Van Vet J, 2026, 37(2) 140-144

Van Veterinary Journal

https://dergipark.org.tr/tr/pub/vanvetj

Cite this article as: **Sengul SA (2026).** Tin (Sn) Levels in Cow's Bulk Tank Milk and Dietary Risk Assessment from Dairy Farms in Industrial and Non-industrial Areas of Hatay Province. *Van Vet J*, 37(2), 140-144. DOI: https://doi.org/10.36483/vanvetj.1816773

ISSN: 2149-3359 **Original Article** e-ISSN: 2149-8644

Tin (Sn) Levels in Cow's Bulk Tank Milk and Dietary Risk Assessment from Dairy Farms in Industrial and Non-industrial Areas of Hatay Province

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Received: 04.11.2025 Accepted: 07.07.2026

ABSTRACT In this study, it was aimed to compare the tin (Sn) concentrations in raw cow milk samples collected from dairy farms located in industrial and non-industrial areas in Hatay, to reveal the relationship of this element with the proximity of industrial areas and consumption of water and feed, and to reveal the health risks that Sn exposure through the consumption of milk may cause in adult male and female in this region. For this purpose, 12 bulk tank milk samples, 12 water samples and 12 feed samples were collected separately from industrial and non-industrial areas. Estimated daily intake (EDI) and target hazard quotient (THQ) parameters were used for non-carcinogenic risk assessment. In milk samples collected from industrial and non-industrial areas, the mean Sn concentrations were found to be 25.743 mg/L and 24.389 mg/L, respectively. In feed and water samples collected from industrial areas, the mean Sn concentrations were found to be 2.762 mg/kg and 0.041 µg/L, respectively, while those from non-industrial areas were found to be 3.857 mg/kg and 0.218 µg/L, respectively. EDI values determined for the Sn element for adult male and female were calculated as 34.55 µg/L bw/day and 39.74 µg/L bw/day, respectively. In addition, THQ values determined for the Sn element in adult male and female were calculated as 0.057590 and 0.066237, respectively. These findings show that industrial activities in the region contribute to the Sn content in milk and that exposure to this element through the consumption of milk is unlikely to cause any health risks.

Keywords: *Industrial, Cow, Tin, Risk assessment, Milk.*

ÖZ

Hatay İlinde Endüstriyel ve Endüstriyel Olmayan Bölgelerdeki Süt Çiftliklerinden Toplanan İneklerin Tank Sütünde Kalay (Sn) Düzeyleri ve Diyet Risk Değerlendirmesi

Bu çalışmada, Hatay ilinde endüstriyel ve endüstriyel olmayan bölgelerde bulunan süt çiftliklerinden toplanan çiğ inek sütü örneklerinde kalay (Sn) konsantrasyonlarının karşılaştırılması, bu elementin endüstriyel alanlara yakınlık ve içme suyu ve yem tüketimi ile olan ilişkisinin ortaya koyulması ve süt tüketimi yoluyla Sn maruziyetinin bu bölgedeki yetişkin erkek ve kadın bireylerde oluşturabileceği sağlık risklerinin ortaya koyulması amaçlandı. Bu amaçla, endüstriyel ve endüstriyel olmayan bölgelerden ayrı olarak 12 adet tank sütü örneği, 12 adet su örneği ve 12 adet yem örneği toplandı. Non-kanserojenik risk değerlendirmesi için tahmini günlük alım (TGA) ve hedef tehlike katsayısı (HTK) parametreleri kullanıldı. Endüstriyel ve endüstriyel olmayan bölgelerden toplanan çiğ inek sütü örneklerinde ortalama Sn konsantrasyonları sırasıyla 25.743 mg/L ve 24.389 mg/L olarak bulundu. Endüstriyel alanlardan toplanan yem ve su örneklerinde ortalama Sn konsantrasyonları sırasıyla 2.762 mg/kg ve 0.041 µg/L düzeyinde, endüstriyel olmayan alanlardan toplanan örneklerde ise sırasıyla 3.857 mg/kg ve 0.218 µg/L düzeyinde tespit edildi. Yetişkin erkek ve kadın bireyler için Sn elementinin belirlenen TGA değerleri sırasıyla 34.55 µg/L vücut ağırlığı/gün ve 39.74 µg/L vücut ağırlığı/gün olarak hesaplandı. Ayrıca yetişkin erkek ve kadın bireylerde Sn elementi için belirlenen HTK değerleri sırasıyla 0.057590 ve 0.066237 olarak hesaplandı. Bulgular, bölgedeki endüstriyel faaliyetlerin sütteki Sn içeriğine katkıda bulunduğunu ve süt tüketimi yoluyla bu elemente maruz kalmanın herhangi bir sağlık riski oluşturmasının olası olmadığını göstermektedir.

