



## PRESENCE OF HEAVY METALS IN RAW BOVINE MILK FROM MACHACHI, ECUADOR

### PRESENCIA DE METALES PESADOS EN LECHE CRUDA BOVINA DE MACHACHI, ECUADOR

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#### Abstract

The presence of heavy metals in milk is an indicator of local environmental contamination. The objective of this investigation was to test raw milk from Machachi, Pichincha Province, Ecuador for the presence of lead, mercury, and arsenic. Fifty eight samples were collected from twenty nine dairy farms with extensive grazing systems located up to one kilometer from the Municipality of Machachi. Machachi is a site of industrial activity and is located near the Panamericana Sur highway. The samples were analyzed using atomic absorption spectrophotometry with a hydride generator (mercury and arsenic) and a graphite furnace (lead). All analyzed samples indicated the presence of lead, with an average abundance of  $0.208 \text{ mg kg}^{-1}$  (range between  $0.0016$  to  $0.719 \text{ mg kg}^{-1}$ ). Of these samples, 98.28% (57/58) contained lead at levels higher than the maximum allowed by the NTE INEN 9,  $0.02 \text{ mg kg}^{-1}$ . Mercury was detected in four samples with a mean abundance of  $0.00009 \text{ mg kg}^{-1}$  (range between  $0.00$  to  $0.002 \text{ mg kg}^{-1}$ ); and arsenic was detected in two samples with a mean abundance of  $0.00003 \text{ mg kg}^{-1}$  ( $0.00$  to  $0.001 \text{ mg kg}^{-1}$ ). Although these levels are very low, they remain worrisome because these carcinogenic elements are capable of accumulating. Based on these results, it can be concluded that lead contamination has occurred in the studied area. Furthermore, the detection of arsenic and mercury, two highly toxic substances, warrants continuous monitoring of the regional milk supply and a search for possible sources of contamination.

**Keywords:** Lead, Arsenic, Mercury, raw milk, Machachi

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**Resumen**

La evaluación de metales pesados en la leche puede considerarse como indicador de contaminación ambiental de un lugar, por lo que el objetivo de la investigación fue determinar la presencia de plomo, mercurio y arsénico en leche cruda de Machachi, Provincia de Pichincha-Ecuador. Se recolectaron 58 muestras provenientes de 29 fincas lecheras con sistema de pastoreo extensivo y ubicadas hasta máximo un kilómetro a la redonda del Municipio de Machachi, donde existe actividad industrial y está cerca de la Panamericana Sur. Las muestras fueron analizadas mediante la técnica de espectrofotometría de absorción atómica por generador de hidruros (mercurio y arsénico) y con horno de grafito (plomo). Todas las muestras analizadas mostraron niveles de plomo, con una media de  $0,208 \text{ mg kg}^{-1}$  (rango entre  $0,0016$  a  $0,719 \text{ mg kg}^{-1}$ ), de las cuales el 98,28% (57/58) contienen niveles superiores a los máximos permitidos por la NTE INEN 9 de  $0,02 \text{ mg kg}^{-1}$ . También se detectó mercurio y arsénico en cuatro y dos muestras de leche, respectivamente, encontrándose en el primer caso en una media de  $0,00009 \text{ mg kg}^{-1}$  (rango entre  $0,00$  a  $0,002 \text{ mg kg}^{-1}$ ) y en el segundo caso un promedio de  $0,00003 \text{ mg kg}^{-1}$  ( $0,00$  a  $0,001 \text{ mg kg}^{-1}$ ), y aunque los niveles son muy bajos, los mismos son bastante preocupantes ya que son capaces de acumularse y ser potencialmente cancerígenos. Con los resultados obtenidos, se concluye que se ha detectado contaminación por plomo en el área estudiada, pero sobre todo por dos sustancias altamente tóxicas (arsénico y mercurio), para lo cual es necesario un monitoreo continuo en la leche, y sobre todo para buscar posibles fuentes de contaminación.

