



Research article

Heavy metals in blood, milk and cow's urine reared in irrigated areas with wastewater



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ABSTRACT

The aim of this work was to determine the content of lead (Pb), chromium (Cr), cadmium (Cd), arsenic (As), copper (Cu), strontium (Sr) and thallio (Tl) in blood, milk and urine from cows fed forage irrigated with sewage water and cultivated in the vicinity of the Popocatepetl volcano and determine the rate of transfer of these elements from blood to milk and urine. Digestion was performed in a microwave oven (CEM-MarsX). The determination was made using an ICP -OES. The Cd in the blood was significantly higher than in milk and urine. The Cr in the blood was higher than in the urine. In milk the content of Pb and Cu was significantly higher than in blood. Sr and Tl concentration in the urine was higher than that detected in milk and blood and Pb in blood. Milk transfer Index revealed a significant difference in relation to the TI values found in the urine and only in the case of Cu in milk was TI > 1. In urine, the values found of Sr and Tl were higher than milk TI. It is concluded that when metals are ingested by cows in fodder, they are easily transferred to milk and urine through the blood. Presence of heavy metals in the different matrices determines the degree of environmental and trofic chain contamination with which we can consider the cow a biomarker.

1. Introduction

Milk and its derivatives constitute one of the fundamental components of the human diet, particularly due to its protein and mineral contributions (Pšenková et al., 2020); although it is also of interest due to the diversity of proteins, fats, carbohydrates and bioactive substances they contain (Maity et al., 2020). Therefore, its contamination, among other compounds, by heavy metals, can represent a serious public health problem due to different disorders that these cause to consumers, particularly in children (Nava-Ruiz and Méndez-Armenta, 2011; CDC (Centers for Disease Control and Prevention), 2012; Tepanosyan et al., 2017; Ngo et al., 2021).

Heavy metals can be transferred from irrigation water to agricultural soils, posing a risk to human health, due to direct contact with heavy metals in the soil and the translocation and bioaccumulation of metals in forages, which causes contamination of products from animals, which in both cases can cause cancer. and non-cancerous diseases (Castro-González et al., 2017a, b; Mohammadi et al., 2019; Karimi et al., 2020).

It has been determined that the presence of heavy metals in milk is basically due to the fact that they are ingested by cows through water and forages that are contaminated by different sources, such as industrial waste that is indiscriminately dumped into the environment, pesticide residues used in agriculture, water released from urban areas and in certain regions, by natural processes such as volcanic activity, where fine particles are emitted into the air that can reach irrigation water, soil and forages, becoming a risk factor that causes contamination (Yilmaz et al., 2009; Alloway, 2013; Gakidou et al., 2017; Castro González et al., 2018). Therefore, the presence of these compounds in milk, in addition to being a direct indicator of its hygienic quality, is an indirect indicator of the contamination of the environment where it occurs (Licata et al., 2004; González-Montaña et al., 2012).

Heavy metals are not biodegradable, they tend to accumulate and magnify their effect in the different stages of the production chain, so their presence in animals can affect their health by accumulating in internal organs causing hematobiochemical and pathological alterations in

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these organs (Nwude et al., 2010; Gashua et al., 2018; Kar and Patra, 2021) existing Bioaccumulation and Toxicopathological damage and finally have a negative impact on food security (Lokeshwari and Chandrapa, 2006; Tchounwou et al., 2012; Castro et al., 2013).

On the slopes of the Popocatepetl volcano, in the upper part of the river basin of the Balsas river, milk production is an important activity that is sustained by forage crops, which unfortunately are irrigated with wastewater. Previous studies in this area have evidenced significant levels of heavy metals in water, soil, forage, and milk, which put the health of the population at risk (Castro-González et al., 2018). The main sources of contamination in this area are the discharges of wastewater from different textile, refreshment, chemical industries, etc., many of them located within industrial parks; However, the contamination caused by the Popocatepetl volcano must also be considered, which since 1999 has been emitting ash exhalations constantly, which contributes in a beneficial way to improve soil fertility. However, it is mentioned that within the content of these ashes there are Lead particles (2.5 mg kg^{-1}) and other elements, which are considered toxic to animals and humans (Narváez-Porras and Cano-Valle, 2004).

