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Determination of Concentrations of Some Toxic Heavy Metals (Cr, Cd and Pb) In Cow's Milk Using Simultaneous Atomic Fluorescence Spectrometer

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Abstract

Heavy metals are considered as the most important contaminations due to the industrialization of countries and have the influence on its existence in milk and milk products. In this study, milk samples were taken from a small household around Debre Tabor Town for determination concentration of Cr, Cd and Pb using AF420. The data obtained as such were subjected to one way ANOVA using Duncan's multiple range tests. The overall mean concentrations of Cd and Cr in all samples were 92.7 ± 0.07 and 54.3 ± 0.01 µg/L respectively whereas concentration of Pb was below detection limit. Significant differences were not observed among the two different days in the sampled cows' milk with respect to their elemental contents.

***Keywords:** Heavy metals; Cow's milk; Simultaneous atomic fluorescence spectrometer*

Introduction

Among Milk is a food of high nutritional value especially for children in many parts of the world and its consumption is suggested by Agriculture Organization of the United Nations (FAO) and United Nations Educational, Scientific and Cultural Organization (UNESCO) as indispensable in the diet of children for the protein contribution it represents [1,2]. In addition to being an important source of essential nutrients, milk is one of the main ways of ingesting metals [3].

There are about 38 micro and trace elements reported to be found in raw milk from different regions around the world [4]. These minerals content in raw cow milk may vary depending on several factors i.e. lactation period of cows, health conditions, seasonal variations, climatic conditions, annual feed composition and environmental contamination [5]. The milk processing conditions may also have effective influence on the contents and retains of minerals in total composition of milk [6].

Heavy metals can be toxic for humans when they are not metabolized by the body and accumulate in the soft tissues. A concern with the metals is their concentration in domestic and industrial waste products because the elements are indestructible [7].

Increased environmental pollution has accelerated the problems of milk contamination and uncertainties about milk qualities. The worldwide milk contamination via environmental pollutants and xenobiotic compounds through cattle feeds like toxic metals, mycotoxin, dioxin and other pollutants are considered to have greater influence on public health [8].

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The presence of heavy metals as lead, cadmium and chromium even in low concentrations leads to metabolic disorders with extremely serious consequences and causing serious problems as it causes many health problems such as weakness, heart failure, cancer and also affects the kidneys [9]. However, it is observed that continuous long term exposures of consumers to heavy metal by consumption of cow milks get less emphasis in developing countries particularly in Ethiopia. Considering the aforementioned issues the study provides a significant importance in terms of public health hazard of Ethiopia. Therefore, the present study was designed to investigate concentration of some toxic heavy metals contaminating cow milk around Debre Tabor Town.

Material and Methodology

Chemicals and reagents

Analytical reagent grade chemicals and deionized water were used to prepare all solutions. Nitric acid (70%) and hydrogen peroxide (30%) were used for cleaning glassware and digesting milk samples throughout this work. Stock standard solutions of Cr, Cd and Pb having concentration 1000 mg/L were prepared from their salts. Intermediate standard solutions of concentration 10 mg/L was prepared by sequential dilution of stock standards.

Apparatus and instruments

All glassware were washed before use with de-ionized water, soaked in nitric acid (20%), then rinsed in de-ionized water and air dried. The glassware was kept in clean place to avoid contamination. Simultaneous atomic fluorescence spectrometer (AF420) was used for the determination Cd, Cr and Pb metals in milk samples. Before analysis of the sample, the instrument was optimized to give maximum signal strength by adjusting the parameters as shown in TABLE 1.

TABLE 1. **Instrumental operating conditions for the determination of metals in cow's milk using AF420.**

Heavy metals	Wavelength (nm)	Flow rate of sheathing gas	Lamp current (mA)	Atomizer Temperature	Carrier flow rate
Chromium	357.9	600 mL/min	100	200	300 mL/min
Cadmium	228.8	600 mL/min	100	200	300 mL/min
Lead	283.3	600 mL/min	100	200	300 mL/min

Sample collection

Sample was collected around Debre Tabor Town, Ethiopia. First polyethylene sampling bottles were soaked in 20% HNO_3 for 24 hours and rinsed with deionized water before collection of raw milk in order to avoid possible contamination. A total of 16 raw milk samples from farmers living around Debre Tabor Town were collected.

All samples were collected during morning milking time, homogenized and stored in an ice box. The samples then, transported to laboratory and immediately kept in a deep freeze (-20°C) until sample preparation carried out [10].

Sample preparation

The milk sample needs to be brought into clear solution for analysis by atomic absorption spectroscopy. For this reason the sample was digested to dissolve the milk and to remove the organic components of the milk. 15 mL of raw cow's milk is treated with 5 mL (70% nitric acid) and 2 mL (30% hydrogen peroxide) and then digested on electric hot plate at 90 and the temperature of this mixture was slowly increased to 120 until brown fumes appeared, indicating completion of oxidation of organic matter [11]. The organic matrix of milk was destroyed and leaving behind the elements in clear solution. After cooling, the clear solution was filtered in to 25 mL volumetric flask and diluted up to the mark with double distillated water. A blank digestion solution was made for comparison, finally the milk samples were directly analyzed for heavy metals by AF420.

