

Presence of arsenic in tissues and biological fluids of cattle exposed to contaminated drinking water

Presencia de arsénico en tejidos y fluidos biológicos de ganado expuesto al consumo de agua contaminada

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ABSTRACT. As concentrations in muscle, liver, milk, and hair of dairy cattle raised in northern Mexico were measured to determine whether the levels exceed those permitted by Mexican regulations (NOM). Furthermore, the possible accumulation of As in samples was evaluated in muscle, liver, milk and hair using atomic absorption spectrophotometry and a two-way ANOVA for unbalanced data was performed. The optimal model was determined and Tukey contrasts for multiple comparisons of means. The As was found in the highest concentrations in hair (6.09-7.13 mg L⁻¹). In general, the concentrations of muscle and liver samples were found to be lower than NOM for human consumption. In milk, samples from G2 exceeded the permitted values, while in G3, only one sample exceeded the limit. No differences were observed between age groups; however, notable variations were identified among the different tissues ($p < .0001$). The findings indicate that the levels of As in tissues samples exceed the standards for human consumption and emphasise the necessity to enhance general environmental monitoring (predominantly water quality) in contaminated areas where cattle are raised for production.

Keywords: Public health, arsenic drinking water, arsenic accumulation, arsenic in milk, unbalanced ANOVA.

RESUMEN. En el norte de México existe una exposición crónica al arsénico que afecta tanto a personas como a los animales. Se midieron las concentraciones de As en músculo, hígado, leche y pelo de ganado lechero criado en estas regiones para determinar cumplimiento de la normativa mexicana (NOM) y analizar la posible acumulación de As en los tejidos. Las muestras de tejido se agruparon según la edad: G1: 0-15 días ($n = 10$), G2: 1-3 años ($n = 10$), G3: 4-6 años ($n = 10$). Se recolectaron 8 muestras de leche de adultos en producción para el grupo 2 y 9 muestras de leche para el grupo 3 con un total de 17 muestras. El análisis de las muestras se efectuó mediante espectrofotometría de absorción atómica. Se realizó un ANOVA de dos vías para datos no balanceados seguido de la prueba de comparaciones múltiples de medias de Tukey. La concentración más se encontró en el pelo (6.09-7.13 mg L⁻¹). Las concentraciones en músculo y hígado se encontraron por debajo de la NOM. En la leche, todas las muestras de G2 excedieron los valores permitidos, mientras que solo una muestra de G3 superó el límite. No hubo diferencias entre los grupos de edad; pero se identificaron variaciones notables entre los diferentes tejidos ($p < .0001$). Los hallazgos indican que los niveles de As en muestras de tejido exceden los estándares para consumo humano y enfatizan la necesidad de mejorar el monitoreo ambiental (principalmente la calidad del agua) en áreas contaminadas donde hay ganado para producción.

Palabras clave: Salud pública, arsénico en agua potable, acumulación de arsénico, arsénico en leche, ANOVA desbalanceado.

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INTRODUCTION

Water scarcity and sanitation are recognised as significant challenges to contemporary society on a global scale. In several cities in northern Mexico, as well as around the world, there has been an alarming increase in the amount of arsenic (As) in the environment, mainly because of exponential population growth (Zhou *et al.* 2019, Ahmed and Hashmi 2022, Bhat *et al.* 2023). It has been determined that economic activities such as livestock farming, agriculture, and mining have been identified as key drivers of excessive exploitation of natural resources. This has resulted in a range of environmental issues, including the accumulation of industrial waste and the overexploitation of aquifers. However, when additional factors, such as volcanic eruptions and other natural phenomena, are taken into consideration, the underlying reasons for this phenomenon become evident. As concentrations have increased at an accelerated rate (Londoño *et al.* 2016, Khosravi-Darani *et al.* 2022).