Anahtar Kelimeler: *Endüstriyel, İnek, Kalay, Risk değerlendirmesi, Süt.*

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INTRODUCTION

Cow's milk is an extremely important nutrient source for humans due to its rich vitamin, mineral, carbohydrate, protein and fat content (Dobrzański et al. 2005). However, in addition to these substances, it also contains many toxic elements that can cause serious health problems (Arianejad et al. 2015). Because of this feature, milk could be an important route of toxic metal intake (Ogut et al. 2016). Industrial activities and increasing urbanization are shown among the important reasons for the contamination of milk with these elements (Usman et al. 2023). For this reason, milk is recognized as an important indicator of environmental pollution (Ogut et al. 2016). Contamination of the production environment can cause these elements to accumulate in foods and cause significant health problems due to their cumulative properties (Arianejad et al. 2015). In addition, consumption of contaminated feed and water and the equipment used in production and storage processes are also shown among different contamination sources (Rezaei et al. 2014; Arianejad et al. 2015).

Tin (Sn), which is widely used in various industrial activities and can accumulate in foods, is considered one of the important potentially toxic elements (PTEs) (Arianejad et al. 2015; Astolfi et al. 2020). The reason for the high levels detected in milk compared to other elements is due to the formation of stable and toxic organic Sn as a result of the element's ability to bind with the carbon in the milk structure. In addition, contamination of milk with this element can be attributed to the proximity of production areas to industrial activities and water and feed consumed by animals (Arianejad et al. 2015). It is known that Sn contamination is quite low in areas far from pollution sources (Harper 2005).

In this study, Sn levels were determined in raw cow's milk, drinking water, and feed collected from farms located in industrial and non-industrial areas in Hatay province. We hypothesized that if concentration of this element is higher in drinking water and feed, it may also be elevated in produced milk. In addition, it was aimed to determine to what extent the emissions of this element into the environment due to industrial activities in the region could affect the levels of Sn in milk. Moreover, the milk produced in the industrial areas was compared with the Sn concentrations in raw cow's milk collected from non-industrial areas, which were thought to be uncontaminated. It was also determined whether exposure to this element causes health risks in adults due to consumption of the milk produced.

MATERIAL AND METHODS

Study Areas and Collection of Samples

The dairy farms where milk, drinking water, and feed samples were collected are presented in Figure 1. Raw milk samples were collected from 24 dairy farms, each of which had no fewer than 50 cattle. Within the scope of the study, a total of 24 raw tank milk samples, each with a volume of 50 mL, were collected from dairy farms located in industrial and non-industrial areas of Hatay province. In this study, 12 farms were classified as close to industrial areas (within 15 km radial distance from industrial units), while 12 farms were grouped as far to industrial areas (areas outside of 15 km distance). Simultaneously with milk sampling, 24 feed samples (total mixed ration) weighing 500 g were collected from the feeds consumed by the cows. The collected feed samples were dried in oven and then homogenized. In addition, a total of 24 drinking water samples were collected in the amount of each 50 mL from each farm. To prevent possible metal contamination, the clean polyethylene sampling bottles (Isolab, Türkiye) used in the collection of milk and drinking water were kept in a 20% nitric acid (HNO_3) (65% purity) (Merck, Germany) solution overnight and then rinsed with ultrapure water (18.2 MΩ/cm resistivity, Millipore, France). All samples were stored at −20°C until further analysis. All samples were obtained during 2021-2022.

