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CHALIGAVA OMARI

**APPLICATION OF MOSSES AS BIOINDICATORS FOR
EVALUATION OF ECOLOGICAL STATE OF AIR IN
DIFFICULT CLIMATIC AND RELIEF CONDITIONS**

166.01. ECOLOGY

Abstract of PhD thesis in environmental science

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The dissertation was performed in the Doctoral School of Natural Sciences of the Moldova State University, and in the Institute of Microbiology and Biotechnology of the Technical University of Moldova.

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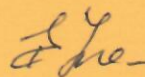
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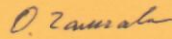
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CONCEPTUAL LANDMARKS OF THE RESEARCH

The importance and relevance of the topic:

Atmospheric air quality is a critical determinant of human health, shaped by anthropogenic emissions and natural factors. According to the World Health Organization, exposure to particulate matter (PM), causes approximately 4.2 million deaths annually. Fine particulate matter is considered the most harmful air pollutant due to its ability to penetrate deeply into the respiratory system and serve as an efficient carrier of toxic substances, including trace metals [1, 2]. The distribution, transport, and deposition of pollutants are strongly influenced by climatic conditions and topographical features. Precipitation, temperature, wind, and atmospheric stability affect wet and dry deposition [3], while relief characteristics, modify air mass circulation, pollutant dispersion, and accumulation zones [4]. As a result, regions with contrasting physico-geographical conditions may exhibit substantially different patterns of pollutant deposition even under comparable emission scenarios.

Heavy metals and metalloids, like Al, As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Sb, V, and Zn are considered critically hazardous even at trace concentrations due to their toxicity and bioaccumulation potential [5]. While some (e.g., Cu, Fe, Zn) are essential micronutrients, others (e.g., Cd, Hg, Pb, As) are toxic at minimal exposure. Their environmental behavior depends on chemical speciation, solubility, and redox state, which influence mobility and bioavailability. Multiple industrial, domestic, agricultural, and other applications, as well as soil erosion, deforestation, and land-use changes, have significantly elevated metal concentrations in airborne PM. Once emitted, metals attach to PM and undergo long-range transport [5].

In mountainous regions, orographic effects enhance precipitation and wet deposition, while valleys act as accumulation zones due to reduced air circulation and temperature inversions. Lowland terrains may be more exposed to long-range transport of pollutants and resuspended dust [6]. These differences raise important questions regarding the comparability of monitoring results across regions with distinct climatic and geomorphological characteristics.

Conventional monitoring typically requires expensive instrumentation, stable power, and skilled personnel, while the sparse distribution of monitoring stations reduces spatial representativeness. Furthermore, data reflect short-term rather than cumulative exposure, highlighting the need for cost-effective complementary approaches. In Republic of Moldova, a network of 17 stations is located in five urban centers [7]. Air samples are collected manually, only three times per day, and analyzed for multiple pollutants, including PM, SO₂, CO, NO₂, soluble sulfates, NO, phenol, and formaldehyde [7]. In Georgia, indicative measurements have

been carried out quarterly across 25 cities, while an automatic network has expanded from 7 to 15 stations since 2019 and now includes heavy metal measurements. Consequently, high operational costs and low station prevalence make traditional monitoring expensive and less informative for assessing pollution spread over large territories.

Biomonitoring using mosses is an adequate low-cost approach applicable for large territories, which increases confidence in estimation. Mosses absorb nutrients and contaminants directly from atmospheric deposition, making them suitable passive samplers. Since 2001, the European biomonitoring program has been coordinated by the International Cooperative Program on Effects of Air Pollution on Natural Vegetation and Crops (UNECE ICP Vegetation) under the Convention on Long-range Transboundary Air Pollution (LRTAP Convention), conducting five-yearly continent-wide surveys [8]. Republic of Moldova, and Georgia joined the program in 2015. Both countries showed higher elemental levels compared to other participating countries in the program [8]. Republic of Moldova and Georgia share several relevant characteristics a temperate zone, influence from the Black Sea, soil erosion as a main source of atmospheric elements, and moss species available. At the same time, these countries have many differences: Republic of Moldova is relatively lowland while Georgia is mountainous. Moldova is predominantly rural with 74% agricultural land, versus only 43% in Georgia [9, 10]. These geographic and economic contrasts provide a valuable framework for comparative analysis.

The aim of the study:

To elucidate the effectiveness of the moss biomonitoring method for assessing the quality of atmospheric air in areas with different physico-geographical conditions: case study of Republic of Moldova and Georgia.

Main objectives:

- Carrying out passive moss biomonitoring in the Republic of Moldova and Georgia, regions characterized by contrasting climatic and relief conditions.
- Determination of the elemental composition of moss samples and comparative analysis of their spatial and temporal variability.
- Identification and differentiation of natural (geogenic) and anthropogenic sources of atmospheric air pollution in the studied regions.
- Assessment of the influence of climatic and topographical factors (precipitation, altitude, slope) on the accumulation of elements in mosses.
- Elaboration of principles for establishing background levels of pollution under different climatic and relief conditions.

- Development of recommendations for the application and interpretation of moss biomonitoring results in regions with contrasting physico-geographical characteristics.

The hypothesis of the research:

Moss biomonitoring captures spatiotemporal variability in atmospheric deposition through the integrated effects of climate and relief on trace element accumulation, and, when coupled with advanced multivariate statistics and supplementary environmental data, enables robust discrimination between geogenic and anthropogenic sources across diverse climatic and geomorphological gradients.

The synthesis of the research methodology and justification of the research methods:

A nationwide assessment of atmospheric heavy metal and metalloid pollution was conducted using moss biomonitoring. This method enabled a spatially comprehensive evaluation of pollution. Initial sampling in Georgia utilized multiple moss species to achieve maximum territorial coverage, while in the Republic of Moldova only one species was used. The elemental analysis of moss tissues was performed using complementary analytical methods: Instrumental Neutron Activation Analysis (INAA), Atomic Absorption Spectrometry (AAS), and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). To isolate the effects of environmental variables on metal accumulation in mosses, a comparison was designed between two countries with distinct climates and geologies. For this controlled comparison, a single moss species was selected to eliminate interspecies variability. Furthermore, to isolate the influence of natural environmental factors and minimize distortion from samples associated with direct anthropogenic activity or local pollution hotspots, the Grubbs' test was applied to identify and exclude statistical outliers. A comprehensive multivariate statistical analysis was performed to extract meaningful patterns from the compositional data. Principal Component Analysis (PCA) served to reduce dimensionality and identify latent factors representing common pollution sources. Associations between elemental concentrations and key environmental variables were evaluated using Spearman's rank correlation coefficient. Finally, the non-parametric Kruskal-Wallis test was applied to detect statistically significant differences in metal accumulation across different geographical or ecological groupings.

The novelty and scientific originality: The novelty of the work consists in the integrated evaluation of moss biomonitoring efficiency under contrasting climatic and relief conditions, using a comparative approach between the Republic of Moldova and Georgia. The study demonstrates, for the first time, the decisive role of topoclimatic factors (precipitation, altitude, terrain) in controlling atmospheric deposition patterns and element accumulation in mosses, beyond the influence of emission sources.

The theoretical importance of the work lies in extending the conceptual framework of biomonitoring by incorporating climatic and relief factors as key drivers of pollutant transport and accumulation. The research contributes to a deeper understanding of the mechanisms of atmospheric deposition, including dry and wet processes, and their interaction with environmental conditions in shaping geochemical patterns in biomonitors.

The applied value of the work consists in the development of methodological recommendations for the interpretation of moss biomonitoring data in regions with different climatic and geomorphological conditions. The results support the establishment of region-specific background levels, improvement of environmental monitoring strategies, and more accurate identification of pollution sources, providing a basis for environmental management and policy development.

1. MOSS BIOMONITORING AS EFFICIENT TOOL OF THE AIR POLLUTION ASSESSMENT

The first chapter presents the scientific foundations of air pollution assessment, beginning with the characterization of atmospheric pollutants as a complex mixture. It details how air pollution, particularly airborne PM, acts as a vector for potentially toxic elements (PTEs) whose health impacts are governed by particle size, from PM₁₀ to ultrafine particles, and metal speciation. It discusses how meteorology, topography, and emission source interactions control the spatial distribution of contaminants, while noting the limitations of traditional, costly monitoring networks. The chapter then establishes the principles of moss biomonitoring as a cost-effective alternative, highlighting the unique biological characteristics of mosses, namely their lack of roots, weakly developed cuticle, and direct uptake from wet and dry deposition, that make them cost-effective instruments for tracking pollution deposition at local, regional, and continental scales within international programs like ICP Vegetation.

2. MATERIALS AND METHODS

The methodological framework developed in this study provides a comprehensive and robust basis for assessing atmospheric pollution using moss biomonitoring. The combination of standardized sampling protocols, complementary methods for elemental analysis, and rigorous quality control ensures the reliability and accuracy of the obtained data.

The comparative design, involving two countries with different climatic and geomorphological conditions, enables the investigation of environmental factors influencing pollutant accumulation. The integration of statistical tools such as correlation analysis,

nonparametric tests, PCA, and Positive Matrix Factorization (PMF) allows for the identification of patterns, relationships, and potential pollution sources within complex datasets. The inclusion of spatial analysis through Geographic Information System (GIS), together with environmental parameters such as climate classification, altitude, slope, precipitation, and population density, enhances the interpretability of the results and supports the evaluation of environmental drivers of pollution distribution. The applied methodology is suitable for large-scale environmental assessment and provides a solid foundation for the analysis and discussion of results presented in the subsequent chapters.

