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DETERMINATION OF LEAD AND CADMIUM IN RAW COW'S MILK BY GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROSCOPY

S. ELATRASH* and N. ATOWEIR

Chemistry Department, Faculty of Science, Garyounis University, BENGHAZI, LIBYA

ABSTRACT

This study describe to evaluate the possible contamination by some heavy metals (Lead and Cadmium) in raw cow's milk. Forty five samples of raw cow's milk collected from various farms in different locations in Benghazi area. The quantitative analysis of lead and cadmium were performed by using graphite furnace atomic absorption spectrometry, for statistical analysis (ANOVA) was used when statistical comparison gave $P < 0.05$, the mean values of lead and cadmium were 3.43, 1.24 µg⁄Kg, respectively. These values were under the permissible limits, indicating that mean intake of heavy metals due to consumption is generally tolerable daily intake is fairly satisfactory. Our results were agreements with the value reported literature.

Key words: Raw cow's milk, Contamination by heavy metals, Lead, Cadmium, Graphite furnace atomic absorption spectroscopy.

INTRODUCTION

Technological progress, various industrial activities and increased road way traffic have caused a significant increases the toxic heavy metals in the environmental and affected all the global air, water and food[1,2]. The presence of toxic elements in the food chain is the result of the environmental pollution and their concentration need to be controlled constantly. Two ubiquitous harmful metals are lead and cadmium[3]. Milk is a basic food in human diet, however, locating cow's may possibly be exposed to high quantity of toxic elements such as Pb & Cd[4]. These toxic elements are introduced in to animals and human organisms mainly by two routs namely: inhalation and ingestion[5,6]. The content of lead and cadmium in milk and dairy products is usually very low except when the animal have consumed feed[3]. The mammary glands are the most physiologically active part of dairy cows, and therefore the input and output of toxic elements in these organisms are clearly reflected in the milk[5].

*Author for correspondence; E-mail: Sateae@yahoo.com

The presence of cadmium and lead in biological fluids such as milk and blood have not been found to possess any evidence that suggest that they may be involved in normal metabolic processes in man. Lead inhibits ferrochelatase, the enzyme responsible for the incorporation of iron in the porphyrin ring resulting in an accumulation of protoporphrin IX in the erythrocyte. The inhibition of α-amino levulinic acid dehydratase activity, decrease in red cell survival, decrease in the rate of globin synthesis and an inhibition of the haeme synthesis are also attributable to lead. Cadmium has been implicated in osteoporosis with impaired general health, lung and kidney damage. Long term chronic exposure to cadmium has been associated with anemia, anosmia, osteomalacia and cardiovascular diseases especially hypertension[7].

Due to growing environmental pollution it's also necessary to determine and monitor the concentration of lead and cadmium in milk, because they can signifantly influence the human and animal health[8]. And permissible levels for these toxic elements to assess compliance with recommended value[9]. Moreover, various international studies have reported particularly levels of lead and cadmium in milk[1,3,4,8,10-12].

Atomic absorption spectroscopy are the most frequently and widely used techniques for the determination of elements levels in milk, dairy products and in other kinds of food.[3] Graphite furnace atomic absorption spectroscopy has become one of the most popular and widely used technique for lead and cadmium determination in foodstuffs, it making possible the direct determination of analytes in difficult matrices, providing high sensitivity, precision and accuracy[14]. The aim of present study is to asses the concentration of toxic elements lead and cadmium raw cow's milk collected from different locations, which monitors the pollution in Benghazi area.

EXPERIMENTAL

Reagent and solution

All reagent were analytical reagent grade. Otherwise stated: Nitric acid 65% (RIEDEL-DEHAËN AG), hydrogen peroxide 30% (EUROSTAR SCIENTIFIC LTD), lead and cadmium standard solution 1000 mg/L obtained from (Fisher Scientific Company. UK), and double distillated water was used for all dilutions.

Instrumentation

A Unicam 989 Qz–Solar system graphite furnace atomic absorption spectroscopy (GFAAS) equipped with autosampler, deuterium background correction system and hallow cathode lamp for lead and cadmium.

Sampling

Forty five samples of raw cow's milk were collected during May to August 2008 from fifteen dairy farms in various locations of Benghazi area. Because the cows in farms have heterogeneous characteristic of animal, age and weight, the samples collected from bulk milk of the farms directly into carefully washed plastic bottles, and then triplicate, all samples frozen at - 20^{o}C until analysis. The sample sites are located in places with different pollution impact (high volume of traffic, industrial plants, dumps and metropolitan area).