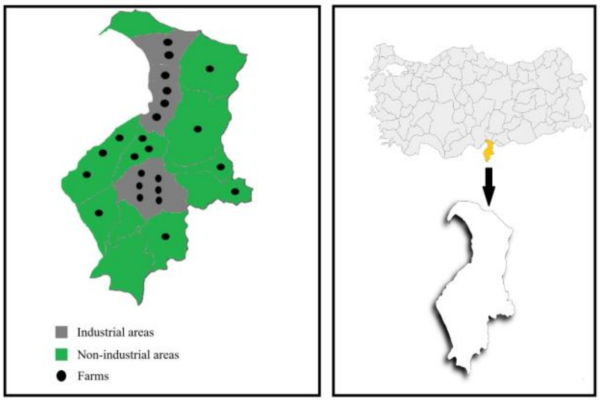

Figure 1: Location of the study area.

Digestion of Milk and Feed Samples

The digestion of milk and feed samples was carried out according to the method described in Nordic Committee on Food Analysis (NMKL) Method 161 (1998). Briefly, 1 mL of milk samples and 0.5 g of feed samples were weighed and placed into polytetrafluoroethylene (PTFE) digestion vessels. Before digestion, to prevent possible contamination, the vessels were kept in HNO_3 solution for 24 h and then rinsed with ultrapure water. Then, 8 mL of HNO_3 (65%) and 2 mL of hydrogen peroxide (H_2O_2) (30%) (Merck, Germany) were added to the samples, and the digestion process was carried out in a microwave oven (MARSXpress, CEM Corporation, USA) according to a four-step procedure (20 min, 800 watts power, 200 °C). When microwave wet digestion was finished, each digested sample was filtered to polyethylene volumetric flasks and then diluted to a final volume of 25 mL with ultrapure water. Diluted samples were storage at 4^{o}C until further analysis. All chemicals and reagents used in the analyses were of analytical grade.

Calibration Standard Preparation and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Analysis

Calibration standard concentrations of 0, 5, 20, 50, and 100 ppb in milk and feed samples and 0, 1, 2, 5, and 10 ppb in water samples were prepared for Sn element and calibration curves were obtained. The levels of Sn in all samples were determined by ICP-MS (Analytik Jena AG, Germany) and autosampler (ASX-560, Teledyne CETAC, USA) according to the TS EN ISO 21424 (2020). The water samples were passed through 25 mm/0.45 µm sterile syringe filters (Isolab, Türkiye). Then, the filtered water samples were acidulated to pH<2 by adding HNO_3 according to USEPA Method 200.8 (1994).

Health Risk Assessment

Estimated Daily Intake (EDI) and Non-carcinogenic Risk Assessment

The EDI and non-carcinogenic target hazard quotient (THQ) used to assess the potential health risk from Sn exposure through consumption of raw cow's milk in adults were calculated according to the equation 1 and 2 given below (USEPA 1995; Usman et al. 2023).

$$EDI = \frac{C \ x \ FIR}{BW} \qquad \text{eq. 1}$$

Here, C is the concentration Sn element (mg/kg), FIR is the milk ingestion rate (kg/person/year) (39.7 kg for adult per year) (Turkish National Dairy Council 2020), BW is the average body weight (78.9 kg for adult male, 68.6 kg for adult female) (TURKSTAT 2022).

$$THQ = \frac{EDI}{RfD_o} \qquad \text{eq. 2}$$

Where EDI is already explained, RfD_o is the oral reference dose (0.6 mg/kg/day for Sn) (USEPA 2016). A THQ value below 1 indicates that there is no consumption-related health risk (USEPA 1995).

Statistical Analysis

Descriptive statistical data were shown as mean±standard deviation (SD). Repeated measures ANOVA were used to evaluate the effect of region on the mean Sn levels in milk samples. One-way analysis of variance was used to evaluate the effect of region on the mean Sn levels in water and feed samples. The relationship between the Sn levels in milk samples and the Sn levels in water and feed samples in each region was calculated by Pearson correlation coefficients. IBM SPSS 23.0 (Armonk, NY: IBM Corp.) package programme was used in all statistical analyses. The value of $p<0.05$ was considered statistically significant.

