



Multivariant Assessment of Metals Using Liverworts as an Appealing Tool in Catchment Sites of Uttarakhand, India

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ABSTRACT

This study aimed to conduct a systematic review to analyze heavy metals seasonal concentrations in Uttarakhand tourist hotspot cities (Almora, Nainital, Ranikhet, Mussoorie, and Dhanaulti). A total of 45 samples of liverwort *Dumortiera hirsuta* were collected from five different cities during winter (Dry deposition) and monsoon (Wet deposition) in the year 2021. The concentrations of Zn, As, Cd, and Pb due to anthropogenic pollution load in the selected locations were analyzed by active biomonitoring using Inductive Coupled Plasma Mass Spectrometry (ICP-MS). Concentration loading of zinc, arsenic, cadmium, and lead was observed to be 79%, 71%, 48%, and 33%, respectively, higher during the dry (winter) season when compared with the monsoon dataset. Multivariant data were analyzed using Principal Component Analysis (PCA) with three components explaining maximum variation in data by factor loading through varimax rotation. The rapid growth and development have connected tourists to the mountain of the western Himalayas. Thus, a monitoring program is needed in these areas for further assessment. So that necessary action can be taken to conserve the eco-sensitive zones of Uttarakhand.

INTRODUCTION

Heavy metals have become a global problem due to their toxicity and adverse environmental and human health effects (Fernandez et al. 2018). The presence of heavy metals in the atmosphere can be attributed to various many natural and human disturbances (Acharya et al. 2017). Metals such as Cd, Cu, Pb, Fe, and Zn are resistant to biological and chemical degradation and have instability and high toxicity, which have adverse effects on organisms. Therefore, a systematic study of these atmospheric elements of great interest (Zhu et al. 2008, Maleki et al. 2014, Singh et al. 2017).

Biomonitoring is a biological technique for measuring the extent of environmental pollution. One can assess the amount and impact of pollution on ecosystems by this method. This procedure is based on the examination of organisms that can accumulate environmental pollution. It provides an opportunity to obtain information on the quantitative and qualitative state of air pollution (Markert et al. 1999).

Heavy metals can break down some of our essential nutrients, and this can lead to lower immune defenses and many health issues. Complications such as headache, abdominal pain, dizziness, vomiting, diarrhea, reproductive

problems failure, gastrointestinal cancer, disruption of hemoglobin biosynthesis and anemia, elevated blood pressure, kidney damage, miscarriage or minor miscarriage, the nervous system, brain damage, reduced learning ability in children, etc. have been observed in several studies (Irfan et al. 2013, Chakraborty et al. 2013, Jaishankar et al. 2014, Engwa et al. 2019).

The objective of the present research was to identify correlations between heavy metal concentrations determined in Liverwort and the seasonal variation of the atmospheric metal residue, which was used in active biomonitoring in the selected cities of Uttarakhand, India. The study was carried out in the western Himalayan region using Liverwort *Dumortiera hirsuta* utilizing Inductive Coupled Plasma Mass Spectrometry to detect the following metals Zn, As, Cd, and Pb.

MATERIALS AND METHODS

Study Area

The study was conducted in five locations selected based on high tourist load during winter snowfall in the western Himalaya (Kumaon region), which covers the catchment sites of Nainital, Almora, and Ranikhet city and northwestern

