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Determination of Zinc, Copper, Iron, Nickel, Chromium, Cadmium and Lead in Milk Samples by Flame and Electrothermal Atomic Absorption Spectrometers§

Argun Türker[1*] and Orhan Acar[1]

[1]*Gazi University, Science Faculty, Department of Chemistry, 06500, Ankara, Türkiye,*

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Abstract: Milk samples consumed in the world contain essential nutrients as well as toxic elements. Quality control is necessary for the production and processing of milk samples. Also, transportation and storage conditions have effects on the composition of samples. In this study, the concentrations of zinc, iron, chromium, nickel, copper, cadmium and lead in fourteen different brands of milk samples (pasteurized and long life) purchased from markets of Turkey in Ankara were determined and evaluated by using flame and electrothermal atomic absorption spectrometers (FAAS, ETAAS). Graphite furnace heating program for Cd and Pb determinations in samples was optimized in the absence or presence of nickel (Ni) matrix modifier. The limits of detection (LOD) for Cd and Pb determined by electrothermal atomic absorption spectrometry (ETAAS) in the presence of Ni matrix modifier were 1.2 and 1.5 µg/L, respectively. The LODs of Cu, Fe, Zn, Cr and Ni determined by flame atomic absorption spectrometry were 18.4, 71.2, 20.8, 2.1 and 2.3 µg/L, respectively. The mean concentrations and standard deviations ($\bar{X}$±s) of Zn, Ni, Cu, Cr, Fe, Cd and Pb were 1.7 ± 0.4 mg/kg, 0.24 ± 0.08 mg/kg, 0.8 ± 0.2 mg/kg, 0.8 ± 0.6 mg/kg, 1.9 ± 0.6 mg/kg, 46 ±5 µg/kg and 28 ± 3 µg/kg, respectively found in fourteen different milk samples. The contents of elements found in samples were compared with permissible limits recommended in the health criteria by the World Health Organization (WHO) and literature values. It was concluded that the tools and materials used in milk production were not caused to heavy metal contamination. The metal concentrations in the milk samples were within the limits of the maximum permissible metal values given by WHO/FAO, other regulations and the Turkish Food Codex.

Keywords: Milk sample; flame and electrothermal atomic absorption spectrometers; Ni matrix modifier; toxic elements.

1. Introduction

Milk is mainly consumed by human beings, especially infants, adults and children, and it is generally utilized in the food industry to prepare other food products. Because of its nutritional importance and widespread consumption worldwide, the determination of the milk's heavy metal contents and quality is necessary. Although milk is a complex bioactive matter for both the growth and the development of human beings, it contains essential microelements such as Cu, Fe and Zn, heavy contaminant metals such as Cd and Pb, which might enter into milk at increasing levels that are harmful

* Corresponding author: e-Mail: argunturker@gazi.edu.tr

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to humans [1, 2]. In Europe, a regulation such as commission regulation (EC) No 1881/2006 has set out maximum levels for contaminants in food [1]. For example, Fe, Cu and Zn are necessary and vital qualities of food to continue suitable metabolic performances for many biological functions in the human body [3]. Still, these elements may also cause ill effects when consumed at higher levels. Moreover, heavy metals such as Pb, Cd, Cr and Ni are toxic even at low concentrations of 10-50 mg/kg (World Health Organization/WHO) [4] due to their toxic effects on humans [5 - 9]. The presence of necessary and heavy toxic metals in milk samples can be due to the animals in some circumstances, such as eating pasture or drinking water from polluted resources such as industrial, agricultural and urban activities on environments [10 - 13]. The metals in milk samples may also come from containers utilized during processing, contaminated water, livestock feed and the environment of milk-producing animals [2, 14].

