PhD THESIS

The impact of exposure to heavy metals on the health and production of sheep in Maramureș

(SUMMARY OF THE PhD THESIS)

PhD student **Florin-Ioan Fechete**

Scientific coordinator **Prof. univ. dr. Radu Lăcătuș**

INTRODUCTION

Minerals are essential components of animals' diets, playing a wide range of roles in the body, including structural processes, physiological functions, enzyme activity, and certain self-regulation mechanisms (GARCÍA-CARMONA ET AL., 2019; MARTÍNEZ-MORCILLO ET AL., 2024). Variations in the mineral content of soil and feed directly influence the mineral status of animals, affecting their productivity and output. Essential dietary elements play a major role in health and growth, while non-essential elements, often classified as potentially toxic, can enter the food chain without fulfilling any direct nutritional role (POURRET ET AL., 2021). Both deficiencies and toxicities of certain minerals can negatively impact human and animal health. Currently, pollution is a global issue, and 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 availability, efficiency, cost-effectiveness, production complexity, and ease of application.

In recent years, there has been increased interest in natural or bio-sorbents, particularly those derived from waste materials (DAKIKY ET AL., 2002). This approach aligns with sustainability and resource conservation principles, offering a more eco-friendly alternative to traditional mineral sorbents.

Sheep wool, an animal fiber based on keratin, has inherent adsorption properties. Numerous studies have explored specific modifications to enhance these properties, using physical, chemical, or combined methods. Wool serves as a chemical indicator reflecting feed quality, nutrition, and environmental factors. Although various factors, including breed, sex, age, physiological state, and health conditions (RAMIREZ-PEREZ ET AL., 2000), may influence the chemical composition of wool, it is evident that the concentration of elements in wool provides valuable information. Sheep and goats have been found to exhibit high levels of Ca, P, Fe, Mn, Zn, Cu, Co, Se, and F in their wool, while concentrations of S and Mo are lower (HUANG & CHEN, 2001).

To understand the transfer of minerals, including both essential and non-essential trace elements, within a soil-feed-animal continuum, a holistic approach is required. This involves analyzing a wide spectrum of elements in various environmental matrices, such as soil, feed, and milk. The mineral content of blood directly influences milk composition, and high concentrations of heavy metals in the blood can disrupt biological processes, including milk composition, leading to health issues and reduced productivity. Additionally, agricultural practices, such as the use of pesticides and fertilizers, can alter the mineral profile of soil and plants.

STRUCTURE OF THE THESIS

The thesis is structured into nine chapters, with each chapter dedicated to a clearly defined topic.

Chapter 1 of the thesis includes information from the specialized literature regarding the orientations and perspectives of sheep farming, the classification of sheep breeds, and the phylogenetic evolution of the species. This chapter also describes the morphophysiological characteristics of the Țurcană sheep breed.

Chapter 2 is dedicated to describing the peculiarities of the digestive system and blood in sheep. It also presents the anatomical elements of the digestive tract, the post-diaphragmatic segment, and the internal structure of the stomach in small ruminants.

Chapter 3 addresses the impact of major pollutants on sheep health. Significant space is dedicated to the main sources of heavy metal contamination and their influence on the metabolism of animals raised in different farming systems, as well as on their productivity. The exposure of animals to certain toxic substances, such as heavy metals or pesticides, is primarily influenced by contaminated feed (KHAN ET AL., 2023). Monitoring animal feed is imperative, especially in areas with polluted soils or industrial zones (USMAN ET AL., 2022). The accumulation of toxic metals in soil, which enter the food chain through plants, poses a direct threat to humans who consume animal-derived products (NĂSTĂSESCU ET AL., 2020). Although it is well known that toxic metals in soil accumulate in animal tissues, the absence of international standards for certain elements in food products poses risks to consumer health.

Chapter 4 outlines the research hypothesis and objectives. Understanding the mechanisms of pollution and heavy metal bioaccumulation can provide tangible support for environmental monitoring efforts and regulatory measures to mitigate pollution effects on ecosystems, livestock, and human health. Sheep of the native Țurcană breed were chosen as pollution biomarkers due to their adaptability to local conditions, which lends ecological validity to the research. By analyzing heavy metal concentrations in sheep wool, the research provides valuable information about 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 using wool as a non-invasive bioindicator. The findings emphasize the need for continuous environmental monitoring and the implementation of regulatory measures to mitigate contamination risks and protect ecological and public health.

Objectives of the thesis: To achieve the proposed goal, the following objectives were established:

- Use sheep wool as a bioindicator to identify the level of environmental contamination with the following heavy metals: copper (Cu), zinc (Zn), and lead (Pb);
- Use sheep wool as a bioindicator to identify the level of environmental contamination with heavy metals such as cobalt (Co), nickel (Ni), and cadmium (Cd);
- Provide a detailed quantification of micronutrient concentrations (^{64}Cu, ^{65}Zn), ultratrace elements (^{52}Cr, ^{59}Co, ^{60}Ni), and heavy metals (^{75}As, ^{111}Cd, ^{201}Hg, ^{208}Pb) in various matrices.

