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# Occurrence, contamination pathways, and risk assessment of potentially toxic elements in cows' milk from industrial and non-industrial areas in Türkiye

# Ocurrencia, vías de contaminación y evaluación del riesgo de elementos potencialmente tóxicos en la leche de vaca procedente de zonas industriales y no industriales de Turquía

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## ABSTRACT

In this study, it was aimed to compare the concentrations of essential and non-essential potentially toxic elements (Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se, and Zn) in milk samples collected at different lactation stages from cows reared in industrial and non-industrial areas in Hatay, to determine the possible contamination pathways of potentially toxic elements in raw milk, and to reveal the potential risk of exposure to these elements through milk consumption in adults. For this purpose, a total of 216 individual raw cows' milk samples, 72 drinking water samples and 72 feed samples were collected from 24 dairy farms. The levels of selected potentially toxic elements were measured using inductively coupled plasma mass spectrometry. Health risk assessment was revealed by calculating the estimated daily intake and target hazard quotient. All elements, except for Cd and Hg, were detected in all analyzed samples. Target hazard quotient values calculated for all elements, except aluminum, were found to be below 1. These results highlight that exposure to Al through milk consumption in this region may lead to health risks in adults

**Key words:** Cow milk; inductively coupled plasma mass spectrometry; potentially toxic elements; risk assessment; Türkiye

## RESUMEN

En este estudio, se tuvo como objetivo comparar las concentraciones de elementos potencialmente tóxicos esenciales y no esenciales (Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se y Zn) en muestras de leche recolectadas en diferentes etapas de lactación de vacas criadas en zonas industriales y no industriales de Hatay, determinar las posibles vías de contaminación de los elementos potencialmente tóxicos en la leche cruda y revelar el riesgo potencial de exposición a estos elementos a través del consumo de leche en adultos. Con este propósito, se recolectaron un total de 216 muestras individuales de leche cruda de vaca, 72 muestras de agua de bebida y 72 muestras de alimento de 24 granjas lecheras. Los niveles de los elementos potencialmente tóxicos seleccionados se midieron mediante espectrometría de masas con plasma acoplado inductivamente. La evaluación del riesgo para la salud se reveló mediante el cálculo de la ingesta diaria estimada y el cociente de riesgo objetivo. Todos los elementos, excepto Cd y Hg, fueron detectados en todas las muestras analizadas. Los valores de cociente de riesgo objetivo calculados para todos los elementos, excepto Al, se encontraron por debajo de 1. Estos resultados destacan que la exposición al aluminio a través del consumo de leche en esta región puede conducir a riesgos para la salud en adultos.

**Palabras clave:** Leche de vaca; espectrometría de masas con plasma acoplado inductivamente; elementos potencialmente tóxicos; evaluación del riesgo; Turquía

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## INTRODUCTION

Cow's (*Bos taurus*) milk, which has high nutritional value, probiotic bacteria potential and also contains protein, vitamin and mineral compounds necessary for growth, immunity and maintenance of physiological functions, is an important food source widely consumed all over the world [1, 2, 3].

However, it is also known that cow's milk is the food with the highest contamination risk due to exposure to toxic non-essential elements through anthropogenic activities and consumption of contaminated foods by animals, in addition to containing essential elements necessary for the maintenance of normal metabolic functions of the body [4].

Although essential for physiological processes in the organism, exposure to high concentrations of essential elements such as copper (Cu), iron (Fe), selenium (Se), and zinc (Zn) in milk can lead to toxic effects, while dietary exposure to non-essential potentially toxic elements (PTEs) such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) even at very low concentrations can cause serious health problems such as cognitive disorders and cardiovascular diseases [5, 6, 7].

Additionally, the cumulative nature of these elements in the food chain and their biological non-degradability make them the most dangerous pollutants [3]. Determining the levels of these contaminants is important both in terms of revealing the hygiene quality of the milk offered for consumption and showing the pollution level of the region where production is carried out [8].

Since it is known that consumption of contaminated milk can cause serious health risks, this situation has made it mandatory to examine the exposure pathways of animals to PTEs [5, 9]. Exposure of lactating cows to high levels of PTEs occurs mainly through consumption of contaminated feed and drinking water and inhalation of pollutants released into the air due to increased industrialization [1, 5, 9].

Industrial activities have been reported to contribute to PTE contamination in milk produced in developing countries [9, 10, 11, 12, 13]. Similar situations have been observed in some regions of rapidly developing industrialized Türkiye, but no data on the interaction of PTE levels in milk produced in Hatay province with the intensive industrial activities in this region have been previously reported [14, 15].

