DOCTORAL THESIS

Combined Effects of Environmental Factors and Nutrition on the Bioaccumulation of Heavy Metals in Equines and the Assessment of Health Risk

(Abstract of the PhD Thesis)

PhD Student **Maria Popescu**

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

Introduction

Environmental contamination with heavy metals represents one of the most severe forms of long-term pollution, with major impacts on ecosystems and the health of living organisms. Due to their chemical stability and high capacity for bioaccumulation, these elements persist for long periods in soil, water, and living organisms, generating toxic effects even at low concentrations (Järup, 2003; Tchounwou și colab., 2012; Jaishankar et al., 2014). Industrial and mining activities constitute important anthropogenic sources of heavy metal release, leading to contamination of air, water, and soils (Kabata-Pendias and Mukherjee, 2007).

Post-closure mining areas continue to affect the environment, as waste dumps and tailings ponds release metals through oxidation processes and acid drainage (Ashraf și colab., 2012). These processes promote the transfer of metals from the abiotic environment into trophic chains, generating a complex ecotoxicological risk (Hu et al., 2019), which extends from soil and water to plants, animals, and humans. Metals such as lead, cadmium, mercury, and arsenic are recognized for their systemic toxicity, being involved in oxidative stress, enzymatic dysfunctions, and neurological impairments (Jomova and Valko, 2011; Koyama et al., 2024). Chronic exposure, even at subclinical levels, produces cumulative effects on health and biological performance (Järup, 2003; Kincaid, 2000).

Biomonitoring allows the evaluation of real exposure to contaminants, integrating long-term exposure and the biological effects of contamination (Budnik and Baur, 2009; Lionetto et al., 2019). Non-invasive biological samples, such as hair, hoof, and blood, are frequently used to assess chronic and recent exposure to heavy metals (Combs et al., 1982; Chojnacka et al., 2006).

Domestic animals are relevant biological models in ecotoxicology, being exposed similarly to humans through water, food, and soil (Roggeman et al., 2013). Horses are of particular interest due to their longevity, direct contact with soil, and the possibility of repeated biological sampling (Dunnett and Lees, 2003; Giannetto et al., 2022), being considered a sentinel species for monitoring contamination. Hair and hoof reflect chronic exposure, while blood and serum indicate recent exposure and metal bioavailability (Asano et al., 2002; Fazio et al., 2020).

In Romania, especially in the northwest, areas with a mining history show significant heavy metal contamination, impacting soils and agroecosystems (Bora et al., 2023). Although there are numerous data on soil and plants, information on metal accumulation in animals is still limited.

The integrated environment–organism approach allows tracing the pathway of metals from source to organism and differentiating between chronic and recent exposure (Kabata-Pendias and Mukherjee, 2007). This fits within the One Health concept, which highlights the link between environmental, animal, and human health (Doğan et al., 2024).

In this context, the thesis aims to evaluate the impact of historical mining activities on the environment and on horses from ecologically exposed areas, correlating environmental parameters with the level of heavy metal bioaccumulation and with the biological response of organisms.

Structure of the thesis

The thesis is structured into nine chapters, each chapter having a clearly defined theme and contributing progressively to the achievement of the research objectives.

Chapter 1 – Sources of heavy metal contamination in agroecosystems and ecotoxicological impact on animals presents the role of anthropogenic activities (mining, industry, intensive agriculture) in loading soils with heavy metals, with examples from mining areas in Romania such as Maramureș, Apuseni or Baia Mare (Bora et al., 2023). The mechanisms by which soil becomes a contamination reservoir are highlighted, the influence of pH on metal mobility (Ashraf et al., 2012) and their transfer to forage plants. The chapter shows that contaminated feed and water represent the main exposure pathways for animals, metals such as lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As) being toxic even at low levels (Tchounwou et al., 2012), justifying the need for biomonitoring in agroecosystems.

Chapter 2 – Bioaccumulation mechanisms and toxicological effects of heavy metals on animals describes the toxicokinetics and toxicodynamics of heavy metals in animals, from exposure and absorption pathways to plasma transport, tissue distribution and long-term retention (Kabata-Pendias and Mukherjee, 2007; Jaishankar et al., 2014). Essential metals (zinc (Zn), copper (Cu), cobalt (Co)), which can become toxic in excess, are differentiated from non-essential ones (Pb, Cd, Hg, As), with important systemic effects (Tchounwou et al., 2012). The chapter highlights tissue tropism (Pb – bone, Cd – liver and kidney, Hg – nervous system) and justifies the use of hair and hoof as biomarkers of chronic exposure (Dunnett and Lees, 2003). The main mechanisms of toxicity (oxidative stress, enzymatic inhibition, genotoxicity, endocrine/immune disturbances) and clinical and subclinical manifestations are presented, emphasizing the need for biomonitoring for early identification of exposure (Jomova and Valko, 2011; Budnik and Baur, 2009).