RESULTS

The Mean Concentrations of Sn Element in Raw Cows' Milk, Feed, and Water Samples Collected from Industrial and Non-industrial Areas

The results of Sn element were determined in milk, feed, and water samples are presented in Table 1.

The mean Sn level in raw milk samples collected from industrial areas was found to be 25.743 mg/L, with values between 22.432 and 28.548 mg/L. The mean Sn level in raw milk samples collected from non-industrial areas was found to be 24.389 mg/L, with values between 19.988 and 33.301 mg/L (p=0.279) (Figure 2).

Table 1: Mean concentrations of Sn in milk, feed, and water samples from industrial and non-industrial areas and risk assessment.

		Areas		EDI		THQ	
		Industrial	Non-industrial	Male	Female	Male	Female
Milk	*N*	12	12	34.55	39.74	0.057590	0.066237
	Mean±SD (mg/L)	25.743±2.039	24.389±3.697				
	Min-Max	22.432-28.548	19.988-33.301				
	p	0.279					
Feed	*N*	12	12				
	Mean±SD (mg/kg)	2.762±1.198	3.857±1.689				
	Min-Max	0.619-4.895	1.844-8.084				
	p	0.081					
Water	*N*	12	12				
	Mean±SD (µg/L)	0.041±0.070	0.218±0.614				
	Min-Max	0-0.197	0-2.157				
	p	0.332					

N: Number of samples. Min-Max: Minimum-Maximum.

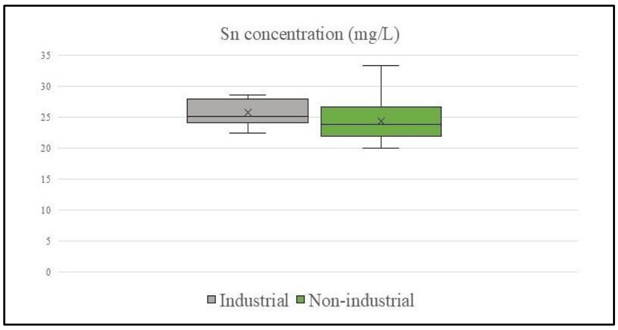

Figure 2: Mean Sn concentrations in raw cow milk.

The mean Sn level in feed samples collected from industrial areas was found to be 2.762 mg/kg, with values between 0.619 and 4.895 mg/kg. The mean Sn level in drinking water samples collected from industrial areas was found to be 0.041 µg/L, with values between 0 and 0.197 µg/L. The mean Sn level in feed samples collected from non-industrial areas was found to be 3.857 mg/kg, with values between 1.844 and 8.084 mg/kg. The mean Sn level in drinking water samples collected from non-industrial areas was found to be 0.218 µg/L, with values between 0 and 2.157 µg/L.

Correlation Between Sn Content in Milk with that in Water and Feed

Correlation coefficients showing the relationship between Sn levels in milk samples and Sn levels in drinking water and feed samples are presented in Table 2. There was no significant correlation between Sn concentrations in milk, drinking water and feed in both regions.

Table 2: Pearson's correlation coefficients between Sn concentrations in milk, feed, and water samples.

	Industrial areas		Non-industrial areas	
Milk	Feed	Water	Feed	Water
r	-0.280	0.568	-0.131	-0.203
p	0.378	0.054	0.684	0.528

r: Correlation coefficients.

Human Health Risk Assessment

The calculated EDI and THQ values for the exposure of adult male and female individuals to the Sn element in milk through consumption are presented in Table 1. Accordingly, the EDI values determined for the Sn element for male and female individuals were calculated as 34.55 µg/L bw/day and 39.74 µg/L bw/day, respectively. According to the non-carcinogenic risk assessment, the THQ values determined for the Sn element in male and female individuals were calculated as 0.057590 and 0.066237, respectively.

