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Seasonal variation in heavy metal build-up in widely occurring bryophytes in Uttarakhand, Western Himalaya

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ABSTRACT

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Bryophytes serve as valuable biological indicators for environmental contamination stemming from a variety of natural and human-induced sources. This study focuses on evaluating recent shifts in air quality, utilizing a passive biomonitoring approach to assess atmospheric metal deposition and the seasonal trends of specific bryophytes as well as substrate in the Kumaon region of Uttarakhand, India, specifically in Nainital, Bhimtal and Mangoli. Bryophyte samples were collected from both the winter and monsoon seasons in 2019, and noteworthy metal concentrations were identified in Nainital, attributed to significant travel activities. Throughout the study duration, the sequence of metal deposition loads was as follows: Zn > Cu > Pb > Cd. The quantitative analysis of these elements in both the vegetative parts and the substrate exhibited an elevation in metal content during the winter season, suggesting that the chosen bryophytes could play a pivotal role in estimating aerial pollution and mineral enrichment in the soil. Such research is of utmost importance, given that developmental endeavours often coincide with detrimental shifts in air quality and adverse impacts of air pollution on human health, agricultural productivity, and natural ecosystems, necessitating vigilant monitoring and mitigation efforts.

Keywords: *Barbula indica*, biomonitoring, metal deposition, *Plagiochasma appendiculatum*, season, *Timmia megapolitana*.

INTRODUCTION

Both active and passive bioindicators find application in conjunction with bryophytes. Numerous prior studies (Muotka & Virtanen 1995, Virtanen et al. 2001, Heino & Virtanen 2006, Scarlett & O'Hare 2006, Rodriguez et al. 2014) exemplify the connections between bryophyte communities and the influencing ecological factors. Bryophytes encompass a diverse array of mosses widely utilized in biomonitoring methodologies, establishing themselves as exceptional tools for tracking climate change effects (Gignac 2001, Varela et al. 2010). Relying solely on precipitation and atmospheric moisture for hydration, bryophytes excel as indicators of air quality. Their capacity to endure desiccation enables them to sustain photosynthesis in such conditions, enabling prolonged exposure to potential toxins at research sites (Proctor & Tuba 2002). Beyond these advantages, bryophytes' uncomplicated nature is evident in their manageable size and minimal requirements (Tingey 1989). With their unique thallus structure characterized by tissue sheets, bryophytes raise intriguing questions about their responsiveness to environmental influences. Obtaining water, nutrients, and essential components primarily from the atmosphere places them in direct interaction with their surroundings, resulting in faster reactions to environmental shifts compared to vascular plants (Alam 2013). Additionally, bryophytes showcase both remarkable sensitivity and notable resilience to diverse hazardous substances, heavy metals, and persistent organic pollutants. Their distinct uptake mechanisms further establish them as exceptional accumulation indicators, as seen in multiple studies (Zechmeister et al. 2003). They serve as highly valuable assets in biological monitoring, employing organisms to furnish insights into the biosphere, including the presence of metals in the atmosphere. Mosses, with their capability to accumulate elements to notably high levels and facilitate the detection of metals present in environment even at extremely low concentrations, hence offer an economical means of gauging potential metal emissions. Consequently, bryomonitoring emerges as an affordable technique for assessing metal release (Alam 2013, Anna et al. 2019). The primary objective of the current study was to assess the degree of metal contamination in the Kumaon region of Uttarakhand. This assessment involved using commonly found bryophytes and substrate as passive biomonitoring tools. The process encompassed sample collection, preparation, and processing.

The investigation of bryophytes in this study serves two main purposes: identifying locations with elevated metal deposition and analyzing seasonal variations in metal concentrations. Employing spectroscopic techniques allows for the quantification of the actual quantities of compounds present in selected bryophytes, enabling the detection of heavy metal deposition in the air (Srivastava et al. 2014).

The data gathered through these methods hold relevance for a comprehensive, long-term study of heavy metal deposition at the research site (Grodzińska & Szarek-Łukaszewska 2001, Lucaciu et al. 2004, Chakrabortty & Paratkar 2006).

MATERIAL AND METHODS

Study area: The entirety of Uttarakhand is comprised of the Kumaon and Garhwal hills. The Kumaon region enjoys favourable weather throughout the year due to its subtropical climate. It experiences three primary seasons: summer, winter, and monsoon. During the winter months spanning from November to February, typical temperatures range from approximately 4°C at the lowest to around 25°C at the highest. The monsoon season, occurring from July to October, witnesses a moderate level of rainfall.

