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Determination of Concentrations of Selected Heavy Metals in Cow's Milk: Borena Zone, Ethiopia

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Abstract Fresh cow's milk samples were collected from four dairy farms of Arero, Gololcha, Odomike, and Semero sites of Borena Zone, Ethiopia and preserved in a deep freezer (-20℃). The milk samples were digested by the optimized microwave digestion method using HNO_3 and H_2O_2. The concentrations of Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb were determined by Flame Atomic Absorption Spectrometer. The elements, Co, Ni, Cd and Pb were not detected in all the milk samples under the study. The concentrations of Cr, Mn, Cu and Zn were 0.845–0.895, 0.411–0.441, 0.087–0.122 and 5.003–6.218 µg/mL, respectively in the milk samples of four sites under study. There is no significant difference in the mean concentrations of Cr and Mn between the milk samples of four farms where as that of Zn is significantly different. The concentrations of heavy metals observed were comparable with some of the reported values in literature. Consumption of beneficial metals through milk is estimated to constitute a minor portion of Recommended Dietary Allowance by FAO, WHO.

Keywords Heavy metals, Fresh cow's milk, Microwave digestion, Flame Atomic Absorption, Spectrometer

1. Introduction

Heavy metals are persistent contaminants in the environment that can cause serious environmental and health hazards. They are released into the environment from natural as well as anthropogenic activities [1, 2, 3, 4]. Some heavy metals like Cu, Fe and Zn are essential to maintain proper metabolic activity in living organisms; others like Pb and Cd are non-essential and have no biological role [5, 6, 7]. However, at high concentrations, even essential metals also cause toxicity to living organisms [8, 9].

Milk and its products are main constituents of the daily diet, especially for vulnerable groups such as infants, school age children and old age people [10, 11]. Milk is excellent sources of calcium, vitamin D, riboflavin, and phosphorus and a good source of protein, potassium, vitamin A, vitamin B-12 and niacin [12, 13]. Milk is contaminated with heavy metals such as zinc, lead, cadmium, selenium, sulphur, iodine and possibly even more dangerous arsenic and cyanide [2]. Due to the growing environmental pollution it is necessary to determine and monitor the levels of heavy metals in milk, because they can significantly influence human health [14, 15, 16]. Many reports indicate the presence of heavy metals in milk and other food products [4, 17, 18]. The determination of trace inorganic constituents in milk is a challenging task due to their complex emulsion like matrices and low concentration levels of the metal ions. Many digestion procedures to oxidize organic matrices of different samples have been reported in literature [19, 20, 21]. The acid digestion procedures are the most popular sample pre-treatment techniques for elemental determination in biological and environmental samples, and acid digestion induced by microwave energy is a well-established method [22, 23].

The determination of heavy metals can be performed by several instrumental techniques [24, 25], including indirect photometric chromatography, ion chromatography, flame atomic absorption spectrometry [26, 27, 28], furnace atomic absorption spectrometry [29], inductively coupled plasma optical emission spectrometry [30, 31], potentiometric stripping [32], capillary zone electrophoresis [33], differential pulse anodic stripping voltammetry [34], mid-infrared spectrometry [35], particle induced x-ray emission [36] and complexometric titration [37]. In this study Flame Atomic Absorption Spectrophotometer was used [38, 39].

The aim of the present study is to determine the concentrations of heavy metals, namely Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb in fresh cow's milk collected from four dairy farms of Arero, Gololcha, Odomike, and Semero sites of Borena Zone, Ethiopia. The obtained mean elemental concentrations were compared with the corresponding values of different countries in the literature and the daily intake of these elements were also compared with the Recommended Dietary Allowance (RDA) values set by

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different international organizations.

2. Materials and Methods

2.1. Description of Study Area

The study was conducted in Abaya district; Borena zone of Oromiya region located 366 km south east of Addis Ababa on the main high way to Moyale in Ethiopia. The study area was selected based on the dependence of farmers on livestock production mainly from cattle and goat. Fresh cow's milk samples were collected from four dairy farms of Arero, Gololcha, Odomike, and Semero sites of the study area.

