Sheep's wool as a bioindicator of heavy metal pollution: Assessing environmental contamination near former mining sites in Romania

[1]Florin I. Fechete, [2]Maria Popescu, [1]Maria C. Lăcătuș, [1]Radu Lăcătuș, [1]Mariana Tataru, [3,4]Rodica I. Matei, [1]Marian S. Mârza, [1]Andrei R. Codea, [1]Iliuță O. Mărcuș, [5,6]Florin D. Bora

[1] Clinical Sciences Department, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania; [2] Equine Clinic, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania; [3] Plastic Surgery Department, University of Medicine and Pharmacy, Cluj-Napoca, Romania; [4] Plastic Surgery Clinic, Spitalul Clinic de Recuperare, Cluj-Napoca, Romania; [5] Viticulture and Oenology Department, Advanced Horticultural Research Institute of Transylvania, Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania; [6] Laboratory of Chromatography, Advanced Horticultural Research Institute of Transylvania, Faculty of Horticulture and Business for Rural Development, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania. Corresponding author: F. D. Bora, boraflorindumitru@gmail.com

Abstract. This study investigates the accumulation of heavy metals, specifically copper (Cu) and zinc (Zn), in sheep's wool from regions near former mining and industrial sites in Romania, including Ferneziu, Firiza, and Tîrlișua. The research aimed to assess the impact of proximity to pollution sources on heavy metal concentrations, using sheep's wool as a bioindicator of environmental contamination. Samples were collected from areas with varying distances from known pollution sources, such as the Herja mine, and analyzed for metal content. The results revealed significantly higher levels of Cu and Zn in sheep's wool from Ferneziu, which is closest to the former mining site, compared to more distant areas like Firiza and Tîrlișua. The findings highlight the strong correlation between industrial activities and the environmental accumulation of heavy metals, posing potential risks to ecosystems, livestock, and human health. The study underscores the importance of ongoing environmental monitoring and suggests the need for regulatory measures to manage heavy metal pollution in agricultural regions. By employing sheep's wool as a cost-effective and reliable bioindicator, this research contributes to a better understanding of the environmental impacts of industrial pollution and provides a basis for future mitigation efforts.

Key Words: agricultural ecosystems, copper and zinc levels, environmental monitoring, industrial impact, mining pollution.

Introduction. Contaminants encompass a wide range of organic and inorganic substances, each with its unique characteristics that influence the appropriate disposal methods. Sorption techniques have emerged as promising solutions for removing contaminants from the environment (Lakherwal et al 2016). Key factors influencing the selection of a sorbent include its availability, efficiency, cost, complexity of production, and ease of application (Nacke et al 2016). In recent years, there has been a growing interest in natural, bio-based sorbents, particularly those derived from waste materials. This approach aligns with the principles of sustainability and resource conservation. While mineral-based sorbents have been traditionally explored, research efforts have increasingly focused on utilizing plant and animal-derived materials as effective sorbents (Nemeș & Bulgariu 2016).

Beyond traditional mineral-based sorbents, research has increasingly explored the potential of plant and animal-derived materials as sustainable and effective alternatives for contaminant removal. These bio-based sorbents offer several advantages, including their abundance, renewability, and potential for reducing the environmental impact of remediation processes (Ghosh & Collie 2014). Through modifications such as activation or functionalization, bio-based sorbents can be tailored to enhance their adsorption capacity and selectivity for specific contaminants. Moreover, they often exhibit lower production costs compared to mineral-based sorbents, making them more economically viable for large-scale applications. A diverse range of plant and animal-derived materials have been investigated as potential sorbents, including agricultural waste (e.g., rice husks, wheat bran, sugarcane bagasse, corn cobs), forestry by-products (e.g., sawdust, wood chips, pine needles), aquatic organisms (e.g., algae, seaweed, aquatic plants), and animal waste (e.g., eggshells, crab shells, chitosan). These materials have demonstrated promising results in removing various contaminants from water and soil, including heavy metals, organic pollutants, and dyes.

Sheep wool, a keratin-based animal fiber, possesses inherent sorption properties. Numerous studies have explored modifications to enhance these properties, employing physical, chemical, and combined approaches. Chemical modification introduces new functional groups, altering the wool's chemical structure and increasing its binding capacity. While chemical treatments can have environmental implications due to chemical usage and wastewater generation, physical-chemical methods, such as plasma treatment, corona charging, or microwave radiation, are generally considered more environmentally friendly as they primarily modify the wool's surface (Hanzlíková et al 2018).

Sheep fleece, a keratin-based animal fiber, serves as a chemical indicator reflecting the quality of both feed and nutrition, as well as environmental factors. While various factors, including breed, sex, age, physiological state, and health conditions (Ramírez-Pérez et al 2000), can influence the chemical composition of wool, it is evident that the concentration of elements within the fleece provides valuable insights. Sheep and goats exhibiting fleece-eating behavior (Shimao Zheng) were found to have elevated levels of Ca, P, Fe, Mn, Zn, Cu, Co, Se, and F in their wool, while concentrations of S and Mo were lower (Huang & Chen 2001). Gabryszuk et al (2000) identified significant differences in Ca, Mg, K, Zn, and Fe concentrations between Booroola and Polish Merino sheep wool. Additionally, Merino sheep exhibited notable variations in Ca, Mg, and Zn content during the perinatal period, resting period, and tupping season.

