Trace Metals in High-Andean Forages: Differential Dietary Exposure in Cattle, Sheep, and Alpacas, and Lack of Non-Carcinogenic Risk

Alberto Arias-Arredondo

albertogilmer@gmail.com

Estación Experimental Agraria Santa Ana, Dirección de Servicios Estratégicos Agrarios, Instituto Nacional de Innovación Agraria

Melina Lopez-Rodriguez

Estación Experimental Agraria Santa Ana, Dirección de Servicios Estratégicos Agrarios, Instituto Nacional de Innovación Agraria

Juancarlos Cruz-Luis

Centro Experimental La Molina, Dirección de Servicios Estratégicos Agrarios, Instituto Nacional de Innovación Agraria

Jose Contreras

Laboratorio de Nutrición Animal y Evaluación de Alimentos, Universidad Nacional de Huancavelica

Hebert Ramos-Acuña

Laboratorio de Nutrición Animal y Evaluación de Alimentos, Universidad Nacional de Huancavelica

Richard Solórzano-Acosta

Centro Experimental La Molina, Dirección de Servicios Estratégicos Agrarios, Instituto Nacional de Innovación Agraria

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Alberto Arias-Arredondo[1*], email: albertogilmer@gmail.com

Melina Lopez-Rodriguez[1,2], email: mlrodriguez1472@gmail.com

Juancarlos Cruz-Luis[3], email: jcruz@inia.gob.pe

Jose Contreras[4], email: jose.contreras@unh.edu.pe

Hebert Ramos-Acuña[4], email: hebert.ramos@unh.edu.pe

Richard Solórzano-Acosta[3], email: investigacion_labsaf@inia.gob.pe

[1]Estación Experimental Agraria Santa Ana, Dirección de Servicios Estratégicos Agrarios, Instituto Nacional de Innovación Agraria, Huancayo, Junín, Peru

[2]Escuela de Formación Profesional de Zootecnia, Facultad de Ciencias Agropecuarias, Universidad Nacional Daniel Alcides Carrión, Cerro de Pasco, Pasco, Peru

[3]Centro Experimental La Molina, Dirección de Servicios Estratégicos Agrarios, Instituto Nacional de Innovación Agraria, Lima, Perú

[4]Laboratorio de Nutrición Animal y Evaluación de Alimentos, Universidad Nacional de Huancavelica, Huancavelica, Peru

***Correspondence:** albertogilmer@gmail.com

ABSTRACT

Trace metal accumulation in forage plants is a growing concern in mountain grazing systems, yet the relative importance of environmental and biological drivers remains poorly understood in the high Andes. This study evaluated trace metal concentrations in soils and cultivated forages along an altitudinal gradient (4,098–4,467 m a.s.l.) in the Junín region of central Peru and assessed potential dietary risks for livestock. Soil properties and concentrations of Zn, Cd, Cu, Mn, Pb, and Cr were determined in soils and four forage species (Avena sativa, Dactylis glomerata, Lolium multiflorum, and Lolium perenne) across eight plots. Dietary exposure was estimated using the Estimated Daily Intake (EDI), while non-carcinogenic risks for cattle, sheep, and alpacas were assessed through the Hazard Quotient (HQ) and Hazard Index (HI). Marked spatial variation in soil properties and trace metal concentrations was observed among plots, indicating that metal accumulation was primarily driven by local soil heterogeneity. Altitude was not associated with trace metal concentrations, and forage species showed no significant differences in metal accumulation ($p > 0.05$). Although sheep exhibited the highest EDI values, all HQ and HI values remained below 1. These findings demonstrate that fine-scale soil heterogeneity is a stronger determinant of trace metal accumulation than altitude or forage species identity in high-Andean forage systems. The study provides new evidence that current trace metal exposure levels do not represent a significant non-carcinogenic risk for cattle, sheep, or alpacas under the evaluated environmental conditions.

KEYWORDS: trace metals, high-Andean grasslands, dietary exposure, non-carcinogenic risk, cattle, sheep, alpacas, soil heterogeneity.

INTRODUCTION

Contamination by trace metals in terrestrial ecosystems has emerged as a growing global environmental concern due to their persistence, potential toxicity, and capacity to bioaccumulate along food chains (Ondrasek et al., 2025; Kosakivska et al., 2020). These elements can enter soil, water, and the atmosphere through both natural processes, such as rock weathering and volcanic activity, and anthropogenic sources, including mining, metallurgical and chemical industries, transportation, and the use of mineral fertilisers (Kosakivska et al., 2020). In livestock systems, ingestion of contaminated forage is the primary pathway of exposure; therefore, forage species play a critical role in transferring metals from soil to herbivores and, ultimately, into the food chain (Afzal and Mahreen, 2024).

This issue is particularly relevant in high-Andean ecosystems, where livestock production relies predominantly on direct grazing of natural and cultivated pastures. These systems operate under extreme environmental conditions, characterised by high altitude, low temperatures, intense solar radiation, and often acidic soils, factors that jointly influence forage productivity and the biogeochemical dynamics of trace metals (Arias-Arredondo et al., 2026; Luo et al., 2024). In this context, the availability and mobility of metals in soil, as well as their uptake by plants, are strongly governed by soil properties such as pH, organic matter content, and texture, in addition to the pronounced spatial heterogeneity that typifies these ecosystems (Chen et al., 2024; Liu et al., 2020).

From a functional perspective, certain elements such as zinc (Zn), copper (Cu), and manganese (Mn) are essential for a wide range of physiological processes in both plants and animals. In contrast, other elements, including cadmium (Cd), lead (Pb), and specific forms of chromium (Cr), have no known biological function and can exert toxic effects even at low concentrations (Hejna et al., 2018; Yadav et al., 2023). The accumulation of these metals in forage arises from the complex interaction among soil properties, environmental conditions, and species-specific traits, with plant uptake and translocation capacity playing a critical role in significantly modulating the dietary exposure of grazing animals (Chen et al., 2024; Su et al., 2023; Liu et al., 2020; Serbula et al., 2012).

At the landscape scale, the distribution of trace metals exhibits high spatial variability, driven by interactions among edaphic, topographic, and climatic factors. Although altitude establishes environmental gradients that can influence metal availability (Guimarães et al., 2024), emerging evidence indicates that local variability and site-specific conditions often exert a stronger influence than the altitudinal gradient alone. This results in heterogeneous distribution patterns and the formation of localised accumulation zones, or “hotspots” (Feng et al., 2024; Victor et al., 2026). Such heterogeneity poses a major challenge for risk assessment, as approaches that rely solely on mean values may underestimate actual exposure in critical areas.

Assessing the risk associated with trace metal exposure in grazing systems requires integrating forage metal concentrations with animal intake parameters. In this context, indicators such as estimated daily intake (EDI), hazard quotient (HQ), and cumulative hazard index (HI) provide robust tools for quantifying exposure and evaluating potentially detrimental non-carcinogenic effects (Afzal and Mahreen, 2024; Shahzad et al., 2025; Romero-Crespo et al., 2023; Farboodi et al., 2026). However, their application in high-Andean ecosystems remains limited and, in many cases, lacks an explicitly spatial perspective.

Despite advances in trace metal research, most studies in high-Andean regions have examined forage nutritional quality, contaminant presence, or risk assessment in isolation, without integrating these components within a spatial and multivariate framework. This limitation constrains the identification of accumulation patterns, the detection of critical exposure zones, and the understanding of the mechanisms regulating metal transfer in grazing systems (Pizarro et al., 2025; Moreno-Aguirre et al., 2024). In this context, the integration of geostatistical tools and multivariate analyses emerges as a key approach for characterising spatial variability and disentangling the interactions among environmental factors, forage species, and nutritional quality (AbdelRahman et al., 2021; Prieto-Amparán et al., 2019).

In light of these considerations, this study aimed to assess the spatial and altitudinal variability of trace metals in high-Andean forage and to integrate this information with estimates of dietary exposure and non-carcinogenic risk in cattle, sheep, and alpacas under grazing conditions. We hypothesised that, although the altitudinal gradient establishes baseline environmental conditions, the final concentrations of trace metals and the associated risks are more strongly governed by local soil heterogeneity and the selective uptake capacity of individual forage species. The main contribution of this study lies in integrating, within a single analytical framework, the spatial variability of trace metals, forage quality, and animal risk assessment in a high-Andean grazing system. This approach provides a more robust basis for ecological interpretation and supports the development of improved monitoring and management strategies for high-mountain livestock systems.

