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Pasteurized Milk Quality Evaluation and Health Risk Assessment of Heavy Metals Using Monte Carlo Simulation and Sensitivity Analysis in Iran Market

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Abstract

Objective

Contamination of milk and its products with toxic heavy metals poses significant risks to food safety and human health. Understanding the extent of heavy metal contamination in milk is essential for assessing health risks. This study aimed to evaluate the quality of pasteurized milk and the health risks associated with heavy metal contamination in the Iranian market.

Methods

A total of 216 pasteurized milk samples were collected from nine prominent commercial brands available in Iranian markets over four seasons. The samples included two types of milk: low-fat (1% fat, n = 108) and full fat (3% fat, n = 108). Testing was conducted in three separate repetitions from winter 2021 through autumn 2022. After preparing the samples and applying the acid digestion method, the levels of heavy metals were measured using inductively coupled plasma mass spectrometry. A combined methods of Monte Carlo simulation and sensitivity analysis was used to estimate the daily intake of heavy metals and assess the associated health risks from consuming milk.

Results

The concentration of Cd was below the detection limit in all milk samples. The average concentration of Pb, Hg, and Cu was higher than the standards set by the World Health Organization (WHO) and Food and Agriculture Organization (FAO) in all seasons. There was no non-carcinogenic risk associated with pasteurized milk exposure in both children and adult groups (HI < 1). However, the carcinogenic risk assessment showed a moderate level of cancer risk through consuming pasteurized milk with an average probability risk distribution level of 1.41×10^{-4} and 6.7×10^{-5} in children and adults, respectively. Therefore, the carcinogenic risk in both groups was within the moderate range. The sensitivity test revealed that As, with an average concentration of 0.051 ± 0.026, is the most influential exposure element in developing carcinogenic risk, with contributions of 0.001 and 0.15 in adults and children.

Conclusion

In general, there was a potential risk of cancer-causing heavy metals through the consumption of pasteurized milk in the target samples. This means that the presence of heavy metals in pasteurized milk may have implications for consumer health because pasteurized milk samples are not safe in terms of the potential cancer risks. Therefore, continuous monitoring of milk and its products for heavy metal content is recommended to maintain food safety, as the results of such monitoring can be used for decision-making to promote human health.

Introduction

Food safety is a major challenge in maintaining the health of the world's population, as it controls and prevents diseases and protects against environmental pollution[1, 2]. According to the World Health Organization and the Food and Agriculture Organization, food safety is defined as "the control of and adherence to standards throughout the process of production, storage, and distribution of food to ensure that the food reaches the consumer in a healthy and beneficial manner"[1]. Using a healthy diet is crucial for preventing non-communicable diseases, such as obesity, diabetes, and cardiovascular diseases, as well as various types of cancer, which are major concerns for human health[3, 4]. Among the various healthy diets, the nutritional value of milk and its effects on health are undeniably significant. This valuable product, which is a complete source of nutrients, is rich in vitamins, minerals, and essential amino acids, and almost single-handedly meets the nutritional needs of all age groups. Therefore, it is rare to find a diet that does not include milk and dairy products [5–7]. According to the latest statistics provided by the World Health Organization and the Food and Agriculture Organization, the per capita consumption of milk in Iran is approximately 25.05 kg per year (0.068 kg per day) per household member. As classified by the Food and Agriculture Organization, the average milk consumption in the Iranian population is moderate compared with other communities[8].

Milk and its derivatives are significant components of the human diet, making them potential carriers of chemical pollutants through contaminated animals or their products. As soon as milk is secreted from the mammary gland, it can be easily exposed to various

pollutants (pesticides, pollutants, drugs, metals, and various environmental contaminants), which not only disrupts the processing of dairy products but can also be recognized as a health risk factor for consumers, as it severely damages public health in the long run. These harmful compounds, all of which result from rapid population growth and industrialization, will eventually manifest in other food products. The current reports confirm this fact and predict it will ultimately affect human life on Earth [9]. Two main sources, including natural and human activities, can cause contamination of pasteurized milk [10, 11]. These factors lead to air and feed pollution, resulting in the inhalation of contaminated air and the feeding of livestock, especially dairy cattle, with feed and water containing heavy metal residues. This leads to the uptake of these elements by the animals, which can be stored in their edible tissues, excreted through milk, and transferred to raw milk—the most important raw material of pasteurized milk [12, 13]. Additionally, during processing, contamination with metals may occur at various stages, including equipment, devices, processing operations, and the use of glazes and resins in machinery—particularly in equipment made of copper, lead, and cadmium, which are sources of contamination. Types of paper, thin cardboard used for packaging, and paints in plastic containers can also contaminate pasteurized milk [14, 15].

Arsenic and heavy metals are more toxic chemical pollutants in body tissues than other harmful substances due to their resistance to react with organic and living ligands [16, 17]. These compounds have various properties, such as toxicity, mutagenicity, and carcinogenicity, and even at very low concentrations, they pose a serious threat to human health[18, 19]. Heavy metals generally refer to a group of metals with high atomic weights and densities that typically exhibit metallic properties such as conductivity and malleability[20, 21]. Although arsenic is often grouped with heavy metals in many contexts due to its toxicological properties, it is technically a metalloid. It exhibits both metallic and non-metallic properties. Arsenic is known for its significant toxicity; exposure can lead to serious health issues, including cancer and cardiovascular diseases. Its toxicity depends on its chemical form, as inorganic arsenic is more toxic than organic forms. Both arsenic and heavy metals pose substantial health risks, but their chemical properties, sources, and environmental behaviors differ[22, 23]. Understanding these differences is essential for accurately assessing health risks and implementing effective safety measures [7, 24]. The International Agency for Research on Cancer has classified cadmium and lead as group 1 and 2 carcinogens, respectively[3]. Furthermore, because of their stability in nature, high heat resistance, biological accumulation in living organisms, and limited biodegradability, heavy metals can accumulate biologically in living organisms and increase their levels in the food chain. The consumption of food contaminated with heavy metals is a major source of entrance into the body and their gradual accumulation [7, 24]. Exposure to heavy metals in milk and dairy products poses serious threats to human health. This concern is especially pressing given the rising incidences of cancer, which suggest that heavy metal contamination may be a contributing factor[25, 26]. Since, presence of heavy metals in dairy products is critical from a public health perspective, highlighting the need for stricter monitoring and health risk assessments by food regulatory authorities.

It is imperative to assess the levels of heavy metals and arsenic contamination in pasteurized milk. Most existing studies have primarily concentrated on the heavy metal concentrations in raw milk, while comprehensive investigations into the quality of commercial milk brands available in the market are notably limited. Since milk constitutes a significant component of the human diet, research into associated health risks—including potential carcinogenic effects—is essential. These risks may vary between children and adults across different seasons, serving as both a direct indicator of the health status of milk and dairy products and an indirect indicator of the contamination levels in raw milk sourced from dairy units. Conducting thorough research to determine the concentrations of heavy metals and arsenic, as well as their daily absorption rates and health implications, is crucial for enhancing food security and informing the development of health programs. Furthermore, this research can contribute to improvements in nutritional and health indicators. Given the absence of a comprehensive assessment of heavy metal levels in milk within the Iranian market and the potential for contamination, this study aims to investigate the presence of heavy and toxic metals in milk products available in Iran.

