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ORIGINAL PAPER

Concentration of trace elements in raw milk from cows in the southeast of Córdoba province, Argentina

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Abstract In recent years, trace elements in cow milk have been considered good bioindicators of pollution in the agricultural environment. The aim of the study was to analyze the correlation between trace element content in livestock drinking water and cow milk from dairy farms located in the southeast of Córdoba province, Argentina. Groundwater is the main source of livestock drinking water. According to the results, trace elements were grouped in three categories: (a) those that were in high concentration in phreatic water and in low concentration in deep wells (As, V); (b) those which showed the opposite trend (Cu, Fe, Mn, Zn); and (c) those that were in very low concentrations in all water samples (Cd, Cr, Ni, Pb, and Se). In elements from group (a), a positive correlation between As content in water and in milk was observed. For elements included in group (b), it was observed a higher concentration in milk samples from farms that use deep wells, related with their higher concentrations in water. Cr, Ni, Pb, and Se milk contents are within the ranges reported in other areas. Soil and forage trace element content may contribute to their presence in milk. Since information about transference of trace elements from environmental matrices to milk is very scarce, at national and international levels, further studies are necessary, including speciation in milk and dairy products, to guarantee food safety.

Keywords Cow milk · Trace elements · Groundwater · Soil and forage

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1 Introduction

Increased awareness on the influence of diet on human health has prompted to produce food of higher quality, rich in nutrients and health promoters (Ataro et al. 2008).

The analysis of production processes and identification of quality markers for milk and dairy products is of great importance for their evaluation and to assure their safeness. Consequently, as a safeguard, one of the main objectives in livestock production is to undertake a careful and thorough assessment of all mechanisms by which feedstuff quality, manufacturing processes, and environmental conditions, including health parameters, can influence milk properties (Vahčić et al. 2010).

Cow milk and its products are basic foods and constitute an important source of nutrients in human diet. Their content of protein, fat, carbohydrates, vitamins and minerals determine their biological and technological properties.

Although the fundamental composition of cow milk is known, its microelement content is generally unknown. It has been reported that the content of the main mineral components, such as Ca, P, K, Na, Mg, Cl, and S, do not vary and undergoes only slight changes depending on the lactation phase and the quality of nutrition, in particular, under the influence of applied mineral additives or environmental conditions, mainly due to chemical pollutants (Dobrzański et al. 2005).

In recent years, there has been a growing interest on microelements, as their presence in food is an indicator of quality, such as processing conditions, environmental pollution, sanitation, and husbandry, and may affect the chemical and functional properties of milk (Ayar et al. 2009). The content of trace elements in cow milk has begun to be more widely studied, particularly in industrialized and polluted regions, since it is considered a good bioindicator of pollution of the agricultural environment (Dobrzański et al. 2005; Elbagermi et al. 2014).

As environmental contaminants, trace metals constitute one of the greatest dangers to human health because they tend to be stable; they persist in the environment and are accumulated in the food chain. To analyze their toxicological aspects, it is necessary to know its concentration in diet, their oxidation state, and chemical form, as well as the quantity of other components that influence their absorption and metabolism (Hughes et al. 2011).

Generally, animals reduce human exposure to trace metals; for instance, levels present in different environmental matrices are higher than those found in food. However, some trace elements have been found in cattle diet at levels tolerated by the animals that could be transferred to their tissues at concentrations not acceptable for human consumption. The elements identified are As, Cd, Cr, Cu, Pb, and Se (NRC 2005; Ayar et al. 2009).

In the case of arsenic (As), its presence in drinking water is one of the most important health problems in the world. Arsenic is a broadly distributed element in the nature and is of high toxicity for living organisms. The Chacopampean plain of central Argentina constitutes one of the largest regions of high As levels in groundwater known. One of the most affected areas is the southeast of Córdoba. In previous studies carried out in this area, groundwater As concentrations were above the recommended limit for livestock drinking water (Pérez-Carrera et al. 2008). This fact motivated a study to estimate an As biotransference factor to milk considering drinking water as the main source of this metalloid (Pérez-Carrera and Fernández-Cirelli 2005; 2008).

The aim of the present work was to analyze the relationship between trace elements content in drinking water and in cow milk from dairy farms located in the southeast of Córdoba province, one of the most important dairy areas of Argentina. These studies may contribute to the knowledge of the influence of livestock drinking water on milk composition regarding elements of toxicological and/or nutritional importance.

