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Effect of Environmental Factors on Heavy Metal Content of Raw Milk

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ABSTRACT

This investigation was conducted to determine the contents of some heavy metals in raw milk samples collected from four different regions: industrial, rural, heavy traffic intensity and agricultural regions around Isparta city, Turkey. A total of 86 raw milk samples were collected from these regions, and the contents of Pb, Cd, Cu, Fe, Zn, As, Sn and Hg of milk samples were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Limit of detection values (LODs) were between 1.00-5 µg/L for the studied elements while recoveries ranged from 91 to 102% and the standard deviations were in the range 1.13 to 4.30%. Pb, Cd, Cu, Fe and Zn were detected in milk while As, Sn and Hg was not detected in milk samples. The highest heavy metal content was found in milk samples collected from the industrial region. According to the statistical analysis, the differences between the regions were statistically significant ($p<0.05$).

Keywords: Contamination**,** Heavy metals, Milk, Inductively Coupled Plasma-Optical Emission Spectroscopy

Çiğ Sütlerin Ağır Metal İçerikleri Üzerine Çevre Koşullarının Etkisi

ÖZ

Bu çalışma, Isparta ili civarında sanayi, kırsal, trafiğin yoğun olduğu ve tarımsal olmak üzere dört farklı bölgeden toplanan çiğ süt örneklerinde bazı ağır metallerin içeriğini belirlemek amacıyla yapıldı. Bölgelerden toplanan 86 çiğ süt örneğinde Pb, Cd, Cu, Fe, Zn, As, Sn, Hg içeriği, İndüklenmiş Eşleşmiş Plazma-Optik Emisyon Spektroskopisi (ICP-OES) tekniği ile belirlendi. Çalışılan elementler için dedeksiyon limitleri, 1.0-5.0 µg/L; geri kazanım değerleri %91-102; standart sapmaları ise 1.13-4.30 arasındadır. Sütlerde tespit edilen elementler, Pb, Cd, Cu, Fe, Zn. As, Sn, Hg örneklerde tespit edilememiştir. En yüksek ağır metal içeriği, sanayi bölgesinde toplanan süt örneklerinde bulunmuştur. Bölgeler arasındaki farklar istatistiksel olarak anlamlı bulunmuştur ($p<0.05$).

Anahtar Kelimeler: Kirlilik, Ağır metal, Süt, İndüklenmiş Eşleşmiş Plazma-Optik Emisyon Spektroskopisi

INTRODUCTION

Milk is very important component of human diet. Dairy products are an important source of many nutrients including calcium, high quality protein, potassium, phosphorus, and riboflavin. In fact, dairy foods provide 73% of the calcium available in the US food supply. This information reveals that it is difficult to meet calcium needs if milk or milk products are not included in the diet. It is known that an adequate intake of calcium throughout life helps to reduce the risk of osteoporosis, hypertension, and possibly some type of cancer like colon cancer [1, 2]. Milk is a complex chemical composition of water, lactose, fat, protein (mostly casein), minerals and vitamins distributed throughout colloidal and soluble phases [3, 4]. Inorganic or aggregated forms of chemical substances (metalloids, heavy metals etc.) in feed and food represent a severe risk for their long-term toxicological effects. Heavy metals are widely dispersed in the environment. The toxicity induced by excessive levels of some of these elements, such as Cr, Cd, Pb and Hg, are well known [5, 6]. The heavy elemental analysis of milk is fundamental important step. Because heavy metals in milk are indicator of environmental contamination. Milk is an important pathway for toxic metal intake and an origin of major nutrients for humans. The presence of over limit of heavy metals in milk may bring important health problems. Pb, Cd, Cu, Fe, Zn, As, Sn, Hg and many of its compounds are very toxic. Heavy metals presence in foods lead to tai–Itai disease that causes severe skeletal changes and often death. They can enter the body by ingestion, inhalation, or resorption through the skin [7].

Milk and most of the dairy products from cows are likely to be exposed to heavy metal contamination during lactation period. These products are important parts of human diet [8]. Milk is affected by environmental factors (pesticides, heavy metals, pollution) reasonably. Different methods have been applied to detect heavy metals contents in milk. The most commonly published techniques for the elemental analysis of milk are atomic absorption spectrometry (AAS) [9-12], inductively coupled plasma optical emission ICP-OES [9-14], inductively coupled plasma mass spectrometry (ICP-MS) [9-12].