Therefore, the primary objectives were as follow: a) to measure the levels of heavy metals (iron, aluminum, nickel, copper, lead, zinc, mercury, cadmium, cobalt, chromium, and arsenic) found in pasteurized milk available on the Iranian market; b) assess the daily intake (EDI), and risk indices, which include hazard quotient (THQ), hazard index (HI), and carcinogenic risk (CR) associated with the consumption of toxic heavy metals in pasteurized milk for two age groups: children and adults, and c) To compare the concentrations of these metallic elements in the samples against both domestic and international safety standards.

Materials and Methods

Sample Collection

The sample size for this study was determined using a factorial design, accounting for the number of seasons, nine commercial brands, milk types, and the number of test repetitions. A total of 216 pasteurized milk samples were collected from nine prominent commercial

brands available in Iranian markets across four seasons: winter, spring, summer, and autumn. The samples consisted of two types of milk—low-fat (1% fat, n = 108) and full-fat (3% fat, n = 108). All selected brands are widely recognized dairy producers that distribute their products throughout all provinces of Iran.To evaluate health and carcinogenic risks associated with ingestion, three testing repetitions were conducted from winter 2021 through autumn 2022. Convenience sampling was utilized, with all samples sourced exclusively from the Iranian market.

Chemicals and Reagents

All acids, reagents, and standard solutions (stock standard, internal standard, and multi-element standard solutions) were purchased from Merck, Germany. To determine the concentrations of Fe, Al, Ni, Cu, Pb, Zn, Hg, Cd, Co, Cr, and As, an inductively coupled plasma-optical emission spectrometer (Spectro Arcoes – 76004555, Germany) was used. All concentrations of arsenic and other elements were reported in milligrams per kilogram. All analyses were done at the Central Laboratory of Ferdowsi University. The detection limit of the inductively coupled plasma-optical emission spectrometer for different elements and the wavelength and recovery rate of the instrument for measuring heavy metals are provided in **(Table S1).**

Sample Preparation

Sample preparation for heavy metal analysis was done using an acid digestion method. Initially, 2 mL of each pasteurized milk sample was added to plastic screw-capped containers (Falcon). Then, 3 mL of concentrated nitric acid (HNO3) (Suprapur®, Merck, Kenilworth, NJ, USA), and 3 mL of hydrochloric acid (HCL) were added to 2 mL of the sample. Indirect heat was then applied in a water bath, and after the initial vapors were removed, the container was sealed, and the samples were placed under a fume hood at room temperature for 24 h. A specific amount of oxygen-free water was then slowly added to each sample. The containers were then heated in a water bath at 90°C for 2 h to complete the digestion process and to obtain a clear liquid. After observing the sediment, the samples were filtered through ashless filter paper. This resulted in a volume of 25 mL in a volumetric flask, which was then shaken and injected into the inductively coupled plasma-optical emission spectrometer[27].

Instrumental Analysis and Method Vaidation

The glassware and plastic bottles used during the analysis were washed in a diluted nitric acid (HNO3) solution for 24 hours, then rinsed with deionized water. The bottles were subsequently dried at room temperature and sterilized. To ensure the overall quality of all heavy metal samples, three repetitions for each element were performed, and calibration curves of the device were plotted using three points. The standard solutions with concentrations of 0.001 ppm, 0.1 ppm, and 10 ppm were used for all heavy metals except mercury (Hg). Heavy metal concentrations were measured using an inductively coupled plasma-optical emission spectrometer (Spectro Arcos – 76004555, Germany), equipped with an air-acetylene flame atomizer and computer Win software, which analyzed all heavy metals. The wavelengths for Al, As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb, and Zn were 396.15, 189.04, 214.43, 228.6, 267.71, 327.39, 259.94, 184.95, 231.6, 220.35, and 213.85 nm, respectively.

Certified Reference Materials (CRMs) with known element concentrations were used as essential tools to ensure the accuracy of heavy metal measurement results via inductively coupled plasma spectrometry. After every set of ten samples, the recovery rate was checked to assess analysis accuracy. Recovery rates for each element were within acceptable ranges (80–120%), as shown in (Table S1). The limits of detection (LODs) for Al, As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb, and Zn in milk were 0.001, 0.014, 0.003, 0.001, 0.015, 0.20, 0.0099, 0.00037, 0.001, 0.01, and 0.01 µg/kg, respectively. The limits of quantification (LOQs) for these elements in milk samples were 0.003, 0.034, 0.11, 0.004, 0.047, 0.71, 0.071, 0.0012, 0.0037, 0.034, and 0.034 mg/kg, respectively (Table S1).

Risk assessment

Daily intake and Non-carcinogenic risk

Risk assessment entails identifying, analyzing, and evaluating risks, with all associated parameters detailed in Table S2. The risk assessment process consists of four stages: hazard identification, dose-response assessment, exposure assessment, and risk characterization. To determine the non-carcinogenic risk of heavy metals, the health risk index (THQ) and non-carcinogenic risk index (HI) were calculated based on the guidelines of the United States Environmental Protection Agency. The hazard index represents the

potential exposure to a substance and the level at which no adverse effect is expected. A hazard index less than or equal to 1 indicates that the odds of adverse effects are minimal[28, 29]. To this end, the daily intake (EDI), hazard quotient (THQ), and hazard index (HI) were calculated using Equations 1, 2, and 3.

(1)

$$EDI == \frac{C \times\ IR \times\ EF \times\ ED}{BW\ \times\ AT}$$

(2)

$$THQ = \frac{EDI}{RFD}$$

(3)

$$HI = \text{▯ THQn}$$

In these equations, EDI represents the daily intake (mg/kg.day), C the concentration of heavy metals in pasteurized milk samples (mg/kg dry weight), IR the milk consumption rate (Kg/day), EF the exposure frequency (days/year), ED the duration of exposure (years), BW the average weight of consumers (kg), and AT the average time (years × days).

Carcinogenic Risk

The carcinogenic risks of arsenic, lead, and mercury were calculated using Eq. 4, where EDI represents the estimated daily intake (mg/kg.day) and OSF denotes the slope factor for carcinogenic elements (mg/kg.day). According to the USEPA, the slope factors for carcinogenicity for arsenic, mercury, and lead were 1.5, 0.0003, and 0.0085 mg/kg, respectively. According to USEPA guidelines, if the estimated carcinogenic risk values are less than 10 − 6, the carcinogenic risk is considered negligible or dismissible. If the values are within the range of 10 − 4 to 10 − 6, the carcinogenic risk is acceptable. However, if the value exceeds 10 − 4, carcinogenic risk is deemed unacceptable[29, 30].

(4) $CR = \text{EDI} \times\ \text{OSF}$

Statistical Data analysis

In the present study, SPSS version 16 was used for data analysis. The Kolmogorov–Smirnov test was used to assess the normality of data. To compare the concentrations of pollutants in pasteurized milk, one-tailed ANOVA tests were used for normal data, and the Kruskal-Wallis test was used for non-normal data. Data were described using descriptive statistics (frequency, mean indices, and standard deviation), and an analysis of variance was used to compare variable changes.

In conventional risk-assessment methods, the risk level is estimated and reported as a point estimate. The point estimate of risk provides limited information about the level of uncertainty and variability around the risk point[31]. The United States Environmental Protection Agency recommends the use of the Monte Carlo simulation method to obtain more accurate information about the risk level or risk ratio. Monte Carlo simulation is based on mathematical statistics and probability theory to achieve a model of uncertainty through random sampling and probability distribution of each input variable. Therefore, in this study, Monte Carlo simulation was used as a probabilistic method to reduce uncertainties. Crystal Ball 11.1.34190 was used with 10,000 iterations and a confidence interval of 97.5% for Monte Carlo modeling. Health risk criteria for consumers at a 97.5% confidence level, risk index, and carcinogenic risk were considered in the simulated model. Another optional application of Crystal Ball is sensitivity analysis, which was used in the present study to determine the weight of each variable in the risk assessment. Risk assessment parameters related to heavy metals in pasteurized milk are shown in (S2 Table)[32, 33].

