

# The Estimation of Heavy Metal Deposition in Kumaon Region by using Moss Biomonitoring Technique

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**Abstract:** Land-based moss has long served as an effective tool for monitoring atmospheric element deposition, yet there remains a paucity of documented cases exploring this phenomenon comprehensively. This research investigates the seasonal variations of heavy metal concentrations, specifically Copper (Cu), Nickel (Ni), and Zinc (Zn), in moss samples collected from catchment sites in the Kumaon region of Uttarakhand, India. Mukteshwar sites are designated as controls due to favorable conditions for *Plagiomnium undulatum* growth, stemming from low population density and minimal atmospheric pollution. Quantitative analysis of metal concentrations utilized Inductive Coupled Plasma Mass Spectrometry (ICP-MS), offering high sensitivity and precision. Principal Component Analysis (PCA) unraveled patterns and relationships within the dataset, while Hierarchical Cluster Analysis (HCA) explored similarities and dissimilarities among study sites. This integrated approach not only quantified metal concentrations but also employed multivariate statistical analyses (PCA and HCA) to interpret complex relationships and variations. Seasonal metal accumulation in *P. undulatum* follows the order of summer > winter > monsoon in 2021. Remarkably high concentrations of Cu (94.4 µg/g) and Ni (99.6 µg/g) were recorded in Nainital, while Zn peaked in Ranikhet (177.9 µg/g). The heightened metal load across surveyed sites is linked to increased gasoline consumption during the summer, driven by tourist activity. During the monsoon, reduced tourist activity and accelerated biomass growth contribute to a rapid reduction in metal percentages in moss. The study enhances understanding of spatial and seasonal dynamics of heavy metal deposition in the Kumaon region, providing valuable insights for environmental monitoring and management strategies. The findings contribute to the broader understanding of metal sources and patterns, aiding in the development of targeted mitigation measures for sustainable environmental practices in the region.

**Keywords:** Biomonitoring, ICP-MS, Heavy metals, *Plagiomnium undulatum*

## 1. Introduction

Bryophytes, encompassing mosses and liverworts, have gained widespread recognition for their effectiveness in monitoring heavy metal pollution. They exhibit the remarkable ability to accumulate and retain pollutants without displaying conspicuous damage (Bellini *et al.*, 2021). The morpho-physiological traits of bryophytes, such as lamina cell shape and cell wall thickness, have been identified as valuable indicators of metal tolerance, further reinforcing their role in biomonitoring (Petschinger *et al.*, 2021). Furthermore, bryophyte communities have proven instrumental in monitoring heavy metal pollution in challenging environments like mines, caves, and rocky desert areas (He *et al.*, 2021). The atmospheric deposition of heavy metals, stemming from processes like incineration, industrial emissions, and agricultural activities, has been extensively studied. Research indicates that atmospheric deposition significantly contributes to heavy metal content in agricultural soil, with industrial and coal combustion being major sources (Shahid *et al.*, 2017). Additionally, atmospheric deposition facilitates the widespread distribution of heavy metals, exacerbating pollution in diverse regions (Zheng *et al.*, 2023). Rainfall-induced deposition of heavy metals emerges as a potential ecological hazard, underscoring the imperative for monitoring and control measures (Sun & Chen, 2016).

Overall, bryophytes, particularly mosses, have been extensively utilized for biomonitoring atmospheric pollution, including heavy metal deposition. They are acknowledged for their exceptional capacity to retain metals and other pollutants (Benitez *et al.*, 2021). Notably, metal concentrations in mosses are influenced by current velocity, emphasizing its importance as a variable in moss-based metal biomonitoring (Croisette *et al.*, 2001). Mosses excel as bio-accumulators of specific heavy metals compared to other plant species, making them superior for monitoring atmospheric heavy metal pollution in urban areas (Jiang *et al.*, 2018). Furthermore, mosses are valuable tools for biomonitoring in diverse environmental settings, including urban ecosystems and for phytoremediation of surface waters (Swisłowski *et al.*, 2022).

Certain moss species, such as *Hypnum cupressiforme*, *Homalothecium lutescens*, *Camphothecium lutescens*, and *Brachythecium glareosum*, have been identified as specific and suitable biomonitoring samples for assessing the variability of trace and macro-elements content (Angelovska *et al.*, 2014). *Plagiomnium undulatum*, a moss species, stands out as a potential bioindicator for atmospheric heavy metal pollution. Studies have demonstrated its effective adsorption of heavy metals from the atmosphere, making it a valuable tool for assessing atmospheric heavy metal deposition (Lee *et al.*, 2005). Moss biomonitoring has proven particularly useful in evaluating heavy metal concentrations in the atmosphere across various regions, encompassing both urban areas and natural ecosystems (Lee *et al.*, 2005; Zhu *et al.*, 2016; Liu *et al.*, 2022). The primary objective of this study is to assess metal deposition in the atmosphere within selected cities in the Kumaon region, employing a cost-effective approach involving moss biomonitoring.