Preparation of samples (sample digestion)

The concentrations in feed samples were analyzed according to the procedures outlined by Peters[15]. The milk sample need to be brought in to clear solution for analysis by atomic absorption spectroscopy. For this reason the sample first digested to dissolve the sample and to remove the fat[6]. 5.00 gr of raw cow's milk is treated with 5 ml (65% nitric acid) and 2 ml (30% hydrogen peroxide) and then digested on electric hot plate at 90ºC and the temperature of this mixture was gradually increased to 120ºC. Until brown fumes appeared, indicating completion of oxidation of organic matter. The organic matrix of milk was destroyed and left the elements in to clear solution, after cooling the clear solution was filtered in to 25 mL volumetric flask and completed to the mark with double distillated water, and a blank digestion solution was made for comparison, finally the milk samples is directly analyzed by GFAAS[6,15,16].

Calibration curve

The concentration measurements for lead and cadmium are determined from a working curve after calibrating the instrument with standards concentration. The range of linearity of the concentration *vs.* absorbance curve is of great importance in determining the elemental concentration of the milk samples. Standard aqueous solutions of different elements obtained from Fisher Scientific Company, UK were used to calibrate the AAS technique.

After the instrument has warmed up and been calibrated with standard solutions, a simple aliquot volume of 20 µL of milk samples, which obtained after wet digestion was injected in to graphite tube with the help of an auto-sampler, argon was used as a purge gas, deuterium background correction and a temperature program of the furnace was optimized to obtain the best signal during the atomization process, the instrumental parameters were adjusted according to the manufacturer's recommendation[17]. The instrumental conditions for the determination of lead and cadmium are provided in Table 1.

The calibration curves for lead (Pb) and cadmium (Cd) are shown in Fig.1 and 2 respectively.

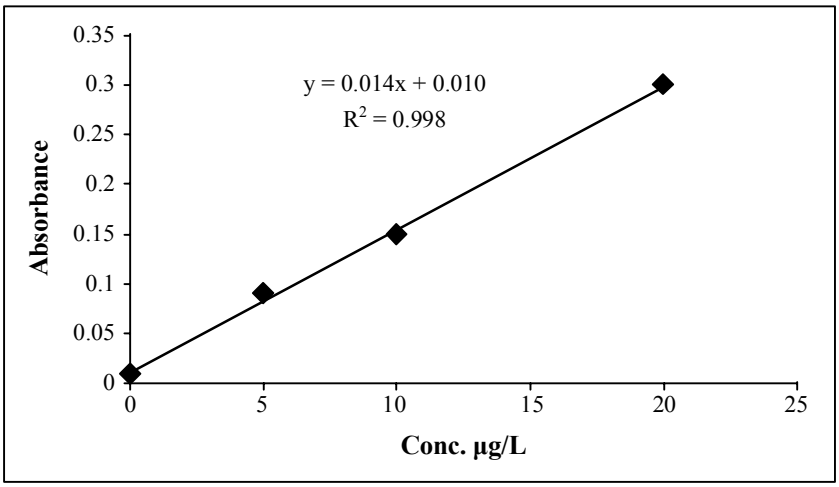

Fig. 1: Calibration curve of lead

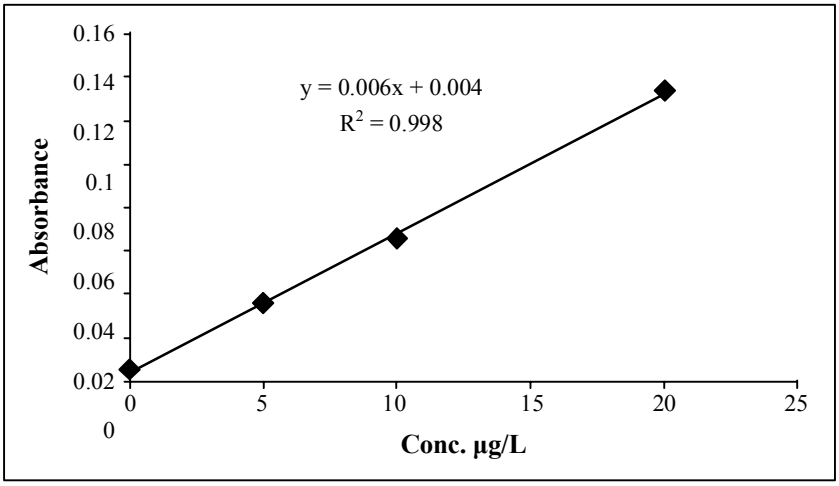

Fig. 2: Calibration curve of cadmium

Statistical analysis

An explorative statistical analysis of the concentration values measured in different farms of samples, the explorative parameters are n, number of sample m, mean r, range and SD, standard deviation.