3. THE ASSESSMENT OF HEAVY METALS DEPOSITION IN GEORGIA USING MOSS BIOMONITORING APPROACH

This chapter presents the results of Georgia's first comprehensive moss biomonitoring program, designed to assess the deposition of trace elements from both geogenic and anthropogenic sources. Two nationwide surveys (2014–2017 and 2019–2023) were conducted, employing analytical techniques like INAA, AAS, and ICP-OES to measure element concentrations. The influence of factors including species type, sampling location, and regional climate was evaluated, while analytical and statistical models, including Spearman correlation, PCA, PMF, and ecological risk indices were calculated to understand the nature, source and potential risks of the pollution. Temporal comparisons were made using the Mann–Whitney U test and by examining contamination factor shifts between periods.

3.1. First Comprehensive Baseline Moss Survey (2014–2017) in Georgia

For the first time in Georgia, a comprehensive baseline dataset for 41 elements was established through the analysis of 120 moss samples (*Hylocomium splendens* (Hedw.) Schimp., *Hypnum cupressiforme* Hedw., and *Pleurozium schreberi* (Brid.) Mitt.), which were collected across nearly the entire country between 2014 and 2017 [11]. Two complementary analytical techniques, INAA and AAS, were employed for the analysis of the collected moss species.

The mean Al content was determined to be 5137 ± 3707 mg/kg, ranging from 759 mg/kg at site No. 23 (Abastumani District, Zekari Pass) to 24500 mg/kg at site No. 114 (Imereti, Chiatura). Peak concentrations for Zr (mean 13.1 ± 10.7 mg/kg), along with As, Th, and U, were found to be proximal to the Tkibuli coal mine (No. 83), reflecting mining-related mobilization. The Chiatura mining region in western Georgia sits within one of the world's largest sedimentary manganese ore basins [12]. In our study, maximum concentrations of Mn (2530, 2470, and 2010 mg/kg) were observed in 2017 at the sites No. 113, 114, and 112. A single As outlier of 83.30 mg/kg at site No. 87 is attributed to the historical arsenic mining complex in Uravi village, where

soil As concentrations reach up to 164,000 mg/kg [13]. The overall mean As concentration was 2.02 ± 7.56 mg/kg (range: 0.18–83.30 mg/kg). Titanium averaged 458 ± 377 mg/kg, peaking near Tkibuli (No. 82) due to coal mining impacts. Fe averaged 3631 ± 2599 mg/kg, with a maximum of 14100 mg/kg in the Borjomi District (No.8) attributed to crustal sources.

CV% ranged from 30–85% for most elements, but was markedly higher for Cu (186%), As (238%), Mn (162%), Ti (105%), and V (104%), indicating non-normal distributions driven by external factors. Strong positive correlations ($r > 0.95$) among Ti-V, Cr-Co-Fe-Ni, and REE groupings suggest common geochemical origins; U and Th showed $r > 0.6$ (Figure 3.1).

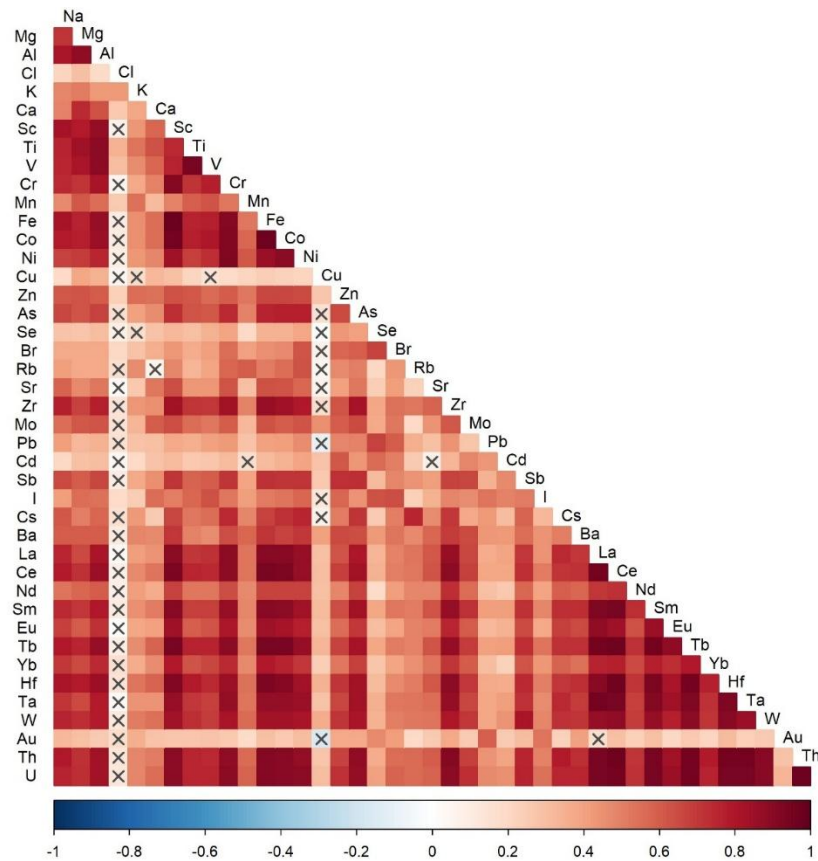


Figure 3.1. Spearman correlation matrix of measured variables from the first moss survey in Georgia.

No statistically significant differences in mean elemental values were found among the species, except for the pair *Hylocomium splendens* vs. *Hypnum cupressiforme* ($p = 0.04$).

The median values determined in this work were found to be in good agreement with those published for Macedonia [14], Bulgaria [15], and Norway [16]. Georgia exhibits the highest median concentrations for Na (581 ± 235 mg/kg), Mg (3060 ± 770 mg/kg), and Al (4295 ± 1500 mg/kg) compared to Macedonia, Bulgaria, and Norway. Georgia also leads in V (9.4 ± 4.12 mg/kg), Cr (7.75 ± 2.65 mg/kg), Fe (2725 ± 1050 mg/kg), Co (1.43 ± 0.61 mg/kg), and Ni (5.56 ± 1.8 mg/kg), with Georgian medians approximately 2–10 times higher than those of comparable

countries. Manganese, however, is highest in Norway (400 mg/kg), followed by Bulgaria (180 mg/kg), North Macedonia (160 mg/kg), and Georgia (141 ± 54.7 mg/kg). For Pb, Bulgaria leads at 10.7 mg/kg, ahead of Georgia (5.57 ± 2.33 mg/kg) and North Macedonia (4.9 mg/kg), while Norway's median is exceptionally low at 0.05 mg/kg.

Potential Ecological Risk Index (PER) assessment showed that Zn, Cr, Pb, Ni, and Cu posed negligible ecological risk, whereas considerable risk was identified for As and Cd, linked to mining activities at Uravi, Kazreti, and Chiatura. Peak Pollution Load Index (PLI) values (Figure 3.2) were recorded at four sampling locations: site No. 38 near arsenic-contaminated waste deposits; site No. 53 near Telavi, influenced by construction material facilities; site No. 64, near asphalt and construction plants; and site No. 12, attributed to local Pleistocene volcanic geology. Secondary clusters of elevated risk were identified near the Kutaisi and Zestafoni industrial plants, Tkibuli coal mines, and the Chiatura mining complex.

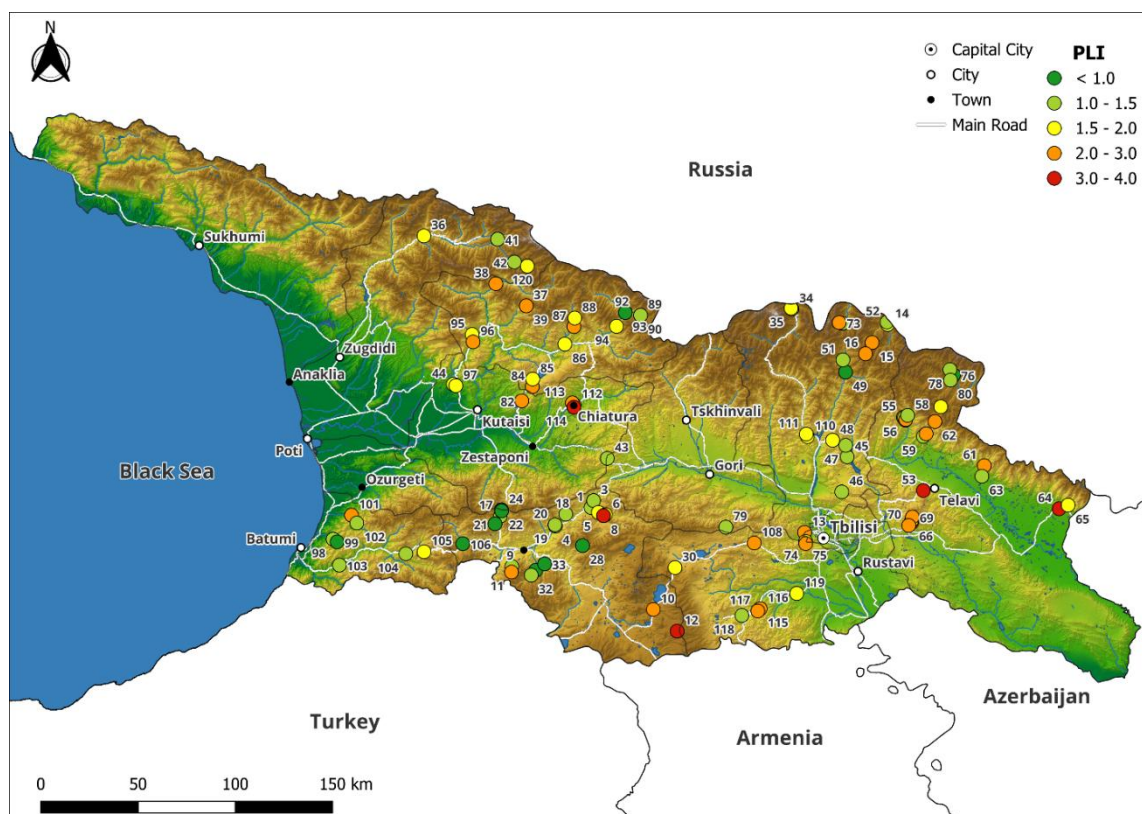


Figure 3.2. Spatial distribution of the Pollution Load Index (PLI) across the first moss survey, Georgia.