Although studies investigating the presence of these elements in environmental and food matrices exist, there remain knowledge gaps regarding their distribution in specific agricultural contexts, especially in areas affected by mining activities.

Chapter 5 focuses on Study I, titled *"Sheep Wool as a Bioindicator of Heavy Metal Pollution: Copper, Zinc, and Lead from Former Mining Sites."* The purpose of this study was to assess the impact of industrial and mining activities on heavy metal accumulation in sheep wool from regions near former mining sites. A total of 144 sheep wool samples were collected from 36 pre-determined locations in four areas: Baia Mare (Ferneziu I, located 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 tailings pond in Tăuții de Sus) and Târlișua (used as a control location to establish baseline contaminant levels). Three replicate samples were taken from each location. The general parameters of the ICP-MS instrument for analysis were determined in this phase. 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 quantified using inductively coupled plasma mass spectrometry (ICP-MS). The study provides compelling evidence that pastures near former mining sites and industrial activities play a significant role in the accumulation of heavy metals, particularly Cu and Zn, in sheep wool. The highest concentrations were observed in Ferneziu, located near the Herja mine, while more distant regions such as Firiza and Târlișua showed relatively lower contamination levels. This clear spatial trend highlights the long-term environmental impact of mining operations and underscores the need for continued monitoring of areas exposed to anthropogenic pollution.

Chapter 6 is dedicated to Study II, titled *"Sheep Wool as a Biological Marker of Environmental Contamination with Heavy Metals: Cobalt, Nickel, and Cadmium from Former Mining Sites."* The study aimed to evaluate heavy metal contamination represented by cobalt (Co), nickel (Ni), and cadmium (Cd) in areas near historical mining sites, particularly the Herja mine in Ferneziu and the Aurul tailings pond in Firiza, using sheep wool as a bioindicator. A total of 144 wool samples were collected from 36 pre-defined locations in four areas: Baia Mare, Ferneziu I, Ferneziu II, Firiza, and Târlișua. The methodology followed the same steps and used the same reagents as Study I. The results revealed a clear relationship between proximity to pollution sources and heavy metal concentrations in sheep wool. For example, in Ferneziu, located approximately 8–12 km from the Herja mine, the concentrations of cobalt (Co) and nickel (Ni) in sheep wool were significantly high. This finding emphasizes the lasting impact of mining activities on environmental health. For example, sample W11-2024 exhibited cobalt levels of 0.67 mg kg^{-1} and nickel levels of 0.38 mg kg^{-1}, indicating significant residual contamination. This finding highlight that, although the distance from the pollution source is several kilometers, heavy metal levels can remain elevated due to the persistence of environmental pollutants. Contamination levels in Ferneziu are variable; in some samples, heavy metal concentrations are lower, suggesting that the degree of pollution is influenced by local factors such as soil composition and environmental conditions. However, the consistently high levels of cobalt and nickel in samples from this area underscore the lasting impact of mining activities on environmental health. Sheep wool samples from Firiza, a locality situated approximately 17 km from the Aurul tailings pond, showed lower cobalt and nickel concentrations compared to those in Ferneziu. For instance, cobalt concentrations ranged between 0.22 and 1.12 mg kg^{-1}, while nickel levels ranged between 0.11 and 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 also affect areas beyond the immediate vicinity of mining activities. This dispersion of pollutants through air and water demonstrates that locations distant from the primary source can also experience some degree of contamination, though its impact diminishes with distance. The reference area, Târlișua, unaffected by mining activities, served as the control area in this study. The significantly lower concentrations of cobalt (0.56 mg kg^{-1}) and nickel (0.18 mg kg^{-1}) in Târlișua compared to Ferneziu and Firiza confirm that the elevated levels in the latter two regions are more likely attributable to anthropogenic sources rather than natural variability.

Chapter 7 presents Study III, titled *"Bioaccumulation of Heavy Metals in Sheep Dairy Products and Their Implications for Human Health."* This study aimed to identify heavy metals in sheep milk, cheese, and serum, as well as in soil and green grass. A total of 576 samples were collected, divided into five categories: soil (144 samples), green grass (144 samples), sheep milk (144 samples), sheep cheese (144 samples), and serum (144 samples). The results showed significant heavy metal contamination of pastures in the Baia Mare area, with copper (Cu) and zinc (Zn) exceeding permitted limits, especially near the former Herja mine. Milk and cheese samples consistently contained heavy metal concentrations, with zinc (Zn) showing significant variability, indicating a tendency for bioaccumulation. Elevated levels were observed, particularly for copper (Cu) and zinc (Zn), which exceeded permissible limits, especially in areas near the former Herja mine, with copper (Cu) reaching values up to 2528.20 mg/kg and zinc (Zn) up to 1821.40 mg/kg. These findings indicate a direct correlation between previous mining activities and the high levels of these metals. Additionally, elevated concentrations of lead (Pb) and cadmium (Cd) were identified in the industrial area of Ferneziu, suggesting that industrial emissions could be a contributing factor to the pollution process. The analysis of fresh grass samples confirmed substantial spatial variability, with zinc (Zn) being the most abundant metal, followed by copper (Cu). It is noteworthy that the pronounced patterns of copper concentration near mining activities raise concerns about the environmental risks associated with heavy metal exposure. Although nickel (Ni) and cobalt (Co) showed lower average levels, their concentrations were higher near contamination sources, necessitating further investigations into the mobility and distribution of these metals. Analyses of sheep milk and cheese samples demonstrated consistent concentrations of heavy metals, with zinc (Zn) showing significant variation, indicating a heightened tendency for bioaccumulation.

