

## Original Article

**Contamination and health risks from heavy metals (Cd and Pb) and trace elements (Cu and Zn) in dairy products**Hamid Reza Ghafari<sup>1</sup> \*Soheil Sobhanardakani<sup>2</sup>

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**Abstract**

**Background and Purpose:** Dairy products are known as an important food source in human diet. This study was carried out to analyze the content of metals (Cd, Cu, Pb, and Zn) in butter and cheese, and evaluates the potential health risks of metals to humans through the consumption of dairy products.

**Materials and Methods:** In this analytical observational study, a total of 32 samples of butter and cheese were collected from the market basket of Hamadan City in 2016. After preparing and processing the samples in the laboratory, the content of metals were determined using inductively coupled plasma-optical emission spectrometer (ICP-OES). Also, all statistical analyses of the collected data were performed using SPSS version 19.0 statistical package according to Shapiro-Wilk test for normality, One Way ANOVA (Duncan Multiple Range Test), Pearson's correlations, and Independent t-test.

**Results:** The results of the current study showed that the mean concentrations ( $\mu\text{g kg}^{-1}$ ) of Cd, Cu, Pb and Zn in butter samples were  $0.83 \pm 0.15$ ,  $6.25 \pm 1.76$ ,  $21.75 \pm 10.94$ , and  $131.35 \pm 9.24$ , respectively, while in cheese samples, they were  $0.70 \pm 0.11$ ,  $39.43 \pm 40.26$ ,  $12.85 \pm 2.41$ , and  $198.08 \pm 10.97$ , respectively. Also, the Target hazard quotient (THQ) values in adults and children through the consumption of butter and cheese were within the safe limits ( $\text{THQ} < 1$ ).

**Conclusion:** Considering the serious contamination of some brands of butter and cheese by Cu and Pb, a control of heavy metals and trace elements levels during the whole production processing of dairy products must be applied.

**Key words:** Food safety; Metal contamination; Health risk index; Milk

**Citation:** Ghafari HR., Sobhanardakani S. Contamination and health risks from heavy metals (Cd and Pb) and trace elements (Cu and Zn) in dairy products. 2017; 5 (3):49-57.

## 1. Introduction

Heavy metals are typically released into the environment from natural and anthropogenic activities. Since they are persistent in the environment, they can cause serious health and environmental effects. Some heavy metals like Cu, Fe, and Zn are essential in maintaining proper metabolic activity in living organisms; while, others like As, Cd, Hg, and Pb are non-essential, and they have no important role in biological systems. At the same time, they can cause toxicity to living organisms at high contents (1-5). According to, dairy products, such as milk, are good sources of fats, proteins, macro and micro elements (Ca, K, P, Cu, Fe, Zn, and Se), and vitamins, therefore, they are known as important components of human diets, and are widely consumed by children and adults, and especially elderly people around the World (3, 6). Nowadays, entering toxic metals to milk and other dairy products via foodstuff, water, as well as manufacturing and packaging processes are serious concerns due to the fact that they can cause adverse health effects to humans (3). Milk and dairy products also become contaminated with heavy metals (2, 7). Cadmium, as a non-essential and very toxic element for human health, accumulates principally in the kidneys and liver organs (8, 9). Copper, on the other hand, is an essential element, but at high amounts, it is very toxic and causes adverse health problems. A maximum intake limit of Cu was set to be at 30 mg/day (10, 11). Lead is also known as one most commonly distributed environmental metal poison, the poisoning of which is considered as the most common environmental health hazard (12, 13), whereas Zinc is one of the important elements for normal growth and development in the human body (14, 15). Health risk

assessment as part of risk analysis process includes some steps, such as hazard identification, dose-response analysis, exposure assessment, and risk characterization, the results of which are quantitative or qualitative explanations of the likelihood of harm associated with exposure to synthetic chemicals. In this regard, the assessment of human health risk requires identification, collection, and integration of information on the health hazards of chemicals, exposure of human to toxins in polluted environmental media, and relationships between exposure duration, dose, and adverse health effects (16). In the current study, for the purpose of safe consumption of dairy products, the presence of trace and toxic heavy metals in the mentioned products and the associated health risks were studied. Since, there has so far been no health risk assessment of the existence of heavy metals in dairy products in Iran, the present study was conducted to determine the potential health risk of Cd, Cu, Pb and Zn concentrations of some popular brands of cheese and butter which were marketed in Hamedan City.