Sheep wool is increasingly recognized as a valuable biomonitor for assessing environmental contamination, particularly for heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg). Wool, a keratin-based fiber, accumulates these metals through both dietary intake and direct exposure to environmental pollutants. Metals from contaminated soil and feed are ingested by sheep and incorporated into wool, while airborne pollutants settle on the wool's surface, especially in industrial areas. Wool’s continuous growth allows it to record both current and historical exposure to pollutants, making it an effective medium for long-term contamination detection. Advanced analytical techniques, such as inductively coupled plasma mass spectrometry (ICP-MS) and atomic absorption spectroscopy (AAS), are employed to measure metal concentrations in wool, revealing significant variations based on geographical location, environmental conditions, and temporal factors. Comparative studies have shown elevated metal levels in wool from industrial regions compared to less polluted areas, highlighting wool's effectiveness in mirroring environmental contamination levels and providing insights into pollution sources and trends. This makes sheep wool a non-invasive, cost-effective tool for environmental monitoring and public health assessments (Markert & Weckert 1996; D’Mello 2003; Tolosana-Delgado et al 2016; Falandysz & Brzostowski 2017).

In a seminal study by D’Mello (2003), the potential of sheep wool as an environmental indicator was thoroughly explored, demonstrating its efficacy in detecting heavy metal contamination in various industrial settings. This research provided compelling evidence that wool samples collected from regions with intense industrial activities, such as smelting and mining, contained significantly elevated levels of heavy metals compared to those from less polluted environments. The study attributed this heightened accumulation to two primary mechanisms: the deposition of airborne pollutants directly onto the wool and the ingestion of contaminants through the sheep's diet. D'Mello's analysis revealed that wool not only reflects current pollution levels, but also serves as a historical record of environmental exposure due to its continuous growth. This property enables the wool to capture and archive data on both past and present contamination, thereby offering a comprehensive view of pollution trends over time. The study underscored the importance of utilizing wool as a non-invasive biomonitoring tool, which provides valuable insights into environmental health and pollution dynamics, making it an effective medium for assessing and tracking environmental contamination in various settings.

Expanding on D'Mello's foundational work, Tolosana-Delgado et al (2016) further refined the use of sheep wool as a biomonitor by employing advanced analytical techniques, specifically inductively coupled plasma mass spectrometry (ICP-MS), to assess metal concentrations in wool from various agro-ecological zones. Their research provided a detailed analysis of metals such as chromium (Cr), nickel (Ni), and zinc (Zn), revealing significant correlations between these metal levels and the intensity of local industrial activities. Tolosana-Delgado et al (2016) found that regions with higher industrial outputs generally had elevated metal concentrations in wool, underscoring the utility of wool in tracking environmental pollution. Additionally, the study identified variations in metal accumulation that were influenced by biological factors, including the breed and age of the sheep. For instance, different sheep breeds exhibited varying capacities for metal uptake and accumulation, while younger or older sheep showed distinct patterns in metal concentration due to differences in wool growth rates and metabolic processes. This variability highlights the necessity for establishing region-specific baseline data to accurately interpret the results of wool analyses. By considering these biological factors alongside environmental conditions, researchers can enhance the precision and relevance of wool-based assessments of heavy metal contamination.

These studies collectively underscore the value of sheep wool as a non-invasive and cost-effective tool for environmental monitoring. The use of wool sampling offers several key advantages in assessing metal pollution. By analyzing wool from different regions, researchers can gain detailed insights into the extent and distribution of heavy metal contamination across various environments. This approach not only identifies current pollution levels, but also tracks temporal changes in contamination, providing a historical perspective on environmental pollution trends.

The ability to monitor and evaluate the accumulation of metals such as Pb, Cd, and As in wool makes it an invaluable resource for understanding the impact of industrial activities on the environment. This information is crucial for developing and refining environmental policies aimed at reducing pollution and mitigating its effects. Furthermore, by providing a clear picture of contamination levels and changes over time, wool analysis aids in assessing the effectiveness of existing pollution control measures and guiding future strategies.

The implications of these findings extend to public health, as understanding heavy metal exposure through wool can inform efforts to protect communities from potential health risks. Elevated metal concentrations in wool can signal areas of concern, prompting further investigation and intervention to prevent adverse health outcomes associated with heavy metal exposure. Overall, the integration of wool analysis into environmental monitoring programs represents a practical and impactful method for enhancing environmental and public health protection.

The primary goal of this research is to assess the impact of industrial and mining activities on the accumulation of heavy metals in sheep's wool, with a particular focus on regions located near former mining sites. By using sheep's wool as a bioindicator, the study aims to evaluate environmental contamination levels, specifically concentrations of metals such as copper, zinc, and lead. This research seeks to provide a deeper understanding of how proximity to pollution sources influences metal accumulation, with the broader objective of informing environmental monitoring efforts and potential regulatory measures to mitigate the effects of heavy metal pollution on ecosystems, livestock, and human health.

Material and Method

Description of the study site. A total of 144 sheep wool samples were collected from 36 predetermined locations across four areas: Baia Mare (Ferneziu I, 8.0-11.5 km from the former Herja Mine; Ferneziu II, 5.5-7.5 km from the former Herja Mine; Firiza, 16.5-17 km from the Aurul settling pond in Tăuţii de Sus), and Tîrlişua (serving as the control site for establishing baseline contaminant levels). Three replicate samples were taken from each location. To establish a reliable baseline for comparison and assess potential pollution, Tîrlişua was selected as an additional control area, as it is devoid of known heavy metal sources and has not been the subject of previous related studies. The study was conducted between May and June, 2024. The spatial distribution of sampling points is detailed in Figure 1.