**Palabras clave:** Plomo, Arsénico, Mercurio, leche cruda, Machachi

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## 1 Introduction

In 2018, Ecuadorian milk production reached ~5 million liters per day, with more than 70% of that being produced in the inter-Andean alley. Pichincha is the largest milk-producing province, with its milk accounting for approximately 16% of the total Ecuadorian milk supply (INEC, 2019). The Canton Mejía has historically been the largest dairy in Pichincha Province, and in all of Ecuador, largely due to its climatic and soil conditions, which are very favorable for livestock development (CIL, 2015). Ecuadorian Technical Standard (NTE) INEN 9 (INEN, zC5C) and Codex Alimentarius (Codex-Alimentarius, 1995) set environmental standards for raw milk, and set the maximum permitted level of lead (Pb) at 0.02 mg/kg. NTE INEN 9 does not indicate the maximum permitted levels for mercury (Hg) or arsenic (As); but NTE INEN 1108, which deals with drinking water, sets a maximum limit of 0.006 mg/L for Hg and 0.01 mg/L for As (INEN, 2011).

The presence of heavy metals in food is a critical public health concern since these elements cause a number of health problems in humans, animals, and agricultural crops (Anastasio et al., 2006). In humans, heavy metals disrupt the functions of the nervous system, the liver, and the kidney; while also promoting mutagenesis and carcinogenesis. In animals, heavy metals can cause a loss of appetite, anemia, reproductive disorders, cancer, and teratogenesis. All of these effects decrease production yield in the long run (González-Montaña, 2009). The primary source of heavy metals is environmental pollution due to the presence of various industrial activities (Zhou et al., 2019). These industrial activities pollute the soil (Ashraf et al., 2019), the water, and the air; and eventually reach people and animals through the food chain. Thus, this pollution greatly harms the health of consumers (Karasakal, 2020). Currently, this contamination is measured through the use of biomonitoring, within which the surveillance of animal products plays an important role (Scaramozzino et al., 2019). In the case of dairy farming, heavy metals can contaminate the water and food of dairy cows, eventually being transferred to their milk (Zhou et al., 2019). This constitutes a major public health problem, especially for vulnerable populations like children (Chirinos-Peinado and Castro-Bedriñana, 2020), be-

cause even low levels of heavy metals incorporated into a diet can lead to chronic illness (Miclean et al., 2019). Thus, monitoring locally-produced raw milk for the presence of heavy metals is a useful indication of environmental contamination in the region (Miclean et al., 2019).

Lead (Pb) is frequently produced by the radioactive decomposition of uranium and actinium, and can be found in the soil (Silva et al., 2010). However, lead can also be introduced into the environment through other avenues such as the use of lead-containing equipment, chemical fertilizers and pesticides, or lead-contaminated water (Litter et al., 2016). Lead is one of the most frequent intoxicants of cattle; even more so in young calves since these animals lack a functional rumen microflora (Perrin et al., 1990). In the case of arsenic, it can be distributed in water, air, and soil. Arsenic is very toxic in its inorganic form, and can cause skin changes and even cancer (WHO, 2018). The presence of mercury is also a matter of importance due to its ability to contaminate and damage the trophic chain. Chronic exposure to this metal can result in asymptomatic toxicity (Rodríguez et al., 2010). Mercury is the only volatile metal, so it is easily absorbed through the skin and lungs. Its presence in the environment is mainly due to human activity, and is commonly introduced by heating systems, mining, and other industrial processes (WHO, 2019).

