean, SD, maximum, minimum.

Elements	Mean	SD	Maximum	Minimum	WHO limit
Fe	0.447	0.043	0.512	0.356	0.5
Cu	0.314	0.077	0.512	0.211	24.2

Zn	0.152	0.025	0.199	0.019	121
Mn	0.065	0.022	0.135	0.025	55.5
Pb	0.008	0.002	0.015	0.005	0.3
Cd	0.026	0.003	0.036	0.021	0.58
Cr	0.158	0.027	0.207	0.117	1.61
As	0.007	0.001	0.01	0.005	0.05

Total heavy metals concentrations were shown in this table from whole country. Iron concentration is (0.447 ± 0.043) mg/kg in which maximum value is 0.512 (mg/kg) and minimum value is 0.356 (mg/kg). Copper concentration is (0.314 ± 0.077) mg/kg in where maximum value is 0.512 (mg/kg) and minimum value is 0.211 (mg/kg). Zinc concentration is (0.152 ± 0.025) mg/kg in which maximum value is 0.199 (mg/kg) and minimum value is 0.019 (mg/kg). Manganese concentration is (0.065 ± 0.022) mg/kg in where maximum value is 0.135 (mg/kg) and minimum value is 0.025 (mg/kg). Lead concentration is (0.008 ± 0.002) mg/kg in which maximum limit is 0.015 (mg/kg) and minimum limit is 0.005 (mg/kg). The concentration of Cd is (0.026 ± 0.003) mg/kg in where maximum value is 0.036 (mg/kg) and minimum value is 0.021 (mg/kg). Chromium concentration is (0.158 ± 0.027) mg/kg in which maximum value is 0.207 (mg/kg) and minimum value is 0.117 (mg/kg). Arsenic concentration is (0.007 ± 0.001) mg/kg in which maximum value found 0.01 (mg/kg) and minimum value found 0.005 (mg/kg). The average value of every parameter was observed to be within allowable range by the World Health Organization's (WHO).

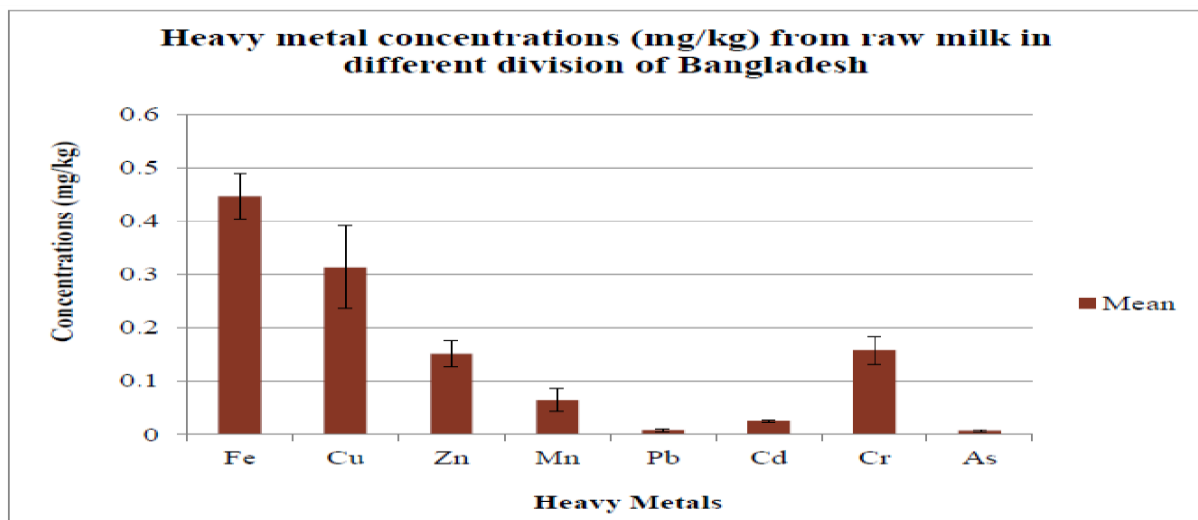


Figure 3.1: Bar diagram of heavy metal concentrations (mg/kg) from raw milk in different division of Bangladesh.

3.1 Iron Concentration

Table 3.2: Concentration of Fe (mg/kg) from raw milk in eight division of Bangladesh.