Determining the presence of toxic heavy metals in milk is vital to health problems in the world, and several countries have established their standard limits for heavy metals [15]. The recommended limits of toxic heavy metals in milk have been reported in several countries, including Poland [16], Pakistan [17], Egypt [18], and Nigeria [19]. The determination of the quality of milk and its validity for human consumption depends on the contents of heavy metals in the milk sample. Because of the increase in environmental pollution, it became necessary to determine the concentrations of heavy metals in milk due to the significant influence on human health [2]. Toxic metals have various effects on health problems such as nervous system illness, genetic mutation [20], respiratory illness, neurological disease, cancer, and cardiovascular, infertility and immune system disease [21], even at low levels. Lead and cadmium may accumulate in tissues and cause diseases such as kidney, central nervous system, anaemia, damage to the liver, etc. [12, 22, 23]. Cadmium is also a carcinogenic element in prostate and lung diseases. In addition, Ni can cause local infections and a variety of cancers of the blood, brain, and bone [3, 8, 12, 22, 24]. Therefore, it is essential to determine the necessary toxic level of the metal concentrations in milk samples.

Several techniques, such as flame and electrothermal atomic absorption spectrometry (FAAS and ETAAS), inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma mass spectrometry (ICP-MS), were utilized in determining the concentrations of Fe, Cu, Zn, Ni, Cr, Pb and Cd in milk samples [10, 25, 26] after performing a previous sample treatment [27 - 30]. Among these techniques, furnace atomic absorption and flame atomic absorption spectrometry are mainly used.

This study aimed to determine the concentrations of essential and toxic metals (Fe, Cu, Zn, Ni, Cr, Pb and Cd) in long life and pasteurized fourteen different brand milk samples purchased from markets in Ankara city and to find out whether the levels of these metals are lower or higher than the recommended maximum permissible levels given by WHO and other regulations for human consumption. Flame and electrothermal atomic absorption spectrometers were used. The contents of metals, which may originate from the environment and/or the tools and materials used during the processing of milk samples, were evaluated in terms of public health.

2. Materials and Methods

2.1. Reagents and Chemicals

Chemicals used in the study were analytical grade reagents, and deionized water (18.3 MΩ/cm) was used in all the experiments. Working standards (Zn, Ni, Cu, Fe, Cr, Cd and Pb) were prepared daily from stock atomic absorption solutions (1000 mg/L for each, Merck, Darmstadt, Germany). In the determinations of Cd and Pb, 1000 mg/L Ni AAS stock solution was used as a matrix modifier. For dissolving milk samples, HNO_3 (65%, m/m) and H_2O_2 (30%, m/m) (Merck, Germany) were used. All glass wares, Teflon beakers, plastic containers and plastic test tubes were soaked overnight in nitric acid (10%, v/v) + 2 mL 30% H_2O_2 mixture, washed later and rinsed with deionized water and dried.

2.2. Dissolving of Samples

Approximately 1.0 g standard reference materials (Whey Powder IAEA-155 and BCR-CRM-150, Milk powder spiked) and 1.0-1.5 g liquid milk samples were transferred into dried beakers. After adding

2 mL of deionized water for mixing samples, 5 mL concentrated HNO_3 and 2 mL H_2O_2 were added to dissolve the samples. Beakers were placed on a hot plate in the fume hood and heated at 160-180 °C to obtain about 3 mL sample solution. After dissolving the samples completely, the solutions were transferred into 15 mL plastic tubes, and the inner surfaces of the beakers were washed with deionized water in plastic tubes. The solutions in the tubes were completed to the mark with deionized water. Blank solutions were also prepared in the same way as milk samples.

2.3. Instrumentation

A Varian AAS240FS model flame atomic absorption spectrometer (FAAS) equipped with a deuterium lamp for background correction and a Varian AA240Z model electrothermal atomic absorption spectrometer (ETAAS) equipped with a Zeeman background correction system (Mulgrave Virginia, Australia) was used. An air-acetylene burner (flow rates of acetylene: 1.4 L/min and air: 13.6 L/min) was used for Zn, Fe, Cu, Cr and Ni atomizations. In the determinations of Cd and Pb by ETAAS, TGA-120 model graphite furnace, pyrolytic carbon tubes (Varian, P/N-63–100,012–00) and PSD-120 model autosampler regulated to 20 µL were used. High-purity argon gas (99.98%, Oksan, Ankara, Turkey) was used as purge and protective in all heating stages of the ETAAS measurements, except in the atomization steps. The integrated mode was utilized for absorbance measurements of elements with FAAS and ETAAS. Absorbance values were evaluated by using the Varian SpectraAA software program. Instrumental parameters of elements were set to the values of the software program recommended by the manufacturer and they were given in Table 1.