Results

Evaluation of heavy metals in pasteurized milk in different seasons

In the present study, 216 pasteurized milk samples were evaluated for heavy metals. The average variations in heavy metal concentrations in pasteurized milk samples collected seasonally from February 2021 to November 2021 were assessed (Table 1).

Table 1
Heavy metals concentration in pasteurized milk reported in different seasons

seasons	Heavy metals levels *(mg/L)*										
	Al	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Ni**	**Pb**	**Zn**
Winter (n = 54)	0.19 ± 0.11	0.045 ± 0.01	ND	0.014 ± 0.002	0.11 ± 0.018	0.23 ± 0.095	0.21 ± 0.11	0.034 ± 0.006	0.037*	ND	3.79 ± 0.44
Spring (n = 54)	0.18 ± 0.19	0.07 ± 0.044	ND	0.028*	0.042 ± 0.029	0.13 ± 0.062	0.13 ± 0.18	ND	0.086 ± 0.01	0.024*	3.1 ± 0.19
Summer (n = 54)	0.02 ± 0.017	0.04 ± 0.025	ND	0.0017 ± 0.0006	0.047 ± 0.018	0.096 ± 0.043	0.12 ± 0.13	ND	ND	0.07 ± 0.06	2.92 ± 0.31
Autumn (n = 54)	0.16 ± 0.057	ND	ND	0.013 ± 0.0014	0.052 ± 0.031	0.46 ± 0.34	0.2 ± 0.16	ND	0.09 ± 0.042	ND	2.95 ± 0.66
Total (n = 216)	0.14 ± 0.094	0.051 ± 0.026	ND	0.014 ± 0.0014	0.065 ± 0.024	0.23 ± 0.13	0.16 ± 0.14	0.034 ± 0.006	0.071 ± 0.026	0.047 ± 0.066	3/.19 ± 0.4
Standard limit	-	0.15	-	-	0.3	0.2	0.37	0.01	0.2	0.02	5
[a]p-value	< 0.001	< 0.001	-	0.053	< 0.001	< 0.001	< 0.001	-	0.34	0.11	< 0.001

ND: not detected N: sample size, * Items that have been detected only once in the measurements; therefore, it is not possible to calculate the standard deviation, ±: standard deviation, a: Statistically significant difference between the concentration of heavy metals in pasteurized milk samples in different seasons based on the Kruskal-Wallis tests.

The results showed that out of the 11 metals, zinc was detected in 100% of samples (n = 216 samples), copper in 97.6% of samples (n = 210), chromium in 93.6% of samples (n = 202), iron in 84.1% of samples (n = 181), arsenic in 71.4% of samples (n = 154), aluminum in 47.6% of samples (n = 102), mercury in 23.8% of samples (n = 51), nickel in 16.7% of the samples (n = 36), lead in 8.7% of samples (n = 19), and cobalt in 8.7% of samples (n = 19). In addition, the level of cadmium was below the detection limit in all samples.

Among the studied metals, variations in Al, As, Fe, Cr, Cu, and Zn in pasteurized milk samples were significantly different at the four time intervals ($p < 0.05$), whereas no significant differences were observed for the other metals such as mercury, cobalt, lead, and nickel ($p > 0.05$). Examination of Al, Cr, Cu, Fe, and Zn levels indicated that the highest levels of these elements were observed in the cold season (winter and autumn), while the highest levels of As were observed during the warm season (spring) (Table 1).

The average concentrations of arsenic and all heavy metals, except for lead (Pb), copper (Cu), and mercury (Hg), remained below the thresholds established by the Iranian National Standard, as well as those set by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) throughout all seasons. However, the study found that copper levels exceeded the permitted limit of 0.2 mg/l during the autumn and winter seasons (Table 1). Among the heavy metals with low identification percentages, the average concentration of lead (Pb) was found to be 0.047 ± 0.066 mg/l in 19 samples, which is higher than the Iranian National Standard limit of 0.02 mg/l during all seasons. Similarly, the average concentrations of mercury (Hg) were above the WHO/FAO standard of 0.01 mg/l in 100% of the identified samples. Additionally, the iron content in eight samples (3.7% of all identified samples) also exceeded the standard limit of 0.37 mg/l (Fig. 1).

No significant variations ($P > 0.05$) were observed in the average concentrations of heavy metals across pasteurized milk samples from both low-fat (1% fat, n = 108) and full-fat (3% fat, n = 108) milk types (Table S3)(Fig S1). The average levels of heavy metals in pasteurized milk samples from nine different commercial brands available in the Iranian market are shown in (Table S4). The average variations in all metals in the nine commercial brands of pasteurized milk were greater than 0.05; therefore, there was no significant difference in the average level of these elements in the nine different commercial brands available in Iranian market.

Risk Assessment

Heavy Metals Daily Intake

Table 2 presents the descriptive statistics of the average daily absorption of heavy metals through the consumption of pasteurized milk. According to the results obtained for both groups (child and adult), the average daily absorption was as follows: Zn > Cu > Fe > Al > Ni > Cr > As > Pb > Hg > Co. Lead, copper, and iron absorbed the highest amounts, while mercury and cobalt absorbed the lowest amounts. Additionally, as the results showed, the average daily absorption of all elements in both research groups (child and adults) is below the permissible daily limit.