Air pollution is composed of fine particulate matter that can combine and transport other toxic substances, such as heavy metals. This enables transportation of these metals through the air and allows them to be deposited in irrigation water, agricultural soils, and grass (Dergham et al., 2012; Alloway, 2013; Yilmaz et al., 2009). In the context of dairy cows, if the animal forages on heavy metal-contaminated materials, those toxic metals are introduced to the animal and eventually are passed into its milk (Miclean et al., 2019). It is essential to study this process (de Oliveira et al., 2017) in order to estimate toxicological effects (Samiee et al., 2019) and safeguard public health (Miclean et al., 2019). Thus far, few studies have investigated the presence of heavy metals in raw milk from Ecuador, despite the utmost importance of this monitoring process in order to prevent food contamination (Kim et al., 2016) and the transfer of heavy metals to consumers (Hashemi, 2018). The objective of this study

was to quantify the levels of lead, arsenic, and mercury in raw milk produced in the largest dairy basin in Ecuador.

## 2 Materials and Methods

### 2.1 Study area location

The study was carried out within the parish of Machachi, Mejía Canton, Pichincha Province, Ecuador. 58 raw milk samples were randomly collected from 29 dairy farming production units (UPAs) with ex-

tensive grazing systems located within one kilometer of the city of Machachi. Machachi is a center of industrial activity with a particular emphasis on steel production, and lies close to the Panamericana Sur highway. The UPAs are all located near -0.510110, -78.567123, and occur at an altitude of approximately 2900 meters above sea level (GeoDatos, 2019). The region experiences temperatures from 12 to 20°C, and is generally characterized as having a cold-temperate climate. The geographical location given was provided using georeferencing based on the global positioning system (GPS), as shown in Figure 1.

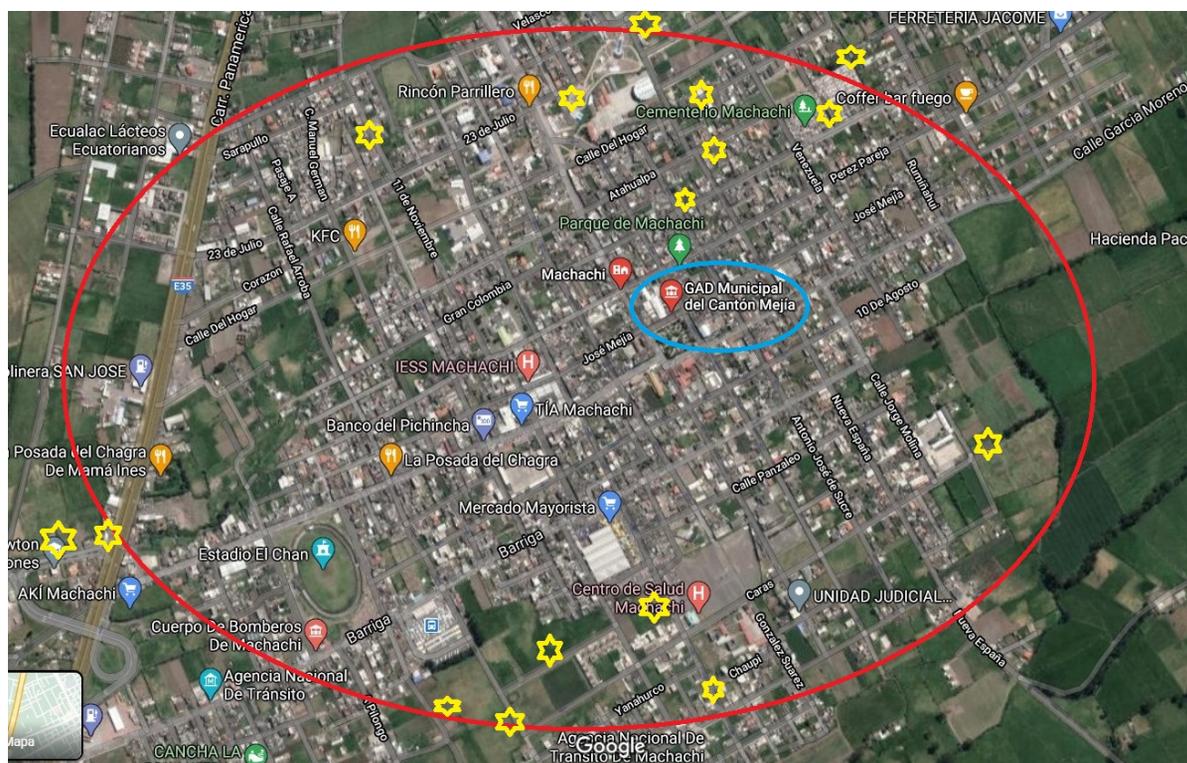


Figure 1. UPAs sampled in the Machachi Parish.