Table 1. Instrumental parameters of elements studied

Element	Wavelength (nm)	Current (mA)	Slit width (nm)
Zn	213.9	5.0	1.0
Fe	372.0	5.0	0.2
Cr	429.0	7.0	0.5
Cu	324.8	4.0	0.5
Ni	352.5	4.0	0.5
Cd	228.8	4.0	0.5
Pb	283.3	10.0	0.5

3. Results and Discussion

3.1. Stabilization Studies of Cadmium and Lead

Electrothermal atomic absorption spectrometry (ETAAS) is primarily used for determining Cd and Pb at low levels in highly complex sample matrixes. Interferences such as spectral or non-spectral in samples can be minimized by optimizing the heating program of the atomizer. For this purpose, (1) 1.5 mL of milk sample solution + 0.5 mL deionized water was added to a 2 mL sample cup, (2) 1.5 mL of milk sample solution + 0.5 mL of 1000 mg/L Ni solution was added to a 2 mL sample cup. The 20 µL solution (sample solution or sample solution + 5 µg Ni) was injected into the atomizer separately. The integrated absorbance values of Cd or Pb versus pyrolysis temperatures were obtained, while other parameters were kept constant. Pyrolysis and atomization curves versus temperature for Cd and Pb obtained in milk sample solutions with and without Ni matrix modifier were shown in Figures 1 and 2. Integrated absorbances obtained are the mean value of three replicate measurements. Integrated absorbance values of Cd and Pb decreased when the temperature applied was higher than 400 °C for Cd and 500 °C for Pb in the absence of Ni modifier. When 5 µg Ni (20 µL of 250 µg/mL Ni) was used in a sample solution as a matrix modifier, the pyrolysis temperatures of Cd and Pb increased up to about 800 °C for both Cd and Pb without losing absorbance values. Temperatures of about 800 °C can remove most of the organic components in milk samples. Thus, a pyrolysis temperature of 800 °C for both Cd and Pb was used in the following studies. Atomization temperatures of analytes were also investigated in the presence or absence of Ni modifier using the previously optimized pyrolysis temperatures and

parameters kept constant. Optimized atomization temperatures were 1800 °C for Cd and 2000 °C for Pb. The Ni was also used as a matrix modifier for determining Cd and Pb in SRMs and milk samples. In the presence of Ni, absorbance values and sensitivity of analytes also increased with the same concentrations of analytes by comparing absorbance signals of analytes without using Ni due to the stabilization effect of Ni on Cd and Pb (Figs. 1 and 2) [31]. An optimized ETAAS heating program for Cd and Pb in the presence of Ni is given in Table 2.

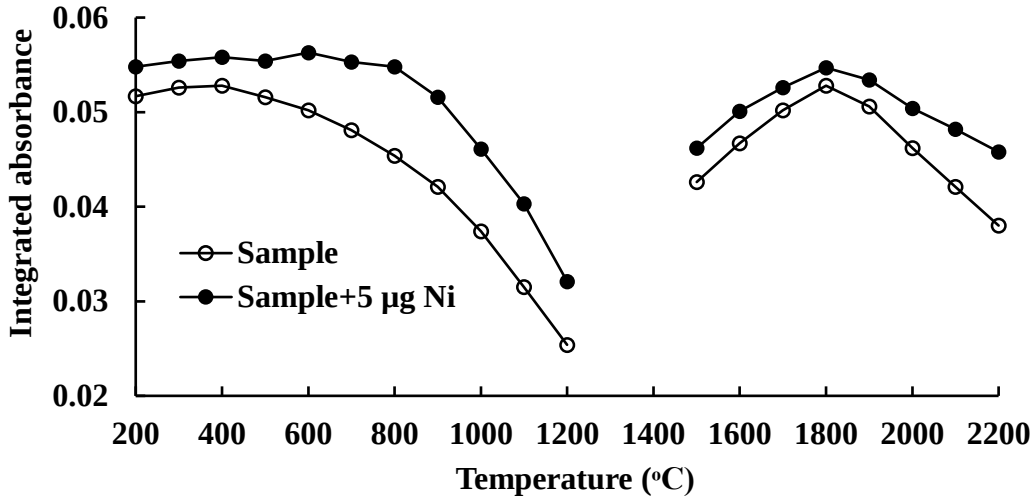

Figure1. Pyrolysis and atomization temperatures of Cd in a sample solution without and with Ni