Table 1: Instrumental conditions for determination of lead and cadmium in raw cow's milk by GFAAS

Parameter	Pb	Cd
Primary wave length	217.0 nm	228.8 nm
Injection volume	20 µL	20 µL
Slit width	0.5 nm	0.5 nm
Lamp type	HCL	HCL
Lamp current	10 mA	8 mA
Back ground correction	D_2 lamp	D_2 lamp
Maximum ash temp	800ºC	300ºC
Atomization temperature	1200ºC	900ºC

Our results were analysed using one-way analysis of variance (ANOVA) to examine statistical significant of difference in the mean concentration of lead and cadmium in different locations, which the samples were collected. assuming that there were significant differences between mean values when statistical comparison gave $P < 0.05$. Statistics were calculated with the SPSS released version 6.0 program for Windows.

RESULTS AND DISCUSSION

The concentration of lead and cadmium in all samples were collected from different locations in Benghazi area are summarized in Table 2. The results were expressed as µg of metal per Kg of milk (µg/Kg) for lead and cadmium.

Table 2: Concentration of lead and cadmium in different milk samples (mean conc. ±SD)

Classification of farms location		Farms	Lead (µg/Kg) mean conc. ± SD	Cadmium (µg/Kg) mean conc. ± SD
Rural regions	1	Sidy Mansur	ND	0.79 ± 0.031
	2	Jarootha	ND	ND
	3	Halees	ND	ND
	4	Sidy Khalifa	ND	0.90 ± 0.024

Cont…

Classification of farms location		Farms	Lead (µg/Kg) mean conc. ± SD	Cadmium (µg/Kg) mean conc. ± SD
	5	El-Abiar	ND	ND
	6	El-Magzaha	ND	ND
	7	El-Gwarsha	ND	0.91 ± 0.032
	8	Buo Atteni	1.53 ± 0.035	1.55 ± 0.046
	9	Sedy Fraj	ND	1.23 ± 0.049
Heavy traffic intensity regions	10	El-Hawari 1	ND	0.93 ± 0.031
	11	Buohedima 1	4.00 ± 0.189	1.64 ± 0.033
	12	Garyounis	4.76 ± 0.319	2.74 ± 0.019
Metropolitan regions	13	El-Hawari 2	ND	0.64 ± 0.032
	14	Joulyana	ND	0.69 ± 0.057
	15	Buohedima 2	ND	1.64 ± 0.032
Total mean			3.43 ± 1.474	1.24 ± 0.602
Range			(4.76-1.53)	(0.64-2.74)

Lead and cadmium are toxic minerals often associated with traffic pollution, therefore variations of their levels in milk from different farms are likely due to the location of meadows in relation to roads[8]. From our result we note, the most examined raw cow's milk samples showed undetectable lead, except in the samples of farms 8, 11 and 12 are 1.53, 4.003, 4.7616 µg/Kg, respectively.

The locations of which collected the samples, show that the farms 11 and 12 are located near busy roads with cars and for this reason can attributed the presence of lead in these samples, while sample of farm 8 due to pesticides used too. The mean concentration value of lead in raw cow's milk samples was 3.43 µg/Kg with the range (4.76-1.53) µg/Kg (n = 45). This value is below the maximum recommended limit of 20 µg/Kg w.w according to EC regulation, indicate that the milk is safe for consumer[18]. The highest concentration of cadmium was seen in samples of farm 12, 11 and 15 is 2.74, 1.64 and 1.64 µg/Kg, respectively, whereas the lowest concentration was seen in samples of farm 13 and 14 are 0.64 and 0.69 µg/Kg, respectively. We note that the farm 12 in Garyounis site which located

near to busy road with cars. While farm 11 in Buohedima, we find that the pesticides are used on the farm 15 is located near a petrol station and these reasons can be that due to the presence of cadmium. The mean concentration of cadmium in raw cow's milk is 1.24 µg/Kg with a range (0.64-2.74) µg/Kg (n = 45). This value is very low and below the maximum recommended limit of 50 µg/Kg w.w[18]. The accuracy of the proposed method was tested by the analysis of spiked milk samples to which a known amount of the trace metals were added. A comparison of heavy metal concentrations (lead and cadmium) in raw cow's milk with reported values from different countries is shown in Table 3.

Table 3: Comparison of heavy metal concentrations (lead and cadmium) in raw cow's milk of different countries

Country	Concentration (µg/Kg)		Reference
	Lead	Cadmium	
Izmit, Turkey	6.83	0.257	12
Taiwan	2.03	0.044	4
Calabria, Italy	1.32	0.02	1
Zagreb, Croatia	42.11	5.31	8
California	91	6.0	10
Santa Fe, Argentine	25	1.47	19
Brazil	40	1000	11
Benghazi, Libya	3.43	1.24	Present study

CONCLUSION

From our results that are obtained from measuring of lead and cadmium (potentially toxic elements) in raw cow's milk, which collected from fifteen locations of Benghazi investigated in this study. All samples show us contain a small concentrations of lead and cadmium, it's not exceed the allowed limit according EU and and Libyan standard specification. Indicting, all locations that are investigated in this study with are not a risk of environmental pollution, and the exposure of these elements by human consuming milk is negligible. We concluded that the health quality of raw cow's milk is very in the spact. And through the variance analysis we concluded that there is an effect of the sites on the concentration of elements in raw cow's milk.