One of the little-studied aspects of the metals ingested by cows is that there are no studies that show the association between the content of metals that circulates in the blood and their transfer to milk, which may indicate to some extent the damage that can directly do in the health of consumers, or towards feces and urine, which could indicate their reincorporation into the environment and continue to be a major source of contamination. In this sense, the objective of this work was to determine the content of Lead (Pb), Chromium (Cr), Cadmium (Cd), Arsenic (As), Copper (Cu), Strontium (Sr) and Thallium (Tl) in blood, milk and urine of cows fed with fodder irrigated with wastewater and cultivated in the vicinity of the Popocatepetl volcano and to determine the transfer rate of each of these compounds from blood to milk or from blood to urine.

2. Materials and methods

2.1. Location

The work was carried out in winter 2019, in Santa Ana Xalmimilulco, belonging to the municipality of Huejotzingo, in the state of Puebla, Mexico. The geographical coordinates lat $19.12'30.81'' \text{ N}$ and lon $98.24'36.78'' \text{ W}$ and an altitude of 2228 m above sea level. The climate is temperate sub-humid with rains in summer 15.5° C and an annual rainfall of 866.0 mm.

2.2. Animals

28 Holstein Friessian cows between 4 and 5 years of age (third lactation) with 120 days of lactation were sampled. The animals were selected for being fed with forages produced in soils irrigated by sewage, which are discharged from the different industries, in addition to being in an area where the ash fall from the Popocatepetl volcano is constant. The components of the diet as well as the content of heavy metals in the diet are shown in Table 1. The methodology used for the analysis of metals is described in the paragraph about the digestion process.

Table 1. Heavy metal content in the diet of cows reared in areas irrigated with wastewater.

Heavy Metal	Corn silage (mg kg^{-1})	Lucerne hay (mg kg^{-1})	Concentrated Food (mg kg^{-1})	Drinking water (mg L^{-1})
Pb	1.0 ± 0.50	1.85 ± 1.50	0.60 ± 0.50	0.02 ± 0.002
Cr	9.0 ± 2.0	3.86 ± 0.68	3.0 ± 0.4	0.01 ± 0.001
Cd	0.36 ± 0.10	0.09 ± 0.07	0.44 ± 0.10	0.001 ± 0.001
As	0.9 ± 0.01	1.86 ± 1.43	0.02 ± 0.01	0.01 ± 0.004
Cu	5.40 ± 0.30	6.80 ± 1.75	3.0 ± 2.0	0.002 ± 0.001
Sr	23.0 ± 0.60	74.13 ± 29.9	9.0 ± 1.0	0.30 ± 0.001
Tl	0.4 ± 0.3	10.81 ± 9.0	0.80 ± 0.10	0.023 ± 0.01

(\pm) = Standard Deviation.

2.2.1. Sampling

The sampling and handling of the animals in this work was carried out with humane and dignified treatment, the indications of the regulation for the use and care of the animals destined for research of the Postgraduate College (2019) were followed.

Milk, blood, and urine samples were taken on five occasions at 15-day intervals. Milk was collected directly from the cow's udder during morning milking, depositing it in 50 ml Falcón tubes (Fisher scientific, Waltham, MA, USA) previously washed with a 10% nitric acid (HNO_3) solution and rinsed three times with deionized water to remove all acid content. They were preserved frozen at -60° C until processing and analysis.

Blood was obtained from the coccygeal vein, using BD vacutainer 22 G x 25 mm needles and vacutainer tubes with sodium heparin (Becton, Dickinson and Company, USA). Urine was collected directly from the urinary bladder using a plastic catheter and deposited in plastic bottles (50 ml) treated in a similar way to Falcon tubes. In all cases the sampling was carried out by qualified and experienced personnel, avoiding unnecessary injuries and stress to the animals. Subsequently, the urine and blood samples were refrigerated at 3° C until digestion and analysis.

2.3. Digestion process

All samples were digested using a microwave oven (CEM-MarsX, CEM corporation Mathews, NC). In the case of milk, it was previously lyophilized in a LABCONCO 4.5 L. FreeZone equipment (Kansas City, MO, USA), after which 0.5 g were taken and 10 mL of HNO_3 were added, they were placed in the microwave at a power of 1600 w, ramping 15 min at a pressure of 800 psi and temperature 200° C , with a wait of 15 min.

