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Using cattle hair to assess exposure to essential trace elements in volcanic soils

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ABSTRACT

Background: In areas where cattle graze outdoors, the environmental availability of trace elements is of utmost importance for an adequate intake. Cattle hair trace elements can be used as exposure biomarkers to assess animal intake and help ensure good nutrition and animal welfare. The aim of this study was to evaluate the environmental exposure to trace elements in cattle grazing outdoors in a volcanic region (São Miguel Island, Azores) and assess the corresponding bioaccumulation factors.
Methods: Twenty-nine samples of pasture topsoil were collected in two areas of São Miguel with different geological characteristics: Picos fissure system (basaltic area) and Povoação volcano (trachytic area). Hair from fifty-four animals (calves and dairy cows) was collected in two grazing sites: Ponta Delgada (Picos fissure system) and Povoação (Povoação volcano). All samples were analysed by inductively coupled plasma mass spectrometry (ICP-MS) after digestion in aqua regia.
Results: Basaltic soils of the Picos fissure system had significantly higher concentrations of most trace elements, except Mo, when compared with the trachytic soils of Povoação volcano. Hair of calves from Povoação showed higher concentrations of most essential trace elements, except Mg, Cu, and Zn, than hair from calves of Ponta Delgada (considered in this study as a control group since they are raised in a barn). On the other hand, hair from dairy cows grazing in Ponta Delgada showed higher concentrations of most essential trace elements, except Mg, As, and Mo, when compared with hair of cows grazing in Povoação.
Conclusions: Cattle hair trace elements are suitable biomarkers for the assessment of the environmental exposure to trace elements in volcanic soils. The results from dairy cows support the uneven distribution of essential trace elements (and their bioavailability) in the different soils of São Miguel, reinforcing the need to graze animals outdoors from an early age. Such practice (grazing cattle outdoors) provides another means of accessing essential trace elements that complemented with feed can reduce the natural depletion of these essential elements in the body due to reproductive phenomena and milk production.

1. Introduction

Farming management is changing as access to pasture for dairy cattle is decreasing in many parts of the world [1]. Conversely, the number of indoor farms is increasing as they aim to recreate outdoor conditions without the associated hazards, but the best farming option for grazing animals is still a mixed system of outdoor and indoor life.

In temperate regions, beef and dairy cattle are kept on pasture for at least part of the year (spring/summer) and, in many cases, in indoor farms during the winter. In the Azores islands, the temperate climate, with mild temperatures and high precipitation [2], and the fertile volcanic soils provide ideal conditions for outdoor farming all year round. According to the Land Occupation Report [3], approximately half of the Azores territory is occupied by agriculture (48.82%) and pasture is the most representative subclass of land occupation (39.63%), surpassing other agricultural activities. The Azores have a cattle inventory of

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approximately 125,000 adult cows, of which 73% are Holstein-Friesian dairy cows [4].

Nonetheless, volcanic islands have specific characteristics that can influence access to pasture and its quality, such as the typical small size of the islands, their irregular topography, the high erosion rates, and the type of soil (explored in this study). This study focuses on the island of São Miguel, the largest of the Azores archipelago and the one with the highest milk production and greatest geological diversity. The geological diversity of São Miguel is reflected in the type of soil, which can be divided into two main groups according to the geochemical composition of the parent rocks (basaltic and trachytic) [5,6]. In São Miguel, as in the rest of the world, agricultural practices are changing but the dairy farming system is still based on pasture and pasture rotation, with most cows grazing freely all year round [7] having additional intake of grass, corn silage and formulated feed. In this farming system, animal nutrition is highly dependent on local geographic and geologic conditions, and great attention has been given to the trace elements (TE) required for crop and forage production. TE are also essential for the healthy development of grazing cattle but are sometimes overlooked. Of all TE, the most important for animal nutrition are: copper (Cu), zinc (Zn), selenium (Se), manganese (Mn), iodine (I), cobalt (Co) and, not to a lesser extent, iron (Fe) and molybdenum (Mo) [8,9]. The adequate intake of these essential TE has positive impacts on foetal development, immune function, bone development, nervous system function, fertility, muscle development, milk production and, skin and hair quality [10]. The lack of TE will not only affect the health of animals but also have important consequences for human health, as meat and milk, and their derived products are some of the main contributors of essential TE [11, 12].

Animal hair is often used as a biological matrix to assess environmental exposure as the specific structure of hair tissue lends it resistance to biological modifications and is sensitive to chemical variations [13]. Therefore, hair composition has been widely used as a biomarker of exposure for different types of mammals [14–16]. Studying the concentration of TE in the hair of grazing cattle allows to evaluate reference values for some nutrients, which can help to ensure better animal welfare [17]. In addition, the hair of grazing animals is a good indicator of environmental pollution by heavy metals, as animals are exposed through ingestion of polluted vegetation, small amounts of soil and, in some cases, drinking water [18].

