Environmental Science and Pollution Research (2024) 31:43903–43912
https://doi.org/10.1007/s11356-024-34060-9

RESEARCH ARTICLE

Toxic element (As, Cd, Pb and Hg) biodistribution and blood biomarkers in Barbaresca sheep raised in Sicily: One Health preliminary study

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Received: 24 January 2024 / Accepted: 17 June 2024 / Published online: 24 June 2024

Abstract
The health of humans, animals and the environment is interconnected. Adopting a One Health approach means intervening promptly to prevent the main diseases that affect animal health to guarantee the safety of livestock production. Exposure to toxic trace elements in sheep can lead to increased accumulation in different biological substrate, developing both acute and chronic diseases in humans and livestock. The aim of this study was to evaluate the bioaccumulation of arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) in Sicilian Barbaresca sheep using the following biological substrates: milk, blood and fleece. An inductively coupled plasma mass spectrometer (ICP-MS) was used for As, Cd and Pb, and a direct mercury analyser (DMA-80) was used for Hg determination. In addition, the role of the haematological parameters as possible indicators of different biodistribution was evaluated. A statistically significant value was observed from our analysed metals in the substrates: arsenic ($p < 0.001$), cadmium ($p < 0.01$), lead ($p < 0.001$) and mercury ($p < 0.0001$). The correlation analysis showed a relationship between milk and blood for arsenic ($p < 0.0001$) and lead ($p < 0.0001$), and no correlation for the metals was observed between milk/blood and the haematological parameters analysed for the low concentration observed in the present study comforting the final consumer.

Keywords Barbaresca sheep · Blood biomarkers · Livestock · One Health · Toxic metals · Bioaccumulation

Introduction

One Health is an integrated and interdisciplinary approach to the design and implementation of actions and policies at the human-animal-environment health interface. However, the role of the environment in this triad has often been overlooked, so it is important to monitor livestock environments. From the perspective of One Health, evaluating the bioaccumulation of toxic elements in animals used for human consumption represents an important challenge for the future in order to protect human health. Trace elements play an essential role in the survival of living organisms; however, if present in high concentrations, they become toxic to humans and animals and are defined as heavy metals. The natural sources of these substances in the environment are surface water, soil, pastures, vegetation and combustion. It is therefore easy for animals in livestock production to take in a high intake of these metals, posing a source of risk to the end consumer (Elafyfy et al. 2023). Heavy metals are non-biodegradable and tend to accumulate in internal organs causing blood-biochemical and pathological changes. They have the capacity to cause toxicity and oxidative deterioration of internal organs due to their chemical properties that allow them to escape cellular control mechanisms and bind to native proteins, DNA and nuclear proteins, inhibiting their biological activity (Jaishankar et al. 2014). Animals are good indicators of environmental toxic metal pollution (Gil and Hernandez 2009; Hernandez et al. 2014; Balali-Mood et al. 2021). Chronic exposure to heavy metals leads to their increased accumulation in various tissues such as the hair or blood (Fazio et al. 2022; Ali et al. 2019,

Responsible Editor: Wei Liu

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2023). Although the heavy metal values found in different biological matrices of different animals are an indicator of exposure to environmental pollutants, the degree of heavy metal contamination is not constant and differs depending on exposure routes, environmental conditions, animal feeding, lactation stage and animal breed (Monteverde et al. 2022). Whole blood gives an idea of short-term exposure to toxic elements. Blood is a less invasive substrate than organs, e.g. kidneys and liver; moreover, haematological variables are closely dependent on environmental changes and are, therefore, considered valid indicators of metal pollution. In addition, routine haematological analysis allows us to monitor the health status of the animal while it is alive (Nava et al. 2023; Altinok-Yipel et al. 2022). Toxic element contamination has been studied extensively in different substrates and species; little information is known about the sheep and goat sector and in particular for the Sicilian Barbaresca sheep, a dual-purpose breed that is a mainstay of livestock farming in Sicily. Optimal zootechnical production is focused on animal and herd management. A correct complete clinical evaluation of the animals is based on the CBC (Complete Blood Count) or haemochromo-cytometric examination or haemogram. The sheep haemogram shows various differences to that of other species due to the size of the red blood cells, which are smaller, as well as the high erythrocyte resistance (Polizopoulou 2010). It is interesting to assess and correlate how different bioaccumulation of pollutants and certain heavy metals evokes a haematological response in this species in relation to anthropogenic loading (Capitão et al. 2022). Blood biomarkers are useful biological indicators that allow us to detect a change in the body's homeostasis due to the exposure to heavy metals through blood tests that can be correlated with their bioaccumulation (Giannetto et al. 2022). The haematological profile is a sensitive index for assessing the metabolism and thus the health condition of humans and animals subjected to 'metal' stress. Indeed, exposure to anthropogenic loading of certain heavy metals in animals could trigger a number of protective mechanisms at the haematological level. These mechanisms could induce changes in blood parameters that tend to bring the organism back to a state of homeostasis. Therefore, the pollution of the environment by anthropogenic loading of heavy metals can be assessed using haematological parameters that reflect the effects of contaminants on organisms exposed to these substances (Fazio et al. 2020). In humans, biomarkers can be used as useful indicators to measure levels of toxic element exposure (Wongsasuluk et al. 2018; Park and Seo 2017). The body defends itself against their bioaccumulation through excretion and elimination of these elements through urine, faeces, sweat, breast milk, hair and nails. These substrates presume a passage of the same metals in the blood and thus an inevitable interaction with haematological parameters. The use of biomarkers brings a number of important advantages, including obtaining an integrated response of the body to the interactions of different toxic effects resulting from exposure to a mixture of several pollutants. Another positive effect is that they allow an immediate response of the body to the effect of the toxic substance by predicting its long-term effects. They are therefore extremely useful in solving the problem of environmental pollution and the concept of One Health, which is an ideal approach to achieving global health. Moreover, in the Mediterranean environment, the sustainable agriculture includes the use of local breeds, disease resistance, forage self-sufficiency, forage quality, milk and cheese quality, typical and/or traditional products. In this context, local breeds appear to be the major component of animal farm biodiversity, thanks to their excellent adaptation to specific environmental conditions. Furthermore, local breeds are linked to traditional products of special qualities and to several practices that are part of cultural heritage. Therefore, every effort to add value to local breeds is important, especially as a contribution to the prospects of their conservation through sustainable use (Di Trana et al. 2015). The aim of the present study is to identify the potential bioaccumulation of some heavy metals (arsenic, cadmium, lead and mercury) in the Sicilian Barbaresca sheep, reared in the semi-wild condition in the province of Tusa (Messina), through the use of biological substrates such as blood, milk and fleece in order to identify their different biodistribution. This experimental investigation also aimed to study the role of haematological parameters as blood biomarkers of the heavy metals studied in this species as indicators of the different biodistribution.

