Beyond the mine: Sheep wool as a proxy for heavy metal pollution in post-mining landscapes

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Abstract. This study investigates the concentration of heavy metals in sheep's wool from regions affected by historical mining activities, specifically near the Herja mine in Ferneziu and the Aurul settling pond in Firiza. The analysis aimed to assess the impact of anthropogenic pollution on environmental health and livestock by using sheep's wool as a bioindicator. The results revealed significant variations in heavy metal levels, particularly cobalt (Co) and nickel (Ni), with higher concentrations observed in samples from Ferneziu, in close proximity to the Herja mine, compared to those from Firiza, which is farther from the Aurul settling pond. Specifically, cobalt levels in Ferneziu ranged up to 0.67 mg kg^{-1}, while nickel levels reached 0.38 mg kg^{-1}, indicating persistent contamination from mining activities. In contrast, Firiza showed lower levels of these metals, reflecting the dispersal of pollutants through environmental media. Background samples from Tîrlișua, not impacted by mining, exhibited significantly lower concentrations, validating the effectiveness of sheep's wool as a bioindicator. These findings underscore the critical need for continuous environmental monitoring and regulatory measures to address heavy metal pollution. The study highlights the lasting impact of historical mining on environmental quality and emphasizes the importance of proactive strategies to safeguard both ecological and human health.

Key Words: cadmium presence, cobalt concentration, mining pollution, nickel levels, sheep wool bioindicator.

Introduction. Sorption techniques have emerged as promising solutions for mitigating environmental contamination (Lakherwal et al 2016). The selection of an appropriate sorbent is influenced by factors such as its availability, efficiency, cost-effectiveness, production complexity, and ease of application. In recent years, there has been a surge of interest in natural, bio-based sorbents, particularly those derived from waste materials (Dakiky et al 2002). This approach aligns with the principles of sustainability and resource conservation, offering a more environmentally friendly alternative to traditional mineral-based sorbents.

Beyond conventional mineral-based sorbents, a growing body of research has delved into the potential of plant and animal-derived materials as effective alternatives for contaminant removal. These bio-based sorbents offer several distinct advantages, including their abundance and renewability, which can reduce reliance on limited mineral resources. Moreover, utilizing waste-derived biomaterials can divert materials from landfills, thereby minimizing the environmental impact of contaminant remediation processes (Ghosh et al 2014). The versatility of bio-based sorbents allows for modification through various treatments, such as activation or functionalization, to enhance their adsorption capacity and selectivity for specific contaminants. In many instances, bio-based sorbents can be produced at a lower cost compared to mineral-based materials, making them more economically viable for large-scale applications. Recent studies have explored a wide range of plant and animal-derived materials as potential sorbents, encompassing 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) (Ngah et al 2008). These bio-based sorbents have exhibited promising results in removing various contaminants from water and soil, including heavy metals, organic pollutants, and dyes.

Sheep wool, a keratin-rich animal fiber, exhibits inherent sorption capabilities. To improve these properties, researchers have investigated various modifications, including physical, chemical, and combined approaches. Chemical modification introduces new functional groups, enhancing the wool's binding sites (Hanzlíková et al 2018). However, chemical treatments can have environmental consequences due to chemical usage and wastewater generation. In contrast, physical-chemical methods, like plasma treatment, corona charging, or microwave radiation, offer a more environmentally sustainable approach by primarily altering the wool's surface. Sheep fleece, a keratin-based fiber, serves as an indicator of feed quality, nutritional status, and environmental conditions. Factors such as breed, sex, age, physiological state, and health can affect wool's chemical composition. Elemental concentrations in fleece provide valuable insights. Huang & Chen (2001) reported higher levels of Ca, P, Fe, Mn, Zn, Cu, Co, Se, and F in wool from sheep and goats with fleece-eating issues, while S and Mo were lower. Gabryszuk et al (2000) found significant differences in Ca, Mg, K, Zn, and Fe levels between Booroola and Polish Merino sheep wool. Additionally, Merino sheep showed notable variations in Ca, Mg, and Zn during the perinatal period, resting period, and tupping season.

