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# Identifying sources of metal exposure in organic and conventional dairy farming

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## HIGHLIGHTS

- Chemometric analysis allow to establish a pattern of metals related to farming.
- In organic farming, the soil ingestion is the main factor affecting metal status.
- In conventional farming, metal profile is determined by the supplementation of feed.
- Mineral supplementation in conventional may not be directly extrapolated to organic farming.

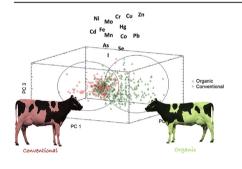
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### ABSTRACT

In humans the main route of exposure to toxic metals is through the diet, and there is therefore a clear need for this source of contamination to be minimized, particularly in food of animal origin. For this purpose, the various sources of toxic metals in livestock farming (which vary depending on the production system) must be taken into account. The objectives of the present study were to establish the profile of metal exposure in dairy cattle in Spain and to determine, by chemometric (multivariate statistical) analysis, any differences between organic and conventional systems. Blood samples from 522 cows (341 from organic farms and 181 from conventional farms) were analysed by inductively coupled plasma mass spectrometry to determine the concentrations of 14 elements: As, Cd, Co, Cr, Cu, Fe, Hg, I, Mn, Mo, Ni, Pb, Se and Zn. In conventional systems the generally high and balanced trace element concentrations in the mineral-supplemented concentrate feed strongly determined the metal status of the cattle. However, in organic systems, soil ingestion was an important contributing factor. Our results demonstrate that general information about the effects of mineral supplementation in conventional farming cannot be directly extrapolated to organic farming and special attention should be given to the contribution of ingestion of soil during grazing and/or ingestion of soil contaminated forage.

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### 1. Introduction

Toxic metals and metalloid compounds can occur in the





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environment as a result of contamination via human activities or they can be derived from natural geological sources. In terms of potentially adverse effects on animal and human health, the heavy metals cadmium, lead and mercury and the metalloid arsenic are among the elements of most concern. In addition, most essential trace elements can cause toxic effects if present at inappropriate concentrations. The main route of exposure to toxic metals in humans (except in heavily polluted areas) is through the diet, and there is a clear need for this source of contamination to be minimized (EC, 2002). Food of animal origin represents an important source of toxic metals in the human diet as meat and milk are extensively consumed and offal contains higher levels of toxic metals than other foods (López-Alonso et al., 2007).

When evaluating the exposure of livestock to toxic elements, it is important to consider that dietary exposure may be strongly influenced by husbandry practices, and minor dietary ingredients and/or supplements, medical treatments, soil and soil-contaminated forage are the main sources of toxic metals (López-Alonso, 2012a). Organic farming aims to reduce environmental contamination by limiting the use of inorganic fertilizers and of mineral supplements in compound feeds widely used in conventional systems; however, local environmental conditions can lead to high exposure to naturally occurring elements, particularly through soil when grazing (López-Alonso, 2012a).

Total element concentrations in the soil and feed do not provide reliable information about this route of transfer, as the bioavailability of toxic elements may be affected by many factors, including interactions between toxic and trace elements (López-Alonso et al., 2004). Moreover, as toxic metal exposure is multifactorial, the main sources of exposure to animals must be identified (i.e. soil, fertilizers, mineral supplements, etc.). These sources may vary depending on the type of production system considered.

Multivariate chemometric techniques provide a useful approach to elucidating the complex relationships in environmental systems characterized by intrinsic variability (natural/ anthropogenic, spatial/temporal, etc.). From this point of view, techniques such as factor analysis, principal component analysis (PCA) and cluster analysis (CA) have been used with the aim of revealing the complex and latent structures residing in chemical information obtained from environmental data (Kaplunovsky, 2005; Hanrahan, 2008). PCA and related techniques have been used to identify sources of contamination and to assess how these contribute to environmental contamination. For example, Barona and Romero (1996) used PCA to study the distribution of metals in soils and relationships between the amounts accumulated in the different soil fractions. Lin et al. (2002) also used factor analysis to characterize the variables affecting the presence of eight heavy metals in soil and the interrelationships between heavy metal contents of soil, the landscape and human activities. Gao and Chen (2012) recently used CA to study the heavy metal pollution status in surface sediments in the coastal Bohai Bay (China). Within the field of agriculture, these techniques have proved useful for estimating both the quality and the geographical origin of a wide variety of products, including wine (Rebolo et al., 2000), alcoholic distillates (Iglesias-Rodríguez et al., 2010), honey (Latorre et al., 2000), potatoes (Herrero-Latorre et al., 2013) and cow's milk (Sola-Larranaga and Navarro-Basco, 2009).