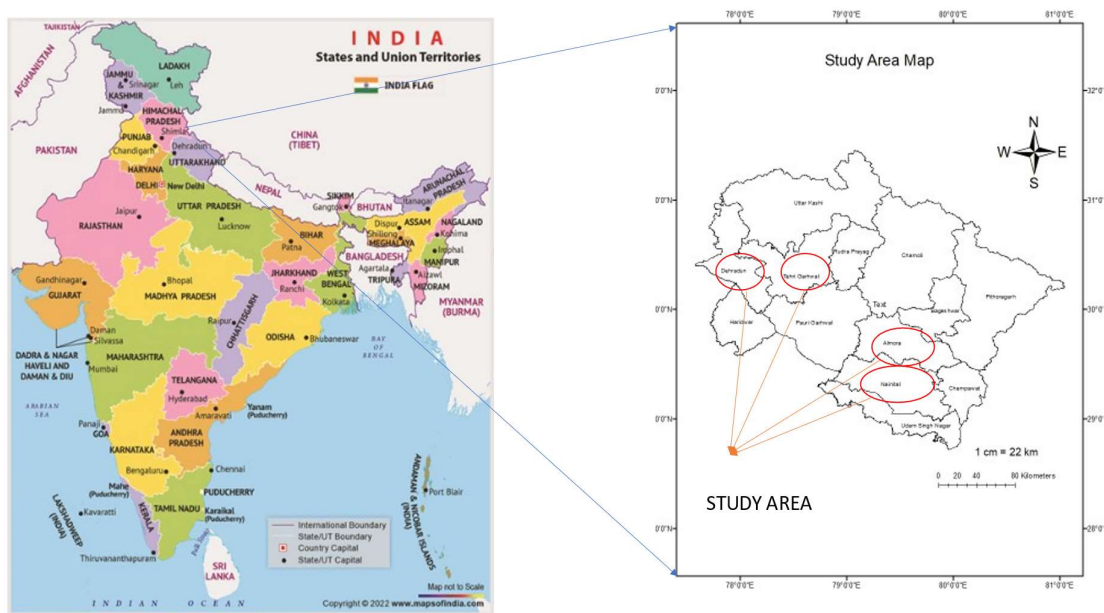


Fig. 1: Map showing different study locations (Almora, Ranikhet, Mussoorie, Nainital, Ranikhet, and Dhanaulti) of Uttarakhand, India.

Table 1: Showing meteorological data along with geo-coordinates of the sampling location.

Sampling locations	Geo-coordinates	Temperature	Altitude
Mussoorie	30°27' N 04°78' E	Max 20°C to 25°C Min -1°C to 7°C	6578 ft
Dhanaulti	30°45' N 78°25' E	Max 20°C to 25°C Min 1°C to 6°C	7500 ft
Almora	29°40' N 79°67' E	Max 15°C to 23°C Min 0°C to 8°C	5387ft
Ranikhet	29°38' N 79°25' E	Max 13°C to 21°C Min 0°C to 7°C	6132 ft
Nainital	29°38' N 79°45' E	Max 16.6°C to 23.5°C Min 1.7°C to 10.7°C	6837 ft

Himalaya (Garhwal region), which covers the city of Mussoorie and Dhanaulti (Fig. 1 and Table 1).

Sample Collection

Liverwort *Dumortiera hirsuta* was collected from the forest of Mukteshwar (control site), which is at an Altitude of 2300 meters (7545 ft). Twelve sampling sites in the city (Based on land use and land cover) at a distance of 0.5 km, 1 km, and 3 km were used for moss transplantation (Srivastava et al. 2014). Green moss was filled into a nylon bag and transplanted to study sites. Each moss bag was suspended 20 cm² above the ground in triplicate. For the dry season (November to February), samples were collected in the month of the first week of February 2021. For the wet season (July to October), samples were harvested in the first week of November 2021. Digestion and metal analysis

were performed on 0.5gm of moss samples to determine the concentration of specific metals using the ICPMS model (Agilent 7900) at IIT Delhi.

Statistical Analysis

Heavy metal concentration data analysis was performed with JMP (SAS) software to understand the statistical significance of the results. PCA was performed in MINITAB-18 to reduce the dimensionality of the data.

RESULTS AND DISCUSSION

The Zn, As, Cd, and Pb concentrations from the control site were found to be non-significant values when compared with the sampling cities dataset during both seasons monsoon 2021 (Wet season) and winter 2021 (Dry season). This analysis