To isolate the impact of land use and grazing systems on mineral profiles, the study focused on farms situated in regions with similar soil and climatic conditions. Sampling of grazing areas took place between May and June 2024, aligning with the typical spring season of the western Mediterranean, marked by mild temperatures, increasing daylight, and moderate precipitation. The soils in Ferneziu and Firiza (Baia Mare, Romania) exhibit diverse pedological features. In the northern and northeastern parts, regosols, eutricambosols, districambosols, and andosols have developed on volcanic deposits, while the southern region predominantly features aluviosols, luvisols, and stagnosols. In contrast, the soil types in Tîrlişua (Bistriţa-Năsăud, Romania), located within the upper Ilişua Valley watershed, have been shaped by a predominantly forested environment. These soils, characterized by active humification, are mainly brown, acidic brown, brown podzolic, and podzolic, reflecting the bioaccumulation processes specific to this region.

Experimental design. A systematic wool sampling protocol was implemented across 36 predetermined locations in four distinct regions, with the aim of assessing mineral profiles and potential environmental contamination. Wool samples were collected from the shoulder area of three sheep per location, using sterilized stainless-steel scissors to obtain 10-20 g of wool. The shoulder was chosen due to its uniform wool growth. Samples were placed in pre-labeled sterile sampling bags, with detailed information including location ID, GPS coordinates, date, and sheep ID recorded on corresponding data sheets. Scissors were sterilized between samples using 70% ethanol to prevent cross-contamination. Environmental conditions and any observations related to sheep health were documented. Samples were stored in a cool, dry environment, with refrigeration utilized if immediate analysis was delayed. The protocol was designed to minimize stress on the sheep and maintain the integrity of the wool samples, ensuring that they were suitable for accurate and reliable laboratory analysis.

Sheep wool samples, accurately weighed at approximately 0.5 g, are prepared for analysis by being placed in Teflon digestion vessels that have been thoroughly cleaned with ultrapure nitric acid (HNO_3, 65%). To each sample, 8 mL of HNO_3 and 2 mL of hydrogen peroxide (H_2O_2, 30%) are added to facilitate complete digestion.

The vessels are sealed and subjected to microwave digestion, following a program of ramping to 120°C in 5 minutes, holding for 10 minutes, followed by a further increase to 180°C over 10 minutes, and a final hold at 180°C for 30 minutes. After cooling, the digested solutions are transferred to volumetric flasks, diluted with deionized water, and stored in labeled containers for analysis. Quality control is maintained by including blank samples and certified reference materials (CRMs) to ensure accuracy and prevent contamination.