Materials and methods

Experimental design

The experiment was carried out in Tusa, province of Messina (Italy: 37°59′N 14°14′E), on a family-run farm from the Nebrodi mountains which is part of non-industrialised part of Sicily. For the experimentation, 25 randomly chosen female sheep in a good state of health were used as a representative sample within a population of 300 animals. The group was homogeneous in terms of age (between 2 and 3 years) and calving order. At the time of the trial, all subjects were in their 6th month of lactation. Prior to the experimental protocol, the physiological parameters of each individual animal, rectal temperature (38.4–39.2 °C), respiratory rate (19–28 rpm) and heart rate (68–80 bpm) were monitored. The animals were kept on a semi-extensive pasture constituted by a silty soil, hilly ground and permanent polyphitic pasture. The pasture area had native trees, such as olive (*Olea europaea*) and chestnut groves (*Castanea*). The animals had free access to water. They remained free in the pasture for most of the day and only lactating sheep were taken to the stalls to receive a supplement of commercial feed if necessary. Prior to the experiment, water samples have been analysed and no mineral excesses or other substances were detected.

Sampling

Protocols and experimentation were reviewed and approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 63/2010/EU. The entire experimental protocol was approved by the standards recommended by the Ethical Committee of University of Messina (06/2023). The experimental protocol involved individual samples of milk, blood and fleece from the evening milking, around 18:00 (hh:mm). Blood samples were obtained during health monitoring, under the supervision of the animal welfare manager and the veterinary doctor from the laboratory of University of Messina. The milk, after complete milking, was collected and transported to the laboratory in special 'falcon' tubes (30 ± 5 ml) according to Liotta et al. (2019) technique. Blood was collected from the jugular vein using a 3.5 ml vacutainer tube containing K3 EDTA (Fazio et al. 2016). Samples were kept at 4 °C. The fleece was obtained from the rump area (30 ± 1.50 mm) using plastic scissors to avoid contamination of the fleece with other metals and collected individually in plastic bags (Tuncer et al. 2017). During sampling, environmental parameters such as temperature (16 °C), sunrise (hh:mm 05:43), sunset (hh:mm 20:18), humidity (97%), ventilation (6 km/h), and AQI (36) established by Legislative Decree 155/2010 were monitored by means of a multiparameter probe (Testo 400). Haematological parameters (RBC, WBC, Hct, HGB, PLT, MCV, MCHC) were processed using an automated system (HeCo Vet C, SEAC, Florence, Italy).

Inductively coupled plasma mass spectrometry (ICP-MS)

The determination of the concentration of the inorganic elements of interest was carried out using a Thermo Scientific iCAP Q-ICP-MS. After mineralisation, the sample arrives at the ICP-MS in liquid form, and with the aid of an autosampler ASX520 (Cetac Technologies Inc., Omaha, NE, USA), it is injected into the instrumentation, where a peristaltic pump brings it to the nebuliser area—coupled to a nebulisation chamber—transforming it into an aerosol. The nebulisation chamber, cooled using Peltier cells to improve instrument performance, then conveys—thanks to a flow of argon acting as a carrier—the aerosol into the torch, a component consisting of two concentric quartz tubes that generates the plasma for atomising the elements. The transfer of the ionised sample from the plasma to the analyser takes place through the interface region; in particular, the passage of ions from the atmospheric pressure zone of the plasma to the vacuum pressure zone of the mass is enabled by a system of cones (sample cone and skimmer cone) and a system of ion lenses, which are essential for achieving high analytical sensitivity. The presence of a collision cell (Q Cell), placed before the analyser, is intended to diminish or eliminate possible polyatomic interference through the use of a so-called collision gas such as helium. The ions are then introduced into the quadrupole mass analyser, from which they will flow to the detector on the basis of their different mass-to-charge ratio (Nava et al. 2023).