Notwithstanding the pervasive environmental degradation, research has been predominantly focused on the contamination of aquifers and surface waters with As, given that ingestion is the predominant exposure route for living organisms, with potentially significant health implications (Ohno *et al.* 2007, Zhou 2019). The World Health Organization (WHO) has established a maximum permissible level of arsenic in drinking water of $10 \mu\text{g L}^{-1}$ (WHO 2001). In contrast, Mexico's NOM-127-SSA1-2021 standard (SS 2021), which was revised in 2021, has been updated to reduce the limit from $25 \mu\text{g L}^{-1}$ to $10 \mu\text{g L}^{-1}$, thereby aligning with international standards. The 2022 National Water Commission (CONAGUA 2025) groundwater quality report comprised a sampling of 775 sites across Mexico, with the objective of determining total As levels. Of these sites, 409 were found to have excellent water quality, with As concentrations below 10 mg L^{-1} . A total of 91 sites were found to have concentrations ranging from 10 to $25 \mu\text{g L}^{-1}$, indicating their potential as viable drinking water sources. Conversely, 155 sites exhibited concentrations greater than $25 \mu\text{g L}^{-1}$, thus being classified as unsuitable for consumption. These findings are consistent with those of previous studies by other researchers (Mancilla-Villa *et al.* 2012, Irunde *et al.* 2022).

The ingestion of contaminated water has been demonstrated to exert a detrimental effect on numerous physiological systems, including the digestive, respiratory, neurological, reproductive, haematopoietic, dermatological, hepatic, renal, muscular, endocrine and immune systems. It has also been demonstrated that this condition can contribute to the development of cancer (Jimenez *et al.* 2016, Jimenez-Villarreal *et al.* 2018, Ro *et al.* 2022, Singh 2023). Humans and animals inhabiting contaminated areas are exposed to arsenic throughout their lives via water, soil, air and food. These animals have been found to produce protein contaminated with As in their soft tissues and bodily fluids, which could significantly contribute to human As levels (Mandal 2017, Rahaman *et al.* 2022).

It has been demonstrated that this substance has an affinity for keratinised tissues, including hair, nails and hooves. Consequently, the presence of arsenic in the body has been demonstrated to affect its likelihood of being detected in soft tissues. The primary route of elimination is urine. However, it is also excreted in milk, saliva, sweat, faeces, and bile, and lost through the desquamation of keratinised cells (Zhou 2019, Das *et al.* 2021). Whilst some authors claim that arsenic does not accumulate in tissues such as muscle, liver, and milk to exceed permissible limits (Islam *et al.* 2017, Hossain *et al.* 2022), others dismiss this idea as dangerous. It is contended by these authors that,

despite the concentration of arsenic in animal protein exceeding permissible limits, the daily intake of these products is low (Azevedo *et al.* 2018, Yakup *et al.* 2018). However, some authors have posited that a genuine risk exists and that further research into the impact of consuming these products is required (Njoga 2021, Davtalabzarghi *et al.* 2023, Morshdy *et al.* 2023).

The international regulations that stipulate maximum permitted levels for arsenic, the Codex Alimentarius CXS 193-1995 establishes limits for certain foodstuffs, specifying 0.1 mg/kg for fat from animal sources and 0.01 mg/kg for water (CAC 1995). However, it should be noted that this document does not provide limits for animal tissue products, such as liver, kidney and muscle. On the other hand, regulation 2023/915 of the European Union (EU) contains no specification regarding limits for organs or meat (EU 2023). Therefore, there is no international standard equivalent to NOM-015-ZOO-1994 (SADER 1994) or NOM-004-ZOO-1994 (SAGARPA 1994). These have been replaced by the National Program for the Control and Monitoring of Toxic Residues in Animal Products, Aquaculture Resources, and Fisheries (SADER-SENASICA 2025). This is because the limits for arsenic are established based on food, not animal tissue.

In Mexico the acceptable limits are defined as 1.4 mg L⁻¹ for liver, 0.7 mg L⁻¹ for muscle, and 0.2 mg L⁻¹ for milk (SENASICA 2026). According with the above, the present study analysed the arsenic concentration in the drinking water of cattle from dairy farms in highly contaminated areas using the Mexican As limits in these tissues, then, the objectives of the study were threefold: firstly, to detect concentrations in different tissues (muscle, liver, milk and hair); secondly, to establish whether levels exceed regulatory limits; and thirdly, to ascertain whether lifetime exposure results in arsenic accumulation in these animals.