### 2.2 Sampling

Approximately 250 ml of raw milk were taken directly from the refrigeration tanks, buckets, or collection containers at each UPA. This sampling operation was repeated again within the span of eight days and was carried out in accordance with NTE INEN-ISO 707: "Milk and dairy products. Guidelines for sampling" (INEN, VdOC). The samples

were transported in thermoses containing frozen gel refrigerants between 2 and 5 oC. Upon arrival at the Laboratory of the Department of Petroleum, Energy, and Contamination (DPEC) of the Faculty of Chemical Engineering of the Central University of Ecuador, the samples were stored at 20°C until analysis.

### 2.3 Analytical procedure

For the determination of Hg and As levels, a hydride generator atomic absorption spectrophotometry technique was used (Rocha, 2011). The determination of Pb levels was carried out using the same atomic absorption spectrophotometry approach, but with a graphite furnace. Technical operations were performed in accordance with Ecuadorian Technical Specification INEN-ISO / TS 6733 (INEN, 2014).

A UNICAM Solar model 9626 spectrophotometer was used. Hollow cathode lamps were used for each element analyzed, with aliquot vaporization being accomplished using an air-acetylene flame. Deionized water was utilized for all analytical processes. Calibration curves were created using certified standards, and blank solutions were prepared and treated identically as samples. The limit of detection (LOD) and the limit of quantification (LOQ) for Pb were 0.1 mg/L and 0.8 mg/L, respectively. For As, the LOD was 0.39  $\mu\text{g/L}$  and the LOQ was 1.19  $\mu\text{g/L}$ . The LOD for mercury was 0.14  $\mu\text{g/L}$  and the LOQ was 0.42  $\mu\text{g/L}$ . All samples were analyzed in triplicate. Respective calibration curves were prepared using the following concentrations: Pb = 0.0, 0.5, 1.5, 3.0, and 5.0 mg/L; Hg = 1.0, 2.5, 5.0, 10.0, 25.0 and 50.0  $\mu\text{g/L}$ ; As = 0, 1, 2, 5, and 10  $\mu\text{g/L}$ .

### 2.4 Statistical analysis

The results are expressed as the mean, the minimum, and the maximum observed concentration of Pb, As, or Hg. A Shapiro-Wilk test was performed to check the data for normality. The results of these

Regarding the abundance of Hg, 6.9% (4/58) of the raw milk samples contained detectable levels. The mean level of mercury was 0.00009 mg/kg, with a minimum value of 0.00 mg/kg, and a maximum value of 0.002 mg/kg (found in the first sampling). No significant differences in mercury abundance were detected between suppliers or between samplings (Table 1). Also, 3.44% (2/58) of analyzed raw milk samples contained detectable levels of As, with a mean value of 0.00003 mg/kg, a minimum value of 0.00 mg/kg, and a maximum value of 0.001 mg/kg. No significant differences in mercury abundance were detected between suppliers or between samplings (Table 1).