Considering that São Miguel Island has an uneven distribution of TE in the soils, due to parent volcanic rocks with different compositions, and that the farming practices still privilege the grazing of animals outdoors, the aim of this study was twofold: i) to assess whether cattle hair TE content is a suitable biomarker of environmental exposure to TE in volcanic soils, and ii) to set the basis for the development of a system for monitoring the status of animals to prevent metabolic disorders.

2. Material and methods

2.1. Study area

São Miguel is the largest (744 km^2) and most populated (137.300 inhabitants) of the nine islands of the Azores, located approximately 1300 km west of mainland Portugal (Fig. 1 inset). The geology of São Miguel is dominated by seven volcanic systems (Fig. 1): three active central volcanoes (Sete Cidades, Fogo and Furnas), characterized by recurrent trachytic explosive eruptions that produced widespread pyroclastic fall deposits; two active fissure systems (Picos and Congro), marked by basaltic eruptions that formed numerous scoria cones and lava flows; and two older extinct volcanic systems (Povoação and Nordeste), which have not erupted in the last 10,000 years [19,20].

The two grazing sites of this study are in the Picos fissure system and the Povoação volcano (Fig. 1). Picos fissure system is a low-lying area that links Sete Cidades and Fogo volcanoes, characterized by two central sub-parallel ridges of scoria cones and lava flows that gently slope to the north and south coasts [21]. On the other hand, Povoação volcano is located between Furnas volcano and Nordeste volcanic system and corresponds to a 6 km-wide sub-circular caldera that is partially open to the sea on the south coast [22].

Soil parent rocks of the Picos fissure system are almost exclusively young (< 5000 years) basaltic lavas and scoria deposits (< 48 wt% SiO_2), while soils of Povoação volcano usually result from young (< 5000 years) trachytic pyroclastic fall deposits (> 61 wt% SiO_2) that were blown by the wind from the neighbouring Furnas volcano and deposited on the Povoação area [5]. Basaltic rocks of the Picos fissure system have

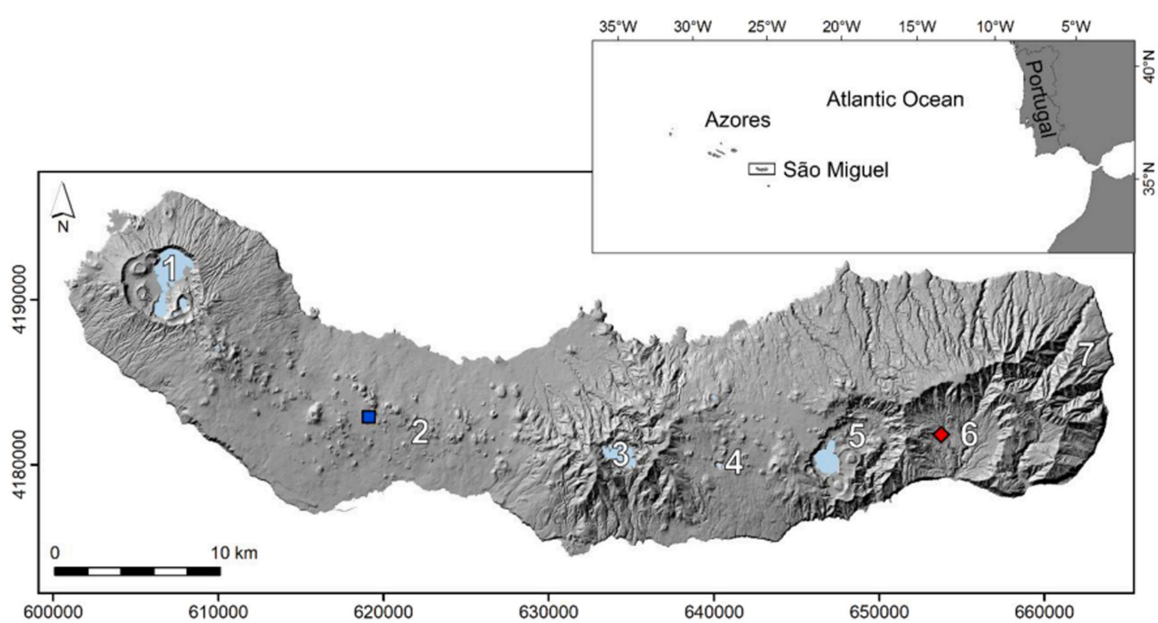

Fig. 1. Digital elevation model of São Miguel (Azores) showing the island's volcanic systems (from west to east: 1 – Sete Cidades volcano; 2 – Picos fissure system; 3 – Fogo volcano; 4 – Congro fissure system; 5 – Furnas volcano; 6 – Povoação volcano; 7 – Nordeste volcanic system; following Gaspar et al., 2015) and location of the two grazing sites (blue square – Ponta Delgada; red diamond – Povoação). Inset shows São Miguel Island and the Azores archipelago in the context of the North Atlantic Ocean.

higher concentrations of major oxides like TiO_2, FeO_{tot}, MgO, CaO, and P_2O_5, and lower concentrations of Na_2O and K_2O, when compared with trachytic rocks that form the soils at Povoação. Regarding TE, and despite some variability, Picos soil parent basalts have much higher concentrations of elements such as Ba, Co, Cr, Cu, Ni, V, Sc, and Sr, and lower concentrations of Rb, Y, Zr, Nb, Mo, Pb, and rare earth elements (except Eu), in comparison with soil parent trachytes at Povoação (Fig. 2; [23,24]), which is in agreement with compositions of such volcanic rocks on other oceanic island settings [25].