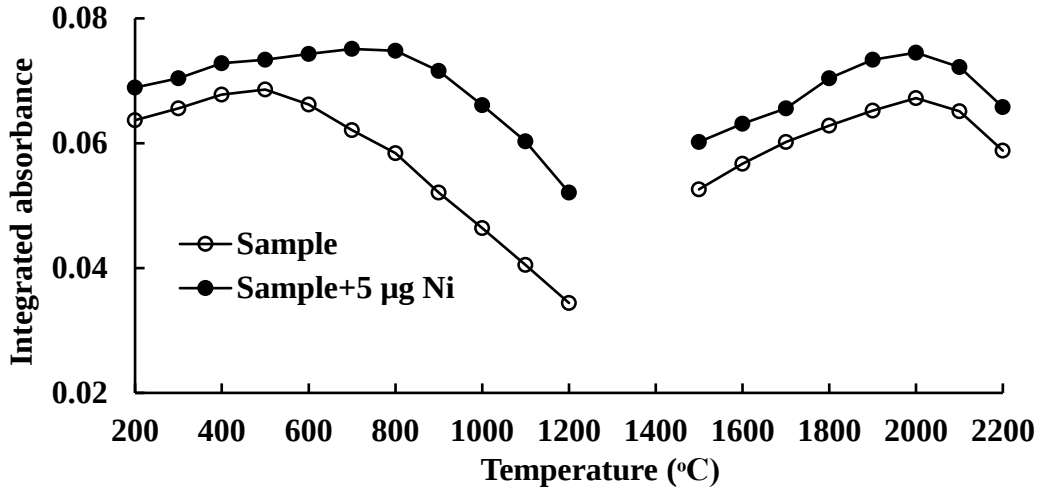

Figure 2. Pyrolysis and atomization temperatures of Pb in a sample solution without and with Ni

Table 2. Optimized ETAAS heating program for Cd and Pb in the presence of Ni

Step	Temperature (°C)		Time (s)		Ar flow rate (L/min)
	Cd	Pb	Ramp	Hold	
Drying I	50	50	5	5	0.3
Drying II	120	120	5	25	0.3
Pyrolysis	800	1000	5	25	0.3
Atomization	1800	2000	1	2	0
Cleaning	2000	2200	2	3	0.3

3.2. Analytical Figures of Merit

The determination of analytes in the dissolved SRMs and milk samples was carried out using the calibration graph method. A calibration curve for each analyte was obtained by plotting absorbance versus concentration of the working standard solution. For the determinations of Zn, Ni, Cu, Cr, Fe, Cd and Pb, AAS standard working solutions were prepared daily by diluting AAS standard stock solutions, and calibration lines were obtained. The Calibration graphs of some elements obtained are given in Figures 3 and 4 as examples. Calibration graph equations for other elements obtained are A = 0.9705C + 0.1291 (R^2 = 0.9989) for Zn; A = 0.0787C + 0.0028 (R^2 = 0.9997) for Cu and A = 0.4802 x C - 0.0005 (R^2 = 1.00) for Cd, where A is absorbance, and C is the concentration of analyte (mg/L for Zn and Cu, µg/L for Cd)