Chapter 3 – The role of horses as sentinel species in ecotoxicological biomonitoring argues for the use of horses as biological models for assessing heavy metal contamination, due to multimodal exposure, longevity and constant diet (Budnik and Baur, 2009; Tchounwou et al., 2012). The biological matrices used in biomonitoring are presented – hair and hoof as indicators of chronic exposure (Dunnett and Lees, 2003; Asano et al., 2002) and blood/serum as indicators of recent exposure (Fazio et al., 2020) – as well as the advantages of the multi-matrix approach. The chapter highlights the role of horses in assessing ecotoxicological risks and within the One Health concept (Lionetto et al., 2019).

Chapter 4 – Working hypothesis and research objectives formulates the general hypothesis according to which mining activities in north-western Romania have durably modified soil and water quality, favoring heavy metal mobility, their transfer into the trophic chain and bioaccumulation in horses, with the appearance of detectable biological responses. The chapter defines three complementary studies: hydrogeochemical evaluation of waters, physico-chemical characterization of soils and analysis of bioaccumulation and hematological response in horses, in order to validate them as a sentinel species.

Chapter 5 – Study I: Hydrogeochemical evaluation of waters from post-closure mining areas in north-western Romania analyzes the long-term impact of historical mining activities on water quality in north-western Romania, by comparing three areas selected according to the degree of anthropogenic influence: areas near tailings ponds, areas with former mining operations and an unaffected control area. The results show that in affected areas the water reaction is slightly acidic, with mean values of 6.1–6.5 pH units, compared to 7.4–7.6 pH units in the control area. Electrical conductivity is high in mining areas, about 840–980 µS/cm, compared to about 390 µS/cm in the control area, and total dissolved solids reach 530–650 mg/l compared to about 270 mg/l, indicating more than double mineralization. Turbidity is much higher in mining areas, with values of 12–17 NTU, compared to about 2 NTU in the control area, reflecting the input of fine particles from mining materials. Dissolved oxygen concentration is lower in affected areas, 4.4–5.5 mg/l, compared to 8.3 mg/l in the control area, and the oxidation–reduction potential is reduced, with values of 208–236 mV compared to over 310 mV. Statistical analysis highlighted a very strong correlation between electrical conductivity and total dissolved solids and inverse relationships between pH and oxidation potential and between dissolved oxygen and mineralization, demonstrating that waters from post-mining areas are more mineralized, less oxygenated and characterized by more reducing conditions, favorable to the mobilization and persistence of contaminants, while the control area shows a stable hydrogeochemical system, close to natural conditions.

Chapter 6 – Study II: Impact of waste dumps and tailings ponds on the physico-chemical properties of soils analyzes how waste dumps and tailings ponds influence the physico-chemical properties of soils in north-western Romania, by comparing three areas selected according to the degree of mining impact: soils from tailings ponds, soils from abandoned mining areas under natural recovery and soils from an unaffected control area. The results show that soils from tailings pond areas have an acidic character, with mean pH of 5.42, low organic matter content, about 1.12%, low cation exchange capacity, about 8.7 cmol(+)/kg, high electrical conductivity, about 478 µS/cm, and high bulk density, 1.54 g/cm^3, indicating chemically unstable soils and strongly structurally degraded. In abandoned mining areas, soils show intermediate values, with pH about 6.11, organic matter about 2.34%, cation exchange capacity about 12.3 cmol(+)/kg and bulk density 1.43 g/cm^3, reflecting an early stage of pedological recovery under the influence of spontaneous vegetation. The control area shows the most favorable conditions, with pH close to neutrality, 6.82, high organic matter, about 3.96%, cation exchange capacity about 17.5 cmol(+)/kg, low electrical conductivity, about 162 µS/cm, and low bulk density, 1.28 g/cm^3, characteristic of stable and fertile soils. Textural distribution indicates a transition from sandy loam soils in the tailings area, favorable to water loss and contaminant mobility, to clay loam soils in the control area, with higher retention capacity. The results demonstrate the existence of a clear gradient of soil degradation and recovery, controlled by the intensity of mining impact and natural stabilization processes, and confirm the role of soil as the main reservoir of contaminants and as a key factor in controlling their mobility in post-mining ecosystems.

Chapter 7 – Study III: Heavy metal bioaccumulation in horses raised in ecologically exposed areas – role of environmental factors analyzes heavy metal bioaccumulation in horses raised in areas with historical mining impact, by comparing three areas selected according to the degree of contamination: areas near tailings ponds, areas with former mining operations and an unaffected control area. The results highlight a clear gradient of exposure, with maximum values in mining areas and minimum in the control area, confirming the active transfer of metals from environmental compartments to the organism.

