



Investigate the Contamination of Raw Milk Heavy Metals by Atomic Absorption Spectrophotometry in Marvdasht

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Abstract

Background and aim: Today, cow's milk is one of the most important and widely consumed animal milks by humans and a unique source of food for all ages. The contamination of milk with heavy metals such as lead, mercury and cadmium is considered as a danger to humans. Heavy metal poisoning has adverse effects on humans that is much more common in childhood. This study was conducted to evaluate the levels of lead, mercury and cadmium in raw milk in Marvdasht.

Materials and Methods: In this study, 30 samples of raw cow milk were collected from milk collection centers in Marvdasht and tested for residual heavy metals such as lead, mercury and cadmium using Atomic Absorption Spectrophotometry.

Results: It was found that the average level of lead was equal to 0.01217 and for cadmium was 0.1340 and mercury was 0.0050 in the samples, which according to the standard level of lead, mercury and cadmium in milk in the codex, it indicates that the average accumulated concentration of lead metals in all samples was within the standard range, but the average concentration of cadmium and mercury indicates that the average concentration exceeds the standard limit.

Conclusion: Measurements of the samples showed that the level of lead was lower and the level of mercury and cadmium was higher than the global standard, so in order to increase the health and food safety for consumers, considering more monitoring, including the city's livestock feed, water consumption, forage cultivation and controlling the pollutants emitted from industrial centers adjacent to livestock are recommended.

Keywords: *Lead, Cadmium, Mercury, Spectrophotometry, Marvdasht*

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Introduction

Today, human being has faced the most serious environmental challenges and problems during his life due to his activities. Excessive population growth has intensified unbalanced industrial growth and over-exploitation of resources, regardless of the crises facing humanity and the damage to the environment. One of these crises is environmental pollution, which can threaten the health of humans and other organisms and increase the loss of vital resources (Manisalidis *et al.*, 2020). Today, food health and safety are one of the most important issues for human beings. The contamination of food used by humans with chemical toxins and toxic metals of preservatives is one of the growing problems in today's world, and among these, milk has been considered more than others as the most nutritious food containing some essential elements such as calcium, magnesium, iron, zinc and copper due to its consumption at all ages, especially in infancy and childhood, and also as a raw material in the preparation of many dairy products and is a key element in food security (Thompson & Darwish, 2019). Heavy metals are one of the most important chemical contaminants in food and their presence in foods with animal origin increases the possibility of human exposure to the effects of metals. Currently, one of the health and environmental issues of industrial societies is the control of heavy metals pollution that among them, arsenic, lead, mercury, cadmium and zinc are more important for human and animal health (Briffa *et al.*, 2020).

Almost all heavy metals in the body remain improper side effects in concentrations above the allowable level that the most common of which are disorders of the nervous system, genetic mutations, mental disorders and carcinogenesis. As far as heavy metals cannot be decomposed in nature like organic pollutants through chemical or biological processes, their stability allows them to be transported by water or air over considerable distances. As a result, their levels in the higher circles of the food chain increase several times. One of the most important problems of heavy metals is their ability to bioaccumulate and disrupt the immune system (Jaishankar *et al.*, 2014). Lead is one of the most important metals in the environment with significant toxicity that its excessive amounts in the body cause learning

disabilities in children (Geier *et al.*, 2017). Mercury is found mainly in foods in the form of methylmercury, which causes neurobehavioral abnormalities as well as impaired perception of children following exposure to methylmercury during pregnancy (Rice *et al.*, 2014). Cadmium, which accumulates in the liver and kidneys due to binding to metallothionein and replacement by bone calcium, impairs kidney function and causes osteoporosis, pulmonary dysfunction and high blood pressure (Bernhoft, 2013).

Milk may be contaminated with various toxins, especially heavy metals, under certain conditions such as water and food contamination of animals, milking process, drug consumption and proximity of livestock to industrial and mining centers, thus leading to the entry of these metals into the human body (Arianejad *et al.*, 2015). Therefore, considering the importance of milk as one of the main foods used by humans and its direct relationship with community health, this study was conducted to investigate the accumulated concentrations of lead, mercury and cadmium in raw milk samples of milk collection centers in Marvdasht.