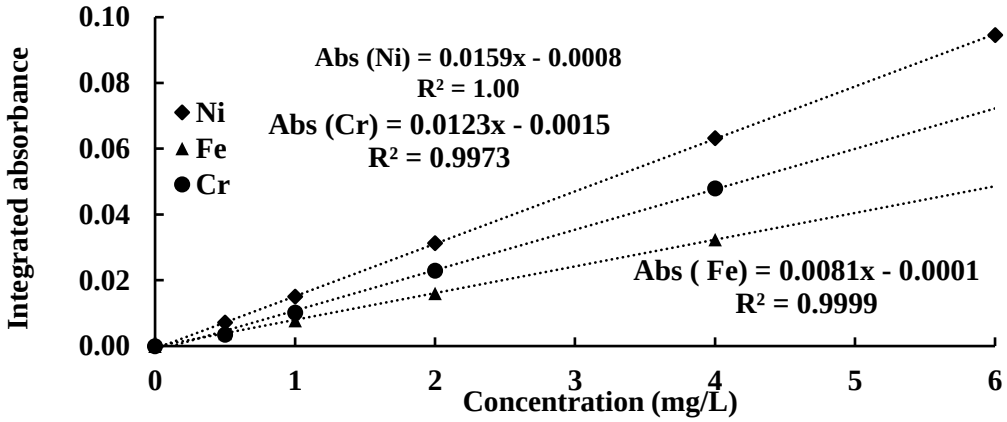

Figure 3. Calibration plots of Ni, Fe and Cr

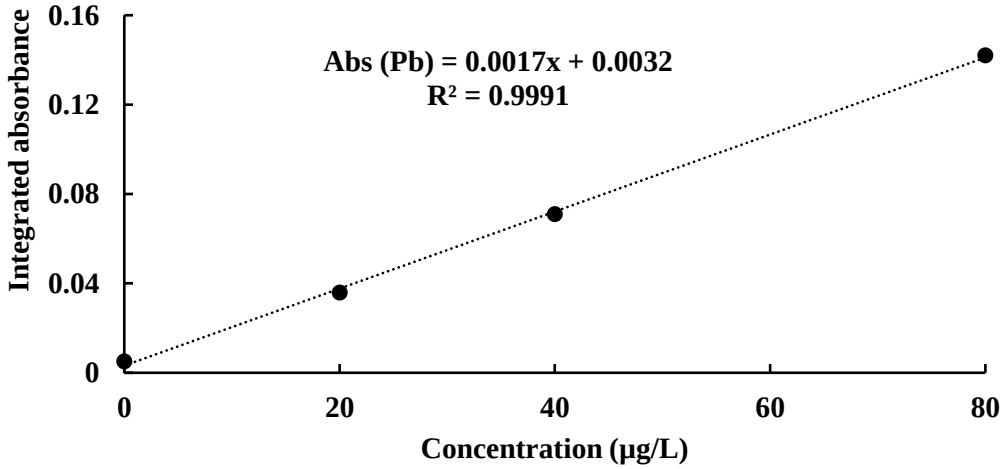

Figure 4. Calibration plot of Pb in the presence of 5 µg Ni

The calibration graphs of Cd and Pb were obtained using their working standard solutions in the presence of Ni modifier. In order to determine the limit of detection (LOD) values for Cu, Fe, Zn, Cr and Ni elements, and for Cd and Pb in the presence of Ni modifier, the absorbance values of blank solutions prepared were measured 10 times. Standard deviations (s_b) of absorbance values were calculated. The LOD was calculated by dividing the three times of standard deviations of blank (s_b) by the slope of the calibration curve (m) (LOD = $3s_b/m$; N = 10). The LOD for Cu, Fe, Zn, Cr, and Ni elements determined by FAAS are 18.4, 71.2, 20.8, 2.1 and 2.3 µg L^{-1}, respectively, and the LOD for Cd and Pb determined by ETAAS in the presence of Ni modifier were 1.2 and 1.5 µg/L, respectively.

Zn, Ni, Cu, Cr and Fe concentrations in standard reference materials (SRMs) (Whey Powder IAEA-155, BCR-CRM-150, milk powder spiked) and milk sample solutions were determined using FAAS. The concentrations of Cd and Pb in SRMs and milk sample solutions were determined using ETAAS. The average concentrations of elements found in SRMs are given in Table 3. As seen in Table

3, average concentrations found in SRMs were in good agreement with certified values. The percent relative errors obtained were below 4%. The relative standard deviations (RSD) of elements (directly related to precision) for 5 replicate measurements for each element obtained were also below 4%. The necessary dilutions were made in the milk samples, and integrated absorbance measurements were made 5 times for each element. Zn, Ni, Cu, Cr, Fe, Cd and Pb concentrations found in the milk samples are given in Table 4.