Table 1 Operating parameters used for the mineralisation of sheep milk samples

Step	Time (min)	Temperature (°C)	Power (W)
1	10	Up to 200 °C	Up to 1000
2	20	200 °C	Up to 1000

Table 2 Operating parameters used for the mineralisation of sheep fleece samples

Step	Time (min)	Temperature (°C)	Power (W)
1	10	Up to 200 °C	Up to 1200
2	12	200 °C	Up to 1200

Sample preparation

In the present work, sampling preparation followed procedure according to Giannetto et al. (2022) and Nava et al. (2023). In order to be able to determine mineral element concentrations, a sample pre-treatment step is required, consisting of a mineralisation or acid digestion process. In the following work, a 'Milestone Ethos One' microwave digestion system was used. Temperature, pressure, time and power parameters are managed via a touchscreen display, equipped with Easy Control software. For the mineralisation of sheep milk samples, approximately 0.5 g was weighed and subsequently digested by adding 7 ml of 65% nitric acid (HNO_3) and 1 ml of 30% hydrogen peroxide (H_2O_2). In the same way, the analytical blank was prepared, a reference mixture quite similar to the samples to be analysed, but without the analytes of interest. The digestion programme was carried out in two stages: the first involved a linear increase in temperature to 200 °C for 10 min at a maximum power of 1000 W, while in the second the temperature was kept constant at 200 °C for 20 min at the same power (Table 1). For the sheep fleece samples, the same amount was weighed as for the milk. However, the reagent volumes and operating conditions of the mineralisation (Table 2) varied, as shown below: 7 ml of 65% nitric acid ($HN O_3$) and 2 ml of 30% hydrogen peroxide (H_2O_2).

The blood samples were also weighed approximately 0.5 g. However, for these matrices, the reagent volumes used for digestion were 8 ml of 65% nitric acid ($HN O_3$) and 2 ml of 30% hydrogen peroxide (H_2O_2). The operating conditions also differed from the milk samples, as shown in Table 3. At the end of the digestion process, all samples were cooled for 20 min and then diluted with ultrapure water to a volume of 25 ml and then filtered through 0.45 µm filters, to be analysed by ICP-MS (Nava et al. 2023).

Calibration procedure

The following standard solutions were used for the ICP-MS analysis: As, Cd, Pb (1000 mg l^{-1} in 2% nitric acid) from Fluka. These were used for the preparation of the calibration curves. Specifically, seven-point calibration curves were obtained using standard solutions in the following concentrations: 0.5, 2.0, 1.0, 5.0, 10.0, 20.0 and 50.0 µg l^{-1}. Standard solutions of ^{45}Sc, ^{73}Ge and ^{115}In (1000 mg l^{-1} in 2% nitric acid, Fluka) were used as online internal standards (at the level of 1.5 mg l^{-1}) so that instrumental drift and variations due to the matrix could be corrected. A Re standard solution at 1000 mg l^{-1} in 2% nitric acid (Fluka) was used as a preparation standard at 0.5 mg l^{-1} to check sample digestion and to correct for volumetric variations. A solution containing 1 µg l^{-1} of ^{7}Li, ^{59}Co, ^{138}Ba, ^{209}Bi, ^{142}Ce, ^{115}In and ^{238}U in 2% HNO_3 + 0.5% HCl (Thermo Fisher Scientific) was used for instrument calibration (Nava et al. 2023). The following isotopes were monitored: ^{75}As, ^{111}Cd, ^{208}Pb. Analyses were performed in triplicate; the operating conditions of the ICP-MS analysis are shown in Table 4. To validate the method in terms of linearity (R^2), sensitivity (LOD and LOQ), and accuracy (% of recovery), three certified matrices were used: Whole Blood Metals Control Level 3 for blood samples, ERM-BD150 (skimmed milk powder) for milk samples, and ERM-DB001 (human hair) for fleece samples (Table 5).

Table 3 Operating parameters used for the mineralisation of sheep blood samples

Step	Time (min)	Temperature (°C)	Power (W)
1	2	Up to 85 °C	Up to 1000
2	4	Up to 135 °C	Up to 1000
3	5	Up to 230 °C	Up to 1000
4	12	230 °C	Up to 1000

Mercury determination by DMA-80

The DMA-80 (Direct Mercury Analyser-Milestone) was used for Hg determination. This system offers the advantage of analysing the sample in a very short time (5 to 10 min) by eliminating any preparation step. The operation of the analyser is based on the principle of thermal decomposition of the sample and subsequent determination by atomic absorption spectrophotometry. The sample (0.1 g for solid samples and 100 µl for liquid samples) is weighed directly into the nickel (solid samples) or quartz cuvettes (liquid samples) housed on the autosampler and introduced into the furnace where it is dried (250 °C for 60 s) and then thermally decomposed in an oxygen current (750 °C for 150 s). In this way, mercury vapours are formed and selectively trapped by a gold amalgamator, while all other products are eliminated. When the amalgamator is subsequently heated, the mercury is released for quantitative determination by atomic absorption at 253.65 nm. The software creates an absorbance/ng graph and returns a concentration in µg/kg or mg/kg calculated from the weight of the sample analysed. Calibration curves were constructed

Table 4 Operating conditions of ICP-MS

Spectrometer	iCAP Q Thermo Scientific with Qcell Reaction System
Nebuliser	Concentric PFA
RF generator	1550 W
Sample depth	5 mm
Interface	Sample and skimmer cones in Ni
Interface pressure	1.89 E + 00 Pa
Argon flow (plasma/auxiliary/carrier)	14/0.8/1.1 l/min
Sample introduction flow	0.93 ml/min
Scanning condition	Number of replicates, 3; dwell time, 1 s
CCT gas flow (He)	4.7 ml/min
Vacuum	< 7.5 E − 07 Pa
Extract lens 1 voltage	1.5 V
Spray chamber temperature	2.7 °C

Table 5 Validation parameters of ICP-MS and DMA-80 methods were performed in terms of linearity, limit of detection (LOD), limit of quantification (LOQ), and accuracy (% of recovery) ($n=6$)