2.2. Chemicals and Reagents

Analytical-reagent grade chemicals and deionized water were used to prepare all solutions. Nitric acid (70%) (Spectrosol, BDH, England) and hydrogen peroxide (30%) (Riedel-de Haen) were used for cleaning glassware and digesting milk samples throughout this work. Stock standard solutions of Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb having concentration 1000 mg/L in 2% HNO_3 were purchased from Buck Scientific Puro-Graphic$_{tm}$ (USA). Intermediate standard solutions of concentration 10 mg/L were prepared by sequential dilution of stock standards. Working standards were prepared from intermediate standards of each metal.

2.3. Apparatus

Table 1. Instrumental operating conditions for determination of heavy metals in milk samples using FAAS

S. No.	Heavy metal	Wave length (nm)	Slit width (nm)	Lamp current (mA)	Sample Energy (eV)
1	Cr	357.9	0.7	2.0	3.567
2	Mn	279.5	0.7	3.0	3.937
3	Co	240.7	0.2	4.5	2.203
4	Ni	232.0	0.2	7.0	2.605
5	Cu	324.7	0.7	1.5	3.884
6	Zn	213.9	0.7	2.0	3.148
7	Cd	228.9	0.7	2.0	3.317
8	Pb	217.0	0.7	3.0	2.470

All glassware were washed before use with deionised water, soaked in nitric acid (30%), then rinsed in deionised water and air dried. The glassware kept in clean place, to avoid contamination. Microwave digestion of milk samples were performed on a BMS-1 (Buck Scientific, German) microwave system. A 210VGP Flame Atomic Absorption Spectrometer (Buck Scientific, USA) (FAAS) equipped with deuterium background corrector and hollow cathode lamps of Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb with air-acetylene flame was used for the determination of their respective metals in milk samples. Before analysis of the sample, the instrument was optimized to give maximum signal strength by adjusting the parameters such as wavelength, slit width, lamp current and sample energy for each element. These optimized conditions of the instrument are given in Table 1.

2.4. Sample Collection

The polyethylene sampling bottles were soaked in 20% HNO_3 for 24 hours and rinsed with deionised water before collection of raw milk in order to avoid possible contamination. The udder of each cow was washed with distilled water before milking. Ten cows from different farmers were randomly selected from each of Arero, Gololcha, Odomike, and Semero sites and one composite sample representative of each site was prepared. A milk sample of 100 ml was collected during morning milking time from each cow and homogenized, and were kept in an ice box. The samples were transported to laboratory and immediately kept in a deep freeze (-20℃) until microwave digestion was carried out [10].

2.5. Sample Digestion and Preparation of Analyte Solution for FAAS

The optimized microwave digestion procedure was selected depending upon the clarity of digests, minimal digestion time, and minimum reagent volume, absence of undigested milk samples, simplicity and low heating temperature. A 3.0 mL of each liquid milk sample was transferred into 60 mL Teflon digestion vessel and then optimized volumes of 6 mL of 70% nitric acid and 1 mL of 30% hydrogen peroxide were added and the mixture was shaken carefully and kept for 10 min before closing the vessel. The samples were subjected to closed vessel microwave digestion at the optimized microwave digestion program in the sequence of 50W, 165℃ (10 min); 80W, 190℃ (20 min); and 0W, 50℃ (10 min). 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. The digest was diluted to 25 mL with deionized water and used for analysis. Digestion of nine reagent blanks were also performed in parallel with the milk samples keeping all the digestion parameters the same.

2.6. Calibration Curve and Measurement of Metal Concentrations

The calibration curves were drawn for Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb using linear regression analysis of the concentrations of the standard solutions versus absorbance values. Four series of working standard solutions (Table 2) of metals were prepared by diluting the intermediate standard solution (10 mg/L) with deionized water. A blank and standards were run in FAAS and four points of calibration curve were established. Each standard solution was measured three times and the mean was plotted. The correlation coefficients of calibration curves are given in Table 2. The correlation coefficient of more than 0.999 showed that there is strong linear relationship between concentrations and absorbance. Each of the sample solutions were aspirated into the flame of the AAS instrument and the absorbance values of the metals was recorded. Concentrations of each metal were determined by interpolation from the calibration curves. Triplicate determinations were carried out on each sample.