## 2. Material method

### Study Area

The study was conducted in the western Himalaya, specifically the Kumaon region, (29.3923° N latitude and 79.7400° E longitude), where specific locations were chosen in each city, namely Nainital, Almora, and Ranikhet. The study area is renowned for its congenial atmosphere that helps revive ailing visitors and remains overcrowded throughout the year due to increasing tourist activity. Moreover, secondary sources of pollution are transportation routes, domestic waste landfills, windblown metal load, industrial sites and agricultural activities. These cities experience a sub-tropical climate, and the weather is pleasant throughout the year. Summers, winters and monsoon constitute the basic seasons. Summers (March-June) are mild and the maximum and minimum temperature ranges from 46°C to 19°C respectively. Winters extend through the months from (November-February) and these months will experience a maximum of around 25°C and a minimum temperature of around 4°C. The monsoon experiences moderate rainfall from July to October.

### Designing of Samples for Mapping

Samples of *Plagiomnium undulatum* were collected during three different seasons of the period of 2021 in accordance with the Indian weather conditions (summer, winter and monsoon). Transplantations of fresh moss were done at equidistance of 0.5, 1 and 3 km on 12 stations (direction wise) within the study area nearly at the same height. This method has been employed in various locations, including urban areas and industrial sites, to assess the presence of heavy metals (Anicic *et al.*, 2008; Zechmeister *et al.*, 2020; Slonina *et al.*, 2021; Saxena *et al.*, 2008). Samples were also collected from the interior of the Mukteshwar (Kumaon) forest cover, which served as a control site for baseline concentration. This area is minimally affected by human activities, maintaining its natural surroundings and potentially diverse flora and fauna.

### Moss Bags Technique

Moss bags were prepared using the moss *Plagiomnium undulatum*, collected using plastic gloves to avoid contamination. 6 gm of fresh moss was placed into a loosely knitted nylon moss bag of 20 cm<sup>2</sup> size only one day before the transplantation. Moss bags were transplanted to different sampling points in triplicate, nearly at an equal height *i.e.* about 4 meters (away from the buildings) ensuring direct and optimal exposure of the samples. After the end of the exposure period of one season (nearly after 122 days or 4 months), these moss transplants were harvested and moss sample was taken from each transplant bag for the metal analysis, whereas in its place fresh moss (in triplicate) was transplanted for the same duration for the next season (four months). The same

process was repeated for every season during entire year (Singh *et al.* 2017; Agostini *et al.*, 2020; Swisłowski *et al.*, 2021; Sorrentino *et al.*, 2021; Anicic *et al.*, 2008).

### Metal Analysis

Metal analysis is performed through quantitative analysis utilizing Inductive Coupled Plasma Mass Spectrometry (ICP-MS) at IIT Delhi. The ICP-MS model employed for this purpose is the Agilent 7900. ICP-MS allows for the simultaneous determination of multiple elements, enabling accurate measurement of trace metal concentrations in the moss samples (Steinnes *et al.*, 1993; Ayrault *et al.*, 2002).

### Statistical Analysis

The concentration data for heavy metals underwent analysis using JMP software from SAS to evaluate the statistical significance of the findings. Subsequently, Principal Component Analysis (PCA) was conducted using MINITAB 18 to reduce the dimensionality of the data, followed by cluster analysis.

## 3. Result and Discussion

Metal concentration data for each season were meticulously measured in  $\mu\text{g/g}$ , and the comprehensive summaries can be found in Tables 1 and 2. Table 1 provides insight into the total variance of the analyzed moss, while Table 2 showcases the metal load for Almora, Nainital, and Ranikhet, utilizing varimax rotation. Figures 1-3 visually depict the seasonal metal loads, offering a nuanced understanding of variations across different seasons. On the other hand, Figures 4-6 in the dendrogram unfold the intricacies of year-wise heavy metal loads, unveiling the correlation coefficient distances between the various heavy metals.

Catchments sites shows significantly higher concentration of Cu, Ni, and Zn than those measured at the baseline and values were found maximum in summer season (Fig. 2a). The sites of Mukteshwar have been considered as control as the conditions in this region is almost ideal for the growth of *Plagiomnium undulatum* because of least population and least atmospheric pollution as compared to the sites in peripheral zone. Seasonal accumulation of metals (Cu, Ni and Zn) in *P. undulatum* was in the order of summer > winter > monsoon in 2021 (Figures 1-3). During the year 2021, there was a notable increase in metal load across all surveyed sites in the Kumaon regions of Uttarakhand. The significant seasonal variation is likely due to increased gasoline consumption during the summer months, driven by a substantial surge in tourist activity (Gerdolet *et al.*, 2000; Saxena *et al.*, 2013; Srivastava *et al.*, 2014). During the monsoon season, tourist activity decreases, and pollutants are washed away by rainfall. Additionally, the accelerated growth (biomass) during the monsoon may contribute to a more rapid reduction in the metal percentage in moss compared to biomass (Singh *et al.*, 2017). The atmospheric deposition of heavy metals is influenced by various sources, including industrial activities, particle deposition, and natural sources, with industrial sources contributing significantly to the accumulation of heavy metals (Yao *et al.*, 2022). This deposition, which includes the absorption of gas-liquid suspension and precipitation, leads to increased heavy metal elements in soil and crops (Wang *et al.*, 2013). Furthermore, changes in industrial sources such as coal combustion, smelting, or waste incinerators, can impact heavy metal deposition, contributing to the observed seasonal variations (Thevenonet *et al.*, 2011).