	Whole Blood Metals Control Level 3				ERM-BD150 (skimmed milk powder)				ERM-DB001 (human hair)			
	LOD (mg/kg)	LOQ (mg/kg)	R^2	Recovery (%)	LOD	LOQ	R^2	Recovery (%)	LOD	LOQ	R^2	Recovery (%)
As	0.004 ± 0.001	0.013 ± 0.003	0.9998	98.50 ± 0.23	0.001 ± 0.000	0.003 ± 0.001	0.9997	97.50 ± 0.20	0.0002 ± 0.000	0.001 ± 0.000	0.9999	101.00 ± 0.41
Cd	0.0002 ± 0.000	0.001 ± 0.000	0.9999	102.00 ± 0.40	0.001 ± 0.000	0.003 ± 0.001	0.9999	99.00 ± 0.25	0.001 ± 0.000	0.003 ± 0.001	0.9998	99.50 ± 0.31
Pb	0.001 ± 0.000	0.003 ± 0.000	0.9999	101.50 ± 0.37	0.0005 ± 0.0000	0.002 ± 0.000	0.9999	102.50 ± 0.43	0.001 ± 0.000	0.003 ± 0.000	0.9998	100.50 ± 0.22
Hg	0.0002 ± 0.0000	0.001 ± 0.000	0.9998	98.00 ± 0.35	0.0002 ± 0.0000	0.001 ± 0.000	0.9997	101.50 ± 0.46	0.001 ± 0.000	0.003 ± 0.000	0.9998	99.10 ± 0.25

using a certified mercury standard at 1000 mg l^{-1} (CZECH Metrology Institute Analytika) (Nava et al. 2023).

Statistical analysis

The data obtained are expressed as mean ± standard deviation (SD). All data were normally distributed (Kolmogorov–Smirnov test $p < 0.05$). One-way ANOVA (analysis of variance) was used to compare the different studied mineral concentrations among substrates. The Pearson correlation coefficient (r) was analysed in order to correlate the concentration of each mineral and the individual substrates (blood, milk and fleece) and to assess a possible relationship of the metal concentrations of each individual substrate with the direct haematological parameters, using the statistical software GraphPad Prism 9v (GraphPad Software, San Diego, Ca, USA). The Bonferroni test was applied for a post hoc comparison. Values of $p < 0.05$ were found to be statistically significant.

The obtained metal levels expressed in their conventional unit were used to assess health risk by calculating the target hazard quotient (THQ) and the hazard index (HI).

THQ developed by the United States Environmental Protection Agency (EPA) was estimated to assess non-carcinogenic human health risks from long-term exposure to metal through consuming milk (EPA 1989). THQ is defined using the following equation (EPA 2004).

$$\mathrm{THQ} = \frac{\mathrm{EF} \times \mathrm{ED} \times \mathrm{Wmilk} \times \mathrm{CMetal}}{\mathrm{RFD} \times \mathrm{WeightBody} \times \mathrm{TA}}$$

EF represent the frequency of exposure (365 days in a year); ED corresponds to the average lifespan of the exposure period (70 years); WMilk is the total dairy intake per day for an adult 0.066 (kg/day) from the Food and Agriculture Organization of the United Nations (FAO) (Ritchie and Roser 2017). CMetal (mg/kg) is the average level of metals in milk samples extracted during the study; WeightBody (kg) corresponds to the average body weight of 70 kg for adults; TA represent the average lifespan of 25,550 days; and RFD corresponds to the reference dose per day for AS (0.0003), Cd (0.001), Pb (0.004) and Hg (0.003), mg/kg body weight/day (Yan et al. 2022).

HI was calculated to assess the overall risk of more than one metal contained in milk. It was calculated using the mean and median THQ of each metal (Boudebbouz et al. 2023). An obtained THQ and HI value lower than 1 suggests negligible non-carcinogenic risk from exposure to the metal via milk consumption and a THQ value greater than 1 could represent a potential non-carcinogenic risk.

Table 6 Mean ± standard deviation of As, Cd, Pb and Hg evaluated within different biological substrates (milk, fleece and blood) in 25 Sicilian Barbary sheep bred in Tusa (Messina). Lowercase letters represent statistical significances observed between substrates

Element	Blood (mg/l) *Mean ± SD*	Milk (mg/l) *Mean ± SD*	Fleece (mg/kg) *Mean ± SD*
As	0.039[ab] ± 0.020	0.017 ± 0.014	0.001[a] ± 0.001
Cd	0.001[b] ± 0.001	0.005 ± 0.002	0.034 ± 0.031
Pb	0.036 ± 0.034	0.010 ± 0.008	0.016 ± 0.011
Hg	0.001[b] ± 0.000	0.001 ± 0.000	0.007[a] ± 0.004

[a]vs milk

[b]vs fleece

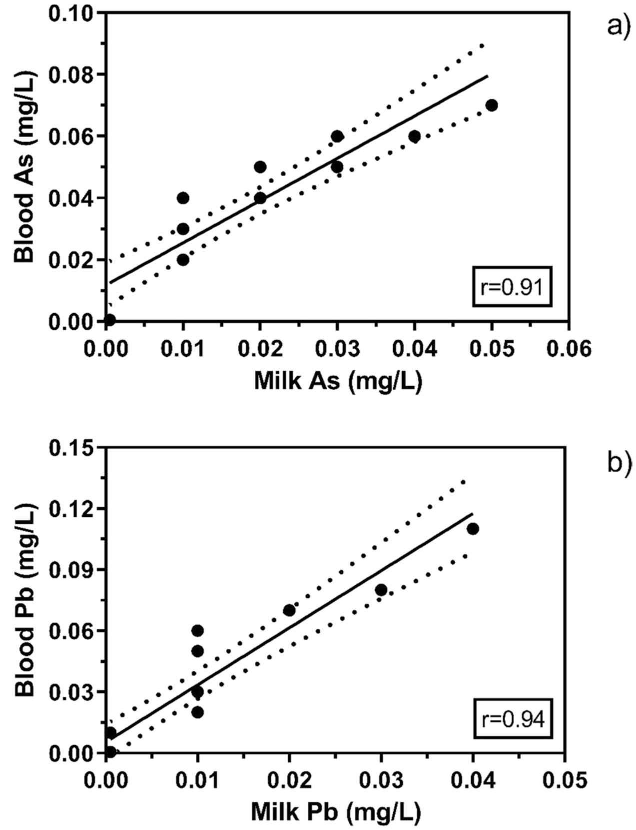

Fig. 1 Positive linear correlation with significant *r* values (Pearson coefficient) between the concentrations of arsenic (As) and lead (Pb) obtained respectively from blood and milk in 25 Sicilian Barbary sheep bred in Tusa (Messina)