Sample design for mapping and identification: Following the typical weather patterns in India, we collected commonly occurring bryophytes, namely *Plagiochasma* sp., *Barbula* sp., and *Timmia* sp., from three distinct regions within the Kumaon hills: Nainital (disturbed region), Bhimtal (buffer region), and Mangoli (undisturbed region) (Figure 1). This collection was undertaken during two separate seasons-winter and monsoon. Through morphological examinations, the specimens were accurately identified. After the completion of one full season's exposure, bryophyte samples were gathered from each site to facilitate subsequent metal analysis.

Sample treatment and chemical analysis: Collected bryophyte samples were carefully placed in plastic bags and transported to the laboratory. Once there, the samples, which had not been washed, underwent a meticulous removal of attached debris and lifeless material. To eliminate adhering dirt particles, a burst of air was employed. The green shoot apexes of bryophytes (measuring 1–2 cm) were subjected to a 48–hour drying process at 40°C in an air oven, followed by homogenization of the samples. For analysis, 0.5 g of the homogenized bryophyte tissue was degraded using HNO_3 at 120°C. The digestion process was considered complete when the liquid turned colourless. Following digestion, the samples were filtered, and the resulting clear solution was diluted to 50 mL using bi-distilled water. Subsequently, the solution was subjected to examination via an atomic absorption spectrophotometer to analyze the presence of Zn, Cu, Pb, and Cd. The concentrations of metals in the plants were expressed as a percentage of their total dry weight. To ensure the absence of extraction-related contamination, a relevant blank (comprising HNO_3 and bi-distilled water) was utilized for verification purposes.