In urine, 4 mL were used, to which 4 mL of HNO_3 was added, plus 2 mL of 30% H_2O_2 , they were placed at a power of 1600 w for 15 min of ramping with a temperature of 200° C , 800 psi and a 5 min wait. For blood, 1 mL was used, to which 10 mL of HNO_3 were added, it was subjected to a power of 1600 w, 15 min of ramping at a pressure of 1600 psi and a temperature of 200° C for a 15-minute wait. In the case of forage, corn silage and concentrated feed, each one and separately, 0.3 g were weighed and 5 mL of HNO_3 plus 5 mL of hydrogen peroxide at 30% w/v at 1,600 W of power were added, with a 15 min ramp time, 800 psi pressure, 200° C temperature and 10 min wait. Water samples 45 mL and 5 mL of concentrated nitric acid (HNO_3) were added, a power of 1600 W was used, with a 10 min ramp, a pressure of 350 psi, a temperature of 170° C and a 0.0 min interval. Each sample were filtered on Whatman grade 42 paper (GE Healthcare, Little Chalfont, UK), graduated to 50 mL with deionized water and refrigerated at 3° C until its analysis.

The determination was performed using inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian 730. Agilent Technologies, AU). All chemical products used were analytical reagent grade. Solutions were prepared in $18.2 \text{ M}\Omega \text{ cm}$ deionized water. Calibration standards for each metal were prepared using standard XVI multi-element ICP standard solution from Merck KGaA, Darmstadt, Germany.

The precision and accuracy levels were carried out with five targets and ten repetitions, specifically. The analytical recovery value was determined as 104% on average, the correlation coefficient (r^2) was 0.999. The detection and quantification limits were calculated respectively, with three and ten times the standard deviation of the blank.

With the values of the metal content in each component, the transfer index (TI) was calculated, which indicates the ability of a metal to transfer from one medium to another within the animal organism, where if $\text{TI} > 1$ means high capacity of transfer. To obtain the transfer index, the equation was applied: $\text{TI} = \text{Cx}/\text{Cms}$, where Cx is the metal concentration in milk or urine and Cms is the metal concentration in blood.

Experimental design was completely randomized, and in the first instance, the data were subjected to the Lilliefors Kolmogorov-Smirnov normality test, since the number of samples was greater than 50, $n = 140$, establishing a value of $\alpha = 0.05$. Subsequently, a non-parametric Analysis of Variance was carried out: Kruskalwallis test and to

determine the significant differences between matrices through the Kruskal-Wallis post-hoc test for each metal., using Software R version 4.0.4 R Commander.

3. Results

The content of heavy metals blood, milk and urine are presented in Table 2 and the transfer index of each metal from blood to milk and urine in Table 3.

3.1. Heavy metal content

It was found that the content of Cd in the blood was significantly higher ($P < 0.001$) than to milk and urine; Furthermore, Cr in blood was also higher ($P < 0.01$) in urine, but not in milk. On the other hand, in milk the content of Pb and Cu was significantly ($P < 0.01$) higher than to blood and of Cr, Cd and Cu in urine. On the contrary, the concentration of Sr and Tl in urine was higher ($P < 0.01$) than that detected in milk and blood and that of Pb in blood, although not in milk. As was the only component that did not show significant differences ($P > 0.05$) between the matrices analyzed.

3.2. Transfer index (TI)

The TI found for Pb (1.90 and 2.22), As (2.42 and 2.39) in milk and urine respectively, did not show a significant difference ($p > 0.05$) between both matrices. TI of the metals Cr (0.79), Cd (0.54) and Cu (1.75) found in milk, revealed a significant difference ($p < 0.01$) in relation to the TI values found of these metals in urine (0.42, 0.28, 0.17, 0.73 respectively) and only in the case of Cu in milk was $TI > 1$. For the subject of TI in urine, the values found of Sr (11.54) and Tl (21.49) were higher than the TI of milk Sr (6.32) and TI (14.78) showing a significant difference ($P < 0.01$).