MATERIALS AND METHODS

Sample collection and classification

The study was conducted in the area where the large aquifer of the Comarca Lagunera is located and included the southwestern portion of the State of Coahuila and the extreme northeast of the State of Durango. The economy of this region is characterised by a high degree of diversification, with the national production of milk, fodder and feedlots being of particular significance. In addition, the region is home to a thriving metallurgical, automotive and food industry. It is regrettable that the execution of these operations has led to a significant level of arsenic contamination in water sources and soil (González-Herrera and García-López 2021, Osuna-Martínez *et al.* 2021). Tissue samples were obtained from female dairy cattle at a slaughterhouse, including muscle tissue, liver and hair. The samples were grouped according to age: Group 1 (0 - 15 days); Group 2 (1 - 3 years); and Group 3 (4 - 6 years). A total of 90 samples were obtained (10 from each group). Moreover, a total of seventeen milk samples were collected from adult subjects in production, yielding a total of eight samples from Group 2 and nine samples from Group 3.

The inclusion criterion was the selection of animals that consumed arsenic-contaminated water from birth to death. A total of ten water samples were collected at random from drinking troughs located in the pens where the study subjects were raised. It was hypothesised that chronic exposure to contaminated water would be indicated by the analysis of samples originating from the same

well. The findings indicated that the concentrations of As were higher than the limits stipulated by NOM 127-SSA1-2021 (mean 0.21 ± 0.02 mg L⁻¹, Table 1), with the maximum permitted level set at 0.01 mg L⁻¹.

Table 1. As concentration (mg L⁻¹) in water from drinking troughs for cattle sampled in this study, using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

	Means
Water 1	0.19
Water 2	0.19
Water 3	0.24
Water 4	0.24
Water 5	0.24
Water 6	0.18
Water 7	0.22
Water 8	0.21
Water 9	0.20
Water 10	0.23

Determination of total arsenic in water samples using the ICP-MS technique

The samples were prepared in accordance with the NMX-AA-051-2020 standard (SCFI 2016). The chemical analysis was conducted within the laboratory setting using the Thermo Scientific iCAP 7000 Series ICP Spectrometer model iCAP 7400 (Duo) from Cambridge, United Kingdom, under the following operating conditions: Temperature: The device operates at an output of 10 000 kW, utilising a radio frequency of 1 350 watts. The auxiliary gas flow rate is specified as follows: The flow rate of the cooling gas is 12 litres per minute, and the gas nebuliser flow rate is 0.5 litres per minute. The pump speed was set at 0.7 L min⁻¹, and the reading was taken in triplicate after 45 seconds of operation and 5 seconds of washing.

Atomic absorption spectrophotometry with hydride generator (acid digestion by wet method)

The digestion process was conducted in accordance with the guidelines stipulated in NOM-015-ZOO-1994 (SADER 1994) and NOM-117-SSA1-1994 (SSA 1994), with certain modifications implemented during the course of the process. A precise quantity of one gram of sample material (liver, milk, muscle, or hair) was meticulously weighed out and placed into a suitable container. To this, 6 mL of nitric acid and 2 mL of peroxide were added. The samples were then placed in a microwave digestion oven at a temperature of approximately 183 °C for a duration of one hour. It is important to note that a blank was incorporated into each iteration. The samples were permitted to cool for a minimum of 8 hours and were subsequently measured with deionised water to 10 mL⁻¹.

Atomic absorption spectrophotometry with hydride generator (Spectrophotometer reading)

The atomic absorption spectrophotometer utilised in this study was a Perkin Elmer Analyst Model 100 hydride generator, manufactured in Waltham, Massachusetts, USA. The experimental procedure was conducted in accordance with the guidelines stipulated in NOM-015-ZOO-1994, a document issued by SADER (1994).