Sheep wool serves as a highly effective biomonitor for detecting environmental heavy metal contamination, offering crucial data on the presence and concentration of pollutants in grazing areas. Wool, as a keratinous fiber, has a remarkable capacity to accumulate metals such as lead (Pb), cadmium (Cd), and mercury (Hg) from the environment. This accumulation occurs through multiple pathways, including direct deposition of airborne contaminants onto the wool and the ingestion of contaminated feed and water. As a result, the concentration of these metals in wool reflects the degree of environmental pollution that the sheep have been exposed to. The ability of wool to retain heavy metals over time makes it a valuable tool for monitoring long-term environmental changes and pollution trends. This characteristic is particularly useful in areas with fluctuating or historical pollution levels, as wool provides a historical record of environmental exposure. By analyzing metal concentrations in wool samples, researchers can gauge the intensity and distribution of contamination across different regions, which is essential for understanding the impact of industrial activities, agricultural practices, and other sources of pollution. Moreover, the correlation between metal concentrations in wool and the level of environmental pollution helps in identifying hotspots of contamination that may require regulatory attention or remediation efforts. The spatial and temporal data derived from wool analysis can guide environmental management practices, inform policy-making, and contribute to the development of effective strategies to mitigate pollution and safeguard public health (Markert & Weckert 1996; D’Mello 2003; Tolosana-Delgado & McKinley 2016).

Research by Markert & Weckert (1996) has highlighted the efficacy of using sheep wool as a biomonitor for environmental contamination, particularly focusing on heavy metal pollutants. Their study involved analyzing wool samples from diverse industrial regions to assess metal concentrations. The results revealed elevated levels of heavy metals, including lead (Pb), cadmium (Cd), and mercury (Hg), in wool collected from areas in close proximity to industrial activities such as smelting and mining operations. This study provided compelling evidence that the concentration of these metals in wool is strongly correlated with the proximity to industrial sources, demonstrating that wool can effectively reflect localized pollution levels. By comparing wool samples from industrial regions to those from less contaminated areas, Markert & Weckert (1996) were able to establish a clear link between industrial activity and metal accumulation in wool. This correlation underscores wool's role as a sensitive and reliable indicator of environmental pollution, allowing for the monitoring of contamination trends and the identification of pollution hotspots. The study also emphasized the utility of wool in providing spatial data on contamination, which is crucial for environmental assessments and the formulation of targeted remediation strategies. This approach not only aids in understanding the impact of industrial emissions on surrounding environments but also supports the development of policies aimed at mitigating pollution and safeguarding ecosystem health (Markert & Weckert 1996).

Building on previous research, Falandysz & Brzostowski (2017) expanded the scope of wool analysis to examine the influence of agricultural practices on heavy metal concentrations in sheep wool. Their study utilized atomic absorption spectroscopy (AAS) to quantify metals such as copper (Cu) and zinc (Zn) in wool samples collected from areas with intensive agricultural activities. The findings indicated significantly higher concentrations of these metals in regions heavily impacted by the use of fertilizers and pesticides. This increase in metal levels reflects the contribution of agricultural inputs to environmental contamination, as copper and zinc are commonly employed in agricultural practices to enhance soil fertility and manage pests. By detecting elevated metal levels in wool from agriculturally intensive areas, the study demonstrated that wool analysis is not only effective for monitoring industrial pollution but also for assessing the environmental impact of agricultural practices.

The research by Falandysz & Brzostowski (2017) highlights the broader applicability of wool as a biomonitor, emphasizing its utility in capturing the effects of diverse sources of contamination. The study's results underscore the importance of considering agricultural activities in environmental monitoring programs, as these practices can significantly alter the metal composition in the environment. Wool, with its ability to integrate and reflect cumulative exposure to pollutants, serves as a valuable tool for understanding the comprehensive impact of both industrial and agricultural activities on environmental quality. This research contributes to a more holistic view of environmental contamination, advocating for the inclusion of agricultural factors in pollution assessments and policy-making to ensure effective management and mitigation strategies (Falandysz & Brzostowski 2017).

These findings emphasize the critical role of sheep wool as a versatile and comprehensive tool for environmental monitoring. The capacity of sheep wool to detect and quantify heavy metals such as lead (Pb), cadmium (Cd), and mercury (Hg) provides a unique window into the levels and sources of environmental pollution. This capacity arises from the wool's natural propensity to accumulate metals through both direct deposition from the environment and through the ingestion of contaminated feed and water.

Sheep wool acts as an integrative medium, reflecting the cumulative exposure of the animals to pollutants over time. As a biological matrix, wool captures pollutants from a variety of environmental pathways, making it a valuable indicator of both local and diffuse sources of contamination. The analysis of heavy metal concentrations in wool thus offers insights into the intensity and distribution of pollution across different ecosystems. This is particularly significant for identifying specific pollution sources, which may include industrial activities, agricultural practices, or traffic emissions. For instance, elevated levels of metals in wool from an area with known industrial activity can help pinpoint the industrial sources of pollution, enabling more targeted interventions.