The highest concentrations of Cu were recorded in mosses at the Nainital site during the summer season, specifically at a distance of 0.5 km to the west (Fig. 2a). Sheoran *et al.* (2021) identified key aerosol sources in Nainital, which include biomass burning, secondary sulfate and nitrate, mineral dust, and long-range transported mixed marine aerosol. Additionally, Panagos *et al.* (2018) delineated various anthropogenic sources contributing to diffuse copper contamination, such as fungicidal treatments, liquid manure, sewage sludge, atmospheric deposition, mining activities, and local industrial contamination. These findings align with those of Felix *et al.* (2015), who emphasized mining operations as potential contributors to metal and metalloid contamination in atmospheric aerosols. Moreover, Loland & Singh (2004) discussed copper contamination in soil and vegetation in agricultural settings, attributing it to the prolonged use of copper fungicides, highlighting yet another potential source of copper contamination in the region.

Ni level was measured maximum in moss collected from Nainital at a distance of 0.5 km to the south during the summer (Fig. 2b). Begum *et al.* (2022) suggested that, Ni is utilized in various applications related to oil

refining and cryogenic processes, including receptacles, devices for reducing pollutants, and systems for water supply and drainage substances or components used in construction. Furthermore, Singh *et al.* (2017) suggested that, accelerated growth of biomass during the monsoon season leads to a decrease in its quantity. The study by Hegde & Kawamura, (2012) observed that the concentrations of organic carbon (OC) and elemental carbon (EC) over Nainital were much lower during the summer compared to winter, indicating a seasonal variation in aerosol composition. Additionally, the enhanced concentration of organic nitrogen (Org-N) during summer is likely attributed to biomass burning sources, as indicated by Hegde *et al.* (2015). This suggests that biomass burning activities during the summer season contribute to the atmospheric Ni concentration in Nainital. Additionally, the research by Gautam *et al.* (2009) indicated that fine-mode pollution particles, such as soot and sulfate, form the bulk of the regional atmospheric loading, leading to dense haze and foggy conditions during winter months, which could indirectly influence the atmospheric Ni concentration during the contrasting summer season.

Zn values were higher at Almora in the summer, at a distance of 0.5 km to the east (Fig. 2a). The concentration of Zn in this area suggests a potential link to agricultural practices, as the inhabitants may use Zn to enhance the growth of crops. Siudeket *et al.* (2015) emphasized the significant contribution of anthropogenic activities, such as metal production, waste incineration, and combustion processes, to the atmospheric deposition of Zn. Weerasundaraet *et al.* (2017) investigated the presence of heavy metals in atmospheric deposition in a congested urban environment, indicating the potential influence of urban activities on Zn atmospheric deposition. These findings affirm that urban areas bear a significant impact from the presence of metals such as zinc and copper, associated with vehicular traffic, coating and automotive sectors, and the deterioration of various materials, including construction metals and materials used for road coverings (Kosior *et al.*, 2018).

The study by Zeng *et al.* (2022) suggests that Cr, Ni, Pb, and Zn may share similar sources, potentially caused by mixed sources of heavy metals, including natural sources, human activities, or atmospheric deposition. The highest atmospheric deposition of heavy metals during the summer season can be attributed to several factors. Sharma *et al.* (2007) also suggested that factors such as abundant precipitation and high air humidity in the summer season could lead to an increase in atmospheric deposition of heavy metals. Siudek& Frankowski (2017) highlighted the impact of anthropogenic sources and biomass burning on the atmospheric budget of trace elements (TEs) during the spring and summer measurements. Shahid *et al.* (2017) stated that atmospheric heavy metals may be absorbed via foliar organs of plants after wet or dry deposition of atmospheric fallouts on plant canopy. Whereas Rezapour *et al.* (2022) depicted that, the greater heavy metal concentrations and the lower water flow during summer contribute to the higher atmospheric deposition of heavy metals during this season. Furthermore, Al-Abbawyet *et al.* (2020) suggested that, the variation in heavy metal accumulation results could be attributed to interactions between factors affecting the concentrations of dissolved metals, such as the absorption or adsorption of ionic metals by phytoplankton and aquatic plants, and fuel burn emissions within the marsh during the summer season.

In the analysis, Principal Component Analysis (PCA) was used to explore metal interrelationships. Table 1 presents the cumulative variance explained by the first three principal components (PCs) based on Kaiser's criterion. For Almora, Nainital, and Ranikhet, the first three components explained 99.9% of cumulative variation. Factor loadings in heavy metal analysis (Table 2) revealed distinct patterns. In Almora, PC1 had positive loading, while PC2 and PC3 showed negative loading for Cu(S) and Ni(W). In Nainital, total variance explained by the first three components was 100.00%, with positive loadings in PC1 and PC2 for various metals and negative loading in PC3 for specific metals. Similarly, for Ranikhet, the total variance explained by the first three components was 100.00%, with positive loadings in PC1 and negative loading in PC2, while PC3 exhibited negative loading for certain metals and positive loading for others (Table 2).