MATERIALS AND METHODS

Study area and site characteristics

The study was conducted in high-Andean grasslands located in the district, province, and region of Junín, central Peru. The study area spans an altitudinal gradient ranging from 4,098 to 4,467 m.a.s.l. (Figure 1), representative of high-Andean *puna* ecosystems characterised by extreme environmental conditions that constrain plant productivity (Yaranga et al., 2023).

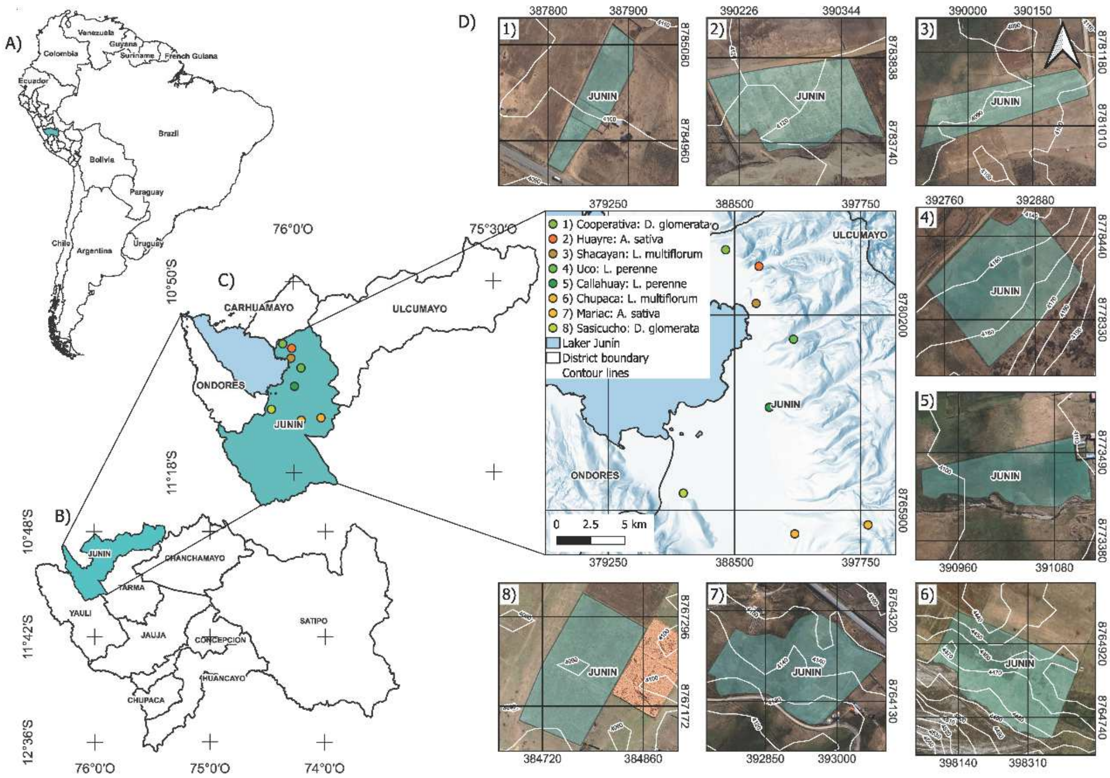

Figure 1. Location of the study area and multiscale spatial distribution of sampling plots in high-Andean grasslands of Junín, Peru. (A) Location of the study area in South America. (B) Location within the Junín region, Peru. (C) Spatial distribution of sampling plots along the altitudinal gradient. (D) Enlarged view of the study plots and their spatial arrangement.

The climate is classified as tundra (ET) according to the Köppen–Geiger classification (Kottek et al., 2006), with marked seasonal variation, including a wet season (October–April) and a dry season (May–September). During the study period (October 2024 to July 2025), maximum temperatures ranged from 10 to 17 °C, while minimum temperatures frequently fell below 0 °C, with recurrent frost. Precipitation was concentrated during the summer months, and relative humidity ranged between 75% and 90%, as shown in Figure 2 (SENAMHI, 2026).

The soils are predominantly classified as Dystric Leptosol–Vitric Andosol (LPd–ANz) and, to a lesser extent, Dystric Regosol–Dystric

Cambisol (RGd–CMd), developed on ridges, hills, and mountainous terrain (SIGMINAM, 2010). These soils exhibit weakly developed profiles (AC or BC horizons) (INRENA, 1996), typical of high-Andean environments, which influences nutrient dynamics and the bioavailability of trace elements within the soil–plant system (Sun et al., 2025; Orellana et al., 2020). The physicochemical properties of the soils analysed are presented in the Results section.

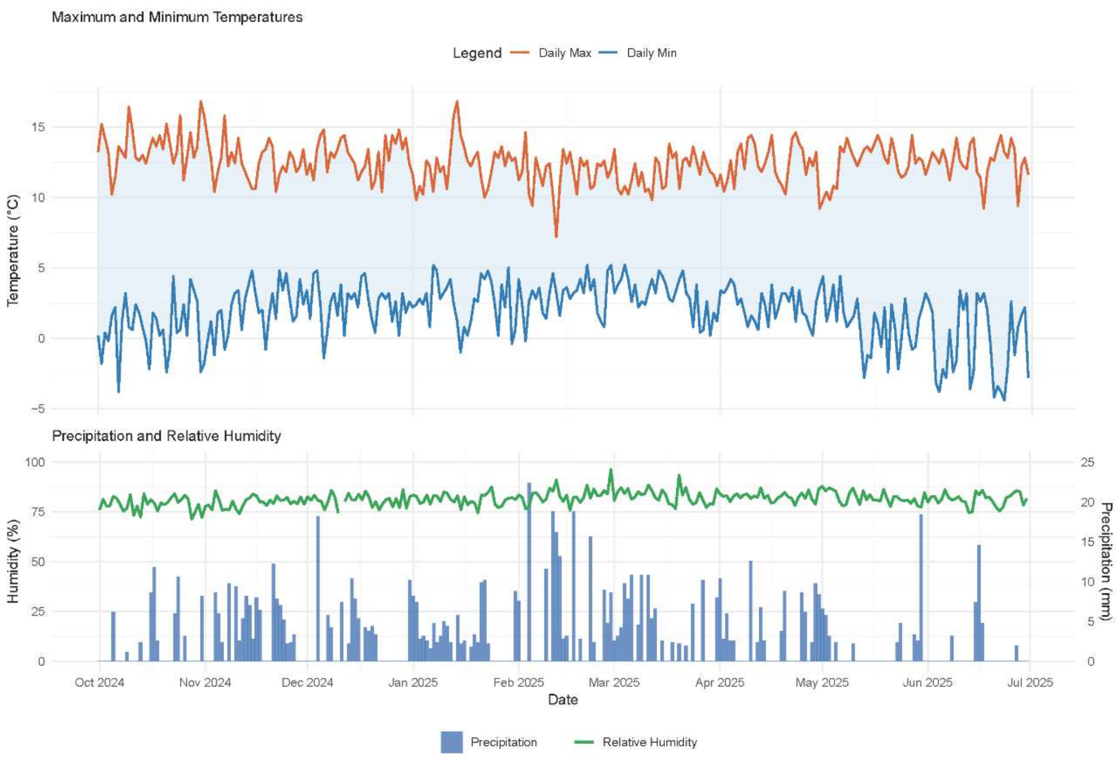

Figure 2. Temporal dynamics of temperature, precipitation, and relative humidity in high-Andean pastures during the study period.

The vegetation is dominated by high-Andean grasses of the *Festuca*, *Jarava*, and *Calamagrostis* genera, which are adapted to low temperatures and seasonal water availability, factors that influence forage quality. The area is primarily used for traditional extensive grazing (sheep, alpacas, and, to a lesser extent, cattle), with patches of cultivated pasture contributing to heterogeneity in vegetation cover.

Collectively, these conditions define a system characteristic of the high-Andean *puna*, in which soil–plant–animal interactions regulate nutrient dynamics and trace-element distribution.