PCA explained 51.86% of cumulative variance across three components. Three clusters were revealed by the analysis. Cluster 1 (Na, Al, Sc, Ti, V, Fe, Co) was defined as geogenic elements with anthropogenic association. Cluster 2, a mixture of geogenic and anthropogenic sources, grouped elements with shared geochemical behavior (Th:U, Hf:Zr, REEs) and included As, whose presence is influenced by nearby arsenic mining. Cluster 3 (Mg, Cl, K, Ca, Mn, Ni, Zn,

Br, Rb, Sr, I, Ba) was composed primarily of crustal elements. Within this cluster, Br and I were attributed to marine aerosol transport, and elevated Mn concentrations were associated with regional manganese mining.

In summary, the weathering and transport of elements from marine and mountainous sources are significantly influenced by predominant wind directions, the orographic effects of the Greater and Lesser Caucasus Mountains, and the distinct climatic zones of Eastern and Western Georgia, affecting the atmospheric accumulation of trace elements by moss species, while crustal rock and soil associations reveal a significant geogenic contribution.

3.2. The Second Moss Survey in Georgia: Validation and Expansion (2019–2023)

During the second moss survey (2019–2023) samples were collected from 95 distinct sites. The dominant species was *Hypnum cupressiforme*; 15 elements were determined by ICP-OES and Hg by DMA-80. The dataset is non-normally distributed and positively skewed, with the highest variability for Pb (CV% = 94%) and Mn (CV% = 74%). Elements like S (26%), Zn (30%), and Sr (31%) showed the lowest CVs, indicating diffuse or uniform sources.

Compared to Armenia [17], Georgia shows generally lower median concentrations. Armenia exhibits notably elevated Ba, Co, Cr, Cu, Fe, and V, with Cr and Fe nearly six and four times higher than Georgia's medians. This is attributable to lower forest coverage in Armenia (11.1% vs. Georgia's 40.6%), enhancing wind erosion and dust loading, and a methodological bias, as Armenian studies predominantly used acrocarpous moss species rather than the recommended pleurocarpous species. In Kazakhstan [18] (using *Hylocomium splendens*) as shown substantially higher median concentrations for most elements — Al five times higher (10550 vs. 2131 mg/kg) — reflecting heavy industrial activity and soil dust contributions.

Comparison with the 2015 Georgian survey indicates a general decline in median metal concentrations. Key decreases include Al (4295→2131 mg/kg), Cr (7.75→3.71 mg/kg), and Fe (2725→1826 mg/kg); Cd and Zn remained stable, while Cu increased from 5.54 to 7.06 mg/kg. Statistical evaluation *via* the Mann-Whitney U test revealed significant differences ($p < 0.05$) between survey periods for all elements except Cd, Pb, and Zn.

Strong correlations among Cr, Co, V, Al, and Fe ($r = 0.74$ – 0.91) indicate a common geogenic origin, while the Cu-Zn-Pb-Cd grouping points to vehicle emissions as a significant anthropogenic source. PCA (Figure 3.3) revealed that PC1 (43.4% of variance) represents a geogenic signature (Al, Co, Cr, Fe, V), with highest loadings near agricultural field sites, reflecting soil dust from farming practices. PC2 (13.3%) reflects anthropogenic pollution — Cd, Hg, Mn, Pb, and Zn — can be emitted from highways, urban areas, and the Zestafoni ferro-alloy plant.

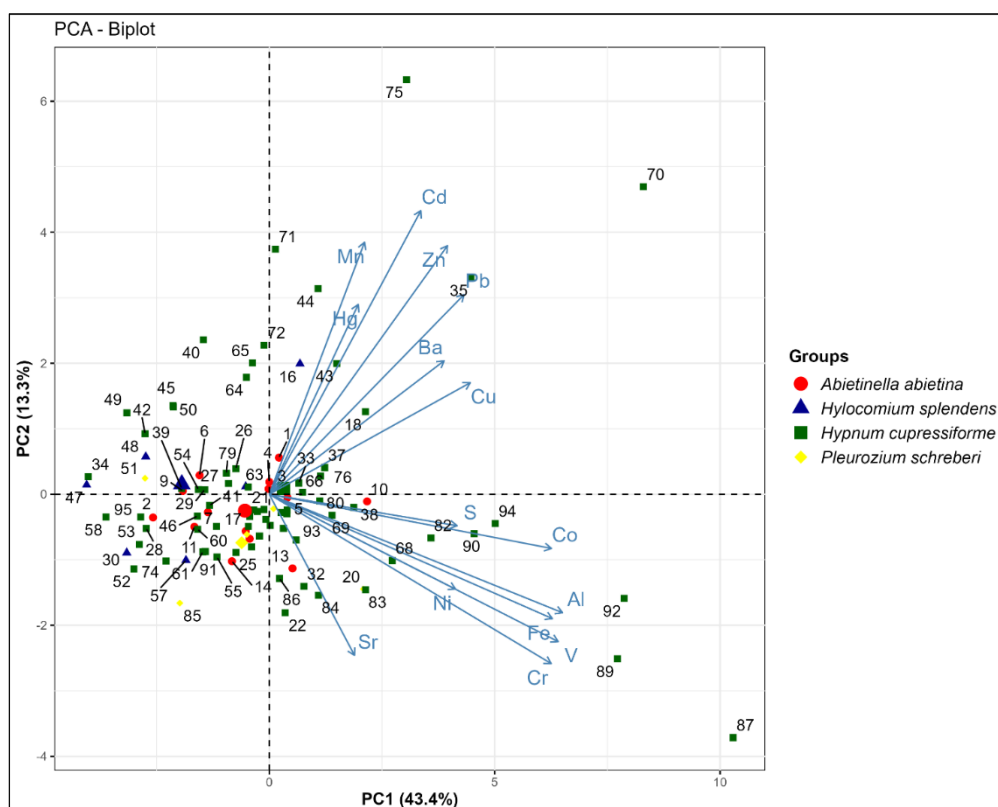


Figure 3.3. Biplot of PC1 and PC2 denote the first two principal components explaining 56.7% of the variance in the data. Each point, distinguished by a unique combination of color and symbol, represents a sample of one of the four species. Arrows emanate from the origin, representing the variables.

PMF supported PCA results and identified three source factors: (1) soil dust (crustal — high Al, V, Cr, Fe, Co loadings); (2) anthropogenic sources — mining and industry (high Hg, S, Cu, Zn); and (3) a mixed geogenic-anthropogenic factor dominated by Mn, Ba, and Cd.

Most median Contamination factors (CFs) declined between surveys, indicating reduced contamination. Copper is a notable exception. the median increased from 1.04 to 1.29, moving from the "No Contamination" threshold firmly into the "Suspected" category. The most pronounced CF decreases were for Cd (1.86→0.98) and Ni (1.69→1.17), both approaching "No Contamination." Ba, Co, Mn, and V also showed substantial reductions. The mean PLI fell from 1.74 to 1.41 and median from 1.50 to 1.34, both within the "Minimal to moderate pollution" category. Mn pollution remains acute near Zestafoni, where the Ferroalloy Plant processes Chiatúra ore. Mn concentrations in mosses near Chiatúra mines were approximately 2.4 times lower than near the Zestafoni plant.

Elevated CFs for Pb near Kutaisi and Lopota are attributed to transport emissions, industry, and near Lopota HPP construction, with upstream sediment enrichment as a contributing factor. The highest PLI values were at sites No. 87 (Heretiskari, PLI = 3.21) and No. 70 (Kutaisi, PLI =

3.06). Kutaisi site is 3 km from an industrial zone with high PM levels, while Heretiskari's high PLI is attributed to wind-transported soil dust and river sediment from an old riverbed.

According to the PER (Figure 3.4), significant risks were posed by Cd and Hg at several sites, with medium Pb risk at two samples. Thus, a significant PER (112) for Cd was found at coastal site No. 65 in Anaklia, can be attributed to dust from port construction debris. Kutaisi and Zestafoni had significant Cd PER scores of 99 and 89, respectively. Site No. 40, where the highest Hg concentration of 0.113 mg/kg lies ~5 km from the Savane quartz sand quarry. An elevated PER of 125 for Hg was associated with sample No. 35 from Rikoti Pass, where anthropogenic Hg emissions are from vehicle exhaust, brake and tire abrasion, and geological disturbance through drilling and blasting during major road construction.