In hair, the best reflection of chronic exposure was observed, with copper values of about 10.18–18.91 mg/kg and zinc of 139.52–186.60 mg/kg in polluted areas, compared to 1.01–1.12 mg/kg copper and 48.56–51.36 mg/kg zinc in the control area. Lead reached up to 7.58 mg/kg and cadmium up to 1.41 mg/kg in mining areas, and nickel, arsenic and chromium showed higher levels than in the control, while mercury was not detected, confirming the long-term integration of contaminants into the hair structure.

The hoof confirmed chronic exposure through high values in contaminated areas, with copper between 4.80 and 11.00 mg/kg, zinc between 108.68 and 176.77 mg/kg and lead up to about 6.60 mg/kg, compared to much lower values in the control area. Nickel and cobalt were also higher in mining areas, and cadmium was not detected, arsenic, chromium and mercury being frequently below the detection limit, showing that the horny structure selectively integrates metals over the long term, especially in the hoof wall.

Serum mainly reflected recent exposure, with relatively similar values between areas for copper and zinc, but with significant differences for nickel and cobalt. Nickel varied between 1.40 and 1.83 µg/l in polluted areas, compared to about 0.17 µg/l in the control area, and cobalt was detected only in contaminated areas, with values of 0.22–0.37 µg/l. Lead, cadmium, arsenic, chromium and mercury were below the detection limit, indicating effective regulation and elimination mechanisms from circulation.

Synovial fluid had limited usefulness for toxic metals, only copper and zinc being constantly detected, with copper between 3.37 and 9.07 µg/l and zinc between 108.87 and 394.63 µg/l in polluted areas. Lead, cadmium, nickel, cobalt, arsenic, chromium and mercury were below the detection limit, suggesting that the joint space is not a major compartment for heavy metal accumulation.

Water samples indicated significant contamination in mining areas, with copper about 3.10–3.50 mg/l, above the legal limit, lead about 0.04 mg/l and nickel 0.10–0.11 mg/l, several times above allowed thresholds. Zinc had high values, close to the upper acceptable limit, and cadmium, arsenic and mercury were below the detection limit. In the control area, all values were below legal limits.

Soil behaved as the main reservoir of contamination, with copper about 74.70 mg/kg in Zone I and 156.71 mg/kg in Zone II, zinc over 350–416 mg/kg and lead and cadmium above allowed limits. Nickel and cobalt showed moderate exceedances, and arsenic, chromium and mercury were below the detection limit. The control area showed values close to natural background.

Forage reflected the transfer of metals from soil and water into the trophic chain. Grass and hay from mining areas had copper about 6.35–12.82 mg/kg, zinc 109–141 mg/kg and lead up to 5.61 mg/kg, tens of times higher than in the control area. Cadmium was present in small amounts, but significantly higher than in the control, and nickel was detected only in contaminated areas. Concentrates from the control area had low and uniform values, confirming that forage is the main link of metal transfer from the environment to horses.

Chapter 8 – General conclusions formulates the final conclusions of the thesis and shows that historical mining activities have produced persistent environmental changes that favor heavy metal mobility and their transfer into the trophic chain. Waters from mining areas are more acidic, less oxygenated and more redox-unstable than in the control area, which maintains a constant risk of metal remobilization. Soils near tailings ponds are acidic, poor in organic matter and with low cation exchange capacity, functioning as the main reservoir of historical contamination. Forages grown on these soils take up metals and transfer them to animals. Exposed horses show progressive metal accumulation, with clear differences between contaminated areas and the control area; hair and hoof are indicators of long-term exposure, while serum and synovial fluid mainly reflect recent exposure. The general conclusion is that only the integrated environment–organism approach allows correct assessment of ecotoxicological risk and confirms the role of horses as sentinel species for biomonitoring post-closure mining areas.

Chapter 9 – Originality and innovative contributions presents the elements of originality of the thesis and shows that the work brings an integrated approach, correlating water, soil, forage and several biological matrices in horses. The chapter highlights the simultaneous use of hair, hoof, serum and synovial fluid to differentiate chronic and recent exposure, an approach rarely applied in Romania. It is shown that the application of inductively coupled plasma mass spectrometry and rigorous analytical validation represent an important methodological contribution. The chapter emphasizes that the thesis provides new data for post-closure mining areas in north-western Romania and demonstrates the value of horses as sentinel species. Finally, the chapter shows that the results can be used for biomonitoring programs, ecotoxicological risk management and ecological rehabilitation.