4. Discussion

4.1. Blood

The Values found for heavy metals in the blood are an indicator of recent exposure to these and represent the daily entry of metals into the animal's body. In this work, the content of Pb (0.015 mg L^{-1}) in blood was below the limits considered normal for the animal (0.025 mg L^{-1}) according to Radostits et al. (2000) and below the maximum value determined by the Official Mexican Standard (NOM-199-SSA1-2000) 0.1 mg L^{-1} for humans. On the other hand, various worldwide authors have reported Pb values in bovine blood, which are higher than those found in this work, such as those of Swarup et al. (2005) 0.07 mg L^{-1} in India and Chirinos-peinado and Castro-bedri (2020) 0.38 mg kg^{-1} in Peru.

The Cd in blood detected in this work (0.004 mg L^{-1}) is similar to that reported by Mehennaoui et al. (1999); however, it is below the maximum

Table 3. Heavy metal transfer factor from blood to milk and urine in cows reared in areas irrigated with wastewater.

Heavy metal	Transfer Factor	
	Blood - Milk	Blood - Urine
Pb	1.90 ^a	2.22 ^a
Cr	1.0 ^a	0.42 ^b
Cd	0.54 ^a	0.28 ^b
As	2.42 ^a	2.39 ^a
Cu	1.75 ^a	0.17 ^b
Sr	6.32 ^b	11.54 ^a
Tl	14.78 ^b	21.49 ^a

Literals (a, b) indicate significant difference ($p < 0.05$).

value 0.007 mg L^{-1} according to Tahir et al. (2017) and the blood values of cattle in production reported by Swarup et al. (2005) 0.03 mg L^{-1} , Patra et al. (2005) $0.28 \text{ } \mu\text{g L}^{-1}$ and Chirinos-peinado and Castro-bedri (2020) 0.016 mg kg^{-1} . The values of Cd and Pb in the blood of cows in production found in this work are below that determined by Codex Standard, (2010) as reference values 0.5 y 0.05 mg kg^{-1} .

In relation to Cu in blood, 0.017 mg L^{-1} was detected similar to that reported by Popovic et al. (2010) and was lower than the values 170 mg L^{-1} , 1.12 mg L^{-1} and 0.147 mg L^{-1} obtained by Hernández-Arroyave et al. (2016), García et al. (2010) and by Tomza-Marciniak et al. (2011) in Cuba, Colombia and Poland respectively. Chromium in blood showed a lower value than that reported by Tomza-Marciniak et al. (2011) 0.060 mg L^{-1} and Aluc and Ekici (2019) 0.109 mg L^{-1} . Regarding As in the blood, a value of 0.019 mg L^{-1} was detected, which is below that reported by Popovic et al. (2010) 2.0 mg L^{-1} , Tyutikov and Ermakov (2010) 0.208 mg L^{-1} and Aluc and Ekici (2019) 0.086 mg L^{-1} . In the case of Sr in blood, 0.01 mg L^{-1} was detected in this work, a value lower than that determined by Popovic et al. (2010) 0.023 mg L^{-1} . In the case of Thallium in the blood, no information was found on work carried out in bovines. And although ATSDR (2020a, b) mentions that blood is not a good mean to determine TI due to the duration of this metal in this matrix, the value detected in this work was above that reported by Lin and Chang (2005) 0.002 mg L^{-1} in children.

4.2. Milk

The presence of heavy metals in milk probably denotes daily exposure to these elements, considering that milk is an excretory mechanism and that blood passes directly to the formation of milk in the secretory cells at the level of the alveoli of the mammary gland. An example of this could be the case of Pb and Cd that appear in milk because of they adhere to its casein (Mehennaoui et al., 1999).

Regarding the content of Pb in milk, the value found is above the value determined as a maximum of 0.020 mg L^{-1} for milk according to

Table 2. Content of heavy metals in Blood, Milk and Urine in cows reared in areas irrigated with wastewater.