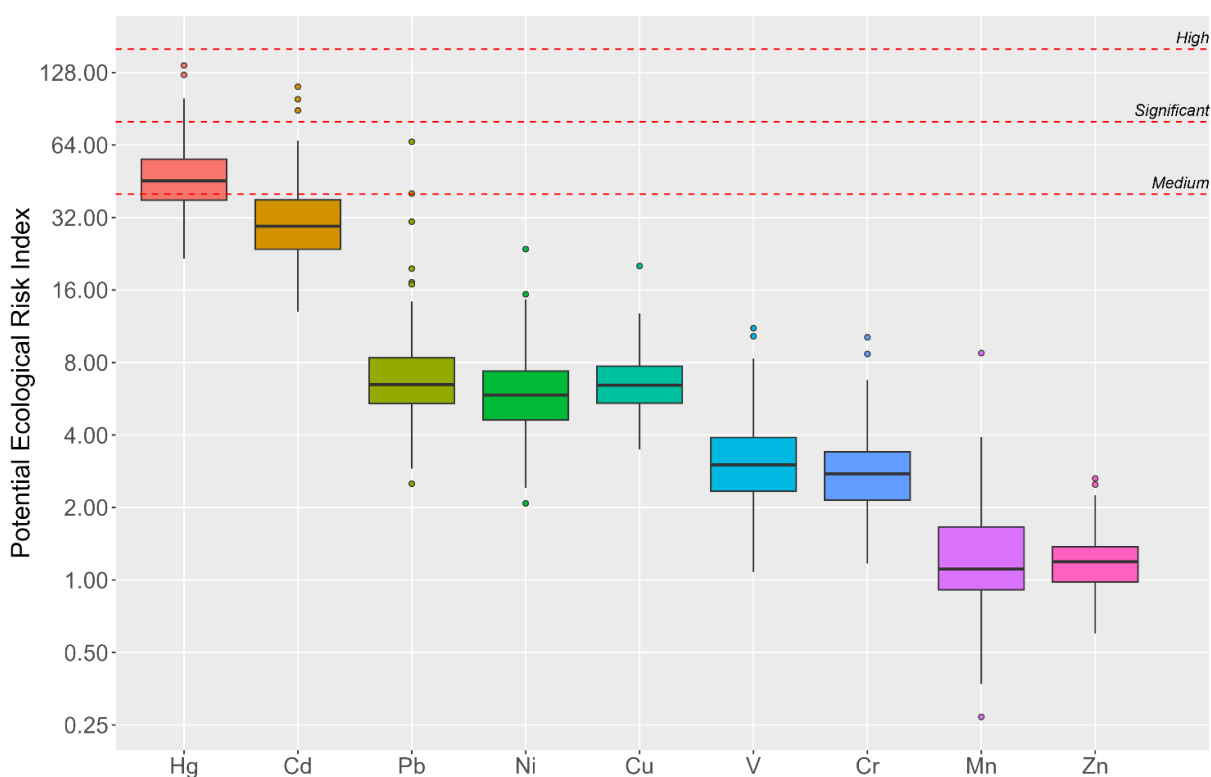


Figure 3.4. Boxplots of Potential Ecological Risk Index (PER) for selected elements accumulated by mosses.

The Ecological Risk Index (RI) indicated low ecological risk across most sampling locations, with moderate risk confined to 16 samples (values 150.5–254.7), the highest at Kutaisi (No. 70) and Rikoti Pass (No. 35).

4. MOSS BIOMONITORING OF ATMOSPHERIC HEAVY METAL DEPOSITION IN THE REPUBLIC OF MOLDOVA

This chapter presents a second national moss biomonitoring survey in Moldova designed to evaluate the spatial distribution and sources of atmospheric deposition of heavy metals and trace

elements. *Hypnum cupressiforme* moss samples were collected from 41 locations nationwide in 2020 and subjected to multi-element analysis *via* NAA and AAS. To interpret the data, descriptive statistics were calculated, and pollution assessment tools such as the CF, PLI, and PER were applied. Source apportionment was performed using factor analysis with Varimax rotation, PCA, and Spearman correlation, while temporal dynamics were investigated by comparing the 2020 data to a previous national survey from 2015/2016 [19], with significant differences tested using non-parametric statistics.

Potassium levels remained remarkably consistent, with the 2020 median (7250 ± 1000 mg/kg) aligning closely with the 2015 value (7100 ± 1500 mg/kg). Minor increases were observed for Al (3400 ± 1200 mg/kg, up from 3120 ± 1100 mg/kg) and Fe (2200 ± 600 mg/kg, up from 2100 ± 900 mg/kg). Calcium experienced a slight decline to 9300 ± 1500 mg/kg from 9900 ± 1100 mg/kg. The most striking reductions were observed for Pb and Cd. The 2020 median for Cd (0.12 ± 0.04 mg/kg) is markedly lower than the 2015 value (0.39 ± 0.08 mg/kg), while Pb fell to 3.1 ± 0.4 mg/kg from 12 ± 2.5 mg/kg. Copper also showed a strong downward trend, falling to 8.7 ± 1.0 mg/kg from 15 ± 3.0 mg/kg. Chromium and As showed more moderate declines, while Zn remained relatively stable (2020: 39 ± 7 mg/kg vs. 2015: 37.2 ± 8.5 mg/kg). Trace elements, such as La, Ce, and Sm, exhibited consistent medians between the two survey periods.

Spatial analysis revealed pronounced spatial variability in Al content, with the highest levels detected near Balti (11700 mg/kg), Comrat (10600 mg/kg), Briceni (9520 mg/kg), and Chisinau (9180 mg/kg) [20]. A comparable pattern was observed for Fe, elevated notably near Edinet (7810 mg/kg), Balti (6930 mg/kg), and Chisinau (6150 mg/kg) [20], attributable to resuspended road dust, lithogenic inputs, coal combustion, and motor vehicle emissions. The highest Zn content was recorded near Cahul (86 mg/kg), followed by Rezina, Vadul lui Voda, and Chisinau, primarily associated with vehicular activities. Elevated Cu content, especially in the northern and central regions, can be attributed to copper-containing pesticides widely used in this predominantly agricultural country. The spatial distribution of Pb indicated greater pollution in the central region, particularly Chisinau, Rezina, Balti, and Drochia [20]. The highest Cd content was detected around Cahul (0.56 mg/kg) and Rezina (0.42 mg/kg). The moss bioindicator data specifically highlight the Balti region, where concentrations of nearly all analyzed elements reached peak national levels, attributable to a high density of industrial point sources including the Combined Heat and Power Plant "North", machinery manufacturers, and significant operations in construction materials, textiles, leather processing, and petroleum storage [20].

Comparisons between the two surveys *via* the Mann–Whitney U test showed no significant differences ($p > 0.05$) for most elements. Significant differences ($p < 0.05$) were observed for Cr,

As, Se, Br, Sb, Cd, Pb, and Cu, all exhibiting lower median values in 2020, potentially attributable to altered bioavailability driven by differing wet and dry deposition rates.

Four factors were extracted from factor analysis, encompassing 84% of the variability (Figure 4.1). Factor 1 (47% of variance) represents a terrigenous source, supported by high loadings of crustal tracer elements (Al, Sc, Ti, Th) and additionally including Na, Cr, Fe, Co, Ni, As, Rb, Sb, Cs, and U. Magnesium, Ca, and Sr are linked to Factor 2 (14% of variance), with mineral extraction as the probable source across Moldova's approximately 900 quarries. Factor 3 (12% of variance) is associated with agricultural activities (Cl, K, Br, Cu), with Br and Cl being common components of fungicides and potassium fertilizers [21]. Factor 4 (11% of variance) presents an anthropogenic fingerprint of fossil fuel combustion, marked by Zn, Sb, Cd, and Pb, with the highest concentrations in the north and north-east of the country.

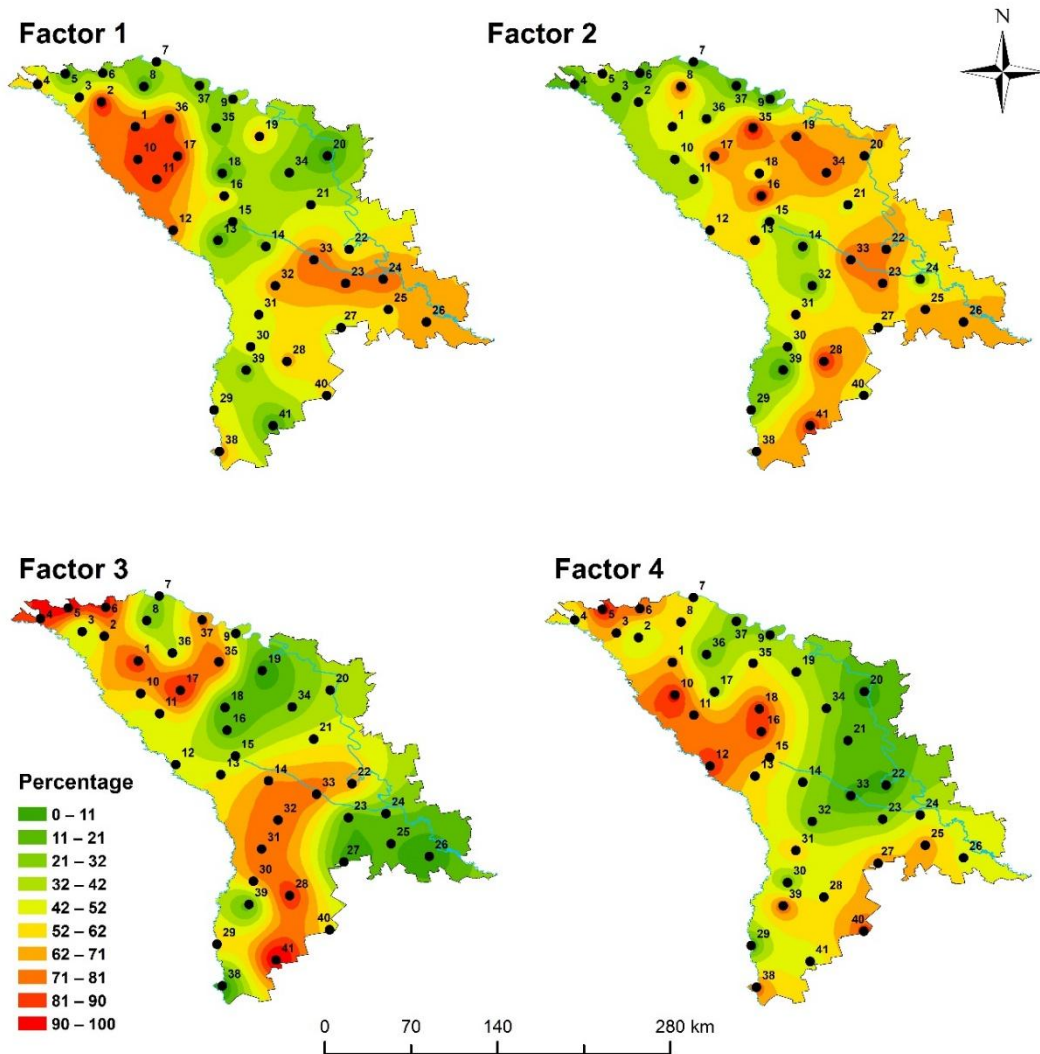


Figure 4.1. Map showing spatial distributions of Factors 1-4.