Experimental design and sampling

Eight representative plots (Mariac, Huayre, Sasicucho, Cooperativa, Chupaca, Shacayan, Uco, and Callahuay) were selected along an altitudinal gradient in the Junín region (Figure 1). These plots correspond to extensively managed cultivated pasture systems representative of the production conditions typical of *puna* ecosystems.

The study was conducted using an observational sampling design with a hierarchical structure, in which the plot was considered the experimental unit. Four forage species of importance in high-mountain systems (*Avena sativa*, *Dactylis glomerata*, *Lolium multiflorum*, and *Lolium perenne*) were evaluated, with two plots selected per species, resulting in a total of eight experimental units.

Five georeferenced sampling points were established within each plot to estimate dry matter production ($t \cdot ha^{-1}$) and capture spatial variability in pasture productivity. For the determination of crude protein, trace metal concentrations (Zn, Cd, Cu, Mn, Pb, and Cr), and derived indices (HQ, HI, and EDI), three points per plot were selected and analysed individually. This approach optimised laboratory analyses while preserving the spatial representativeness of the samples.Sampling was conducted on the edible portion of the forage (aboveground biomass) to accurately reflect grazing conditions. Samples were collected manually and transported under appropriate conditions for subsequent laboratory processing. At each sampling point, geographic coordinates (UTM: Easting and Northing) and altitude were recorded using a global positioning system (GPS), enabling the incorporation of spatial data into the analysis and the assessment of patterns associated with the altitudinal gradient.

Each sample was assigned a unique identification code incorporating the species, plot, and sampling point, ensuring full traceability throughout the analytical process.

Determination of productivity, nutritional parameters, and trace metals

The collected forage samples were processed in the laboratory to determine productivity and nutritional parameters, as well as trace metal concentrations. Prior to analysis, samples were oven-dried at 65 °C until constant weight was achieved, then ground to a fine, homogeneous powder.

Dry matter (DM) yield was estimated using 1 m^2 quadrat sampling. Five subsamples of aboveground biomass were collected from each plot and weighed in the field to determine fresh weight (FW). Based on the dry matter content (DM%), biomass yield was expressed as tonnes of dry matter per hectare ($t \cdot ha^{-1}$) using the following equation:

$$DM = FW \times \frac{DM\%}{100} \times 10 \quad (1)$$

Where DM is the dry matter yield ($t \cdot ha^{-1}$), FW is the fresh weight ($kg \cdot m^{-2}$), and DM% is the dry matter content (%). The factor 10 represents the conversion from kilograms per square meter to tonnes per hectare.

For nutritional and trace metal analyses, three sampling points per plot were selected and analyzed individually. Crude protein content was determined using the Kjeldahl method, based on total nitrogen, applying a conversion factor of 6.25 in accordance with widely accepted official methods for food and forage analysis (Latimer, 2023).

Trace metal concentrations (Zn, Cd, Cu, Mn, Pb, and Cr) were determined following microwave-assisted acid digestion of the dried and ground samples, in accordance with EPA Method 3051A, a widely validated protocol for elemental extraction from environmental matrices (US EPA, 2007). Subsequently, element concentrations were quantified using microwave plasma–atomic emission spectrometry (MP-AES), a technique widely employed for multi-element analysis in biological and environmental samples.

Results were expressed as mg·kg^{-1} on a dry matter basis. Analytical quality control included the use of procedural blanks, certified reference materials, and duplicate analyses, in accordance with international guidelines to ensure the precision and accuracy of the measurements (Gray et al., 2025).

Soil physicochemical properties and trace metal concentrations

Soil sampling was conducted in each plot by collecting multiple subsamples, which were homogenised to obtain a representative composite sample for each experimental unit. Samples were collected at a depth of 0–20 cm, corresponding to the topsoil layer, where the highest root and biological activity develops in grazing systems.

Samples were subsequently air-dried, sieved, and prepared for laboratory analysis. Soil physicochemical properties were determined, including pH, electrical conductivity (EC), organic matter (OM), available phosphorus (P), available potassium (K), texture, and trace metal concentrations.

Soil pH was determined following EPA Method 9045D (US EPA, 2004a), while electrical conductivity was measured in accordance with ISO 11265 (ISO, 1994). Organic matter content was determined by dry combustion following ISO 10694 (ISO, 1995).

Available phosphorus was determined using the Olsen method (Olsen and Sommers, 1982) for neutral to alkaline soils and the Bray and Kurtz method (Bray and Kurtz, 1945) for acidic soils, according to soil pH. Available potassium was measured following extraction with ammonium acetate and quantified by microwave plasma–atomic emission spectrometry (MP-AES). Soil texture was determined using the hydrometer method (Bouyoucos, 1962). All procedures were conducted in accordance with the Mexican Official Standard NOM-021-RECNAT-2000 (SEMARNAT, 2002).

Total trace metal concentrations (Zn, Cd, Cu, Mn, Pb, and Cr) were determined following microwave-assisted acid digestion of the samples in accordance with EPA Method 3051A (US EPA, 2007). Element concentrations were subsequently quantified using microwave plasma–atomic emission spectrometry (MP-AES).

Assessment of dietary exposure and non-carcinogenic risk

Exposure to trace metals through forage consumption was assessed by estimating the Estimated Daily Intake (EDI), based on metal concentrations measured in plant material and representative intake rates and body weights for different animal species (cattle, sheep, and alpacas), following methodologies widely applied in environmental and food risk assessment (US EPA, 2012).

The EDI ($mg \cdot kg^{-1}$ body weight$\cdot day^{-1}$) was calculated using the following equation:

$$EDI = \frac{C \times DMI}{BW} \qquad (2)$$

Where C is the metal concentration in forage ($mg \cdot kg^{-1}$ dry matter), DMI is the daily dry matter intake ($kg \cdot day^{-1}$), and BW is the animal body weight (kg).

The body weight (BW) and dry matter intake (DMI) values used in the calculations were derived from ranges reported for ruminant species in comparable production systems, taking into account typical high-Andean livestock conditions (NRC, 2005; Van Soest, 1994). These values were selected to reflect the prevailing management and feeding practices in the study area. Mean values of 475 kg and 11.0 $kg \cdot day^{-1}$

were assumed for cattle, 52.5 kg and 1.35 kg·day^{-1} for sheep, and 65 kg and 1.30 kg·day^{-1} for alpacas.

Non-carcinogenic risk was estimated using the Hazard Quotient (HQ) (US EPA, 2004b), defined as the ratio of the Estimated Daily Intake (EDI) to the maximum tolerable levels (MTL) in the diet reported for each metal in cattle, in accordance with NRC (2005):

$$HQ = \frac{EDI}{MTL} \quad (3)$$

Cumulative risk was assessed using the Hazard Index (HI), calculated as the sum of the individual hazard quotients:

$$HI = \sum HQ_i \quad (4)$$

Where HQ_i represents the hazard quotient for each individual metal.

HQ and HI values were interpreted such that values < 1 indicate negligible non-carcinogenic risk, whereas values > 1 suggest the potential for adverse effects on animal health (US EPA, 2012; Emurotu et al., 2024).

Statistical, spatial, and multivariate analysis

Statistical analyses were conducted using R version 4.4.2 (R Core Team, 2024), employing specialised packages for data modelling and visualisation, including *lme4*, *lmerTest*, *ggplot2*, and *vegan*. Descriptive statistics (mean ± standard error) were calculated for production and nutritional variables, as well as for trace metal concentrations.

The effect of forage species on the assessed variables was analysed using linear mixed-effects models (LMMs), with species included as a fixed effect and plot as a random effect to account for spatial variability among sampling units. The significance of fixed effects was evaluated using analysis of variance (ANOVA) on the mixed models, applying

Satterthwaite's approximation for degrees of freedom as implemented in the *lmerTest* package, with a significance level of $p < 0.05$. Models were fitted independently for each response variable.

The effect of the altitudinal gradient on trace metal concentrations was assessed using linear regression models, with altitude treated as a continuous variable. These analyses were complemented by regression diagnostics and graphical visualisation of trends.

Data visualisation was performed using boxplots, scatterplots, and faceted representations after restructuring the dataset into long format.

Spatial analysis of trace metal concentrations and the cumulative hazard index (HI) was performed using inverse distance weighting (IDW) interpolation based on UTM coordinates (Zone 18S). Interpolated surfaces were generated at a spatial resolution of 5 m using a power parameter ($p = 2$) for visualisation purposes. The resulting surfaces were clipped to plot boundaries and presented as thematic maps. Given the limited number of sampling points, the interpolation results were interpreted as exploratory representations of spatial variability.