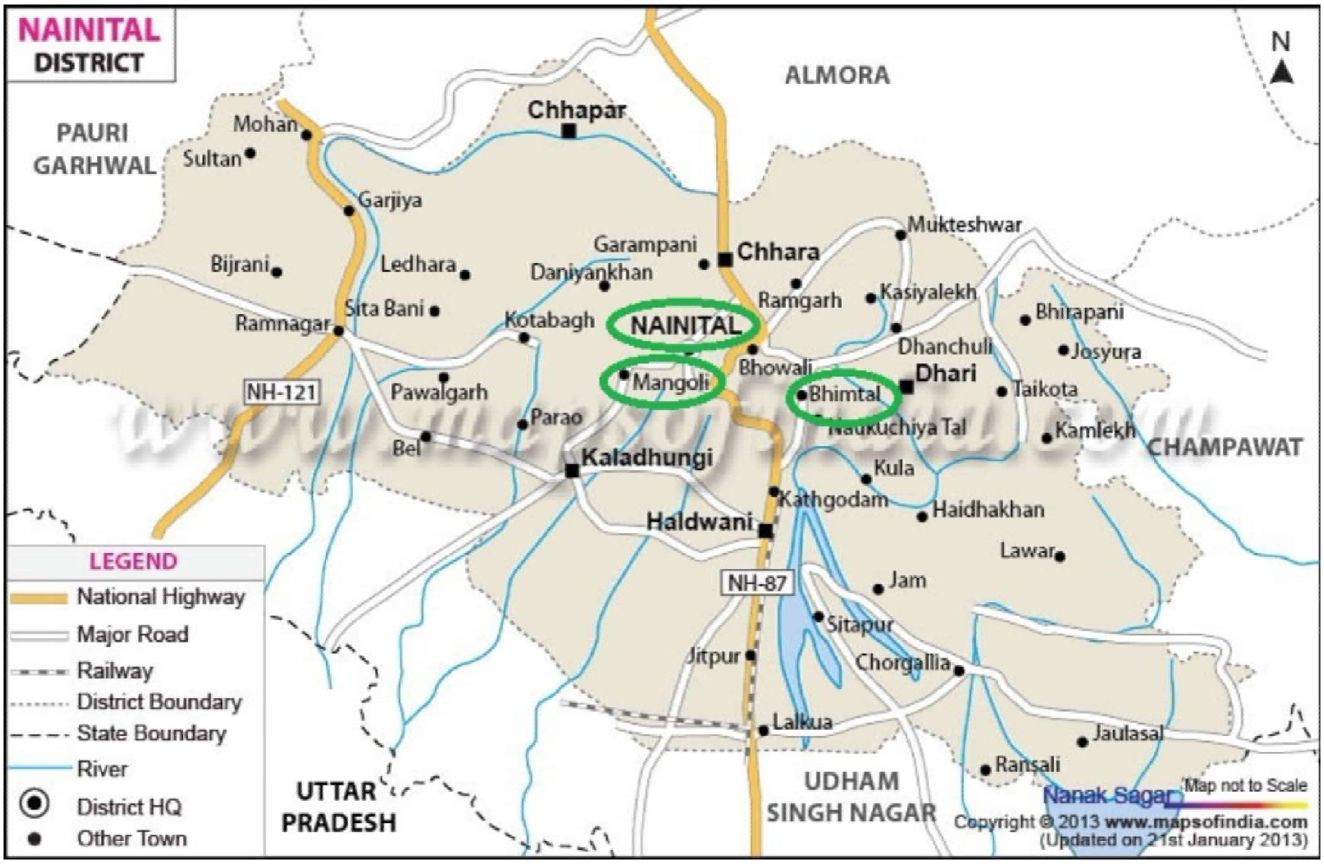

Figure 1. Map showing different sites of sample collection in North-west Himalayan region

Statistical analysis: The complete dataset underwent thorough statistical evaluation, with all analyses carried out using three sets of selected bryophyte transplants to ensure robustness. To isolate the individual effects of each treatment, the collected data underwent one-way analysis of variance (ANOVA), allowing for the assessment of variations in metal concentrations across different seasons.

RESULTS

The outcomes have been succinctly presented in tables 1 and 2, showcasing the metal concentrations for each season expressed as µg/g dry weight ±SE, while figures 2 to 4 provide visual representations of the data. The average values for Zn, Cu, Pb, and Cd were significantly elevated in comparison to the baseline values observed at the control site. For comparative purposes, sites in the undisturbed zone were employed as the control due to their reduced human activity and lower air pollution levels, creating conditions highly conducive to bryophyte growth. The accumulation of metals followed the sequence of winter followed by monsoon (Zn, Cu, Pb, and Cd).

DISCUSSION

Significant increases in metal burden were observed across all construction sites in Kumaon region of Uttarakhand. However, a noteworthy seasonal trend, possibly influenced by the surge in tourist activities leading to higher gasoline consumption during both summer and winter, was evident (Gerdol et al. 2000, Saxena et al. 2013, Srivastava et al. 2014). The monsoon season, characterized by reduced tourism and the cleansing effect of rain on pollutants, contrastingly witnessed a decrease in pollution levels.

Furthermore, it is plausible that the monsoon's influence could accelerate growth (biomass) at a higher rate than usual, consequently lowering the proportion