Application of the Wilcoxon test to identical sampling sites revealed significant ($p < 0.05$) shifts for Cr, As, Sb, Cd, Pb, and Cu. A general downward trend was evident, with reductions most

substantial for Pb (75% decrease) and Cd (66% decrease) (Figure 4.2). Moderate declines were recorded for Cu (43%), and V and Cr (31% each), while Zn showed a slight increase of 8.9%.

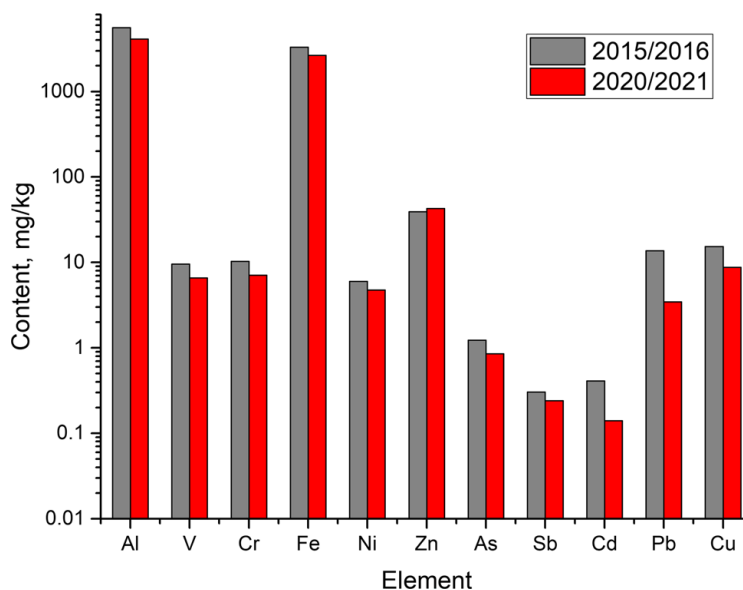


Figure 4.2. Comparison of the results from two moss surveys in Moldova

PCA identified key gradients and clusters in the previous and current Moldavian moss surveys. The consistent strong correlations for specific element groups in both surveys indicate persistent common emission sources. In 2015, distinct correlation clusters were identified: one for Cu and Sb, and another for Ni, Cr, Fe, Al, V, and As. The 2020 survey identified Chisinau as a probable source for Cu, Ni, and Sb, with additional correlations for V-Al near Comrat and As-Fe-Cr near Edineț and Bălți. These sources are attributed to resuspended soil particles, thermal power generation, vehicular transport, and industrial activity [20].

The median content of Al in Moldavian mosses (3400 mg/kg) is substantially higher than in any neighboring country. Elements such as As, Ni, V, and Cr are also found in their highest concentrations within Moldova. Conversely, Moldova records the lowest median concentration of Cd among all countries surveyed (0.1 mg/kg), considerably lower than Ukraine (0.31 mg/kg) and Poland (0.21 mg/kg). Similarly, the median Pb concentration in Moldova (3.1 mg/kg) is among the lowest in the region. Elevated mass fractions of Al, Fe, Ni, V, and As in Moldovan moss are derived not only from anthropogenic sources but also from soil particles, promoted by relatively dry weather, low precipitation, and widespread cropland.

The correlation results (Figure 4.3) are highly consistent with the factor analysis. In the group of 19 elements, strong positive correlations among Al, Si, Ti, and rare earth elements suggest a common control by aluminosilicate minerals.

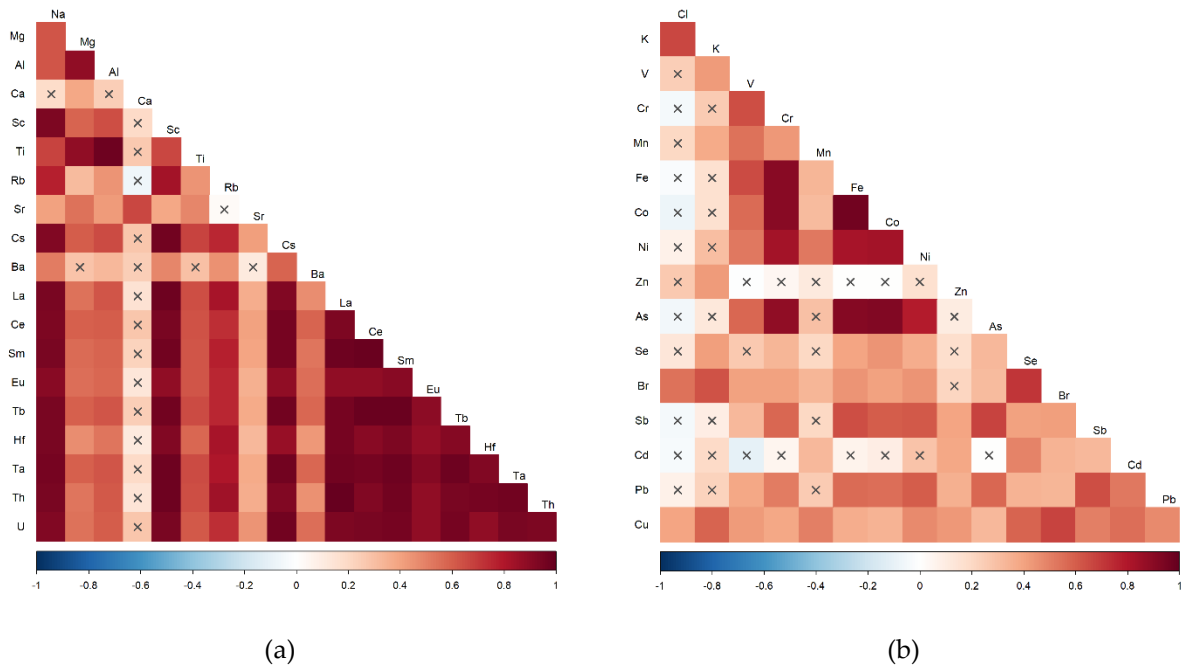


Figure 4.3. Spearman correlation matrix of element content in mosses in Moldova: (a) 19 elements of natural origin, (b) 16 elements with mixed natural and anthropogenic sources.

The Cd–Zn–Pb–Sb–Cu assemblage is particularly indicative of anthropogenic pressure, with these elements commonly linked to non-ferrous metal smelting, road traffic, and long-range atmospheric transport, while Sb serves as a tracer for brake pad wear and Cu for vehicle-related emissions [22, 23].

5. CONTRASTING ENVIRONMENTAL DRIVERS OF POTENTIALLY TOXIC ELEMENT ACCUMULATION

This chapter outlines a cross-country comparative analysis combining moss survey data from Georgia and Moldova [20, 24] to examine how environmental parameters influence elemental deposition. The investigation was controlled for biological bias by exclusively selecting *Hypnum cupressiforme* samples, and statistical outliers linked to local pollution hotspots were removed to focus on regional signals. The study then assessed how factors such as altitude, average annual precipitation, and topographic slope, as well as categorical variables such as Köppen-Geiger climate classification, elevation zones, terrain type, and population density, affect the composition, using multiple statistical techniques including correlation analysis, non-parametric significance testing, and PCA.

Grubbs' outlier test ($\alpha = 0.05$) was applied to exclude sites with anomalously high concentrations attributable to point sources, reducing the dataset from 70 to 55 samples in Georgia and from 41 to 30 samples in Moldova.

The correlation matrix (Figure 5.1) for the adjusted dataset revealed strong positive intercorrelations ($r = 0.54\text{--}0.86$) for Al, Co, Cr, Fe, Ni, and V, suggesting a common natural origin related to crustal materials or soil dust [20]. In contrast, topographic and climatic variables — average precipitation, slope, and altitude — were negatively correlated ($r = -0.42$ to -0.61) with Zn, Ba, Cr, Al, Cu, Fe, and Sr.

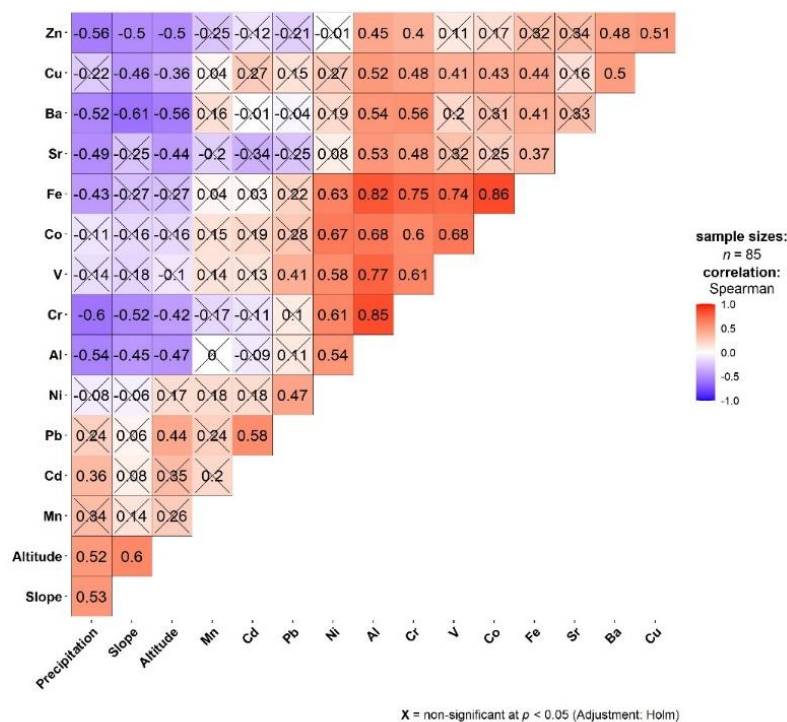
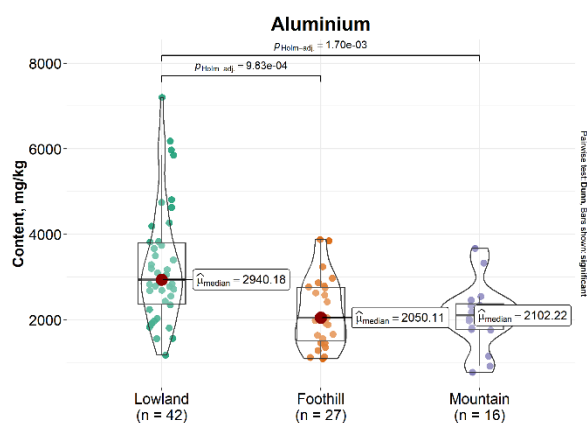
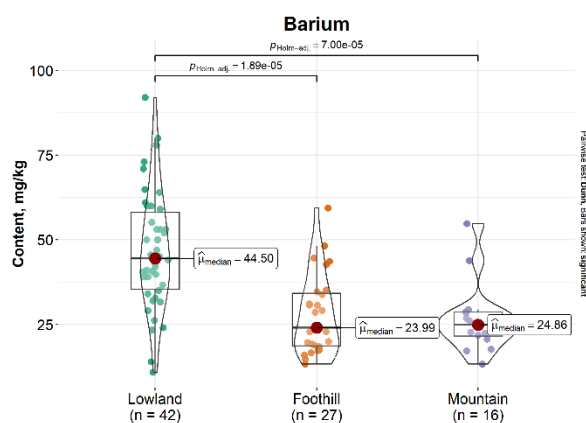