2.2. Topsoil sampling and chemical analysis

A total of 29 samples of pasture topsoil were collected in two areas of São Miguel with different geological characteristics: 18 composite samples collected in the Picos fissure system (a typical basaltic area), and 11 composite samples collected in Povoação volcano (representative of a trachytic area). Topsoil composite samples were collected between April and July 2018 following the GEMAS field protocol [26]. After the removal of surface litter, five samples (a 1 ×1 m square with four collecting points in the vertices and one collecting point in the centre) were collected using a spiral hand auger up to a maximum depth of 10 cm. The composite soil samples were sieved down to 2 mm and dried in an oven at 60 °C until constant weight was achieved.

A total of 65 elements were determined at Activation Laboratories Ltd. (Canada), a laboratory accredited to standards ISO/IEC 17025:2017 and ISO 9001:2015, with a package that includes digestion in aqua regia, using a combination of concentrated hydrochloric and nitric acids to dissolve the samples and induce elemental leaching for subsequent analysis and quantification by inductively coupled plasma mass spectrometry (ICP-MS). Analytical quality control was assured by analysis of four certified reference materials for elemental composition, DH-1a, GXR-4, GXR-6, OREAS 45d, as well as four blanks per element for one batch of 29 samples (see supplementary material 1 A). For statistical calculations, concentrations below the lower detection limit were considered equal to that limit.

In this work, only the elements with significant differences in the soils of the two geological areas were considered (*i.e.*, Al, Fe, Mg, Mn, Ba, Cd, Co, Cr, Cu, Mo, Ni, Pb, Sr, Ti, V, and Zn).

2.3. Animals and diet

The hair samples were obtained from Holstein-Friesian calves and adult dairy cows. The Ponta Delgada grazing site, located in the Picos fissure system, included 12 adult cows, while the Povoação grazing site, located in the Povoação volcano, included 14 calves and 14 adult cows. Calves from Ponta Delgada (14 animals), unlike all others, were raised in a naturally ventilated barn with no access to grazing pasture or to fresh pasture grass and, therefore, are here considered a control group. Information regarding the cattle's general health and feeding habits was obtained through questionnaires to the farmers. Regarding the animals' diet, calves and dairy cows from both sites ingested feeds with different specifications (see supplementary material 2).

2.4. Cattle hair collection and chemical analysis

Cattle hair samples were collected in the summer period (July 2021). The hair was cut from two parts of the animals: the back of the head and the last third of the tail and the switch. During hair sampling, animals were head-locked individually at the feeder and released after each sampling. To avoid differences related to hair colour, only homogenous, white-coloured hairs were selected. Hair was cut as close to the skin as possible while preventing skin damage and hair follicle removal. The scissors used to cut the hair were cleaned and disinfected with 70% isopropyl alcohol between animals. Each hair sample was immediately placed in an individually labelled Ziploc® bag and stored in an opaque container. Hair samples remained stored in an opaque container at room temperature until analysis to reduce exposure to light. The hair was washed with purified water, as often as necessary, to remove faeces, until the washing water came out clear. Hair samples were dried at room temperature and subsequently cut into small pieces; the scissors used were also cleaned and disinfected with 70% isopropyl alcohol between samples. For each hair sample, a total mass of 2.0 g was transferred to an opaque container and shipped for analysis.

A total of 22 elements classified as essential TE (Co, Cr, Cu, Fe, Mn, Mo, Se and Zn) and non-essential TE (Al, As, Ba, Cd, Cs, Hg, Mg, Ni, Pb, Sn, Sr, Ti, U, and V) were quantified at Activation Laboratories Ltd. (Canada) with a package that includes open-vessel digestion (with HNO_3 /H_2O_2 (2:1)) heated to 95 °C and analyses by ICP-MS. Analytical quality control was assured by analysis of certified reference material for elemental composition, IV-STOCK-1643, as well as duplicate samples (two independent measurements on four samples) and one blank per element for one batch of 54 samples (see supplementary material 1B). However, in this work, only the elements with significant differences between the soils of the two grazing sites were considered (see results section). For all elements that yielded values below the detection limit, the limit value was considered the result.

2.5. Bioaccumulation factor

Bioaccumulation factor (BAF) was considered to correspond to the intake of a TE from the environment, by all possible routes (*e.g.,* respiration, diet, dermal), and from the soil [27]. It should be noted that the calves from Ponta Delgada grazing site were not considered, as these animals were confined in a barn and do not reflect adequate environmental exposure.

The equation defined by USEPA [28] was followed,

$$\text{Total BAF} = [\text{M}]\ \text{Hair sample}/[\text{M}]\ \text{Soil sample}\cdot$$

BAF values > 1 indicate that the accumulation in the organism is greater than that of the medium (*e.g.,* soil) from which the TE was taken.