2 Materials and methods

2.1 Study area

The study area is located in the southeast of Córdoba province, Argentina, between 62° 33′ and 62° 57′, west longitude and 32° 12′ and 32° 50′, south latitude, in the rural area belonging to four counties: Bell Ville, Morrison, Cintra, and San Antonio de Litín (Fig. 1). Water, soil, forage, and milk were taken from 30 small (10–20 animals) and medium (100–120 animals) dairy farms.

2.2 Sampling and samples preparation

2.2.1 Water

Water samples (500 mL) were collected in polyethylene bottles rinsed with 10% nitric acid and deionized water prior to collection. Samples were cooled (but not frozen) for

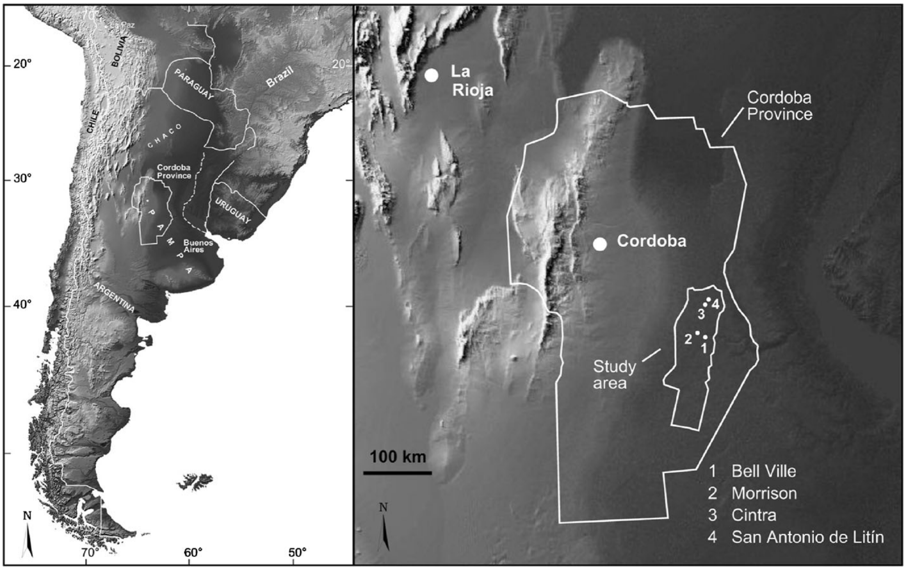

Fig. 1 Maps of Córdoba province in Argentina (*left*) and of the study area (*right*) in the southeast of Córdoba

their transportation to the laboratory (APHA 1993). All measurements were performed in duplicate with relative errors below 1.0%.

2.2.2 Milk

Raw milk samples (500 mL) from three animals were collected at each small farm while five animals were chosen at each medium-sized farm. The animals were randomly selected. Samples were placed into polyethylene bottles that were rinsed with 10% nitric acid in deionized water prior to collection. Samples were cooled (but not frozen) for their transportation to the laboratory. Milk samples (approximately 100 g from each) were placed into 400-mL heat-resistant glass beakers and dryly reduced to ash as previously described by Cervera et al. (1994) and Pérez-Carrera and Fernández-Cirelli (2005). White ash was moistened with a reagent grade water, dissolved in 10 mL 6 M HCl, filtered through Whatman No. 1 paper into a 25-mL volumetric flask, and diluted to original volume with 6 M HCl. Duplicate controls were prepared by treating the ash solution with the same digestion procedure.

2.2.3 Soil

Soil samples were air dried, sieved through a 2-mm sieve mesh, and pulverized in an agate mortar. For trace element content determination, 0.1 g of soil were subjected to acid digestion with 10 mL of HNO_3 (c) and 10 mL of H_2SO_4 (c). After the complete oxidation of organic matter, and production of sulfur trioxide fumes, the samples were cooled and diluted to 50 mL with deionized water.

2.2.4 Forage

All forage samples were washed with H_2O_d and ultrapure water to removed soil particles. The disintegration of the vegetable samples was performed by microwave digestion in closed cup. The following scheme digestion was performed: 10 mL HNO_3 and 1 mL H_2O_2 per 0.5 g forage sample. There were 2 cycles, 2 min at 250 W and 5 min at 750 W. The samples were diluted to 50 mL with deionized water.