Figure 5.1. Spearman Correlation matrix between the elements of the entire adjusted data set.

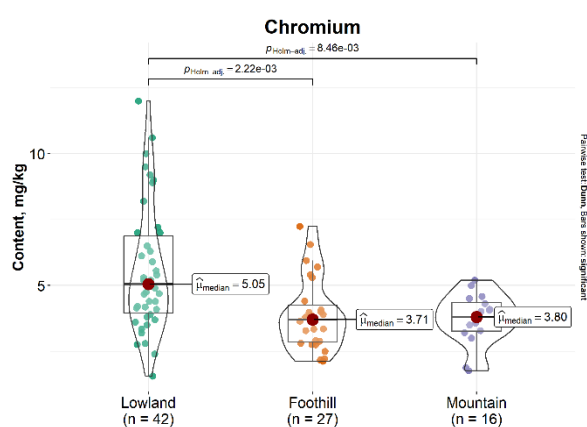
The Kruskal–Wallis test indicated significant differences in elemental concentrations among lowland, foothill, and mountain zones (Figure 5.2). Al, Ba, Cr, Cu, Pb, and Zn differed significantly between lowland and both foothill and mountain areas, whereas Cd and Sr showed significant differences only between lowland and mountain zones. Median concentrations were typically greater in lowland zones, with the exception of Pb and Cd, which displayed higher values at higher elevations.



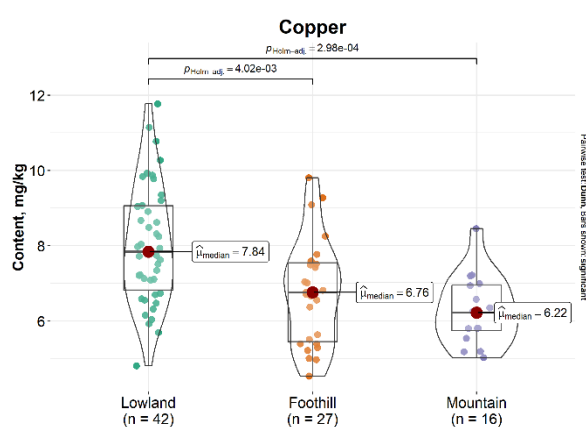
(a)



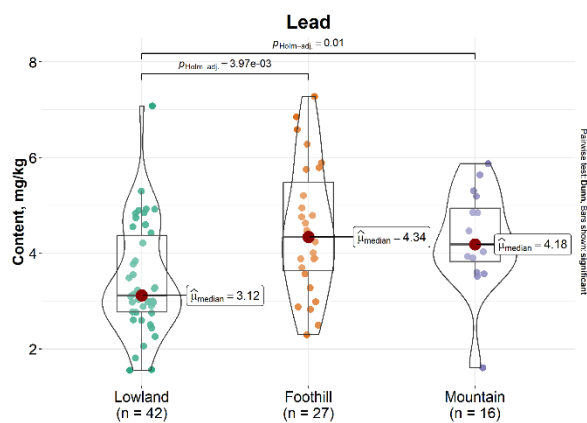
(b)



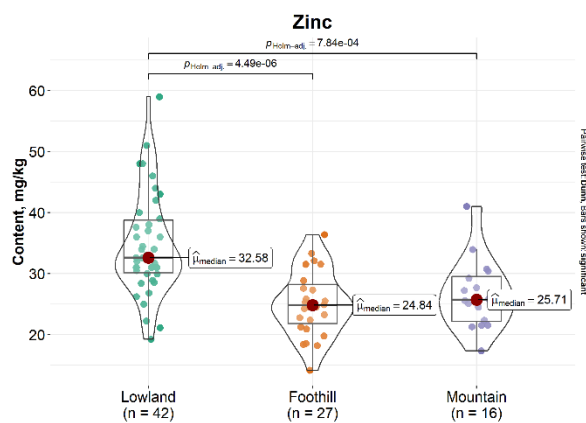
(c)



(d)



(e)



(f)

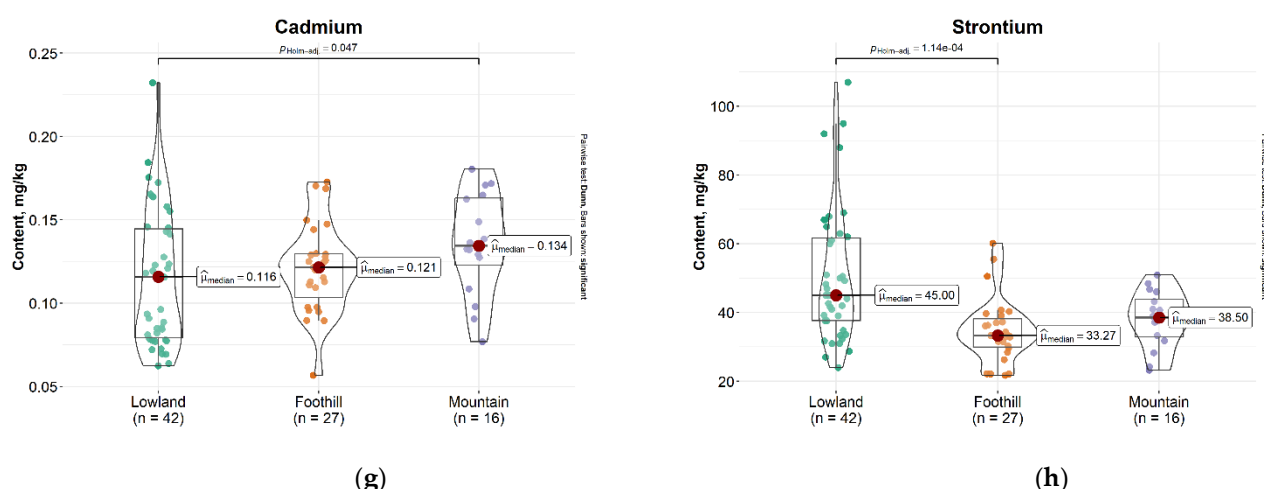


Figure 5.2. Boxplots of elemental content across elevation zones. Significant pairwise differences (Kruskal–Wallis with Dunn’s post-hoc, $p < 0.05$) are indicated with connecting lines.

Comparison across climate zones demonstrated significant differences for Ba between Dfa (Cold, no dry season, hot summer) and Dfb (Cold, no dry season, warm summer), Cd between BSk (Arid, steppe, cold) and Cfa (Temperate, no dry season, hot summer), and Pb, Sr, and Zn between Cfa and Dfa. The BSk-associated samples were characterized by elevated median concentrations of Al, Co, Cr, Fe, Sr, and Zn, alongside comparatively low concentrations of Cd and Pb, possibly attributable to wind erosion and local lithological characteristics.

Despite the absence of significant differences in analogous environmental parameters between the countries, distinct patterns in elemental composition were observed: Moldovan samples were enriched in Al, Ba, Cr, Sr, and Zn, whereas Georgian samples showed higher concentrations of Cd, Mn, and Pb.

PCA on the dataset (Figure 5.3) showed that the first two components capture 58.7% of the total variance (PC1: 40.3%, PC2: 18.4%). PC1 is mainly associated with Fe, Cr, Al, Co, V, Ni, Cu, and Ba, while PC2 is largely governed by Pb, Cd, Sr, and Zn. The pronounced contribution of lithophilic elements to PC1 implies a dominant influence of geogenic sources, largely attributable to natural weathering in Moldovan samples. Such processes are enhanced by pronounced soil erosion and atmospheric suspension of mineral particles, driven by limited vegetation cover and arid climatic conditions [20]. The comparatively low loading of copper on PC1 indicates reduced linkage to geogenic sources, suggesting an anthropogenic contribution, as Cu is extensively applied as a fertilizer and fungicide in Moldova.

The PCA biplot shows that altitude, slope degree, and precipitation align closely with the positive direction of PC2, where Pb and Cd also display positive correlations, indicating that higher elevations, steeper terrain, and increased precipitation are associated with enhanced accumulation

of these metals. This relationship is mechanistically explained by orographic lifting and enhanced precipitation that efficiently removes atmospheric pollutants [25].