Validation of optimized procedure (method recovery)

The recovery of metals was studied by spiking known amount of standard solution to milk samples. The percentage recovery of the analyte element was calculated using equation below:

$$\%\text{ Recovery} = \frac{C_M\ in\ spiked\ sample - C_M\ in\ sample}{C_M\ added\ for\ sample} x100\%$$

Where, c_M =concentration of metal of interest.

The analyses were performed with three repetitions for each metals and the average value were considered as the final concentration. The recovery rates for Cr, Cd and Pb were 98.21%, 99.01% and 95.76% respectively.

Results and Discussion

Calibration curve

The range of linearity of concentration Vs absorbance curve is of great importance in determining the elemental concentration of the milk samples and ensures the accuracy of the simultaneous Atomic Fluorescence Spectrometer (AF420) and to establish that results of the determination were true and reliable. The qualities of results obtained for heavy metals analysis using AF420 are seriously affected by the calibration and standard solution preparations procedures. Four series of working standard solutions of each metal were prepared by diluting the intermediate standard solution (10 mg/L) with de-ionized water. A blank and standards were run in AF420 and four points of calibration curve were established. The correlation coefficient of more than 0.995 showed that there is strong linear relationship between concentrations and absorbance. Concentrations of each metal were determined by interpolation from the calibration curves. Triplicate determinations were carried out on each sample.

Analytical method detection limit

Method detection limit is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically three times the noise level but not necessarily quantitated as an exact value. For the present study, replicate analyses for six blank samples for all elements were performed and the pooled standard deviation of the six blank samples was calculated. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank by three. As can be seen from TABLE 2, the method detection limit of each element is above the instrument detection limit

TABLE 2. **Series of working standards and correlation coefficients of the calibration curves for the determination of metals in milk using AF420.**

Heavy metals	**Concentration of standards, (μg/L)**	**Correlation coefficient**
Cr	1.5, 2.5, 3.5, 4.5	0.995
Cd	1.5, 2.5, 3.5, 4.5	0.996
Pb	1.5, 2.5, 3.5, 4.5	0.997

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Analysis of raw cow milk samples for metal level

The samples were analyzed using AF420 for the determination of Cr, Cd and Pb at their corresponding wavelength. The concentrations of these metals in milk samples were obtained in μg/L after calibration as recorded in TABLE 3 below.

TABLE 3. **Method and instrumental detection limits for the determination of heavy metals in cow's milk samples ($n = 6$).**

Metals	**IDL (μg/L)**	**MDL (μg/L)**
Cr	0.05	<0.01
Cd	0.01	<0.001
Pb	0.05	<0.01
MDL=Method Detection Limit and IDL=Instrumental Detection Limit		

The average heavy metal concentrations in the fresh cow's milk collected from farmer around Debre Tabor Town are presented in TABLE 4. Among the three elements analyzed, two elements (Cr and Cd) were detected whereas the other one (Pb) was below their corresponding method detection limit. Probably this is because there are no industries and vehicle emissions which are the basic sources of this toxic heavy metal around the study area. The feeds and the water which the cows use may are also free from this toxic metal.

TABLE 4. **Concentration of heavy metals in milk samples (μg/L).**

Samples	**Minerals concentration in raw cow's milk (μg/L)**		
	Cr	**Cd**	**Pb**
Milk	54.3 ± 0.01	92.7 ± 0.07	ND
Note: ND=not detected at p<0.05			

Comparison of the present study with the reported data

There are broad variations in the published data for the elemental concentrations of cow's milk of different countries as shown in TABLE 5. The concentration of Cr in the present study (54.3 ± 0.01 µg/L) is high compared with the corresponding values of other countries and less with that of Bangladesh (539 ± 0.013 µg/L and 373 ± .008). This showed that the cow's milk in the present study area is rich in chromium.

The concentration of Cd (92.7 ± 0.07 µg/L) is also higher compared to the respective values of remaining countries except Pakistan (1970 ± 0.40) and Egypt (288) in the literature.

TABLE 5. **Comparison of the elemental concentration (µg/L) of raw cow's milk of present study with the literature values of the other countries.**

Country	**Cr**	**Cd**	**Pb**	**Reference**
Bangladesh	373 ± .008	24 ± .009	3146 ± 1.081	[12]
Bangladesh	539 ± 0.013	47 ± 0.026	12 ± 0.001	[12]
Egypt	NR	288	4404	[13]
Egypt	30	86	66	[14]
Pakistan	NR	1970 ± 0.40	680 ± 0.15	[14]
India	40	1	2	[15]
USA	30	10	14	[15]
Libya	NR	1	3	[16]
Iran	NR	30 ± 0.3	1290 ± 6.0	[17]
Ethiopia	54.3 ± 0.01	92.7 ± 0.07	BDL	Present study
NR=Not reported, BDL=Below Detection Limit				

Conclusion

Even though milk samples tested did not contain higher amounts of minerals in reference to permitted levels, except for Cd in the study area, the amounts of heavy metals consumed is still a matter of health concern since many other locally produced food items may have remarkable amount of contamination. In addition, in some industrial areas due to the air pollution and subsequent contamination of animal feed and waters, the levels of heavy metals in milk and other dairy products may be increasing. More specifically, high amounts of Cd might be potentially hazardous to the people living there. It is suggested that control and monitoring of water and feed for livestock and use of appropriate and clean containers in transit of raw milks may be helpful for production of healthier milks.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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