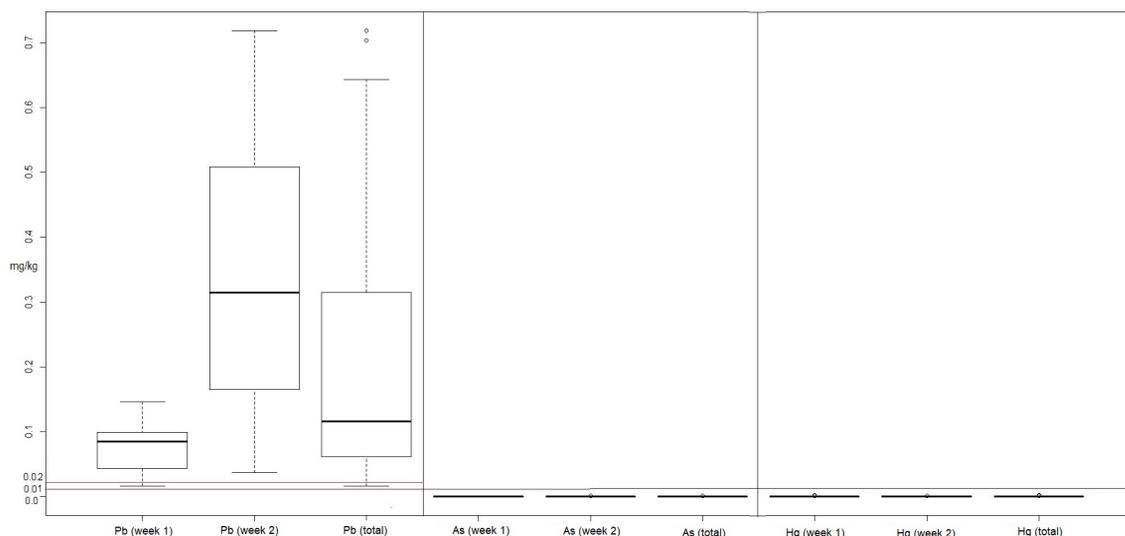
tests indicated that the data were not normally distributed ( $p \leq 0,05$ ). For this reason, non-parametric statistical tests were used (Celis De La Rosa and Labrada, 2014). A Kruskal-Wallis test was used to compare the heavy metal levels in raw milk between suppliers. A Wilcoxon test was used to compare data from the first and second samples taken from a given supplier. The open source statistical software RStudio version 1.2.5019 [RStudio Inc. Boston, MA, USA] was utilized to conduct all statistical tests. For all analyses, a significance threshold of  $p = 0,05$  was adopted.

## 3 Results

The minimum, mean, and maximum observed abundances of Pb, Hg, and As in sampled raw milk from Machachi is shown in Table 1 and Figure 2. In the case of Pb, 100% (58/58) of the raw milk samples had levels of lead above the limit of detection for this heavy metal. Of these, 98.28% (57/58) contained lead at levels higher than 0.02 mg/kg, the maximum limit established by NTE INEN 9 and the Codex Alimentarius. The mean value of lead observed in sampled raw milk was 0.208 mg/kg, with a minimum value of 0.0016 mg/kg (found in the first sample), and a maximum value of 0.719 mg/kg (from the second sample). There were no significant differences ( $p \geq 0,05$ ) observed comparing raw milk from the 29 suppliers sampled, but there were significant differences ( $p \leq 0,05$ ) between raw milk from the first sampling and raw milk from the second sampling (Table 1).

## 4 Discussion

All raw milk samples indicate the presence of Pb and 98.28% of them had lead levels higher than 0.02 mg/kg, the maximum allowed by NTE INEN 9 and the Codex Alimentarius. However, of graver concern is the presence of As and Hg in milk. Although the vast majority of samples did not contain either of these heavy metals, the fact that they were detected at all is alarming since even minute amounts are toxic to human health. Mercury, which can occur naturally (Bernhoft, 2012), has been released into the environment for centuries as the result of anthropogenic activities. Mercury has been shown to have harmful effects on various human tissues and

**Figure 2.** Box diagram of Pb, Hg, and As abundances in analyzed raw milk.

organs (Rocha et al., 2012), and also possesses genotoxic potential. As a result of this, mercury may be involved in carcinogenesis, but further studies are needed (Yang et al., 2020). In humans, arsenic exposure increases the risk of lung, skin, and bladder cancer, particularly when said exposure is chronic (Khairul et al., 2017). Arsenic is also linked to numerous other pathological conditions, which are collectively known as arsenicosis (Bjørklund et al., 2018).