Division	Mean	SD	Maximum	Minimum	P value
Dhaka	0.443	0.046	0.511	0.361	
Chittagong	0.427	0.038	0.487	0.356	

Rajshahi	0.446	0.044	0.509	0.381	0.012499
Rangpur	0.460	0.032	0.509	0.391	
Barishal	0.455	0.032	0.511	0.403	
Khulna	0.443	0.048	0.512	0.356	
Sylhet	0.478	0.026	0.512	0.441	
Mymensingh	0.458	0.048	0.504	0.378	

The greatest Fe concentration was determined in Sylhet division (0.478 ± 0.026 mg/kg), while the lowest was reported in Chittagong division (0.427 ± 0.038 mg/kg). Every parameter's Fe maximum values column exceeded WHO's recommended limits. The limits exceeded in every division except Chittagong division. The concentration of Fe in every division was in the order Sylhet > Rangpur > Mymensingh > Barishal > Rajshahi > Dhaka > Khulna > Chittagong. The iron approved limit by WHO for is 0.5 mg/kg.[15] Fe concentration in raw milk from 64 areas differed statistically significantly ($P < 0.05$).

3.2 Copper Concentration

Table 3.3: Concentration of Cu (mg/kg) from raw milk in eight divisions of Bangladesh.

Division	Mean	SD	Maximum	Minimum	P value
Dhaka	0.294	0.076	0.481	0.211	0.0000012
Chittagong	0.291	0.069	0.452	0.233	
Rajshahi	0.393	0.075	0.507	0.266	
Rangpur	0.301	0.072	0.432	0.221	
Barishal	0.280	0.059	0.381	0.211	
Khulna	0.315	0.044	0.396	0.221	
Sylhet	0.319	0.085	0.452	0.243	
Mymensingh	0.351	0.098	0.512	0.253	

The concentration of copper in each sample is listed in Table 3.3 by division. The maximum Cu concentration was observed in Rajshahi milk (0.393 ± 0.075 mg/kg), whereas the minimum was reported in Barishal milk (0.280 ± 0.059) mg/kg. Rajshahi > Mymensingh > Sylhet > Khulna > Rangpur > Dhaka > Chittagong > Barishal was the order of Cu concentration in each division. When compared to the copper permitted limit of 24.2 mg/kg Cu content differences in raw milk from 64 districts were highly significant ($P < 0.05$).

3.3 Zinc Concentration

Table 3.4: Concentration of Zn (mg/kg) from raw milk in eight division of Bangladesh.

Division	Mean	SD	Maximum	Minimum	P value
Dhaka	0.156	0.022	0.188	0.119	0.395329
Chittagong	0.151	0.021	0.188	0.122	
Rajshahi	0.151	0.021	0.188	0.122	
Rangpur	0.152	0.034	0.188	0.019	
Barishal	0.145	0.015	0.176	0.122	
Khulna	0.146	0.031	0.188	0.019	
Sylhet	0.158	0.030	0.199	0.127	
Mymensingh	0.164	0.013	0.185	0.142	

The zinc content in our investigation is shown in Table 3.4. Mymensingh had the most zinc concentration (0.164 ± 0.013 mg/kg) while Barishal had the least value (0.145 ± 0.015 mg/kg). The study samples all had results below the permitted limit of 121 mg/kg. Mymensingh > Sylhet > Dhaka > Rangpur > Chittagong > Rajshahi > Khulna > Barishal had the maximum Zn concentration in raw milk. The difference in Zn levels in raw milk between 64 districts was insignificant ($P > 0.05$).

3.4 Manganese Concentration

Table 3.5: Concentration of Mn (mg/kg) from raw milk in eight division of Bangladesh.

Division	Mean	SD	Maximum	Minimum	P value
Dhaka	0.061	0.024	0.128	0.033	0.010626
Chittagong	0.057	0.016	0.089	0.033	
Rajshahi	0.067	0.024	0.12	0.036	
Rangpur	0.064	0.016	0.089	0.036	
Barishal	0.074	0.019	0.112	0.025	
Khulna	0.075	0.027	0.135	0.033	
Sylhet	0.059	0.013	0.085	0.041	
Mymensingh	0.057	0.018	0.089	0.036	

The highest Mn concentration (0.075 ± 0.027) mg/kg was observed in Khulna division while lowest was found in Chittagong (0.057 ± 0.016) mg/kg and Mymensingh division (0.057 ± 0.018) mg/kg. The Mn concentration in every division was followed by the order Khulna > Barishal > Rajshahi > Rangpur > Dhaka > Sylhet > Chittagong > Mymensingh. The limit for Manganese is 55.5 mg/kg that is approved by WHO.[15] The Mn concentration in raw milk of 64 districts differed statistically significantly ($P < 0.05$).

3.5 Lead Concentration

Table 3.6: Concentration of Pb (mg/kg) from raw milk in eight division of Bangladesh.