Milk is an important pathway for toxic metal intake and an origin of major nutrients for humans. The determination of trace heavy element contents of milk as daily drink for all peoples which being a complex food has great value. The heavy elemental analysis of milk is fundamental important step. Because heavy metals in milk are indicator of environmental contamination. We have investigated to determine the contents of some heavy metals in raw milk samples collected from four different regions around Isparta a province of Turkey.

MATERIAL and METHODS

Materials

48 raw milk samples (Isparta and districts), 12 samples each from the industrial, heavy traffic regions, agricultural region and rural region with three repetitions were used to investigate the effect of environmental pollution on the heavy metal content of raw milk. Milk samples (1 liter) were taken with milking machines with the help of manufacturers. Samples were taken from the cows of the same barn. The samples were immediately transported in ice boxes to the laboratory and were processed within 2 hours.

Reagents

The single component standards of lead (Pb), cadmium (Cd), copper (Cu), iron (Fe), zinc (Zn), arsenic (As), tin (Sn), and mercury (Hg) (each one with the content of 1000 ppm, Manchester New Hampshire). For the decomposition, analytical grade 65% (w/v) HNO_3 and 30% (w/v) H_2O_2 was used (Sigma Aldrich, Darmstadt, Germany).

Sample preparation

Prior to analysis, the samples were homogenised. Approximately 0.5 g of the sample was accurately weighed into an acid washed TPFA digestion tube and 6 mL of HNO_3 (65%, w/v) and 2 mL H_2O_2 (30%, w/v) were added [15]. The microwave oven condition lines are listed in Table 1.The maximum total output of the microwave generator was 1450 W and the maximum pressure in the digestion tube was 45 bar. The digest was transferred into a 50 mL acid washed volumetric flask and the flask was filled up with demineralised water and stored in a polypropylene container. Each sample was decomposed into four replicates. Two water blanks were run with each batch of samples.

Table 1. Microwave oven condition lines

Step	Time	Temperature
1	15	110°C
2	15	110°C

Equipment

Measurements was carried out with a sequential, axialy viewed Perkin Elmer Optima 8000 ICP-OES equipped with a meinhard nebulizer, a glass cyclonic spray chamber and ICP WinLab software Data System. The samples werede composed in a microwave decomposition apparatus Milestone Stard D (Sorisole, Italy). Demineralised water was taken from a Sartorious Arium Ultrapure water purification system (Göttingen, Germany).

The ICP–OES Method

The measurement conditions were optimised based on the signal-to-back ground ratio of the least sensitive element (Mn). The measurement conditions are listed in Table 2. The analyte emission was based on taking the difference of measured emission intensity on the top of the peak and back ground near the peak. All detection limits given by the ICP–OES software were based on three times the Standard deviation of the background counts. Including the washing time between samples, the total time for analysis was approximately 7 min.

Statistical Analysis

Limit of detection (LOD), limit of quantification (LOQ), linearity of calibration, intra-and inter-day accuracy, precision and recovery were estimated for the validation of this method. Each element concentration was measured in three replicates. Samples with a mount below the LOD were not detectable. The extraction recovery and intraday precision of this method were determined by spiking blank milk with each compound in three replicates; they were extracted as previously described. The inter-day precision and recovery were assessed by analyzing the target elements spiked at 5 different days.

Table 2. Optimised operating conditions for the determination of constituents in milk by ICP-OES

Rf power (W)	1450
Injector:	Alumina 2 mm i.d.
Sample tubing:	Standard 0.76 mm i.d
Drain tubing:	Standard 1.14 mm i.d.
Quartz torch:	Single slot
Sample capillary:	PTFE 1 mm i.d.
Sample vials:	Polypropylene
Source equilibrium delay:	15 sec
Plasma viewing:	Axial
Processing mode:	Peak area
Gases:	Argon and Nitrogen
Shear Gas:	Air
Replicates:	3
Pb (nm)	220.356
Cd (nm)	226.502
Cu (nm)	324.757
Fe (nm)	259.943
Zn (nm)	213.857
Hg (nm)	253.652
As (nm)	188.979
Sn (nm)	189.926

RESULTS

Analytical Results

When applied to milk samples the proposed method showed good results. The calibration curves for all the species studied showed good linear correlation coefficients (r^2>0.999), independent of the method used for sample preparation. The detection limits (LODs) (calculated as 3 times the standard deviation from 10 measurements of the blank) which are obtained for each method, are presented in Table 3. Recoveries, intra- and inter-day precisions are shown in Table 4.