Results

The concentrations (mean ± standard deviation SD) of As, Cd, Pb and Hg within milk, fleece and blood in sheep from a semi-wild condition are given in Table 6 together with their significances.

A statistically significant difference among substrates was found for the levels of As ($p < 0.0001$), Cd ($p < 0.01$), Pb ($p < 0.001$) and Hg ($p < 0.0001$). The Pearson correlation coefficient showed a positive correlation of As ($p < 0.0001$) between blood and milk and for Pb ($p < 0.0001$) between blood and milk as shown in Fig. 1. No correlation was found for Cd and Hg between blood and milk.

Blood biomarkers were described as mean ± standard deviation (RBC 8.10 ± 0.89; WBC 12.57 ± 3.76; HCT 34.51 ± 3.57; HGB 8.24 ± 0.83; PLT 270.72 ± 133.2). Linear correlation with *r* values (Pearson coefficient) between the studied blood biomarkers (RBC, WBC, Hct, HGB and PLT) and the concentrations of analysed metals in the different biological matrices (blood, milk and fleece) showed no statistical differences as reported in Fig. 2. The THQ and HI results of each analysed metal suggested a negligible non-carcinogenic risk of metals in milk as expressed in Table 7.

Discussion

Analyses of the bioaccumulation of certain heavy metals (arsenic, cadmium, lead and mercury) in different biological matrices (blood, milk and fleece) of Sicilian Barbaresca sheep showed data that are certainly comforting for consumers. In fact, for all the analysed metals, no risk to human health emerged as the analysed concentrations are below the acceptable values for meat and milk consumption in compliance with Regulation (EU) 2023/915. The resulted highest value of THQ for adult milk consumers was observed for Pb; nevertheless, THQ and HI for the analysed elements were all in a relatively safe range. It should, however, be pointed out that among the biological matrices analysed for bioaccumulation of heavy metals in Sicilian Barbaresca sheep, arsenic presented a significantly higher concentration in blood than in milk and fleece, with a lower value in fleece than in all the other matrices (blood and milk), as expressed in Table 6. It is noteworthy that for arsenic, a positive correlation was also found between milk and blood concentrations (Fig. 1), which points to a direct relationship between the concentrations of this metal that easily pass from the blood into the milk during lactopoiesis. Cd and Hg did not show a correlation between milk and blood probably due to their different biodistribution through tissues.

This experimental finding is not surprising as blood and milk represent biological substrates that are better identified in acute responses to bioaccumulation from toxic element exposure than fleece. It is well known that differences in concentrations in different substrates depend on the organism's exposure time to the anthropogenic load of heavy metals, and biological matrices such as blood and milk represent good indicators of acute-type bioaccumulation for these elements (Monteverde et al. 2022; Nava et al. 2023). Keratinised structures such as hair and nails previously studied in humans for the bioaccumulation of trace elements (Ahmed

Fig. 2 Linear correlation with *r* values (Pearson coefficient) between the studied blood biomarkers (RBC, WBC, Hct, HGB and PLT) and the concentrations of the different heavy metals (arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) in the different biological matrices (blood, milk and fleece) in 25 Sicilian Barbary breed sheep bred in Tusa (Messina). The different shades represent positive and negative correlations

Table 7 Calculation of target hazard quotient (THQ) and hazard index (HI) for As, Cd, Pb and Hg in milk for adults in the Mediterranean area

	As	Cd	Pb	Hg
THQ	0.05	0.004	0.21	0.002
Mean THQ	0.067			
Median THQ	0.027			

et al. 2016), but also hoof, tail and mane manes analysed in horses (Fazio et al. 2022; Aragona et al. 2024) seem to play an important role in the chronic bioaccumulation of heavy metals. In humans, in fact, hair and nails have the ability to provide retrospective information on the exposure status of the population and are considered as an important indicator of environmental pollution. Hair and nails are mainly composed of fibrous proteins in the form of keratin that make them stable and robust. Their composition and trophic activity are closely dependent on blood and element transport capabilities (Sahoo and Sahu 2022). Keratinised tissues, such as fleece, are for this reason particularly useful and valuable in terms of biomonitoring environmental exposure to anthropogenic metal loading (Kehagia et al. 2011; Powolny et al. 2023).