Hierarchical Cluster Analysis (HCA) revealed atmospheric heavy metal similarities and differences. Dendrograms (Figure 4-6) visually display hierarchical relationships. In Figure 4, clusters 1 and 2 show associations between metals, and cluster 3 highlights the outlier Cu(S). In Nainital (Fig. 5), Zn(W) stands alone in cluster 2, while other metals form links in clusters 1 and 3. For Ranikhet (Fig. 6), cluster 1 associates Cu(M), Zn(M), Cu(W), and Ni(M), cluster 2 includes Cu(S) and Zn(S), and cluster 3 encompasses the remaining metals.

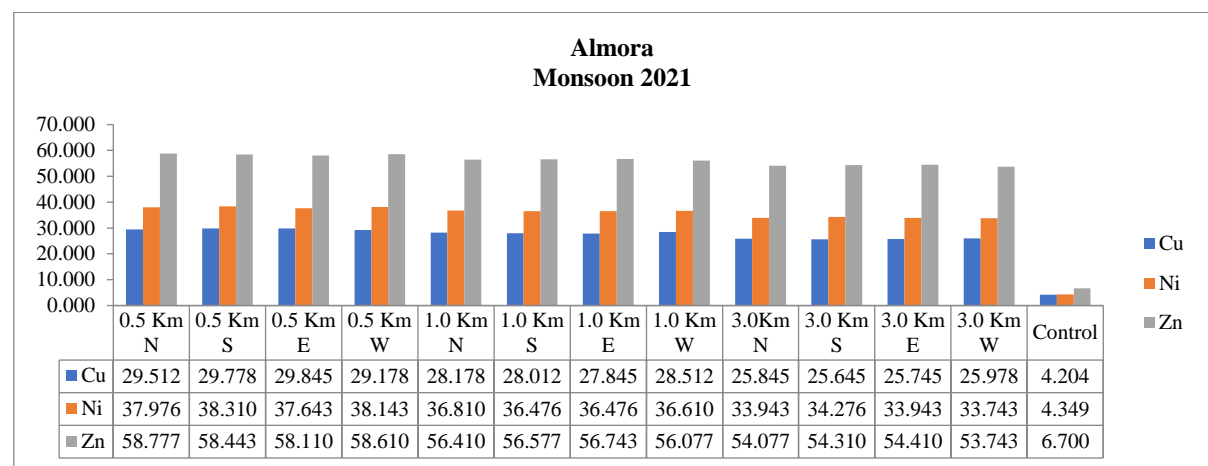


Figure 1a: The Concentration of Metals during Monsoon Season 2021 in Almora.

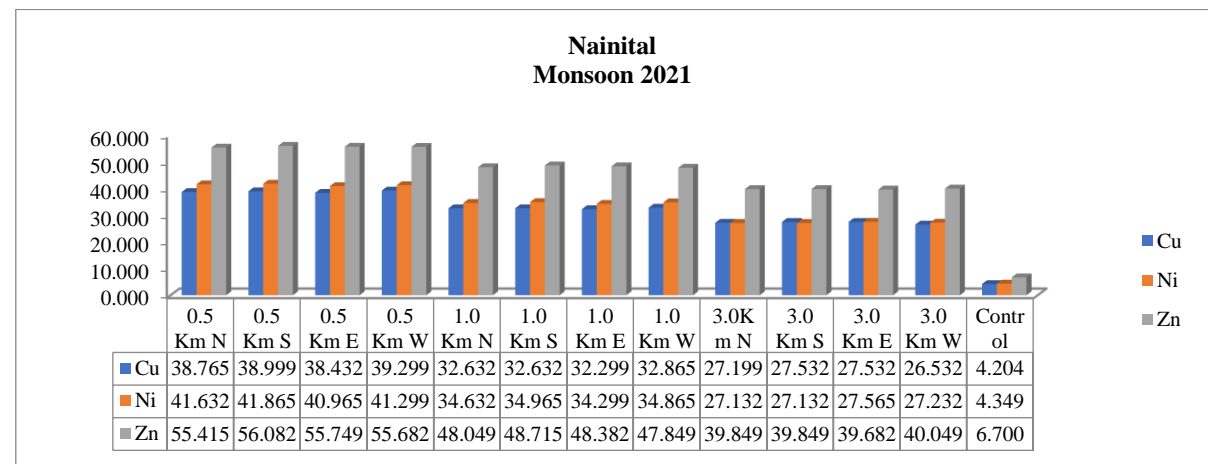


Figure 1b: The Concentration of Metals during Monsoon Season 2021 in Nainital.