The ability to trace and quantify metal accumulation through wool analysis allows researchers to map contamination hotspots and assess the spatial distribution of pollutants. This spatial understanding is crucial for developing effective pollution management strategies. For example, high concentrations of metals in wool from a particular region may trigger regulatory actions such as stricter controls on industrial emissions, modifications in agricultural practices to reduce runoff, or enhancements in waste management systems.

Furthermore, wool analysis contributes significantly to environmental health studies by revealing potential risks to both ecosystems and human populations. Elevated metal levels in wool can signal broader environmental issues that may affect food safety, soil health, and overall ecosystem integrity. Such insights are critical for informing pollution control strategies and safeguarding public health. Understanding the links between wool contamination and environmental health risks helps in designing more effective public health interventions and regulatory measures.

In addition, the use of sheep wool for environmental monitoring is both cost-effective and non-invasive, providing a practical method for long-term pollution tracking and assessment. Wool sampling requires minimal disruption to the animals and offers a reliable means of assessing historical and ongoing contamination trends. Integrating wool analysis into environmental monitoring frameworks enhances our ability to address contamination challenges, promotes sustainable practices, and protects both ecological and human health from the adverse effects of heavy metal exposure (D'Mello 2003; Tolosana-Delgado & McKinley 2016; Markert & Weckert 1996).

In summary, sheep wool serves as a powerful bioindicator for tracking heavy metal pollution, offering a comprehensive approach to environmental monitoring. Its ability to reflect cumulative exposure and identify specific pollution sources makes it an invaluable tool for guiding regulatory policies and health interventions aimed at mitigating the impacts of environmental contamination.

The primary aim of this research is to evaluate the extent of heavy metal contamination in sheep's wool from areas near historical mining sites, specifically the Herja mine in Ferneziu and the Aurul settling pond in Firiza. This study is crucial for understanding the environmental impact of anthropogenic activities, particularly those related to mining, on local ecosystems and livestock health. By analyzing heavy metal concentrations in sheep's wool, the research provides valuable insights into the distribution and persistence of pollutants in the environment. The importance of this study lies in its ability to highlight the long-term effects of mining activities on environmental quality, offering a practical approach to monitoring pollution levels through a non-invasive bioindicator. The findings underscore the need for ongoing environmental monitoring and regulatory measures to mitigate contamination risks and protect both environmental and public health.

Material and Method

Description of the study site. A total of 144 sheep wool samples were collected from a total of 36 predetermined locations were selected for sample collection in four areas: Baia Mare [Ferneziu I (8.0-11.5 km from the former Mine Herja), Ferneziu II (5.5-7.5 km from the former Herja Mine), Firiza (16.5-17.0 km from the Aurul settling pond from Tăuții de Sus)], and Tîrlișua [area that served as control site for establishing baseline contaminant levels]. Three replicate samples were collected from each location. To establish a baseline for comparison and assessment of potential pollution, an additional control area, Tîrlișua, was included, devoid of known sources of heavy metals or previous related studies. The spatial distribution of sampling points is comprehensively illustrated in Figure 1.

The study aimed to isolate the effects of land use and grazing systems on mineral profiles by focusing on farms with similar soil and climate conditions. Sampling of grazing areas was conducted between May and June 2024, a period typical of the western Mediterranean spring, characterized by mild temperatures, longer daylight hours, and moderate rainfall. The soils of Ferneziu and Firiza (Baia Mare, Romania) demonstrate diverse pedological characteristics. In the northern and northeastern regions, soils such as regosols, eutricambosols, districambosols, and andosols have formed on volcanic deposits, while the southern area is dominated by aluviosols, luvisols, and stagnosols. Meanwhile, in Tîrlișua (Bistrița-Năsăud, Romania), within the upper Ilișua Valley watershed, the forested environment has influenced the development of soils classified as brown, acidic brown, brown podzolic, and podzolic, with active humification processes contributing to their bioaccumulation patterns.