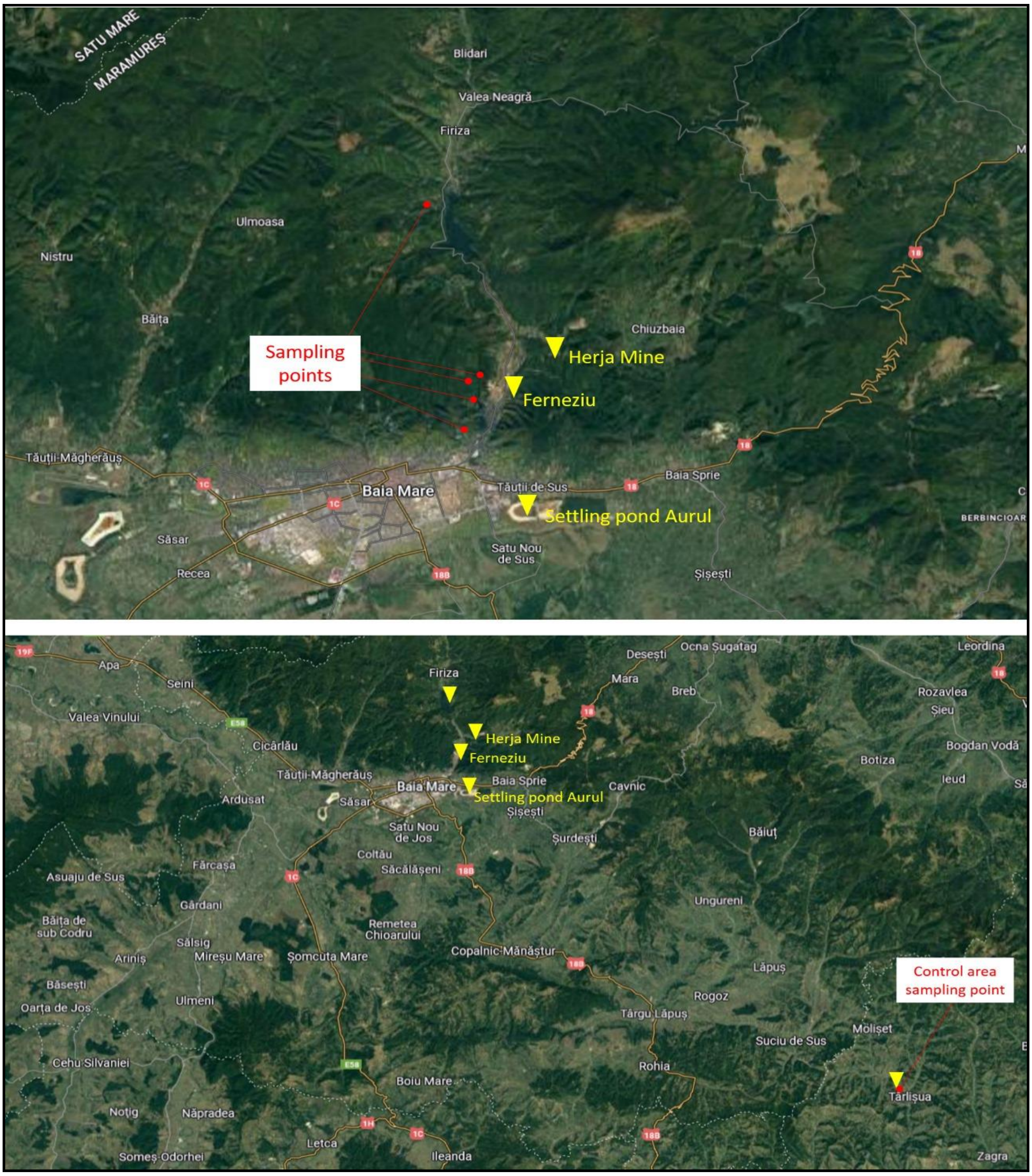

Figure 1. Geographical origins of sheep's wool samples.

General ICP-MS instrumental parameters of analysis. Concentrations of micronutrients (copper-64, zinc-65), ultratrace elements (chromium-52, cobalt-59, nickel-60), and heavy metals (arsenic-75, cadmium-111, mercury-201, lead-208) were quantified using inductively coupled plasma mass spectrometry (ICP-MS). An iCAP Q ICP-MS instrument (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an ASX-520 autosampler, a micro-concentric nebulizer, Ni sampler and skimmer cones, and a peristaltic sample delivery pump was employed for the analysis. Samples were introduced into the ICP-MS plasma using a nebulizer connected to a cyclonic spray chamber. A standard ICP-MS torch with a 1.5 mm diameter injector was utilized. To ensure accurate and reliable results, the ICP-MS underwent a stabilization period of at least 45 minutes after startup. During this time, experimental conditions were verified, and mass calibration was performed. A short-term stability test was conducted using a tuning standard solution containing Ba, Bi, Ce, Co, In, Li, and U (each at 1.0 μg L^{-1}) in a

2% HNO_3 + 0.5% HCl matrix. This auto-tuning process optimized the plasma sampling zone for high sensitivity, optimized ion optics voltages, and minimized the formation of cluster and doubly charged ions. Daily optimization was performed to maintain maximum sensitivity for M+ ions. The formation of double ionization and oxides was monitored using the Ba^{2+}/Ba^{+} and Ce^{2+}/CeO^{+} ratios, respectively, ensuring they remained below 2%. Argon (Ar 5.0) and helium (He 6.0) carrier gases with a purity of 99.99% (Messer, Austria) were used. All samples were analyzed in triplicate, with each analysis consisting of seven replicates.

Reagents and equipment. All reagents, including ultrapure nitric acid (HNO_3, 65%), ultrapure hydrogen peroxide (H_2O_2), and deionized water from a Milli-Q Integral Ultrapure Water System, were of high purity and sourced from Merck or Sigma-Aldrich (Darmstadt, Germany). Teflon digestion vessels, constructed from modified polytetrafluoroethylene (TFM-PTEE) and cleaned with HNO_3 prior to each use, were utilized for the triplicate mineralization of samples such as soil, grass, and dairy products, with up to six vessels per run. Flasks were pre-treated with 5M HNO_3 and thoroughly rinsed with deionized water. A high-precision KERN ADB 100-4 balance was employed for sample weighing and solution preparation.

Quality control of the chemical analyses. Following the guidelines of Commission Regulation (EU) No. 2016/582, limits of detection (LoDs) and quantification (LoQs) for the analyzed elements were determined using the standard deviation (σ) of 20 blank solution measurements. LoD was set at 3σ and LoQ at 10σ. To ensure analytical accuracy, a multi-element internal standard solution containing indium, scandium, and praseodymium at 10 ng mL^{-1} was used. Repeatability was evaluated with the Horwitz ratio (HorRat), with all values confirmed to be below 2. Calibration standards were prepared at five concentrations (2.5, 5, 10, 25, and 50 μL). Precision and accuracy were assessed through spiking experiments, with precision expressed as the percent relative standard deviation (RSD%) of triplicate analyses.

Statistical analysis. Data acquisition and descriptive statistics (mean, median, SD) were performed with Microsoft Excel 365 and Addinsoft version 15.5.03.3707. Precision was reported as SD. Statistical analysis was conducted using IBM SPSS Statistics version 24, with replicate measurements (n=3) averaged. Two-way ANOVA assessed variable impacts on heavy metal concentrations in sheep's wool, followed by Duncan's multiple range test, with significance set at $\alpha \leq 0.005$.

Results and Discussion. The analysis of heavy metal concentrations in sheep's wool from regions near former mining sites, such as Ferneziu and Firiza, provides a crucial window into the environmental health of these areas, illustrating how industrial activities have lasting effects on local ecosystems. Sheep's wool, a readily accessible and non-invasive biological sample, serves as a practical bioindicator for assessing the presence and concentration of environmental pollutants, particularly heavy metals. Wool has a high affinity for metal ions, allowing it to accumulate and reflect the chemical profile of the surrounding environment. In this study, the analysis of metals like Cu and Zn in sheep's wool reveals distinct patterns of contamination that can be directly linked to the sheep's proximity to industrial pollution sources (Table 1).