Table 2. Series of working standards and correlation coefficients of the calibration curves for the determination of metals in milk using FAAS

S. No.	Metal	Concentration of standards, mg/L	Correlation coefficient
1	Cr	0.050, 0.500, 1.000, 2.000	0.9996
2	Mn	0.035, 0.100, 0.200, 0.300	0.9993
3	Co	0.060, 0.200, 0.400, 0.600	0.9999
4	Ni	0.060, 0.200, 0.600, 1.000	0.9997
5	Cu	0.008, 0.200, 0.400, 0.600	0.9995
6	Zn	0.008, 0.100, 0.500, 1.000	0.9994
7	Cd	0.015, 0.040, 0.060, 0.100	0.9993
8	Pb	0.050, 0.100, 0.200, 0.300	0.9993

2.7. Method Validation

The recovery of metals was studied by spiking known amounts of standard solution to samples. The amounts added were selected (5-7%), so that they would not cause significant deviation from the amounts naturally found in the milk [10]. Accordingly 500 µL of 0.04 mg/L of Cr, 0.023 mg/L of Mn, 0.005 mg/L of Cu and 0.4 mg/L of Zn were added to 3 mL of milk samples and digested with the same procedure of microwave digestion for milk samples. After diluting the spiked samples to 100 mL with deionized water, they were analyzed with the same procedure followed for the analysis of milk samples. Triplicate samples were prepared and triplicate readings were obtained. The recoveries of the spiked samples were calculated using the following formula.

$$\text{Percentage recovery} = \frac{\text{Concentration in spiked sample} - \text{Concentration in sample}}{\text{Amount spiked}} X\ 100$$

2.8. Method and Instrument Detection Limits

Method detection limit is the lowest analyte concentration that can be distinguished from fluctuations in a blank, which usually corresponds to average of the blank signal plus three times standard deviation of the blank [40]. In order to determine the method detection limits nine blank samples were digested following the same procedure as the milk samples and each of the blank samples were analyzed for Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb by using FAAS. The standard deviation for each element was calculated from the nine blank measurements to determine method detection limit of the instrument. Instrument detection limits are directly obtained from the instrument manual for all the elements under study. The values for both are shown in Table 3. It can be observed that the method detection limit of each element is somewhat above the instrument detection limit.

Table 3. Method and instrumental detection limits for the determination of heavy metals in cow's milk samples

Heavy metals	MDL (mg/Kg)	IDL (mg/Kg)
Cr	0.072	0.040
Mn	0.042	0.030
Co	0.064	0.050
Ni	0.105	0.050
Cu	0.008	0.005
Zn	0.089	0.005
Cd	0.016	0.010
Pb	0.069	0.040

MDL= Method Detection Limit
IDL = Instrumental Detection Limit

3. Results and Discussion

3.1. Evaluation of Analytical Figures of Merit

In this study the precision of the results was evaluated by the standard deviation of the triplicate samples (n = 3), analyzed under the same conditions. Most of the results in both the spiked (Table 4) and real sample (Table 5) showed very less standard deviation indicating high precision. The accuracy and validity of optimized procedure was evaluated by analyzing the digests of spiked samples. The recoveries of the detected metals in the spiked milk samples were 92.50%, 95.65%, 100% and 96.86% for Cr, Mn, Cu and Zn, respectively (Table 4). These values show that the method is very accurate and valid for the determination of heavy metals under study.

3.2. Mean Concentration of Heavy Metals in Cow's Milk

The average heavy metal concentrations in the fresh cow's milk from the four farms of Borena Zone are presented in Table 5. Among the eight elements analyzed, four elements (Cr, Mn, Cu and Zn) were detected where as the other four (Pb, Cd, Co, and Ni) were below their corresponding method detection limit. From the detected elements, the highest mean concentration obtained was for Zn (5.592 ± 0.092 mg/Kg) and lowest for copper (0.109 ± 0.006 mg/Kg) among all the studied farms of Borena zone. The mean concentrations of heavy metals detected in the milk samples in the present study can be arranged in the order of Zn (5.592 ± 0.092) > Cr (0.868 ± 0.026) >Mn (0.427 ± 0.018) > Cu (0.109 ± 0.006) in mg/Kg.