The fact that Hatay is close to international transportation arteries and markets, that there are ports belonging to public and private companies operating in the region, and that there are six organized industrial zones as well as organized industrial zones where establishment and expansion activities are still ongoing are important in terms of monitoring and evaluation of cow's milk produced in the region where industrial activities are carried out in terms of PTE contamination levels and exposure risk assessment [16].

The aim of this study was to compare PTE concentrations in milk samples collected at different lactation stages from cows raised in industrial and non-industrial areas in Hatay province, to determine the possible contamination pathways of PTEs into raw milk and to evaluate the risk of consumption of this milk for the adult population.

## MATERIALS AND METHODS

### Study area and sample collection

The study location was in industrial and non-industrial areas of Iskenderun and Antakya of Hatay province, Eastern Mediterranean region, Türkiye. To collect cow milk, feed, and drinking water samples, 12 dairy farms used to within 15 km radial distance from industrial units and 12 dairy farms far to industrial areas (areas outside of 15 km distance) were selected.

All samples were collected between 2021 and 2022. In order to collect milk samples, a total of 72 cows were randomly selected, with three cows chosen from each of the 24 designated dairy farms. Raw milk samples were collected three times from each selected cow during the I., II., and III. stages of lactation, resulting in a total of 216 raw cow milk samples.

Additionally, a total of 72 drinking water samples (each with a volume of 50 mL, collected directly from the water troughs into clean polyethylene sampling bottles) and a total of 72 feed samples (each consisting of 500 g of roughage and concentrated feed) were collected simultaneously with the collection of milk samples. All samples were stored in a −20 °C freezer (Bosch, Germany) until analysis.

### Microwave digestion of milk and feed samples

Milk and feed samples were subjected to acid digestion using a MARSXpress microwave digestion system (CEM Corporation, USA) according to the method described in Nordic Committee on Food Analysis (NMKL) Method 161 [17]. 1 mL of homogenized milk sample or 0.5 g of feed sample was transferred into polytetrafluoroethylene (PTFE) tubes. Eight mL of concentrated nitric acid ($HNO_3$, 65 % purity) (Merck, 100456, Germany) and 2 mL of hydrogen peroxide ($H_2O_2$, 35 % purity) (Merck, 108600, Germany) were added to the samples, and the milk and feed samples were digested by microwave at a range of settings (20 min, 800watt power, 200 °C). After microwave digestion, digested samples were filtered through 25 mm/0.45 µm syringe filters (Macherey-Nagel, CHROMAFIL Xtra, Germany) to a 50 mL polypropylene tubes (Isolab, Türkiye), and diluted to a final volume of 25 mL with ultrapure water (Millipore Milli-Q IQ 7000, France). Afterwards, the diluted samples were stored in a 4 °C freezer (Bosch, Germany) until analysis.

### Inductively coupled plasma mass spectrometry analysis

The potentially toxic elements concentrations in milk and feed samples were determined using ICP-MS (Analytik Jena AG, Germany) with an autosampler (ASX-560, Teledyne CETAC, USA) in accordance with TS EN ISO 21424 [18]. The concentrations of PTEs in water samples were determined using ICP-MS and an autosampler according to the method described in USEPA Method 200.8 [19]. Briefly, water samples were filtered through 25 mm/0.45 µm syringe filters into polypropylene tubes to achieve a volume of 25 mL. Filtered water samples were mixed with 1% (v/v) $HNO_3$ prior to ICP-MS analysis.

## Estimated daily intake

In order to determine the exposure of individuals to PTEs through milk consumption, estimated daily intake (EDI) values were calculated based on the contamination levels determined in milk samples [2].

$$EDI = \frac{C_{PTE}\ x\ W_{milk}}{BW(kg)}$$

Where $C_{PTE}$ is average PTE concentration in raw milk (µg/L); Wmilk is average daily milk consumption (kg/day); BW is average body weight of an adult individual (kg). The average body weight of an adult individual in Türkiye is considered to be 73.7 kg [20]. According to the Dairy Sector Statistics in the World and Türkiye published by the Turkish National Dairy Council [21], the average per capita milk consumption in Türkiye in 2019 was 39.7 kg.