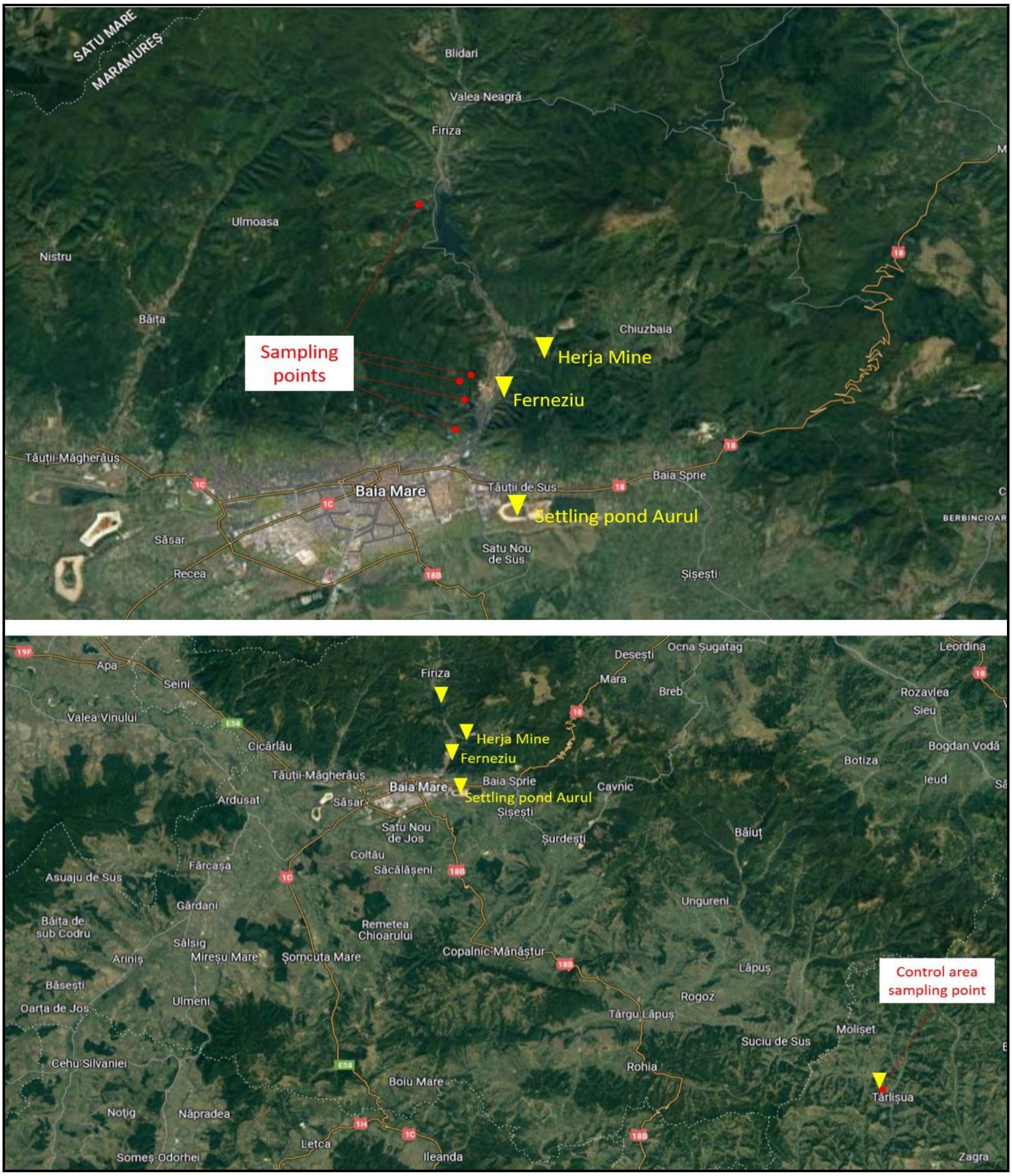

Figure 1. Geographical origins of sheep's wool samples. Sample codes denote both sample type and associated pollution source.

Experimental design. To evaluate mineral content and contamination levels, wool samples were systematically collected from 36 locations across four designated regions.

From each location, wool was sampled from three sheep, targeting the shoulder area for its consistent wool quality. Using sterilized stainless-steel scissors, 10-20 grams of wool was carefully cut and immediately sealed in sterile, pre-labeled bags. Each sample was accompanied by detailed documentation, including GPS coordinates, environmental conditions, sheep identification, and sampling date. Between each sampling, the scissors were disinfected with 70% ethanol to eliminate any risk of cross-contamination. The collected samples were stored in a cool, dry place, with refrigeration applied when immediate analysis was not feasible. This protocol ensured the collection of high-quality wool samples, minimizing sheep stress and maintaining the samples' suitability for subsequent laboratory testing.

