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RESEARCH ARTICLE

The assessment of lead concentration in raw milk collected from some major dairy farms in Iran and evaluation of associated health risk

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Abstract
Milk is one of the most consumed sources among people, especially children. hence, its contamination with heavy metals can pose a serious risk to children. Therefore, this study aimed to measure the lead concentration as one of the most dangerous heavy metals in the raw milk of several major animal husbandries in Tehran province from Iran. A total of 57 raw milk samples were collected from different regions of Tehran province. The lead contents were measured using a graphite furnace atomic absorption spectrometer. To evaluate the risk of the samples and hazard quotient (HQ) were calculated. The results showed that HQ for all samples was lower than 1 which was found within the acceptable level. Because the absorption of Pb is higher in children and this metal has a cumulative property in the body, even its small weekly intake can be dangerous in long-term consumption.

Keywords Dairy farms · Heavy metals · Lead · Milk · Risk assessment

Introduction

Heavy metals in most countries of the world have always been a major concern for regulatory agencies, as there is a risk of exposure to heavy metals through air, soil, water, and food may pose a serious risk to human health. Among heavy metals, lead (Pb) and cadmium are the most concerned because they are toxic and stable, do not biodegrade easily, and are easily transported to the food chain [1, 2]. According to the International Agency for Research on Cancer (IARC), Pb is in group 2 A, which is known as a probable carcinogen to humans [3]. High intake of Pb causes chronic and acute poisoning in humans and its accumulation in the body causes damage to some tissues and pathophysiological changes in many organ systems including, renal, central nervous, hematopoietic, and immune system (Fig. 1).

Cow's milk contains the most important micro- and macro-nutrients including fatty acids, amino acids, vitamins, and minerals [4]. It can also be an important source of heavy metals. Because cows often graze in open areas, their forage and water consumption are prone to high metal contamination. These heavy metals can also accumulate in milk and beef and enter the human food cycle [5, 6]. Because children have lower body weight, they are more vulnerable, and even low concentrations of Pb can be dangerous to them [7].

Due to the toxicity of Pb, many regulatory agencies have defined limits for this metal in raw milk. According to the Codex Committee on contaminants in foods [8], European Union regulation (EU) [9], and Iran National Standard [10], 20 µg/kg is defined as the maximum permitted amount of Pb in milk. In the last decade, many studies in Iran [11–18] and in the world [19–23] have detected Pb in raw milk. A number of studies indicated that Pb contamination of raw milk is a serious issue for researchers and regulatory agencies. Therefore, researchers in recent years have paid more attention to the presence of Pb in raw milk. This study aimed to measure the lead residues in the raw milks of several major

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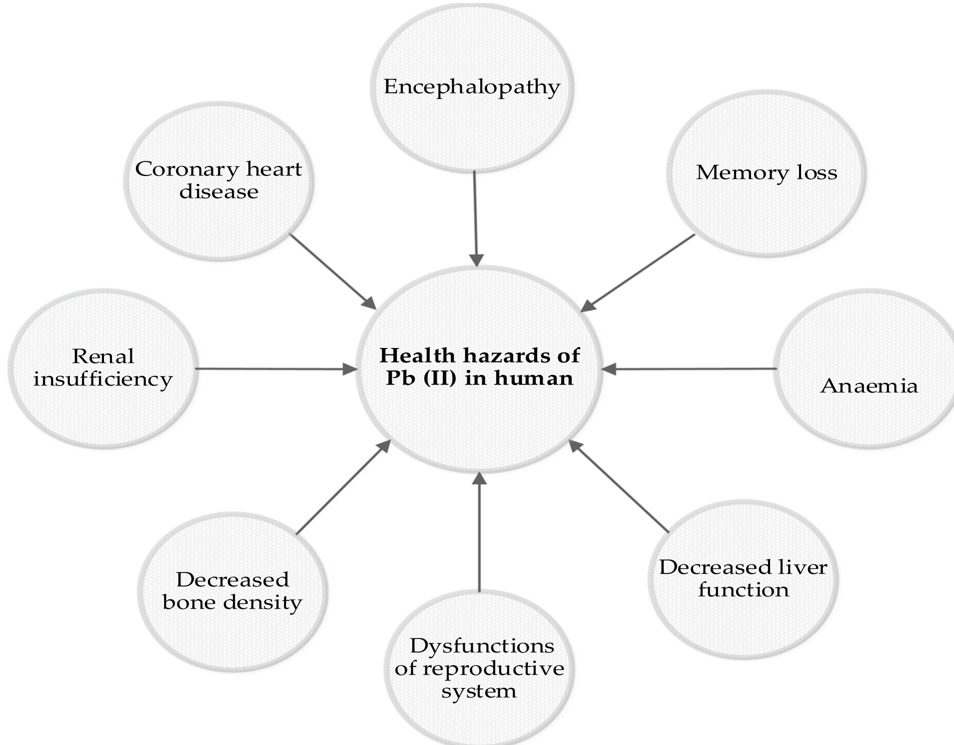