According to the Wilks' test, country is the qualitative variable that contributes most to explaining the distances between individuals in the PC1–PC2 biplot, followed by elevation zones, land cover, climate, and terrain, in decreasing order of importance. This hierarchical ordering confirms that national-level geological factors have the strongest influence, followed by progressively finer-scale environmental gradients.

According to the PCA results, individuals m8, m19, m13, m34, m41, and m35 cluster on the negative axis, distinguished by high Sr and Zn loadings coupled with low contributions from Cd, Pb, altitude, precipitation, and slope. According to Kabata-Pendias et al. [21], the highest Sr concentrations are typically observed in heavy loamy soils formed under hot and arid conditions. In Moldova, soil erosion on sloped landscapes with erodible loamy soils creates conditions favorable for mobilizing Sr-rich soil particles [26]. Carbonate chernozems, characterized by loamy texture, are especially vulnerable to erosion and distributed mainly in the south and southeast, where BSk climatic conditions prevail.

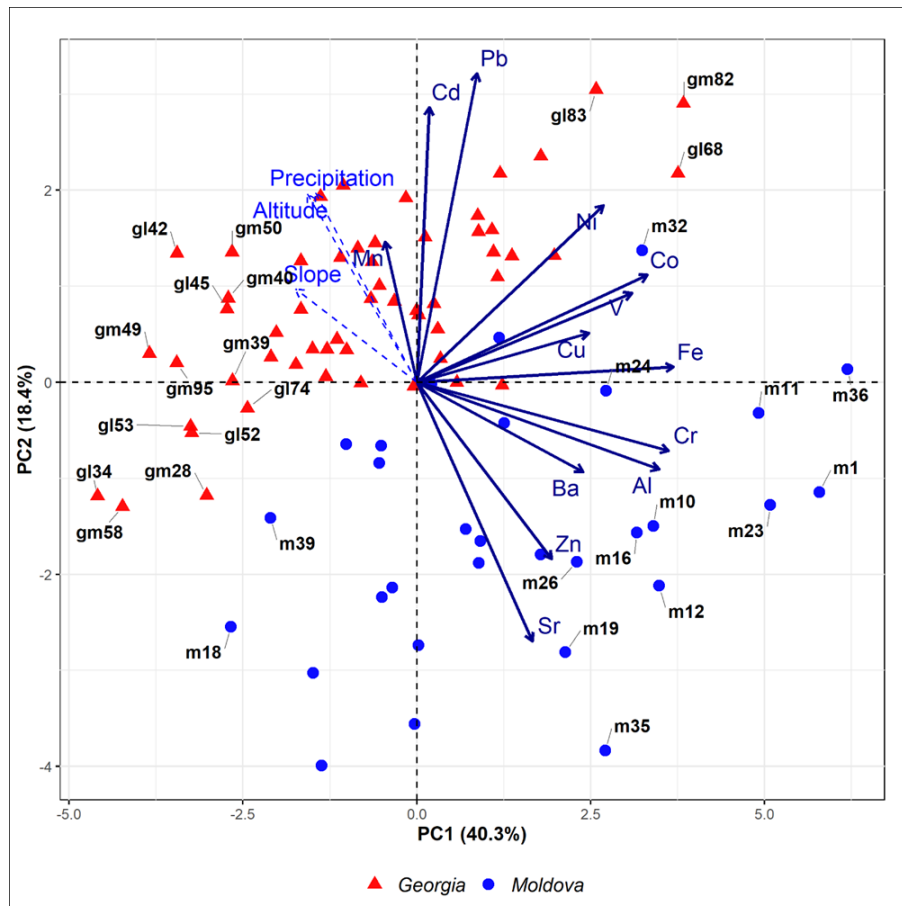


Figure 5.3. Biplot of the first two principal components express 58.7% of the variance in the data. Samples are marked with unique combination of color and symbol, dependent on the country. Variables appear as arrows from the origin, with qualitative variables shown as dashed arrows.

CONCLUSIONS AND RECOMMENDATIONS

The present study confirms that moss biomonitoring represents an effective and reliable approach for assessing atmospheric deposition in regions characterized by contrasting physico-geographical conditions. Based on large-scale surveys conducted in Georgia (2014–2023) and the Republic of Moldova (2020-2021), the research established baseline elemental concentrations, identified major pollution sources, and, importantly, demonstrated the significant role of climatic and geomorphological factors in controlling trace element accumulation in mosses.

1. Elemental accumulation patterns reflect both environmental conditions and emission sources. The results revealed distinct spatial patterns of elemental accumulation associated with differences in relief, climate, and land use. Georgian moss samples showed higher concentrations of lithogenic elements (Al, Sc, Ti, V, Cr, Fe, Co) and rare earth elements, reflecting the influence of mountainous terrain, complex geology, and enhanced weathering and erosion processes. In contrast, Moldovan samples exhibited elevated levels of Al, Fe, Ni, V, Cr, Sr, Zn, and As, which are consistent with lowland topography, dry climatic conditions, and intensive agricultural activity promoting soil resuspension and dry deposition processes.
2. Temporal trends indicate a general improvement in atmospheric deposition quality, with localized persistence of pollution sources. Comparative analysis between survey periods demonstrated a general decrease in median concentrations for most elements in both countries. In Moldova, substantial reductions were observed for Cr, As, Sb, Cd, Pb, and Cu between 2015 and 2020, while in Georgia, decreasing trends were recorded between 2014–2017 and 2019–2023, with Cd and Zn remaining relatively stable and Cu showing a slight increase. These trends reflect changes in emission intensity and environmental management, although persistent hotspots indicate ongoing local sources.
3. Multivariate analysis confirms the coexistence of geogenic and anthropogenic sources. Principal Component Analysis, Factor Analysis, and Positive Matrix Factorization identified three main source categories: (i) geogenic sources related to soil dust and crustal materials (Al, Fe, Cr, Co, V, rare earth elements); (ii) anthropogenic sources associated with traffic, industrial activity, and fossil fuel combustion (Pb, Zn, Cd, Cu, Hg); and (iii) mixed sources, including agricultural inputs (Cu, Br, Cl, K in Moldova) and mining-related contamination (Mn, As in Georgia). These findings highlight the complexity of atmospheric deposition systems and the need for integrated source interpretation.
4. Climatic and relief factors represent key controlling variables of atmospheric deposition. The study demonstrates that elemental accumulation in mosses is strongly influenced by

environmental conditions, particularly precipitation, altitude, and terrain characteristics. Lithogenic elements (Al, Cr, Ba, Zn) were negatively correlated with precipitation and altitude, indicating their dominance in regions characterized by dry conditions and active soil resuspension. Conversely, elements such as Cd and Pb were positively associated with altitude and precipitation, reflecting enhanced wet deposition and orographic effects in mountainous areas. Significant differences in elemental concentrations across elevation zones and Köppen–Geiger climate classes further confirm that climatic and geomorphological factors play a decisive role in shaping biomonitoring results.

5. Spatial analysis and pollution indices identify localized contamination hotspots within broader environmental gradients. The Pollution Load Index and Contamination Factor analyses revealed moderate to severe pollution in specific areas of Georgia, including the Chiatura manganese mining complex, the Zestafoni Ferroalloy Plant, and arsenic-contaminated regions such as Uravi and Tsana. In the Republic of Moldova, elevated concentrations were observed in urban and industrial areas such as Chisinau and Balti. Ecological risk assessment indicated predominantly low to moderate risk at the national scale, with localized increases associated with industrial and mining activities.
6. Methodological framework supports reliable comparative biomonitoring under different environmental conditions. The use of *Hypnum cupressiforme* as a common species, combined with the application of outlier filtering, enabled the identification of regional-scale patterns by minimizing the influence of local point sources. This approach allowed a clearer evaluation of environmental drivers, particularly climate and relief, while maintaining the comparability of datasets between countries.

Scientific contribution: integration of environmental drivers into biomonitoring interpretation. The study demonstrates that moss biomonitoring can be used not only to assess pollution levels but also to identify the environmental processes controlling atmospheric deposition. It provides evidence that climatic variability and relief complexity significantly modulate pollutant transport, deposition, and accumulation, which must be considered for accurate environmental assessment.

Practical recommendations:

1. Integration of environmental context into biomonitoring interpretation. The interpretation of moss biomonitoring data should explicitly account for climatic and geomorphological conditions. In dry, low-relief regions, elevated concentrations of lithogenic elements may reflect soil resuspension rather than direct pollution, whereas in mountainous and humid regions, increased accumulation of certain elements may be associated with wet deposition and

orographic processes. Ignoring these factors may lead to misinterpretation of pollution levels and sources.

2. Development and application of region-specific background values. Background concentrations established using the 10th percentile approach should be applied as reference values adapted to the specific physico-geographical conditions of each region. These values should be periodically updated to reflect environmental changes.
3. Implementation of standardized and comparable monitoring frameworks. Future biomonitoring programs should ensure methodological consistency, including the use of a single or comparable moss species, standardized sampling density, and harmonized analytical protocols, to allow reliable comparisons across regions with different environmental conditions.
4. Targeted environmental management of identified pollution sources. Mitigation strategies should focus on key pollution hotspots, including mining areas (Chiatura, Uravi), industrial facilities (Zestafoni, Kutaisi, Balti), and urban traffic emissions, as well as on reducing diffuse inputs from agricultural practices.

Expansion of monitoring to capture environmental variability. Future studies should extend spatial and temporal coverage to include a wider range of climatic zones, relief types, and land-use systems, enabling a more comprehensive understanding of atmospheric deposition dynamics in heterogeneous environments.