Chapter 8 presents the general conclusions of the thesis. The findings provide convincing evidence that pastures near former mining sites and industrial areas play a crucial role in heavy metal contamination, particularly Cu, Zn, Co, Ni, Pb, and Cd. The use of sheep wool as a bioindicator proves to be an effective tool for evaluating environmental contamination, offering a non-invasive method for monitoring pollution over time. The highest concentrations were observed in Ferneziu, a locality situated near the Herja mine, while more distant regions, such as Firiza and Târlișua, exhibited comparatively lower levels of contamination. This clear spatial trend highlights the long-term impact of mining operations on the environment and the need for ongoing monitoring of areas exposed to anthropogenic pollution. The use of sheep wool as a bioindicator proves to be an extremely effective tool for assessing environmental contamination. The wool's ability to accumulate heavy metals from soil, water, and atmospheric sources represents a non-invasive means of tracking pollution levels over time. This method provides a crucial early warning system, enabling researchers and decision-makers to identify high-risk areas before pollution causes more severe effects on ecosystems, livestock, and human populations. Analyses of sheep milk and cheese samples revealed the presence of consistent concentrations of heavy metals, with zinc (Zn) showing significant variation, indicating an increased tendency for bioaccumulation. Serum analysis revealed a complex interplay of heavy metal concentrations in the sheep under study. Zinc (Zn) was the predominant metal, followed by copper (Cu) and lead (Pb), while other heavy metals were present at negligible levels.

Chapter 9 is dedicated to the originality and innovative contributions of the thesis.

BIBLIOGRAPHY

1. Dakiky M., Khamis M., Manassra A., Mereb M., (2002). Selective absorption of chromium (VI) in industrial wastewater using low-cost abundantly available absorbents. Advances in Environmental Research 6(4):533-540.
2. García-Carmona, M.; García-Robles, H.; Turpín Torrano, C.; Fernández Ondoño, E.; Lorite Moreno, J.; Sierra Aragón, M.; Martín Peinado, F.J. (2019). Residual Pollution and Vegetation Distribution in Amended Soils 20 years after a Pyrite Mine Tailings Spill (Aznalcóllar, Spain). Sci. Total Environ, 650, 933–940.
3. Huang Y., Chen H., (2001). Studies on the pathogenesis of Shimao Zheng (fleence-eating) in sheep and goats. Veterinary Research Communications 25(8):631-640.
4. Khan, Z., Muhammad, F., Ahmad, K., Alrefaei, A., Ahmad, T., Ejaz, A., et al. (2023). Evaluation of potential ecological risk assessment of toxic metal (lead) in contaminated meadows in the vicinity of suburban city: soil vs forages vs livestock. Braz. J. Biol. 83, e272087. doi:10.1590/1519-6984.272087.
5. Lakherwal D., Rattan H.P., Singh H.P., (2016). Kinetic studies of absorption process of chromium ions from aqueous solution. International Journal of Advance Research 4:545-550.
6. Martínez-Morcillo S., Barrales I., Pérez-López M., Rodríguez F.S., Peinado J. S., Míguez-Santiýan M. P., (2024). Mineral and potentially toxic element profiles in the soil-feed-animal continuum: Implications for public, environmental, and livestock health in three pasture-based sheep farming systems. Science of The Total Environment 919:170860.
7. Năstăsescu, V., Mititelu, M., Goumenou, M., Docea, A. O., Renieri, E., Udeanu, D. I., et al. (2020). Heavy metal and pesticide levels in dairy products: evaluation of human health risk. Food Chem. Toxicol. 146, 111844. doi:10.1016/j.fct.2020.111844.
8. Pourret, O.; Bollinger, J.C.; Hursthouse, A. (2021). Heavy Metal: A Misused Term? Acta Geochim. 40, 466–471.
9. Ramírez-Pérez A.H., Buntinx S.E., Tapia-Rodríguez C., Rosiles R., (2000). Effect of breed and age on the voluntary intake and the microminerals status of non-pregnant sheep. 1. Estimation of voluntary intake. Small Ruminant Research 37(3):223-229.
10. Usman, M., Murtaza, B., Natasha, N., Imran, M., Abbas, G., Amjad, M., et al. (2022). Multivariate analysis of accumulation and critical risk analysis of potentially hazardous elements in forage crops. Environ. Monit. Assess. 194 (2), 139. doi:10.1007/s10661-022-09799-8.