DISCUSSION AND CONCLUSION

In our study, it was observed that the mean Sn levels detected in cow milk collected from industrial areas were higher than the levels detected in milk collected from non-industrial areas. This situation may be thought to be due to the higher Sn emissions to the environment in areas where industrial activities are maintained. In industrial areas, Sn is released into the atmosphere as a result of the emissions of waste disposed of by incineration. The remoteness of contamination sources ensures that environmental levels of Sn are generally low (Harper 2005). There are a limited number of studies in the literature on Sn content in cow's milk. Therefore, the determination of this element's levels in milk, the assessment of exposure-related risks, and the examination of its correlation with environmental factors are considerably important. Usman et al. (2023) reported that they detected mean Sn levels in cow's milk collected from regions where mining activities are maintained in Northwestern Nigeria in the range of 0.090 to 0.130 mg/kg. In a study of 100 cow's milk samples in China, Chen et al. (2020) reported that the mean Sn concentration was found to be 97.6 µg/kg. In Iran, Rezaei et al. (2014) were found the mean Sn level in milk to be 4.8 µg/L. In another study conducted on cow's milk in Egypt by Enb et al. (2009), the mean Sn level was determined to be 0.003 mg/kg. In a study of 238 raw cow's milk samples in Italy by Stocco et al. (2019), the mean Sn level was reported to be 271 µg/kg. Birghila et al. (2008) determined the mean Sn level to be 0.10 ppm in their study on raw cow's milk in Romania. Gabryszuk et al. (2008) found the mean Sn concentrations in raw cow's milk collected from conventional and organic farms in Poland to be in the range of 12.3-15.4 µg/L. Again, the mean Sn levels detected in raw cow's milk collected from organic and conventional farms in Southern England by Qin et al. (2021) are 2.68 µg/kg and 2.66 µg/kg, respectively. Ogut et al. (2016) did not detect Sn in raw milk collected from industrial and rural areas in Türkiye. It was observed that the reported mean values were lower than the levels detected in the present study. This may be attributed to the fact that the industrial activities maintained in our study area and the Sn-contaminated drinking water and feed consumed by animals contribute more to the Sn content in milk compared to other study areas. It was reported by Astolfi et al. (2020) and Toscano et al. (2023) in Italy and Newton et al. (2023) in Southeast England that Sn element was not detected in cow milk. This may be thought to be due to the fact that the lack of industrial activities in these regions, unlike our study region, significantly reduced Sn contamination in the milk produced. In contrast to our current results, in a similar study conducted by Arianejad et al. (2015) in Iran, it was observed that the mean Sn levels in raw cow milk collected from industrial areas were lower than the levels detected in milk produced in traditional farms. It is noteworthy that, in the present study as well, elevated Sn contamination was observed not only in milk produced in industrial areas but also in milk produced in areas far from industrial activities. This suggests that the observed Sn contamination in milk produced in areas far from industrial activities may originate from milk storage equipment.

EDI and THQ values were determined to reveal the health risk assessment of exposure to the Sn element through the consumption of milk. It was determined that the THQ values determined for the Sn element in milk for adult male and female individuals were lower than 1. This indicates that exposure to the Sn element through the consumption of milk produced in the region does not pose a risk for adult male and female individuals and that their consumption is safe. In the literature, there is only 1 study that has investigated whether exposure to Sn element through milk consumption poses a health risk. In the non-carcinogenic risk assessment conducted by Usman et al. (2023), it was reported that the THQ values calculated for Sn element in cow's milk consumed in different regions were all less than 1 and that exposure to this element through milk consumption in these regions would not cause any health risk in adults.

This study is the first report showing the possible contamination sources relationship with Sn content in milk produced in the region and evaluating the potential health risk associated with the consumption of milk in adults. In the study, according to the non-carcinogenic risk assessment, it was revealed that the consumption of raw milk does not pose any toxicological risk in the Hatay province. However, since elements have a cumulative property, it is thought that regularly food analyses should be done to evaluate the toxicological risk.

CONFLICTS OF INTEREST

The author reports no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea/Concept: SAŞ
Audit/Consultancy: SAŞ
Data Collection and/or Processing: SAŞ
Analysis and/or Interpretation: SAŞ
Article Written by: SAŞ
Critical Review: SAŞ

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