## Non-carcinogenic risk assessment

Non-carcinogenic risk assessment was determined using target hazard quotient (THQ). If the THQ value is less than 1, the exposed populations is unlikely to experience adverse health effects. However, values greater than 1 indicate a potential health risk [22].

$$THQ = \frac{EFr\ x\ ED_{tot}\ x\ F_{IR}\ x\ C_{PTE}}{RfD_o\ x\ BW_a\ x\ AT_n}\ x\ 10^{-3}$$

Where EFr is exposure frequency (365 days/year); $ED_{tot}$ is exposure duration (70 years for adults); $F_{IR}$ is food ingestion rate (kg/person/day); $C_{PTE}$ is average PTE concentration in raw milk (mg/L); $RfD_o$ is oral reference dose of PTEs (mg/kg/day); BWa is average body weight (73.7 kg for adults); and $AT_n$ is average exposure time. The determined $RfD_o$ values for aluminum (Al), As, Cr, Cu, Fe, Ni, Pb, Se, and Zn were taken as 0.0004 mg/kg/day, 0.0003 mg/kg/day, 0.003 mg/kg/day, 0.04 mg/kg/day, 0.7 mg/kg/day, 0.02 mg/kg/day, 0.004 mg/kg/day, 0.005 mg/kg/day, 0.3 mg/kg/day, respectively [23, 24, 25].

## Statistical analyses

Descriptive statistical data were shown as mean±standard deviation (SD). Repeated Measures ANOVA was used to evaluate the effect of lactation stage and region on the average PTE levels in milk samples. One-way analysis of variance was used to evaluate the effect of region on the average PTE levels in water and feed samples. The relationship between the PTEs levels in milk samples and the PTEs levels in water and feed samples in each region was calculated by Pearson correlation

**TABLE I**

***Mean concentrations of essential potentially toxic element in raw milk samples from industrial and non-industrial areas***

| Essential PTEs | Region | Lactation Stage | | | Mean±SD | P | |
|---|---|---|---|---|---|---|---|
| | | 1st stage | 2nd stage | 3rd stage | | Lactation | Region |
| Cr (µg/L) | I | 193.16 ± 12.94 | 219.14 ± 11.11 | 200.97 ± 16.12 | 209.58 ± 8.21$^{AC}$ | 0.174 | 0.018 |
| | II | 218.30 ± 20.62 | 196.33 ± 8.87 | 166.10 ± 7.70 | 193.65 ± 7.75$^{BC}$ | | |
| | III | 251.02 ± 33.88 | 222.49 ± 19.9 | 207.02 ± 27.77 | 223.63 ± 9.14$^{A}$ | | |
| | IV | 189.33 ± 19.5 | 207.58 ± 14.43 | 176.85 ± 13.76 | 187.40 ± 7.80$^{B}$ | | |
| | **Mean±SD** | 212.69 ± 12.06 | 209.64 ± 7.01 | 188.36 ± 9.08 | | | |
| Cu (µg/L) | I | 151.61 ± 13.3 | 114.78 ± 7.41 | 127.13 ± 12.41 | 129.44 ± 7.63$^{A}$ | 0.102 | 0.014 |
| | II | 115.88 ± 11.85 | 113.54 ± 5.00 | 132.63 ± 12.78 | 122.91 ± 8.11$^{A}$ | | |
| | III | 129.93 ± 12.75 | 120.46 ± 13.50 | 110.82 ± 7.95 | 121.54 ± 10.21$^{A}$ | | |
| | IV | 111.67 ± 11.21 | 99.54 ± 11.58 | 88.19 ± 6.84 | 101.18 ± 8.05$^{B}$ | | |
| | **Mean±SD** | 123.43 ± 7.31 | 110.39 ± 5.90 | 116.41 ± 6.39 | | | |
| Fe (mg/L) | I | 4.95 ± 0.33 | 6.58 ± 0.32 | 6.13 ± 0.27 | 5.96 ± 0.21$^{A}$ | 0.302 | <0.001 |
| | II | 6.25 ± 0.54 | 6.30 ± 0.46 | 5.18 ± 0.46 | 5.65 ± 0.19$^{AC}$ | | |
| | III | 5.13 ± 0.30 | 5.63 ± 0.36 | 5.35 ± 0.21 | 5.22 ± 0.22$^{BC}$ | | |
| | IV | 4.37 ± 0.34 | 4.51 ± 0.46 | 4.53 ± 0.39 | 4.45 ± 0.17$^{B}$ | | |
| | **Mean±SD** | 5.13 ± 0.20 | 5.61 ± 0.22 | 5.23 ± 0.20 | | | |
| Se (µg/L) | I | 180.30 ± 18.54 | 202.35 ± 13.51 | 158.42 ± 13.65 | 178.57 ± 12.45$^{A}$ | 0.044 | 0.002 |
| | II | 178.14 ± 18.07 | 160.16 ± 15.70 | 159.75 ± 13.37 | 174.26 ± 13.13$^{A}$ | | |
| | III | 164.24 ± 19.33 | 188.06 ± 15.03 | 146.41 ± 15.36 | 163.83 ± 11.39$^{A}$ | | |
| | IV | 118.81 ± 12.77 | 121.35 ± 19.10 | 126.25 ± 13.51 | 122.53 ± 8.69$^{B}$ | | |
| | **Mean±SD** | 160.90 ± 37.91$^{a}$ | 166.29 ± 11.24$^{a}$ | 144.25 ± 7.11$^{b}$ | | | |
| Zn (mg/L) | I | 10.65 ± 1.14 | 9.10 ± 0.88 | 8.20 ± 0.72 | 9.85 ± 0.62 | 0.202 | 0.191 |
| | II | 10.36 ± 0.90 | 9.56 ± 0.75 | 11.52 ± 1.47 | 9.37 ± 0.70 | | |
| | III | 11.80 ± 1.97 | 13.46 ± 2.07 | 8.63 ± 1.36 | 11.31 ± 1.02 | | |
| | IV | 8.74 ± 1.36 | 10.60 ± 1.69 | 10.87 ± 1.81 | 11.16 ± 0.69 | | |
| | **Mean±SD** | 11.48 ± 1.24 | 12.00 ± 1.14 | 9.38 ± 1.02 | | | |