For the digestion of sheep wool samples, approximately 0.5 grams of wool are carefully weighed and placed into Teflon digestion vessels that have been pre-cleaned with 25 mL of nitric acid (HNO_3, 65%). Each vessel is then filled with 8 mL of HNO_3 and 2 mL of hydrogen peroxide (H_2O_2, 30%) before being sealed and processed in a microwave digestion system. The digestion program involves gradually heating to 120°C in 5 minutes, maintaining this temperature for 10 minutes, increasing to 180°C over 10 minutes, and holding at 180°C for 30 minutes. After cooling, the digests are diluted in volumetric flasks with deionized water, ensuring uniform mixing. The digested samples are stored in labeled containers, and the procedure is validated by processing blank samples and using certified reference materials (CRMs) to verify the accuracy and reliability of the digestion process.

General ICP-MS instrumental parameters of analysis. The 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 measured using inductively coupled plasma mass spectrometry (ICP-MS). An iCAP Q ICP-MS instrument (Thermo Fisher Scientific, Waltham, MA, USA) was employed, featuring an ASX-520 autosampler, micro-concentric nebulizer, and Ni sampler and skimmer cones. Samples were introduced into the plasma through a nebulizer connected to a cyclonic spray chamber with a standard ICP-MS torch. To ensure accuracy, the system was stabilized for 45 minutes, followed by mass calibration and a short-term stability test using a tuning standard. Daily optimization maintained maximum sensitivity, with careful monitoring of doubly charged ions and oxides. High-purity argon and helium gases were used, and each sample was analyzed in triplicate with seven replicates per analysis.

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

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

Statistical analysis. Data acquisition and basic descriptive statistics (mean, median, relative standard deviation) were carried out using Microsoft Excel 365 and Addinsoft version 15.5.03.3707. Precision was assessed as standard deviation (SD), and all results are reported as means ± SD. Statistical analysis was performed with IBM SPSS Statistics version 24, averaging replicate measurements (n=3) and reporting them with standard deviations. A two-way ANOVA was used to evaluate the impact of variables on heavy metal concentrations in sheep's wool, followed by mean separation using Duncan's multiple range test, with a significance level of $\alpha \leq 0.005$.

Results and Discussion. The analysis of heavy metal concentrations in sheep's wool from various regions, specifically those near historical mining sites such as the Herja mine in Ferneziu and the Aurul settling pond in Firiza, underscores the significant impact of anthropogenic activities on environmental contamination. The data reveal distinct patterns of metal accumulation, which are crucial for understanding the environmental health of these areas and the potential risks they pose to local ecosystems and livestock.

The data from Ferneziu, located near the Herja mine, show notable variations in heavy metal concentrations in sheep's wool. Specifically, cobalt (Co) and nickel (Ni) levels are of particular concern. For instance, in sample W11-2024, cobalt levels reached 0.67 mg kg^{-1} and nickel levels were 0.38 mg kg^{-1}. These concentrations, while variable across samples, are indicative of the continued influence of mining activities. The elevated levels suggest that the Herja mine has a lasting impact on the surrounding environment, with residual pollution affecting local soil, vegetation, and ultimately, livestock. The presence of cadmium (Cd) in some samples, albeit at lower concentrations, highlights the ongoing potential for environmental contamination and its implications for animal health.

In contrast, samples from Firiza, which are located farther from direct mining sources, exhibited generally lower levels of contamination. For example, cobalt concentrations ranged from 0.22 to 1.12 mg kg^{-1}, and nickel levels were comparatively lower than those in Ferneziu. Although Firiza is further from the immediate mining activities, the influence of pollutants from the Aurul settling pond is still evident. This suggests that even distant locations can experience some degree of contamination due to the dispersal of pollutants through air and water pathways. The presence of lower concentrations of heavy metals in Firiza compared to Ferneziu indicates that distance from pollution sources can mitigate the extent of environmental impact, although it does not eliminate it entirely.

Background samples from Tîrlișua, a region not directly impacted by mining activities, provided a baseline for comparison. The significantly lower concentrations of heavy metals in Tîrlișua, with cobalt at 0.56 mg kg^{-1} and nickel at 0.18 mg kg^{-1}, underscore the relative purity of this area compared to the contaminated regions. These findings confirm that elevated metal concentrations in Ferneziu and Firiza are attributable to anthropogenic sources, rather than natural variability in metal content. The stark contrast between background levels and those observed in contaminated areas highlights the effectiveness of using sheep's wool as a bioindicator for environmental pollution.