It is clear that chromium is detected in all the samples collected from four different farms and the concentrations varied from 0.845 to 0.895 mg/Kg. When compared with the other detected elements, the average concentration of chromium from all the sites was high (0.868 ± 0.026 mg/Kg) next to zinc. This shows that, the cow's milk in the study area is rich in chromium. Although Cr and other microelements are essential to maintain the metabolic systems of human body, they can lead to poisoning at higher level [41]. It can cause stomach upsets and ulcers, convulsions, kidney and liver damage, and even death. The toxicity of chromium depends on its chemical form, with hexavalent chromium (VI) compounds having a toxic, mutagenic and even carcinogenic nature, and the trivalent chromium (III), which prevails in foods having a low toxicity. Tolerance limit of Cr in milk is 0.3 mg/kg [41]. The daily intake of chromium in milk from the present study was 45.14 μg/day while the recommended/ permissible value for chromium (III) is 50-200 μg/day [9].

The average concentration of manganese in milk sample from all the sites is 0.427 ± 0.018 mg/kg. Exposure to high concentration of manganese is associated with impaired neurological and neuromuscular control, mental and emotional disturbances (muscle stiffness, and lack of coordination). Exposure to very high doses result in impaired male fertility, birth defects, and impaired bone development. Chronic inhalation of high levels of Mn has been associated with a neurodegenerative disorder characterized by both central nervous system abnormalities and neuropsychiatric disturbances. The brain is particularly susceptible to excess Mn. In humans, it has been postulated that there is a spectrum of neurobehavioral and neurophysiological effects associated with Mn toxicity, including both subclinical and clinical symptoms [42]. The estimated safe and adequate Daily Dietary intake of manganese is 2-5 mg/day for adults and 2.5-25 μg/kg bodyweight for infants [43]. Evaluations of standard diets from the USA, the United Kingdom and the Netherlands revealed average daily intakes of 2300 – 8800 μg of manganese per day [44]. In the present study, the daily intake of manganese was very low (22.20 μg/day) and it is far below the recommended value which is 2000-5000 μg/day [26].

In the case of copper, the mean concentrations in the present study were found in the range of 0.087 to 0.122 mg/kg. The average concentration of copper is the lowest (0.109 ± 0.006 mg/Kg) among all the sites. This indicates that the cow's milk of this zone is poor source of copper. Copper is an essential trace element that plays a vital role in the physiology of animals for foetal growth and early post-natal development. Excess copper in the body leads to Wilson's disease which is characterized by deficiency of ceruloplasmin [45]. The toxic limit of copper in cow's milk is 0.4 mg/L [46]. The concentration of copper in the present study is below this toxic limit. The daily intake of copper in the present study is 5.67 μg/day which is very low compared to the recommended daily intake of copper 2000-3000 μg/day [9].

In this study, zinc is present in all the milk samples with concentrations ranging from 5.003 to 6.218 mg/kg. The average concentration of zinc from all the sites is 5.592 ± 0.092 mg/kg. Taking too much of zinc into the body through food, water, or dietary supplements can affect health. The Recommended Dietary Allowances (RDAs) for zinc is 11 mg/day for men and 8 mg/day for women. If large doses of zinc (10–15 times higher than the RDA) are taken by mouth even for a short time, stomach cramps, nausea, and vomiting may occur. Ingesting high levels of zinc for several months may cause anemia, damage the pancreas, and decrease levels of high-density lipoprotein (HDL) cholesterol. In cow's milk of Borena zone the daily intake was determined to be 290.78 μg/day while the recommended daily intake is 12000 – 15000 μg/day [9]. Therefore, the concentration of zinc in the milk of present study is less than the internationally recommended level.