Division	Mean	SD	Maximum	Minimum	P value
Dhaka	0.008	0.002	0.014	0.005	0.242659
Chittagong	0.007	0.002	0.014	0.005	
Rajshahi	0.008	0.002	0.012	0.005	
Rangpur	0.009	0.002	0.014	0.005	
Barishal	0.008	0.002	0.013	0.005	
Khulna	0.009	0.003	0.015	0.005	
Sylhet	0.007	0.002	0.012	0.005	
Mymensingh	0.007	0.018	0.014	0.005	

The table 3.6 shows the lead concentration in all samples from various divisions. Maximum concentration of Pb was observed in milk of Rangpur (0.009 ± 0.002) mg/kg and Khulna (0.009 ± 0.003) mg/kg respectively. Minimum was found in Mymensingh (0.007 ± 0.018) mg/kg, Sylhet (0.007 ± 0.002) mg/kg, and Chittagong (0.007 ± 0.002) mg/kg. The Pb concentration in every division was in the order Khulna > Rangpur > Dhaka > Barishal > Rajshahi > Chittagong > Mymensingh > Sylhet. When compared with the permissible limit of lead is 0.3 mg/kg. The difference in Pb levels in raw milk between 64 districts was insignificant ($P > 0.05$).

3.6 Cadmium Concentration

Table 3.7: Concentration of Cd (mg/kg) from raw milk in eight division of Bangladesh.

Division	Mean	SD	Maximum	Minimum	P value
Dhaka	0.025	0.003	0.036	0.021	0.242659
Chittagong	0.027	0.004	0.035	0.021	
Rajshahi	0.026	0.004	0.035	0.021	
Rangpur	0.025	0.003	0.033	0.021	
Barishal	0.025	0.003	0.036	0.021	
Khulna	0.025	0.003	0.032	0.021	
Sylhet	0.025	0.002	0.029	0.021	
Mymensingh	0.026	0.004	0.035	0.021	

The highest Cd concentration (0.027 ± 0.004) mg/kg was found in Chittagong division while the lowest was found in Dhaka (0.025 ± 0.003) mg/kg, Rangpur (0.025 ± 0.003) mg/kg, Barishal (0.025 ± 0.003) mg/kg, Khulna (0.025 ± 0.003) mg/kg and Sylhet (0.025 ± 0.002) mg/kg division. The Cd concentration in every division was followed by this order: Chittagong > Rajshahi > Mymensingh > Rangpur > Dhaka > Sylhet > Khulna > Barishal. The cadmium approved limit by WHO is 0.58 mg/kg.[16] The variation in Mn concentration in raw milk across 64 districts was statistically not significant ($P > 0.05$).

3.7 Chromium Concentration

Table 3.8: Concentration of Cr (mg/kg) from raw milk in eight division of Bangladesh.

Division	Mean	SD	Maximum	Minimum	P value
Dhaka	0.156	0.027	0.207	0.117	0.031646
Chittagong	0.163	0.028	0.205	0.122	
Rajshahi	0.158	0.021	0.191	0.119	
Rangpur	0.163	0.025	0.203	0.128	
Barishal	0.153	0.034	0.205	0.117	
Khulna	0.143	0.023	0.191	0.117	
Sylhet	0.166	0.031	0.201	0.132	
Mymensingh	0.172	0.022	0.207	0.142	

The concentration of chromium in each sample is listed in Table 3.8 for each division. Mymensingh milk has the maximum Cr value (0.172 ± 0.022) mg/kg. Khulna has the minimum concentration (0.143 ± 0.018 mg/kg). Mymensingh was followed by Sylhet, Chittagong, Rangpur, Rajshahi, Dhaka, Barishal and Khulna in terms of Cr concentration. Cr in milk has a maximum daily dietary intake limit of 1.61 mg/kg.[16] The variation in Cr concentration in raw milk was significant ($P < 0.05$) among 64 districts..

3.8 Arsenic Concentration

Table 3.9: Concentration of As (mg/kg) from Raw milk in eight division of Bangladesh.

Division	Mean	SD	Maximum	Minimum	P value
Dhaka	0.007	0.001	0.01	0.005	0.792511
Chittagong	0.007	0.001	0.01	0.005	
Rajshahi	0.007	0.001	0.009	0.005	
Rangpur	0.007	0.001	0.01	0.005	
Barishal	0.006	0.001	0.01	0.005	
Khulna	0.007	0.001	0.01	0.005	
Sylhet	0.007	0.001	0.01	0.005	
Mymensingh	0.007	0.001	0.01	0.005	

The concentration of Arsenic was found same (0.007 ± 0.001) mg/kg in every division except Barishal division (0.006 ± 0.001) mg/kg. There were no deviations from the WHO's recommended maximum values for any parameter, including the maximum values column (Table 3.1). The Arsenic limit by WHO is 0.05 mg/kg.[16] Raw milk As concentrations from 64 different administrative areas did not differ statistically significantly ($P > 0.05$).