Statistical analyses

R (R Core Team 2025) with the Integrated Development Environment for R, RStudio (Posit team 2026), and the R-packages dplyr (Wickham 2023), emmeans (Lenth and Piaskowski 2025), forcats (Wickham 2025a), ggplot2 (Wickham 2016), lubridate (Grolemund and Wickham 2011), papaja (Aust and Barth 2025), purrr (Wickham and Henry 2025), readr (Wickham *et al.* 2024a), stringr (Wickham 2025b), tibble (Müller and Wickham 2025), tidyr (Wickham *et al.* 2024b), tidyverse (Wickham 2019), nlme (Pinheiro 2011), kableExtra (Zhu *et al.* 2022), and tinylabels (Barth 2025) were used for all analyses.

Following the testing of normality and homogeneity of variances, a mixed-effects analysis was conducted, with the following covariance matrices for the model being tested: Result ~ Part * Group, data = dts, correlation = CovMat (form = ~1 | Id)). The data set is then analysed using a covariance matrix, with the tissue and group variables acting as fixed effects and the subject as a random effect. The latter model demonstrated superior performance, with AIC: 347.4, BIC: 386, LogLink: -158.7, whilst the other structure of variance and covariances criteria selection values were AIC: 402.2, BIC: 428, LogLink: -191.1. The variance-covariance structures tested were: Autoregressive process of order 1; Continuous autoregressive process (AR(1) process for a continuous time covariate; Compound symmetry structure corresponding to a constant correlation; Exponential spatial correlation; Gaussian spatial correlation; Linear spatial correlation; Rational quadratics spatial correlation; Spherical spatial correlation; and General correlation matrix, with no additional structure.

RESULTS

As illustrated in Table 2, the mean concentrations of As were observed to vary among different tissues and milk samples across various age groups. The highest concentrations were detected in hair, followed by liver, and the lowest concentrations were observed in muscle, with the range of values varying from 6.09 to 7.13 mg L⁻¹, 0.21 to 0.78 mg L⁻¹, and 0.19 to 0.38 mg L⁻¹, respectively. In the case of milk, the concentrations were found to be comparable to those observed in muscle.

Table 2. Means and standard deviation of arsenic (mg L⁻¹) for tissues and milk in Holsteins cows raised in a farm in northern Mexico, according its age group.

	Hair	Liver	Muscle	Milk
0 – 15 days	6.53 ± 2.82	0.21 ± 0.19	0.30 ± 0.62	-
1 – 3 years	6.09 ± 3.43	0.33 ± 0.18	0.19 ± 0.30	0.39 ± 0.11
4 – 6 years	7.13 ± 2.44	0.78 ± 0.74	0.26 ± 0.28	0.14 ± 0.05

The analysis of the milk revealed the presence of As, with concentrations that exceeded the permissible limits stipulated by the National Program for the Control and Monitoring of Toxic Residues in Animal Products, Aquaculture Resources, and Fisheries. In the case of bovines aged 1 - 3 years, all samples demonstrated elevated concentrations, ranging from 0.25 to 0.55 mg L⁻¹. In contrast, among bovines aged 4 - 6 years, a single sample exceeded the permissible limit, with a concentration of 0.22 mg L⁻¹.

With regard to the tissues analysed, four of the muscle samples showed values above the limit: one sample from calves (0 - 15 days old: 2.00 mg L⁻¹), two samples from cows aged 1 - 3 years and one more from cows aged 4 - 6 years. In the final three samples, the values ranged from 0.73 to 0.78 mg L⁻¹. Finally, in the liver, only two samples from cows aged 4 - 6 years exceeded the permissible limit, with values of 1.47 and 2.47 mg L⁻¹. As demonstrated in Table 3, the AIC, BIC and log Likelihood values are presented for comparison of the various variance and covariance structures. It is evident that the General correlation matrix, devoid of any additional structure, emerges as the optimal choice, with values of 347.38, 386.00 and -158.69, respectively.

Table 3. AIC, BIC and log Likelihood values for Variance and Covariance Structure Matrices tested for model selection.