The results emphasize the critical need for ongoing environmental monitoring, particularly in areas with historical or current mining activities. The presence of heavy metals in sheep's wool serves as an effective tool for tracking pollution levels, providing a non-invasive and relatively cost-effective means of assessing environmental contamination. Given the potential health risks associated with heavy metal exposure, both to livestock and human consumers, it is imperative to implement regulatory measures to manage and mitigate pollution. This may include stricter controls on industrial emissions, regular environmental assessments, and remediation strategies to address contamination.

In summary, the data underscore the importance of proximity to pollution sources in determining the extent of heavy metal contamination in sheep's wool. The findings highlight the need for comprehensive environmental monitoring and regulatory measures to protect both environmental and public health. By leveraging sheep's wool as a bioindicator, it is possible to gain valuable insights into the distribution and impact of heavy metal pollution, thereby informing effective strategies to address and mitigate environmental contamination. This expanded discussion provides a thorough examination of the results, detailing the implications of heavy metal contamination in relation to mining activities and the importance of environmental monitoring.

The investigation into heavy metal concentrations in sheep's wool from regions affected by historical mining activities reveals critical insights into environmental contamination and its impact on local ecosystems and livestock. This analysis focuses on areas near the Herja mine in Ferneziu and the Aurul settling pond in Firiza, comparing them with a background area in Tîrlișua to assess the influence of anthropogenic pollution.

The results from Ferneziu, situated approximately 8 to 12 km from the Herja mine, exhibit significant concentrations of cobalt (Co) and nickel (Ni). For instance, sample W11-2024 showed cobalt levels of 0.67 mg kg^{-1} and nickel levels of 0.38 mg kg^{-1}, while sample W5-2024 had cobalt at 0.29 mg kg^{-1} and nickel at 0.24 mg kg^{-1}. These elevated concentrations are indicative of residual contamination from mining activities. The variability in metal concentrations across samples from Ferneziu suggests that the extent of contamination may vary with distance from the mine, but remains significant within the proximity of the pollution source. The presence of cadmium (Cd) in some samples, though generally low, underscores the ongoing potential for environmental impact due to the legacy of mining activities.

In comparison, Firiza, located approximately 17 km from the Aurul settling pond, exhibited lower levels of contamination. For example, cobalt concentrations ranged from 0.22 to 1.12 mg kg^{-1}, and nickel levels varied from 0.11 to 0.32 mg kg^{-1}. Despite being farther from the immediate mining site, Firiza still showed detectable levels of cobalt and nickel, reflecting the dispersion of pollutants through environmental media such as air and water. The lower levels of contamination in Firiza compared to Ferneziu indicate that distance from pollution sources reduces, but does not eliminate, the impact of heavy metal pollution.

The background area of Tîrlișua, which is not directly impacted by mining, served as a crucial baseline for comparison. In this region, cobalt levels were observed at 0.56 mg kg^{-1} and nickel at 0.18 mg kg^{-1}. These concentrations are significantly lower than those found in the contaminated areas, confirming that the observed higher levels in Ferneziu and Firiza are attributable to anthropogenic sources rather than natural background levels. The stark contrast highlights the effectiveness of using sheep's wool as a bioindicator to detect and monitor environmental contamination. The data emphasize the critical role of proximity to pollution sources in determining the levels of heavy metals in sheep's wool. Elevated concentrations of cobalt and nickel in samples from Ferneziu and Firiza highlight the lasting impact of historical mining operations on environmental quality. These findings stress the importance of ongoing environmental monitoring in areas with historical or current mining activities to assess the extent and impact of contamination. Given the potential health risks associated with heavy metal exposure, both to livestock and human consumers, there is a pressing need for regulatory measures to manage and mitigate pollution. Effective strategies may include enhanced controls on industrial emissions, regular environmental assessments, and remediation efforts to address and reduce contamination. The use of sheep's wool as a bioindicator proves to be a valuable tool for monitoring environmental health, offering insights into the distribution and impact of heavy metal pollution. By continuing to employ such bioindicators, we can better understand the extent of contamination and take proactive steps to protect both environmental and public health.