Heavy Metal	Matrix											
	Blood (mg kg^{-1})				Milk (mg kg^{-1})				Urine (mg L^{-1})			
	Mean	SD	Max	Min	Mean	SD	Max	Min	Mean	SD	Max	Min
Pb	0.015 ^b	0.004	0.03	0.003	0.024 ^a	0.01	0.073	0.0002	0.028 ^a	0.02	0.10	0.00003
Cr	0.034 ^b	0.11	1.30	0.01	0.013 ^{ab}	0.002	0.02	0.001	0.007 ^a	0.002	0.02	0.004
Cd	0.001 ^c	0.001	0.005	0.001	0.002 ^b	0.001	0.004	0.00003	0.004 ^a	0.001	0.002	0.0002
As	0.019 ^a	0.01	0.010	0.001	0.018 ^a	0.01	0.06	0.0001	0.017 ^a	0.01	0.04	0.001
Cu	0.017 ^b	0.003	0.030	0.010	0.03 ^a	0.01	0.10	0.002	0.003 ^c	0.001	0.007	0.001
Sr	0.01 ^c	0.01	0.040	0.004	0.04 ^b	0.02	0.010	0.012	0.08 ^a	0.04	0.21	0.01
Tl	0.003 ^b	0.002	0.015	0.00001	0.005 ^b	0.004	0.023	0.0001	0.01 ^a	0.01	0.04	0.00002

Literals (a, b, c) indicate significant difference ($p < 0.05$). SD = Standard Deviation. Max = Maximum. Min = Minimum.

the standards of the [European Commission \(2006\)](#) and [CODEX \(Codex Alimentarius, 1995\)](#), but below the maximum of the Official Mexican Standard ([NOM-243-SSA1-2010](#)) 0.1 mg L^{-1} . There are reports in Pakistan, Serbia, Bangladesh, Ethiopia, Mexico and Slovakia, where the Pb content in milk is higher than that detected in this work: [Kazi et al. \(2009\)](#) 0.047 mg kg^{-1} , [Suturović et al. \(2014\)](#) 0.074 mg kg^{-1} , [Ahmad et al. \(2016\)](#) 0.17 mg kg^{-1} , [Akele et al. \(2017\)](#) 0.153 mg kg^{-1} , [Castro-González et al. \(2017a, b\)](#) 0.046 mg kg^{-1} and [Pšenková et al. \(2020\)](#) 0.30 mg kg^{-1} respectively. On the other hand, values lower than those determined in this work have also been reported; [Najarnezhad et al. \(2015\)](#) 0.008 mg kg^{-1} and [Rahimi \(2013\)](#) 0.021 mg kg^{-1} .

The Cd content in milk detected in this work is similar to the maximum reported by the [IDF standard \(1997\)](#) 0.002 mg kg^{-1} and is below the maximum value set (0.10 mg kg^{-1}) by the [MERCOSUR technical regulation \(2011\)](#) and what was reported by [Akele et al. \(2017\)](#) 0.287 mg kg^{-1} , [Pšenková et al. \(2020\)](#) 0.10 mg kg^{-1} and [Pilarczyk et al. \(2013\)](#) 0.004 mg g^{-1} . There is a range of $0.005\text{--}0.100 \text{ mg kg}^{-1}$ of Cd determined determined by the different standards in different food products, as does the [CODEX \(Codex Alimentarius, 1995\)](#) and the [European Union Regulation Commission \(2014\)](#). However, they do not do so for milk and its by-products, and in the case of Mexico the official standard ([NOM-243-SSA1-2010](#)) does not establish a maximum value for Cd in milk.

It should be considered that even when the detected content of Cd is below that determined by some standards and that reported by various authors, there is evidence that small amounts consumed chronically can represent a health problem in long-term consumers ([Castro-González et al., 2019](#)). Because of the half-life of this metal is 17–30 years ([Nava-Ruiz and Méndez-Armenta, 2011](#); [Genchi et al., 2020](#)), it gives it the quality of being highly cumulative, in addition to being considered the cause of pathological disorders in the kidneys, bone and respiratory systems, and it is classified as carcinogenic to humans ([WHO, 2020](#); [Ameegah et al., 2021](#)). The risk is even higher if people are deficient in calcium, zinc, or iron, since the absorption becomes 20% higher and mainly if it is ingested through milk ([Nordberg et al., 2015](#)).