Exposure to trace metals was estimated using the Estimated Daily Intake (EDI), evaluated by forage species, animal type (cattle, sheep, and alpaca), and metal. Differences in EDI among forage species were analysed using linear mixed-effects models consistent with those previously described; the results are presented in Supplementary Table S1. To facilitate relative comparisons among metals, EDI values were normalised to a 0–1 range for graphical representation only.

The hazard quotient (HQ) and cumulative hazard index (HI) were calculated for each metal and forage species, and differences among them were evaluated using linear mixed-effects models. The corresponding results are presented in the Supplementary Table S2.

The relationships between production and nutritional variables and trace metal concentrations were assessed using redundancy analysis (RDA), with altitude and species included as explanatory variables. Prior to analysis, variables were standardised (mean = 0, standard deviation = 1) to ensure comparability. Model significance was evaluated using permutation tests (n = 999), and results were visualised through biplots. Additionally, a variance partitioning analysis was conducted to quantify the relative contributions of the evaluated factors.

RESULTS

Soil physicochemical properties and trace metal concentrations

Soil physicochemical properties and trace metal concentrations exhibited marked variability across the high-Andean plots (Table 1). Soil pH ranged from slightly acidic to neutral (5.1–7.2), while electrical conductivity varied between 3.7 and 15.2 mS·m^{-1}. Organic matter content was high across all plots (68.5–254.3 g·kg^{-1}), with particularly elevated values observed at Cooperativa.

Table 1. Soil physicochemical properties and trace metal concentrations across sampling plots in high-Andean grasslands (Junín, Peru).

Plot	**Altitude**	**Species**	**pH**	**EC**	**OM**	**P**	**K**	**Texture**	**Zn**	**Cd**	**Cu**	**Mn**	**Pb**	**Cr**
Mariac	4,140	*A. sativa*	7.2	13.7	89.9	17.3	635.1	Sandy loam	19.0	0.3	11.6	508.8	56.3	5.9
Huayre	4,128	*A. sativa*	5.2	3.7	105.7	294.1	119.2	Sandy loam	13.2	0.3	2.7	555.1	34.7	11.9
Sasicucho	4,107	*D. glomerata*	5.6	6.6	118.9	59.7	110.4	Loam	21.1	0.3	2.6	575.8	50.6	15.0

Cooperativa	4,102	*D. glomerata*	5.7	15.2	254.3	94.1	476.3	Sandy loam	11.5	0.4	6.0	242.2	183.3	2.3
Chupaca	4,467	*L. multiflorum*	5.1	4.2	105.3	351.0	162.3	Sandy loam	13.4	0.4	5.3	461.4	19.8	7.5
Shacayan	4,098	*L. multiflorum*	5.1	4.7	82.4	128.3	111.3	Sandy loam	10.3	2.6	11.3	3398.0	95.2	18.6
Uco	4,162	*L. perenne*	5.6	5.4	68.5	7.7	88.6	Sandy loam	8.9	0.5	1.5	308.1	24.5	8.3
Callahuay	4,108	*L. perenne*	5.6	5.9	69.6	3.1	105.3	Loam	12.5	0.2	4.4	341.9	48.3	7.6

Altitude: meters above sea level (m.a.s.l.); EC: electrical conductivity ($mS \cdot m^{-1}$); OM: organic matter ($g \cdot kg^{-1}$); P and K: available phosphorus and potassium ($mg \cdot kg^{-1}$); Zn, Cd, Cu, Mn, Pb, and Cr: trace metal concentrations ($mg \cdot kg^{-1}$). Soil texture was determined using the textural triangle method. Values correspond to a single composite sample per plot and are presented for descriptive purposes.

Regarding available nutrients, substantial variability was observed in phosphorus (3.1–351.0 $mg \cdot kg^{-1}$) and potassium (88.6–635.1 $mg \cdot kg^{-1}$), indicating pronounced soil heterogeneity among plots. Regarding trace metals, manganese exhibited the highest concentrations, with a maximum value recorded at Shacayan (3398.0 $mg \cdot kg^{-1}$). Elevated concentrations of lead were also detected at Cooperativa (183.3 $mg \cdot kg^{-1}$), as well as cadmium at Shacayan (2.6 $mg \cdot kg^{-1}$). Collectively, these findings highlight marked spatial heterogeneity in both soil physicochemical properties and trace metal distribution.

Forage yield, nutritional quality, and trace metal concentrations

Forage productivity and nutritional quality varied among species; however, no statistically significant differences were detected according to the linear mixed-effects model (Table 2). Dry matter yield was highest in *Avena sativa* (12.03 ± 4.25 t·ha^{-1}), whereas perennial grasses exhibited lower values, particularly *Dactylis glomerata* and *Lolium perenne*. Similarly, crude protein content ranged from 15.08 ± 3.38% to 20.11 ± 1.64%, with *Avena sativa* showing the highest mean values (Table 2).

Table 2. Productive, nutritional, and trace metal characteristics of high-Andean forage species.

Variable	***Avena sativa***	***Dactylis glomerata***	***Lolium multiflorum***	***Lolium perenne***	**F**	***p***
Dry matter yield (t·ha^{-1})	12.03 ± 4.25	1.23 ± 0.32	2.57 ± 0.55	1.53 ± 0.15	1.21	0.412
Crude protein content (%)	20.11 ± 1.64	17.75 ± 1.54	15.08 ± 3.38	17.63 ± 1.46	0.90	0.457
Zn (mg·kg^{-1})	26.78 ± 2.39	51.90 ± 3.30	39.05 ± 4.36	27.88 ± 1.91	5.37	0.069
Cd (mg·kg^{-1})	1.13 ± 0.60	1.00 ± 0.49	1.16 ± 0.64	0.62 ± 0.12	0.20	0.892
Cu (mg·kg^{-1})	1.56 ± 1.01	5.37 ± 1.32	1.78 ± 1.31	1.45 ± 0.65	2.64	0.186
Mn (mg·kg^{-1})	71.09 ± 25.99	149.40 ± 14.55	158.16 ± 19.43	221.54 ± 29.99	2.44	0.205
Pb (mg·kg^{-1})	5.30 ± 1.26	4.12 ± 2.07	5.16 ± 1.43	2.58 ± 0.69	0.75	0.535
Cr (mg·kg^{-1})	0.07 ± 0.04	0.31 ± 0.13	0.77 ± 0.57	0.26 ± 0.05	0.81	0.550

Values are presented as mean ± standard error. Differences among species were evaluated using linear mixed-effects models, with species included as a fixed effect and plot as a random effect. F and *p* values correspond to the effect of species on each variable.

Regarding mineral composition, foliar metal concentrations also varied among species (Figure 3). Zinc showed a tendency towards differences among species (F = 5.37, $p = 0.069$), with higher concentrations observed in *Dactylis glomerata*, whereas *Avena sativa* and *Lolium perenne* exhibited comparatively lower values (Table 2). For the remaining elements (Cd, Cu, Mn, Pb, and Cr), no significant differences were detected among species ($p > 0.05$).

Although no significant effects of species were detected ($p > 0.05$), consistent interspecific patterns were observed in micronutrient accumulation. For instance, *Lolium perenne* exhibited the highest mean Mn concentration, whereas *Dactylis glomerata* showed comparatively higher Cu levels. In contrast, Pb concentrations were relatively low and uniform across species.