Table 1

Concentration of heavy metals in sheep's wool from Ferneziu, Firiza (Maramureș region), and Tîrlișua (Bistrița-Năsăud), Romania (mg/kg dry weight)

Areas sample code year of harvest	*Distance from source of pollution (~)*	*Sampling depth (surface)*	*Cu M.P.L.**	*Zn M.P.L.**	*Pb M.P.L.**	*As M.P.L.**	*Cr M.P.L.**
Serum sheep's samples exposed to anthropogenic sources of heavy metals pollution							
Ferneziu	Near (10-12 km) the Herja Mine from Ferneziu						
The sheepfold was located approximately 8 km from the former Herja mine in Ferneziu							
W_1-2024 2024			2.99±2.99	35.95±5.13	0.10±0.08	BLD	BLD
W_2-2024 2024			0.96±0.34	30.66±15.17	0.12±0.02	BLD	BLD
W_3-2024 2024			0.98±0.30	20.20±3.49	BLD	BLD	BLD
W_4-2024 2024			3.36±0.33	40.63±15.60	BLD	BLD	BLD
W_5-2024 2024			4.37±1.61	64.09±16.93	BLD	BLD	BLD
The sheepfold was located approximately 11.5 km from the former Herja mine in Ferneziu							
$W_{6\text{-}2024}$ 2024			1.96±0.79	16.31±5.12	0.12±0.02	BLD	BLD
$W_{7\text{-}2024}$ 2024			3.40±0.59	53.96±30.90	0.13±0.03	BLD	BLD
$W_{8\text{-}2024}$ 2024			0.32±0.21	72.55±5.94	0.13±0.02	BLD	BLD
$W_{9\text{-}2024}$ 2024			1.27±0.72	16.45±5.06	0.15±0.05	BLD	BLD
$W_{10\text{-}2024}$ 2024			3.59±3.38	56.63±23.88	0.14±0.02	BLD	BLD
$W_{11\text{-}2024}$ 2024			4.28±0.65	14.45±4.51	0.13±0.02	BLD	BLD
$W_{12\text{-}2024}$			2.12±0.52	47.86±7.56	0.12±0.02	BLD	BLD

2024					
Ferneziu	Near (6-7 km) the Herja Mine from Ferneziu				
The sheepfold was located approximately 5.5 km from the former Herja mine in Ferneziu					
$W_{13-2024}$ 2024	1.01±0.39	26.09±17.25	BLD	BLD	BLD
$W_{14-2024}$ 2024	0.57±0.32	36.18±12.31	BLD	BLD	BLD
$W_{15-2024}$ 2024	2.46±0.85	17.89±6.07	0.13±0.02	BLD	BLD
$W_{16-2024}$ 2024	2.65±1.72	29.95±5.25	0.12±0.01	BLD	BLD
$W_{17-2024}$ 2024	0.24±0.13	47.56±6.06	BLD	BLD	BLD
$W_{18-2024}$ 2024	1.08±0.46	40.83±8.12	BLD	BLD	BLD
The sheepfold was located approximately 7.5 km from the former Herja mine in Ferneziu					
$W_{19-2024}$ 2024	0.78±0.70	26.90±17.28	0.12±0.02	BLD	BLD
$W_{20-2024}$ 2024	3.22±0.06	115.69±14.87	BLD	BLD	BLD
$W_{21-2024}$ 2024	1.02±0.40	73.25±15.52	BLD	BLD	BLD
$W_{22-2024}$ 2024	0.32±0.21	70.39±20.21	BLD	BLD	BLD
$W_{23-2024}$ 2024	2.60±1.43	63.73±9.91	BLD	BLD	BLD
$W_{24-2024}$ 2024	1.24±0.67	51.12±12.51	BLD	BLD	BLD
Firiza	Near (17 km) the settling pond mining (decant pond) Aurul from Tăuții de Sus				
The sheepfold was located approximately 16.5 km from the settling pond mining (decant pond) Aurul from Tăuții de Sus					
$W_{25-2024}$ 2024	0.80±0.40	39.87±24.97	0.13±0.02	BLD	BLD
$W_{26-2024}$ 2024	1.53±0.63	68.72±4.73	0.01±0.01	BLD	BLD
$W_{27-2024}$ 2024	1.69±1.40	54.86±16.99	0.13±0.02	BLD	BLD
$W_{28-2024}$	0.41±0.38	84.02±5.87	BLD	BLD	BLD