Table 3. In this research obtained data of LOD (µg/L) and r^2

Element	LOD	r^2
Pb	5.0	0.999
Cd	2.5	0.999
Cu	2.5	0.999
Fe	2.5	0.999
Zn	2.5	0.999
Hg	1.0	0.999
As	5.0	0.999
Sn	2.5	0.999

Table 4.Intra-day and inter-day precisions and average recovery [mean ± Standard Deviation (SD) (%)]

Compounds	Precision Intra-day (n=5) (µg/L)			Precision Inter-day (n=5) (µg/L)			Average Recovery (mean ± SD (%))
	Standard Concentration			Standard Concentration			
	10.00	25.00	50.00	10.00	25.00	50.00	
Pb	10.11	25.98	50.09	10.99	25.95	50.97	98±2.22
Cd	10.05	25.02	50.01	10.98	24.99	50.90	99±2.15
Cu	10.97	25.11	50.05	10.89	24.95	50.01	97±4.30
Fe	10.20	25.25	50.12	10.03	25.02	50.03	100±1.13
Zn	10.11	25.04	50.08	9.97	24.98	49.97	101±1.98
Hg	9.99	25.03	50.02	9.95	24.96	49.98	102±4.23
As	10.13	25.22	50.10	9.93	24.95	49.96	91±1.95
Sn	10.01	24.96	49.99	9.95	24.97	49.99	98±2.15

Pb, Cd, Cu, Fe and Zn in Raw Milk Samples

The results of the analysis for milk are presented in Tables 5. The highest heavy metal content was found in the milk samples collected from industrial region followed by agricultural region, traffic intensive region and rural region. The highest Pb content was determined in traffic intensity region (0.077 mg/kg) followed by industrial intensive (0.062 mg/kg), agricultural region (0.035 mg/kg) and rural (0.021 mg/kg) regions, respectively. According to the statistical analysis, the differences between the regions were statistically significant ($p<0.05$). The highest Cd content was determined in industrial intensive (0.007 mg/kg) followed by traffic intensity region (0.0053 mg/kg). Cd not detected in agricultural and rural region. According to the statistical analysis, the differences between the regions were statistically significant ($p<0.05$). The highest Cu content was determined in rural region (1.13 mg/kg) followed agricultural region (0.95 mg/kg), traffic intensity region (0.46 mg/kg) and industrial region (0.44 mg/kg), respectively. According to the statistical analysis, the differences between the regions were statistically significant ($p<0.05$). The highest Fe content was determined in industrial region (6.02 mg/kg) followed agricultural region (4.59 mg/kg), traffic intensity region (3.48 mg/kg) and rural region (1.98 mg/kg), respectively. According to the statistical analysis, the differences between the regions were statistically significant ($p<0.05$). The highest Zn content was determined in industrial region (4.92 mg/kg) followed agricultural region (4.76 mg/kg), traffic intensity region (4.04 mg/kg) and rural region (2.20 mg/kg), respectively. According to the statistical analysis, the differences between the regions were statistically significant ($p<0.05$).

Table 5. Pb, Cd, Cu, Fe and Zn in contents of raw milk samples from different regions