These structures can store heavy metals for a longer time than blood, urine and serum and thus serve as good indicators in populations exposed to bioaccumulation (Chirinos-Peinado and Castro-Bedriñana 2020). In addition to being useful biomarkers, keratinised structures of humans and animals have numerous other advantages, e.g. ease of sampling, transport, stability, high concentration of elements and no need for special storage (Kovacik et al. 2017). Not all heavy metals showed the same type of bioaccumulation; in fact our data showed a significantly higher concentration in fleece than in blood for cadmium and mercury. We have no data in the literature in sheep for these elements, but probably compared to the other metals they seem to accumulate more in the body with a greater biodistribution in the fleece. As for lead, this metal did not show statistically significant variations between the different matrices (blood, milk and fleece) studied in Sicilian Barbaresca sheep. These data are in disagreement with what was observed by Chirinos-Peinado et al. (2022) in the bovine species, where lead concentrations were higher in milk than in blood. The authors, however, monitored a group of animals reared in an industrial area with the possibility of environmental contamination and thus of the plant trophic chain (food chain through the soil–plant-animal system), which resulted in bioaccumulation in milk with risk for the consumer (Chirinos-Peinado et al. 2022; Elafyfy et al. 2023). Anthropogenic loading from heavy metals in the environment certainly determines the biodistribution and bioaccumulation of these elements in both humans and animals. Motas et al. (2021), in a study conducted in breast milk, found high concentrations of arsenic, lead and mercury in a group of women living in industrial areas with high anthropogenic risk. The study area and anthropogenic activity strongly influence toxic element contamination in the Mediterranean area (Motas et al. 2021). Bioaccumulation of heavy metals through milk consumption can lead to various toxic effects in a variety of body tissues and organs, especially because Cd is classified by the International Agency for Research on Cancer as carcinogenic to humans (Group 1) (IARC 2021) and Pb as probably carcinogenic to humans (Group 2 A) (IARC 2021).

Heavy metals have mutagenicity, teratogenicity, and carcinogenicity; they induce poor body conditions, reduced reproduction rate, and lead to immunosuppression in domestic animals even at lower dosages. Toxic elements may affect kidney function as well as the cardiovascular and nervous systems and damage different organs such as the reproductive system, nervous system, respiratory system, the liver, the gastrointestinal tract, and the endocrine system (Aragona et al. 2024). Milk synthesis, carried out by the secreting cells located in the mammary alveoli, is a peculiar activity of the udder and is supported by the continuous supply of nutrients from the blood. The mammary alveolus is a functional unit of milk production and is formed by a layer of milk-secreting cells that ball up a hollow centre. The function of the mammary alveoli is to remove nutrients from the blood, transform these nutrients into milk and release its contents into the glandular lumen for use by the infant through sucking. It is evident, therefore, that there is a close connection between the biological matrices we have studied (blood and milk), which represents a way for pollutants such as heavy metals to be removed from the body through the chemical characteristics of milk (Chirinos-Peinado and Castro-Bedriñana 2020). Lead and cadmium may be present in milk when their anthropogenic load increases as they adhere to casein, the main protein present in milk. According to CODEX (Codex Alimentarius 2023) standards, the lead content in milk must not exceed 0.020 mg/l. The cadmium content in milk must have a maximum limit of 0.050 mg/l according to CODEX standards (Codex Alimentarius 2023) and the European Union Regulatory Commission (2014), but today these values have been updated in Regulation (EU) 2023/915. Maximum limits for arsenic are not indicated, but its presence could be a health risk because as it inhibits DNA replication with reduced enzyme repair. It prevents enzyme activity at the mitochondrial level with inhibition of dehydrogenase; at the same time, it stimulates adenosine triphosphatase activity, deactivating oxidative phosphorylation, which causes deterioration of tissue respiration and consequently causes toxicity (Maity et al. 2020; Gakidou et al. 2017). Exposure to arsenic at the cellular level generates nitric oxide and superoxide anions that cause the formation of hydroxyl radicals and with this comes oxidative stress, lipid peroxidation and DNA damage (Flora et al. 2008; Lai et al. 2008; Obinaju 2009). With regard to the possible role of haematological parameters, assessed in the Sicilian Barbaresca sheep, as blood biomarkers in response to the bioaccumulation of heavy metals in the different matrices studied (blood, milk and fleece), it emerges that no significant correlation was found between haematological parameters and the concentration of each metal (arsenic, cadmium, lead and mercury) present in the biological matrices studied (Figs. 1 and 2). The blood biomarkers, for these analysed toxic elements concentrations, which are extremely low in the organic matrices studied, do not appear to play a role as 'sentinel cells' for the Barbaresca sheep reared in Sicily. From literature, it appears that lead can influence the number of red and white blood cells in rats (Balali-Mood et al. 2021), while in sheep, lead and mercury at higher concentrations can significantly influence the number of red blood cells and haemoglobin concentration (Kovacik et al. 2017). However, we do not know whether an increased bioaccumulation of the heavy metals in this species is able to stimulate the blood biomarkers showing results that can be correlated with the different biological matrices. It must also be considered that the geographical area (Tusa) used for this biomonitoring survey for heavy metals in sheep is 'a zootechnical environment' that is potentially not very susceptible to anthropic toxic element loading because it is far from industrial sites. The presence of livestock settlements in areas with a higher anthropogenic impact for heavy metals appears to increase bioaccumulation and biodistribution as previously observed for other species in literature (Powolny et al. 2023).

The present study is preliminary; this certainly represents a limitation with regard to the depth of the analysis. The study acknowledges the need for further research to validate and extend the results. Furthermore, although the study provides valuable insights into the bioaccumulation of heavy metals in Barbaresca sheep, the long-term effects of heavy metal exposure on animal health or the specific mechanisms of bioaccumulation in different biological matrices should be addressed in more detail. Likewise, it might be useful to explore in depth other potential health impacts or ecological implications of heavy metal contamination in the sheep population studied.

Conclusions

In conclusion, we can state that this research, although preliminary, has allowed us to focus on a widespread Sicilian livestock farming reality for the protection of numerous consumers against the bioaccumulation and biodistribution of certain toxic elements.