2.6. Statistical analysis

Continuous variables, such as the TE concentration in soil and in hair were compared by a Mann-Whitney *U* test. The age of the animals (in months) did not present normality in the Kolmogrov-Smirnov test, so this variable was also compared by a Mann-Whitney *U* test. All statistical analyses were performed using IBM SPSS Statistics 27.0 for Windows.

3. Results

3.1. Animals and diet

Regarding the characterization of the animals, significant differences were observed in the age of the calves; calves from Povoação grazing site were significantly older than those from Ponta Delgada (Table 1). As for the animals' diet, our results demonstrated that farmers from the two sites apply different feeding plans. On what concerns calves, the only observed differences were between grass and grass silage proportion, but combined they represented the same intake by the animals (grass + grass silage = 8 kg/animal/day) (Table 1). Considering that calves from Ponta Delgada were enclosed in a barn and do not graze freely like calves from Povoação, differences in the intake of fresh pasture grass were expected, as well as of grass silage, which is higher in animals from Ponta Delgada.

The diet management of dairy cows is significantly different between sites: cows in Ponta Delgada are fed with significantly more fresh pasture grass (22 kg/animal/day in Ponta Delgada *vs.* 18 kg/animal/day in Povoação) and feed (9 kg/animal/day in Ponta Delgada *vs.* 5 kg/animal/day in Povoação), while in Povoação cows are fed with significantly more grass silage and have no corn silage intake (Table 1). Still,

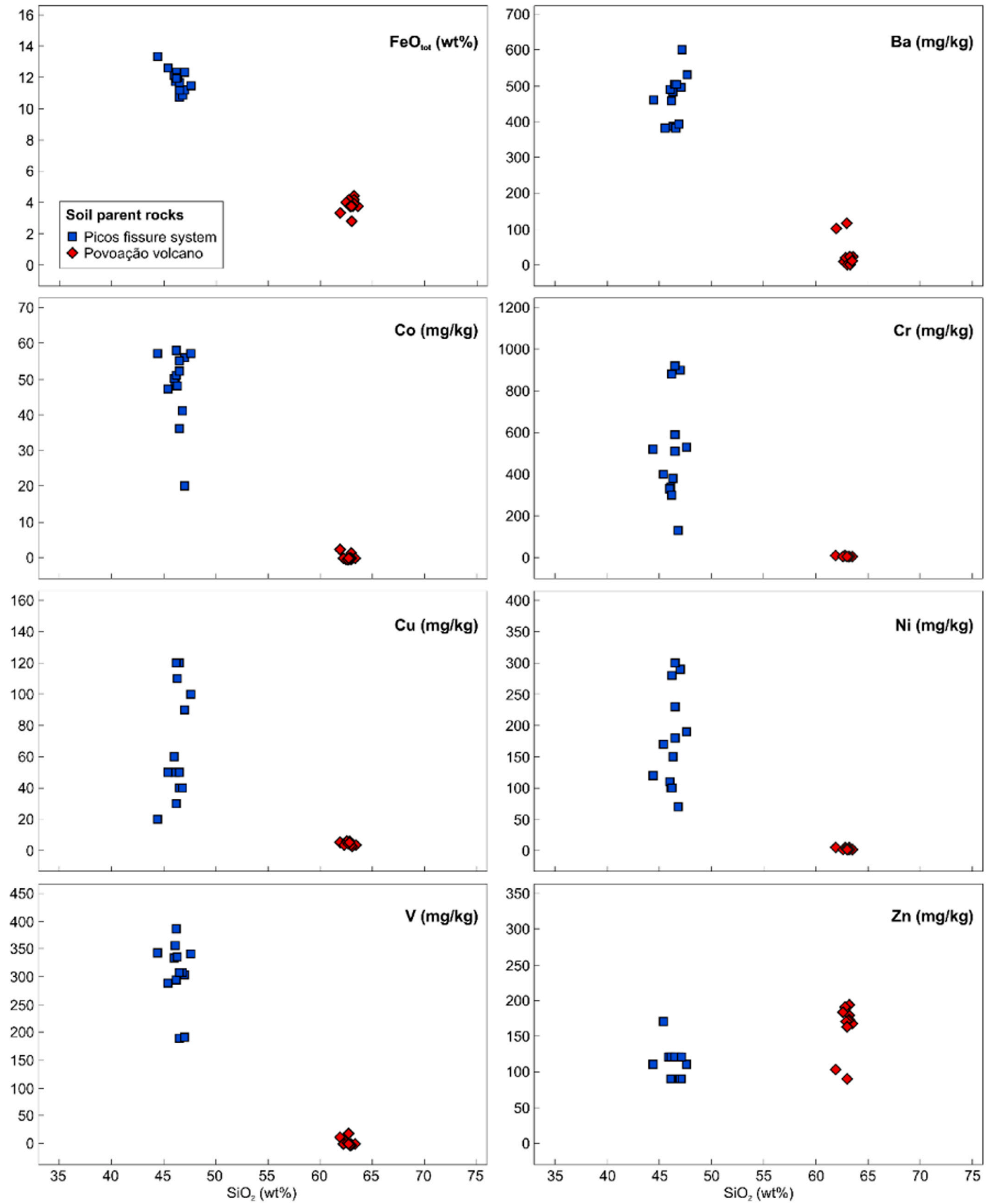

Fig. 2. Bulk rock geochemistry of soil parent rocks from Picos fissure system (basaltic lavas and scoria deposits) and Povoação volcano (trachytic pyroclastic fall deposits blown from Furnas volcano). Variation diagrams of selected elements plotted *vs.* SiO_2. Data sourced from Zanon (2015) and Jefferey et al. (2016).