Table 4. Analytical results obtained for the validation of the optimized procedure

S. No.	Metal	Amount spiked mg/Kg	Conc. in sample, mg/Kg (SD)	Conc. in spiked sample, mg/Kg (SD)	Recovery (%)
1	Cr	0.040	0.102 (0.003)	0.139 (0.002)	92.50 (3.00)
2	Mn	0.023	0.049 (0.002)	0.071 (0.002)	95.65 (2.90)
3	Cu	0.005	0.010 (0.001)	0.015 (0.001)	100.00 (3.85)
4	Zn	0.400	0. 601 (0.008)	0.988 (0.013)	96.86 (3. 27)

Table 5. Average concentration, mg/Kg (SD, n = 3) of metals in milk samples collected from different farms of Borena zone

S. No.	Heavy metals	Study sites			
		Arero	Gololcha	Odomike	Semero
1	Cr	0.845 (0.032)	0.847 (0.028)	0.895 (0.013)	0.884 (0.032)
2	Mn	0.441 (0.019)	0.411 (0.019)	0.424 (0.021)	0.431 (0.011)
3	Co	<MDL	<MDL	<MDL	<MDL
4	Ni	<MDL	<MDL	<MDL	<MDL
5	Cu	0.112 (0.008)	0.087 (0.004)	0.115 (0.003)	0.122 (0.007)
6	Zn	5.880 (0.096)	5.003 (0.063)	6.218 (0.099)	5.267 (0.108)
7	Cd	<MDL	<MDL	<MDL	<MDL
8	Pb	<MDL	<MDL	<MDL	<MDL

<MDL = below Method Detection Limit

Among the eight elements analyzed in this study, the elements cadmium, cobalt, nickel and lead were below the method detection limits in all the milk samples of study area. Probably this is because there are no industries and vehicle emissions which are the basic sources of these toxic heavy metals around the study area. Since it is almost rural area, there is no exposure to these toxic heavy metals. The feeds and the water which the cows use are also free from these toxic metals.

The detected heavy metals Cr, Mn, Cu and Zn, can be found naturally in food. They are essential elements and thus, these metals can be found in cow's milk since it is one of the most important types of food. The significance of variation between samples was analyzed using one-way ANOVA. The mean concentrations of both Cr and Mn are not significantly different ($P > 0.05$ at 95% confidence interval) between any two sample sites. Similarly Cu does not differ significantly between Arero/Odomike, Arero/Semero and Odomike/ Semero. This may be due to the same geographical location and same climatic conditions.

Concentration of Zinc showed significant difference ($P > 0.05$ at 95% confidence interval) between any two sample sites. Similarly mean concentration of copper, differ significantly ($P < 0.05$ at 95% confidence interval) between the milk samples of Arero/Gololcha, Gololcha/Odomike, Gololcha/Semero. This difference match with the fact that topography of Gololcha farms where cattles graze is somewhat highland compared to the other three. The significance differences for mean concentration of Zn indicate that, the accumulation of Zn in soil, grass at which the cows grassed in each sites may be different. The concentrations of the elements present in milk depend on atmospheric deposits-soil-cattle feed-milk chain.

3.3. Comparison of Results of the Present Study with Literature Values

There are wide variations in the published data for the elemental concentrations of cow's milk of different countries as shown in Table 6. The concentration of Cr in the present study (0.868±0.026 mg/kg) is high compared with the corresponding values of other countries except that of Nigeria (1.568 ± 2.08 mg/L). This showed that the cow's milk in the present study area is rich in chromium.

The concentration of Mn (0.427 ± 0.018 mg/kg) is also comparable with that of China (0.600 ± 0.150 mg/kg) but it is higher compared to the respective values of remaining countries in the literature.

The result of Cu (0.109 ± 0.006 mg/kg) is also closer to the report made by Dobrazanski *et al.* in Poland (0.089 ± 125.14 mg/L) and comparable with the value reported for Egypt (0.140 ± 0.116 mg/kg). It is less than the values of Croatia, China and Nigeria whereas very much high compared to the value reported for Saudi Arabia.

Zinc concentration (5.592 ± 0.092 mg/kg) is almost same as the reported value of Nigeria (5.521 ± 13.900 mg/L) and comparable with the value reported for Ethiopia (4.923 ± 0.217). It is higher compared to that of Egypt, Poland and Croatia whereas very much lower compared to the China.