Metal	Industrial region		Rural region		Traffic intensity region		Agricultural region	
	Sample no	mg/kg	Sample no	mg/kg	Sample no	mg/kg	Sample no	mg/kg
Pb	1	0.068	1	0.051	1	0.075	1	0.045
	2	0.075	2	0.032	2	0.086	2	0.034
	3	0.078	3	0.012	3	0.063	3	0.044
	4	0.058	4	ND	4	0.095	4	0.039
	5	0.074	5	0.024	5	0.082	5	0.027
	6	0.090	6	0.035	6	0.071	6	0.043
	7	0.059	7	0.014	7	0.086	7	0.055
	8	0.043	8	0.017	8	0.067	8	0.033
	9	0.038	9	0.019	9	0.091	9	0.018
	10	0.061	10	0.035	10	0.057	10	0.021
	11	0.058	11	0.013	11	0.083	11	0.032
	12	0.053	12	ND	12	0.074	12	0.033
	Average	0.062	Average	0.021	Average	0.077	Average	0.035
	Minimum	0.043	Minimum	ND	Minimum	0.057	Minimum	0.018
	Maximum	0.090	Maximum	0.051	Maximum	0.095	Maximum	0.055
Cd	1	ND	1	ND	1	ND	1	ND
	2	0.025	2	ND	2	0.020	2	ND
	3	0.018	3	ND	3	0.021	3	ND
	4	ND	4	ND	4	ND	4	ND
	5	0.024	5	ND	5	ND	5	ND
	6	ND	6	ND	6	0.015	6	ND
	7	0.011	7	ND	7	ND	7	ND
	8	ND	8	ND	8	ND	8	ND
	9	0.016	9	ND	9	ND	9	ND
	10	ND	10	ND	10	0.008	10	ND
	11	ND	11	ND	11	ND	11	ND
	12	ND	12	ND	12	ND	12	ND
	Average	0.007	Average	ND	Average	0.0053	Average	ND
	Minimum	ND	Minimum	ND	Minimum	ND	Minimum	ND
	Maximum	0.025	Maximum	ND	Maximum	0.021	Maximum	ND
Cu	1	0.45	1	0.65	1	0.54	1	0.88
	2	0.87	2	1.18	2	0.48	2	1.38
	3	0.12	3	0.87	3	0.32	3	1.17
	4	0.25	4	1.23	4	0.65	4	0.59
	5	0.24	5	0.98	5	0.29	5	0.76
	6	0.56	6	1.73	6	0.34	6	0.97
	7	0.34	7	0.87	7	0.46	7	1.06
	8	0.42	8	1.45	8	0.43	8	0.68
	9	0.51	9	0.75	9	0.48	9	0.87
	10	0.53	10	1.63	10	0.19	10	0.97
	11	0.44	11	0.88	11	0.65	11	1.35
	12	0.65	12	1.37	12	0.78	12	0.81
	Average	0.44	Average	1.13	Average	0.46	Average	0.95
	Minimum	0.12	Minimum	0.65	Minimum	0.19	Minimum	0.59
	Maximum	0.87	Maximum	1.73	Maximum	0.78	Maximum	1.38
Fe	1	4.56	1	2.46	1	2.44	1	4.56
	2	6.34	2	2.15	2	3.57	2	3.45
	3	5.22	3	3.32	3	3.55	3	5.69
	4	6.45	4	1.29	4	4.42	4	6.71
	5	4.47	5	2.05	5	3.54	5	5.73
	6	5.78	6	2.73	6	4.75	6	2.90
	7	8.10	7	0.96	7	3.52	7	4.68
	8	7.99	8	1.68	8	5.88	8	3.56
	9	6.53	9	2.26	9	2.79	9	3.65
	10	5.58	10	1.51	10	2.04	10	4.49
	11	6.44	11	0.83	11	2.12	11	3.97
	12	4.87	12	2.55	12	3.23	12	5.92
	Average	6.02	Average	1.98	Average	3.48	Average	4.59
	Minimum	4.47	Minimum	0.96	Minimum	2.04	Minimum	2.90
	Maximum	8.10	Maximum	3.32	Maximum	5.88	Maximum	6.71
Zn	1	3.45	1	2.46	1	4.45	1	3.86
	2	5.61	2	1.34	2	3.96	2	4.71
	3	5.18	3	2.20	3	4.75	3	2.76
	4	7.33	4	1.87	4	4.51	4	5.48
	5	4.25	5	2.73	5	4.38	5	5.53
	6	4.43	6	2.62	6	4.87	6	4.89
	7	5.65	7	3.95	7	5.11	7	5.70
	8	7.67	8	1.75	8	2.78	8	3.08
	9	2.90	9	1.55	9	3.67	9	4.66
	10	4.82	10	2.73	10	2.09	10	4.54
	11	4.56	11	0.97	11	3.43	11	5.99
	12	3.21	12	2.33	12	4.55	12	6.03
	Average	4.92	Average	2.20	Average	4.04	Average	4.76
	Minimum	3.21	Minimum	1.34	Minimum	2.09	Minimum	2.76
	Maximum	7.67	Maximum	3.95	Maximum	5.11	Maximum	6.03