## 2. Material and Methods

In the current study, for analyzing the content of heavy metals (Cd and Pb) and tracing the elements (Cu and Zn) in dairy products consumed in Hamedan City, a total of 16 pasteurized butter products from four major brands and 16 pasteurized cheese products from four major brands were collected from dairy shops in 2016. The samples were then immediately transported to the laboratory in a cooler with ice packs, and were stored at -20 °C until analysis. Dairy product samples (2 g) were digested with the mixture of HNO<sub>3</sub> and HClO<sub>4</sub> (4:1 v/v) and heated at 70 °C for 2 hours until a transparent solution was

obtained (17-19). After digestion, the samples were filtered through Whatman filter paper No. 42, and diluted to a suitable concentration. Three blank samples, substituted by de-ionized distilled water, were run simultaneously with each batch of digestion (18). Working standard solutions of Cd, Cu, Pb, and Zn were also prepared by dilution of certified standard solutions to desired content. The heavy metals and trace elements content in the digested samples were then measured using inductively coupled plasma-optical emission spectrometry (710-ES Varian, Australia) at wavelengths (nm) of 226.50 for Cd, 324.75 for Cu, 196.03 for Pb and 206.20 for Zn with three replications.

The statistical analysis of the collected data were performed using SPSS version 19.0 statistical package consisted of a first Shapiro-Wilk test for normality, followed by one-way ANOVA, which was conducted to examine the statistical significance of differences in the mean concentration of Cd, Cu, Pb, and Zn analyzed in dairy samples. Also, the concentration of the analyzed metals in butter and cheese samples was compared through independent sample t-test. Finally, to observe if there was a correlation between the metals in the different dairy samples, the 2-tailed test of Pearson correlation was performed.

The daily intake of metals depends on both the metal content in food and the daily food consumption. In addition, the body weight of a human being can influence the tolerance of contaminants. The EDI ( $\text{mg kg}^{-1} \text{ day}^{-1}$ ) of metals was computed according to Equation 1 (18, 20).

$$EDI = \frac{C_{metal} \times W_{food}}{BW} \quad (1)$$

, here  $C_{metal}$  represents the content of heavy metals in contaminated foods ( $\text{mg kg}^{-1}$ ),  $W_{food}$  indicates that the daily average consumption of food (g per day) and  $BW$  represent the body weight (70000 g for adults and 15000 g for children) (21-23). Hence, the average daily consumption of dairy products in Iran was considered to be 139 g (24).

The Target Hazard Quotient for the local inhabitants via the consumption of contaminated dairy products was evaluated based on the food chain and the reference oral dose (RfD) for each metal (0.001, 0.04, 0.0035, and  $0.30 \text{ mg kg}^{-1} \text{ day}^{-1}$  for Cd, Cu, Pb, and Zn, respectively) (25-28). In this regard, the  $THQ < 1$  meant that the exposed population was assumed to be safe (18, 29). The THQ of metals was calculated by Equation 2 (18).

$$THQ = \frac{EDI}{RfD} \quad (2)$$

The total THQ (TTHQ) of heavy metals for the dairy products was also calculated according to Equation 3:

$$TTHQ = THQ (\text{toxicant 1}) + THQ (\text{toxicant 3}) + \dots + THQ (\text{toxicant } n) \quad (3)$$

### 3. Results

The concentrations of Cu, Cd, Pb and Zn in the analyzed butter and cheese samples are presented in Table 1. Among the analyzed butter samples, Cd was detected in a range of  $0.60$  to  $0.90 \mu\text{g kg}^{-1}$ , while Cu was in a range of  $4.70$  to  $8.70 \mu\text{g kg}^{-1}$ , and Pb was observed to be in a range of  $14.70$  to  $38.00 \mu\text{g kg}^{-1}$ . Zn was also found to be in the range of  $123.00$  to  $142.70 \mu\text{g kg}^{-1}$ . Also, among the analyzed cheese samples, Cd was detected in a range of  $0.60$  to  $0.80 \mu\text{g kg}^{-1}$ , Cu was in a range of  $17.00$  to  $99.70 \mu\text{g kg}^{-1}$ , Pb was in a range of

10.00 to 15.00  $\mu\text{g kg}^{-1}$ , and Zn was in a range of 189.30 to 212.00  $\mu\text{g kg}^{-1}$ .

Comparing the heavy metal concentrations in the studied dairy products with the maximum permissible limits ( $\mu\text{g kg}^{-1}$ ) (2.60, 10.0, 20.0, and 328.0 for Cd, Cu, Pb, and Zn, respectively) established by Codex Alimentarius Commission and International Dairy Federation(18, 30), showed that only the mean content of Cu in cheese samples was higher than MPL.