Table 3. Determination of metals in standard reference materials (Whey Powder IAEA-155 and BCR-CRM-150, Milk powder spiked)

SRM No.	Element	Certified Value (µg/kg)	Found[a] (µg/kg)	Relative Error (%)	RSD, % $(\frac{s}{\bar{x}})$x100
IAEA- 155	Zn	34300 ± 1400	35000 ± 1200	2.04	2.76
	Ni	540 ± 100	532 ± 20	-1.48	3.02
	Cu	570 ± 110	561 ± 24	-1.58	3.44
	Cr	590 ± 70	604 ± 21	2.37	2.80
	Fe	62000 ± 12000	60000 ± 1850	-3.22	2.48
	Cd	16.0 ± 3.5	16.4 ± 0.6	2.50	2.94
	Pb	104 ± 32	100 ± 4	-3.85	3.22
BCR-CRM-150	Ni	61.5 ± 3.14	63 ± 3	2.44	3.83
	Cu	2230 ± 80	2150 ± 63	-3.59	2.36
	Fe	11800 ± 602	12200 ± 500	3.39	3.30
	Cd	21.8 ± 1.4	22.6 ± 1.0	3.67	3.56

[a]Average of 5 measurements with 95% confidence level, ($\bar{x}$± 2.78 x s/ $\sqrt{N}$).

3.3. Comparison of Results with Regulations and Other Studies

The average concentrations of some elements given in Table 4 were compared with the maximum permissible values given by the FAO/WHO and other studies. The FAO/WHO expert committee reported that the allowable range of Cd is 10 - 50 µg/kg (ppb) in most foods [26, 32]. In the case of Pb, according to the EC Regulation, the maximum residue limits (MRL) in raw and fat milk were 20 µg/kg and 100 µg/kg , respectively [26, 33]. In Egypt, the standard limits for lead and cadmium in milk are 0.3 and 0.05 mg mL^{-1} [8, 15]. The average lead concentration in this study was nearly similar to the FAO/WHO limit (0.0205 mg/kg (ppm)). In the Turkish Food Codex Communiqué on Determination of the Maximum Levels of Certain Contaminants in Foodstuffs [34], the maximum amount of cadmium allowed in foodstuffs has been reported as 0.01 - 1.0 mg/kg for various foods. The mean concentration of cadmium value (0.046 ±0.005 mg/kg) detected in 14 milk samples in the study is within these limits. The mean concentration of chromium in 14 milk samples (0.8 ± 0.6 mg/kg given in Table 4) was found to be lower than the values found by Temurci (Usta) (1.613 mg/kg) [35].

It was observed that the mean concentration of Cu obtained in the study (0.8 ± 0.2 mg/kg given in Table 4) was within the limits of 0.05 - 50 mg/kg reported for various foods in the mentioned Turkish Food Codex [34]. The mean concentration of iron in milk samples (1.9 ± 0.6 mg/kg given in Table 4) was found to be lower than the values found by Temurci (Usta) (52.194 mg/kg) [35]. The mean nickel level determined in milk samples was 0.24 ± 0.08 mg/kg (Table 4). In the Communiqué on Determination of the Maximum Levels of Certain Contaminants in Foodstuffs [34], nickel levels for various foods have been specified as 0.1 - 0.2 mg/kg, and there is no restriction on the amount of nickel found in milk samples within the determined limits. The upper levels of nickel and copper from dietary sources are 0.3 – 1.0 and 3 - 10 mg mL^{-1}, respectively [8]. Therefore, the concentrations of necessary and heavy toxic metals were in agreement with the FAO / WHO limits and the Turkish Food Codex [34].

Table 4. Zn, Ni, Cu, Cr, Fe, Cd and Pb concentrations found [a] in milk samples.