Material and methods

Sampling and preparation

In order to determine the contamination of raw milk with lead, cadmium and mercury, 30 milk samples were collected from Marvdasht centers before mixing and cooling and stored at -20 °C.

These samples were tested and analyzed according to AOAC (Association of Official Analytical Chemists) method. Approximately 500 cc of milk was collected from each farm in sterile containers and transferred to the laboratory near ice and kept at -20 °C until the experiment. It should be noted that all glassware used in sampling and testing was washed with 10% nitric acid (Merck, Germany). During the experiment, milk samples were placed in the laboratory for 48 hours. According to AOAC reference method, 5 cc of each sample was transferred to porcelain dishes (Crucible) that had been previously washed with acid and the container containing the sample was then placed in a 90 °C oven for 12 hours to remove moisture. The dishes were heated on a flame and then placed in an electric oven at 250 °C and every hour, 50 degrees Celsius

was added to the oven temperature until the temperature reached 550 degrees Celsius. At this stage, the dishes were taken out of the oven and placed in the environment for 6 hours. In the last step and after cooling the dishes, the ashes of the samples were dissolved in nitric acid medium and the resulting solution was brought to a volume of 250 ml.

Extraction

In a separating funnel, 5 ml of 10% nitric acid was added to the prepared sample solution as tampon, and for every 10 ml of solution, one drop of 1% alcoholic Bromocresol green (BioChem, France) was added and the pH of the solution was adjusted to 5.4 using the concentrated and dilute solution of 4% ammonia (BioChem, France). Then, 5 ml of 2% ammonium pyrrolidine f dithiocarboxylic acid solution (Merck, Germany) was added to the mixture and the container containing the mixture was shaken for 10 to 15 minutes until the cadmium metal become complexed with the above solution, then 5 to 10 ml of normal butyl acetate solution (Kimia Tehran Acid, Iran) was added to the contents of the dishes and the solutions were shaken again in the separating funnel for one minute. At this stage, the aqueous phase was discarded and the organic phase containing the complex was tested.

Drawing a calibration curve

At this stage, the organic phase containing the complex was poured into the places for the atomic absorption device and then the device automatically removed the blanks and standards and the corresponding curve was drawn. After drawing the calibration curve, the samples were taken automatically by the device and their amount was calculated in terms of mg/kg using the curve.

Preparation for measuring mercury

Preparation for measuring mercury was done as follows:

At room temperature, some milk was poured into microwave cells and hydrogen peroxide (cciran, Iran) and nitric acid (Merck, Germany) were added. The cell was then placed in the microwave and a special mercury program was performed. After digestion, the sample was taken out and made to volume. To the

prepared sample, some concentrated solution of Potassium dichromate (AfraChem, Iran) and hydrogen chloride (AfraChem, Iran) were added and then brought to the desired volume. The prepared sample was injected by atomic hydride device into atomic absorption device. The concentration shown by the device was reported by comparison with the calibration curve.

Data analysis

In this study, the results of the experiments were analyzed using SPSS software. First, the data normality test (using Kolmogorov-Smirnov KS test) was performed, then ANOVA and Tukey post hoc test at the level of $p < 0.05$ were used to examine the significant difference between the means and to compare the amount of lead, cadmium and mercury in milk with the standard amount of t-test.

Results

Table 1 shows the distribution of selected samples based on collection center and the type of livestock. The descriptive statistics of continuous research variables are also presented in Table 2 which includes indicators (mean, standard deviation, minimum and maximum) related to each of the research variables. According to the results and the statistical value of the test obtained from Table 3, the amount of lead and cadmium has a normal distribution and mercury has an abnormal distribution.