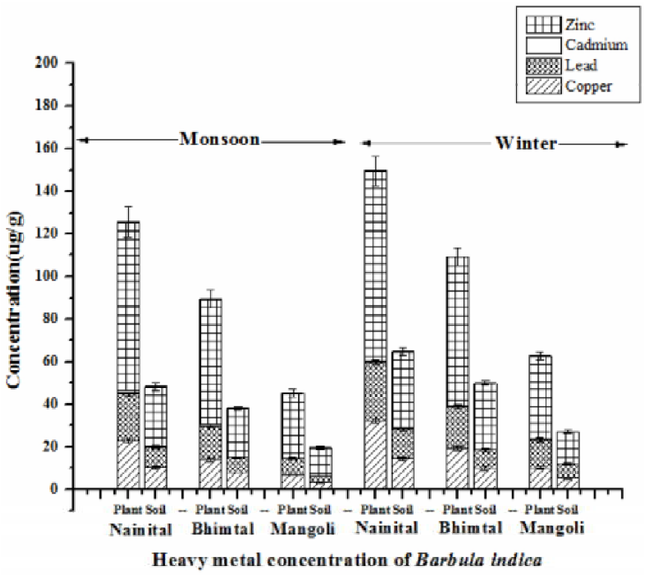

Figure 2. Heavy metal concentration of *Barbula indica* in two different seasons

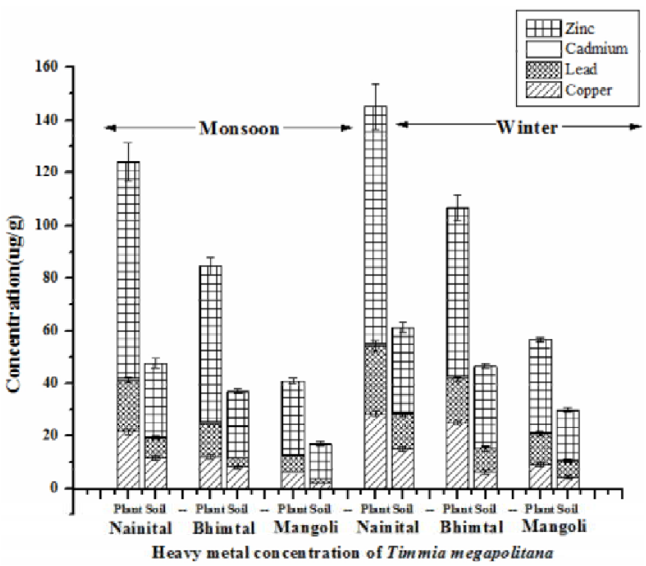

Figure 3. Heavy metal concentration of *Timmia megapolitana* in two different seasons

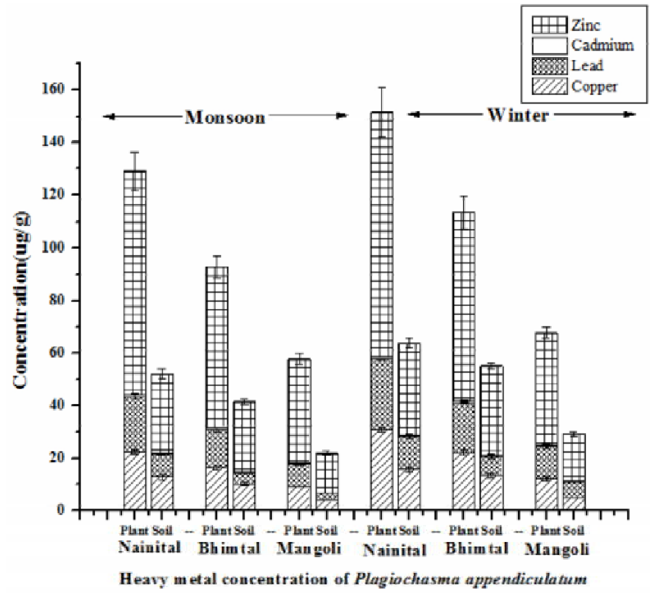

Figure 4. Heavy metal concentration of *Plagiochasma appendiculatum* in two different seasons

of metals within the moss relative to biomass. Zinc levels exhibited elevation in bryophytes situated close to rural areas and along highways near roads. This phenomenon could be attributed to vehicle wear and tear, with a relatively smaller contribution from exhaust emissions. In comparison to the baseline, substantial increases in zinc concentrations within bryophytes were observed. Notably, Nainital exhibited consistently higher zinc readings throughout the year. This could potentially be linked to agricultural practices in these regions where residents employ zinc to enhance crop growth, as these areas showed discernible concentrations of the element. This observation aligns with the findings of Otvos et al. (2003), suggesting the presence of zinc in pesticides and fungicides. As per Pearson et al. (2000) and Poikolainen et al. (2004), significant copper levels observed near roads could stem from factors like high engine wear and tear, household waste, laundry discharge, and discarded kerosene oil in residential areas (Loppi & Bonini 2000). It's also worth considering that the presence of copper in agricultural zones could be attributed to its essential role in fungicides, agricultural practices, and the incineration of solid waste (Gerdol et al. 2000, Otvos et al. 2003).

Another potential source of copper in rural regions could be the utilization of copper sulphate ($CuSO_4$) mixed with kerosene. This mixture is made available at a reduced cost by the Indian government to individuals falling below the poverty line (BPL). The present findings align with those of Lopez et al. (1997), who similarly observed an elevated copper (Cu) level in densely populated areas. The current study reveals an inverse relationship between lead (Pb) content and proximity to the road within an urban context. Locations with catchment areas display notably higher concentrations compared to the baseline, with peak values identified in Nainital. This pattern indicates that the notable increase in Pb levels can be attributed to vehicular emissions.