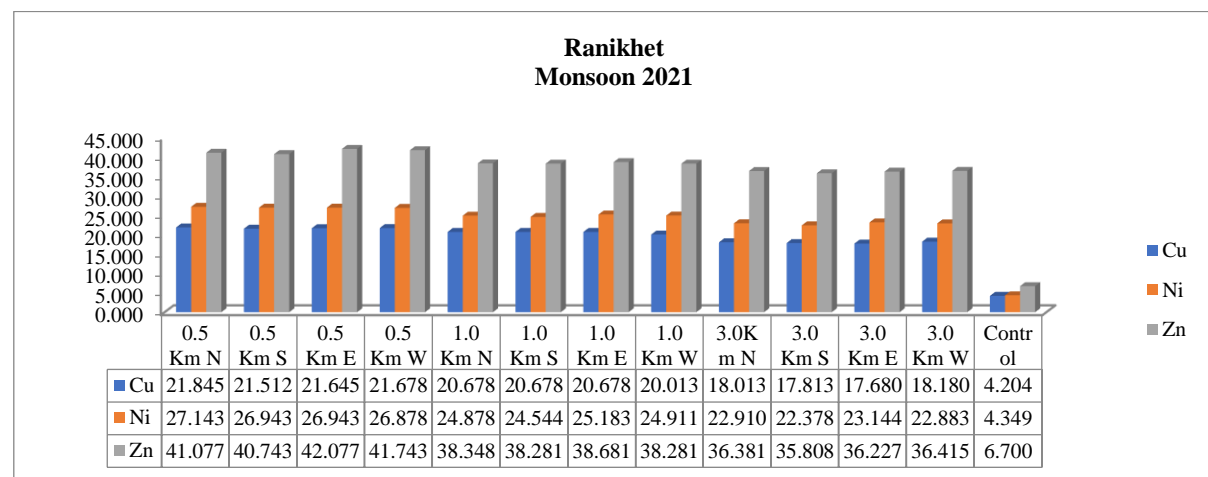


Figure 1c: The Concentration of Metals during Monsoon Season 2021 In Ranikhet.

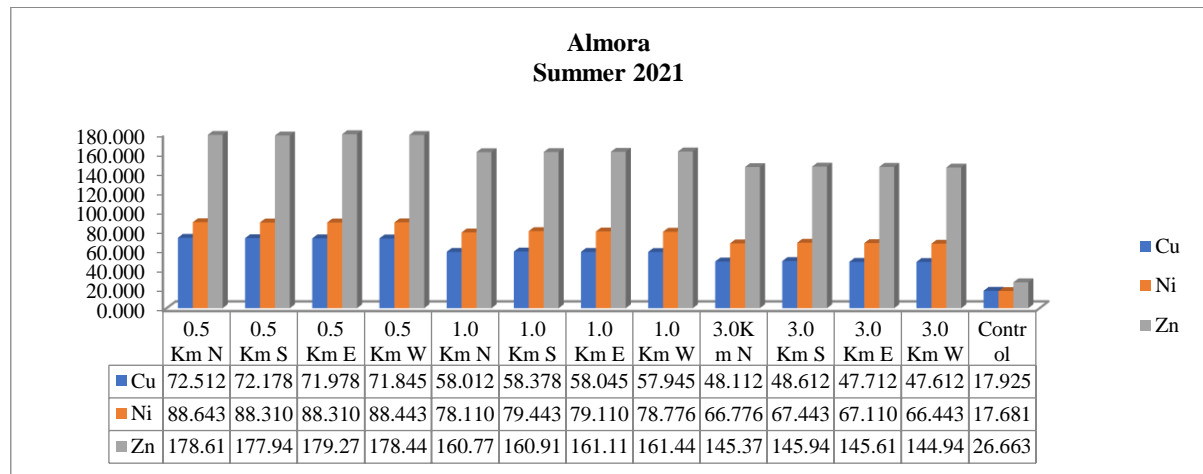


Figure 2a: The Concentration of Metals during Summer Season 2021 in Almora.

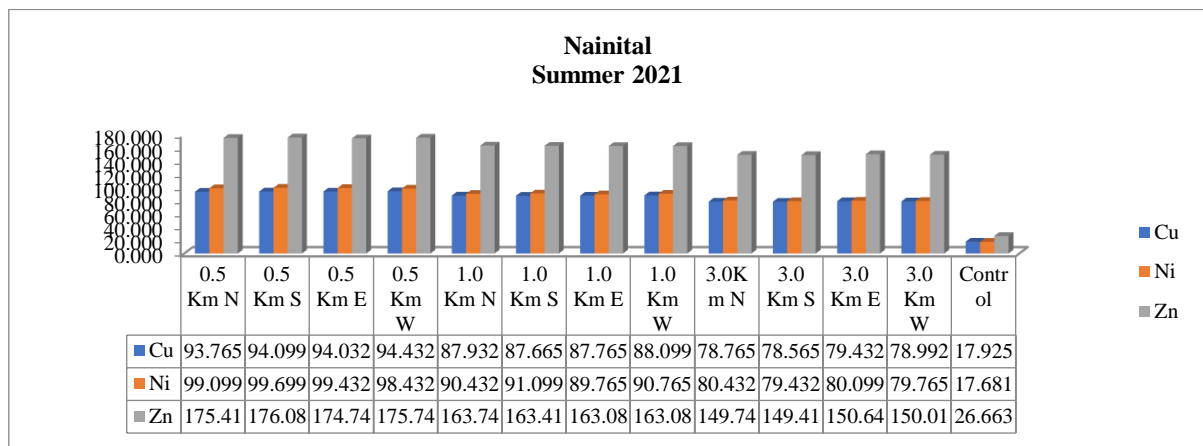


Figure 2b: The Concentration of Metals during Summer Season 2021 In Nainital.