The results presented in Table 2 also indicated that the THQ values in adults and children via the consumption of both butter and cheese were lower than 1 (safe limits).

The Pearson's correlations analyses were then performed between metal concentrations in

dairy product samples to understand the relationships between them.

The results showed that in butter samples, there were found positive correlations between Cu and Zn ( $r = 0.738, P < 0.01$ ), and negative correlations between Pb and Zn ( $r = -0.610, P < 0.05$ ). While, in cheese samples, there were positive correlations found between Cu and Zn ( $r = 0.762, P < 0.01$ ) and also between Pb and Zn ( $r = 0.870, P < 0.01$ ).

At the same time, the results of independent t-test showed that there were significant differences ( $p < 0.05$ ) in the contents of Cu, Pb, and Zn between butter and cheese samples. While, the content levels of Cd did not differ significantly ( $p > 0.05$ ) between the samples of dairy products.

**Table1.** Heavy metals and trace elements concentration (mean  $\pm$  SD) of the butter and cheese samples ( $\mu\text{g kg}^{-1}$ )

Brand	Metal Concentration			
	Cd	Cu	Pb	Zn
<b>Butter</b>				
1	0.90 $\pm$ 0.12a*	5.30 $\pm$ 1.50a	38.00 $\pm$ 1.73c	123.00 $\pm$ 0.00a
2	0.90 $\pm$ 0.15a	6.30 $\pm$ 0.50b	18.30 $\pm$ 0.58b	135.00 $\pm$ 4.00b
3	0.90 $\pm$ 0.23a	4.70 $\pm$ 1.50a	16.00 $\pm$ 1.00a	124.70 $\pm$ 3.51a
4	0.60 $\pm$ 0.17a	8.70 $\pm$ 1.50c	14.70 $\pm$ 0.58a	142.70 $\pm$ 1.53c
Min.	0.90	4.70	14.70	123.0
Max.	0.60	8.70	38.0	142.70
Mean	0.83	6.25	21.75	131.35
S.D.	0.15	1.76	10.94	9.24
<b>Cheese</b>				
1	0.80 $\pm$ 0.25a	17.00 $\pm$ 2.00a	11.70 $\pm$ 0.58b	189.30 $\pm$ 5.13a
2	0.60 $\pm$ 0.10a	18.30 $\pm$ 1.53a	14.70 $\pm$ 0.58c	201.70 $\pm$ 2.08b
3	0.60 $\pm$ 0.15a	99.70 $\pm$ 8.39b	15.00 $\pm$ 0.00c	212.00 $\pm$ 5.30c
4	0.80 $\pm$ 0.21a	22.70 $\pm$ 5.13a	10.00 $\pm$ 0.00a	189.30 $\pm$ 1.15a
Min.	0.60	17.00	11.70	189.30
Max.	0.80	99.70	15.0	212.00
Mean	0.70	39.43	12.85	198.08
S.D.	0.11	40.26	2.41	10.97

\* The letters (a, b, and c) as presented in Table 1, represent the statistical differences (statistical grouping) among different brands of dairy products based on the mean concentration of metals according to Duncan multiple range test ( $p = 0.05$ )

Before the intervention, the mean of adherence to exercise programs, drug treatment, and diabetic diet were calculated to be 15.53, 23.92, and 48.20, respectively, in Podcast group, while the mean of adherence to the same factors in Pamphlet group were equal to 14.48, 24.04, and 46.54, respectively. The independent t-test showed no significant difference between adherence to exercise programs, drug treatment, and diabetic diet in aural training and pamphlets groups before the intervention (P = 0.265, P = 0.936, and P=0.523). After the intervention, the mean of adherence to exercise programs, drug treatment and diabetic diet increased as follows: Podcast group (20.00, 23.95 and

57.12); Pamphlet group (21.26, 25.40 and 55.59).

However, the results of independent t-test showed no significant difference between the adherence rate to exercise programs, drug treatment, and diabetic diet in Podcast and Pamphlet groups (P = 0.371, P= 0.319 and P = 0.378), as shown in Table 2.