2.3 Reagents

All reagents were of analytical grade. Working solutions were prepared by appropriate serial dilution of commercially available multielement standard stock solutions (PerkinElmer. Atomic Spectroscopy Standard No. 9300281) using ultrapure water provided by a Milli-Q water purification System (Millipore, Bedford, MA, USA).

Certified reference materials for verification of the calibration procedure and validation of the analytical method were used for each type of studied matrices. In the case of water, TM 26.2, TMDA 51.2, TMDA 52.2, and TMDA 54.3 from the National Water Research Institute of Canada (NWRI) were used. In the case of soils, WQB CRM-3 were used (NWRI). For forage, NIST-1570a was used, from the National Institute of Standards and Methods (NIST), and, in the case of milk, NIST-8435 was used.

To avoid possible matrix interference internal standard technique was used. This technique was performed using Yttrium (1 mg. L^{-1}) as internal standard according to Milburn, 1996.

2.4 Trace elements analysis

Trace elements content in water, milk, soil, and forage was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer, Optima 2000) following standardized methods (APHA 1993). Determinations were performed in duplicate being the relative error <1.0% for all of them. In the case of As, due to its generally low concentrations in cow milk, it was used as a highly sensitive hydride generator coupled to ICP-OES (PerkinElmer 1979) for its determination in collected milk samples following the procedure previously described by Cervera et al. (1994). All samples were analyzed in duplicate with a relative error below 1.0%.

2.5 Statistical analysis

The average, standard deviation, and minimum and maximum of trace elements concentrations in milk samples from dairy farms using phreatic water or deep wells for drinking water were calculated using the statistical package Statistica for Windows (ver. 5.1, 1997, Statsoft, USA).

To compare and validate the concentration of trace elements founded with the certificated values, an accuracy test was used. This test was based on the calculation of the recovery percentage (trueness). The results are showed in Supplementary Tables SI and SII. For those elements which values were not determinate in certified reference, it was used the internal standard using yttrium (1 mg.L^{-1}) previously described in Section 2.3. Certified concentrations of As, Cd, Cr, Ni, and V for milk (NIST-8435); Cd, Cr, Cu, Pb, and V in soil (WQB CRM-3); and Cr, Fe, and Pb in forage (NIST-1570a) were not determined since reference materials were not available, therefore those data should be considered as preliminary.

3 Results

In the studied area, groundwater is the main source of livestock drinking water. Phreatic water wells account for 60% of the analyzed samples. The majority of phreatic water wells were found between 3- and 8-m depth, with extremes of 2- and 15-m depth. The remaining 40% of wells ranged in depth from around 80- to 150-m depth (deep wells).

Trace element content in cow's drinking water is shown in Tables 1 and 2. According to these results, trace elements may be grouped in three categories: (a) those that are in high concentration in phreatic water and in low concentration in deep wells (As, V); (b) those which show the opposite trend (Cu, Fe, Mn, Zn); and (c) those that are in very low concentrations in all water samples from the study area (Cd, Cr, Ni, Pb, and Se).

In previous studies, correlations between As content both in phreatic water and deep wells, and its content in milk allowed us to calculate a biotransference factor to milk (Pérez-Carrera and Fernández-Cirelli 2005). Taking into account those results, we have

Table 1 Trace elements concentrations (μg.L^{-1}) in samples obtained from phreatic water

	Maximum	Minimum	Average	Standard deviation	NRC[†]
As	4550	71	1282	1430	50
Cd	<0.5[‡]	<0.5[‡]	–	–	5
Cr	<5[‡]	<5[‡]	–	–	100
Cu	22.4	<4	6.2[§]	6.8[§]	1000
Fe	1405	<5	154[§]	415.3[§]	2000
Mn	175.4	<2	28.1[§]	55.3[§]	50
Ni	<3[‡]	<3[‡]	–	–	250
Pb	<12[‡]	<12[‡]	–	–	15
Se	<5 [‡]	<5[‡]	–	–	–
V	5661.4	125.2	2079.5	1891	100
Zn	129.8	25.8	64	28.2	5000

[†] Guideline level (μg.L^{-1}) for dairy cattle drinking water (NRC 2005)

[‡] Detection limit

[§] To calculate the average and standard deviation it was considered half the value of the detection limit

analyzed milk from dairy farms that used phreatic water and deep wells to study their composition and trace elements content.

