Food Anal. Methods (2010) 3:85–89
DOI 10.1007/s12161-009-9092-1

Stable Lead in Milk and Derivates

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Received: 18 February 2009 / Accepted: 29 May 2009 / Published online: 11 June 2009

Abstract Lead is a heavy metal widely distributed in soil since it is constantly produced by the radioactive decay series of uranium (^{238}U), actinium (^{235}U), and thorium (^{232}Th). The purpose of this research was to determine lead concentrations in fresh milk and derivates of the farms located in the municipalities of Pedra and Venturosa, in the state of Pernambuco, Brazil. The concentration of lead varied from 3 to 90 µg L^{-1} in fresh milk, from 20 to 1,050 µg kg^{-1} in curdled cheese, and from 6 to 20 µg L^{-1} in milk serum. Seven samples of fresh milk and one of curdled cheese presented concentration limits higher than those established by the Brazilian Ministry of Health.

Keywords Stable Lead · Dairy Cows · Fresh Milk · Curdled Cheese · Milk Serum

Introduction

In 1975, the Garanhuns Project, conducted by NUCLEBRAS (Brazilian Nuclear Enterprises) and the CPRM (Minerals recurses and Research Company), identified 263 radioactive anomalies on a radiogeological prospection of 35,000-km^2 area between the Brazilian states of Pernambuco, Alagoas, and Sergipe. In this area, rocks samples collected at the municipalities of Pedra and Venturosa, in the semi-arid region of the state of Pernambuco, showed levels up to 22,000 and 100 mg kg^{-1} to uranium and thorium, respectively (Costa et al. 1976). A more recent study carried out on the milk-producing farms of these mentioned municipalities found elevated concentrations of ^{238}U and ^{232}Th in rock and soil samples (Santos et al. 2006). The biggest dairy farms on the state of Pernambuco are located in these municipalities that are together responsible by around 10% of the milk production in the referred state.

Lead is a chemical element considered the heavy metal of higher natural occurrence in soil, with concentrations varying from 1 to 200 µg g^{-1}, widely distributed in the earth's crust. Natural lead is a mixture of four stable isotopes: Pb-208 (51% to 53%), Pb-206 (23.5% to 27%), Pb-207 (20.5% to 23%), and Pb-204 (1.35% to 1.5%). The Pb-206 and Pb-208 are the final products of the natural radioactive decay of ^{238}U and ^{232}Th, respectively (Jaworowski 1969). Therefore, soil has a very important contribution on the ingestion of lead by dairy milk cows. The consumption of soil adhered to plants used as fodder is the principal way of internal contamination by lead to this animals (Strojan and Phillips 2002), also being responsible for the transference of this element content on fodder to the milk (Parkpian et al. 2003).

The cow's milk and its derivates are foodstuffs very much consumed by human beings, mainly by children. The presence of lead in the milk brings severe harm to its quality, posing risks to the human health (Patra et al. 2008). Thus, some researches (Tokuşoglu et al. 2004; Ay and Karayünlü 2008; Tajkarimi et al. 2008) have shown the necessity of monitoring lead in fresh milk samples and milk in cartons, with the aim of knowing the need to restrict the contamination and preserve collective health. In Brazil, researches were also carried out to determine lead concen-

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trations in fresh milk (Okada et al. 1997) and food samples (Da-Col et al. 2009).

Due to the elevated toxicity of lead to human beings, even at a trace level, the world sanitary authorities established limits of concentration of this metal in milk since the consumption of these foodstuffs via ingestion of the element by human is considered important (Sharma et al. 1982). In Brazil, the Ministry of Health reviewed the tolerance levels of lead in foodstuffs and lowered the acceptability levels of lead in the majority of alimentary products. The levels for milk and cheese are, respectively, 50 μg L^{-1} (0.05 mg L^{-1}) and 1,000 μg kg^{-1} (1.0 mg kg^{-1}; BRASIL 1990). In this context, the objective of the present work was to determine lead concentrations in samples of fresh milk, milk serum, and curdled cheese produced at farms localized in an area with anomalous levels of uranium and thorium in the municipalities of Pedra and Venturosa. The results obtained were compared with the limits established by the Brazilian Health Ministry.