Table 2
Estimated Daily Intake (EDI) of heavy metals

Metals	Seasons								PTDI	MTDI
	Winter		Spring		Summer		Autumn			
child	adult	child	adult	child	adult	child	adult			
Al	$3.5\times10^{-4}\pm 2.06\times10^{-4}$	$1.65\times10^{-4}\pm 9.5\times10^{-5}$	$3.3\times10^{-4}\pm 3.45\times10^{-4}$	$1.6\times10^{-4}\pm 1.64\times10^{-4}$	$3.8\times10^{-5}\pm 3.13\times10^{-5}$	$1.8\times10^{-5}\pm 1.5\times10^{-5}$	$3.03\times10^{-4}\pm 1.03\times10^{-4}$	$1.4\times10^{-4}\pm 4.9\times10^{-5}$	0.14	-
As	$8.1\times10^{-5}\pm 1.96\times10^{-5}$	$3.9\times10^{-5}\pm 9.36\times10^{-6}$	$1.3\times10^{-4}\pm 8.04\times10^{-5}$	$6.03\times10^{-5}\pm 3.8\times10^{-5}$	$7.2\times10^{-5}\pm 4.5\times10^{-5}$	$3.4\times10^{-5}\pm 2.14\times10^{-5}$	ND	ND	0.0021	0.0018
Co	$2.6\times10^{-5}\pm 4.14\times10^{-6}$	$1.25\times10^{-5}\pm 1.97\times10^{-6}$	5.04×10^{-5}	2.4×10^{-5}*	$3.0\times10^{-6}\pm 1.04\times10^{-6}$	$1.4\times10^{-6}\pm 4.95\times10^{-7}$	$2.34\times10^{-5}\pm 2.5\times10^{-6}$	$1.1\times10^{-5}\pm 1.2\times10^{-6}$	0.5	0.3
Cr	$2.1\times10^{-4}\pm 3.3\times10^{-5}$	$1.02\times10^{-4}\pm 1.6\times10^{-5}$	$7.6\times10^{-5}\pm 5.16\times10^{-5}$	$2.8\times10^{-5}\pm 2.6\times10^{-5}$	$8.4\times10^{-5}\pm 3.2\times10^{-5}$	$4.01\times10^{-5}\pm 1.5\times10^{-5}$	$9.4\times10^{-5}\pm 5.55\times10^{-5}$	$4.5\times10^{-5}\pm 2.6\times10^{-5}$	0.003	0.0028
Cu	$4.2\times10^{-4}\pm 1.7\times10^{-4}$	$2.02\times10^{-4}\pm 8.1\times10^{-5}$	$2.4\times10^{-4}\pm 1.1\times10^{-4}$	$1.1\times10^{-4}\pm 5.3\times10^{-5}$	$1.7\times10^{-4}\pm 7.8\times10^{-5}$	$8.2\times10^{-5}\pm 3.7\times10^{-5}$	$8.4\times10^{-4}\pm 6./2\times10^{-4}$	$4.0\times10^{-4}\pm 1.3\times10^{-4}$	0.5	0.05
Fe	$3.76\times10^{-4}\pm 2.1\times10^{-4}$	$1.8\times10^{-4}\pm 8.1\times10^{-5}$	$2.36\times10^{-4}\pm 3.2\times10^{-4}$	$1.1\times10^{-4}\pm 1.5\times10^{-4}$	$2.3\times10^{-4}\pm 2.4\times10^{-5}$	$1.1\times10^{-4}\pm 1.14\times10^{-4}$	$3.66\times10^{-4}\pm 2.9\times10^{-4}$	$1.9\times10^{-4}\pm 36\times10^{-5}$	0.8	0.8
Hg	$6.2\times10^{-5}\pm1.1\times10^{-5}$	$2.9\times10^{-5}\pm5.3\times10^{-6}$	ND	ND	ND	ND	ND	ND	0.00057	-
Ni	6.66×10^{-5}*	3.2×10^{-5}*	$1.5\times10^{-4}\pm 1.8\times10^{-5}$	$7.4\times10^{-5}\pm 8.6\times10^{-6}$	ND	ND	$1.6\times10^{-4}\pm 7.6\times10^{-5}$	$7.8\times10^{-5}\pm 3.6\times10^{-5}$	0.005	0.043
Pb	ND	ND	4.3×10^{-5}	2.06×10^{-5}*	$1.3\times10^{-4}\pm 1.2\times10^{-4}$	$6.08\times10^{-5}\pm 5.6\times10^{-6}$	ND	ND	0.0035	-
Zn	$6.8\times10^{-3}\pm$	$3.25\times10^{-4}\pm$	$5.6\times10^{-3}\pm$	$2.6\times10^{-3}\pm$	$5.3\times10^{-3}\pm$	$2.5\times10^{-3}\pm$	$5.3\times10^{-3}\pm$	$2.5\times10^{-3}\pm$	1	0.3

PTDI: Provisional tolerable daily intake value, MTDI: Maximum tolerable daily intake, ND: not detected; ±: standard deviation, * Items that have been detected only once in the measurements, therefore, it is not possible to calculate the standard deviation

Metals	Seasons								PTDI	MTDI
	Winter		Spring		Summer		Autumn			
child	adult	child	adult	child	adult	child	adult			
	7.98×10^{-4}	3.8×10^{-4}	3.5×10^{-4}	1.6×10^{-4}	5.7×10^{-4}	2.7×10^{-4}	1.2×10^{-3}	5.7×10^{-4}		

PTDI: Provisional tolerable daily intake value, MTDI: Maximum tolerable daily intake, ND: not detected; ±: standard deviation, * Items that have been detected only once in the measurements, therefore, it is not possible to calculate the standard deviation

Non-carcinogenic Risk

In the present study, the non-carcinogenic health risk and hazard index (HI) of heavy metals associated with the consumption of pasteurized milk were calculated for two age groups: adults and children. The hazard index for the consumption of pasteurized milk in all seasons was below the risk threshold of 1 for both adults and children. Additionally, the hazard index had the highest value in spring and winter, and decreased in summer and autumn (Table 3).

PTDI values: Fe(0.8 mg/kg BW/day); Al(0.14 mg/kg BW/day); Ni(0.3 mg/kg BW/day); Cu(0.5 mg/kg BW/day); Pb(0.0035mg/kg BW/day); Zn(1 mg/kg BW/day); Hg(0.00057 mg/kg BW/day) (for inorganic mercury); Cd(0.0008 mg/kg BW/day); Co(0.005 to 0.04); Cr(0.05 to 0.2); As(0.0021 mg/kg BW/day); ND: not detected, ±: standard deviation, * Items that have been detected only once in the measurements, therefore, it is not possible to calculate the standard deviation.

Cancer Risk

In this study, the levels of cancer risk associated with heavy metals through the consumption of pasteurized milk were calculated for two age groups: adults and children. The average changes in cancer risk through the consumption of pasteurized milk for child and adults in all seasons of study was determined to be between 1.4×10^{-4} to 6.7×10^{-5}, indicating that the cancer risk in all seasons of study did not fall within a safe range ($CR \geq 1\times10^{-4}$). Additionally, the cancer risk showed that the spring season had the highest value, followed by the winter and summer seasons, which had the lowest levels (Table 4).

Table 4
Carcinogenic risk of heavy metals from pasteurized milk consumption in the two populations of child and adults

Seasons	Ages	As	Hg	Pb	TCR
winter	child	$1.2\times10^{-4}\pm2.95\times10^{-5}$	$1.86\times10^{-8}\pm3.35\times10^{-9}$	ND	$1.22\times10^{-4}\pm2.95\times10^{-5}$
	adult	$5.8\times10^{-5}\pm1.4\times10^{-5}$	$8.87\times10^{-9}\pm1.6\times10^{-9}$	ND	$5.8\times10^{-5}\pm1.4\times10^{-5}$
Spring	child	$1.9\times10^{-4}\pm1.2\times10^{-4}$	ND	3.67×10^{-7}*	$1.90\times10^{-4}\pm1.2\times10^{-4}$
	adult	$9.05\times10^{-5}\pm5.74\times10^{-5}$	ND	1.75×10^{-7}*	$9.06\times10^{-5}\pm5.74\times10^{-5}$
Summer	child	$1.08\times10^{-4}\pm6.74\times10^{-5}$	ND	$1.08\times10^{-6}\pm1.02\times10^{-6}$	$1.09\times10^{-4}\pm6.84\times10^{-5}$
	adult	$5.14\times10^{-5}\pm3.2\times10^{-5}$	ND	$5.17\times10^{-7}\pm4.84\times10^{-7}$	$5.19\times10^{-5}\pm3.26\times10^{-5}$
Autumn	child	ND	ND	ND	ND
	adult	ND	ND	ND	ND
Total	child	$1.40\times10^{-4}\pm7.25\times10^{-5}$	$1.86\times10^{-8}\pm3.35\times10^{-9}$	$7.26\times10^{-7}\pm1.02\times10^{-6}$	$1.41\times10^{-4}\pm7.35\times10^{-5}$
	adult	$6.66\times10^{-5}\pm3.45\times10^{-5}$	$8.87\times10^{-9}\pm1.6\times10^{-9}$	$3.46\times10^{-7}\pm4.84\times10^{-7}$	$6.70\times10^{-5}\pm3.50\times10^{-5}$

ND: not detected, ±: standard deviation, * Items that have been detected only once in the measurements, therefore, it is not possible to calculate the standard deviation

Uncertainty analysis

The carcinogenic risk and risk index of consuming pasteurized milk were evaluated using the appropriate confidence intervals (97.5%) through 10,000 iterations using Crystal Ball. In health risk assessment, all measures should be considered conservatively. Therefore, in this study, the 97.5 percentile was considered as the action threshold. As shown in (Fig. 2), with 97.5% confidence, the risk index for child lies within the range of 1.04×10^{-1} – 3.36×10^{-1}, and for adults, with 97.5% confidence, it lies within the range of 4.96×10^{-1} – 1.60×10^{-1}, which is lower than the permissible level set by the United States Environmental Protection Agency (HI > 1). Additionally, with 97.5% confidence, the carcinogenic risk of consuming pasteurized milk for children lies within the range of 3.20×10^{-4} – 5.54×10^{-5}, and for adults, with 97.5% confidence, it lies within the range of 1.53×10^{-4} – 2.63×10^{-5}, which falls within the average range suggested by the United States Environmental Protection Agency ($10^{-4}\times$ to $\times10^{-6}$).