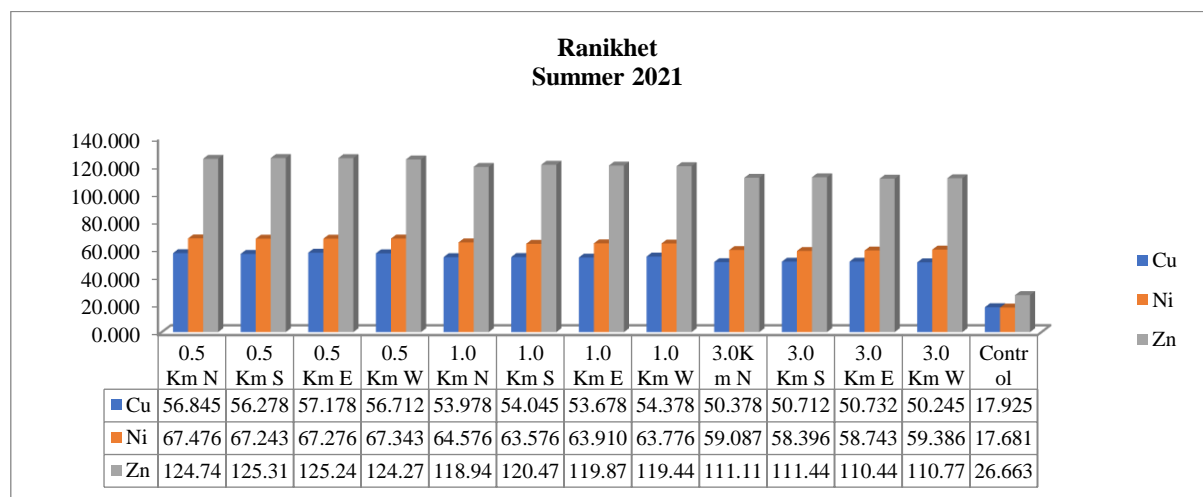


Figure 2c: The Concentration of Metals during Summer Season 2021 in Ranikhet.

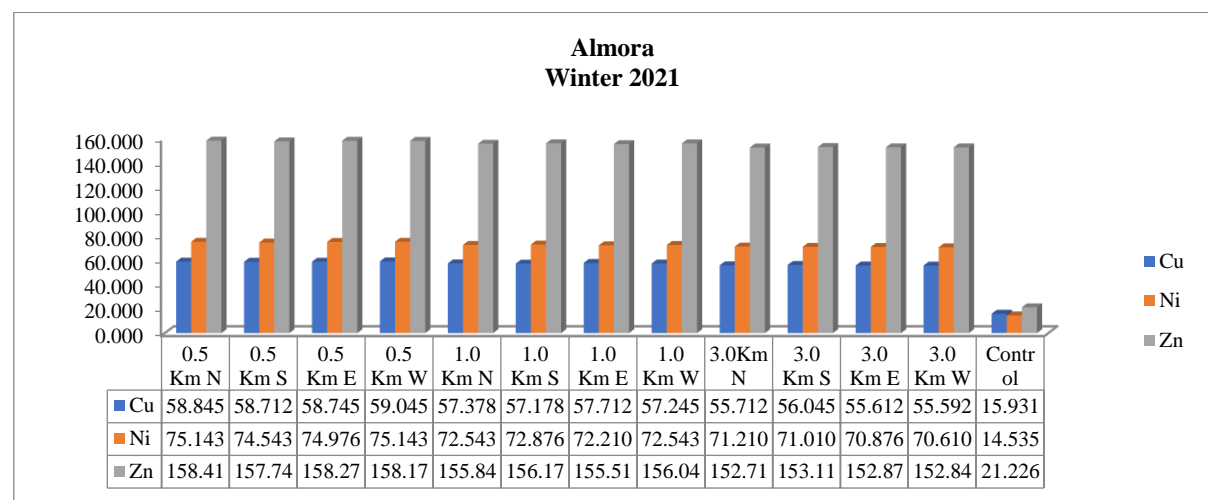


Figure 3a: The Concentration of Metals during Winter Season 2021 in Almora.

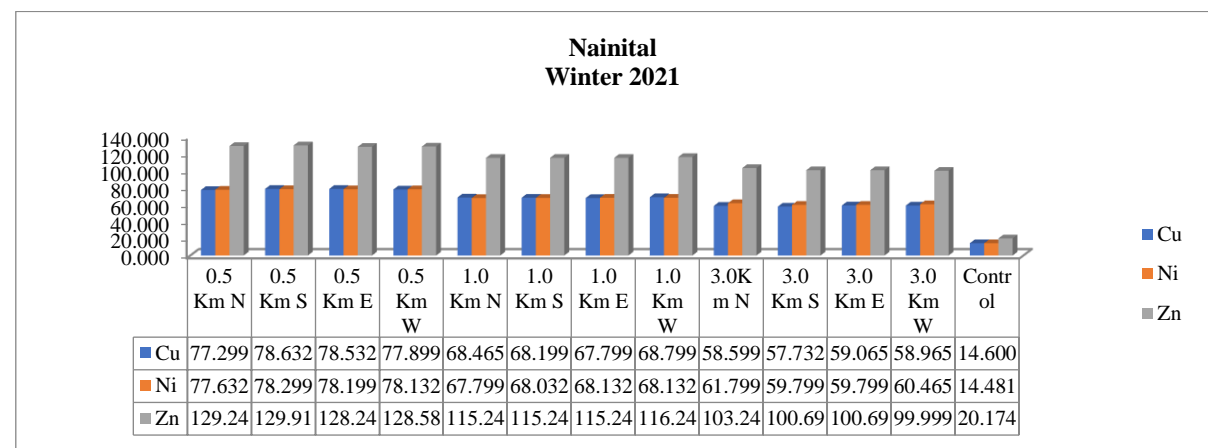


Figure 3b: The Concentration of Metals during Winter Season 2021 in Nainital.