Inferential review of Data

Table 4 and Figure 1 show the mean and standard deviation of lead in milk samples taken from 5 livestock centers of Marvdasht and Table 5 and Figure 2 show the mean and standard deviation of cadmium in milk samples taken from 5 livestock centers of Marvdasht. Also, Table 6 shows the mean and standard deviation of mercury in milk samples taken from 5 dairy centers of Marvdasht.

Study the significant difference between the amount of lead in milk with the standard amount

In order to answer this question, one-sample t test was used. As it is seen in table 7, the Zero hypothesis saying that the amount of lead in raw milk is equal to 0.02 has not been confirmed. Given the smallest an

Type of livestock	Traditional Semi- industrial	Milk Collection Center					Total
		Firuzi	Amir Abad	Emad Abad	Faruq	Seyedan	
		3	4	5	7	8	27
		1	2	0	0	0	3
	Total	4	6	5	7	8	30

Table 1. Frequency distribution based on type of livestock * Milk collection center

	No.	Minimum	Maximum	Mean	Standard Deviation
Lead	30	0.006	0.023	0.01217	0.004579
Cadmium	30	0.007	0.024	0.01340	0.004207
Mercury	30	0.00	0.02	0.0050	0.00682
Valid N (list wise)	30				

Table 2. Descriptive statistics of research variables in general.

	N	Kolmogorov-Smirnov Z	Asymp. Sig. (2-tailed)
Lead	30	0.916	0.371
Cadmium	30	0.573	0.898
Mercury	30	2.017	0.001

Table 3. Test the normality of the data.

Livestock	No.	Mean	Standard Deviation	F Statistics	P Value
Firuzi Center	4	0.00950	0.001915		
Amir Abad Center	6	0.01567	0.003266		
Emad Abad Center	5	0.01880	0.003194	17.497	0.000
Faruq Center	7	0.00929	0.002430		
Seyedan Center	8	0.00925	0.001669		
Total	30	0.01217	0.004579		

Table 4. Mean and standard deviation of lead content in milk samples taken from 5 livestock centers of Marvdasht.

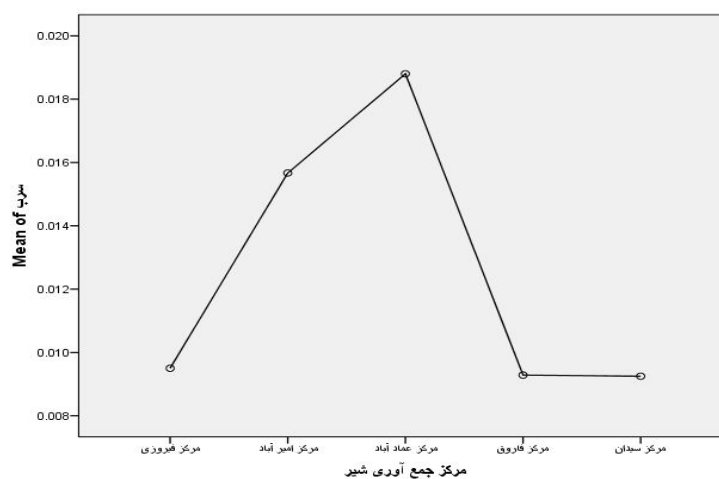


Diagram 1. Diagram of the mean amount of lead in raw milk collection centers.

Livestock	No.	Mean	Standard Deviation	F Statistics	P Value
Firuzi Center	7	0.00975	0.001708	6.693	0.001
Amir Abad Center	5	0.01600	0.003578		
Emad Abad Center	5	0.01840	0.003912		
Faruq Center	3	0.01200	0.003109		
Seyedan Center	6	0.01138	0.002825		
Total	30	0.01340	0.004207		

Table 5. Mean and standard deviation of cadmium in milk samples taken from 5 livestock centers of Marvdasht.