In general, the concentrations of metals detected in the present study were more or less comparable with the reported literature values. However, relatively higher concentration of Cr, Mn and Zn, relatively lower concentrations of Cu were observed in this study in comparison to the reported values.

3.4. Recommended Daily Allowance of Heavy Metals

The daily intake of the metals depends on both the concentration and the amount of food consumed. The daily dietary intake of milk for an average Mumbai (India) population is 113g. The reported values of daily milk consumption in USA and Spain are 224g and 124g, respectively [9]. However, in Ethiopia the daily consumption of milk is very low. It is considered to be 53 mL/day which is calculated from 19 Kg per capita per year [49]. The value given per year is changed to per day to know the approximate daily intake in Ethiopia. Since density of whole milk is very close to the density of water, that is 1.0002 g/mL compared to water 1.0 g/mL. Thus it is possible to assume that 53 g is equal to 53 mL of whole milk. Assuming a value of 53 mL of milk consumption per day, the daily intake of the detected heavy metals from samples of the present study are determined and depicted in Table 7. The 4th column shows the Recommended Dietary Allowance (RDA) as set by different international organizations [9].

From the Table 7, it is observed that the daily intakes of detected elements in the study area were less than the recommended/permissible levels set by different international organizations. The daily intake of Cr is very close to the recommended value whereas Mn, Cu and Zn daily intakes constitutes a small portion of RDA as per FAO and WHO standards. Any way milk is not a significant part of the Ethiopian diet particularly for adults and this deficiency can be compensated by intake of these metals through other staple Ethiopian food items.

4. Conclusions

In conclusion, the heavy metals such as (Pb, Ni, Co and Cd) were not detected in the cow's milk of Borena zone even though they were reported in the cow's milk in several studies. The heavy metals Cr, Cu, Mn and Zn were detected in milk samples of Borena zone. The concentrations of Cr, Mn, Cu and Zn were 0.845–0.895, 0.411–0.441, 0.087–0.122 and 5.003–6.218 μg/mL, respectively in the milk samples of four sites under study. The concentrations of heavy metals observed were comparable with some of the reported values in literature. The results obtained for detected elements in the present study were also compared with international daily intake guidelines of different international organizations for food and were found to be below the levels allowed.

Table 6. Comparison of the elemental concentrations of fresh cow's milk of present study with the literature values of the other countries

Country	Cr	Mn	Cu	Zn	Reference
Egypt (mg/kg)	0.034 (0.014)	0.056 (0.038)	0.140 (0.116)	3.146 (1.081)	[10]
Saudi Arabia (mg/kg)	0.003 (0.400)	NR	0.005 (0.600)	0.944 (2.400)	[9]
Ethiopia (mg/kg)	0.036 (0.004)	NR	NR	4.923 (0.277)	[47]
Nigeria (mg/L)	1.568 (2.080)	0.219 (0.090)	0.214 (0.230)	5.521 (13.900)	[26]
Poland (mg/L)	0.088 (59.33)	0.102 (25.63)	0.089 (125.14)	3.163 (710.61)	[48]
China (mg/kg)	0.280 (0.090)	0.600 (0.150)	0.420 (0.130)	30.400 (2.600)	[6]
Croatia (mg/kg)	NR	NR	0.380 (0.120)	0.510 (0.160)	[38]
Ethiopia (mg/Kg)	0.868 (0.026)	0.427 (0.018)	0.109 (0.006)	5.592 (0.092)	Present study

The values in the parenthesis are SD's
NR= Not reported

Table 7. Comparison of daily intakes of metals from 53 mL milk by Ethiopian population with recommended/ permissible values

Element in fresh milk	Concentration (µg /mL)	Daily intake (µg /day)	Recommended/ permissible value (µg/day)	References
Chromium	0.868	45.14	50 - 200	[47, 9]
Manganese	0.427	22.20	2000-5000	[26]
Copper	0.109	5.67	2000 - 3000	[47, 9]
Zinc	5.592	290.78	12000 - 15000	[47, 9]

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