Table 1
Description of the study animals [mean ± SE for age (in months) and diet (in kg/day/animal)].

	Calves			Cows		
	Ponta Delgada (n = 14)	Povoação (n = 14)	*p*-value[a]	Ponta Delgada (n = 12)	Povoação (n = 14)	*p*-value[a]
Age	9.21 ± 0.51	12.2 ± 0.78	0.005	62.2 ± 7.70	61.8 ± 7.33	0.860
Grass	0	5	< 0.001	22	18	< 0.001
Grass silage	8	3	< 0.001	14	47	< 0.001
Corn silage	0	0	1	20	0	< 0.001
Feed	2.5	2.5	1	9	5	< 0.001

[a] *p*-value for group comparisons by Mann-Whitney *U* test for all the continuous variables.

considering that fresh pasture grass, grass silage, and corn silage were produced in the same areas, the total daily intake (56 kg/animal/day in Ponta Delgada *vs.* 65 kg/animal/day in Povoação) evidence that dairy cows consume food produced in the grazing sites in a similar amount. This evidences that there may only be differences in its nutritional composition, since the soils in question, and according to the existing literature, may have an irregular distribution of TE.

3.2. Chemical elements in the soil and hair

Most chemical elements in the studied topsoil samples showed significant differences between basaltic (Picos fissure system) and trachytic (Povoação volcano) areas (Table 2). Regarding essential TE, higher concentrations were consistently found in basaltic soils, except Mo, which showed higher values in trachytic soils (Table 2).

As for cattle hair, our results showed that calves from Povoação have significantly higher concentrations of most elements, except for Mg, Cu, and Zn, when compared to the calves from Ponta Delgada (Table 2); still, calves from Ponta Delgada were closed inside the barn and therefore did not have access to TE in the soil like the calves from Povoação. Regarding the hair of adult cows, we observed an inverse tendency: cows from Ponta Delgada have significantly higher concentrations of most TE, except Mg and Mo, when compared with Povoação cows (Table 2).

When comparing calves and cows from the same grazing site, we observed that in the case of Ponta Delgada there are significant differences in the concentrations of all TE, except Mo, which has a similar concentration in calves and cows. Our results also demonstrate that for the considered essential TE, cows always present higher concentrations of these elements in the hair when compared with calves (Table 2). In the case of animals grazing in Povoação, there are no significant differences in Mg, Ba, Cu, Mn, V, and Zn concentrations in the hair of calves and cows. Regarding the essential TE, only Mo and Zn have higher concentrations in the hair of the cows of Povoação when compared with the calves of the same grazing site (Table 2).

Table 2
Trace element (TE) concentration in basaltic (Picos fissure system) and trachytic (Povoação volcano) soils, and TE concentration in cattle hair by age (calves *vs.* cows) and grazing site (mean ± SE for continuous variables).