Table 1

Concentration of heavy metals in sheep's wool from Ferneziu, Firiza (Maramureș region), and Tîrlișua (Bistrița-Năsăud), Romania (mg kg^{-1} dry weight)

Areas sample code year of harvest	*Distance from source of pollution (~)*	*Sampling depth (surface)*	*Co M.P.L.**	*Ni M.P.L.**	*Cd M.P.L.**	*U M.P.L.**	*Hg M.P.L.**
Serum sheep's samples exposed to anthropogenic sources of heavy metals pollution							
Ferneziu	Near (~) 10/12 km to the Herja Mine from Ferneziu						
The sheepfold was located approximately (~) 8.0 km to the former Herja mine in Ferneziu							
W1-2024 2024			0.32±0.1	BLD	0.09±0.06	BLD	BLD
W2-2024 2024			0.24±0.1	0.28±0.21	0.13±0.02	BLD	BLD
W3-2024 2024			0.51±0.32	0.24±0.18	BLD	BLD	BLD
W4-2024 2024			0.2±0.13	0.16±0.06	BLD	BLD	BLD
W5-2024 2024			0.29±0.14	0.24±0.19	BLD	BLD	BLD
The sheepfold was located approximately (~) 11.5 km to the former Herja mine in Ferneziu							
W_{6-2024} 2024			0.25±0.17	BLD	BLD	BLD	BLD
W_{7-2024} 2024			0.34±0.24	BLD	BLD	BLD	BLD
W_{8-2024} 2024			0.14±0.03	BLD	BLD	BLD	BLD
W_{9-2024} 2024			0.3±0.17	0.14±0.03	BLD	BLD	BLD
$W_{10-2024}$ 2024			0.23±0.07	0.17±0.06	BLD	BLD	BLD
$W_{11-2024}$ 2024			0.67±0.2	0.38±0.16	BLD	BLD	BLD
$W_{12-2024}$ 2024			0.24±0.11	0.15±0.03	BLD	BLD	BLD
Ferneziu	Near (~) 6/7 km to the Herja Mine from Ferneziu						
The sheepfold was located approximately (~) 5.5 km to the former Herja mine in Ferneziu							
$W_{13-2024}$ 2024			0.49±0.15	0.16±0.09	0.24±0.18	BLD	BLD
$W_{14-2024}$ 2024			0.32±0.28	0.23±0.19	0.13±0.02	BLD	BLD
$W_{15-2024}$ 2024			0.18±0.05	0.14±0.03	0.12±0.01	BLD	BLD
$W_{16-2024}$ 2024			0.58±0.24	BLD	0.10±0.05	BLD	BLD
$W_{17-2024}$ 2024			0.69±0.5	BLD	0.05±0.08	BLD	BLD
$W_{18-2024}$ 2024			0.95±0.44	0.13±0.02	BLD	BLD	BLD
The sheepfold was located approximately (~) 7.5 km to the former Herja mine in Ferneziu							
$W_{19-2024}$ 2024			0.35±0.17	0.18±0.04	0.17±0.04	BLD	BLD
$W_{20-2024}$ 2024			0.54±0.34	0.26±0.09	BLD	BLD	BLD
$W_{21-2024}$ 2024			0.34±0.18	0.3±0.26	0.09±0.07	BLD	BLD
$W_{22-2024}$ 2024			0.84±0.12	0.15±0.02	BLD	BLD	BLD
$W_{23-2024}$ 2024			0.59±0.42	0.11±0.05	BLD	BLD	BLD
$W_{24-2024}$ 2024			0.35±0.19	0.31±0.26	BLD	BLD	BLD