Materials and Methods

Criteria for Selection of Samples

Twelve samples of fresh milk were collected from nine farms located near the anomalous area. The selection criteria were the nearness of the farms to the occurrence of uranium and the quantity of milk produced. The farms chosen in the present study are the biggest producers of milk in the region studied, which have a daily production of approximately 6,000 L. Three samples of curdled cheese and milk serum were collected from three principal dairy product manufacturers in the municipalities of Pedra and Venturosa, which are located near the mineralization of uranium. There are no industries or paved roads in the areas where the farms are located, besides the three dairy manufacturers included in the study. The shortest distance between any of the farms included in the study to a paved road is approximately 10 km, and the nearest industry to the area of the study is about 30 km. The selection criteria for choosing those manufacturers were the quantity of curdled cheese and milk serum produced and their proximity to the occurrence of uranium. The dairy industries chosen in the present study are the biggest producers of curdled cheese and milk serum in the studied region. Several farms nearby, including the nine farms evaluated in the present study, supply milk to the three manufacturers of dairy products investigated in this research. All farms are located on the area of uranium and thorium mineralization. This region is characterized mainly by subsistence agriculture systems; therefore, there are very few trucks that pass through the dirt roads that connect the farms. The samples were collected in June 2002 (rainy period), December 2003 (dry period), and May 2004 (rainy period). The possibility of lead contamination during the milking process in the farms is minimal since practically all farmers use polyethylene containers to store milk before sending it to the dairy manufacturers. A few of the larger farms use mechanical milking systems according to the standard procedures (BRASIL 1980), therefore without materials that could contaminate the milk with lead. Besides, the facilities of the dairy manufacturers do not have processes that could expose the milk to materials that could allow for lead contamination.

Treatment of Samples

From each farm, 5 L of fresh milk were collected from the storage tank of the daily milk production. The milk samples were collected in polyethylene containers, and immediately, 5 mL of acetic acid and 10 mL of 37% formaldehyde were added (IRD, Instituto de Radioproteção e Dosimetria 1983). Dry material was obtained after heating to 80°C during 48 h. Next, the material was taken to the furnace, and the temperature was raised gradually up to 300°C and then left for 48 h at this temperate until ashes were obtained (IRD, Instituto de Radioproteção e Dosimetria 1983). Twenty liters of milk serum was collected in each dairy manufacturer, with polyethylene recipients directly from the storage tanks, and immediately, 10 mL of acetic acid and 20 mL of 37% formaldehyde were added (IRD, Instituto de Radioproteção e Dosimetria 1983). After evaporation, the milk serum samples were submitted to the same treatment as given to the milk samples. Three kilograms of curdled cheese was collected and placed to dry at 80°C, until a constant weight was obtained. After this procedure, the others treatment are similar for the milk and serum samples. After these procedures, 10 g of ashes was taken for digestion.

Determination of Lead

The digestion procedure was based on the method developed by Akagi et al. (1995). Each sample, weighing approximately 20 mg, was transferred into a volumetric flask of 50 mL, to which 1 mL of HNO_3, 1 mL of $HClO_4$, 1 mL of H_2SO_4, and 1 mL of high-purity water (18 MΩ) were added and heated to 90°C on hotplate for 30 min. After cooling, the digested sample was completed up to 50 mL with high-purity water (18 MΩ). The analyses were carried out by using a Varian AA220-FS atomic absorption spectrometer with a flow injection system. The precision and the accuracy of lead determination were checked using the certified reference material (trace elements in milk) from the Food Analysis Performance Assessment Scheme.