Sensitivity analysis

Sensitivity analysis was used to determine the effect of various variables, such as metal concentration, consumption of pasteurized milk, duration of exposure, and body weight, on the risk index and carcinogenic risk associated with the consumption of pasteurized milk in the two populations of children and adults. Sensitivity analysis can determine the extent to which a variable affects the outcomes. As the results of the sensitivity analysis showed (Fig. 3), the health risk associated with consuming pasteurized milk in children and adults is influenced by arsenic concentration by 61.6%, and other parameters have an impact of less than 15%. The sensitivity analysis results, illustrated in Fig. 3, indicate that the carcinogenic risk associated with consuming pasteurized milk is significantly influenced by arsenic levels. For children and adults, the average arsenic concentration is 0.051 ± 0.026, which accounts for 89.1% and 88.9% of the risk, respectively. In contrast, other parameters have an impact of less than 15%.

Discussion

As environmental pollution increases and contaminants enter the food chain, it is crucial to examine the types of pollutants and the hygiene of food products. The current literature indicates a significant gap in comprehensive studies assessing levels of heavy metals and their health and carcinogenic risks in pasteurized milk from various commercial brands in the Iranian market. Most existing research has focused on a limited number of metals. Therefore, this study aims to investigate the presence of heavy metals in pasteurized milk and evaluate the associated human health risks linked to the consumption of these products from different commercial brands available in Iran.

The results of this study showed significant differences in the concentrations of some heavy metals present in pasteurized milk during different seasons (Al, As, Cr, Cu, Fe, and Zn). Notably, significant differences in the concentrations of metals (Al, Cr, Cu, Fe, and Zn) were evident in cold seasons (winter and autumn) compared with the other seasons, indicating higher concentrations in the cold seasons. This increase is likely due to heavy rainfall and the subsequent washing of various industrial wastes, leading to the entrance of these pollutants into groundwater and surface water, and consequently contaminating agricultural products through irrigation with polluted water. The accumulation of these environmental pollutants in dairy animals owing to the consumption of contaminated water and agricultural products is likely to increase[34]. Therefore, climatic changes in the cold season can be considered a probable cause of the higher levels of heavy metals [10, 11].

Previous studies conducted in cities of Gorgan, Mashhad, Tabriz, Tehran, Isfahan, and Ahvaz during the years 2013 and 2020 showed that seasonal variations led to changes in the concentrations of various elements. The cold seasons proved to have the highest concentrations of elements, which can be attributed to changes in weather and rainfall during these seasons, as proven by the present study too[12, 13]. The results of this study indicated that mercury was only detectable in winter, with levels exceeding the standards set by the WHO and FAO. One possible explanation for this is that during the winter season, livestock have reduced access to the open environment, which may lead to an increased reliance on fish meal in animal feed. Fish meal is a significant source of mercury contamination in milk[16]. During the warm seasons, the levels of arsenic (As) were found to be significantly different compared to other seasons. One possible reason could be the greater dietary diversity of animals, increased water consumption, and irrigation of fields with urban wastewater during the warm seasons. These factors may contribute to a rise in the concentration of As in soil, water, and plants [35, 36]. Furthermore, lead was found in pasteurized milk during the warm seasons (spring and summer). Possible reasons for the presence of Pb in pasteurized milk during the warm seasons could be attributed to increased water consumption during these seasons[37, 38]. In contaminated areas, some Pb in soil enters groundwater reserves. Additionally, because the primary storage location of lead in plants is the leaves, the increased consumption of leafy and fresh forage in the summer season could be another factor leading to the increased lead intake in the body and subsequently increased lead levels in milk [36, 39]. However, this difference was not statistically significant. Additionally, lead (Pb) was detected in pasteurized milk during the warmer months of spring and summer. The presence of Pb could be linked to the increased water consumption during these seasons. In contaminated areas, lead

from the soil can seep into groundwater reserves. Moreover, since the primary storage location for lead in plants is the leaves, the higher intake of leafy and fresh forage during the summer may also contribute to increased lead absorption in the body, resulting in elevated lead levels in milk. However, it is important to note that this difference in lead levels was not statistically significant.

The results showed that, of the 11 metals examined in the present study, the levels of Hg, Pb, and Cu exceeded the standards permission limits, whereas the levels of iron, chromium, arsenic, nickel, and zinc remained within the standard limits. However, the Cd levels in this study were lower than the detection limit of the instrument. The present findings showed that Pb has been identified as exceeding the standard limits during the warm seasons (summer and spring). This could be due to the excessive consumption of fresh forage and contaminated water, particularly in industrial areas. Lead is a common chemical pollutant emitted into the air and water as a result of industrial activities. Other influential factors include the natural presence of Pb as a component of the Earth's crust, which is exacerbated by industrial activities[34]. Industrial activities have surged, significantly increasing the deposition of heavy metals in the environment and contaminating food products. Several studies report that elevated levels of lead (Pb) in pasteurized milk may result from dairy cattle consuming water and forage sourced near highways, industrial zones with Pb smelting and extraction plants, or urban areas where crops are irrigated with municipal wastewater or fertilized with contaminated materials. Additionally, alkyl additives found in burned gasoline release lead into the atmosphere, leading to elevated lead concentrations in some plants growing alongside roads, as well as in soil and air [40, 41]. This contamination of air, water, soil, and plants with the toxic heavy metal results in the introduction of lead (Pb) into the food chain. Furthermore, lead can contaminate milk through the equipment, storage tanks, and machinery used during processing [11, 42].

Several studies conducted in Iran between 2014 and 2020 revealed that the concentration of lead in pasteurized milk and dairy products exceeds the permissible level [38, 42, 43]. This contamination can be attributed to the supply of raw milk from farms located near industrial areas, which affects the quality of water and feed for livestock [13, 44]. The lead concentrations reported in these studies ranged from 0.066 to 0.10 mg/l, exceeding the maximum allowable limit. Additionally, other studies focusing on raw milk samples from livestock units in Iran indicated lead concentrations varying from 0.013 to 2.72 mg/l[9, 37, 45–47].