**Table 2.** Estimated Daily Intake of metals (EDI, mg kg<sup>-1</sup> day<sup>-1</sup>) and Target Hazard Quotient (THQ) for individual heavy metal caused by butter and cheese

	Cd	Cu	Pb	Zn
<b>Butter</b>				
Adults				
EDI	1.65E-06	1.24E-05	4.32E-05	2.61E-04
THQ	1.65E-03	3.10E-04	1.23E-02	8.70E-04
Children				
EDI	7.69E-06	5.79E-05	2.01E-04	1.22E-03
THQ	7.69E-03	1.45E-03	5.76E-02	4.06E-03
<b>Cheese</b>				
Adults				
EDI	1.39E-06	7.83E-05	2.55E-05	3.95E-04
THQ	1.39E-03	1.96E-03	7.29E-03	1.32E-03
Children				
EDI	6.49E-06	3.65E-04	1.19E-04	1.84E-03
THQ	6.49E-03	9.13E-03	3.40E-02	6.12E-03

**4. Discussion**

Cadmium is a non-essential trace element that gradually accumulates inside human body, and especially in kidneys. Thus, in people affected by Cd poisoning, the occurrence of kidney stones is increased. The toxicity of Cd may lead to acute pulmonary odema, acute gastroenteritis, hypertension, and may also

cause a case called Itai-Itai disease (31). Natural or anthropogenic origins, such as atmospheric deposition in soils, and chemical fertilizers are the major resources of the presence of Cd in milk and dairy products (18). In the present study, the Cd levels in butter and cheese samples were in the ranges of 0.60-0.90 µg kg<sup>-1</sup>, and 0.60-0.80 µg kg<sup>-1</sup>

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with an average concentration of  $0.83 \pm 0.15 \mu\text{g kg}^{-1}$  and  $0.70 \pm 0.11 \mu\text{g kg}^{-1}$ , respectively. These findings come against with the level obtained by Abdulkhaliq et al. for white cheese consumed in West Bank, Palestine ( $29.78 \pm 19.60 \mu\text{g kg}^{-1}$ ) (4), Meshref et al. for butter ( $57.0 \pm 5.0 \mu\text{g kg}^{-1}$ ), kareish cheese ( $90.0 \pm 9.0 \mu\text{g kg}^{-1}$ ) consumed in Egypt (18), and Al-Ashmawy et al. for kareish cheese ( $87.0 \mu\text{g kg}^{-1}$ ) (20). Ayar et al. reported that the mean content of Cd for butter and white cheese consumed in Turkey were 15.0 and  $12.0 \mu\text{g kg}^{-1}$ , respectively (2). On the contrary, higher levels of Cd contamination in kinds of cheese and butter consumed in Egypt were reported 10.0-1120 and  $439 \mu\text{g kg}^{-1}$ , respectively (31, 32). Copper as an essential trace element is necessary for the integrity of cardiovascular system, adequate growth, neuronendocrine function, elasticity of the lungs, and Fe metabolism. It is also mentioned in the literature review that milk and dairy products are considered a very poor source of Cu; however, except in conditions with severe malnutrition, Cu deficiency is uncommon (18). The results of the current study showed that Cu concentrations in butter and cheese samples were found in the ranges of  $4.70\text{-}8.70 \mu\text{g kg}^{-1}$  and  $17.0\text{-}99.7 \mu\text{g kg}^{-1}$  with an average content ( $\mu\text{g kg}^{-1}$ ) of  $6.25 \pm 1.76$  and  $39.43 \pm 40.26$ , respectively. This reported Cu level was much lower than those observed in kinds of cheese consumed in Egypt ( $90.0\text{-}1030.0 \mu\text{g kg}^{-1}$ ) (31),  $2.0\text{-}530.0 \mu\text{g kg}^{-1}$  for Kareish cheese marketed in Egypt (18), and  $610\text{-}1230 \mu\text{g kg}^{-1}$  for white cheese consumed in West Bank, Palestine (4). Regarding Cu ( $1500\text{-}3300 \mu\text{g kg}^{-1}$ ) for kareish cheese, a higher level was also reported by Deeb (33). On the other hand, Meshref et al. and Enb et al. reported that the mean levels of Cu for butter were 600.0 and  $1472 \mu\text{g kg}^{-1}$ ,

respectively (18, 32). Also, the mean levels of Cu for butter consumed in Brazil and Egypt were determined to be  $32.0 \pm 12.0 \mu\text{g kg}^{-1}$  and  $907 \mu\text{g kg}^{-1}$ , respectively (32, 34).