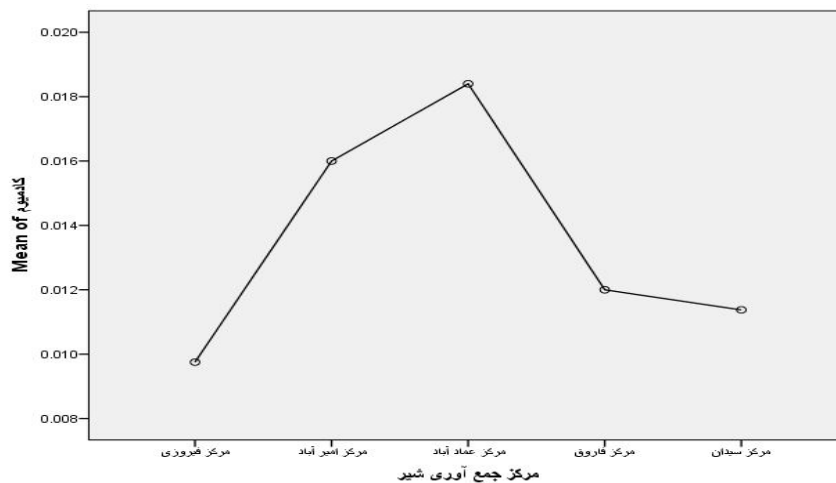


Diagram 2. Diagram of the mean amount of cadmium in raw milk collection centers.

Livestock	No.	Mean	Standard Deviation	Chi-square Statistics	P Value
Firuzi Center	4	0.0025	0.00500	3.997	0.406
Amir Abad Center	6	0.0067	0.00816		
Emad Abad Center	5	0.0080	0.00837		
Faruq Center	7	0.0014	0.00378		
Seyedan Center	8	0.0063	0.00744		
Total	30	0.0050	0.00682		

Table 6. Mean and standard deviation of mercury in milk samples taken from 5 livestock centers of Marvdasht.

largest values of the 95% confidence interval of the mean difference, that both of which are negative, it is clear that the average lead content is less than the test value (allowable threshold).

Investigate the significant difference between the amount of cadmium in milk and the standard amount

According to P-value and level of significance of that is seen in Table 8, the Zero hypothesis saying that the amount of Cadmium in livestock milk is equal to 0.001 has not been confirmed. Given the smallest and largest values of the 95% confidence interval of the mean difference, that both of which are positive, it is clear that the average Cadmium content is more than the allowable threshold, 0.001.

Investigate the significant difference between the amount of mercury in milk with the allowable threshold

According to the P-value and meaningful level seen in Table 9, the existence of a significant difference between the amount of mercury in milk and the amount tested (allowable threshold) is confirmed.

Discussion

The mean concentrations of cadmium, lead and mercury in raw cow milk samples were 0.01340, 0.1217 and 0.0050 mg/kg, respectively. The concentration of cadmium in any of the studied samples was not higher than the maximum

	Test Value = 0.02				95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Lead	-9.369	29	0.000	-0.007833	-0.00954	-0.00612

Table 7. Test results of comparing the average amount of lead in the milk of livestock with the tested amount.

	Test Value = 0.001				95% Confidence Interval of the Difference	
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
Cadmium	16.145	29	0.000	0.012400	0.01083	0.01397

Table 8. Test results of comparing the average amount of Cadmium in the milk of livestock with the tested amount.

	Category	N	Observed Prop.	Test Prop.	Asymp. Sig. (1-tailed)
	> 0	12	0.40		
	Total	30	1.00		

Table 9. Test results of comparing the average amount of Mercury in the milk of livestock with the tested amount.

concentration determined by codex. The results obtained in this study are same as the reports from Paclovic *et al.* and Ayer *et al.* and the reported results are in the range of results obtained from the present study (Ayar *et al.*, 2009; Pavlovic *et al.*, 2004). Despite the limited studies and reports on the status of cadmium and other heavy metals in the milk of the southern regions of the country, there are reports of the presence and amount of these metals in the milk of the north of the country.

