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# **ORIGINAL ARTICLE**

# Analysis and Health Risk Assessment of Arsenic and Zinc in Ghee Consumed in Kermanshah City, Western Iran using Atomic Absorption Spectrometry

# Soheil Sobhanardakani<sup>\*1</sup>, Lobat Taghavi<sup>2</sup>

<sup>1</sup> Department of the Environment, Hamedan Branch, Islamic Azad University, Hamedan, Iran

<sup>2</sup> Department of the Environmental Pollution, Science and Research Branch, Islamic Azad University, Tehran, Iran

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	ABSTRACT: This study was carried out for analysis and non-carcinogenic risk assessment of			
KEYWORDS	arsenic (As) and zinc (Zn) in some of consumed brands of ghee marketed in Kermanshah City,			
	western Iran in 2015. For this purpose, 12 samples of four popular brands of ghee were analyzed			
Arsenic;	for levels of As and Zn after digestion with acids using atomic absorption spectrometry in 3 repli-			
Zinc;	cations and health index was obtained. The results showed that the mean concentrations of As and			
Ghee,	Zn in ghee samples were 0.37 $\pm$ 0.12 and 272.83 $\pm$ 81.55 µg/kg, respectively. The mean concentra-			
Health risk assessment;	tion of As and Zn in ghee samples were lower than WHO maximum permissible limits (100 $\mu$ g/kg			
Food safety	for As and 10000 $\mu$ g/kg for Zn). In addition, health risk assessment showed that no potential risk			
	for children and adult by consume of the ghee samples. According to the results, although con-			
	sumption of studied ghee has not adverse effect on the consumers' health, but concerning to the			
	grazing the livestock on soils polluted by heavy metals due to the increased use of agricultural			
	inputs by farmers, industrial development and establishment of pastures near the roads, it is very			
	important to take the appropriate measures during the production process and the treat products			
	before selling them to markets by companies.			

# INTRODUCTION

Oils and fats are essential part of human daily diet. The human body uses oils and fats in the diet for energy source, structural component, make powerful biological regulators and metabolic reactions. Oils and fats contain fatty acids, which are susceptible to attack by a number of agents such as oxygen, light, metals, etc. [1, 2].

Because heavy metals are potential to human health problems, today a growing interest in assessing the levels of trace heavy metals in foodstuffs is increased.

\* Corresponding author: s\_sobhan@iauh.ac.ir (Soheil Sobhanardakani).

The ingestion of food is an obvious means of exposure to heavy metals, not only because many metals are natural ingredients of foodstuffs but also because of environmental contamination and contamination during food processing technologies, packaging, traveling and storage. Therefore, concern about quality of food is increasing around the world [3, 4].

Low or high trace element unbalances can be lead to several diseases. Metals, such as Cu, Fe, Mn and Zn, are essential metals since they play important and vital roles in biological systems, but they can also produce toxic effects at high levels. Whereas nonessential metals, such as As, Cd, Cr, Hg, and Pb are toxic and harmful, even in low contents. The toxic metals are characterized as having no demonstrated biological requirement in humans, and exposure is associated with recognizable toxicity. In addition, intensity of toxicity increases with increases in dosage [5-7].

Arsenic is a widely distributed metalloid regarded as human carcinogen from extremely low levels of exposure and having no possible beneficial metabolic functions for humans. The combustion of fossil fuel for production of energy and smelting of non-ferrous metals are two significant processes that lead to As contamination of the environment, especially air pollution. Other sources of As are arsenical pesticides, manufacture and wood preservatives such as chromated copper arsenate [8].

Zinc is an essential structural and functional element for numerous cellular processes which oftencatalyzing reactions, binding to substrates by favoring various reactions through the mediation of redox or oxidation–reduction reactions, via reversible changes in the oxidation state of the metal ions. Despite, Zn is critical for growth, but this element may play an important role in cancer etiology and outcome and harms some physiological activities like breathing [9-11].

Risk analysis is a process that combine risk assessment, risk management and risk communication. Risk assessment, includes of scientific analyses, the results of which are quantitative or qualitative explanations of the likelihood of harm associated with exposure to a chemical compounds. In this regard, the human health risk assessment requires identification, collection and integration of information on the chemicals health hazards, exposure of human to the chemical and relationships between exposure, dose and adverse effects [12]. On the other hand, a human potential health risk assessment is the process to estimate the nature and possibility of adverse health effects in humans who may be exposed to toxins and chemicals in polluted environmental media, now or in the future and includes hazard identification, dose-response assessment, exposure assessment and risk characterization steps [13].