These results are consistent with the conclusions of Westerlund (2001), Adachi & Tainosho (2004), and Vidovic et al. (2005). The study underscores that despite the availability of lead-free fuel; lead (Pb) is still widely dispersed, maintaining elevated values. This observation could be attributed to the fact that although Pb levels are reduced per liter of gasoline, a 40–fold surge in traffic leads to a corresponding 40–fold increase in gasoline consumption, subsequently elevating Pb concentrations along roadways. Reports from various regions also suggest that vehicular traffic remains a primary contributor to atmospheric Pb levels (Loppi & Bonini 2000). Lead (Pb) remains non-biodegradable, persisting in sediments, soils, and dust. The highest cadmium (Cd) levels were observed in Nainital. Although cadmium's surface leaching rate is comparatively higher, it possesses a lower rate of metal contamination compared to other metals. The substantial divergence in its distribution pattern compared to other metals implies potential distinct sources, aligning with earlier findings by researchers (Scharova & Suchara 1998, Grodzińska & Szarek-Łukaszewska 2001). The

Table 1. Evaluation of heavy metals present in selected bryophytes and their substratum in monsoon season, year 2019

S.No.	Plant Name	Study site	Metal concentration (µg/g dry weight) ± SE			
			Copper	Lead	Cadmium	Zinc
1.	*Plagiochasma appendiculatum* Lehm. & Lindenb.	**Nainital**				
		Plant	22.42±0.99[c]	20.86±0.97[b]	0.90±0.05[c]	85.03±7.20[d]
		Soil	12.62±0.90[b]	08.97±0.54[a]	0.37±0.02[c]	30.08±1.96[c]
		Bhimtal				
		Plant	16.08±0.81[b]	14.87±0.82[b]	0.78±0.04[b]	61.08±4.21[d]
		Soil	10.06±0.80[b]	04.16±0.01[a]	0.26±0.02[a]	27.06±0.99[c]
		Mangoli				
		Plant	09.06±0.02[b]	08.78±0.60[a]	0.38±0.02[a]	39.48±1.99[c]
		Soil	04.07±0.01[a]	02.54±0.01[a]	0.21±0.01[a]	15.06±0.91[b]
2.	*Barbula indica* (Hook.) Spreng.	**Nainital**				
		Plant	23.06±1.02[c]	21.56±0.98[b]	0.87±0.04[b]	80.04±7.01[d]
		Soil	10.26±0.79[b]	09.65±0.68[a]	0.35±0.02[b]	28.05±1.84[c]
		Bhimtal				
		Plant	14.05±0.78[b]	15.76±0.92[b]	0.73±0.03[b]	58.82±4.16[c]
		Soil	08.02±0.02[a]	06.86±0.05[a]	0.21±0.02[a]	23.02±0.96[c]
		Mangoli				
		Plant	07.07±0.02[a]	07.45±0.59[a]	0.31±0.02[a]	30.42±1.86[c]
		Soil	03.01±0.01[a]	03.23±0.02[a]	0.19±0.02[a]	13.07±0.87[b]
3.	*Timmia megapolitana* Hedw.	**Nainital**				
		Plant	21.43±0.98[c]	19.87±0.93[b]	0.80±0.04[c]	82.09±7.20[d]
		Soil	11.42±0.80[b]	07.75±0.58[a]	0.32±0.02[a]	28.06±1.80[c]
		Bhimtal				
		Plant	12.05±0.79[b]	12.56±0.09[b]	0.72±0.02[b]	59.04±3.20[c]
		Soil	08.07±0.72[a]	03.45±0.02[a]	0.24±0.02[a]	25.07±0.90[c]
		Mangoli				
		Plant	06.05±0.02[a]	06.54±0.15[a]	0.34±0.02[a]	27.87±0.97[c]
		Soil	02.06±0.01[a]	01.51±0.01[a]	0.20±0.02[a]	13.07±0.83[b]

Table 2. Evaluation of heavy metals present in selected bryophytes and their substratum in winter season, year 2019