The low concentration of toxic metals analysed, although comforting, should not deter us from continuing this research in the future and deepening it, using different environmental monitoring sites for heavy metals under different climatic conditions and certainly using different 'blood biomarkers' so as to obtain an integrated response of the organism to the interactions of the different toxic effects resulting from exposure to a mixture of several toxic substances. The present results confirmed that blood and milk were the most relevant biological substrates due to their ability to concentrate minerals compared to fleece. It would be useful to further investigate the correlation of these substances with blood biomarkers that provide us with an immediate response of the body to the effect of the toxic substance and predict its long-term effects contributing to the real and potential risk to which all of the consumers could be exposed.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection and analysis were

performed by Francesco Fazio, Francesca Aragona, Vincenzo Nava. The first draft of the manuscript was written by Francesco Fazio and Francesca Aragona and Elif Doğan, Salvatore De Caro and Alessandro Zumbo commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding The authors declare that no funds, grants, or other support was received during the preparation of this manuscript.

Declarations

Ethical approval Protocols and experimentation were reviewed and approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 63/2010/EU. The entire experimental protocol was approved by the standards recommended by the Ethical Committee of University of Messina (06/2023).

Consent to participate All authors have given consent to submit the manuscript.

Consent for publication All authors approved the version to be published.

Competing interests The authors declare no competing interests.

References

Ahmed AS, Elgharabawy RM, Hatem A, Ahmed HA, Barghashe SS (2016) Human hair and nails as bio-indicator of heavy metals contamination by hair dye exposure among population in Saudi Arabia. World J Pharm Med Res 2(6):130–137

Ali MU, Liu G, Yousaf B, Ullah H, Abbas Q, Munir MAM, Irshad S (2019) Biomonitoring and health risks assessment of trace elements in various age- and gender-groups exposed to road dust in habitable urban-industrial areas of Hefei, China. Environ Pollut 244:809–817. https://doi.org/10.1016/j.envpol.2018.10.084

Ali MU, Wang C, Li Y et al (2023) Heavy metals in fish, rice, and human hair and health risk assessment in Wuhan city, central China. Environ Pollut 328:121604. https://doi.org/10.1016/j.envpol.2023.121604

Altinok-Yipel F, Yipel M, Altuğ N (2022) Element concentrations in horse blood and relation between age, gender, breed, hematological and biochemical parameters. J Appl Biol Sci 16:434–446. https://doi.org/10.5281/zenodo.7114040

Aragona F, Cicero N, Nava V et al (2024) Blood and hoof biodistibution of some trace element (lithium, copper, zinc, strontium and lead) in horse from two different areas of Sicily. J Trace Elem Med Biol 82:127378. https://doi.org/10.1016/j.jtemb.2023.127378

Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR, Sadeghi M (2021) Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. Front Pharmacol 13(12):643972. https://doi.org/10.3389/fphar.2021.643972

Boudebbouz A, Boudalia S, Bousbia A, Gueroui Y, Boussadia MI, Chelaghmia ML, Zebsa R, Affoune AM, Symeon GK (2023) Determination of heavy metal levels and health risk assessment of raw cow milk in Guelma Region Algeria. Biol Trace Elem Res 201(4):1704–1716. https://doi.org/10.1007/s12011-022-03308-1

Capitão C, Martins R, Santos O, Bicho M, Szigeti T, Katsonouri A, Bocca B, Ruggieri F, Wasowicz W, Tolonen H, Virgolino A (2022) Exposure to heavy metals and red blood cell parameters in children: a systematic review of observational studies. Front Pedriat 10:921239. https://doi.org/10.3389/fped.2022.921239

Chirinos-Peinado DM, Castro-Bedriñana JI (2020) Lead and cadmium blood levels and transfer to milk in cattle reared in a mining area. Heliyon 6(3):e03579. https://doi.org/10.1016/j.heliyon.2020.e03579

Chirinos-Peinado D, Castro-Bedriñana J, Ríos-Ríos E et al (2022) Lead and cadmium bioaccumulation in fresh cow's milk in an intermediate area of the Central Andes of Peru and risk to human health. Toxics 10:317. https://doi.org/10.3390/toxics10060317

CODEX ALIMENTARIUS CXC 1–1969 / Rev. 2023 (EN). https://certifico.com/index.php?option=com_acymailing&ctrl=archive&task=view&mailid=91008.

Di Trana A, Sepe L, Di Gregorio P et al (2015) The role of local sheep and goat breeds and their products as a tool for sustainability and safeguard of the Mediterranean environment. In: Vastola A (ed) The sustainability of agro-food and natural resource systems in the Mediterranean basin. Springer International Publishing, Cham, pp 77–112

Elafify M, Marwa ET, Sallam KI, Sadoma NM, Abd-Elghany SM, Abdelkhalek A, El-Baz AH (2023) Heavy metal residues in milk and some dairy products with insight into their health risk assessment and the role of Lactobacillus rhamnosus in reducing the lead and cadmium load in cheese. Food Chem Adv 2:100261

EPA, 1989. Guidance manual for assessing human health risks from chemically contaminated, fish and shellfish Washington

EPA, 2004. Risk assessment guidance for superfund. Human Health Evaluation Manual (Part A). vol. I.