In this study, Hg levels were detected only during the winter months and were found to exceed the limits set by the WHO and FAO. These results differ from those reported by Yaryar et al., Paclovic, and Ayer et al., whose findings indicated that mercury levels in milk samples were below the permissible limits[38, 45]. This increase may stem from Hg released during mining and coal combustion, which contributes to airborne mercury that contaminates surface runoff and wastewater treatment plants. Industrial effluents can significantly contaminate soil, particularly when used for irrigation [14, 15]. Additionally, Hg-containing pesticides exacerbate soil contamination due to mercury's high absorption rate. This poses risks to the food chain, notably for lactating animals consuming contaminated plants, leading to bioaccumulation[48]. The mercury levels in pasteurized milk are also influenced by fishmeal in animal feed, depending on the fish species and habitat. This underscores the complex link between environmental contamination and agricultural practices [14, 16, 37, 49].

In the present study, a higher concentration of copper in pasteurized milk than the standard range can occur for several reasons. One possible explanation is the use of copper alloys in certain equipment during the production, storage, and packaging processes of pasteurized milk [50, 51]. Copper, like other heavy metals, can enter the food chain through industrial activities and polluted water and soil [40, 41, 45]. Based on 13 studies conducted in Iran between 2013 and 2025 in the cities of Mashhad, Tabriz, Tehran, Isfahan, Ahvaz, Gorgan, Shahrood, Kerman, Sirjan, Arak, and Hamedan, most results indicated lower concentrations of lead, copper, and mercury in pasteurized milk samples consumed, which contradicts the present findings [5, 6, 52–56]. Studies conducted in Libya, Egypt, Pakistan, and Brazil between 2015 and 2020 showed that the levels of Pb, Cu, and Hg in pasteurized milk exceeded the standard range. This was attributed to the use of alkyl lead additives in burnt gasoline and the use of sewage for agricultural purposes, which may directly affect drinking water or accumulate lead in the soil, feed, and vegetables accessible to animals [7, 57–62]. They also reported the equipment used in the processing and packaging of pasteurized milk as a contaminating factor, similar to the results of the present study. They reported that although natural and human sources can lead to the entrance of heavy metals into milk, the main factor in the contamination of pasteurized milk occurs during processing in factories. This includes equipment and tools used in collecting and processing pasteurized milk, as well as packaging materials, such as colors used in plastic containers, which can contaminate pasteurized milk [7, 57–62].

Studies conducted in Iran on raw milk samples from Lorestan revealed that the average concentrations of Pb, Cd, Cu, and Zn were 142 ± 149 µ/l, 2720 ± 2790 µ/l, 18.5 ± 10.4 µ/l, and 674 ± 3072 µ/l, respectively[9]. These values significantly exceeded the WHO standards. In contrast, another study analyzing the concentrations of Pb and Cd in 100 raw milk samples from Shahrekord found levels of 0.060 mg/l and 0.002 mg/l, respectively, both within the permissible limits established by international standards [47, 55, 56]. The

contradictions in the results regarding heavy metals such as Pb, Hg, Cd and Cu concentrations in pasteurized milk may be attributed to various factors, including differences in sample sources, methodological techniques, environmental factors (pollution sources and regulatory differences), and temporal changes [9, 55]. However, these contradictions highlight the complexity of environmental health studies. Therefore, it is essential to consider the setting of each study to interpret the reasons behind these discrepancies effectively.

In this study, the levels of Fe, Cr, As, Ni, and Zn were within the standard range. However, studies have shown that the transfer of heavy metals from water and contaminated food to milk is low because, while feeding cattle with heavy metal-contaminated fodder, the majority of these metals are not directly transferred to the milk of dairy cows. In fact, the cow's body acts as a biological filter. A large portion of ingested metals are not absorbed, and the absorbed amounts are initially transferred to bones, liver, and kidneys and accumulate there. Then, a small number of metals are transferred from these tissues to milk. However, because of their high dispersion, heat resistance, chemical stability, and accumulation capacity in body tissues, the continued consumption of contaminated milk can lead to the accumulation of metals and can cause acute or chronic poisoning[63, 64]. As the present findings showed, it can be concluded that the presence of heavy metals in pasteurized milk available in the Iranian market may have health implications for consumers. Therefore, in this study, a risk assessment model was used to complete the calculations and obtain a comprehensive and accurate conclusion.

Health and carcinogenic risk assessments

Milk is considered an important component of human diet because of its immunological and nutritional properties. The significance of this dietary substance lies in the presence of vitamins, minerals, and proteins that are necessary for the health and growth of body. The importance of consuming milk and dairy products in a healthy diet, especially among vulnerable age groups in society such as children and the elderly needs to be highlighted. Therefore, determining the levels of heavy metals and assessing the health and carcinogenic risks associated with heavy metal pollution are of particular importance to ensure consumer health. Among the heavy metals of interest, Cd, As, Pb, and Hg are among the most dangerous that can cause adverse effects in humans and pose the risk of developing certain cancers with long-term exposure to these metals. These elements have spread widely in the environment either naturally or as a result of human activities, and they can enter the human food chain [65–67].

According to the U.S. Environmental Protection Agency, the expected number of years a person is expected to be exposed to a pollution source is 70, which can represent the average life expectancy of the general population[68]. Regarding the effects that manifest as cancer, all exposure to risk factors throughout one's life accumulate and can ultimately lead to the emergence of cancer at any time of life. This is because non-cancerous effects occur only during exposure. Therefore, the time taken for cancerous effects to emerge differs from the time taken for non-cancerous effects to emerge[69]. As the experiments showed, the EPA introduced a dose-response relationship for the effects of arsenic on human health, considering differences in age, gender, and individual sensitivities to arsenic exposure, and it does not introduce any threshold for arsenic-induced cancer[70].

As the results showed in, the estimated health risk index or risk for all age groups through the consumption of pasteurized milk in all seasons is less than 1. Therefore, there are no chances of the non-carcinogenic effects of heavy metals following the consumption of pasteurized milk.

Few studies have ever assessed the health risks of heavy metals in relation to pasteurized milk. Studies conducted in Gorgan, Tehran, Shahrood, and Hamedan during the years 2014–2020, which assessed the non-carcinogenic health risk in pasteurized milk, showed that the health risk for all measured metals was less than one[6, 13, 44, 71]. Furthermore, in studies conducted in Egypt and China in 2014 and 2022, respectively, the health risk of heavy metals was less than the threshold of one[7, 72], which is consistent with the present study.

In contrast, a study in Egypt in 2019 reported a risk index higher than 1, which is contrary to the results of the present study[61]. Based on the results presented in this study and comparing the levels of cancer risk with the maximum acceptable risk proposed by the U.S. Environmental Protection Agency, the results showed that the cancer risk in all samples of pasteurized milk lies within the moderate range ($1\times10-4$ to $1\times10-6$). The average changes in cancer risk due to the consumption of pasteurized milk in the child and adult populations were $1.4\times10-4$ to $6.7\times10-5$, respectively. This means approximately 1 in every 10,000 children and approximately 7 in every 100,000 adults are at risk of developing cancer through consuming pasteurized milk contaminated with heavy metals. Although the results of the present study showed that the levels of lead, mercury, and copper exceeded the standard limits, the sensitivity analysis results regarding the health risk and carcinogenicity show that arsenic in child and adults has the highest level of contamination. This is because arsenic is more toxic and has greater carcinogenic effects than lead and mercury. Therefore, the

reference dose for arsenic has been studied less than other heavy metals. Additionally, the cancer slope factor estimated for arsenic is higher than other metals, indicating its greater contribution to sensitivity analysis, while other metals have less than 15% impact[73]. Only Sharifi et al. evaluated the carcinogenic risk associated with lead in pasteurized milk samples, which reported acceptable levels, consistent with the present findings[6]. Therefore, further studies are needed to assess the carcinogenic risk of heavy metals in pasteurized milk samples in Iran.