Fig. 1 Health hazards from consuming Pb contaminated foodstuffs (Ara and Usmani 2015; Kumar et al. 2020)

animal husbandries in Tehran province from Iran. Also, the risk of Pb in adults and children was evaluated according to the daily milk intake.

Materials and methods

Sampling

Milk samples were collected in February and March 2020 from 19 animal husbandries from different regions of Tehran province. These farms were recognized as the most important suppliers of raw milk to dairy factories in Tehran province. Three raw milk samples were collected from each animal husbandry where milk was stored at 4 °C in storage tanks. In general, 57 samples were collected in this study. Upon collection, all samples were placed into clean, acid-washed polyethylene bottles, labeled, and stored at -18 ˚C until analysis [24].

Chemicals and standards

Pb standard, H2O2, Nitric oxide, and ultrapure water were purchased from Merck (Darmstadt, Germany). Standard solutions of Pb at 1000 mg/L concentrations were prepared and used in order to obtain calibration curves. In a 1000 mL volumetric flask, 1 g of Pb was dissolved in 7 mL of nitric oxide and the volume increased up to 1000 mL [24].

Preparation of test samples

Milk samples stored at -18˚C were thawed at room temperature and shaken vigorously using a Vortex-Genie 2 mixer. First, 2 g of milk samples were weighed in digestion vessels. Then, 5 mL of nitric acid and 2 mL of hydrogen peroxide were added. After that, the samples were placed in a microwave oven. The microwave program was set on one step at low power for a few minutes, then, one or more steps a high power were used to prevent the occurrence of sudden pressure peaks inside the digestion vessels. Microwave program was done in four steps including: (1) power:

Table 1 The parameters of human health risk assessment [11, 25]

Parameter	Meaning	Unit	Age groups	
			Adult	Child
HQ	Hazard Quotient	-	-	-
C	Concentration of metal	mg kg^{-1}	-	-
EF	Exposure Frequency	days year^{-1}	365	365
ED	Exposure Duration	years	30	6
F_{IR}	Food Ingestion Rate	mL day^{-1}	175	239
BW	Body Weight	kg	70	15
AT	Average Time	days	ED×365	ED×365
RFD	Reference Dose	mg kg^{-1} day^{-1}	3.50E-03	3.50E-03