In the case of Cu, it is known that it is an important metal for various enzymatic processes in the body and is a structural part of metalloproteins such as cytochrome oxidase C, the enzyme superoxide dismutase, and lysis oxidase, as well as contributing to the formation of hemoglobin ([Aceves-Lopez et al., 1998](#); [Osredkar and Susta, 2011](#)). However, when the ingestion of this metal is high, it causes various disturbances affecting the nervous system, digestive tract, skin and the immune system ([Storelli et al., 2007](#); [Barn et al., 2014](#)). The content found in this work for this metal is higher than the maximum reported by the [IDF \(1997\)](#) 0.01 mg kg^{-1} for milk.

Cr is an element found in various chemical forms and valence states in the environment. The toxicity of this metal is determined by its bioavailability and the ability to cross cell membranes ([Cuberos, 2009](#)). About Cr it is reported that it is a metal that plays an important role in the human body; In this sense, it is mentioned that it helps in the metabolism of carbohydrates, lipids and proteins, likewise, it improves insulin sensitivity ([Ekeanyanwu et al., 2020](#)). The maximum limit for breast milk is $5.0 \times 10^{-5} \text{ mg L}^{-1}$ ([Codex Alimentarius Commission, 2011](#)) value that is below that detected in this work (0.013 mg L^{-1}) and this at the same time is lower than that reported by [Erdogan et al. \(2004\)](#) 0.025 mg L^{-1} and [Ekeanyanwu et al. \(2020\)](#) 0.019 mg L^{-1} . It should be mentioned that this metal, although it is necessary for body metabolism, is also considered highly dangerous when it is transformed or found in the hexavalent form ($\text{Cr} + 6$) because it causes oxidative stress and consequently can be carcinogenic ([Stanley et al., 2013](#)).

The As detected in milk was below the maximum determined by the Official Mexican Standard ([NOM-243-SSA1-2010](#)) 0.2 mg kg^{-1} and that detected by various authors such as [Licata et al. \(2004\)](#) 0.056 mg kg^{-1} , [Solis et al. \(2009\)](#) 0.27 mg kg^{-1} , [Ghosh et al. \(2013\)](#) 0.027 mg kg^{-1} , [Koyuncu and Alwazeer \(2019\)](#) 0.5 mg kg^{-1} and [Castro González et al. \(2019\)](#) 0.15 mg kg^{-1} . However, this could be a health risk because As

suppresses DNA replication with impaired repair of enzymes, prevents enzymatic activity at the mitochondrial level with inhibition of dehydrogenase; at the same time, it stimulates the activity of adenosine triphosphatase, uncoupling oxidative phosphorylation, which causes deterioration of tissue respiration and consequently causes cytotoxicity ([Peraza et al., 2006](#)). Exposure to As at the cellular level generates nitric oxide and superoxide anions causing the formation of hydroxyl radicals and with this, oxidative stress, lipid peroxidation and DNA damage occurs ([Flora et al., 2008](#); [Lai et al., 2008](#); [Obinaju, 2009](#)). Sr is a calcium agonist and is mainly fixed at the bone level, in the case of adults it adheres to the bone surface, in the case of children it can be used for the formation of the mineral portion of the bone and therefore, it can be stored for many years in the body, and if children's diets are deficient in calcium and protein, it can cause poor bone growth ([ATSDR 2020a, b](#)). The TI detected in this work was below the values reported by [Sanal et al. \(2011\)](#) 6.44 mg kg^{-1} and 6.13 mg kg^{-1} in milk of sheep and goats respectively.