The presence of heavy metals in raw milk could be the result of the dairy farms being located in an area with industrial activity that is also near an important highway (González-Montaña et al., 2019). Heavy metal levels are known to be higher in such areas (Zhou et al., 2019), and indeed heavy metal accumulation has been previously reported in alfalfa from industrially active regions (Rezaeian et al., 2020). Contamination can be introduced through various avenues including drinking water, pastures, and/or the soil itself. Determining the source of heavy metal contamination is critical to its containment and other studies have noted contamination in both the silage (Pb) and the drinking water (As and Hg) consumed by dairy cows (Zhou et al., 2017, 2019).

In Ecuador, very few studies have investigated the presence of heavy metals in milk. In one study using 20 samples of raw milk from the Arenillas Canton, El Oro Province (Ayala and Romero,

2013), Hg and As were detected (mean abundance of 0.01035 mg/kg) in 100% of the samples. While this does not coincide with the results from the present study, this contrast can perhaps be explained due to the fact that Arenillas is a mining sector unlike Machachi. With regards to Pb contamination, El Oro data demonstrated a mean abundance of 0.011 mg/kg (range of 0.006 to 0.018 mg/kg). This level of lead contamination is less than that found in the data presented herein. One possible explanation could be the presence of lead in river water that supplies the irrigation channels used by the herds. Furthermore, a study from the city of Guayaquil, found high levels of Pb contamination in powdered milk ( $5.450 \pm 2.474$  mg/kg), but no Pb was detected in pasteurized and ultrapasteurized milks from the same study.

Variability in such data is common worldwide. A meta-analysis of 72 investigations from 37 countries demonstrated that heavy metal levels varied greatly. Some of the highest Pb concentrations were observed in Brazil, Croatia, Egypt, Mexico, Nigeria, Palestine, Serbia, and Turkey. Likewise, higher levels of As and Pb were observed in milk produced using traditional systems (such as that employed in Machachi) compared to milk produced using organic systems. In the case of Hg, all levels were observed to be below the minimum risk threshold regardless of production system (Zwierzchowski and Ametaj, 2018).

**Table 1.** Minimum, mean, and maximum observed abundances of Pb, Hg, and As in sampled raw milk.

Value	Lead (mg/kg)			Mercury (mg/kg)			Arsenic (mg/kg)		
	First sampling	Second sampling	Total	First sampling	Second sampling	Total	First sampling	Second sampling	Total
Minimum	0.16	0.037	0.016	0	0	0	0	0	0
Mean	0.07631	0.339	0.20764	0.0001	3E-05	9E-05	0	0.0001	3E-05
Maximum	0.146	0.719	0.719	0.002	0.001	0.002	0	0.001	0.001
P value	1.302e-06*	0.979*	0.3045*	0.052**	0.1609*	0.492**		0.1609*	0.492**

\* Kruskal Wallis test between the 29 providers

\*\* Wilconox test between the samplings

If the results of this study are compared with other investigations from across Latin America, the variability remains large even within the same country. In the case of Brazil, Pb contamination was found exceeding the set limits in both raw milk and milk derivatives (Silva et al., 2010; de Oliveira et al., 2017; de Vasconcelos et al., 2019; Gomes et al., 2013). In Puebla, Mexico, the analysis of raw milk samples revealed the presence of Pb at a mean abundance of 0.03 mg/kg, and As at a mean abundance of 0.12 mg/kg. In both cases, heavy metal contamination exceeded the maximum allowed under the Codex Alimentarius, but occurred below the limit set by Mexican law. Samples of dairy derivatives analyzed in the same study also contained Pb and As. Therefore, both raw milk and dairy derivatives from this study may represent a health risk for consumers (Castro-González et al., 2018a).