In another study conducted by Rahimi and Derakhshesh (2010) in order to obtain the average percentage of cadmium recycling in the tested samples, 93.84% was reported. The results showed that the average and mean concentrations of cadmium in 109 samples of raw cow milk collected from 12 provinces of Iran were 0.0059 and 0.0052 mg/L, respectively. In another study conducted by Jeng *et al.* in Taiwan, the concentrations of lead and cadmium in 107 samples of milk from different farms were measured by an atomic absorption spectrometer with a graphite furnace and the results showed that the concentrations of lead and cadmium are 2.03 and 0.044 ng/ml, respectively and the mean of lead and cadmium in raw milk of Kazerun is higher than the values reported in this study (Jeng *et al.*, 1994). In a study conducted by Husain *et al.* in Kuwait, the amount of lead in the milk of farms around Kuwait was measured by an atomic absorption device with a graphite furnace and the mean of lead in these

samples was reported to be 43 ng/ml which is lower than the milk tested in the present study (Husain *et al.*, 1996). In a study by Rodrigues *et al.* in 1999 on the raw milk in England, the Cadmium and lead levels were reported to be 14.82 and 4.88 µg/L, respectively that the lead concentration is lower than the average lead in the tested raw milk, but the concentration of cadmium is higher than the average of cadmium in the raw milk tested (Rodrigues *et al.*, 1999).

In a study conducted by Sobhanardakani and Tizhosh (2016) in Lorestan province on 24 raw milk samples from 8 livestock units, these results were reported: The concentrations of zinc, lead, cadmium and copper were 3072, 2720, 104 and 142 µg/kg, respectively. In a study aimed at investigating the elements of lead and cadmium in the milk of cows living in an industrial and mining area of Otarias in Spain, it was shown that the concentration of lead in micrograms per kilogram is 0.71-1.16 and the average concentration of cadmium is less than 2 micrograms per kilogram (González-Montaña *et al.*, 2012). In 1997, Hinikaar *et al.*, conducted a study on four types of milk (raw milk, pasteurized milk, dry milk and breast milk) for contamination with metals such as nickel, lead, copper and cadmium by atomic absorption method and the results of this study showed lead contamination in 2.84% of the samples and cadmium contamination in 4.61% of them. Mean levels of lead was 1.2 ppm, nickel 42 ppm, cadmium

14 ppm, and copper 34 ppm (Hinikaar *et al.*, 1997). In 2014, samples of raw milk produced in Lorestan Livestock Industrial Town were studied and the results showed that the mean concentrations of elements in micrograms per liter in raw milk samples for zinc, lead, cadmium and copper were 674 ± 3072 , 2720 ± 2790 , 18.5 ± 104 and 142 ± 149 , respectively, and all were much higher than the standards of the World Health Organization (Sobhanardakani and Tizhosh, 2016). In another study on the amount of lead and cadmium contamination in 100 milk samples in Shahrekord, the results showed that the average concentration of lead in raw milk was 60.72 ppb and in the case of cadmium was 2.87 ppb, which in all samples in terms of lead and cadmium contamination is within the allowable range (Bonyadian *et al.*, 2006). In a study conducted by Erdinc and Saldamli in Turkey, the amount of lead and cadmium in the milk of different farms was measured by atomic absorption device with graphite furnace. The results of this study showed that the concentrations of lead and cadmium are 12.07 and 1.82 ng/ml, respectively (Erdinc and Saldamli, 2000).

In another study conducted by Saldamli and Erdinc in Turkey, the amounts of lead and cadmium in the milk of various farms were measured by a graphite furnace atomic absorption spectrometer. The results of this study showed that the concentrations of lead and cadmium in milk samples were 12.07 and 1.82 ng / ml, respectively, which is much lower than the values obtained in raw milk (Erdinc and Saldamli, 2000). Mitiovic *et al.*, (2012) also reported in Bulgaria that pasteurization would cause a slight decrease in lead and cadmium levels. Also, in Spain, Moreno-Rojas *et al.*, (1999) examined the amounts of lead and cadmium in 10 samples of raw milk, 10 samples of pasteurized milk and 10 samples of sterilized milk, using an atomic absorption device with a graphite furnace to study the effect of heat pasteurization and sterilization in milk on lead and cadmium content. The results showed that there was no statistically significant difference in lead and cadmium levels during pasteurization and sterilization heat. Therefore, they stated that heating could not reduce the amount of lead and cadmium in milk samples. According to the results of various studies, pasteurization heat does not have much effect on the amounts of heavy metals, but different stages that milk goes through for pasteurization and sterilization,