	AIC	BIC	logLik
Autoregressive process of order 1	402.23	427.97	-191.11
Continuous autoregressive process AR(1)	402.24	427.98	-191.12
Compound symmetry structure for a constant correlation	402.24	427.98	-191.12
Exponential spatial correlation	402.24	427.98	-191.12
Gaussian spatial correlation	402.24	427.98	-191.12
Linear spatial correlation	402.24	427.98	-191.12
Rational quadratics spatial correlation	402.24	427.98	-191.12
Spherical spatial correlation	402.24	427.98	-191.12
General correlation matrix, with no additional structure	347.38	386.00	-158.69

Regarding the analysis of variance in the model, the fixed effects corresponded to the Tissues and the Groups, and the random effect corresponded to the observational unit. As illustrated in Table 4, the analysis of variance (ANOVA) reveals a discrepancy between at least one tissue, as well as an interaction between the tissue and the group. This finding indicates a potential difference in the data. The statistical procedure employed was "Simultaneous Tests for General Linear Hypotheses and Multiple Comparisons of Means: Tukey Contrasts" test was utilised, as it is widely regarded as the optimal approach for post hoc analysis for the outcomes of mixed models (Table 5).

Table 4. ANOVA results

Source	df	F-value	p-value
(Intercept)	1	170.708	<.001
Part	3	166.499	<.001
Group	1	0.077	0.782
Part:Group	3	6.932	<.001

Table 5. Simultaneous Tests for General Linear Hypotheses.
Multiple Comparisons of Means: Tukey Contrasts.

	Estimate	Std. Error	z value	Pr(> z)
Liver – Hair	-6.04	1.10	-5.50	0.00
Milk – Hair	-5.03	2.09	-2.40	0.07
Muscle – Hair	-5.61	1.10	-5.11	0.00
Milk – Liver	1.01	2.07	0.49	0.96
Muscle – Liver	0.43	1.05	0.41	0.98
Muscle – Milk	0.58	2.07	-0.28	0.99

DISCUSSION

This study lends further credence to the hypothesis that chronic exposure to arsenic through contaminated water results in accumulation in different tissues of exposed cattle, a result that is consistent with the findings of several previous studies. The mean values found in hair (6.09 and 7.13 mg L⁻¹) demonstrate that this tissue is the primary site of deposition, which is consistent with the strong affinity of the metalloid for keratinised matrices and validates the proposal by authors to utilise this tissue as a biomarker for chronic exposure (Draghi *et al.* 2024).

On the other hand, concentrations in muscle and milk were lower, although in both cases samples were detected that exceeded the maximum permissible limits established for human consumption. The presence of high concentrations of arsenic in milk is of particular significance, due to its high consumption by vulnerable populations, especially infants and children. As demonstrated in previous studies, milk has been shown to serve as a route for the excretion of arsenic, in addition to its role as a significant source of exposure in cases where contamination levels exceed the established regulatory limits (Castro-Bedriñana *et al.* 2023, Souto *et al.* 2025). The chronic dietary intake of arsenic, even at low concentrations, has been associated with a number of adverse health effects, including carcinogenicity, endocrine disruption, neurotoxicity, and alterations in immune and cardiovascular function (Rahaman *et al.* 2022, Singh *et al.* 2023).

In cows aged 4 to 6 years, only one sample exceeded the permitted value, but in animals aged 1 to 3 years, all samples exceeded the regulatory limit, reinforcing the potential risk associated with continued consumption. In contrast, although muscle tissue is not traditionally considered a major accumulation site, the detection of values above regulatory limits suggests that under chronic exposure conditions, arsenic can reach levels of concern. This aligns with reports indicating that dietary exposure through animal protein may contribute significantly to total arsenic intake in exposed populations (Azevedo *et al.* 2018, Njoga *et al.* 2021).

The absence of significant differences between groups (0 - 15 days; 1 - 3 years; 4 - 6 years), despite an expectation of accumulation with age, suggests that As storage is not strictly linear with age under constant exposure. In order to facilitate a comprehensive analysis, it is imperative to consider the following potential scenarios: The presence of substantial inter-individual variability, which obscures the discernment of age trends, is attributable to the rapid distribution and continuous excretion of As, predominantly via renal excretion and in secretions such as milk (Beene *et al.* 2022).