S.No.	Plant Name	Study site	Metal concentration (µg/g dry weight) ± SE			
			Copper	Lead	Cadmium	Zinc
1.	*Plagiochasma appendiculatum* Lehm. & Lindenb.	**Nainital**				
		Plant	30.78±1.02[c]	26.87±0.14[c]	0.99±0.16[c]	93.02±9.16[d]
		Soil	15.54±0.95[b]	12.75±0.90[b]	0.42±0.12[b]	35.07±2.01[c]
		Bhimtal				
		Plant	22.07±1.02[b]	18.67±0.12[b]	0.82±0.14[c]	72.03±6.16[d]
		Soil	13.32±0.92[b]	07.45±0.84[a]	0.34±0.21[a]	34.08±0.99[c]
		Mangoli				
		Plant	12.03±0.92[b]	12.64±0.90[b]	0.41±0.06[b]	42.64±1.99[c]
		Soil	05.04±0.03[a]	05.62±0.06[a]	0.30±0.19[a]	18.07±0.94[b]
2.	*Barbula indica* (Hook.) Spreng.	**Nainital**				
		Plant	32.04±1.01[c]	27.56±0.99[c]	0.93±0.15[c]	89.01±6.81[d]
		Soil	14.54±0.81[b]	13.74±0.93[b]	0.40±0.29[b]	36.07±2.03[c]
		Bhimtal				
		Plant	19.07±0.98[b]	19.56±0.96[b]	0.79±0.12[b]	69.86±4.02[d]
		Soil	10.05±0.89[a]	08.56±0.82[a]	0.29±0.19[a]	30.98±0.97[c]
		Mangoli				
		Plant	10.06±0.87[a]	13.45±0.94[b]	0.39±0.05[b]	38.87±1.78[c]
		Soil	05.07±0.03[a]	06.89±0.62[a]	0.21±0.17[a]	15.05±0.89[b]
3.	*Timmia megapolitana* Hedw.	**Nainital**				
		Plant	28.45±1.02[c]	25.67±1.98[c]	0.89±0.22[c]	90.14±8.82[d]
		Soil	14.75±0.85[b]	13.63±0.82[b]	0.35±0.12[b]	32.54±1.92[c]
		Bhimtal				
		Plant	25.04±0.82[c]	16.69±0.95[b]	0.83±0.19[c]	64.08±4.89[d]
		Soil	06.04±0.75[a]	09.12±0.90[a]	0.30±0.11[b]	31.05±0.99[c]
		Mangoli				
		Plant	09.04±0.70[a]	11.67±0.91[b]	0.37±0.14[b]	35.44±0.95[c]
		Soil	04.09±0.60[a]	06.43±0.70[a]	0.27±0.10[b]	19.04±0.88[b]

involvement of coloured polythene bags, household waste, discarded plastics, and kitchenware could all contribute to community-wide cadmium contamination. Urban areas may encounter additional Cd sources through the production of coloured plastic bags and the inclusion of cadmium compounds in paints and enamels. There's also the possibility of mining-related contaminants, including Cd, being present in gasoline. Another contributor to this rise might be service-oriented businesses dealing with metals (Alam 2013).

The majority of elements demonstrated strong inter-elemental correlations, implying a shared origin or similar behaviour during long-range atmospheric transport throughout the experiment. Bryophytes, known for their resilience to harsh environments and resistance to various toxic compounds that affect other plant species, serve as exceptional indicators for a broad spectrum of contaminants. Environmental pollutants in the form of aqueous liquids, gases, or particles accumulate on these stationary mosses. These uniquely adaptable species possess physiological traits suitable for medium- and long-term environmental research (Teixeira et al. 2022, Joshi & Alam 2023).

CONCLUSION

The collective results strongly suggest that vehicular activity might contribute to the metal presence, potentially linked to increased tourist activity during winter seasons, a trend often observed in biomonitoring studies, though the precise role is yet to be fully established. Moreover, the elevated metal burden is associated with diverse factors such as municipal waste, agricultural practices; open burning of solid waste and laundry discharge. This study advocates for the utilization of specific bryophytes as biomonitoring agents through a biomapping approach, which proves highly effective in quantifying atmospheric metal loads.

Despite the decrease in lead content per liter of gasoline, the growth in the number of automobiles has amplified lead consumption, consequently leading to a surge in atmospheric lead concentrations. Bryophytes, characterized by their simplicity, totipotency, and rapid reproductive rate, make them an ideal choice for pollution-related investigations. It's important to acknowledge that climate variables should also be considered, as they could modify the impact of metal exposure.

This investigation not only sheds light on the extent of contamination within the study area and the seasonal variability in metal pollution but also delves into the distribution of the selected metals, highlighting potential sources. The chemical analysis of the employed bryophytes underscores that biomonitoring is a swift and cost-effective approach for gauging heavy metal deposition in both the ambient air and terrestrial ecosystem. Furthermore, bryophytes serve as a valuable supplementary tool in monitoring, offering potential for biomonitoring studies focusing on similar sources of metal pollution. They can also serve as a warning mechanism to alert local communities about potentially hazardous metal accumulation.

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