I, II, III, and IV represent the Iskenderun industrial areas, Antakya industrial areas, Iskenderun non-industrial areas, and Antakya non-industrial areas, respectively. PTE: Potentially toxic element, Cr: Chromium, Cu: Copper, Fe: Iron, Se: Selenium, Zn: Zinc. $^{a}$, $^{b}$ : Different letters in the same line indicate significant difference between regions (P < 0.05). $^{A}$ , $^{B}$ , $^{C}$ : Different letters in the same column indicate significant difference between regions (P < 0.05) coefficients. IBM SPSS Version 23.0 (IBM Corp., Armonk, USA) package programme was used in all statistical analyses [26]. The statistical significance limit was accepted as P < 0.05.

## RESULTS AND DISCUSSION

The levels of essential PTEs determined in milk samples are presented in TABLE I. It was determined that Cr, Cu, Fe, Se, and Zn levels were found to be in the range of 166.10-251.02 µg/L, 88.19-151.61 µg/L, 4.37-6.58 mg/L, 118.81-202.35 µg/L, and 8.20-13.46 mg/L in regions and all lactation stages, respectively.

In terms of Cr, Cu, Fe, and Se levels, region IV was found to be significantly lower than the other regions (P < 0.05). However, there was no significant difference between regions in terms of Zn levels. In addition, in terms of Se element, it was determined that the 3rd lactation stage was significantly lower than the other lactation stages (P < 0.05).

The levels of non-essential PTEs determined in milk samples are presented in TABLE II. It was determined that Al and As levels were found to be in the range of 4.68-7.47 mg/L and 40.09-64.86 µg/L in regions and all lactation stages, respectively. In terms of Al and As levels, region IV was found to be significantly lower than the other regions (P < 0.05). It was determined that Ni and Pb levels were found to be in the range of 215.09-415.95 µg/L and 0.94-1.62 mg/L in regions and all lactation stages, respectively. In terms of Ni and Pb levels, region IV was found to be significantly higher than the other regions (P < 0.05).

Cd and Hg elements were not detected in milk, water, and feed samples from regions. The levels of essential and non-essential PTEs detected in drinking water and feed samples are presented in TABLES III and IV, respectively.

**TABLE II**

***Mean concentrations of non-essential potentially toxic element in raw milk samples from industrial and non-industrial areas***