In another study, carried out on 160 raw milk samples from Puebla and Tlaxcala, Mexico; Pb levels were found to range from  $0.039 \pm 0.02$  to  $0.059 \pm 0.05$  mg/kg, while As levels ranged from 0.029 to 0.039 mg/kg. In this region, the soil receives industrial wastewater, and pastures are mainly composed of alfalfa. These Pb levels exceed the European and FAO limits, but both the Pb and As abundances comply with Mexican regulations (Pb 0.1 mg/kg and 0.2 mg/kg for As). It is possible that these quantities were observed due to the fact that alfalfa may facilitate bioaccumulation of the heavy metals, ultimately enabling their transfer to milk (Castro-González et al., 2018b).

In a recent Peruvian study, it was determined that heavy metals can be transferred from blood to cow's milk, with Pb values in milk being 54% higher compared to levels in blood. The Pb levels observed in this study were 29 times higher than

those allowed by the Codex Alimentarius, and can be attributed to mining wastes that pollute the environment (Chirinos-Peinado and Castro-Bedriñana, 2020).

When comparing data from studies at other latitudes, results continue to vary depending on the locale. For example, in raw milk from nine regions of Korea, Cd and Pb concentrations did not exceed the limits set by Korean standards (Kim et al., 2016). In a study carried out in Asturias, Spain, raw milk from 7 dairy farms (36 samples in total) was analyzed. Arsenic was detected in only 4 samples with a mean value of  $0.01845 \pm 0.00689$  mg/kg. No Hg was found in any sample (González-Montaña et al., 2019). In milk from six Indonesian goat farms, Pb was found at levels between 50 and 80 mg/kg, while As was found at levels between 70 and 110 mg/kg (Wanniatie et al., 2019). In raw milk from Turkey, 70% of analyzed samples had Pb at levels 2.5 times higher than that allowed by European standards. In addition, 100% of the samples exhibited As contamination at levels above the maximum allowed threshold (Koyuncu and Alwazeer, 2019).

In a different study from Turkey, 90 milk samples were analyzed and failed to demonstrate any detectable levels of Pb. However, As was detected in 94.45% of the samples, albeit at levels below established standards (Totan and Filazi, 2020). A study from Romania sought to evaluate the risk of heavy metal transmission from forage to milk, but did not report the risk to public health (Miclean et al., 2019). Both Hg and As were found in raw milk from Arak, Iran; but at levels lower than the standards suggested by the Codex Alimentarius. In that study, the mean concentration of Hg was significantly higher ( $p < 0,05$ ) in milk produced on traditional farms as compared to milk produced on industrial farms

(Arianejad et al., 2015).

Even at levels below the maximum thresholds set by regulations, both Hg and As have the potential to be carcinogenic, and have the capability to accumulate further. Based on the data presented herein, heavy metal contamination remains a distinct possibility and continuous monitoring is recommended to protect public health. In order to fully address the possibility of contamination in the surveyed area, future efforts should investigate possible sources of these heavy metals, such as drinking water, irrigation water, forages, and food received by dairy cows in the area.

## 5 Conclusions

In the present investigation, 100% of analyzed raw milk samples were positive for the presence of Pb; with 98.28% of samples having Pb levels above the maximum limit set by NTE INEN 9 and the Codex Alimentarius (0.02 mg/kg). With regards to Hg and As, 6.9% and 3.44% of samples contained this heavy metal, respectively and while the vast majority of analyzed samples did not contain any Hg or As, or only showed low levels of these heavy metals, these data remain very worrisome. Based on the data presented, heavy metal contamination remains a distinct possibility and continuous monitoring is recommended to protect public health. Future efforts should be aimed at investigating possible sources of these heavy metals, such as drinking water, irrigation water, forages, and the feed that dairy cows in the area receive.

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