Limitations

In assessing risk, other potential limitations cannot be measured, which may affect the credibility of estimated values. For example, 1) body weight and daily milk consumption were estimated according to the U.S. Environmental Protection Agency standard; 2) risk assessment based on the cancer slope factor index was only calculated for lead, arsenic, and mercury, as there is currently no specific cancer slope factor for other heavy metals; 3) the cancer slope factor is considered constant for all individuals, yet in reality, it can vary among individuals; 4) the risk assessment model was only based on heavy metal concentrations, but milk can also contain other chemical contaminants; and 5) this study focuses exclusively on milk as part of the daily food basket. Therefore, caution is necessary when interpreting the findings, as the actual risk level may be higher than the estimated values when considering the entire daily food intake. Additionally, one limitation is the inability to compare milk brands based on production location, due to a lack of comparative studies. Understanding how geographical factors may influence the safety and quality of milk products is essential. Future research should include a wider range of brands from various production sites for a more comprehensive assessment. Moreover, although cadmium (Cd) has been detected in other surveys in Iran, its concentration in this study was below the detection limit. This discrepancy may be due to the sensitivity of our detection methods or the actual Cd levels in the samples. The reasons for this variance are uncertain; therefore, further studies should expand the sample size and use more sensitive detection techniques to ensure accurate measurement of Cd concentrations.

Conclusion

The results showed that out of the 11 heavy metals studied, cadmium was undetectable in any of the samples, and 3 metals (lead, mercury, and copper) exceeded the national and international standards in pasteurized milk. Regarding the health risk assessment, the results showed that the health risk index through the consumption of pasteurized milk was lower than the threshold level 1 for adults and children. Additionally, the study showed that changes in cancer risk through the consumption of pasteurized milk for children and adults ranged from 4.1×10^{-6} to 7.6×10^{-7}, placing the risk in the moderate range for all the samples of interest. There are potential limitations in the risk assessment that may affect the estimated values to some extent; therefore, the actual risk level in the consumed pasteurized milk in Iran may be higher than the estimated values of this study. With a continuous increase in pollutants, there is a possibility of increased risk over time. Therefore, continuous monitoring of pasteurized milk for heavy metal content and chemical residues is recommended to ensure food safety, as the results of such monitoring can be used as a basis for decision-making to promote human health and safety.

Declarations

Competing interests

The author(s) report no conflicts of interest in this work.

Data Availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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Author Contributions

The authors' contributions are as follows: B. Tavakoly Sany contributed to methodology; Z. Alinezhad and B. Tavakoly Sany contributed to resources; Z. Alinezhad contributed to Data collection, Sample collection and writing—original draft preparation; M. Hashemi contributed to writing—review and editing; M. Hashemi and M. boskabadi contributed to visualization; Z. Alinezhad and B. Tavakoly Sany contributed to table editing; B. Tavakoly Sany and S. Khanzadi contributed to supervision; M. boskabadi contributed to Formal Analysis. All authors gave final approval of the final version and are accountable for this work.

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Table 3

Table 3 is available in the Supplementary Files section.

Figures

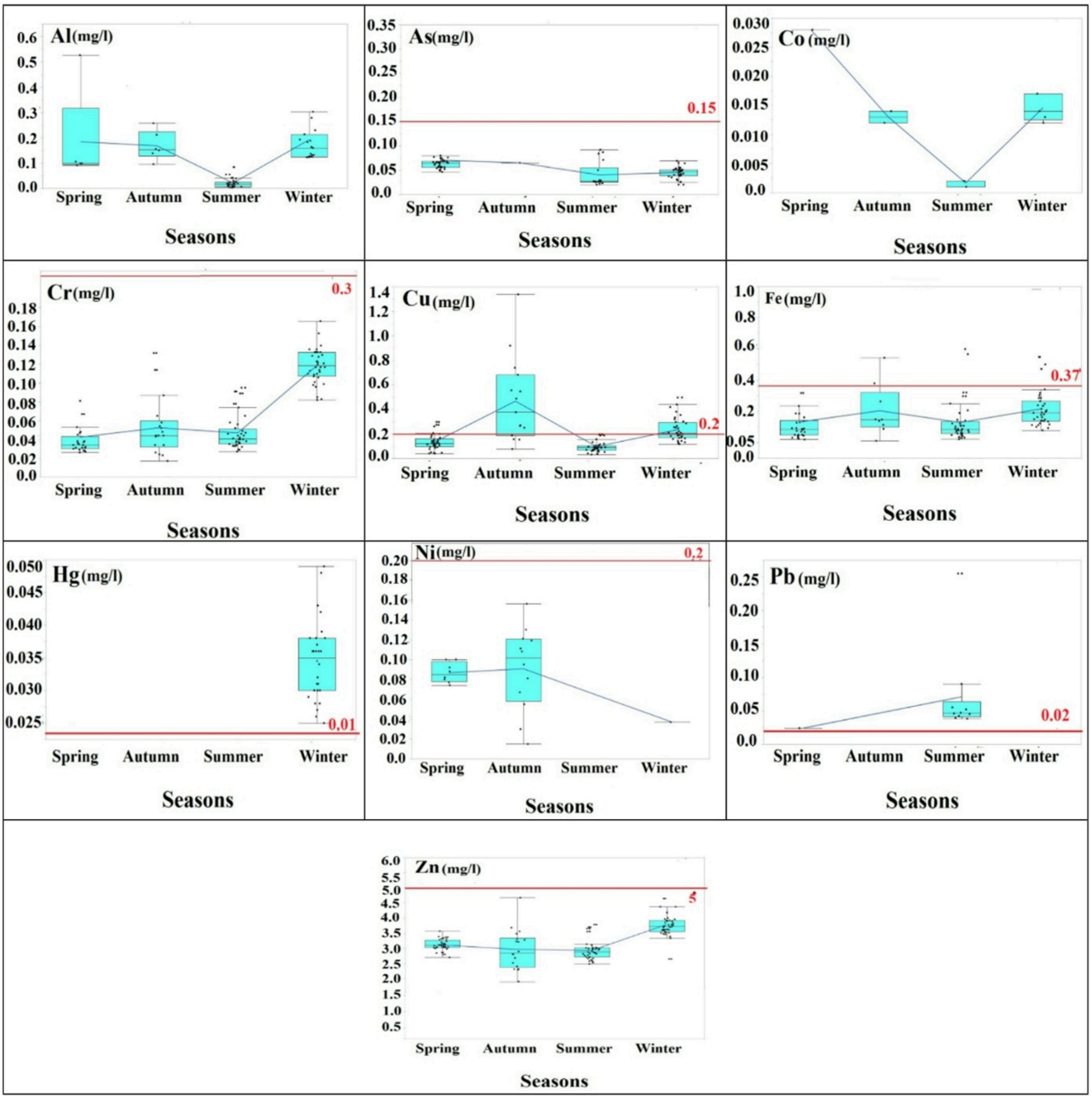


Figure 1

The concentration of heavy metals (mg/kg) in pasteurized milk and compared with the standard