| Non-essential PTEs | Region | Lactation Stage | | | Mean±SD | P | |
|---|---|---|---|---|---|---|---|
| | | 1st stage | 2nd stage | 3rd stage | | Lactation | Region |
| Al (mg/L) | I | 6.72 ± 0.33 | 6.70 ± 0.26 | 6.41 ± 0.28 | 6.53 ± 0.21[A] | 0.266 | < 0.001 |
| | II | 7.03 ± 0.38 | 7.03 ± 0.23 | 6.21 ± 0.19 | 6.59 ± 0.20[A] | | |
| | III | 7.47 ± 0.75 | 7.18 ± 0.61 | 6.18 ± 0.37 | 6.93 ± 0.19[A] | | |
| | IV | 4.68 ± 0.41 | 5.09 ± 0.50 | 5.38 ± 0.47 | 4.62 ± 0.22[B] | | |
| | **Mean±SD** | 6.33 ± 0.24 | 6.29 ± 0.26 | 5.89 ± 0.17 | | | |
| As (µg/L) | I | 59.75 ± 4.41 | 52.13 ± 2.66 | 64.86 ± 3.54 | 56.62 ± 2.30[A] | 0.703 | < 0.001 |
| | II | 57.89 ± 2.77 | 63.43 ± 3.20 | 53.32 ± 2.96 | 58.42 ± 1.92[A] | | |
| | III | 57.91 ± 2.28 | 48.46 ± 2.30 | 53.05 ± 3.49 | 54.96 ± 2.48[A] | | |
| | IV | 40.09 ± 3.67 | 44.83 ± 3.62 | 43.71 ± 3.21 | 42.96 ± 2.78[B] | | |
| | **Mean±SD** | 53.88 ± 1.95 | 52.00 ± 2.07 | 54.00 ± 1.84 | | | |
| Ni (µg/L) | I | 261.65 ± 21.45 | 215.09 ± 7.34 | 300.93 ± 27.25 | 262.44 ± 21.69[B] | 0.315 | 0.003 |
| | II | 304.22 ± 39.31 | 342.57 ± 38.86 | 247.74 ± 14.02 | 295.90 ± 21.10[B] | | |
| | III | 306.41 ± 40.13 | 284.93 ± 35.72 | 292.73 ± 29.38 | 307.91 ± 23.16[B] | | |
| | IV | 341.51 ± 52.90 | 415.95 ± 60.54 | 273.79 ± 49.76 | 345.73 ± 21.16[A] | | |
| | **Mean±SD** | 305.66 ± 22.43 | 322.53 ± 21.92 | 281.22 ± 16.70 | | | |
| Pb (mg/L) | I | 0.94 ± 0.07 | 1.11 ± 0.06 | 1.06 ± 0.04 | 1.01 ± 0.05[B] | 0.808 | < 0.001 |
| | II | 0.98 ± 0.08 | 1.33 ± 0.17 | 1.16 ± 0.12 | 1.16 ± 0.04[C] | | |
| | III | 0.94 ± 0.10 | 1.15 ± 0.09 | 1.11 ± 0.10 | 1.06 ± 0.02[B] | | |
| | IV | 1.34 ± 0.11 | 1.55 ± 0.07 | 1.62 ± 0.10 | 1.47 ± 0.07[A] | | |
| | **Mean±SD** | 1.20 ± 0.06 | 1.29 ± 0.05 | 1.13 ± 0.07 | | | |

I, II, III, and IV represent the Iskenderun industrial areas, Antakya industrial areas, Iskenderun non-industrial areas, and Antakya non-industrial areas, respectively. PTE: Potentially toxic element, Al: Aluminum, As: Arsenic, Ni: Nickel, Pb: Lead. [A], [B], [C]: Different letters in the same column indicate significant difference between regions (P < 0.05).

**TABLE III**

***Mean concentrations (µg/L) of essential and non-essential potentially toxic element in water samples***

| | Industrial Areas | | Non-industrial Areas | | |
|---|---|---|---|---|---|
| | I | II | III | IV | P |
| **Essential PTEs** | | | | | |
| Cr | 13.22 ± 3.09[bc] | 18.04 ± 2.70[b] | 27.97 ± 4.00[a] | 7.54 ± 1.98[c] | < 0.001 |
| Cu | 4.60 ± 1.21[b] | 2.56 ± 0.53[b] | 3.37 ± 0.66[b] | 8.74 ± 2.54[a] | 0.019 |
| Fe | 24.11 ± 1.04[b] | 22.11 ± 0.91[b] | 24.50 ± 1.50[b] | 29.33 ± 1.97[a] | 0.004 |
| Se | 4.76 ± 0.94[b] | 9.50 ± 1.07[a] | 2.17 ± 0.11[c] | 3.59 ± 0.40[bc] | < 0.001 |
| Zn | 99.02 ± 34.59 | 88.54 ± 19.12 | 20.66 ± 4.22 | 83.82 ± 16.54 | 0.071 |
| **Non-essential PTEs** | | | | | |
| Al | 6.81 ± 0.81 | 5.36 ± 0.57 | 5.14 ± 0.44 | 4.62 ± 0.29 | 0.053 |
| As | 2.23 ± 0.15 | 1.82 ± 0.16 | 1.89 ± 0.13 | 1.98 ± 0.15 | 0.242 |
| Ni | 3.78 ± 0.51 | 3.78 ± 0.43 | 2.99 ± 0.38 | 3.19 ± 0.32 | 0.416 |
| Pb | 1.58 ± 0.23 | 0.90 ± 0.18 | 1.35 ± 0.17 | 1.07 ± 0.15 | 0.083 |