Results and Discussion

The accuracy of the results was checked by running three replicates of the certified reference material. The recuperation of the lead was 95% with a relative standard deviation of 2.5%. In the calculation of the detection limits and quantification, the Brazilian Agency of Sanitary Surveillance (ANVISA) definitions were used (ANVISA 2003). The detection limits and quantification for lead were 1 and 3 µg, respectively. The fresh milk, milk serum, and curdled cheese samples were analyzed in triplicate, and the mean concentrations are found in Table 1. Seven milk samples (see Table 1) presented lead concentrations higher than the limit established by the Brazilian Ministry of Health, which is 50 µg L^{-1} (0.05 mg L^{-1}; BRASIL 1990). On the F-7 farm is located the principal radioactive anomaly of ^{238}U and ^{232}Th (Santos et al. 2005; Santos et al. 2006). According to these authors, the ^{238}U and ^{232}Th anomalies found in the soils of the F-8 farm were coming from the existing anomalous rock outcrops on the F-7 farm. In the area of the present study, no anthropogenic alterations were found to justify the presence of lead in the environment.

It was verified that the sample collection procedure and storage of milk and milk serum carried out on the F-1 to F-9 farms were in accordance with the Ministry of Agriculture Legislation (BRASIL 1980) that defines the following: Every recipient used in the milk sampling or storing in deposit should be made of stainless steel, aluminum, or tinned iron, of perfect finishing, without faults. This suggests that the presence of lead in the fresh milk, milk serum, and curdled cheese samples presented in Table 1 was coming only from the natural occurrence of this element. Thus, the radioactive isotopes ^{238}U and ^{232}Th can be considered as principal productive sources of lead in the soils of the Pedra and Venturosa municipalities. For Parkpian et al. (2003), soil ingestion is principally responsible for the presence of lead in cow's milk.

The lead concentration values of the farms that exceed the limits established by the Brazilian Ministry of Health, as shown in Table 1, are found at an interval of 10 to 350 µg

Table 1 Mean concentration of Pb in fresh milk, milk serum and curdled cheese samples

Local	Latitude	Longitude	Year	Concentration of Pb		
				Milk (µg L^{-1})	Serum (µg L^{-1})	Cheese (µg kg^{-1})
F-1	8° 49′ 16″	37° 00′ 24″	2002	7±1[a]		
F-1	8° 49′ 16″	37° 00′ 24″	2003	<LQ[b]		
F-2	8° 48′ 30″	37° 00′ 51″	2003	61±1		
F-2	8° 48′ 30″	37° 00′ 51″	2004	<LQ		
F-3	8° 55′ 9″	37° 00′ 59″	2003	64±3		
F-4	8° 49′ 9″	37° 00′ 42″	2004	<LQ		
F-5	8° 44′ 16″	37° 00′ 41″	2003	92±1		
F-6	8° 2′ 14″	37° 00′ 32″	2003	8±1		
F-6	8° 2′ 14″	37° 00′ 32″	2004	64±2		
F-7	8° 39′ 10″	37° 00′ 19″	2004	130±15		
F-8	8° 39′ 20″	37° 00′ 18″	2004	110±8		
F-9	8° 53′ 39″	37° 00′ 44″	2004	54±3		
DM-1	8° 47′ 18″	37° 00′ 54″	2004		16±4	
DM-2	8° 48′ 25″	37° 00′ 38″	2004		11±1	
DM-3	8° 52′ 19″	37° 00′ 54″	2004		6±2	
DM-1	8° 47′ 18″	37° 00′ 54″	2004			1,100±1
DM-1	8° 47′ 18″	37° 00′ 54″	2004			393±1
DM-1	8° 47′ 18″	37° 00′ 54″	2004			745±1
DM-2	8° 48′ 25″	37° 00′ 38″	2004			227±3
DM-2	8° 48′ 25″	37° 00′ 38″	2004			<LQ[b]
DM-3	8° 52′ 19″	37° 00′ 54″	2004			640±3
DM-3	8° 52′ 19″	37° 00′ 54″	2004			206±1
DM-3	8° 52′ 19″	37° 00′ 54″	2004			127±14

[a] Standard deviation (95% confidence)