Bibliography

1. Asano, R.; Suzuki, K.; Otsuka, T.; Otsuka, M.; Sakurai, H. Concentrations of Toxic Metals and Essential Minerals in the Mane Hair of Healthy Racing Horses and Their Relation to Age. Journal of Veterinary Medical Science 2002, 64, 607–610,
2. Ashraf, M.A.; Maah, M.J.; Yusoff, I. Chemical Speciation and Potential Mobility of Heavy Metals in the Soil of Former Tin Mining Catchment. ScientificWorldJournal 2012, 2012.
3. Bora, F.D.; Babeș, A.C.; Călugăr, A.; Jitea, M.I.; Hoble, A.; Filimon, R.V.; Bunea, A.; Nicolescu, A.; Bunea, C.I. Unravelling Heavy Metal Dynamics in Soil and Honey: A Case Study from Maramureș Region, Romania. Foods 2023, 12, 3577,.
4. Budnik, L.T.; Baur, X. The Assessment of Environmental and Occupational Exposure to Hazardous Substances by Biomonitoring. Dtsch Arztebl Int 2009, 106, 91–97.
5. Chojnacka, K.; Górecka, H.; Górecki, H. The Effect of Age, Sex, Smoking Habit and Hair Color on the Composition of Hair. Environ Toxicol Pharmacol 2006, 22, 52–57,.
6. Combs, D.K.; Goodrich, R.D.; Meiske, J.C. Mineral Concentrations in Hair as Indicators of Mineral Status: A Review. J Anim Sci 1982, 54, 391–398,
7. Doğan, E.; Fazio, F.; Aragona, F.; Nava, V.; De Caro, S.; Zumbo, A. Toxic Element (As, Cd, Pb and Hg) Biodistribution and Blood Biomarkers in Barbaresca Sheep Raised in Sicily: One Health Preliminary Study. Environmental Science and Pollution Research 2024, 31, 43903–43912,
8. Dunnett, M.; Lees, P. Trace Element, Toxin and Drug Elimination in Hair with Particular Reference to the Horse. Res Vet Sci 2003, 75, 89–101,

9. Fazio, F.; Cicero, N.; Piccione, G.; Giannetto, C.; Licata, P. Blood Response to Mercury Exposure in Athletic Horse From Messina, Italy. J Equine Vet Sci 2020, 84, 102837.

10. Fazio, F.; Gugliandolo, E.; Nava, V.; Piccione, G.; Giannetto, C.; Licata, P. Bioaccumulation of Mineral Elements in Different Biological Substrates of Athletic Horse from Messina, Italy. Animals 2020, Vol. 10, Page 1877 2020, 10, 1877.

11. Giannetto, C.; Fazio, F.; Nava, V.; Arfuso, F.; Piccione, G.; Coelho, C.; Gugliandolo, E.; Licata, P. Data on Multiple Regression Analysis between Boron, Nickel, Arsenic, Antimony, and Biological Substrates in Horses: The Role of Hematological Biomarkers. J Biochem Mol Toxicol 2022, 36, e22955,

12. Hu, G.; Bakhtavar, E.; Hewage, K.; Mohseni, M.; Sadiq, R. Heavy Metals Risk Assessment in Drinking Water: An Integrated Probabilistic-Fuzzy Approach. J Environ Manage 2019, 250, 109514.

13. Jaishankar, M.; Tseten, T.; Anbalagan, N.; Mathew, B.B.; Beeregowda, K.N. Toxicity, Mechanism and Health Effects of Some Heavy Metals. Interdiscip Toxicol 2014, 7, 60–72

14. Järup, L. Hazards of Heavy Metal Contamination. Br Med Bull 2003, 68, 167–182,

15. Jomova, K.; Valko, M. Advances in Metal-Induced Oxidative Stress and Human Disease. Toxicology 2011, 283, 65–87.

16. Kabata-Pendias, A.; Mukherjee, A.B. Trace Elements from Soil to Human. Trace Elements from Soil to Human 2007, 1–550.

17. Kincaid, R.L. Assessment of Trace Mineral Status of Ruminants: A Review. J Anim Sci 2000, 77, 1–10, doi:10.2527/JAS2000.77E-SUPPL1X.

18. Koyama, H., Kamogashira, T., & Yamasoba, T. (2024). Heavy Metal Exposure: Molecular Pathways, Clinical Implications, and Protective Strategies. Antioxidants, 13(1), 76.

19. Lionetto, M.G.; Caricato, R.; Giordano, M.E. Pollution Biomarkers in Environmental and Human Biomonitoring. Open Biomark J 2019, 9, 1–9,.

20. Roggeman, S.; Van Den Brink, N.; Van Praet, N.; Blust, R.; Bervoets, L. Metal Exposure and Accumulation Patterns in Free-Range Cows (Bos Taurus) in a Contaminated Natural Area: Influence of Spatial and Social Behavior. Environmental Pollution 2013,

21. Tchounwou, P.B.; Yedjou, C.G.; Patlolla, A.K.; Sutton, D.J. Heavy Metal Toxicity and the Environment. EXS 2012, 101, 133–164