	Soils			Calves			Cows			Calves *vs.* Cows	
TE[a]	Basaltic	Trachytic	*p*-value[b]	Ponta Delgada	Povoação	*p*-value[c]	Ponta Delgada	Povoação	*p*-value[d]	Ponta Delgada *p*-value[e]	Povoação *p*-value[f]
Fe	3.16 ± 0.88	1.29 ± 0.26	< 0.001	31.2 ± 11.9	367.9 ± 89.0	< 0.001	339.84 ± 47.06	114.25 ± 189.6	< 0.001	< 0.001	0.004
Mg	0.56 ± 0.35	0.09 ± 0.04	< 0.001	1000.1 ± 147.9	523.2 ± 79.8	< 0.001	410.58 ± 46.17	559.53 ± 49.63	0.046	< 0.001	0.425
Al	3.93 ± 0.91	2.30 ± 0.38	< 0.001	49.3 ± 14.9	646.8 ± 130.7	< 0.001	350 ± 62.97	108.73 ± 21.83	< 0.001	< 0.001	< 0.001
Ba	144.92 ± 51.96	33.13 ± 5.43	< 0.001	1.19 ± 0.36	2.23 ± 0.77	0.164	3.79 ± 0.48	1.39 ± 0.29	< 0.001	< 0.001	0.533
Cd	0.39 ± 0.08	0.25 ± 0.10	0.004	0.0006 ± 0.0003	0.06 ± 0.012	< 0.001	0.02 ± 0.002	0.02 ± 0.002	0.290	< 0.001	< 0.001
Co	13.58 ± 7.28	1.66 ± 0.61	< 0.001	0.07 ± 0.008	0.08 ± 0.016	0.839	0.34 ± 0.04	0.04 ± 0.005	< 0.001	< 0.001	0.046
Cr	32.62 ± 14.86	6.75 ± 3.06	< 0.001	0.02	0.16 ± 0.14	0.769	0.21 ± 0.03	0.09 ± 0.02	< 0.001	< 0.001	< 0.001
Cu	76.50 ± 8.36	4.60 ± 2.06	< 0.001	9.01 ± 0.21	7.99 ± 0.16	< 0.001	10.11 ± 0.33	7.48 ± 0.39	< 0.001	0.014	0.505
Mn	1461.11 ± 221.45	874.88 ± 222.10	< 0.001	29.1 ± 4.58	107.2 ± 18.5	< 0.001	49.34 ± 5.05	66.44 ± 8.60	0.146	0.014	0.112
Mo	1.97 ± 0.70	2.92 ± 0.58	0.014	0.49 ± 0.12	0.28 ± 0.05	0.401	0.34 ± 0.05	0.93 ± 0.16	< 0.001	0.511	< 0.001
Ni	47.53 ± 36.06	4.90 ± 3.02	< 0.001	2.53 ± 2.37	0.15 ± 0.001	0.734	0.37 ± 0.05	0.11 ± 0.03	< 0.001	0.007	< 0.001
Pb	25.61 ± 17.63	8.57 ± 3.26	0.002	0.53 ± 0.02	0.68 ± 0.04	0.002	0.29 ± 0.04	0.09 ± 0.01	< 0.001	< 0.001	< 0.001
Sr	74.75 ± 34.97	17.88 ± 5.69	< 0.001	3.37 ± 0.59	11.68 ± 1.90	< 0.001	6.15 ± 0.88	5.10 ± 0.64	0.425	0.024	0.003
Ti	0.38 ± 0.12	0.11 ± 0.03	< 0.001	9.58 ± 2.43	35.6 ± 6.14	< 0.001	50.10 ± 7.72	5.00 ± 0.92	< 0.001	< 0.001	< 0.001
V	67.03 ± 28.30	9.13 ± 0.90	< 0.001	0.29 ± 0.03	0.31 ± 0.04	0.946	1.18 ± 0.15	0.10 ± 0.02	< 0.001	< 0.001	0.983
Zn	154.83 ± 49.81	83.2 ± 8.29	0.002	114.6 ± 4.19	97.19 ± 5.91	0.007	260.29 ± 38.11	108.37 ± 4.78	< 0.001	< 0.001	0.146

[a] The TE concentration in the soil is expressed in mg/kg, with the exception of Fe, Mg, Al and Ti, which are represented in % and Hg, respectively, in µg/kg. The TE concentration in cattle hair is expressed in mg/kg.
[b] *p*-value for group comparisons (soils) by Mann–Whitney *U* test.
[c] *p*-value for group comparisons (calves) by Mann–Whitney *U* test.
[d] *p*-value for group comparisons (cows) by Mann–Whitney *U* test.
[e] *p*-value for group comparisons (Ponta Delgada) by Mann–Whitney *U* test.
[f] *p*-value for group comparisons (Povoação) by Mann–Whitney *U* test.

3.3. Bioaccumulation factor

The BAF was not calculated for the calves of Ponta Delgada as these animals were raised inside a barn, without access to the grazing area or to fresh pasture grass. BAF > 1 was found for Cu in both calves (1.74) and cows (1.63) from Povoação, and for Zn in calves (1.17) from Povoação and cows from both Povoação (1.30) and Ponta Delgada (1.68) grazing sites (Table 3), indicating that the accumulation in the organism is greater than that of the medium (*e.g.,* soil) from which the TE were taken.

4. Discussion

The analysis of cattle hair is often used as a method to assess the TE status in animals [29–31]. Among the TE studied in this work, Co, Cr, Cu, Fe, Mn, Mo, and Zn are considered essentials for ruminants, as they are required for different biochemical and physiological functions [32]. Therefore, these essential TE are usually added to animal feed to meet nutritional requirements [33,34].

Previous studies have described an irregular distribution of most TE in the volcanic soils of São Miguel Island, with basaltic soils being richer in most elements [5,6]; the lack of some essential TE in the trachytic soils of São Miguel may weaken the health of grazing animals, leading to the development of several diseases.

Considering the results of this study, we observe that the environment plays a crucial role in the possible intake of essential TE by grazing cattle. This role is visible in two main aspects. We observed that calves from Ponta Delgada, although living in a basaltic area (the Picos fissure system) where soils are richer in most of the essential TE, show lower concentrations of these elements in the hair than the calves from Povoação. This most probably occurs because these animals are closed inside a barn and, therefore, do not have access to natural sources of intake of TE, which are the consumption of fresh grass, small portions of soil, and by dermal exposure. Dairy cows from Ponta Delgada show, in general, higher concentrations of TE than cows from Povoação. As there is a natural depletion of essential elements in dairy cows due to reproductive phenomena and milk production (average 40 L per day), these results reveal that in the case of cows from Ponta Delgada this depletion is much smaller than in cows from Povoação, because the area where they graze (the basaltic Picos fissure system) offers better concentrations of these essential elements, allowing to reduce the losses suffered. This demonstrates that animal age (calves *vs.* dairy cows) and soil type, and therefore the geochemical composition of parent rocks, are relevant factors for the intake of essential TE. It also reinforces the need for adjusted feeding plans, especially in agricultural areas where soils have lower concentrations of essential TE, particularly regarding adult lactating animals.