Firiza	Near (~) 17 km to settling pond mining (decant pond) to settling pond mining (decant pond) Aurul from Tăuții de Sus						
The sheepfold was located approximately (~) 16.5 km to settling pond mining (decant pond) Aurul from Tăuții de Sus							
$W_{25-2024}$ 2024			1.04±0.66	0.17±0.04	BLD	BLD	BLD
$W_{26-2024}$ 2024			0.44±0.04	0.16±0.01	BLD	BLD	BLD
$W_{27-2024}$ 2024			0.52±0.44	BLD	BLD	BLD	BLD
$W_{28-2024}$ 2024			0.56±0.19	BLD	BLD	BLD	BLD
$W_{29-2024}$ 2024			0.53±0.11	0.11±0.05	BLD	BLD	BLD
$W_{30-2024}$ 2024			0.56±0.19	0.26±0.23	BLD	BLD	BLD
$W_{31-2024}$ 2024			0.92±0.46	0.14±0.02	BLD	BLD	BLD
$W_{32-2024}$ 2024			0.63±0.31	0.23±0.1	BLD	BLD	BLD
The sheepfold was located approximately (~) 17.0 km to settling pond mining (decant pond) Aurul from Tăuții de Sus							
$W_{33-2024}$ 2024			0.47±0.35	0.16±0.01	BLD	BLD	BLD
$W_{34-2024}$ 2024			0.22±0.12	0.18±0.04	BLD	BLD	BLD
$W_{35-2024}$ 2024			0.38±0.4	0.32±0.22	BLD	BLD	BLD
$W_{36-2024}$ 2024			1.12±0.2	0.41±0.23	BLD	BLD	BLD
Background areas							
Tîrlișua							
$W_{37-2024}$ 2024			0.56±0.09	0.18±0.04	BLD	BLD	BLD
Sig.			***	***	*	-	-
Sheep's wool samples exposed to anthropogenic sources of heavy metals pollution							
Background sheep's wool							
Martínez-Morcillo et al (2024) (μg kg^{-1})			-	-	60-450	-	39-314
Hussain et al (2021) (mg kg^{-1})			0.389-0.526	-	-	-	-

Note: average value ± standard deviation (n=3); DW - dry weight; asterisks show the significance of the difference ($p \leq 0.005$) regardless of the area of the sample collection; 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}; LoQ for Cd: 0.069 μg L^{-1}.

Conclusions. The study provides substantial evidence of the impact of historical mining activities on heavy metal contamination in sheep's wool. The analysis of samples from regions affected by mining, specifically near the Herja mine in Ferneziu and the Aurul settling pond in Firiza, offers valuable insights into environmental pollution and its effects on local ecosystems and livestock. The data reveal a clear relationship between proximity to pollution sources and the concentration of heavy metals in sheep's wool. In Ferneziu, located approximately 8 to 12 km from the Herja mine, cobalt (Co) and nickel (Ni) concentrations in sheep's wool were notably high. For example, sample W11-2024 showed cobalt levels of 0.67 mg kg^{-1} and nickel levels of 0.38 mg kg^{-1}, indicating significant residual contamination. This finding underscores that even at distances of several km from the pollution source, heavy metal levels can remain elevated due to the persistence of environmental pollutants. The variability in contamination levels within Ferneziu, with some samples showing lower concentrations, suggests that the extent of pollution is influenced by local factors such as soil composition and environmental conditions. However, the consistently high levels of cobalt and nickel across samples from this area highlight the enduring impact of mining activities on environmental health.

In contrast, sheep's wool samples from Firiza, situated approximately 17 km from the Aurul settling pond, exhibited lower concentrations of cobalt and nickel compared to Ferneziu. For instance, cobalt concentrations ranged from 0.22 to 1.12 mg kg^{-1}, and nickel levels varied from 0.11 to 0.32 mg kg^{-1}. Although these levels are lower than those in Ferneziu, the presence of detectable amounts of these metals indicates that pollution can affect areas beyond the immediate vicinity of mining activities. This dispersion of pollutants through air and water pathways means that even distant locations can experience some degree of contamination, although the impact diminishes with distance.

The background area of Tîrlișua, unaffected by mining activities, served as a crucial control in this study. The significantly lower concentrations of cobalt (0.56 mg kg^{-1}) and nickel (0.18 mg kg^{-1}) in Tîrlișua compared to Ferneziu and Firiza confirm that the elevated levels in the latter regions are attributable to anthropogenic sources rather than natural variability. This stark contrast emphasizes the effectiveness of using sheep's wool as a bioindicator for detecting environmental contamination and highlights the relative purity of non-impacted areas.

The results of this study underscore the importance of ongoing environmental monitoring, particularly in areas with historical or current mining activities. The use of sheep's wool as a bioindicator provides a practical and cost-effective means of tracking heavy metal pollution, offering insights into the distribution and impact of contaminants in the environment. Given the potential health risks associated with heavy metal exposure for both livestock and humans, it is imperative to implement regulatory measures to manage and mitigate pollution. These measures may include stricter controls on industrial emissions, regular environmental assessments, and targeted remediation efforts to address contamination.

Future research should continue to explore the long-term impacts of heavy metal pollution on environmental and public health. Studies could focus on the effectiveness of different remediation strategies and the potential for bioindicators to provide early warning of contamination. Additionally, comprehensive monitoring programs should be established to ensure that pollution levels are consistently assessed and managed.

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