Due to its low rate of elimination, lead has a cumulative and severe toxic effect. The major resources for the presence of Pb in milk and dairy products may be due to environmental sources, such as waste disposal, atmospheric deposition, urban effluent, and vehicle exhausts (18). The important symptoms of Pb toxicity include ulcerative stomatitis, decreases in sexual drive, abnormalities in sperm number, a blue gingival, unpotance, and sterility. Also, women exposed to Pb poisoning may suffer from menstrual disorders with spontaneous abortion and abnormal ovarian cycle (31). The obtained results of the current study showed that the mean concentrations of Pb in butter and cheese samples were found in the ranges of  $14.70\text{-}38.0 \mu\text{g kg}^{-1}$  and  $10.0\text{-}15.0 \mu\text{g kg}^{-1}$ , with an average content ( $\mu\text{g kg}^{-1}$ ) of  $21.75 \pm 10.94$ , and  $12.85 \pm 2.41$ , respectively. Lead levels in the literature have also been reported in the mean of  $80.0 \pm 16.0 \mu\text{g kg}^{-1}$  in the butter consumed in Brazil (34),  $430.0 \pm 29.0 \mu\text{g kg}^{-1}$  and  $490.0 \pm 21.0 \mu\text{g kg}^{-1}$  for Kareish cheese and butter marketed in Egypt (18),  $224 \mu\text{g kg}^{-1}$  for butter marketed in Egypt (32), and in the range of  $80\text{-}1480 \mu\text{g kg}^{-1}$  for the Kareish cheese consumed in Egypt (31). On the other hand, the Pb levels of Italian fresh cheese were recorded to be  $470.0 \mu\text{g kg}^{-1}$  (7). In one other study, Ayar et al. reported that Pb contents in butter and white cheese were  $116.0 \mu\text{g kg}^{-1}$  and  $920.0 \mu\text{g kg}^{-1}$ , respectively (2). Zinc is necessary for the functional performance of the immune system and physiological processes, and it may also be involved in the activity and the structure of about 300 enzymes responsible for protein

synthesis and nucleic acid, cellular differentiation and replication, sexual maturation, and insulin secretion. Also, it has been proved that chronic Zn exposure resulted in leucopenia, anemia, diarrhea, and gastrointestinal diseases (18, 35). It should be noted that Zinc contents in butter and cheese samples were found in the ranges of 123.00-142.70  $\mu\text{g kg}^{-1}$ , and 189.30-212.00  $\mu\text{g kg}^{-1}$ , with an average content of  $131.35 \pm 9.24 \mu\text{g kg}^{-1}$ , and  $198.08 \pm 10.97 \mu\text{g kg}^{-1}$ , respectively. In this regard, Fahmy and Abdel-Fattah (36) reported that Zn content in butter samples was  $86.0 \mu\text{g kg}^{-1}$ , which was lower than those obtained in the current study. Also, these values were lower than the levels obtained by Leggli et al. (34), Meshref et al. (18), and Enb et al. (32) who determined the mean levels of Zn ( $\mu\text{g kg}^{-1}$ ) 1360.0, 5980.0, and 19086 for butter, respectively. Inversely, higher contents of Zn in cheese (33660-63410  $\mu\text{g kg}^{-1}$ ) were reported by Maas et al. (37). Regarding kareish cheese, higher levels of Zn equal to 3402-17570  $\mu\text{g kg}^{-1}$  and 24900-35900  $\mu\text{g kg}^{-1}$  were reported by Meshref et al. (18) and Deeb (33), respectively.

The target hazard quotient has also been recognized as a useful parameter for the assessment of risks associated with the consumption of metal contaminated food (29). According to the results of the present study, the Total THQ values (TTHQ) of heavy metals for adults and children through the consumption of butter were found to be  $1.51\text{E-}02$  and  $7.10\text{E-}02$ , respectively, which were below the safe limit ( $\text{TTHQ} < 1$ ) for both age groups. Also, the TTHQ values for adults and children through the consumption of cheese were documented to be  $1.20\text{E-}02$  and  $5.57\text{E-}02$ , respectively, which were below the safe limit ( $\text{TTHQ} < 1$ ) similar to butter samples for both groups of consumers. Thus,

it could be concluded that all consumers including adults and children have no potential health risk through consuming dairy products (butter and cheese) from the study area. Similarly, Meshref et al. (18) reported that the THQ for daily exposure of Cd, Cu, Pb and Zn through the consumption of Kareish cheese and butter marketed in Egypt were below the safe limit ( $\text{THQ} < 1$ ).

### Conflict of Interests

The authors have no conflict of interest.

### Acknowledgements

The authors are grateful to the Hamedan Branch, Islamic Azad University for providing facilities to conduct and accomplish this study.

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