Ghee is clarified butter made from pasture fed dairy cow butter. Ghee, if made appropriately, will reduce small intestine and colon inflammation and assist restoring intestinal permeability. There are several anticarcinogenic and health attributes found in ghee. In addition, ghee contains antioxidants, linoleic acid, and fat-soluble vitamins. On the other hand, lipids and the high amount of phospholipids in ghee assist in the absorption of fat-soluble vitamins specifically cephalin and makes it highly antioxidant [14].

Since the metal pollution in environment can be harmful to human health, it is necessary to understand and control the hazard levels of pollution in foodstuffs. Therefore, as regards to date, there is no information available on heavy metal accumulation in ghee, this study aimed to analysis and non-carcinogenic risk assessment of As and Zn in ghee marketed in Kermanshah City in 2015 through atomic absorption spectroscopy.

#### MATERIALS AND METHODS

#### Reagents

All reagents and standard stock solutions used were purchased from Merck (Germany). The concentrated HNO<sub>3</sub> was pure, specific for trace analysis, and was diluted to 10% (v/v) concentration with deionized water. The blank consisted of the 10% dilute HNO<sub>3</sub> and deionized water.

## Sampling

According to the Cochran's sample size formula, 12 samples of four popular brands of ghee were taken from some food supply markets in Kermanshah City, western Iran for analysis of their As and Zn content.

#### Samples preparation and Analysis

One gram of each samples were weighed into separate conical flasks. Five mL of concentrated HNO3 was then added and the contents heated at 70-80 °C for 2-3 h, on a hot plate. Heating was continued at about 150 °C overnight, 3-5 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and 30% H<sub>2</sub>O<sub>2</sub> (each) was added occasionally and continuous heating further allowed to completely decomposing the organic matter, until obtaining clear solutions. All contents of the flasks were evaporated and the semidried mass was dissolved in a small amount (approx. 5 mL) of deionized water, filtered through Whatman 42 paper (Sigma-Aldrich), and made up to a final volume of 25 mL in volumetric flasks with 2N HNO<sub>3</sub> [15,16]. Finally the concentrations of As and Zn determined using a Shimadzu atomic absorption model Aa680 (Shimadzu, Japan) with three replications.

# STATISTICAL ANALYSIS

Data were statistically analyzed using one sample *t* test to comparing the mean concentrations of As and Zn with WHO maximum permissible limits using the SPSS statistical package version 20 (Chicago, IL, USA).

## Human Health Risk Assessment

Estimated average daily intakes (EADIs) of a metal in food and food consumption assumption were used to survey long-term health risks to consumers. For each type of exposure, the EADI was computed by using the equation 1:

$$EADI = \frac{C \times F}{W \times D} \tag{1}$$

where

C= the concentration of metal in each commodity (mg/kg); F= mean annual intake of food per person; D= number of days in a year (365); and W= the mean body weight (70 kg for adult and 15 kg for children) [17, 18].

The World Health Organization has set values for toxicity, termed Acceptable Daily Intakes (ADIs) for a large number of chemicals, including some essential trace elements [19]. The health risk indices were obtained by dividing the EADI by the acceptable daily intakes (ADI) (mg/kg/day) established by FAO/WHO Codex Committee [17-20]. The HI was computed by using the equation 2:

$$HI = \frac{EADI}{ADI} \tag{2}$$

When the HI >1; the food involved is considered a risk to the concerned consumers. When the HI <1, the food involved is considered as acceptable (no concern) to the concerned consumers [18].

# **RESULTS AND DISCUSSION**

The concentrations of As and Zn in the analyzed ghee samples are presented in Table 1. The percentage of metals contamination of ghee samples was reached 100%. The range ( $\mu$ g/kg) found for As and Zn were 0.257-0.570 and 178.0-413.0, respectively. Besides, the mean concentrations of As and Zn were lower than WHO maximum permissible limits (MPL) (100  $\mu$ g/kg for As, and 10000  $\mu$ g/kg for Zn) [21].