I, II, III, and IV represent the Iskenderun industrial areas, Antakya industrial areas, Iskenderun non-industrial areas, and Antakya non-industrial areas, respectively. PTE: Potentially toxic element, Cr: Chromium, Cu: Copper, Fe: Iron, Se: Selenium, Zn: Zinc, Al: Aluminum, As: Arsenic, Ni: Nickel, Pb: Lead. The results were expressed as a mean±standard deviation (SD). [a], [b], [c]: Different letters in the same line indicate significant difference between regions (P < 0.05)

**TABLE IV**

***Mean concentrations of essential and non-essential potentially toxic element in feed samples***

| | Industrial Areas | | Non-industrial Areas | | |
|---|---|---|---|---|---|
| | I | II | III | IV | P |
| **Essential PTEs** | | | | | |
| Cr | $13.06 \pm 1.22^{a}$ | $8.92 \pm 1.78^{b}$ | $8.05 \pm 1.28^{b}$ | $9.00 \pm 1.10^{b}$ | 0.049 |
| Cu | $25.72 \pm 0.97^{b}$ | $24.05 \pm 1.21^{b}$ | $27.04 \pm 1.48^{b}$ | $30.75 \pm 1.84^{a}$ | 0.008 |
| Fe | $283.78 \pm 19.09^{a}$ | $200.66 \pm 6.83^{b}$ | $203.55 \pm 9.78^{b}$ | $214.03 \pm 10.33^{b}$ | < 0.001 |
| Se | $1.74 \pm 0.06^{a}$ | $1.69 \pm 0.09^{b}$ | $2.20 \pm 0.10^{a}$ | $1.85 \pm 0.09^{a}$ | < 0.001 |
| Zn | $170.55 \pm 7.93^{b}$ | $134.91 \pm 7.57^{bc}$ | $154.59 \pm 9.40^{c}$ | $187.50 \pm 13.57^{a}$ | 0.002 |
| **Non-essential PTEs** | | | | | |
| Al | $378.78 \pm 38.33^{a}$ | $233.46 \pm 19.73^{b}$ | $273.75 \pm 37.16^{b}$ | $212.90 \pm 25.77^{b}$ | 0.001 |
| As | $713.16 \pm 29.12^{a}$ | $672.93 \pm 16.79^{a}$ | $714.16 \pm 25.17^{a}$ | $556.34 \pm 14.06^{b}$ | < 0.001 |
| Ni | $34.00 \pm 5.67^{a}$ | $13.93 \pm 2.36^{b}$ | $18.45 \pm 5.38^{b}$ | $32.93 \pm 5.26^{b}$ | 0.004 |
| Pb | $13.05 \pm 0.71^{a}$ | $8.53 \pm 0.43^{b}$ | $8.30 \pm 0.47^{b}$ | $7.94 \pm 0.55^{b}$ | < 0.001 |

I, II, III, and IV represent the Iskenderun industrial areas, Antakya industrial areas, Iskenderun non-industrial areas, and Antakya non-industrial areas, respectively. PTE: Potentially toxic element, Cr: Chromium, Cu: Copper, Fe: Iron, Se: Selenium, Zn: Zinc, Al: Aluminum, As: Arsenic, Ni: Nickel, Pb: Lead. The results were expressed as a mean±standard deviation (SD). Mean concentration of As was expressed as µg/kg, other elements were expressed as mg/kg. $^{a}$, $^{b}$, $^{c}$: Different letters in the same line indicate significant difference between regions ($P < 0.05$)

Studies revealing the presence and potential sources of essential and non-essential PTEs in milk produced in Türkiye are limited. One of the strong aspects of the study is that the farms where cow's milk is collected are close to industrial zones. In this study, the highest average As, Cu, Fe, and Se levels were recorded in lactating cows' milk reared around industrial units. It can be said that this situation is due to the industrial activities maintained in the region.

As is a potential cumulative toxic compound released into the environment due to industrial emissions and affecting milk production plants [27]. As in the present study, there are similar studies showing that As contamination in the milk of cows reared close to the areas where industrial activities are maintained is caused by industrial emissions [7, 11, 13, 15, 28].

Essential elements such as Cu, Fe, Se, and Zn are necessary for the maintenance of physiological functions in the body [10]. However, exposure to these elements at high levels can lead to adverse health effects [27]. High concentrations of Cu and Fe detected in other studies, as in the current study, have been attributed to environmental pollution.

Castro-González *et al*. [4] in Mexico and Sai-Chaithanya *et al*. [29] in India reported Cu contamination in the milk of cows grazing in areas where wastewater is used. It was also reported by Iftikhar *et al*. [9] in Pakistan, Shahbazi *et al*. [10] in Iran, and Monteverde *et al*. [30] in Italy that the average Cu levels in milk produced in areas where industrial activities were maintained were higher than those in milk produced in non-contaminated areas, as in the present study, and it can be argued that this is due to emissions from maintained industrial activities and climate factors such as wind in the region.

