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Determination of Lead Residue in Raw Cow Milk from Different Regions of Iran by Flameless Atomic Absorption Spectrometry

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Abstract: Milk and dairy product are important components of human food the presence of toxic metals in the food chain is the result of environmental pollution and their concentrations need to be constantly controlled. The objective of this study was to detect the level of lead residue in to cow and goat milk samples in Iran. From September 2010 to July 2011 a total of 109 bovine bulk milk samples were collected from 12 commercial dairy herds, in different regions of Iran and analyzed according to the AOAC method. Concentration of lead determined by the graphite furnace atomic absorption spectrometry. The mean and standard deviation concentration of lead in raw cow milk were 13.45 6.41. Our data were within the normal ranges. The results of this study showed that the concentration of lead in 11% of cow milk samples were higher than the maximum permitted level in codex (0.02 μg/l). In conclusion, the result of this study showed the importance of periodically monitoring the residue levels of lead in milk in different regions of Iran.

Key words: Milk · Cow · Lead · Iran · Heavy Metal

INTRODUCTION

The world-wide contamination of milk with undesirable substances via animal feeds, heavy metals, mycotoxins, dioxins and similar pollutants is considered to be of great concern to public health due to their toxic effects on humans and wildlife. Recent report according to Semaghiul *et al*. [1] indicated that good quality measurements are essential to control and often play a vital role in maintaining products and process quality, both in manufacturing, trade and in research. In recent years, there has been a growing interest in microelements as their presence in foods is the indicator of qualitative parameters such as processing conditions, environmental pollution, sanitation and husbandry and may affect the chemical and functional properties of milk [2].

Lead is one of the most abundant heavy metals in the environment, often coexist in a polluted environment [3] and are mostly implicated in human and animal poisoning [4]. Lead poisoning is one of the most frequently reported causes of poisoning in farm livestock; with cattle being most commonly affected [5]. Lead is known to induce reduced cognitive evelopment and intellectual performance in children and increased blood pressure and cardiovascular disease in adults [6]. A variety of exposure routes allow toxic heavy metals predominantly lead to enter the food chain of farm animals, the commonest being the contaminated feed and water, lead paint, lead batteries, lead shot, automobile emissions, aluminum paints, textile, metallurgy and petrochemical based industries, combustion of coal and mineral oil, smelting, mining, alloy processing, paint industries, aluminum processing, phosphate fertilizer plant, lead-zinc smelter or coal mining areas [7-9].

Milk and dairy products are important components of human food. Thus, contamination of milk and dairy products by toxic metals can be a possible health risk to human population [8, 10, 11]. The presence of toxic metals in the food chain is the result of environmental pollution and their concentrations need to be controlled constantly. The composition of the mineral fraction of milk and milk products has been frequently considered,

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but only a few published investigations deal with minor and trace elements, despite their importance in nutrition or in food [12].

The purpose of this survey was to detect the level of lead residue in to cow milk samples in 11 provinces, Iran using graphite furnace atomic absorption spectrometry.

MATERIALS AND METHODS

Milk Samples: Overall, 43 bovine herds were randomly selected in 11 provinces, Iran. From September 2010 to July 2011 a total of 109 bovine bulk milk samples were collected from 11 commercial dairy herds in different regions of Iran (Table 1). The animals whose milk samples collected for this study were clinically healthy and the milk samples showed physical (color, pH and density) consistency. The samples were immediately transported to the laboratory in a cooler with ice packs and were frozen at -20°C until analysis. Clean, acid washed polyethylene bottles were used to contain the samples.

Apparatus: A Perkin Elmer Model 4100 atomic absorption spectrometer equipped with a GTA Graphite furnace and deuterium background corrector was used. Pyrolytic-coated graphite tubes with a platform were used and signals were measured as peak height mode.

Reagents: All reagents and solvents were of analytical reagent grade unless otherwise stated. Double distilled water was used for the preparation of solution. The stock solutions of metals (1000 mg/L) were obtained by dissolving appropriate metal salts (Merck, Darmstadt, Germany). All the plastic and glassware were soaked in nitric acid for 15 min and rinsed with deionized water before use.