[b] Limit of quantification

L^{-1} determined by Marengo and Aceto (2003) in fresh milk samples from the province of Cuneo, in Italy. Studies carried out by Patra et al. (2008) analyzed samples of cow's milk from farms localized in the proximity of different industrialized units and determined mean elevated concentrations varying from 250 to 850 μg L^{-1} (0.25 a 0.85 μg mL^{-1}). It is verified that these values are of the same order of magnitude as those of the F-7 and F-8 farms, as shown in Table 1, which result from the lead in the soil derived from the decay of the uranium and thorium anomalies in these two farms. Tajkarimi et al. (2008) determined mean concentrations of lead in fresh milk varying from 1.5×10^{-4} to 2.34×10^{-2} μg L^{-1} (1.5 to 23.4 ng L^{-1}), in regions of Iran. It is observed that these values are at least three orders of magnitude lower than those presented in Table 1. In the community of Navarra, in the north of Spain, Sola-Larrañaga and Navarro-Blasco (2009) determined lead concentrations in fresh milk varying from 0.55 to 18.7 μg L^{-1}. The maximum value is an order of magnitude lower than the values found on the F-7 and F-8 farms, as can be seen in Table 1. In Brazil, Okada et al. (1997) determined lead concentrations varying from 10 to 200 μg L^{-1} (0.01 and 0.2 mg L^{-1}) in fresh milk samples from an area with anthropogenic environmental contamination in the Vale do Paraíba, in São Paulo. The value of 200 μg L^{-1} is on the same order of magnitude as those found in the F-1 and F-8 farms, as shown Table 1.

Within each farm, lead concentrations in fresh milk samples were usually higher in the samples collected in 2003, compared to samples collected in 2004, with the exception of F-6, where lead was higher in 2004. These differences among years may have been derived from differences in the location where the cows were grazing during the period of sampling during each year since the soils of the different pastures within a given farm may vary in lead content.

In the semi-arid region of the state of Pernambuco, the milk serum is a yellowish liquid that is obtained on the fabrication of curdled cheese. In this region, the serum milk is intensively used in the fabrication of milk based drinks, mainly yogurt. In the studied region, there are only three dairy manufacturers that were designated in Table 1 as DM-1, DM-2, and DM-3. As can be seen in this table, the concentration of lead in the milk serum samples were lower than the limit established by the Brazilian Ministry of Health for milk, that is, 50 μg L^{-1} (0.05 mg L^{-1}; BRASIL 1990). Data on levels of lead in milk serum samples are very scarce in scientific literature. However, studies carried out by Jeng et al. (1994) in six Taiwan cities determined lead concentrations in milk serum varying from 0.98 to 4.45 μg L^{-1} (0.98 to 4.45 ng mL^{-1}). These values were lower than those presented in Table 1.

As shown in Table 1, only one sample of curdled cheese (dairy manufacturers D-1) presented lead concentrations higher than 1,000 μg kg^{-1} (1.0 mg kg^{-1}), established by the Brazilian Ministry of Health (BRASIL 1990). In the literature, works evaluating the presence of lead in curdled cheese were not found. However, researches carried out by Mendil (2006) in Turkey determined concentrations of lead in diverse kinds of cheese, whose values varied from 140 to 1,100 μg kg^{-1} (0.14 to 1.1 μg g^{-1}). These values are in the same interval of concentration as those presented in Table 1.

Conclusions and Recommendations

Approximately 60% of the farms studied showed concentrations of lead in milk higher than the limit established by the Brazilian Ministry of Health. To avoid impacts on collective health, the elaboration of a monitoring program for the quality of milk produced in the studied area, regarding the contamination by lead, is necessary, particularly for farms 7 and 8. Also, the importance of a constant surveillance of limits must be emphasized in the milk producing farms, mainly those that are localized on uranium and thorium anomalies, to avoid acute intoxication in the population of the region, principally of children, big consumers of milk. The toxicity effects of lead can appear over time if urgent corrective measures are not taken in the productive processes of the farms that present the higher concentrations of lead in milk.

Acknowledgment This research was supported by Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE).

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