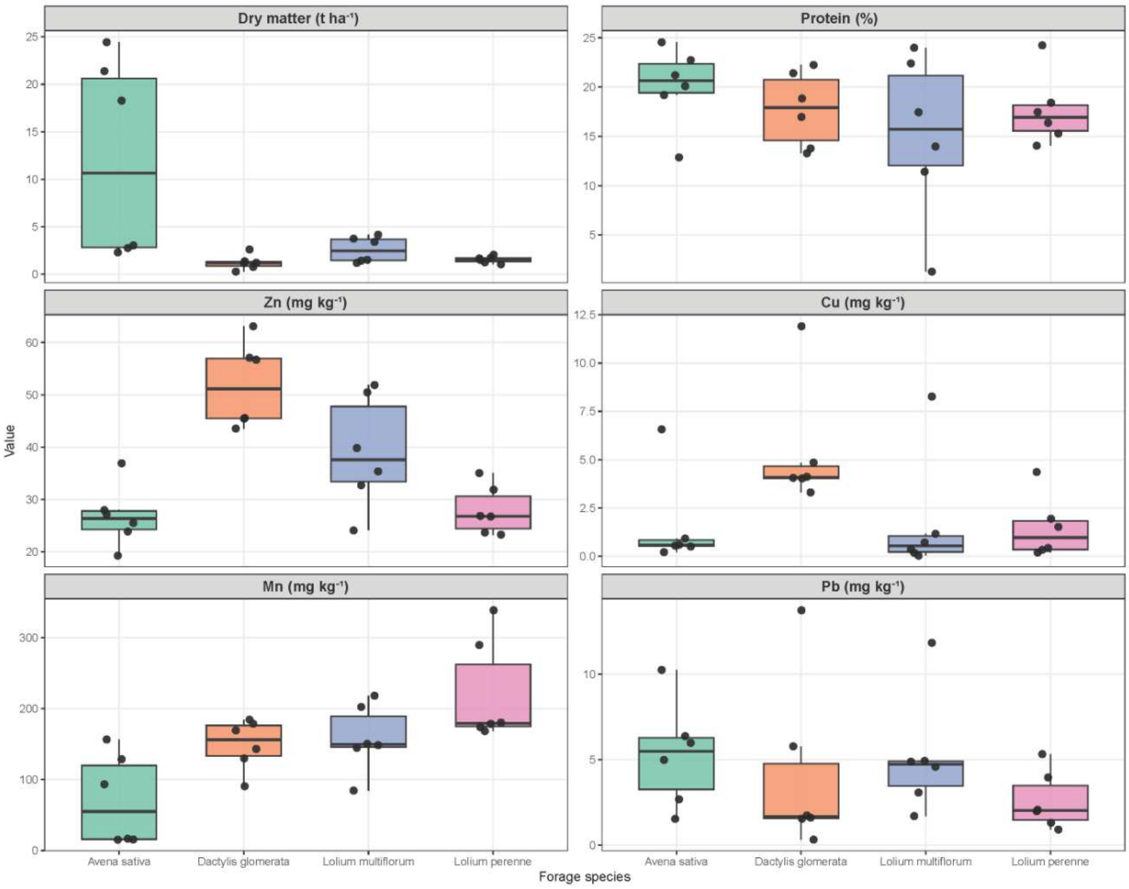

Figure 3. Variation in forage productivity, crude protein content, and selected trace metal concentrations among forage species. Boxplots represent the median and interquartile range, while points indicate individual observations.

Intraspecific variability and data distribution patterns are illustrated in Figure 3, where boxplots display the dispersion of individual values for productivity, crude protein, and trace metal concentrations across forage species.

Spatial distribution of trace metals in cultivated forages

Spatial interpolation of trace metal concentrations enabled the visualisation of distribution patterns within the evaluated plots (Figure 4). Overall, the distributions of chromium (Cr), cadmium (Cd), and lead (Pb) were heterogeneous, revealing fine-scale variability and localised zones of higher accumulation (“hotspots”). For Cr, a relatively homogeneous pattern was observed across most plots, characterised by gentle gradients and low-to-moderate concentrations. However, localised increases were identified in certain specific areas, indicating small-scale concentration variability within plots. In contrast, Cd exhibited greater spatial heterogeneity than Cr, with more pronounced contrasts between low- and high-concentration areas within the same plot. Distinct zones with elevated levels of these trace metals were identified, suggesting localised accumulation processes potentially associated with soil texture, organic matter content, or topographic variation.

Lead (Pb) exhibited an intermediate pattern, characterised by a heterogeneous distribution that was less pronounced than that of Cd. In several plots, distinct gradients and areas of moderate accumulation were observed, indicating spatial variability within plots. Collectively, these results demonstrate that trace metal distribution in high-Andean grasslands is strongly influenced by local-scale environmental variability. The presence of zones with relatively elevated concentrations within small spatial extent plots underscores the importance of accounting for spatial heterogeneity when assessing forage quality and estimating livestock exposure risk.

However, given the exploratory nature of inverse distance weighting (IDW) interpolation, the resulting maps should be interpreted as approximate representations of spatial variability and should not be considered robust spatial inferences.

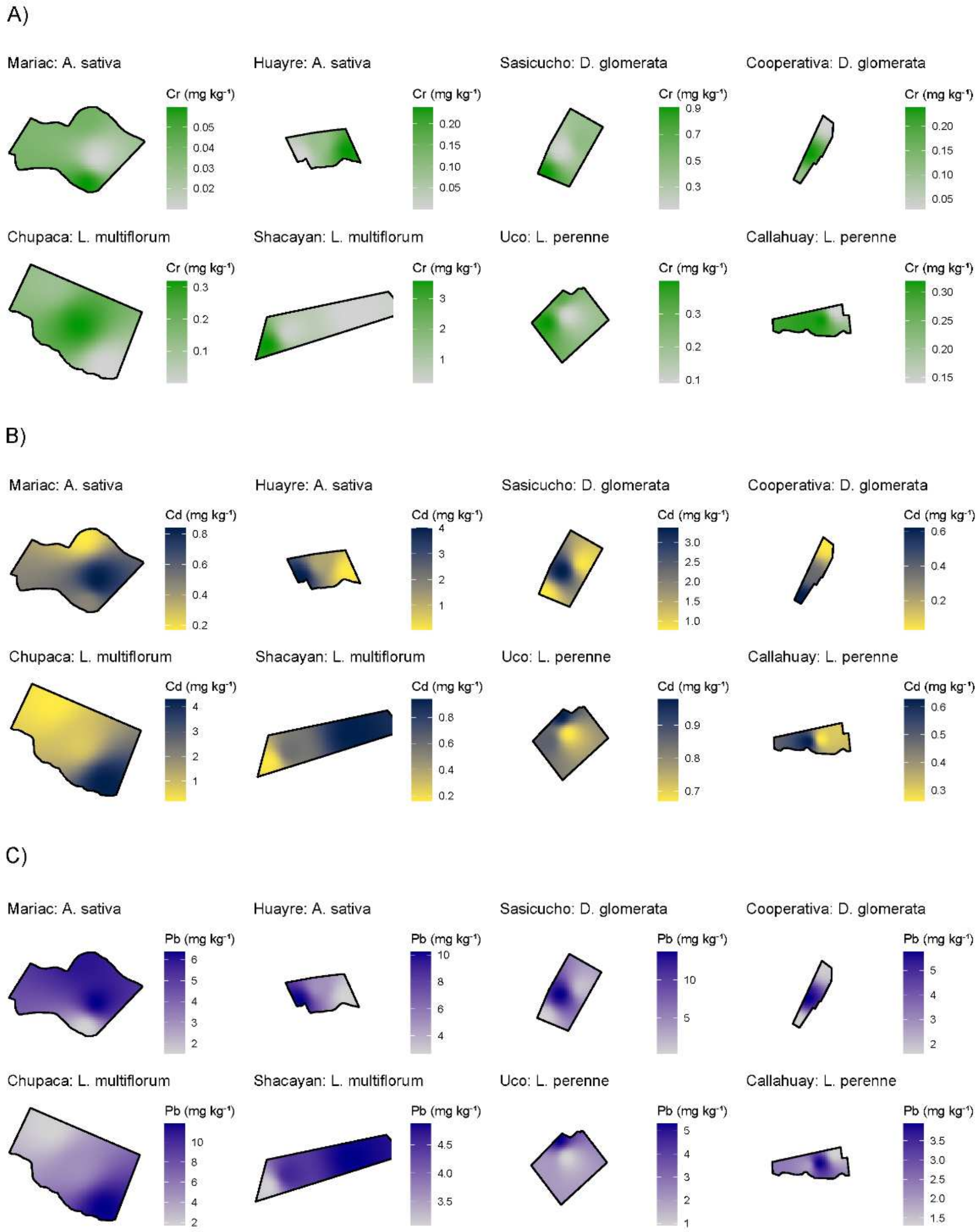

Figure 4. Spatial distribution of chromium (Cr), cadmium (Cd), and lead (Pb) in high-Andean forage species based on spatial interpolation.