2024						
$W_{29-2024}$ 2024		1.32±0.78	34.66±12.16	0.11±0.01	BLD	BLD
$W_{30-2024}$ 2024		1.31±0.21	52.63±6.61	BLD	BLD	BLD
$W_{31-2024}$ 2024		0.42±0.15	76.53±10.63	BLD	BLD	BLD
$W_{32-2024}$ 2024		1.59±0.92	61.83±5.52	BLD	BLD	BLD
The sheepfold was located approximately 17 km from the settling pond mining (decant pond) Aurul from Tăuții de Sus						
$W_{33-2024}$ 2024		0.56±0.14	80.53±21.67	BLD	BLD	BLD
$W_{34-2024}$ 2024		0.67±0.19	41.99±16.38	BLD	BLD	BLD
$W_{35-2024}$ 2024		1.19±1.01	13.98±1.69	BLD	BLD	BLD
$W_{36-2024}$ 2024		0.33±0.21	36.17±12.34	BLD	BLD	BLD
Background areas						
Tîrlișua						
$W_{37-2024}$ 2024		1.14±0.21	53.89±7.07	0.10±0.01	BLD	BLD
	Sig.	***	***	***	-	-
Sheep's wool samples exposed to anthropogenic sources of heavy metals pollution						
Background sheep's wool						
Martínez-Morcillo et al (2024) (mg kg^{-1})		2.72-13.0	81-178	60-1450 (µg kg^{-1})	60-450 (µg kg^{-1})	-
Patkowska-Sokoła et al (2009) (mg kg^{-1})		5.30-10.30	73.62-88.80	-	-	-

Note: average value ± standard deviation (n=3); DW - dry weight; M.P.L. - maximum permissible limit; currently, there are no national or international regulations governing the concentration of heavy metals in sheep's wool; BLD - below the detection limit (LoQ): LoQ for Pb: 0.231 µg L^{-1}.

The proximity of sheepfolds to pollution sources, particularly former mining operations such as the Herja mine in Ferneziu, plays a critical role in the levels of heavy metals detected in wool samples. Mining activities, both historical and contemporary, are known to release large quantities of metals into the environment through the extraction, processing, and disposal of ores. Over time, these metals can persist in the soil, water, and air, leading to long-term contamination that can affect local flora and fauna.

The data clearly indicate that sheep reared closer to these pollution sources exhibit higher concentrations of Cu and Zn in their wool compared to those grazing further away. For instance, wool samples collected from Ferneziu, located just 5–12 km from the former Herja mine, contained significantly elevated levels of Cu, with values ranging from 0.32 to 4.37 mg kg^{-1}. Zn concentrations were even higher, reaching up to 115.69 mg kg^{-1} in some samples. This pattern suggests that the sheep are exposed to high levels of these metals through direct environmental contact, including grazing on contaminated soil, ingestion of metal-laden plants, and drinking from polluted water sources. Furthermore, atmospheric deposition from mining operations or wind-borne dust could contribute to the accumulation of these metals in the sheep's environment, thereby increasing their exposure (Table 1).

The significant variation in metal concentrations across different wool samples within the same geographic region also highlights the heterogeneous nature of contamination. Factors such as the specific location of grazing areas, topography, and hydrological patterns can influence the distribution and deposition of metals. For example, lower-lying areas or those near water bodies may experience greater metal runoff and accumulation, leading to higher contamination levels. This variability underscores the complex dynamics of metal pollution in mining regions and the importance of spatially detailed environmental monitoring to capture the full extent of contamination.

Cu and Zn are essential trace elements for both animals and plants, playing vital roles in various physiological processes, including enzyme function and protein synthesis. However, elevated levels of these metals can be toxic, particularly in environments where long-term exposure occurs. In the context of environmental health, the presence of high concentrations of Cu and Zn in sheep's wool serves as an indicator of anthropogenic contamination. These metals are commonly associated with mining activities, as Cu ores often contain Zn and other heavy metals that are released during extraction and processing.

The elevated Cu levels observed in Ferneziu sheep wool are concerning because Cu toxicity can have detrimental effects on livestock. Chronic exposure to high levels of Cu can lead to Cu poisoning, which affects the liver and other organs. Sheep are particularly susceptible to Cu accumulation due to their unique metabolism, which allows them to store large amounts of Cu in their liver. If Cu levels exceed the sheep's ability to detoxify and excrete the metal, it can lead to liver damage, jaundice, and in severe cases, death. The high variability in Cu concentrations in Ferneziu wool samples suggests that some sheep may be at greater risk of Cu toxicity than others, depending on their specific grazing locations and environmental conditions.

Similarly, Zn, while essential in small amounts, can become toxic at elevated concentrations. High levels of Zn can interfere with the absorption of other essential minerals such as Fe and Cu, leading to deficiencies and health problems in livestock. The remarkably high Zn concentrations found in some Ferneziu wool samples (up to 115.69 mg kg^{-1}) indicate significant environmental contamination, likely stemming from the historic mining operations. Zinc contamination in the environment can persist for decades, as the metal is relatively stable and does not easily degrade. This poses long-term risks to the health of grazing animals and the broader ecosystem.

The accumulation of heavy metals in sheep’s wool not only reflects the exposure of livestock to contaminated environments but also signals broader risks to the entire ecosystem. Heavy metals, once introduced into the environment, can have cascading effects through the food chain. Plants absorb metals from contaminated soils, and animals that graze on these plants, such as sheep, further concentrate the metals in their tissues. Predators and humans who consume contaminated animal products can also be affected, leading to biomagnification, where metal concentrations increase at higher trophic levels.

In areas near mining sites, such as Ferneziu and Firiza, the long-term exposure of vegetation and soil to metal pollution can lead to the degradation of local biodiversity. Plants that are sensitive to heavy metal toxicity may decline in abundance, leading to shifts in species composition and ecosystem function. Additionally, soil microorganisms that play critical roles in nutrient cycling and soil health may be disrupted by the presence of toxic metals, further compromising the resilience of the ecosystem.