In reference to Vanadium (V) content, all of the samples from phreatic water were above the guideline level recommended for cattle drinking water, while 8% of the samples from deep wells are above that value. All the other trace elements were below the recommended values both in phreatic water and deep wells.

Table 2 Trace elements concentrations (μg.L^{-1}) in samples obtained from deep wells

	Maximum	Minimum	Average	Standard deviation	NRC[†]
As	79	29	56	16	50
Cd	<0.5[‡]	<0.5[‡]	–	–	5
Cr	<5[‡]	<5[‡]	–	–	100
Cu	46.1	<4	7.7[§]	12.7[§]	1000
Fe	3982.4	14.7	865.9	1291.4	2000
Mn	684.6	25.1	232.1	209.6	50
Ni	<3[‡]	<3[‡]	–	–	250
Pb	<12[‡]	<12[‡]	–	–	15
Se	<5[‡]	<5[‡]	–	–	–
V	274.5	<4	26.3[§]	78.2[§]	100
Zn	513.9	16.2	157.3	150.9	5000

[†] Guideline level (μg.L^{-1}) for dairy cattle drinking water (NRC 2005)

[‡] Detection limit

[§] To calculate the average and standard deviation it was considered half the value of the detection limit

The mineral content of milk is about 0.7% of its dry matter. This fraction has a high nutritional and technological importance, in particular, the contribution of Ca and P. In raw milk, approximately 65% of Ca, 60% of Mg, and 50% of P are associated with casein, while Na and K are in solution (DeGaris and Lean 2008). The results of the analysis of major elements in raw milk samples are shown in Table 3.

The concentrations of trace elements in milk samples are summarized in Table 4, corresponding to dairy farms using phreatic water (A) or deep wells (B), respectively.

Regarding elements included in group (b), Cu, Fe, and Mn concentrations in milk samples from dairy farms using water from deep wells were between the ranges determinate in milk samples from dairy farms using phreatic water. In the case of Zn concentration, there were higher values obtained in milk samples from dairy farms using deep wells. No significant differences were found (ANOVA, Kruskal-Wallis).

In Table 5, the concentrations of the different trace elements determined in this work are compared with that obtained by other authors.

Since trace elements are present in milk, although in low concentrations, but are at non detectable or detectable in very low levels both in phreatic water and in deep wells, soil, and forage in the studied dairy farms were analyzed for trace elements.

Soil content of Cd (<0.5 to 28 mg.kg^{-1}), Cr (42.8 to 108.2 mg.kg^{-1}), Cu (31.5 to 93 mg.kg^{-1}), Pb (7.8 to 55 mg.kg^{-1}), Se (<0.1 to 0.8 mg.kg^{-1}) and Zn (61 to 129.5 mg.kg^{-1}) were in the range reported for non contaminated agricultural soil in a few countries around the world (Kabata-Pendias and Mukherjee 2007).

In alfalfa, concentrations of Cd were below the detection limit of the method, and Cu (8.2 to 15.2 μg.kg^{-1}) was below the phytotoxicity level (de Souza Silva et al. 2014). Zinc concentrations (20.2 to 56.8 μg.g^{-1}) were in the range reported in literature (Rafique et al. 2006).

Pb levels in soil and alfalfa (<1.0 to 8.3 μg.g^{-1}) are low due to the lack of industrial pollution in the study area. Since Se levels were low in the studied soil, the concentrations in forage were below the detection limit.

4 Discussion

According to the results obtained in groundwater samples, the trace elements could be grouped in three groups, previously described, (a) As and V that appears in high concentrations in phreatic water samples; (b) Cu, Fe, Mn, and Zn present in high concentrations in deep wells; and (c) those elements that are in low concentrations in all samples.

Considering As concentration, all the samples from phreatic water and 80% of deep wells were above the level of 50 μg.L^{-1} proposed by the National Research Council for cow's drinking water (NRC 2005). Vanadium (V) usually occurs together with As and F in groundwater in extensive areas of Argentina. Its importance in the animal nutrition has not been demonstrated yet (Pérez-Carrera et al. 2015).