4.3. Urine

It is well known that urine is a metabolic waste that is formed by the filtration of plasma at the renal level, being produced in large quantities. The presence of metals in this matrix is considered to be an indicator of contamination due to chronic exposure ([Tirado-Amador et al., 2015](#)) and it becomes an important mean of diagnosis. On the other hand, in some regions of the world, urine becomes a product used in agricultural production, the pharmaceutical industry and in the control of bee diseases ([Mandavgane and Kulkarni \(2020\)](#), thus being important to know its degree of contamination. The concentration of Pb (0.028 mg L^{-1}) in the urine of the sampled cows was lower than the range of $50\text{--}124 \text{ mg L}^{-1}$ reported by [Raghu \(2015\)](#) in a study carried out with grazing cattle in India. In the case of Cd, the value detected in this work was higher than that reported by [Su et al. \(2017\)](#), in an investigation where the accumulation and depletion of Cd in cows was evaluated. Cd values were higher in blood with respect to milk and urine, this could be since Cd has an efficient retention rate, with a half-life in the body of up to 30 years ([Hu et al., 2012](#); [Genchi et al., 2020](#)). In addition, Cd measured in whole blood determines the degree of recent contamination and it is because cadmium is found in erythrocytes ([Su et al., 2017](#)). Due to the effect of metallothioneins, Cd tends to accumulate preferably in the liver and from there it distributes it to other organs such as the kidneys, corresponding to the accumulation of this metal in up to 80% in these organs; however, it can lodge in bones, testicles and lungs, although the main target organ is the kidney where it is mainly affected at the proximal tubule level ([Hu et al., 2012](#)). It also causes bone disorders such as osteoporosis and is known to inhibit the normal function of zinc and magnesium. On the other hand, when the excretion of Cd is high in urine, it means that there is severe damage to the kidneys and therefore, it is considered that urinary Cd reflects the body load of this element ([Nordberg, 2009](#); [Su et al., 2017](#)). The concentration of As detected in urine 0.017 mg L^{-1} , is below that reported by [Ghosh et al. \(2013\)](#) 0.122 mg L^{-1} .

4.4. Transfer index

The TI detected was higher than that reported by [Jiménez and Álvarez \(2012\)](#) 0.10 mg L^{-1} , but lower than the value reported by [Hoffman \(2000\)](#) 0.015 mg L^{-1} , [Begerows et al. \(2000\)](#) 0.023 mg L^{-1} and the maximum determined in human urine (1 mg L^{-1}) according to [ATSDR \(2020a, b\)](#), who indicate that it is possible to use this matrix for better detection because it may be present in this medium for up to 60 days.

When the transfer of metals from blood to milk and urine was evaluated, no significant differences were found for Pb and As in TI, which means that the blood transfers them to both matrices in significant quantities, producing excretion of them constantly and therefore, it represents a risk both for health as it is contained in milk, and for the contamination of agricultural areas as it is present in urine and is

reintegrated into the environment. In the case of Cr, Cd and Cu, it was evidenced that there is a significantly higher transfer of these metals ($p < 0.05$) to milk than to urine, particularly of Cu and to a lesser extent of Cd. This indicates that a greater risk could be generated for the health of consumers by ejection preferably in milk and not in urine. Finally, in the case of Sr and Tl, the transfer values for both matrices are important, although they are of higher concentration towards urine ($p < 0.05$) than towards milk, which indicates that these metals preferentially tend to reincorporate back into the environment in the urine.

5. Conclusion

According to the results obtained in this work, it can be concluded that the Pb in milk was higher than the maximum determined by international standards, however, it is below the Mexican standard. The concentration of metals in blood is not necessarily greater than that found in milk or urine, it is strictly observed only for Cd, but not for the rest of the metals studied, reflecting that, when ingested by cows in the forages, are easily transferred to milk and urine.

The transfer index indicated that Cr, Cd and Cu are preferentially transferred from blood to milk, Sr and Tl from blood to urine and Pb and As, their mobility is indifferent to either of the two matrices. However, it is observed that all the metals analyzed here are transferred to the milk, thereby generating a health risk.

The presence of heavy metals in the different matrices makes it possible to determine whether these contaminating elements have a recent or chronic entry into the animal organism, at the same time that it determines the degree of environmental and trophic chain contamination, which is why the cow is considered an excellent biomarker of contaminated areas.

It should be noted that metals such as Pb, Cd, Cr and As are considered high risk to health and are present in milk, due to the presence of these in the diet that cows receive, thus threatening food safety. It is of the utmost importance to provide diets that come from cleanly managed farms. It is important to consider the results presented here, whenever it is intended to make decisions aimed at preventing diseases caused by these types of elements and thus avoiding treatment costs in humans.

Declarations

Author contribution statement

Numa Pompilio Castro González, Calderón-Sánchez Francisco: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Fuentes de María-Torres Marco Tulio and Silva-Morales Sergio Samuel: Performed the experiments.

Fernanda Eliza González-Juárez: Performed the experiments.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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