Fazio F, Giangrosso G, Marafioti S et al (2016) Blood haemogram in Ovis aries and Capra hyrcus : effect of storage time. Can J Anim Sci 96:32–36. https://doi.org/10.1139/cjas-2015-0106

Fazio F, Gugliandolo E, Nava V, Piccione G, Giannetto C, Licata P (2020) Bioaccumulation of mineral elements in different biological substrates of athletic horse from Messina Italy. Animals 10(10):1877. https://doi.org/10.3390/ani10101877

Fazio F, Aragona F, Piccione G, Arfuso F, Giannetto C (2022) Lithium concentration in biological samples and gender difference in athletic horses. J Equine Vet Sci 117:104081. https://doi.org/10.1016/j.jevs.2022.104081

Flora SJ, Chouhan S, Kannan GM, Mittal M, Swarnkar H (2008) Combined administration of taurine and monoisoamyl DMSA protects arsenic induced oxidative injury in rats. Oxid Med Cell Longev 1(1):39–45. https://doi.org/10.4161/oxim.1.1.6481

Gakidou E, Afshin A, Abajobir AA et al (2017) Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet 390:1345–1422. https://doi.org/10.1016/S0140-6736(17)32366-8

Giannetto C, Fazio F, Nava V, Arfuso F, Piccione G, Coelho C, Gugliandolo E, Licata P (2022) Data on multiple regression analysis between boron, nickel, arsenic, antimony, and biological substrates in horses: the role of hematological biomarkers. J Biochem Mol Toxicol 36(2):e22955. https://doi.org/10.1002/jbt.22955

Gil F, Hernandez A (2009) Significance of biomarkers in applied toxicology. In: Ballantyne, B.M.T.C., Syversen, T. (Eds.), Gen Appl Toxicol, third ed.; 2. John Wiley & Sons, pp. 847e858. 6 vols

Hernandez AF, Gil F, Tsatsakis A (2014) Biomarkers of chemical mixture toxicity. In: Gupta, R. (Ed.), Biomarkers in Toxicol. Academic Press, pp. 655e670

IARC. (World Health Organization/International Agency for Research on Cancer) 2021; https://www.iarc.who.int.

Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN (2014) Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol 7(2):60–72. https://doi.org/10.2478/intox-2014-0009

Kehagia K, Bratakos S, Kolovou M, Potiriadis C (2011) Hair analysis as an indicator of exposure to uranium. Radiat Prot Dosimetry 144(1–4):423–426. https://doi.org/10.1093/rpd/ncq324

Kovacik A, Arvay J, Tusimova E, Harangozo L, Tvrda E, Zbynovska K, Cupka P, Andrascikova S, Tomas J, Massanyi P (2017) Seasonal variations in the blood concentration of selected heavy metals in sheep and their effects on the biochemical and hematological parameters. Chemosphere 168:365–371. https://doi.org/10.1016/j.chemosphere.2016.10.090

Lai R, Wang Y, Li X, Yu RA (2008) Effect of selenium and arsenic on oxidative stress, DNA oxidative damage and repair in HepG2 cells. Wei Sheng Yan Jiu 37(6):645–648 (**Chinese PMID: 19238993**)

Liotta L, Randazzo CL, Russo N et al (2019) Effect of molasses and dried orange pulp as sheep dietary supplementation on physico-chemical, microbiological and fatty acid profile of Comisana ewe's milk and cheese. Front Nutr 6:1. https://doi.org/10.3389/fnut.2019.00001

Maity S, Bhat AH, Giri K, Ambatipudi K (2020) BoMiProt: a database of bovine milk proteins. J Proteomics 215:103648. https://doi.org/10.1016/j.jprot.2020.103648

Monteverde V, Camilleri G, Arfuso F, Pennisi M, Perillo L, Patitò G, Gioia G, Castronovo C, Piccione G (2022) Heavy metal levels in milk and serum of dairy cows from different farms located near an industrial area. Animals 12(19):2574. https://doi.org/10.3390/ani12192574

Motas M, Jiménez S, Oliva J, Cámara MA, Pérez-Cárceles MD (2021) Heavy metals and trace elements in human breast milk from industrial/mining and agricultural zones of southeastern Spain. Int J Environ Res Public Health 18(17):9289. https://doi.org/10.3390/ijerph18179289

Nava V, Licata P, Biondi V, Catone G, Gugliandolo E, Pugliese M, Passantino A, Crupi R, Aragona F (2023) Horse whole blood trace elements from different Sicily areas: biomonitoring of environmental risk. Biol Trace Elem Res. https://doi.org/10.1007/s12011-023-03889-5

Obinaju BE (2009) Mechanism of arsenic toxicity and carcirogenesis. Afr J Biochem Res 3(5):232–237

Park K, Seo E (2017) Toenail mercury and dyslipidemia: interaction with selenium. J Trace Elem Med Biol 39:43–49. https://doi.org/10.1016/j.jtemb.2016.07.005

Polizopoulou ZS (2010) Haematological tests in sheep health management. Small Rum Res 92(1–3):88–91. https://doi.org/10.1016/j.smallrumres.2010.04.015

Powolny T, Scheifler R, Raoul F, Coeurdassier M, Fritsch C (2023) Effects of chronic exposure to toxic metals on haematological parameters in free-ranging small mammals. Environ Pollut 317:120675. https://doi.org/10.1016/j.envpol.2022.120675

Ritchie H, Roser M (2017) Meat and dairy production. Our World in Data

Sahoo BP, Sahu HB (2022) Assessment of metal pollution in surface water using pollution indices and multivariate statistics: a case study of Talcher coalfield area India. Appl Water Sci 12:223. https://doi.org/10.1007/s13201-022-01743-y

Tuncer SS, Şireli D, Dellal G (2017) Comparative analysis of various fleece characteristics of norduz and zom sheep. J Anim Plant Sci 27:2017–2763

Wongsasuluk P, Chotpantarat S, Siriwong W, Robson M (2018) Using hair and fingernails in binary logistic regression for bio-monitoring of heavy metals/metalloid in groundwater in intensively agricultural areas, Thailand. Environ Res 162:106–118. https://doi.org/10.1016/j.envres.2017.11.024

Yan M, Niu C, Li X et al (2022) Heavy metal levels in milk and dairy products and health risk assessment: a systematic review of studies in China. Sci Total Environ 851:158161. https://doi.org/10.1016/j.scitotenv.2022.158161

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