Altitudinal trends in trace metal concentrations

The relationships between altitude and trace metal concentrations in forage species are presented in Figure 5. Across the evaluated altitudinal range (4095–4484 m.a.s.l.), no significant associations were detected between altitude and trace metal concentrations ($p > 0.05$). Although slight positive trends were observed for Zn, Cd, Mn, and Pb, the high variability among plots suggests that local environmental factors may exert a stronger influence than altitude per se on metal accumulation.

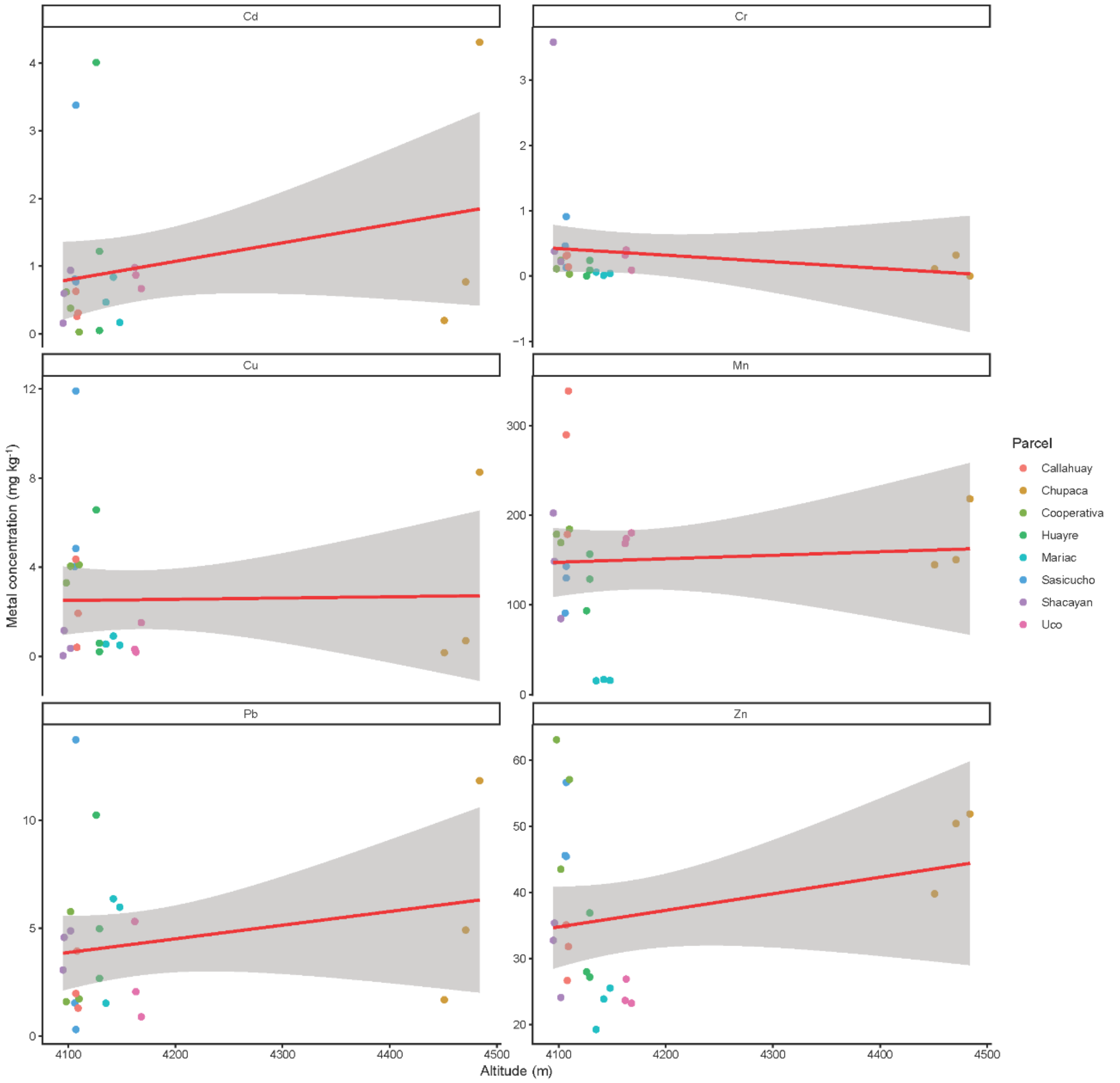

Figure 5. Relationship between altitude and trace metal concentrations in forage species across grazing plots in high-Andean ecosystems. Points represent individual samples colored by plot. Red lines indicate linear regression fits with 95% confidence intervals.

Estimated daily intake (EDI) of trace metals in grazing livestock

The estimated daily intake (EDI) of trace metals varied according to forage species and livestock type (Figure 6A). Overall, the highest exposure levels were observed in sheep, followed by alpacas and, to a lesser extent, cattle, reflecting differences in the dry matter intake-to-body-weight ratio. Among the metals evaluated, zinc (Zn) exhibited the highest EDI values across all forage–animal combinations, with *Dactylis glomerata* consistently contributing to higher intake levels. A similar pattern was observed for manganese (Mn), with relatively elevated values primarily associated with *Lolium perenne* and *Lolium multiflorum*. In contrast, EDI values for chromium (Cr) were consistently low across all species, while cadmium (Cd) and lead (Pb) showed intermediate and relatively uniform levels among forages. At the animal level, sheep exhibited the highest intake rates for most metals, followed by alpacas, whereas cattle showed the lowest values. This pattern was consistent across all forage species evaluated and is attributable to physiological differences and variation in nutritional requirements among production systems.

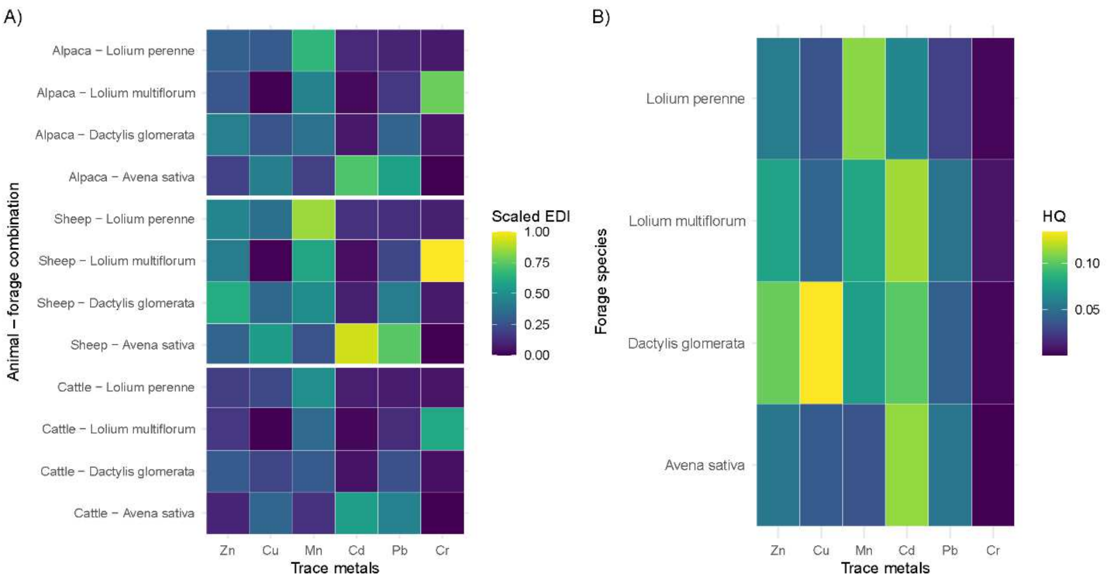

Figure 6. Estimated daily intake (EDI) and hazard quotient (HQ) of trace metals in grazing livestock. (A) Scaled EDI values of trace metals for different animal–forage combinations. (B) Hazard quotient (HQ) associated with the consumption of forage species. Values were normalised to facilitate relative comparisons across metals and forage species.

Despite the observed variability, no statistically significant differences in EDI were detected among forage species for any of the evaluated metals (*p* > 0.05; Table S1). Nevertheless, consistent trends suggest that forage composition may influence the accumulation and transfer of trace metals to livestock. Collectively, these findings indicate that, although exposure to trace metals through forage consumption varies among forage species and livestock types, the estimated intake levels remain within comparable ranges across production systems. These results also highlight the potential role of certain forage species as modulators of trace metal intake in high-Andean ecosystems.