Table 1: Lead residues (µg/l) in cow milk samples from 11 centers of provinces, Iran

City	Number of samples	Mean	Standard deviation
Isfahan	12	17.17	6.40
Tabriz	8	13.68	3.19
Sanandaj	6	7.58	2.53
Tehran	10	20.13	9.19
Yazd	5	13.04	3.96
Ghom	11	13.50	4.05
Rasht	7	10.04	3.39
Shiraz	9	14.46	6.72
Ahvaz	10	18.03	4.88
Mashhad	18	12.96	4.32
Kerman	8	6.39	2.80
Yasuj	5	5.16	2.25
Total	109	13.45	6.41

Digestion and Determination of Lead: When the samples were to be analyzed, the milks held at room temperature (28°C) for 48h to reduce the pH below 4.6 and separate the casein and fat. Each sample was centrifuged at 1000 rpm for 10 min, supernatant mixed with 5ml 65% HNO_3 and heated to 90°C, after which 20 ml of de-ionized water was added. Lead was measured by atomic absorption spectrometry (AAS; PERKINELMER Model 4100-Germany) equipped with a GTA graphite furnace and with deuterium background corrector was used. The lead absorbance was measured at 15mA of lamp current, 0.5 nm slit width and the peak height mode of the wave lengths used was 283.3nm. As a standard solution for recovery tests, 1000 ng/l of lead solution (Aldrich co. catalog No.20/7233) was diluted with 1% HNO_3 to obtain 10, 20 and 40 ng/ml and it was also done for milk samples to prepare the spiked samples. The mean recoveries of acid-diluted lead from these three selected concentration were 92 5% and for milk-spiked samples was 87%. The instrument detection limit was 0.01ng.

Statistical Analysis: Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, Washington, USA) for analysis. SPSS 16.0 statistical software (SPSS Inc., Chicago, Illinois, USA), was used for ANOVA and Student's t-test analysis; differences were considered significant at values of $p < 0.05$.

RESULTS AND DESCUSION

Mean concentration of lead in cow milk samples from various sites are shown in Table1. Lead concentration in raw cow milk samples ranged from 2.10 to 39.40 µg/l with the mean valves was 13.45 µg/l. The lead content of raw cow milk samples showed significant differences ($P < 0.05$) among various sites (Table1). It seemed that the lead level was variable in Iran. According to these data, lead levels in four sets of samples, which were from Tehran, Shiraz, Ahvaz and Isfahan, were higher that other regions, especially Tehran; the indicated region, seem to be more industnalized that other regions. The high levels of lead in milk may result from industrial air pollution in these regions. It seems that there should be more research in regard to lead contamination sources, to control the lead residue levels in milk, based on the pertinent FAO/WHO guidelines.

In the present study the overall concentration level of lead in load in cow milk sample is in agreement with those reported in literature from other countries including

Turkey, Croatia and Czech [13] and was lower than those reported by Ayer *et al*. [2], Susuki *et al*. [14], Bulinski *et al*. [15], Paclovic *et al*. [16], Enb *et al*. [17], Qin *et al*. [18] and Jigam *et al*. [19]. Our results, however, was higher than those reported by Patra *et al*. [9], Larsen and Rasmussen, [20], Rubio *et al*. [21], Rodrigues *et al*. [22] and Tajkarimi *et al*. [23]. The differences could be due to several factors including analytical techniques employed, samples size, season of the year, livestock management.

Lead is among the main metals present in the environmental which has major toxic effect. Lead residues in milk are of particular concern because milk is largely consumed by infants and children [23, 24] and the determination of lead level in milk is particularly attended by international organization [25]. The mean, median and about 80% of the samples were less than the newly established codex standard (20 µg/l). However, 11% of cow and milk samples, respectively, were higher than the maximum permitted level in Codex standard.

The presence of lead in milk samples from such areas could also be due to other factors such as transhumance along roads and/or motorways, fodder contamination, climatic factors, such as winds and the use of pesticide compounds. One of the most important sources of lead contamination in milk is water, especially in more contaminated areas [25] so water testing should be one of the important topics for future study. Therefore, it is necessary to monitor this metal over time for better clarification of its presence in milk from the studied areas. In conclusion, the results of the percent study showed the importance of periodically monitoring the level of lead in milk and other diary products in different regions of Iran.

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