The obtained results are comparable with those reported by Taverna et al. (2001), in raw milk from dairy farms from another milk basin located at the east of our study area. The average concentration of Ca, P, K, and Mg were within the range considered appropriate for dairy cattle. These results confirm the assessment previously reported by Dobrzański et al. (2005) that the content of main mineral

Table 3 Macromineral concentration in analyzed milk samples (g.kg^{-1})

Element	Minimum	Maximum	Average	SD
Ca	0.7	1.6	1.03	0.26
P	0.9	1.3	1.06	0.12
Na	0.3	1.2	0.6	0.25
K	1.0	2.7	1.5	0.36
Mg	0.06	0.2	0.09	0.03

components is not diverse and may undergo only slight changes in different breeding and environmental conditions.

As expected, higher As concentrations were determined in milk samples from dairy farms using the phreatic water than the ones from those using deep wells. Although V is related to As due to their common origin in water, it shows a distinctive behavior regarding its possible transference to milk. In spite of its high concentration in water, V presence in milk samples could be determined only in 23% of the analyzed samples from dairy farms using phreatic water. In addition, none of the analyzed milk samples from dairy farms using deep wells were above the detection limit from V.

Copper (Cu) is a cofactor of many cuproenzymes and several proteins are involved in its metabolism (Miranda et al. 2009). Cu concentration in bovine milk reported in the literature is very variable (Table 5). In the analyzed samples at this work, Cu concentration was within the reported values in literature.

Table 4 Trace element concentrations in milk (ng.g^{-1}) from dairy farms that use drinking water obtained from the phreatic aquifer (A) and from deep wells (B)

	A			B		
Elements	Minimum	Maximum	Mean ± SD‡	Minimum	Maximum	Mean ± SD‡
As	0.7	11	4 ± 2.8	0.2	7.8	3.5 ± 2
Cr	<3.5†	72.8	38 ± 20.6‡	33	64.7	54 ± 9
Cu	9	90	26.2 ± 20.5	10	68	38.5 ± 19.5
Fe	245	3297	855.3 ± 737	364	2790	966 ± 734
Mn	5.2	95	21 ± 18	11	87	29 ± 22
Ni	22	149.6	48 ± 25	29.2	93.5	48.3 ± 16.4
Pb	<5†	51	23 ± 13‡	<5†	64	24 ± 17.3‡
Se	<5†	13.6	5.3 ± 3.8 ‡	<5†	18	9.4 ± 5.1‡
V	<2†	8.9	2.5 ± 2.2‡	<2†	–	–
Zn	300	2900	1800 ± 800	510	3995	2300 ± 1360

† Detection limit of the methodology

‡ To calculate the mean and standard deviation it was considered half the value of the detection limit

Iron (Fe) content in milk samples are comparable to the values reported in raw milk by Arellano et al. (2014) and within the range reported for pasteurized milk (Soares et al. 2010). The average level of Fe is higher than the average value reported in other studies by Rodríguez-Rodríguez et al. (2001) and NRC (2005), Table 5. These results indicate that high Fe content in drinking water and food, as it has been determined in this work, may affect Fe milk content.

Manganese (Mn) is a cofactor of mutase antioxidant enzyme and its elevated content could reduce Fe absorption. The average level of Mn found in this study is an order of magnitude lower that the reported in the literature (Table 5) and show differences between dairy farms using phreatic water and those using deep wells, although they are not statistically significant.

The average content of zinc (Zn) in milk (Tables 4) was lower than it was previously reported (Table 5). Zn deficiency reduces the immune response and the integrity of epithelia. It has been shown that Zn supplementation reduces the number of somatic cells in bovine milk (Pechová et al.. 2006). The study area is one of the most important dairy areas in Argentina, where 27.5% of milk production is characterized by less than 100,000 CFU.mL^{-1}; however, the concentration of somatic cells was greater than 400,000 cells.mL^{-1}(data not shown), which could be associated with Zn deficiency.

Among those elements which were in very low concentration in water samples, group (c), Cd was not detected in any of the milk samples (detection limit: 3 ng.g^{-1}).

Chromium (Cr) plays a crucial role in glucose metabolism, in which it enhances insulin activity (NRC 2005; Miranda et al.. 2009). The content of Cr in milk samples is within the range reported by Licata et al. (2004) and Soares et al. (2010) in a dairy area in Calabria, Italy (Table 5). However, the main concentrations of Cr in milk samples were lower than those previously reported by Ataro et al. (2008).

The content of Ni in milk samples was, in all cases, below the range reported by Coni et al. (1994, 1995) in Italy, and that reported for pasteurized milk in Brazil by Soares et al. (2010), Table 5.