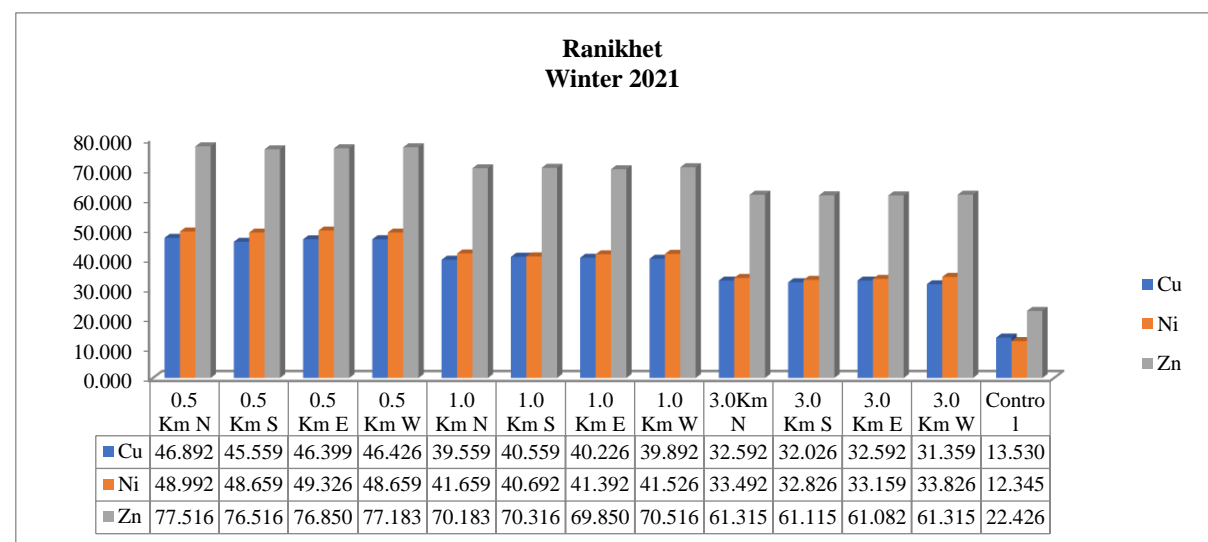


Figure 3c: The Concentration of Metals during Winter Season 2021 in Ranikhet.



Table 1: The Total Variance of Almora, Nainital and Ranikhet for Analyzed Moss during 2021.

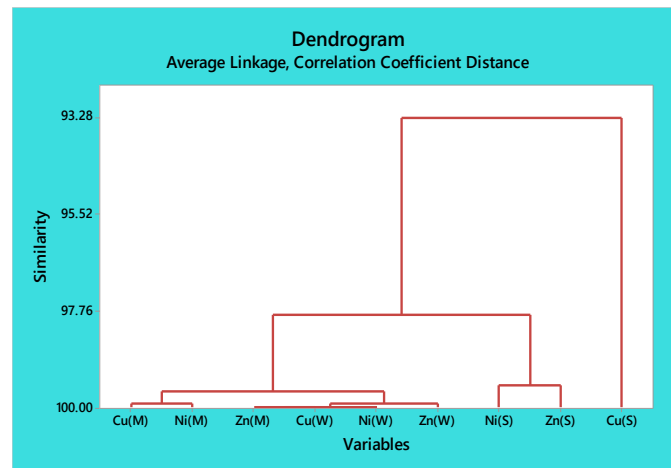
Components	Initial Eigen value			Extraction sum of squared loadings				Rotation sums of squared loadings		
		Total	% of variance	Cumulative	Total	% of variance	Cumulative	Total	% of variance	Cumulative
Almora	1	0.636	0.960	0.960	0.636	0.960	0.960	5.466	0.607	0.607
	2	0.355	0.040	0.999	0.355	0.040	0.999	3.523	0.391	0.998
	3	0.005	0.001	100.000	0.005	0.001	100.000	0.008	0.001	0.999
	4	0.001	0.000	100.000						
	5	0.000	0.000	100.000						
	6	0.000	0.000	100.000						
	7	0.000	0.000	100.000						
	8	0.000	0.000	100.000						
	9	0.000	0.000	100.000						
Nainital	1	8.725	0.970	0.970	8.725	0.970	0.970	5.171	0.575	0.575
	2	0.260	0.029	0.999	0.260	0.029	0.999	3.814	0.424	0.999
	3	0.007	0.001	0.999	0.007	0.001	0.999	0.008	0.001	100.000
	4	0.002								
	5	0.001								
	6	0.001								
	7	0.000								
	8	0.000								
	9	0.000								
Ranikhet	1	8.896	0.988	0.988	8.896	0.988	0.988	4.553	0.506	0.506
	2	0.096	0.011	0.999	0.096	0.011	0.999	4.438	0.493	0.999
	3	0.003	0.000	100.000	0.003	0.000	100.000	0.005	0.001	100.000
	4	0.001		100.000						
	5	0.000		100.000						
	6	0.000		100.000						
	7	0.000		100.000						
	8	0.000		100.000						
	9	0.000		100.000						

Table 2: Factor Loadings for The Almora, Nainital And Ranikhet Using (Varimax Rotation).