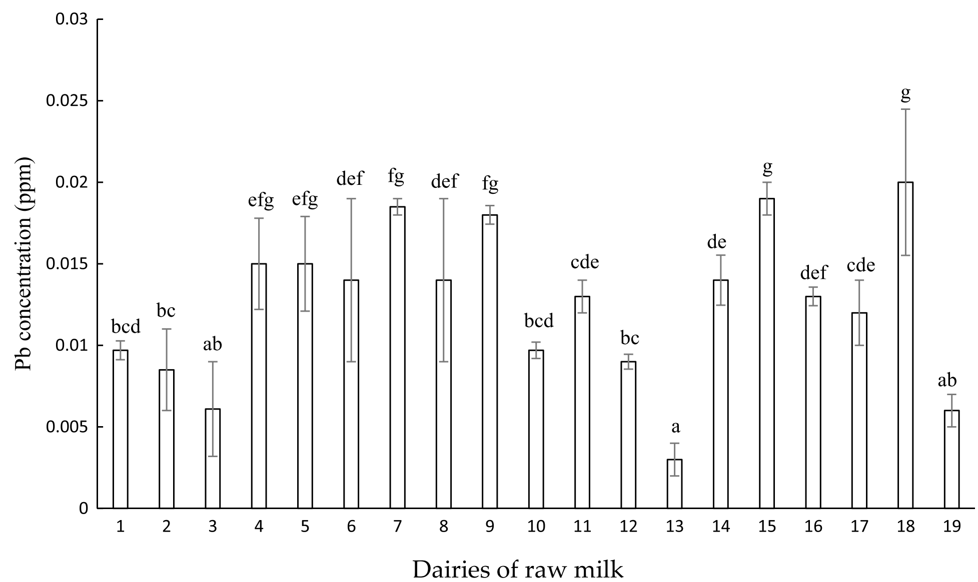

Fig. 2 Pb concentrations of the raw milk samples (ppm)

250 W, time: 3 min; (2) power: 630 W, time: 5 min; (3) power: 500 W, time: 22 min; (4) power: 0 W, time: 15 min. This program was valid as long as the full capacity of the microwave oven was used. The digested vessels were taken from the microwave oven and were allowed to cool completely. It was finally reached the volume of 25 mL [17].

Table 2 Comparison of Pb concentrations of raw milk samples to other studies

Location (province)		Year study	Sample	Method of detection*	Metal concentration (Mean±SD, ppm)	Reference
Present study		2020	cow's milk	GFAAS	0.012 ± 0.005	-
Iran	Isfahan	2018	bovine, ovine, caprine, buffalo and camel milks	GFAAS	0.0117 ± 0.001	[29]
	East Azerbaijan	2018	cow's milk	ICP-OES	0.010 ± 0.001	[30]
	Khuzestan	2016	bovine milk	GFAAS	0.047 ± 0.003	[14]
	Shahroud	2015	cow's milk	GFAAS	0.038 ± 0.001	[27]
	Hamadan	2014	cow's milk	ICP-OES	0.032 ± 0.020	[11]
	Lorestan	2014	cow's milk	GFAAS	0.003 ± 0.001	[15]
	Khorram-abad	2014	cow's milk	GFAAS	0.002 ± 0.002	[31]
	Zabol	2014	cow's milk	GFAAS	0.009 ± 0.002	[32]
	Iran	2013	cow's milk	Volta metric	0.014 ± 0.002	[33]
	West Azerbaijan	2012	cow's milk	GFAAS	0.007 ± 0.001	[17]
	Fars	2012	cow's milk	FAAS	0.010 ± 0.004	[34]
	Northeast Iran	2012	cow's milk ewe milk	GFAAS	0.0129 ± 0.006 0.0149 ± 0.007	[18]
	Arak	2011	cow's milk	GFAAS	0.016 ± 0.002	[12]
	Isfahan	2008	cow's milk	FAAS	0.0102 ± 0.007	[13]
Romania		2019	cow's milk	AAS	0.12 ± 0.044	[35]
Mexico		2019	cow's milk	ICP-OES	0.024 ± 0.01	[36]
China		2017	cow's milk	ICP-MS	0.0012 ± 0.001	[37]
Croatia		2014	cow's milk goat milk	GFAAS	0.011± 0.008 0.015± 0.025	[21]
Korea		2012	cow's milk	ICP-MS	0.003 ± 0.000	[38]

*GFAAS = Graphite furnace atomic absorption spectrometric, FAAS = Flame atomic adsorption spectroscopy, ICP-AES = Inductively coupled plasma atomic emission spectroscopy, ICP-OES = inductively coupled plasma optical emission spectrometry, ICP-MS = Inductively coupled plasma mass spectrometry

Table 3 Hazard quotients (HQ) for Pb intake through raw milk

HQ	Sources of raw milks																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Adult	0.007	0.006	0.004	0.011	0.011	0.010	0.013	0.010	0.012	0.007	0.009	0.006	0.002	0.010	0.013	0.009	0.008	0.014	0.004
Child	0.044	0.038	0.027	0.068	0.068	0.064	0.084	0.064	0.082	0.043	0.058	0.041	0.014	0.064	0.085	0.058	0.054	0.089	0.027

Pb analysis

A graphite furnace atomic absorption spectrometer (Perkin Elmer/HGA 6002, USA) equipped with an autosampler was used for the determination of Pb in milk samples. A hollow element cathode lamp was used, operating at 283.3 nm wavelength. Graphite tubes coated pyrolytically were used. The program of the atomic absorption spectrometer was defined in four steps, including 1) T: 130˚C, ramping time: 10 s, holding time: 30 s; 20 T: 450˚C, ramping time: 15 s, holding time:10 s; 3) T: 1900˚C, ramping time: 0 s, holding time: 4 s; 4) T: 2500˚C, ramping time: 2 s, holding time: 2 s [21].