Discussion

Several things can contaminate milk and milk products. Heavy metals, mycotoxins, and veterinary medicine residues are some of the causes. Heavy metals are one of the most dangerous and complex contaminants.[19, 20, 21, 22] Contamination occurs mostly as a result of pollutants being ingested by animals drinking milk or contamination occurring during the manufacture of milk.[23] Due to their endurance, toxicity to the environment, bioaccumulation, etc., heavy metals are a significant source of pollution.[24, 25, 26, 27] The maximum Fe content was (0.478 ± 0.026) mg/kg to (0.427 ± 0.038) mg/kg in our study. In another study, it was found that camel milk has the most Fe (1.580 ± 0.53 mg/kg) and sheep milk has the least (0.592 ± 0.321 mg/kg).[18] Our raw milk concentration is lower than what Abdulkhaliq et al.[28] and Ahmad et al.[18] In comparison to this investigation, Meshref et al. found a much higher level of Fe in milk and milk derivatives.[29] In our investigation, Cu contents in Bangladeshi raw milk varied from (0.393 ± 0.075) mg/kg to (0.280 ± 0.059) mg/kg. According to Ahmad et al., Cu concentration was found in a maximum amount in Buffalo milk (0.222 ± 0.010 mg/kg), while the minimum concentration was observed in the camel milk (0.06 ± 0.040 mg/kg).[18] In milk, the values of Cu are closely correlated with those reported by Ahmad.[18] In the investigation by Meshref et al., the Cu levels

approached the upper limits.[29] With the exception of buffalo, Pilarczyk et al. found lower Cu

levels in cows.[30] Lutfullah et al. discovered decreased Cu levels in all species except buffalo, which is consistent with the results of the present investigation.[31] In our study, the maximum Zn content was (0.164 ± 0.013) mg/kg, and the minimum was (0.145 ± 0.015) mg/kg in our study. Camel milk had the highest Zn concentration (5.150 ± 0.021) mg/kg and sheep milk had the lowest Zn concentration (3.113 ± 0.072) mg/kg, which is greater than our study.[18] In comparison to this investigation, Lutfullah et al. observed Zn at a maximum level in milk, whereas we found Zn minimally in this investigation in raw milk.[31] According to our research, the greatest Mn concentration was (0.075 ± 0.027) mg/kg, while the smallest concentrations were (0.057 ± 0.016) mg/kg and (0.057 ± 0.018) mg/kg. Manganese concentrations in cow milk have never been documented before.[17] In our investigation, the maximum Pb content was (0.009 ± 0.002) mg/kg, and the minimum Pb content was (0.009 ± 0.003) mg/kg. Raw milk in the district of Dhaka contained a greater concentration of lead (0.26) mg/L than raw milk in the district of Chittagong (0.11) mg/L. Ahmad et al.[4] found a value that is greater than ours. However, another study found that Pb levels in milk samples from all animals tested were below the detection limit.[18] In Bangladeshi divisions, the Cd readings extended from (0.027 ± 0.004) mg/kg to (0.025 ± 0.002) mg/kg. Cadmium levels were highest in buffalo milk (0.117 ± 0.086) mg/kg and less in sheep milk (0.001) mg/kg, both of which were higher than in our study. The Cd levels found in Egypt by Meshref et al.[29] are higher than those observed in this study. Pilarczyk et al. discovered lower Cd levels in cows, which is similar to our findings.[30] With the exception of buffalo and camel milk, which contain significant levels of heavy metals such as Cd, Abdelkhalek et al. found lower Cd levels in their study.[31] The maximum Cr level was (0.172 ± 0.022) mg/kg, while the minimum level was (0.143 ± 0.018) mg/kg, according to our research. The highest Cr concentration was reported in goat milk (1.152 ± 0.045) mg/kg and the minimum value was found in camel milk (0.024 ± 0.013) mg/kg. It is greater than our research.[18] According to our study, the highest As concentration was (0.007 ± 0.001) mg/kg while (0.006 ± 0.001) mg/kg was the lowest level of the research. The maximum values column of each parameter complied with WHO's acceptable limits.

Conclusion

In the human body, heavy metals play important functions. Food is the primary source of these heavy metals. The investigation was done to investigate whether the levels of heavy metals were within the permitted thresholds and to determine their concentration. Specific amounts of iron and all metals were found in our sample, all of which were below WHO guidelines. But in the Sylhet division, iron exceeds its permitted values. Future studies are needed for more specific confirmatory tests, such as NIR.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We thank the department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, Jahangirnagar University and Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh for supporting this work.

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