such as fat separation, homogenization, etc. may reduce the amount of these metals in milk. According to the results of the present study, the amount of lead and cadmium in raw and pasteurized milk produced in Marvdasht is in line with international standards and there will be no risk of accumulation of these metals in the body for milk consumers and finally, the high level of lead in areas with traditional livestock is debatable, and a significant relationship should be found between this level and the distance to the road and industrial and workshop areas, such as power plants and petrochemicals.

Conclusion

According to the results of the present study, the concentration of lead, mercury and cadmium in milk samples is close to the standard level, therefore, it does not have a risk to consumers. However, it is observed that the milk of some farms is more contaminated, so it needs further investigation. Regionally, farms near roads and industrial centers are more polluted, so some arrangements need to be made for the future. It is also necessary to conduct similar studies with a wider range to measure heavy metals in milk and milk products. It is also necessary to conduct similar studies with a wider range to measure heavy metals in milk and milk products and be monitored regularly. Finally, the high level of lead in areas with traditional livestock is debatable and a significant relationship should be found between this level and the distance to the road and industrial areas such as power plants and petrochemicals.

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Conflict of interest

There is no conflict of interest between the authors.

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بررسی آلودگی فلزات سنگین شیر خام با طیف سنجی نوری جذب اتمی در

شهرستان مرودشت

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چکیده

زمینه و مقدمه: امروزه شیر گاو یکی از مهمترین و پرمصرف ترین شیرهای حیوانی مورد تغذیه توسط انسان و یک منبع منحصر فرد از مواد غذایی برای تمام سنین است. آلودگی شیر به فلزات سنگین مانند سرب، جیوه و کادمیوم یک خطر برای انسان به شمار می آید. مسمومیت با فلزات سنگین موجب اثرات نامطلوبی بر روی انسان می شود که در دوران کودکی بسیار شایعتر است. این مطالعه به منظور بررسی سطح سرب، جیوه و کادمیوم در شیر خام شهرستان مرودشت انجام گرفت.

مواد و روش‌ها: در این مطالعه تعداد ۳۰ نمونه شیر خام گاو از مراکز جمع آوری شیر شهرستان مرودشت جمع آوری و از نظر باقیمانده فلزات سنگین سرب، جیوه و کادمیوم با استفاده از روش طیف سنجی نوری جذب اتمی مورد آزمایش قرار گرفتند.

یافته‌ها: مشخص شد که میانگین سطح سرب برابر با ۰/۰۱۲۱۷، برای کادمیوم ۰/۰۱۳۴۰ و برای جیوه ۰/۰۰۵۰ در نمونه ها بود که با توجه به حد استاندارد فلزات سرب، جیوه و کادمیوم شیر در کدکس، نشان دهنده آن است که میانگین غلظت تجمع یافته فلزات سرب در تمامی نمونه ها در حد مجاز بوده اما میانگین غلظت فلز کادمیوم و جیوه بیانگر تجاوز غلظت میانگین از حد مجاز می باشد.

نتیجه گیری: اندازه گیری نمونه ها نشان داد که سطح سرب کمتر و سطح جیوه و کادمیوم بیشتر از حد استاندارد جهانی بوده بنابراین با هدف افزایش میزان سلامت و ایمنی غذایی برای مصرف کنندگان، در نظر گرفتن نظارت‌های بیشتر از جمله نظارت بر خوراک مصرفی دام های شهرستان، آب مصرفی، محل کشت علوفه و کنترل آلاینده های منتشر شده از مراکز صنعتی مجاور دامداری ها توصیه می شود.

واژه‌های کلیدی: سرب، جیوه، کادمیوم، طیف سنجی نوری، مرودشت

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