The concentrations of Se in milk samples (Table 4), are within the range reported in bovine milk by other authors (Licata et al. 2004; Dobrzański et al. 2005) and lower than that reported by Ayar et al. (2009)

Lead (Pb) concentrations are in accordance with the reported values in non contaminated areas around the world (Ataro et al. 2008; Patra et al. 2008; Ayar et al. 2009). These results relate to the fact that concentrations of this element in water and forage in this area are below the limit considered safe for ruminants. Contrariwise, milk Pb concentrations reported by Soares et al. (2010) in industrial polluted areas are higher (mean: 230 ng.g^{-1}).

Arsenic levels in soil (2.1 to 8.2 mg.kg^{-1}) are in accordance with the concentrations reported in non contaminated areas (Nriagu et al. 2007; Kabata-Pendias and Mukherjee 2007). In alfalfa (*Medicago sativa*) leaves, the most often used forage in the area, As levels ranged between 0.1 and 1.5 μg.g^{-1}. No reference values were found for comparison, but they are below the ranges reported by Bundschuh et al. (2012) on the leaves used for livestock fodder in México.

The low levels of Pb and Se determined in soil and forage may suggest the existence of another source for these elements to explain their occurrence in milk, which requires further studies.

Table 5 Microelements and trace elements concentrations in milk (ng.g^{-1}) from groups (a)–(c) compared with other works

Trace element	Concentration		Reference
	Min–max	Mean	
Group (a)			
As	14–57	–	Dobrzański et al. 2005
	<0.2–80	20	Ayar et al. 2009
	0.2–11	4	This work
V	44.1–151.2	–	Dobrzański et al., 2005
	23.4–42	–	Ataro et al. 2008
	<2–8.9	2.5	This work
Group (b)			
Cu	<0.13–737.6	2	Licata et al. 2004
	7.2–357.8	51.8	Sola-Larrañaga and Navarro-Blasco 2009
	100–2550	1730	Soares et al. 2010
	9–90	20.5	This work
Fe	196–1030	500	Rodríguez-Rodríguez et al. 2001
	1457–2321	1859	Arellano et al. 2014
	245–3297	855.3	This work
Mn	14–614	231	Coni et al. 1994 and 1995
	130–221	169	Arellano et al 2014
	5.2–95	25	This work
Zn	2370–6800	4540	Rodríguez-Rodríguez et al. 2001
	3564–8229	6150	Brodziak et al. 2011
	27,140–42,870	34,330	Arellano et al. 2014
	300–3995	2050	This work
Group (c)			
Cr	<1.5–82	–	Licata et al. 2004
	186–371	–	Ataro et al. 2008
	3–179	79	Soares et al. 2010
	<3.5–72.8	38	This work
Ni	162–712	–	Coni et al. 1994 and 1995
	2–210	73	Soares et al. 2010
	22–149.6	48	This work
Pb	<0.14–19.7	–	Ataro et al. 2008
	90–110	103	Ayar et al. 2009
	<5–64	23	This work
Se	11.7–53.4	–	Dobrzański et al. 2005
	230–350	232	Ayar et al. 2009
	<5–18	5.3	This work

5 Conclusions

Taking into account the data obtained in this work and considering it from a toxicological point of view, trace elements content in milk samples are similar to those reported in non contaminated areas and seemingly do not represent a risk for human health.

A correlation between drinking water and milk As content was observed. A similar trend may be seen for Fe, Cu, Mn, and Zn. These last elements are also present in animal food (mainly forage), which make an important contribution to their milk content, whereas in the former case, water is by far the main source of As in milk.

According to our results, there is no transference of V from drinking water to cow milk. Soil and forage trace elements content may contribute to their presence in milk. Nevertheless, concentrations determined in the present study are within the range reported for non polluted areas.

Since information about transference of trace elements from environmental matrices to milk is very scarce, not only at a national but at an international level, further studies on this subject are necessary, including speciation in milk and dairy products, to guarantee food safety.

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Compliance with ethical standards

Conflict of interest The authors on this manuscript, Alejo L. Peréz Carrera, Ph.D.; Mrs Flavia E. Arellano; and Alicia Fernández Cirelli declare that they have no conflict of interest.

Statement of human and animal rights This article does not contain any studies with human or animals subjects performed by any of the authors.

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