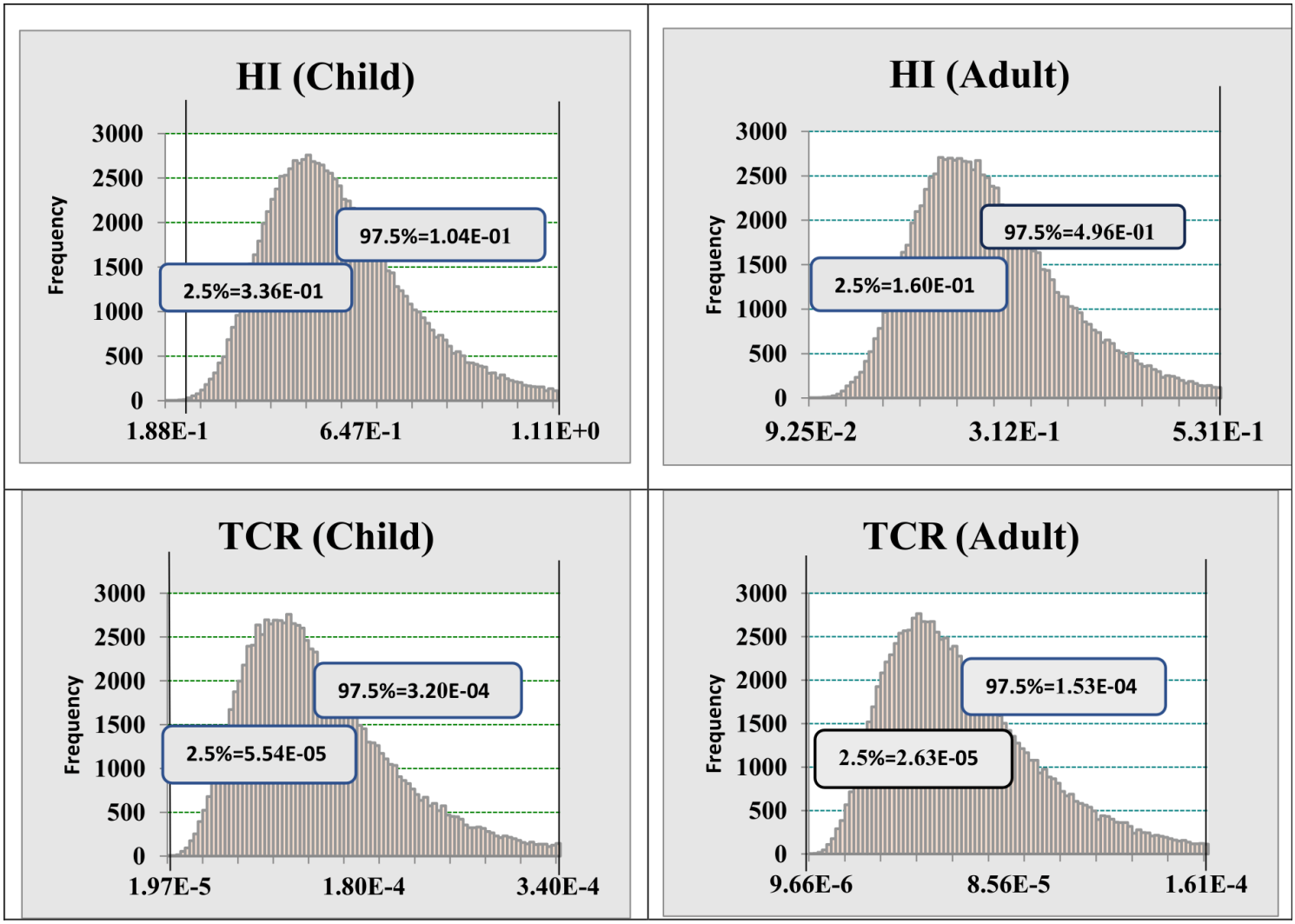


Figure 2

The cumulative distribution of non-carcinogenic and carcinogenic risk index of heavy metals in child and adults through Pasteurized milk consumption

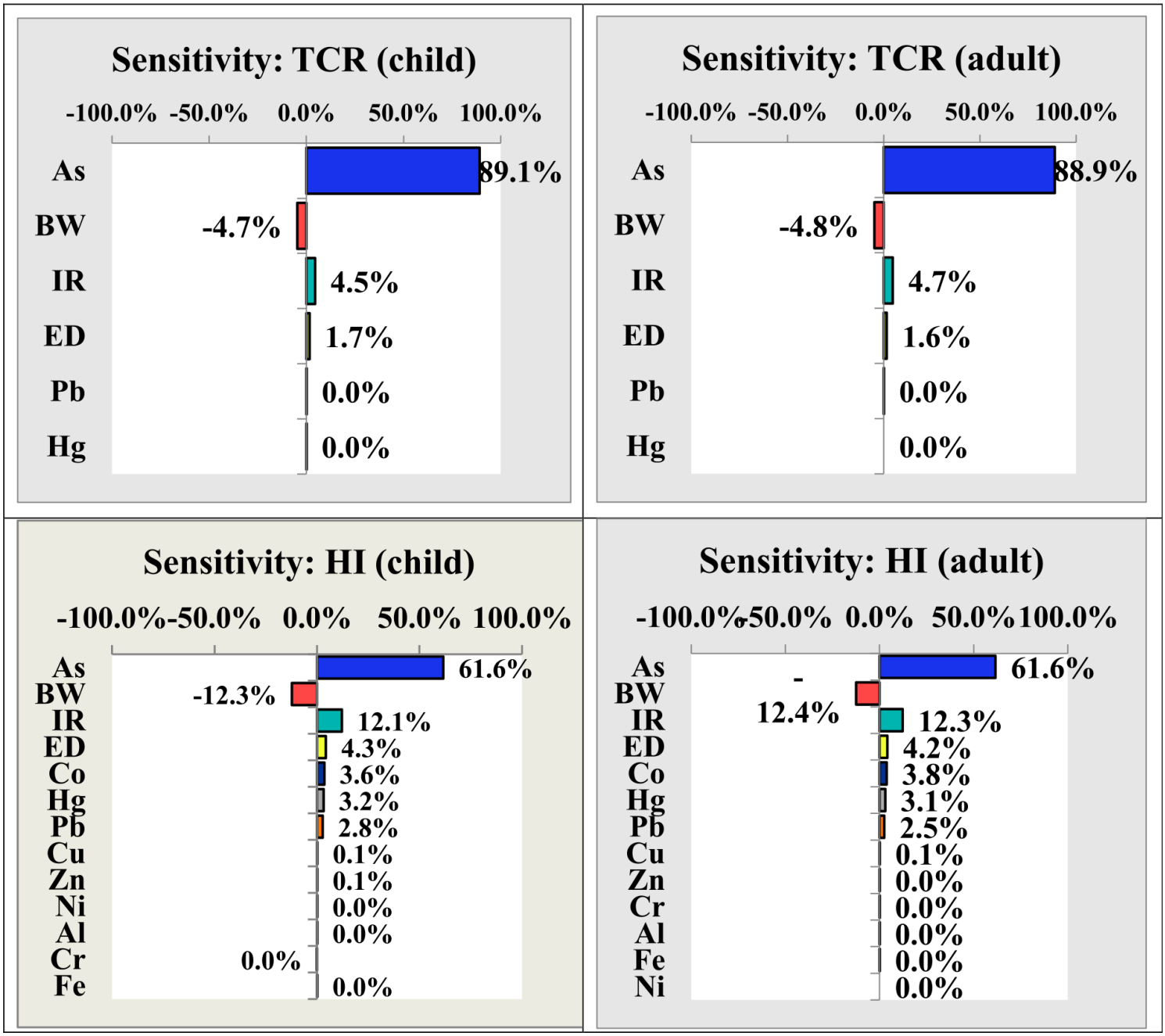


Figure 3

The effect of different variables on non-carcinogenic and carcinogenic risk index caused by Pasteurized milk consumption in child and adults

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement.docx
- Table3.docx