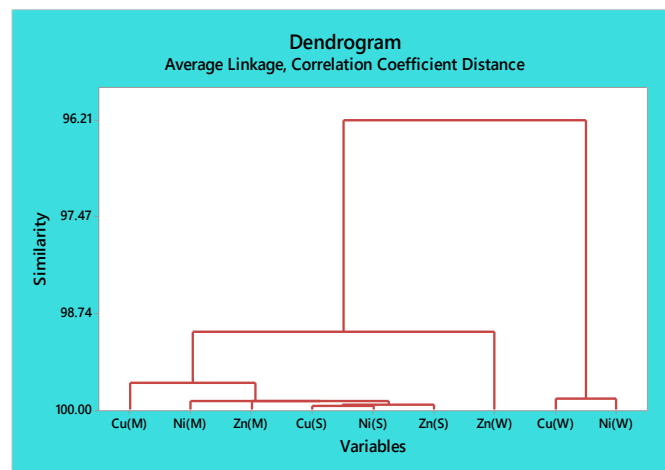
Components	Almora			Nainital			Ranikhet		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Cu(M)*	0.800	-0.597	-0.050	0.784	0.617	0.066	0.779	-0.626	-0.031
Ni(M) *	0.827	-0.56	-0.041	0.814	0.579	-0.021	0.818	-0.576	0.013
Zn(M) *	0.857	-0.515	-0.008	0.845	0.534	-0.035	0.762	-0.647	0.005
Cu(S) *	0.433	-0.901	0.017	0.836	0.549	0.002	0.615	-0.788	0.009
Ni(S) *	0.626	-0.777	-0.058	0.828	0.560	0.016	0.663	-0.748	0.002
Zn(S) *	0.725	-0.688	-0.011	0.852	0.522	0.024	0.582	-0.813	-0.014
Cu(W) *	0.867	-0.499	-0.007	0.533	0.845	0.021	0.746	-0.665	-0.011
Ni(W) *	0.870	-0.493	0.009	0.539	0.841	-0.021	0.715	-0.697	-0.056
Zn(W) *	0.891	-0.453	-0.006	0.704	0.71	0.018	0.688	-0.725	-0.018

\*Where (M) is monsoon, (S) is summer and (W) is winter season.

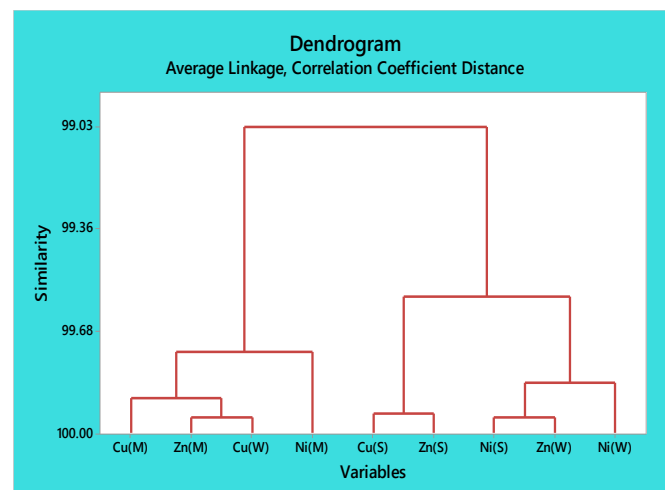




**Figure 4: Dendrogram for Heavy Metal Concentration in Almora City during Monsoon, Summer and Winter Season 2021**



**Figure 5: Dendrogram for Heavy Metal Concentration in Nainital City during Monsoon, Summer and Winter Season 2021**



**Figure 6: Dendrogram for Heavy Metal Concentration in Ranikhet City during Monsoon, Summer and Winter 2021.**

#### 4. Conclusion

Overall, the findings indicate that automobiles are likely the primary source of metals, which could be attributed to the increased tourist activities throughout summer and winter. Municipal waste, agricultural operations, open burning of solid waste, and laundry activities are substantial contributors to the escalating metal burden. This study advocates the use of specific bryophytes as effective biomonitors through a biomapping approach, proving to be a valuable tool for gauging atmospheric metal loads. Bryophytes, with their simplicity, totipotency, and rapid reproduction rate, emerge as ideal organisms for pollution-related investigations. Furthermore, climate factors should be considered, as they can influence the effects of metals. The investigation sheds light on contamination levels in the study area, revealing seasonal variations in metal pollution. The study delves into the distribution of selected metals, discussing potential sources. Chemical analysis of bryophytes demonstrates that biomonitoring is a cost-effective and swift approach for assessing heavy metal deposition in the ambient air and terrestrial ecosystem. The influence of climate on metal impact should be acknowledged in biomonitoring surveys, although its specific significance remains uncertain. Additionally, open burning encompasses various waste disposal methods beyond laundry and municipal waste, with solid waste and agricultural practices identified as significant pollution sources.

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