The objectives of the present study were to establish the profile of metal exposure in dairy cattle in Spain and to evaluate (by chemometric analysis) any differences between organic and conventional systems.

#### 2. Material and methods

#### 2.1. Sample collection and preparation

Blood samples (n = 522) were obtained from organically and conventionally raised cows in northern Spain, between November 2011 and June 2012. All organic dairy farms in the study area (22) farms), which supply almost 80% of all the organic milk produced in Spain, were sampled (n = 341). A total of 10 conventional farms representative of the dairy sector were sampled in the area (n = 181). Briefly, in all organic farms in the study the cows were grazed (mean = 44.0%) and received a low proportion of the dry matter (DM) intake as concentrate feed (15.6%). On the contrary, conventionally managed cows received more intensive diets (34.9% of concentrate feed) and were either grazed or were kept indoors and fed total mixed rations (TMR). The herd size was similar in both types of farms (mean milking cows per herd ranging between 47 and 52), although milk production was higher on the conventional farms (on average a 305-d-corrected milk yield of 9040 kg, relative to the corresponding value of 7122 kg on the organic farms).

Blood samples (10 mL) were taken from the coccygeal vein (with minimal stress) of cows after the morning milking, during routine visits to the farms between 2 weeks prior to the expected calving date and the peak lactation period (estimated at 90 days). Dietary information was recorded and samples of feedstuff (3 per farm) and soil (1 per farm) were collected for mineral analysis. All samples were refrigerated immediately and transported to the laboratory. Serum was obtained by centrifuging the blood samples at 3000 g for 15 min, within 4 h of collection. Triplicate subsamples of serum were stored at -20 °C until analysis. All sample collection and other experiments involving the cattle followed Spanish standards for the protection of animals used for experimental and other scientific purposes. The experimental design was assessed by the Bioethical Committee of the University of Santiago de Compostela (Spain), which also verified and granted permission for the procedures.

For the subsequent ICP analysis, the serum samples were treated as follows. Two mL of serum were cold digested with 2.5 mL of nitric acid (69% w/v) for 1 h. An aliquot (0.5 mL) of hydrogen peroxide (33% w/v) was added to the serum samples, which were then placed in a thermostatic block at 120 °C for 1 h to complete digestion. Once cooled, the digested samples were diluted to 10 mL with Milli-Q ultrapure water and analysed by ICP. All samples were analysed in duplicate. For total iodine determination, samples were prepared by a high temperature alkaline extraction procedure with tetramethylammonium hydroxide (TMAH) (25% w/v), according to the British-adopted European Standard (European Committee for Standardization, 2007).

#### 2.2. Analytical methods

Serum samples were analysed by inductively coupled plasma mass spectrometry (ICP-MS; VG Elemental PlasmaQuad SOption) to determine the concentrations of the following 14 elements: arsenic [As], cadmium [Cd], cobalt [Co], chromium [Cr], copper [Cu], iodine [I], iron [Fe], lead [Pb], manganese [Mn], mercury [Hg], molybde-num [Mo], nickel [Ni], selenium [Se] and zinc [Zn]. All samples were analysed in duplicate. An analytical quality control program was applied throughout the study. The readings obtained for blank samples (n = 36) processed alongside the serum samples were subtracted from the sample readings. The limits of detection in the acid digest were calculated as three times the standard deviation of the reagent blanks. Analytical recovery of the elements from standard reference material (SRM) (1598a, bovine serum, n = 16), processed simultaneously to the samples, was also determined. The results show acceptable agreement between the measured and

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