Table 1. Concentrations (mean concentration $\pm$ S.D.) of heavy metals in the ghee samples (µg/kg)

Metal	Ghee Sample				Min	Max	Mean concentration ±S.D.	
	Brand 1	Brand 2	Brand 3	Brand 4		111UA	Fred concentration 2012	
As	0.36±0.02 <sup>b</sup>	$0.27 \pm 0.01^{a}$	$0.55 \pm 0.02^{\circ}$	0.29±0.01 <sup>a</sup>	0.26	0.57	0.37±0.12	
Zn	210.0±7.55 <sup>a</sup>	277.67±11.50 <sup>b</sup>	396.0±19.31°	207.67±30.53 <sup>a</sup>	178.0	413.0	272.83±81.55	

Vertically, letters a, b and c show statistically significant differences (P < 0.05).

The systemic health risk assessment associated with heavy metals encountered in ghee samples are summarized in Table 2. The results showed that the EADIs calculated ranged between  $4.92 \times 10^{-8}$  to  $1.69 \times 10^{-4}$ 

mg/kg/day, while the hazard indices (HI) ranged from  $2.34 \times 10^{-5}$  to  $5.63 \times 10^{-4}$  for the analyzed metals and indicating no direct hazard to human health, in spite of their presence in the food.

Table 2. Acceptable and estimated dail	y intakes and health index for metals found i	n ghee samples
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Metal	ADI (mg/kg/day) [22, 23]	Mean Concentrations	EADI (mg/kg/day) (Children)	HI (Children)	EADI (mg/kg/day) (Adult)	HI (Adult)	
As	0.0021	3.7E-04	2.30E-07	1.10E-04	4.92E-08	2.34E-05	
Zn	0.30	2.73E-01	1.69E-04	5.63E-04	3.62E-05	1.21E-04	

World researches for content of heavy metals in the oils and fats especially ghee is narrow and mainly refer to some of them As and Zn. As regards that once the heavy metals fall in human body do not decompose but are accumulated and in certain cause of adverse health effect, that's why it is necessary to pay great attention when livestock grazing on soils polluted by heavy metals.

Literature reports few data about the presence of heavy metals in ghee. Ahmad et al. determinedZn in ten different brands of ghee and reported that in all samples average concentrations of Zn was below the WHO permissible limits [2]. Khan et al. analyzed of Zn in Vanaspati ghee consumed in Pakistan and reported that average concentrations of Zn was below the WHO permissible limits [24]. Average content of Zn in ghee was 46.0±3.0 µg/kg [25]. Ilyas et al. analyzed 10 different brands of ghee for residual Ni content and reported that an average concentration of Ni was above the WHO permissible limits [1]. Latif et al. evaluated the value of different brands of banaspati ghee found in the local Peshawar market and reported that Zn was observed in the range of 19.0 to 13000 µg/kg [26].

The EADI of heavy metals was compared with the oral reference dose (RfD) or provisional tolerable

weekly intake (PTWI) to survey the potential health risks. In accordance with the standard methods (USEPA), the risk of chronic-toxic effects is explained as the ratio of the dose resulting from exposure to site media to the dose that is believed to be safe, even in sensitive individuals such as children and elderly. If the HI <1, no significant risk of chronic-toxic effects exists. If the HI >1, chronic-toxic effects may occur. The chronic-toxic effects tend to increase with increased HI. On the other hand, the HI expresses the combined chronic-toxic effects of multiple metals [27, 28]. As shown in Table 2, HI values of As and Zn for children and adults are less than 1.

Here, the average HI value was 7.22 E-05 for adults and 3.36E-04 for children. Therefore, target population might have no potential significant health risk through only consuming ghee from the study area. However, the non-carcinogenic risks were greater for children than for adults.

# CONCLUSIONS

This study for the first time in Iran was carried out for health risk assessment of As and Zn in some of consumed brands of ghee marketed in Kermanshah City to provide some baseline information for further research in this field. The metals contamination in ghee samples was reached 100%. However, the average concentrations of As and Zn was lower than MPL. Although these metals have toxic potential, but the detrimental impact will be apparent only after years of exposure. Therefore monitoring of toxic metals in ghee is essential in order to prevent excessive build-up of these pollutants in the human food chain. Finally, taking the appropriate measures during the production process and the treat products before selling them to markets by companies is recommended.

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