* ND: Not detected

The highest heavy metal content was found in the milk samples collected from industrial region followed by agricultural region, traffic intensive region and rural region. The highest Pb content was determined in traffic intensity region (0.077 mg/kg) followed by industrial intensive (0.062 mg/kg), agricultural region (0.035 mg/kg) and rural (0.021 mg/kg) regions, respectively. According to the statistical analysis, the differences between the regions were statistically significant ($p<0.05$). The highest Cd content was determined in industrial intensive (0.007 mg/kg) followed by traffic intensity region (0.0053 mg/kg). Cd not detected in agricultural and rural region. According to the statistical analysis, the differences between the regions were statistically significant ($p<0.05$). The highest Cu content was determined in rural region (1.13 mg/kg) followed agricultural region (0.95 mg/kg), traffic intensity region (0.46 mg/kg) and industrial region (0.44 mg/kg), respectively. According to the statistical analysis, the differences between the regions were statistically significant ($p<0.05$). The highest Fe content was determined in industrial region (6.02 mg/kg) followed agricultural region (4.59 mg/kg), traffic intensity region (3.48 mg/kg) and rural region (1.98 mg/kg), respectively. According to the statistical analysis, the differences between the regions were statistically significant ($p<0.05$). The highest Zn content was determined in industrial region (4.92 mg/kg) followed agricultural region (4.76 mg/kg), traffic intensity region (4.04 mg/kg) and rural region (2.20 mg/kg), respectively. According to the statistical analysis, the differences between the regions were statistically significant ($p<0.05$).

DISCUSSION

Milk is an ideal source of macro elements, such as calcium, potassium, phosphorus. Moreover, microelement even heavy metal can be found in milk. However, heavy metals such as As, Cd, Hg and Pb have no beneficial effects on human [16]. The results of this study showed that no As, Sn, Hg were detected in any region of the investigation. In this research, the differences among these regions were statistically significant ($p<0.05$). However, Cd, Fe and Zn amounts of the milk collected from industrial region were higher. This results shows that industry may be effect on environmental pollution. Similar results were obtained in some studies [17-20].

The maximum limit of Pb content in raw milk was determined as: 0.02 mg/kg in Turkey [21]. But, average Pb value of all regions above the maximum limit. Similar results were obtained in some studies [3, 8].

Average Cd values of the raw milk samples varied between 0.000–0.007 mg/kg (Table 5). The highest average values were in industrial region (0.025 mg/kg) while the lowest value was in the milk from rural region and agriculture region. Similar results were obtained in some studies [22, 23].

Average Cu values of the raw milk samples varied between 0.12–1.13 mg/kg (Table 5). The highest average values were in rural region (1.73 mg/kg). This is using of metal equipment can caused in rural region. In another study, similar results have obtained [17].

Average Fe values of the raw milk samples varied between 1.98–6.02 mg/kg (Table 5). The highest average values were in industrial region (8.10 mg/kg) while the lowest value was in the milk from rural region (0.96 mg/kg). Similar results were obtained in some studies [17, 20].

Since many biological and environmental samples contain low levels of zinc, it is easy to contaminate samples [24]. Zinc contents of the raw milk samples varied between 2.30–6.46 mg/kg (Table 5). The highest average values were in industrial region (7.67 mg/kg) while the lowest value was in the milk from rural region (1.34 mg/kg). In another study, similar results have obtained [17].

As a result, average recoveries of studied heavy metals were higher than 90% (Table 4). Thus, this method seemed sensitive, precise and accurate for determining studied heavy metals in milk.

CONCLUSION

In conclusion food consumption had been identified as the major pathway of human exposure to heavy metals, and consuming foodstuff threatens the health of the population. In this study, it has been determined that heavy metal contamination identified in many milk samples from different regions. The elevated levels could be related to contamination during industry processing, environmental pollution and pesticides. Lactating animals exposed to high concentrations of toxic metals such as Pb, Cd, Cu, Fe, Zn, As, Sn, Hg, accumulate these metals in their milk which becomes a health hazardous to consumers. In addition, the very low concentrations of heavy metals in milk indicate that the exposure of these elements on humans consuming milk is negligible.

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