REFERENCES

1. P. Licata, D. Trombetta, M. Cristani, F. Giofre, D. Martino, M. Calo and F. Naccari, Levels of Toxic and Essential Metals in Samples of Bovine Ailk from Various Dairy Farms in Calabria, Italy, Environ. Int., **30** (2004) pp. 1-6.

2. T. Zamir and S. A. Hussain, Determination of Lead and Cadmium Level in Powdered Milk in Quette (Pakistan) by Atomic Absorption Spectrometry, J. Biol. Sci., **1(5)**, 412-413 (2001).

3. I. Pavlović, M. Sikirić, L. Havranek, N. Plavljanić and Brajenović, Lead and Cadmium Levels in Raw Cow's Milk from an Industrialised Croatian Region Determined by Electrothermal Atomic Absorption Spectrometry, Czech J. Animal Sci., **49(4)**, 164-168 (2004).

4. S. L. Jeng, S. J. Lee and S. Y. Lin, Determination of Cadmium and Lead in Raw Milk by Graphite Furnace Atomic Absorption Spectrophotometer, J. Dairy Sci., **77(4)**, 945-949 (1994).

5. A. Ayar, D. Sert and N. Akın, The Trace Metal Levels in Milk and Dairy Products Consumed in Middle Anatolia-Turkey, Environ. Monitoring and Assessment, **152(1-4)** (2007) pp. 1-12.

6. S. M. Farid, M. A. Enani and S. A. Wajid, Determination of Trace Elements in Cow's Milk in Saudi Arabia, J. King Abdul Aziz University- Engg. Sci., **15(2)**, 131-140 (2004).

7. A. O. Lawal, S. S. Mohammed and D. Damisa, Assessment of Levels of Copper, Cadmium and Lead in Secretion of Mammary Gland of Cows Grazed on Open Fields, Science World J., **1(1)**, 7-10 (2006).

8. M. Sikirić, N. Brajenović, I. Pavlović, J. L. Havranek and N. Plavljanić, Determination of Metals in Cow's Milk by Flame Atomic Absorption Spectrophotometry, Czech J. Animal Sci., **48 (11)**, 481-486 (2003).

9. R. M. Tripathi, R. Raghunath, V. N. Sastry and T. M. Krishnamoorthy, Daily Intake of Heavy Metals by Infants through Milk and Milk Products, The Science of the Total Environment, **227** (1999) pp. 229-235.

10. J. C. Bruhn and A. A. Franke, Lead and Cadmium in California Raw Milk, J. Dairy Sci., **59(10)**, 1711-1717 (1976).

11. I. A. Okada, A. M. Sakuma, F. D. Maio, S. Dovidauskas and O. Zenebon, Evaluation of Lead and Cadmium Levels in Milk in Environmental Contamination in the Vale do Paraíba Region, Southeastern Brazil, Rev. Saude Publica, **31(2)** (1997) pp. 140-134.

12. Ü. Ay and S. Karayünlü, Modification in Direct Analysis Method: Metal Levels in Raw Milk at the Region of Izmit by Graphite Furnace Atomic Absorption Spectrophotometer, Int. J. Food Sci. Technol., **43**, 326-329 (2008).

13. C. Cabrera, M. L. Lorenzo and M. C. Lopez, Lead and Cadmium Contamination in Dairy Products and its Repercussionon Total Dietary Intake, J. Agricult. Food Chem., **43**, 1605-1609 (1995).

14. J. C. P. D. Mattos, A. M. Nunes, A. F. Martins, V. L Dressler and E. M. D. M Flores, Influence of Citric Acid as Chemical Modifier for Lead Determination in Dietary Calcium Supplement Samples by Graphite Furnace Atomic Absorption Spectrometry, Spectrochimica Acta Part B., **60** (2005) 687-692.

15. J. B. Peters (Ed.), Recommended Methods of Manure Analysis (2003).

16. S. J. Hashwell, Atomic Absorption Spectroscopy, Elsevier, New York (1991).

17. Unicam-Atomic Absorption Methods Manual, Unicam Limited (1994).

18. CE Regulation 2001/466: Off. J. Eur. Comm. (2001), L 039, 21.

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