Consistent with the present study, Forcada *et al*. [7] reported that the average Fe level in milk samples collected from farms close to industrial zones in Spain was higher than that in milk samples collected from farms far from industrial zones. Similarly, Simsek *et al*. [14] reported that the aveage Fe level in cow's milk collected from farms close to industrial zones in Türkiye was 4.27 mg/kg, while it was 1.01 mg/kg in milk collected from rural areas. Similarly, different studies on milk from cows reared on farms near industrial areas have shown that environmental contamination contributes to the Fe content in milk [29, 30].

The high level of Se detected in raw milk in industrial areas in this study is consistent with findings from other studies. In a recent study in Spain similar to the current study, it was reported that the average Se level in cow's milk collected from farms near industrial areas was determined to be 28.7 µg/kg, and this value was higher than the average value determined in farms far from the contaminated area [7].

This supports that the presence of the industrial activities significantly increases environmental contamination with Se, which is reflected in the higher levels of Se contamination in the milk produced. No studies regarding the monitoring of PTE concentrations in the milk of lactating cows were found in the literature. Although cows raised in the same area are exposed to the same level of environmental pollution, they may have differences in transferring elements to their milk [12].

It has been reported that besides environmental pollution, the lactation periods of cows also have a significant effect on this variability [31]. In this study, it can be considered that the reason for the higher average Se concentrations in cow's milk in the 2nd lactation stage is due to the animals being exposed to high amounts of Se through drinking water, feed, or air during this period. When lactating cows are fed with contaminated feeds, the transfer of exposed elements to milk varies considerably [32]. As a result, it can be concluded that the lactation stage affects the element concentrations in cow's milk.

Correlation coefficients showing the relationship between essential and non-essential PTE levels in milk samples and essential and non-essential PTE levels in water and feed are presented in TABLE V.

In the reported studies, findings showing the link between PTE contents in cow's milk and the regions where the milk is produced have been revealed. Various studies have also reported that the levels of PTE in the feed and drinking water consumed by animals may contribute to levels of PTE in milk [9, 33].

In the current study, positive correlations were observed between Pb concentrations in region I and Cu concentrations in region II in the collected milk and water samples. Similarly, positive correlations between Pb and Cu concentrations detected in raw cow's milk and water samples were reported in a study conducted by Abdou *et al*. [34]. Therefore, it can be said that the concentrations of these elements in drinking water in the specified regions contribute to their concentrations in milk. In the present study, as seen with between Pb levels in feed and milk in region III, a positive correlation between Pb levels in silage and milk have also been found by Iftikhar *et al.* [9] and Zhou *et al.* [12].

These findings reveal that feed plants grown in PTE-contaminated soils accumulate these elements at high levels and can contribute to their content in milk. Similar to the results by Zhou *et al*. [6], in the current study as well, the Al concentrations in milk in region II can be attributed to the Al concentrations in the water and feed. PTE levels in milk vary depending on their concentrations in animal feeds [9]. It was determined that the feeds consumed by animals in region I contained the highest level of Ni and that this significantly contributed to the Ni content in milk when looking at its correlation between milk and feed.

The EDI and THQ values calculated for essential and non-essential PTEs in milk samples are presented in TABLE VI. The ranking of EDI values of essential and non-essential PTEs was determined as Zn > Fe > Al > Pb > Ni > Cr > Se > Cu > As. According to the non-carcinogenic risk assessment, it was reported that the THQ values of essential and non-essential PTEs were in the range of 0.004604-23.54171, and the order of the determined values was Al > Pb > As > Cr > Zn > Se > Ni > Fe > Cu. In milk samples, THQ values of all PTEs except Al element were found to be below 1. This indicates that exposure to Al through milk consumption may pose potential adverse health risks to human health.

**TABLE V**

***Correlation coefficients between the essential and non-essential potentially toxic element concentrations in milk with that in drinking water and animal feed***