For sheep and other livestock, chronic exposure to contaminated environments can result in subclinical health effects that may not be immediately apparent, but can reduce overall vitality, productivity, and reproductive success. For instance, the presence of high levels of Cu and Zn in their wool suggests that these animals are regularly exposed to environments that exceed their physiological thresholds for metal detoxification. Over time, this could lead to cumulative health impacts, including weakened immune systems, reduced growth rates, and lower reproductive performance.

The findings from this study also have important implications for human health, particularly in agricultural communities, where livestock plays a key role in food production. Sheep are commonly raised for both wool and meat, and contamination of the wool can be an indicator of potential contamination in other tissues, such as muscle and fat, which are consumed by humans. If heavy metals are accumulating in wool, it is likely that these metals are also present in other parts of the animal, raising concerns about food safety and the potential for heavy metal exposure through the consumption of contaminated meat.

In addition to direct consumption, heavy metals can also enter the human food chain through secondary routes, such as contaminated milk from dairy sheep or goats grazing in polluted areas. Heavy metal contamination in dairy products can pose significant risks to human health, particularly for vulnerable populations such as children, pregnant women, and the elderly, who are more susceptible to the toxic effects of metals like copper and lead.

Given these risks, the use of sheep's wool as a bioindicator provides a valuable early warning system for environmental and food safety concerns. By monitoring heavy metal levels in wool, it is possible to detect contamination before it becomes widespread in the food supply, allowing for timely interventions to protect both livestock and human health. However, the absence of regulatory guidelines for heavy metal concentrations in wool, as noted earlier, highlights a critical gap in environmental and public health policy. The establishment of standards for acceptable levels of heavy metals in wool and other livestock products could help safeguard against the long-term impacts of industrial pollution on agricultural systems and human health.

In the Ferneziu region, which is located near the former Herja mine, Cu concentrations in sheep's wool ranged from 0.32 to 4.37 mg kg^{-1}, with notable variability across samples. These values reflect the influence of the mine's legacy on the local environment, as Cu is commonly associated with mining activities due to its use in industrial processes and its presence in mineral deposits. The highest Cu concentrations were observed in samples closest to the mine, indicating that the source of pollution is likely contributing directly to the contamination of the surrounding ecosystem. Elevated Cu levels in sheep's wool suggest that the animals are exposed to higher-than-normal concentrations of this metal through ingestion of contaminated feed, water, or soil, as well as through direct contact with polluted environments. Zn concentrations in Ferneziu were even more pronounced, with some samples reaching up to 115.69 mg kg^{-1}, far exceeding background levels typically found in uncontaminated regions. Zn, like Cu, is often associated with industrial activities, particularly metal smelting and mining, which can release large amounts of Zn into the air, water, and soil. In sheep's wool, the high Zn concentrations reflect significant environmental exposure. This metal is essential in small amounts for biological functions, but at elevated levels, it can have toxic effects on both livestock and wildlife. The broad range of Zn concentrations (from 16.31 to 115.69 mg kg^{-1}) observed across different samples may be due to variability in the sheep's grazing patterns, their proximity to contaminated water sources, and the distribution of pollutants in the soil. While Cu and Zn were the most prevalent contaminants detected, Pb was found in trace amounts in a few samples, but generally at low concentrations (ranging from 0.10 to 0.15 mg kg^{-1}). Pb is a well-known toxic metal, often released into the environment through mining, industrial processes, and the use of leaded gasoline in the past. Although its concentrations in these samples are not alarmingly high, the presence of Pb in even small amounts raises concerns about long-term exposure, especially in areas with a history of mining. Pb bioaccumulates in organisms, meaning that chronic exposure, even at low levels, can lead to adverse health effects, particularly in animals like sheep that graze in contaminated areas over long periods (Table 1).

Interestingly, As and Cr were below detectable levels (BLD) in nearly all samples, which suggests that these metals are not currently significant contaminants in the study regions. This could be due to the specific geochemical characteristics of the Herja mine area, where Cu and Zn may dominate the metal pollution profile. Alternatively, it is possible that historical pollution from As and Cr has decreased over time due to remediation efforts or natural attenuation processes, although this requires further investigation (Table 1).

The Firiza region, located further from the former Herja mine and other pollution sources, still exhibited considerable levels of Zn contamination, with concentrations reaching up to 84.02 mg kg^{-1} in some samples. However, Cu concentrations in Firiza were generally lower than those observed in Ferneziu, with a maximum value of 1.69 mg kg^{-1}. This suggests that, while Zn contamination may be more pervasive, affecting areas even at a distance from direct pollution sources, Cu contamination is more localized. The distribution of Zn in this region could be attributed to atmospheric deposition of Zn particulates from industrial sources, as well as the natural distribution of Zn in the soil and water systems.

Despite being further from direct pollution sources, Firiza's sheep wool still showed significant heavy metal accumulation, underscoring the far-reaching impacts of mining activities and industrial pollution. Given the presence of Zn and Cu at greater distances from the mine, it is possible that local hydrology, prevailing wind patterns, and soil composition are contributing to the dispersal of these metals over larger areas than initially expected. This finding is particularly concerning as it suggests that livestock and, by extension, human populations in surrounding areas may be exposed to these contaminants through indirect means, such as through contaminated food chains or water supplies. In contrast, sheep’s wool samples from the Tîrlișua region, which was used as a background site due to its distance from industrial pollution, showed markedly lower levels of heavy metals. Cu concentrations in Tîrlișua were generally low, with values of around 1.14 mg kg^{-1}, and Zn levels were moderate at approximately 53.89 mg kg^{-1}. These results are consistent with the expected background levels of heavy metals in an area not heavily impacted by industrial activities. The comparison with polluted sites like Ferneziu and Firiza highlights the extent to which anthropogenic activities can elevate metal concentrations in the environment. The relatively clean profile of Tîrlișua serves as a baseline for assessing the degree of contamination in regions closer to mining operations, reinforcing the role of industrial pollution in influencing the heavy metal content in sheep’s wool (Table 1).