Sample No	Zn (mg/kg)	Ni (mg/kg)	Cu (mg/kg)	Cr (mg/kg)	Fe (mg/kg)	Cd (µg/kg)	Pb (µg/kg)
1	1.68 ± 0.08	0.23 ± 0.01	1.12 ± 0.05	2.13 ± 0.08	2.82 ± 0.11	40 ± 2	32 ± 2
2	2.12 ± 0.02	0.17 ± 0.01	1.05 ± 0.03	1.48 ± 0.07	2.09 ± 0.10	45 ± 2	27 ± 1
3	2.00 ± 0.07	0.15 ± 0.01	0.80 ± 0.03	2.08 ± 0.09	2.52 ± 0.10	49 ± 2	29 ± 1
4	1.19 ± 0.03	0.19 ± 0.01	0.54 ± 0.02	0.93 ± 0.04	2.27 ± 0.11	46 ± 2	25 ± 1
5	1.42± 0.05	0.17 ± 0.01	1.07 ± 0.05	0.65 ± 0.03	1.45 ± 0.07	52 ± 3	23 ± 1
6	1.44 ± 0.06	0.18 ± 0.01	0.92 ± 0.04	0.50 ± 0.02	1.28 ± 0.06	44 ± 2	28 ± 1
7	1.25 ± 0.05	0.19 ± 0.01	0.35 ± 0.02	0.50 ± 0.02	1.18 ± 0.05	48 ± 3	29 ±2
8	1.60 ± 0.05	0.20 ± 0.01	0.94 ± 0.03	0.42 ± 0.02	2.18 ± 0.10	44 ± 2	29 ± 1
9	2.35 ± 0.11	0.25 ± 0.01	0.46 ± 0.02	0.49 ± 0.02	2.72 ± 0.09	54 ± 3	33 ± 2
10	2.33 ± 0.11	0.43 ± 0.02	1.07 ± 0.04	0.43 ± 0.02	2.09 ± 0.08	47 ± 3	29 ± 2
11	1.99 ± 0.08	0.30 ± 0.02	0.84 ± 0.03	0.42 ± 0.03	2.48 ± 0.10	45 ± 2	22 ± 1
12	2.29 ± 0.09	0.32 ± 0.02	1.10 ± 0.05	0.23 ± 0.01	0.59 ± 0.02	34 ± 2	34 ± 2
13	1.35 ± 0.06	0.36 ± 0.02	0.56 ± 0.02	0.34 ± 0.02	1.28 ± 0.06	53 ± 3	29 ± 1
14	1.16 ± 0.05	0.18 ± 0.01	0.95 ± 0.04	0.23 ± 0.01	1.65 ± 0.07	42 ± 2	26 ± 1
$\bar{x} \pm s$ [b]	1.7 ± 0.4	0.24 ± 0.08	0.8 ± 0.2	0.8 ± 0.6	1.9 ± 0.6	46 ±5	28 ± 3

[a]Mean of 5 measurements of samples with 95% confidence level, $\bar{x} \pm 2.78 \times s/\sqrt{N}$).

[b]Mean and standard deviation of the results were obtained from 14 different milk samples.

It was observed that the mean concentration of Cu obtained in the study (0.8 ± 0.2 mg/kg given in Table 4) was within the limits of 0.05 - 50 mg/kg reported for various foods in the mentioned Turkish Food Codex [34]. The mean concentration of iron in milk samples (1.9 ± 0.6 mg/kg given in Table 4) was found to be lower than the values found by Temurci (Usta) (52.194 mg/kg) [35]. The mean nickel level determined in milk samples was 0.24 ± 0.08 mg/kg (Table 4). In the Communiqué on Determination of the Maximum Levels of Certain Contaminants in Foodstuffs [34], nickel levels for various foods have been specified as 0.1 - 0.2 mg/kg, and there is no restriction on the amount of nickel found in milk samples within the determined limits. The upper levels of nickel and copper from dietary sources are 0.3 – 1.0 and 3 - 10 mg mL^{-1}, respectively [8]. Therefore, the concentrations of necessary and heavy toxic metals were in agreement with the FAO / WHO limits and the Turkish Food Codex [34].

4. Conclusion

In this study, the concentration levels of essential and toxic heavy metals (Fe, Zn, Cu, Cr, Ni, Cd, and Pb) in long-life and pasteurized milk samples and standard reference materials (Whey Powder IAEA-155 and BCR-CRM-150, Milk powder spiked), were determined by FAAS and ETAAS. The concentrations of analytes found in SRMs are in good agreement with certified values. Furthermore, the percent relative error and percent relative standard deviation of analytes found were below 4%. These values indicate that accuracy and precision are analytically acceptable. As a result, it was concluded that the tools and materials used in milk production were not exposed to heavy metal contamination since the metal concentrations in the milk samples were within the limits of the maximum permissible metal values given by WHO/FAO and the other regulations and the Turkish Food Codex. This study of the concentration levels of the necessary and toxic heavy metals in milk samples will become a reference for future studies on the subject.

ORCID
Argun Türker: 0000-0002-5876-9512
Orhan Acar: 0000-0002-0969-2627

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