Variability in metabolic pathways (hepatic methylation capacity) (Punshon *et al.* 2015, Zubair and Martyniuk 2019, Hirano 2020, Thomas 2021) hypothesise that early exposure through placental transfer can equalise levels between groups. Recent studies on biomarkers in livestock have demonstrated that the response to and storage of As may be more dependent on local factors, such as the species of As present in water (Chakraborti *et al.* 2018, Rahaman *et al.* 2022), the total exposure load or environmental conditions (Osuna-Martínez *et al.* 2021), and the characteristics of the forage, than on exposure time alone (Alvarez-González *et al.* 2024, Draghi *et al.* 2024).

Despite the absence of statistically significant variations associated with age, substantial disparities were observed among the various tissues, particularly between hair and muscle, as well as between hair and liver ($p < .0001$). Conversely, no substantial disparities were observed between muscle and liver tissues. However, findings that exceeded the permissible limits were documented in both cases, irrespective of the subject's group affiliation. This finding indicates that, despite the liver's elevated metabolic activity leading to higher As concentrations, muscle also demonstrated accumulation levels that exceeded the established parameters. This phenomenon could be attributed to the potential experience of atypical accumulation processes by certain species, which may be associated with variations in methylation activity, oxidative stress, or prior tissue damage (García-Alvarado *et al.* 2018, Hirano 2020, Xu *et al.* 2009).

Statistical analysis was performed using a mixed model of fixed effects (tissue and age group) and random effects per observational unit. The covariances that were selected by the information criteria (AIC, BIC and logLik) were found to be the general correlation matrix. This indicated a correlation of measurements within the same individual, which was relevant between measurements of different matrices.

In this study, the total As is determined, yet no distinction is made between inorganic species (As (III), As(V) and organic/methylated forms. Consequently, while milk, meat, and liver samples with results exceeding the permissible limits have been documented, the implementation of speciation would facilitate a more precise estimation of the actual risk. This does not negate the dietary risks associated with consuming contaminated foodstuffs, particularly in light of the documented exceedances of water quality limits as stipulated by national and international regulations. Consequently, the implementation of regulatory controls is imperative, encompassing systematic monitoring of animal drinking water, analysis of food matrices, and assessment of dietary exposure for local populations (Kretchik 2002).

Management recommendations. In view of these findings, it is imperative to implement evidence-based strategies with the objective of reducing arsenic (As) exposure in livestock production systems. The objective can be accomplished through the utilisation of treatment systems founded upon adsorption, membrane filtration, and hybrid materials. The efficacy of these systems in the removal of arsenic from contaminated water sources has been demonstrated (Bolisetty *et al.* 2021). It is recommended that environmental regulations be strengthened and aquifer monitoring programs be implemented with a view to preventing further deterioration of environmental quality. This is achieved with a view to mitigating arsenic contamination associated with anthropogenic activities such as mining, agriculture and industrial processes, as well as with natural geochemical processes (Bhat *et al.* 2023, Patel *et al.* 2023).

As a complementary strategy, the reduction of arsenic bioavailability in livestock should also be considered. Recent studies have indicated that certain adsorbent compounds, such as bentonite, have the capacity to bind to arsenic in the gastrointestinal tract of cattle, thereby limiting its absorption and subsequent transfer to tissues and milk (Fuentes-de Marco *et al.* 2024). This approach may be particularly valuable in regions where the immediate recovery of contaminated water sources is not possible. In such situations, alternative water management strategies may include the use of treated water, the mixing of contaminated sources with low-arsenic water, and the ensuring that livestock drinking water meets standards similar to those established for human consumption.

CONCLUSION

The present study corroborates earlier findings that chronic exposure to As through contaminated water results in accumulation in various tissues of exposed cattle. The mean values found in hair (6.09–7.13 mg L⁻¹) demonstrate that this tissue constitutes the primary deposition site, in accordance with the high affinity of the metalloid for keratinized matrices. Highlighting that the levels of arsenic detected in milk exceeded permissible limits in most samples is extremely important, since it is a significant risk for human consumption, particularly among vulnerable populations. Although concentrations in muscle and liver were generally lower, the presence of samples that exceeded regulatory limits indicates that these tissues may also contribute to dietary exposure. These findings are consistent with the objectives of the study and emphasize the need to strengthen environmental monitoring, particularly water quality, as well as implement effective control strategies to reduce arsenic exposure in livestock production systems and protect public health.

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