The findings from this study align with previous research on heavy metal contamination in sheep’s wool. For instance, Martínez-Morcillo et al (2024) reported similar ranges for Cu and Zn in sheep wool from other industrially impacted areas, with Cu levels ranging from 2.72 to 13.0 mg kg^{-1} and Zn concentrations between 81 and 178 mg kg^{-1}. These results suggest that the levels observed in Ferneziu and Firiza are consistent with known industrial contamination patterns. Likewise, Patkowska-Sokoła et al (2009) documented Cu concentrations of 5.3 to 10.3 mg kg^{-1} and Zn levels of 73.62 to 88.80 mg kg^{-1} in sheep wool from other regions, further supporting the conclusion that the metal levels observed in this study are influenced by anthropogenic activities. The wide range of metal concentrations observed across the different regions has important implications for both environmental monitoring and public health. Sheep’s wool can serve as an effective bioindicator of environmental pollution, reflecting the levels of heavy metals in the ecosystem. Given that sheep are often used in rural and agricultural areas, their exposure to contaminated environments can have direct consequences for food safety, particularly if these metals accumulate in other tissues, such as muscle or milk, which are consumed by humans. Despite the clear evidence of heavy metal contamination in sheep’s wool, there is currently no national or international legislation specifically regulating the concentrations of heavy metals in wool. This gap in the regulatory framework is concerning, as wool contamination could be indicative of broader environmental pollution that may affect other agricultural products and livestock. The absence of specific guidelines for heavy metal levels in wool highlights the need for increased awareness and potentially the development of monitoring programs to track environmental contamination in agricultural regions. Future research should focus on expanding the scope of monitoring to include other tissues, such as milk and meat, to assess the full impact of environmental pollution on food safety and public health.

Conclusions. The study provides compelling evidence that proximity to former mining sites and industrial activities plays a critical role in the accumulation of heavy metals, particularly Cu and Zn, in sheep's wool. The highest concentrations were observed in Ferneziu, which is located near the Herja mine, while more distant regions like Firiza and Tîrlișua exhibited comparatively lower levels of contamination. This clear spatial trend highlights the lasting environmental impact of mining operations and the need for ongoing monitoring of areas exposed to anthropogenic pollution.

The use of sheep's wool as a bioindicator proves to be a highly effective tool for assessing environmental contamination. Wool’s ability to accumulate metals from soil, water, and atmospheric sources offers a non-invasive means to track pollution levels over time. This method provides a crucial early warning system, allowing researchers and policymakers to identify high-risk areas before the contamination has more severe effects on ecosystems, livestock, and human populations.

The results of this study also emphasize the need for the development and implementation of regulatory frameworks to manage heavy metal pollution, particularly in agricultural regions that are vulnerable to contamination from industrial legacies. Despite the lack of existing legislation governing metal concentrations in wool, the findings suggest that introducing such standards could be crucial for protecting agricultural productivity and ensuring food safety. This would also safeguard the health of livestock, reduce the risk of bioaccumulation in the food chain, and mitigate the broader ecological impact of heavy metal pollution.

Furthermore, the study highlights the importance of adopting a multidisciplinary approach to environmental health, integrating environmental science, agriculture, and public health to address pollution challenges. By doing so, policymakers and stakeholders can ensure a more sustainable and resilient future for agricultural communities and ecosystems impacted by industrial contamination.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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Authors:
Florin Ioan Fechete, Clinical Sciences Department, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania, e-mail: florin-ioan.fechete@student.usamvcluj.ro
Maria Popescu, Equine Clinic, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania, e-mail: maria.popescu@usamvcluj.ro
Maria Caroline Lăcătuș, Clinical Sciences Department, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania, e-mail: caroline-maria.lacatus@student.usamvcluj.ro
Radu Lăcătuș, Clinical Sciences Department, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania, e-mail: radu.lacatus@usamvcluj.ro
Mariana Tataru, Clinical Sciences Department, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania, e-mail: mariana.tataru@usamvcluj.ro
Rodica Ileana Matei, Plastic Surgery Department, University of Medicine and Pharmacy, 8 Victor Babes St., 400012 Cluj-Napoca, Romania; Plastic Surgery Clinic, Spitalul Clinic de Recuperare, 46-50 Viilor St., 400347 Cluj-Napoca, Romania, e-mail: irmatei@yahoo.com
Marian Sorin Mârza, Clinical Sciences Department, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania, e-mail: sorin.marza@usamvcluj.ro
Andrei Răzvan Codea, Clinical Sciences Department, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania, e-mail: razvan.codea@usamvcluj.ro
Iliuță Ovidiu Mărcuș, Clinical Sciences Department, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania, e-mail: marcusovi18@gmail.com
Florin Dumitru Bora, Viticulture and Oenology Department, Advanced Horticultural Research Institute of Transylvania, Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăștur Street, 400372 Cluj-Napoca, Romania, e-mail: florin-dumitru.bora@usamvcluj.ro

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