Human health risk assessment

Hazard quotient (HQ), exposure frequency (EF), exposure duration (ED), food ingestion rate (F_{IR}), body weight (BW), average time (AT), and reference dose (RD) for Pb were calculated according to Sobhanardakani et al. (2018) and Dashtizadeh et al. (2019) for two different age groups including children and adults in the Iran population [11, 25] (Table 1). The hazard quotient was calculated using the following formula [26].

$$HQ = \frac{EF \times ED \times FIR \times C}{RFD \times w \times AT} \times 10^{-3} \quad (1)$$

Statistical analysis

Data were analyzed using statistical analysis of variance (ANOVA) followed by Duncan's comparison test to calculate the significant differences (p<0.05) between the means using the SPSS (ver. 24) software. The measurements were performed in triplicate. The graphs were drawn using the Excel software (version 2016). The concentrations of Pb in milk samples were expressed as the minimum and maximum, mean ± standard deviation (SD).

Results and discussion

Figure 2 shows Pb concentrations in raw milk samples. In all animal husbandries, the Pb concentration was lower than the maximum permitted level in raw milk (20 µg/kg according to Codex, EU, and Iran National Standard. The highest amount of Pb was reported in animal husbandry of 15 and 18, with the amounts of 0.19 and 0.02 mg/mL, respectively. The lowest amount of Pb was 0.003 mg/mL.

Various studies in Iran have previously investigated the concentration of Pb in raw milk (Table 2). In some studies including the study of Norouzirad et al. (2018) [14], Madani-Tonekaboni et al. (2019) [27], Sobhanardakani et al. (2018) [11], Pajohi-Alamoti et al. (2017) [11], and Radmehr et al. (2020) [28], the concentration was higher than the permissible limit, which might have caused by several reasons. For example, in the study of Norouzirad et al., sampling was done from animal husbandries near oil fields, which can be considered as one of the possible reasons for contamination of fodder and livestock water and as a result, high Pb contamination in raw milk. Moreover, the vicinity of animal husbandries to industrial areas or fodder supplying areas could be the reason. In our study, animal husbandries located near industrial areas had higher levels of lead in their raw milk than the others. From the permissible level point of view, results of other researchers were in range and in line with our results.

As shown in Table 3, HQ was calculated for each animal husbandry. According to the United States Environmental Protection Agency (USEPA) [29], if the HQ is less than 1, it means that there is no health risk for humans. The results of this study showed that HQ in all of the trials was lower than 1. Therefore, there was no carcinogenic health risk for the adult and children population as a result of milk consumption. Although the amount of HQ was in the acceptable range, in all cases the HQ value for children was higher than for adults. These findings are consistent with Qu et al. (2018), Liang et al. (2015), Yasotha et al. (2020), Islam et al. (2015), and Anita et al. (2010) [4, 30–33]. Reasonably, due to higher milk consumption in children as well as their lower body weight compared to adults, the health risk in children is higher. Also, studies have shown that the absorption rate of heavy metals in adults and children is 10% and 50%, respectively [34]. Moreover, immature digestive tract, kidney, and blood-brain barrier contributed to the increased body burden of toxic metals in the children, and hence, they were considered as high-risk groups [35].

Conclusions

In general, the results showed that the Pb values in all 19 animal husbandries were below the permissible limits (0.02 mg/kg). The results of the risk assessment showed that the HQ for all samples was less than 1, representing that carcinogenic hazards do not threaten children and adults. However, the amount of HQ in children was higher than in adults. Since the absorption of Pb is higher in children and this metal has a cumulative property in the body, therefore, its small amount can be dangerous for the health of children in the long term. In order to prevent Pb over-contaminations in raw milk, it is necessary to consider more Pb monitoring programs in animal feed and water and to use processing methods for reducing Pb in animal feed. Special attention should also be given to the location of animal husbandries because industrial areas can cause high Pb pollution.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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