Hazard quotient (HQ) and cumulative hazard index (HI)

Analysis of the hazard quotient (HQ) for trace metals showed that all values remained below the critical threshold of 1 across the different forage species evaluated (Figure 6B; Table S2), indicating negligible non-carcinogenic risk to grazing livestock. Nevertheless, relative variations among metals and forage species were observed, revealing consistent patterns of differential accumulation.

Among the evaluated metals, cadmium (Cd) and zinc (Zn) exhibited the highest HQ values, particularly in *Dactylis glomerata* and *Lolium multiflorum*, consistent with their elevated estimated intake levels. In contrast, chromium (Cr) showed consistently low HQ values across all species, indicating a negligible contribution to overall risk. Manganese (Mn) and copper (Cu) presented intermediate HQ values with moderate variability among forage species, whereas lead (Pb) levels remained comparatively low and stable across species.

The cumulative hazard index (HI), calculated as the sum of the individual HQ values, also remained below 1 across all evaluated species (Figure 7), confirming the absence of significant risk associated with combined exposure to trace metals. Nevertheless, differences were observed in the spatial distribution of HI at the plot level (Figure 7), with localised areas exhibiting higher values, particularly in zones with elevated concentrations of Cd and Pb.

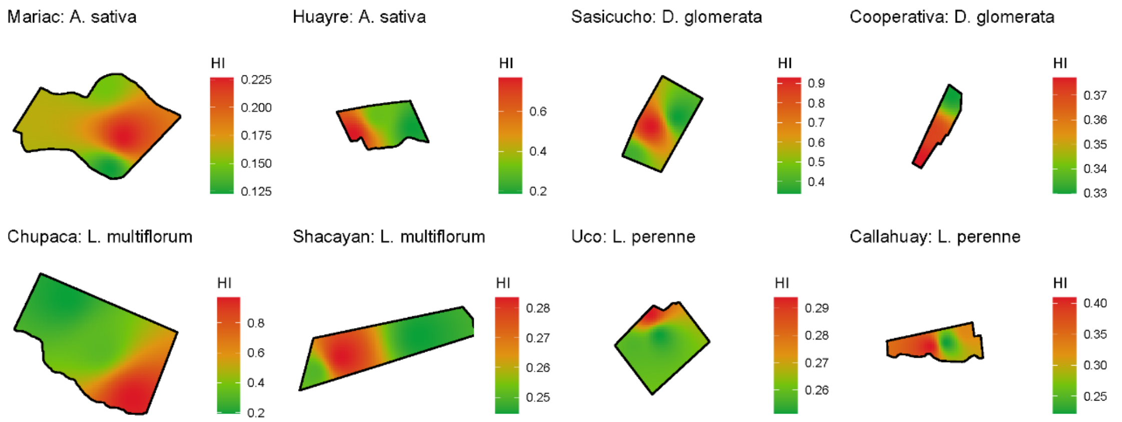

Figure 7. Spatial variability of the cumulative hazard index (HI) associated with trace metal intake in high-Andean pastures. Maps represent the interpolated distribution of HI across plots with different forage species, revealing spatial heterogeneity and localised zones of relatively greater risk.

The spatial interpolation maps generated for the HI enabled the identification of variability patterns within plots, including localised “hotspots” of relative risk. These patterns suggest that local environmental factors modulate the availability and accumulation of trace metals in forage, generating fine-scale heterogeneity that is not fully explained by forage species alone. However, given the exploratory nature of the inverse distance weighting (IDW) interpolation method, these results should be interpreted as an approximate visualisation of the spatial distribution of risk rather than as robust spatial inferences.

Despite these spatial variations, no statistically significant differences in HQ or HI values were detected among forage species ($p > 0.05$; Table S2). However, the observed trends suggest that certain species, such as *Dactylis glomerata*, may contribute more consistently to relatively higher exposure to trace metals.

Collectively, these findings indicate that, although the risk associated with trace metal intake in high-Andean grazing systems is low, spatial variability and interspecific differences among forage species should be considered in more detailed risk assessments, particularly under local conditions where environmental factors may favour metal accumulation.

Multivariate relationships among forage characteristics, trace metals, and environmental factors

Redundancy analysis (RDA) enabled the assessment of the combined relationships among nutritional composition, trace metal concentrations, forage species, and environmental factors. The first two axes explained a substantial proportion of the total variability (RDA1: 17.7%; RDA2: 11.4%), indicating moderate ecological structuring driven by environmental and biological gradients (Figure 8A). The ordination revealed clear differentiation among forage species. *Dactylis glomerata* was primarily associated with higher concentrations of Zn and Cu, as well as relatively elevated altitude values, suggesting a greater capacity to accumulate these elements under high-Andean conditions. In contrast, *Lolium multiflorum* showed a closer association with altitude but a weaker affinity for specific metals, indicating a more generalised response to this environmental gradient. Conversely, *Lolium perenne* was associated with comparatively lower metal concentrations and positioned at the opposite end of the gradient, reflecting reduced accumulation of these elements.

Production and nutritional variables exhibited distinct multivariate patterns. Dry matter yield and crude protein were positioned in the opposite direction to most trace metals, suggesting an inverse relationship between forage productivity/nutritional quality and metal accumulation. Among the evaluated elements, Mn and Cd displayed similar distribution patterns, indicating possible co-variation in their behaviour, whereas Cr exhibited a more independent distribution within the multivariate space.

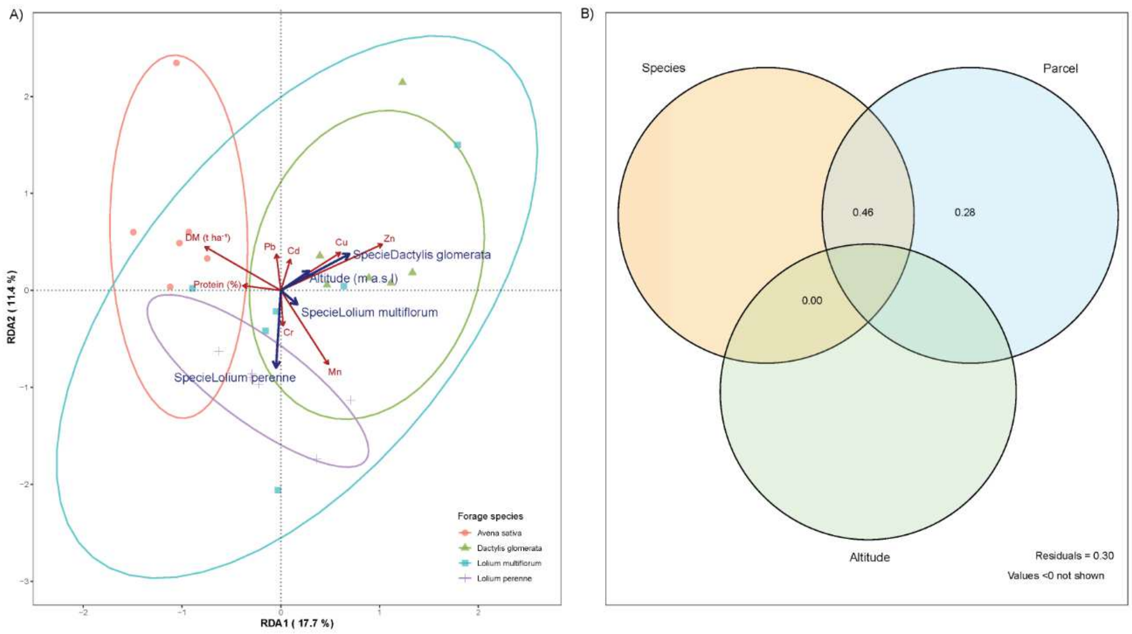

Figure 8. Redundancy analysis (RDA) and variance partitioning of nutritional variables, trace metals, and environmental factors in high-Andean forage species. (A) RDA ordination diagram showing the association among nutritional variables (dry matter and crude protein), trace metals (Zn, Cu, Mn, Cd, Pb, and Cr), altitude, and forage species. (B) Variance partitioning analysis showing the relative contribution of species, plot, and altitude to the observed variability, as well as their interaction.