| | | Industrial Areas | | | | Non-industrial Areas | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | I | | II | | III | | IV | |
| Milk | | Water | Feed | Water | Feed | Water | Feed | Water | Feed |
| **Essential PTEs** | | | | | | | | | |
| Cr | *r* | 0.075 | -0.346 | -0.153 | 0.065 | -0.083 | 0.077 | 0.243 | -0.581 |
| | P | 0.599 | 0.010 | 0.274 | 0.643 | 0.560 | 0.621 | 0.089 | < 0.001 |
| Cu | *r* | 0.009 | 0.258 | 0.438 | -0.100 | 0.014 | 0.154 | 0.074 | -0.178 |
| | P | 0.953 | 0.076 | 0.004 | 0.471 | 0.929 | 0.284 | 0.628 | 0.208 |
| Fe | *r* | 0.171 | -0.223 | 0.061 | 0.100 | -0.060 | 0.530 | -0.341 | -0.082 |
| | P | 0.218 | 0.137 | 0.677 | 0.479 | 0.672 | <0.001 | 0.012 | 0.553 |
| Se | *r* | -0.237 | 0.079 | 0.178 | 0.232 | 0.225 | 0.210 | 0.220 | 0.254 |
| | P | 0.344 | 0.571 | 0.321 | 0.091 | 0.209 | 0.128 | 0.218 | 0.063 |
| Zn | *r* | -0.453 | -0.056 | -0.130 | -0.253 | -0.176 | 0.200 | 0.023 | -0.023 |
| | P | 0.059 | 0.699 | 0.526 | 0.073 | 0.484 | 0.174 | 0.903 | 0.904 |
| **Non-essential PTEs** | | | | | | | | | |
| Al | *r* | 0.141 | 0.217 | 0.523 | 0.331 | -0.036 | 0.023 | 0.091 | -0.248 |
| | P | 0.308 | 0.142 | < 0.001 | 0.018 | 0.798 | 0.873 | 0.568 | 0.070 |
| As | *r* | -0.282 | -0.346 | 0.143 | 0.018 | 0.269 | -0.159 | 0.082 | 0.146 |
| | P | 0.082 | 0.016 | 0.350 | 0.895 | 0.151 | 0.270 | 0.597 | 0.296 |
| Ni | *r* | 0.218 | 0.314 | 0.123 | -0.078 | -0.046 | -0.077 | -0.001 | -0.340 |
| | P | 0.133 | 0.023 | 0.384 | 0.583 | 0.748 | 0.624 | 0.996 | 0.012 |
| Pb | *r* | 0.644 | -0.171 | 0.097 | -0.068 | 0.178 | 0.315 | 0.185 | 0.047 |
| | P | 0.002 | 0.234 | 0.765 | 0.634 | 0.580 | 0.024 | 0.510 | 0.734 |

I, II, III, and IV represent the Iskenderun industrial areas, Antakya industrial areas, Iskenderun non-industrial areas, and Antakya non-industrial areas, respectively. PTE: Potentially toxic element, Cr: Chromium, Cu: Copper, Fe: Iron, Se: Selenium, Zn: Zinc, Al: Aluminum, As: Arsenic, Ni: Nickel, Pb: Lead. A value of P < 0.05 was considered statistically significant. r: Correlation coefficients

**TABLE IV**

***Estimated daily intake (mg/L bw/day) and target hazard quotient values of essential and non-essential potentially toxic element in milk samples***

| | EDI | THQ |
|---|---|---|
| Essential PTEs | | |
| Cr | 0.35 | 0.117026 |
| Cu | 0.18 | 0.004604 |
| Fe | 13.01 | 0.018588 |
| Se | 0.23 | 0.046837 |
| Zn | 16.37 | 0.054559 |
| Non-essential PTEs | | |
| Al | 9.42 | 23.54171 |
| As | 0.08 | 0.267868 |
| Ni | 0.88 | 0.043998 |
| Pb | 1.83 | 0.456485 |

EDI: Estimated daily intake. bw: Body weight. THQ: Target hazard quotient. PTE: Potentially toxic element, Cr: Chromium, Cu: Copper, Fe: Iron, Se: Selenium, Zn: Zinc, Al: Aluminum, As: Arsenic, Ni: Nickel, Pb: Lead

## CONCLUSION

This is the first study conducted in Türkiye on the contamination of cow's milk with essential and non-essential PTEs from drinking water and feed consumption and proximity to industrial activities, and the assessment of health risks that could arise from exposure to these elements through milk consumption in adults. The study revealed that exposure to PTEs, except for the Al element, did not lead to non-carcinogenic health risks for individuals in the consumption region.

However, although it has been stated that exposure to Al element through milk consumption may cause health issues in the short term, it should be known that other PTEs may cause serious health issues in the long term due to their cumulative effects. Additionally, the study only assessed the risk arising from milk consumption. However, it should also be considered that PTE exposures that may occur as a result of consuming different foods may lead to health risks.

Therefore, in order to ensure food safety, it is recommended to determine permitted safe levels for PTEs not only in raw cow's milk but also in all foods. In addition, considering the toxic effects of carcinogenic elements such as Pb, As, Cd, Hg, and Al, particularly the areas where industrial activities are maintained and possible contamination sources should be regularly monitored.


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### Conflict of interest statement

The authors have no relevant financial or non-financial interests to disclose.


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