Table 3
Bioaccumulation factor.

TE	Calves	Cows	
	Povoação	Ponta Delgada	Povoação
Fe	0.029	0.011	0.009
Mg	0.581	0.073	0.622
Al	0.028	0.009	0.005
Ba	0.067	0.026	0.042
Cd	0.240	0.051	0.080
Co	0.048	0.025	0.024
Cr	0.024	0.006	0.013
Cu	**1.737**	0.132	**1.626**
Mn	0.123	0.034	0.076
Mo	0.096	0.173	0.318
Ni	0.031	0.008	0.022
Pb	0.079	0.011	0.011
Sr	0.653	0.082	0.285
Ti	0.032	0.013	0.005
V	0.034	0.018	0.011
Zn	**1.168**	**1.681**	**1.303**

Overall, cows grazing in Ponta Delgada have significantly higher concentrations of essential TE in the hair when compared with cows from Povoação (Table 2). Mn made an exception, which is the only essential TE that does not show significant differences in cows from both grazing sites, and Mo, which is present in significantly higher concentrations in cows grazing in Povoação.

Regarding Mn, animals from both grazing sites have an adequate intake, given that the minimum requirement for calves is 20 mg/kg and for lactating cows is 40 mg/kg [35]. However, it should be noted that Mn is present in higher concentration in basaltic soils but not in the hair of calves and cows from Ponta Delgada, when compared with animals from Povoação. This may be because Mn in animals depends, among other factors, on the type of chemical compounds in which this element is present in plants and the antagonistic action of some elements, including Fe [36]. As excessive Fe content in the soil affects the bioavailability of Mn to plants [13], the differences observed in the hair of animals from both grazing sites were expected, as basaltic soils have significantly higher concentration of Fe that limits the availability of Mn on fresh grass and some of the byproducts obtained from the soil, such as grass silage and corn silage, that are also part of their diet.

Molybdenum is naturally present in higher concentrations in trachytic soils than in basaltic soils, which can be explained by the pedogenesis of parent volcanic rocks with different geochemical compositions, as trachytic rocks are known to have higher Mo contents when compared with basaltic rocks [37,38]. Our results for dairy cows bring forward this difference and reinforce the notion that cattle hair reflects the environmental availability of essential TE.

On what concerns the other essential TE, cows from Ponta Delgada have a higher intake when compared to animals from Povoação. This difference was expected for Co, as previous studies have demonstrated that basaltic soils have higher concentrations of Co when compared to trachytic soils [5,6]. The Co deficiency evidenced in the hair of animals from Povoação (see Table 2; minimum requirement 0.10 mg/kg [35]) can lead to symptoms of vitamin B_{12} deficiency, such as reduced milk production, growth, and fertility, as well as rough haircoat, weight loss, and anaemia [39,40].

The higher concentrations of Cr detected in the hair of cows from Ponta Delgada may be due to the combined effect of two types of factors: geogenic factors, as basaltic soils from the Picos fissure system have high values of Cr (Table 2), which are inherited from the parent basaltic rocks (see Fig. 2), and anthropogenic factors, such as the release of Cr into the environment through sewage and fertilizers [41]. In Ponta Delgada area, where agricultural production is more intense, the continuous use of pesticides and fertilizers may increase the concentration of Cr in the soils [42,43] and, consequently, in cattle hair. The higher values observed in our study are well above the values determined in other studies, which found Cr concentrations in the mane and tail hair of horses ranging from 0.017 to 0.060 mg/kg [44].

Copper, another essential TE for cattle, is part of several enzymes and proteins, thus playing important metabolic roles. Cattle is known to be tolerant to Cu poisoning and the results of our study reveal much higher concentrations (10.11 mg/kg) than those described by Perillo et al., [45], where mean values ranged from 0.20 to 3.07 mg/kg. Our results follow the tendency observed in other studies [17,46], where the recorded values were above 8 mg/kg and within the adequate requirement for calves and cows [35]. Calves and cows from Ponta Delgada have higher concentrations of Cu in the hair than the animals from Povoação, being overall related to the availability of Cu in the soil. However, the magnitude of the difference in Cu concentration between animals from the two grazing sites does not directly reflect that observed in the soils, as basaltic soils have much higher contents of Cu when compared with trachytic soils (76.50 mg/kg *vs.* 4.60 mg/kg, respectively). This difference may be due to the interrelationship with Fe, which at high concentrations (like in basaltic soils) is known to reduce Cu absorption [47]. This explains why in cows from Ponta Delgada the bioaccumulation factor for Cu is inferior to 1, while for the animals from Povoação (calves and cows) the bioaccumulation factor is higher than 1, despite the much lower concentrations of this essential TE in trachytic soils.