Variance partitioning analysis (Figure 8B) revealed that the largest proportion of explained variability was associated with spatial factors (plot), followed by forage species identity, whereas altitude contributed less. However, a notable interaction between species and plot was observed, suggesting that species responses to trace metal accumulation are strongly dependent on spatial context. Collectively, the evaluated factors explained a moderate proportion of the total variability, leaving a residual component attributable to other unmeasured factors. These findings demonstrate that the distribution and accumulation of trace metals in high-Andean systems are governed not by a single driver, but by the interaction of multiple environmental and biological processes.

Collectively, these results suggest that trace metal accumulation in high-Andean ecosystems is driven by complex interactions among environmental factors, species-specific characteristics, and spatial heterogeneity, rather than by isolated individual effects.

DISCUSSIONS

The results of this study indicate that trace metal accumulation in forage species from high-Andean grasslands is influenced more strongly by local-scale environmental heterogeneity than by plant species identity. Although differences in productivity and nutritional composition were observed among species, no statistically significant differences were detected in trace metal concentrations or risk indices (HQ and HI), suggesting that edaphic and spatial factors play a dominant role in regulating the dynamics of these elements in high-mountain systems. Collectively, these findings support the notion that edaphic availability regulates the transfer of trace metals within the soil–plant–animal system, a pattern widely documented in studies addressing metal dynamics in soils and vegetation (Vega et al., 2010; Liu et al., 2022).

The marked variability observed in soil physicochemical properties, particularly pH, organic matter content, and nutrient availability, is consistent with previous reports for mountain ecosystems characterised by a high spatial heterogeneity (Kousar et al., 2025). Such heterogeneity directly influences the mobility and bioavailability of trace metals. For instance, soils with higher organic matter content tend to promote metal complexation and retention, whereas acidic conditions increase metal solubility and plant availability (Zhang et al., 2018; Chen et al., 2024). In this context, the high variability observed for elements such as Cd and Pb suggests the presence of microenvironments with contrasting soil conditions that regulate their dynamics at the local scale.

Despite this soil variability, the evaluated forage species showed no significant differences in trace metal accumulation, suggesting a relatively conservative response among grasses regarding the uptake of these elements. Nevertheless, the interspecific trends observed may be associated with differences in uptake, exclusion, and translocation mechanisms, as well as root system characteristics and physiological strategies for nutrient acquisition (Nascimento et al., 2014; Ferreira et al., 2022). Moreover, interactions with soil microorganisms may modulate metal availability and their incorporation into plant biomass (Jing et al., 2007; Ma et al., 2016), reinforcing the concept that trace metal dynamics are governed by complex soil–plant–microorganism processes.

Spatial analysis revealed a heterogeneous distribution of trace metals within plots, including localised accumulation zones (“hotspots”), particularly for Cd and Pb. Similar patterns have been widely reported in natural and agricultural soils, where fine-scale variability is influenced by factors such as soil texture, topography, and the distribution of organic matter (Duan et al., 2024; Solórzano et al., 2025; Xu et al., 2025). Although the analysis was conducted using an exploratory inverse distance weighting (IDW) interpolation approach, the results identified relevant spatial trends and reinforced the importance of accounting for intra-plot heterogeneity in trace metal assessments. In this context, the absence of significant relationships with the altitudinal gradient suggests that local-scale variability may overcome the influence of broader environmental gradients, as previously reported for mountain ecosystems (Naud et al., 2019; Jia et al., 2022).

From the perspective of animal exposure, estimated daily intake (EDI) values varied among livestock types, with sheep and alpacas exhibiting higher relative exposure levels than cattle. This pattern is explained by differences in the dry matter intake-to-body weight ratio, which increases the relative intake of trace metals in smaller animals (Van Soest, 1994; NRC, 2005). Despite these differences, HQ and HI values remained below critical thresholds, indicating that current exposure levels do not pose a significant risk to animal health, consistent with findings reported in other pastoral systems (Afzal and Mahreen, 2024).

However, the spatial variability observed in HI values suggests the presence of localised areas with relatively higher risk within plots. Although these patterns arise from an exploratory spatial analysis, several studies have emphasised the importance of incorporating spatial heterogeneity into environmental risk assessments (Wu et al., 2021; Shahzad et al., 2025). In this context, the occurrence of hotspots associated with Cd and Pb may have relevant implications under scenarios involving land-use change or shifts in environmental conditions.

From an ecological and productive perspective, these findings suggest that forage quality in high-Andean systems is closely linked to soil and spatial variability. This implies that grassland management should focus not only on forage species selection but also on managing soil physicochemical properties. Strategies aimed at enhancing organic matter content and regulating soil pH may help reduce trace metal bioavailability (Melo et al., 2022; Birghila et al., 2023). Furthermore, identifying areas with higher metal accumulation could facilitate the implementation of site-specific management practices, thereby optimising the sustainability of livestock production systems.

This study presents certain limitations that should be acknowledged. The sample size and spatial resolution constrain the robustness of the inferences, particularly within the context of exploratory spatial analysis. Furthermore, the assessment was restricted to a limited number of trace metals and forage species and did not account for temporal variability or the bioavailable fraction of the evaluated elements. Future research should therefore expand the number of sampling sites, incorporate seasonal evaluations, and consider additional biological processes, such as microbial dynamics, to achieve a more comprehensive understanding of trace metal dynamics in these systems (Newsome and Falagán, 2021; Nicolaidis Lindqvist et al., 2019).

Overall, the results demonstrate that trace metal accumulation in high-Andean systems arises from complex interactions among edaphic, spatial, and biological factors. Although the risk associated with the intake of these elements was low under the evaluated conditions, the pronounced heterogeneity of the system highlights the importance of accounting for local-scale variability when assessing forage quality and developing sustainable management strategies for high-mountain livestock systems.

CONCLUSIONS

The results of this study indicate that trace metal accumulation in forage species from high-Andean grasslands was primarily driven by local-scale soil heterogeneity and spatial variability rather than by plant species identity or the altitudinal gradient. Although differences in productivity and nutritional quality were observed among forage species, trace metal concentrations did not vary significantly, suggesting a relatively consistent plant response under comparable environmental conditions and reinforcing the predominant role of soil properties in regulating the bioavailability of these elements. Furthermore, the spatial distribution of trace metals revealed heterogeneous patterns and localised accumulation zones (“hotspots”), highlighting the need to incorporate fine-scale spatial variability into forage quality assessments. In this context, the absence of significant associations with altitude suggests that local environmental factors exert a stronger influence than broader altitudinal gradients on trace metal dynamics in these systems.

From the perspective of animal exposure, estimated daily intake (EDI) values and non-carcinogenic risk indices (HQ and HI) remained below critical thresholds under the evaluated conditions, indicating a low risk to grazing livestock. However, sheep and alpacas exhibited higher relative exposure than cattle due to their greater dry matter intake-to-body weight ratio, which proportionally increases trace metal intake. Moreover, the observed spatial variability suggests the potential presence of localised areas with relatively higher exposure, underscoring the importance of incorporating spatial approaches into risk assessment. Collectively, these findings demonstrate that the sustainable management of high-Andean grasslands must extend beyond forage species selection and integrate the management of soil properties and spatial heterogeneity. In particular, practices aimed at optimising organic matter content and regulating soil pH may help reduce trace metal bioavailability and improve forage quality. Finally, strengthening continuous monitoring and promoting studies that incorporate temporal variability, bioavailable metal fractions, and higher spatial resolution will be essential for improving risk assessment and enhancing the sustainability of high-mountain livestock systems.

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AUTHOR CONTRIBUTION

A.A-A.: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – Original Draft. M.L-R.: Methodology, Investigation, Validation, Writing – Original Draft. J.C-L.: Supervision, Funding acquisition, Validation, Writing – Review & Editing. J.C.: Formal analysis, Data curation, Validation, Writing – Review & Editing. H.R-A.: Formal analysis, Data curation, Validation, Writing – Review & Editing. R.S-A.: Supervision, Funding acquisition, Validation, Writing – Review & Editing.

All authors read, reviewed, and approved the final manuscript.

DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

DECLARATIONS

All authors have read, understood, and have complied as applicable with the statement on “Ethical responsibilities of Authors” as found in the Instructions for Authors.

COMPETING INTERESTS

The authors declare no competing interests.

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