Suggestions for future research:

1. Expand spatial and temporal coverage: Future surveys should include remote and sensitive ecosystems (e.g., alpine zones in Georgia, protected areas in Moldova) to establish true background levels. Repeat surveys at 5-year intervals are essential to track the effectiveness of pollution control measures and assess the impact of climate change on deposition patterns.
2. Integrate complementary monitoring approaches: Combining moss biomonitoring with passive air samplers, deposition collectors, and soil analysis would provide a more comprehensive understanding of atmospheric pollution dynamics. Isotopic fingerprinting (e.g., Pb, Sr isotopes) could enhance source apportionment accuracy.
3. Investigate species-specific accumulation mechanisms: Controlled experiments should examine the physiological and morphological factors governing elemental uptake in different moss species. This would improve the comparability of studies using different biomonitors.
4. Assess health implications: Future research should link moss-derived deposition data with human biomonitoring and health studies in polluted areas (Kutaisi, Chisinau, mining

regions) to evaluate the public health impacts of air pollution and support evidence-based policy development.

5. Develop predictive models: Integrating moss biomonitoring data with GIS-based environmental variables (topography, climate, land use) and atmospheric transport models would enable spatial prediction of deposition patterns and identification of regions vulnerable to future contamination. This approach could be extended to other countries in the Black Sea basin with similar physico-geographical conditions.

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LIST OF PUBLICATIONS RELATED TO THE THESIS

2. Articole în reviste științifice

2.1. în reviste din bazele de date Web of Science și SCOPUS

1. ZINICOVSCAIA, I., HRAMCO, C., **CHALIGAVA, O.**, YUSHIN, N., GROZDOV, D., VERGEL, K., DUCA, G. Accumulation of Potentially Toxic Elements in Mosses Collected in the Republic of Moldova. In: *Plants*. 2021, vol. 10, no. 3, pp. 471. DOI: 10.3390/plants10030471.
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3. Articole în lucrările conferințelor și altor manifestări științifice

3.3. în lucrările manifestărilor științifice incluse în Registrul materialelor publicate în baza manifestărilor științifice organizate din Republica Moldova

1. **CHALIGAVA, O.** Moss biomonitoring of air pollution in the vicinity of mines: Georgia case study. In: *Abstract Book “Technical Scientific Conference of Undergraduate, Master, PhD*

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3a. Teze în lucrările conferințelor și altor manifestări științifice

3.2a. în lucrările manifestărilor științifice incluse în alte baze de date acceptate de către ANACEC

1. **CHALIGAVA, O.**, ZINICOVSCAIA, I., PESHKOVA, A., YUSHIN, N., FRONTASYEVA, M.V., VERGEL, K., GROZDOV, D. Evaluation of air quality in Georgia based on moss survey 2019-2022 results. In: *The 36th Task Force Meeting of the UNECE ICP Vegetation*, February 13-15, 2023. Hosted by UNECE.
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ADNOTARE

Chaligava Omari, „Aplicarea mușchilor ca bioindicatori pentru evaluarea stării ecologice a aerului atmosferic în condiții climatice și de relief dificile” teză de doctor în științe ale mediului, Chișinău, 2026

Structura tezei: Adnotare (în engleză și română), Introducere, Cinci capitole, Concluzii și recomandări, Bibliografie cu 365 surse, 26 figuri, 18 tabele, 140 pagini de text de bază. Rezultatele obținute au fost publicate în 15 lucrări științifice, inclusiv 6 articole în reviste indexate WoS/Scopus.

Cuvinte-cheie: Biomonitorizarea cu mușchi, depuneri atmosferice, elemente potențial toxice, evaluarea calității aerului, factori climatici, relief și topografie, Republica Moldova, Georgia, metode analitice

Scopul tezei. Elucidarea eficacității metodei de biomonitorizare cu mușchi pentru evaluarea calității aerului atmosferic în zone cu diferite condiții fizico-geografice: studiu de caz pentru Republica Moldova și Georgia.

Obiectivele studiului sunt: Efectuarea biomonitorizării pasive cu mușchi în Republica Moldova și Georgia, regiuni caracterizate prin condiții climatice și de relief contrastante; Determinarea compoziției elementare a probelor de mușchi și analiza comparativă a variabilității lor spațiale și temporale; identificarea și diferențierea surselor naturale (geogene) și antropogene de poluare a aerului atmosferic în regiunile studiate; Evaluarea influenței factorilor climatici și topografici (precipitații, altitudine, pantă) asupra acumulării elementelor în mușchi; Elaborarea principiilor de stabilire a nivelurilor de fond ale poluării în diferite condiții climatice și de relief; elaborarea recomandărilor pentru aplicarea și interpretarea rezultatelor biomonitorizării cu mușchi în regiuni cu caracteristici fizico-geografice contrastante.

Noutatea și originalitatea științifică: Noutatea lucrării constă în evaluarea integrată a eficienței biomonitorizării cu mușchi în condiții climatice și de relief contrastante, utilizând o abordare comparativă între Republica Moldova și Georgia. Studiul demonstrează, pentru prima dată, rolul decisiv al factorilor topo climatici (precipitații, altitudine, teren) în controlul modelelor de depunere atmosferică și acumulare a elementelor în mușchi, dincolo de influența surselor de emisie.

Rezultatul obținut care contribuie la soluționarea unei probleme științifice importante constă în identificarea și fundamentarea factorilor climatici și geomorfologici ca determinanți - cheie ai depunerii atmosferice și ai acumulării elementelor în mușchi, printr-o analiză comparativă a regiunilor cu condiții fizico-geografice contrastante (Republica Moldova și Georgia), ceea ce a condus la o diferențiere mai clară între contribuțiile geogene și antropogene și la o interpretare mai exactă a datelor de biomonitorizare, în contextul aplicării biomonitorizării cu mușchi pentru evaluarea calității aerului în diverse condiții climatice și de relief.

Semnificația teoretică a tezei constă în extinderea conceptului de biomonitorizare prin încorporarea factorilor climatici și de relief ca factori cheie ai transportului și acumulării poluanților. Cercetarea contribuie la o înțelegere mai profundă a mecanismelor depunerii atmosferice (depunerea uscată și umedă), și a interacțiunii acestora cu condițiile de mediu în modelarea tiparelor geochimice în biomonitori.

Valoarea aplicativă a tezei: constă în dezvoltarea de recomandări metodologice pentru interpretarea datelor de biomonitorizare cu mușchi în regiuni cu condiții climatice și geomorfologice diferite. Rezultatele susțin stabilirea unor niveluri de fond specifice regiunii, îmbunătățirea strategiilor de monitorizare a mediului și identificarea mai precisă a surselor de poluare, oferind o bază pentru managementul de mediu și politică de dezvoltare.

Implementarea rezultatelor științifice: Rezultatele obținute în urma acestui studiu au fost incluse în raportul ICP Vegetation, contribuind astfel la dezvoltarea rețelei europene de monitorizare a metalelor grele în Europa și alte state.

ANNOTATION

Chaligava Omari, Application of mosses as bioindicators for evaluation of ecological state of air in difficult climatic and relief conditions. PhD thesis in ecology sciences, Chisinau, 2026

Structure of the thesis: Abstract (in Romanian and English), introduction, five chapters, conclusions and recommendations, bibliography from 365 sources, 26 figures, 18 tables and 140 pages of main text. The results of the dissertation are reflected in 15 scientific publications, including 6 articles in peer-reviewed journals, indexed in WoS/Scopus.

Keywords: Moss biomonitoring, atmospheric deposition, potentially toxic elements, air quality assessment, climatic factors, relief and topography, Republic of Moldova, Georgia, analytical techniques

The aim of the thesis. To elucidate the effectiveness of the moss biomonitoring method for assessing the quality of atmospheric air in areas with different physico-geographical conditions: case study of Republic of Moldova and Georgia.

The objectives of the study are: carrying out passive moss biomonitoring in the Republic of Moldova and Georgia, regions characterized by contrasting climatic and relief conditions; determination of the elemental composition of moss samples and comparative analysis of their spatial and temporal variability; identification and differentiation of natural (geogenic) and anthropogenic sources of atmospheric air pollution in the studied regions; assessment of the influence of climatic and topographical factors (precipitation, altitude, slope) on the accumulation of elements in mosses; elaboration of principles for establishing background levels of pollution under different climatic and relief conditions; development of recommendations for the application and interpretation of moss biomonitoring results in regions with contrasting physico-geographical characteristics.

The novelty and scientific originality: The novelty of the work consists in the integrated evaluation of moss biomonitoring efficiency under contrasting climatic and relief conditions, using a comparative approach between the Republic of Moldova and Georgia. The study demonstrates, for the first time, the decisive role of topoclimatic factors (precipitation, altitude, terrain) in controlling atmospheric deposition patterns and element accumulation in mosses, beyond the influence of emission sources.

A result that contributes to the solution of a scientific problem consists in the identification and substantiation of climatic and geomorphological factors as key drivers of atmospheric deposition and element accumulation in mosses, through a comparative analysis of regions with contrasting physico-geographical conditions (Republic of Moldova and Georgia), which led to improved differentiation between geogenic and anthropogenic contributions and to more accurate interpretation of biomonitoring data, in the context of applying moss biomonitoring for air quality assessment under diverse climatic and relief conditions.

The theoretical importance of the work lies in extending the conceptual framework of biomonitoring by incorporating climatic and relief factors as key drivers of pollutant transport and accumulation. The research contributes to a deeper understanding of the mechanisms of atmospheric deposition, including dry and wet processes, and their interaction with environmental conditions in shaping geochemical patterns in biomonitors.

The applied value of the work consists in the development of methodological recommendations for the interpretation of moss biomonitoring data in regions with different climatic and geomorphological conditions. The results support the establishment of region-specific background levels, improvement of environmental monitoring strategies, and more accurate identification of pollution sources, providing a basis for environmental management and policy development. **The implementation of the results:** The obtained results of this study were included in ICP Vegetation survey report, thereby contributing to the European monitoring network for heavy metals across Europe and beyond.

CHALIGAVA OMARI

**APPLICATION OF MOSSES AS BIOINDICATORS FOR
EVALUATION OF ECOLOGICAL STATE OF AIR IN
DIFFICULT CLIMATIC AND RELIEF CONDITIONS**

166.01 ECOLOGY

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