Iron takes part in several biological redox processes due to its interconversion between ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions [41,48]. Our results show that the hair of animals from both grazing sites (except calves from Ponta Delgada) has concentrations much higher than required (50 mg/kg; [35]) but are still below the maximum tolerable level (1000 mg/kg; [35]). These results may be related to the use of some fertilizers that can increase Fe concentration. Calves from Ponta Delgada, on the other hand, because they are closed in a barn, do not have access to fresh pasture grass and, therefore, are not so easily subject to the possible intake of fertilizers along with the feed, as the fertilizers are usually applied just at the beginning of the development of the grass and corn.

Zinc is a very important essential element for animals and humans, as it intervenes in physiological and regulatory processes and is necessary for the metabolic activity of numerous metalloenzymes [49,50]. The requirement of Zn for calves is not determined, but for adult cows is 30 mg/kg, with a maximum tolerable level of 500 mg/kg [35]. Our findings report concentrations of Zn in the hair of dairy cows much higher than the adequate requirement (108.3 mg/kg and 260.29 mg/kg, for Povoação and Ponta Delgada, respectively); still, the observed values are below the maximum tolerable level and, therefore, intoxication-related health problems are not expected. Our study also showed that for Zn the bioaccumulation factor is > 1 in cows from Ponta Delgada and Povoação and, in calves from Povoação, indicating that the intake of this element derives essentially from its availability in the soil. Also, there is another factor, besides Zn availability in the soils, that can contribute to a higher BAF: the island's climate with warm and humid periods creates the perfect conditions for ruminants to develop hepatogenous photosensitization caused by the fungus *Pithomyces chartarum* and, to avoid this condition cattle are routinely protected by supplementing their feed with zinc oxide or using a slow release intraruminal zinc bolus [51].

Although Ni is not considered an essential nutrient for calves and cows, recent advances in ruminant nutrition have led to the establishment of Ni requirements, as it is essential for bacteria that exert an effect on the microbial ecosystem of the rumen [33,52]. Our results evidence that cows from Ponta Delgada have higher concentrations of this element in the hair when compared with the animals from Povoação; this can be related to local geographic and geologic conditions as basaltic soils are much richer in Ni than trachytic soils. Still, since there are no minimum requirements for Ni intake, even a small amount, as observed in the animals from Povoação, might be enough to ensure a normal function of the bacteria in the rumen.

It is well known that the concentrations of TE in the environment vary greatly and that they are influenced by a series of factors of natural or anthropogenic origin, thus there is an ever-present risk that these changes may cause ecological imbalance in the most diverse environments [53]. In a region like the Azores, where volcanism shapes the soils and agriculture is the main economic sector [also shaping the elemental composition of soils [42]], mineral supplementation in animal production is of great importance to prevent any inadequate supply of essential TE, which often leads to poor animal health, adversely affecting immunity and reproduction. Likewise, TE exposure in grazing cattle also has important consequences for humans as milk, milk products, meat and meat products are some of the main contributors of TE in human nutrition.

Despite the limitations of this study [i) calves from Ponta Delgada were raised inside a barn, without access to the grazing area or pasture grass, which did not allow for comparison between calves from both grazing sites, as well as comparison between calves and cows from Ponta Delgada; and ii) animals had an intake of essential TE from other sources than just the environment (*e.g.* feed)] and the fact that the reference used in the bioaccumulation factor (soil-aqua regia extract) may sometimes not reflect the bioavailability of the trace elements in the soil, our results demonstrated that cattle hair can be successfully used as a biomarker to assess the intake of TE in animals grazing in volcanic soils.

5. Conclusion

This study demonstrates that cattle hair TE can be successfully used as exposure biomarkers to assess TE status in volcanic soils. Our results reinforce the uneven distribution of essential TE in the soils of São Miguel, due to different geochemical compositions of the parent volcanic rocks, which contributes to an unbalanced intake by animals grazing outdoors and consequently a poor health status. The data obtained also demonstrate that grazing the animals outdoors, from an early age, provides another means of accessing essential TE in addition to feed, which can reduce the natural depletion of these essential elements in the body due to reproductive phenomena and milk production.

In regions where the economic core is based on agriculture, the development of geo-environmental studies, such as ours, is of utmost importance to assess the availability of essential TE and the influence of geo-environmental factors on its cycle. The information obtained will be fundamental for farmers, as it can have an impact on the general health and welfare of grazing animals.

CRediT authorship contribution statement

Diana Linhares: Conceptualization; Formal analysis; Investigation; Methodology; Writing – original draft; Review & editing. **Adriano Pimentel:** Investigation; methodology; Writing – review & editing. **Diogo Gaspar:** Investigation. **Patrícia Garcia:** Funding aquisition; Formal analysis; Investigation; Writing – review & editing. **Armindo Rodrigues:** Conceptualization; Funding aquisition; Investigation; Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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