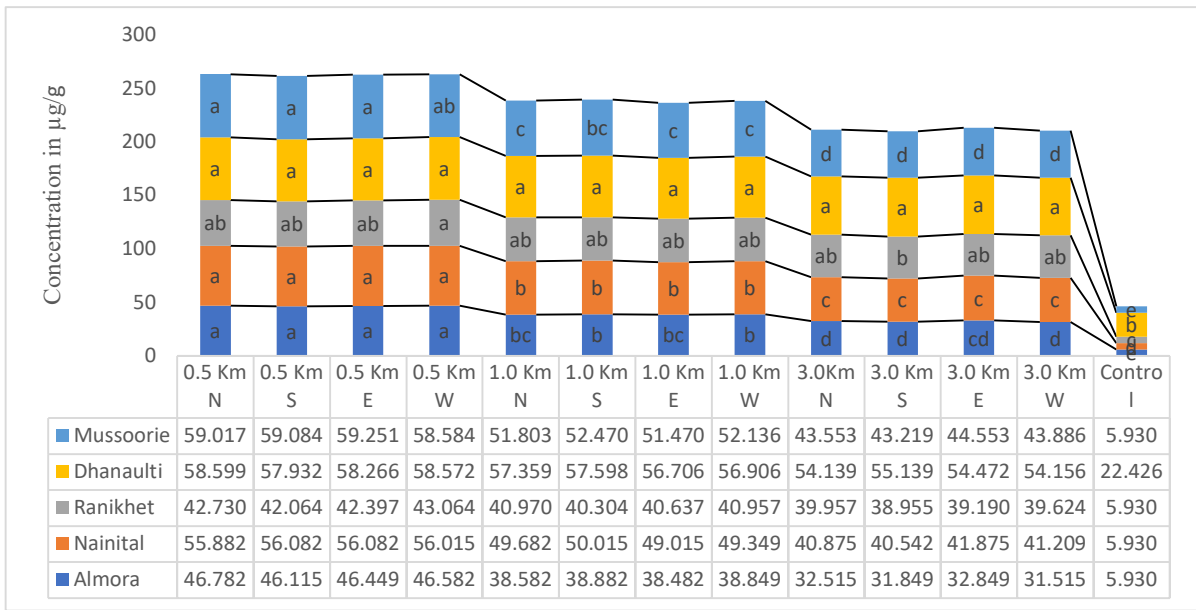


Fig. 2a: The concentration of zinc during Monsoon 2021 in Mussoorie, Dhanaulti, Ranikhet, Nainital, and Almora.

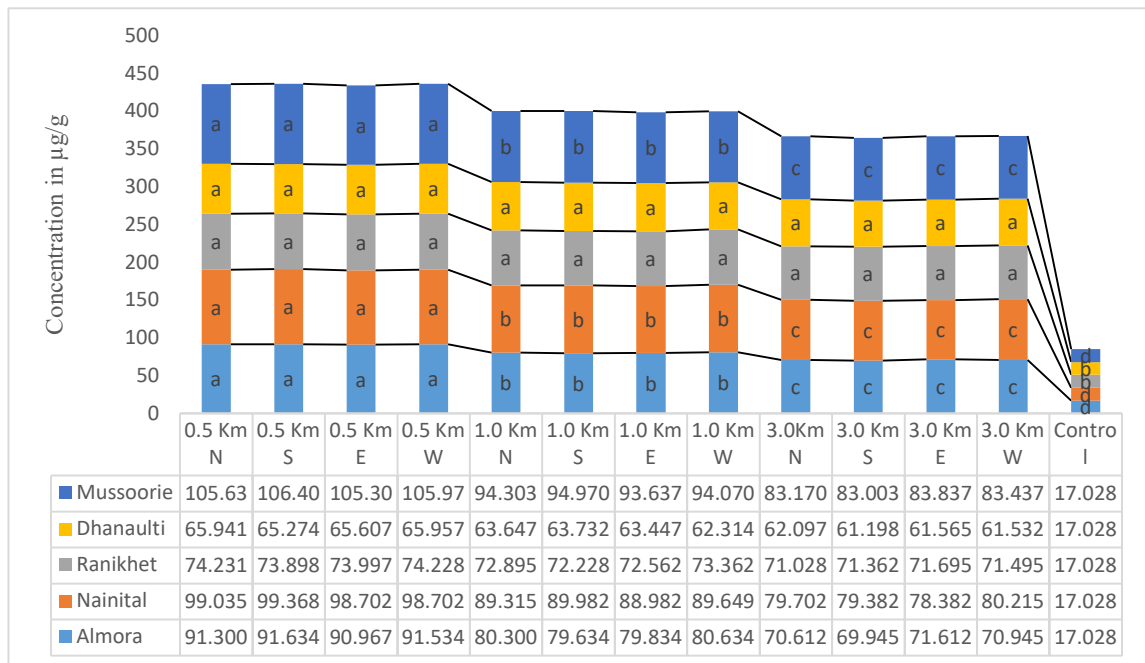


Fig. 2b: The concentration of zinc during Winter 2021 in Mussoorie, Dhanaulti, Ranikhet, Nainital, and Almora.

was conducted using the active biomonitoring method *Dumortiera hirsuta* samples collected at distances of 0.5 km, 1 km and 3 km in the east, west, north and south direction.

Zinc: Zinc results exhibited non-significant values at 0.5 km and 3 km during monsoon 2021 compared to its baseline (control) concentration (Fig. 2a). Zn is considered a biologically

active and living element, so it usually makes a complex with other environmental elements and can break down some essential nutrients (Stefanidou et al. 2006). The highest accumulation of zinc (59.251 µg.g⁻¹ DW) was observed at 0.5 km in the Mussoorie catchment site, and non-significant values were also recorded at the same distance

to north (59.017 $\mu\text{g.g}^{-1}$) and south (59.084 $\mu\text{g.g}^{-1}$). Declining concentration was measured in Almora city of Kumaon region towards 3 km west (31.515 $\mu\text{g.g}^{-1}$), and non-significant values were observed at the north

(32.515 $\mu\text{g.g}^{-1}$), south (31.849 $\mu\text{g.g}^{-1}$) and east (32.849 $\mu\text{g.g}^{-1}$) (Fig. 2a). However, researchers believed that Zn could originate from the abrasion of traffic lights and barriers (Carrero et al. 2012).

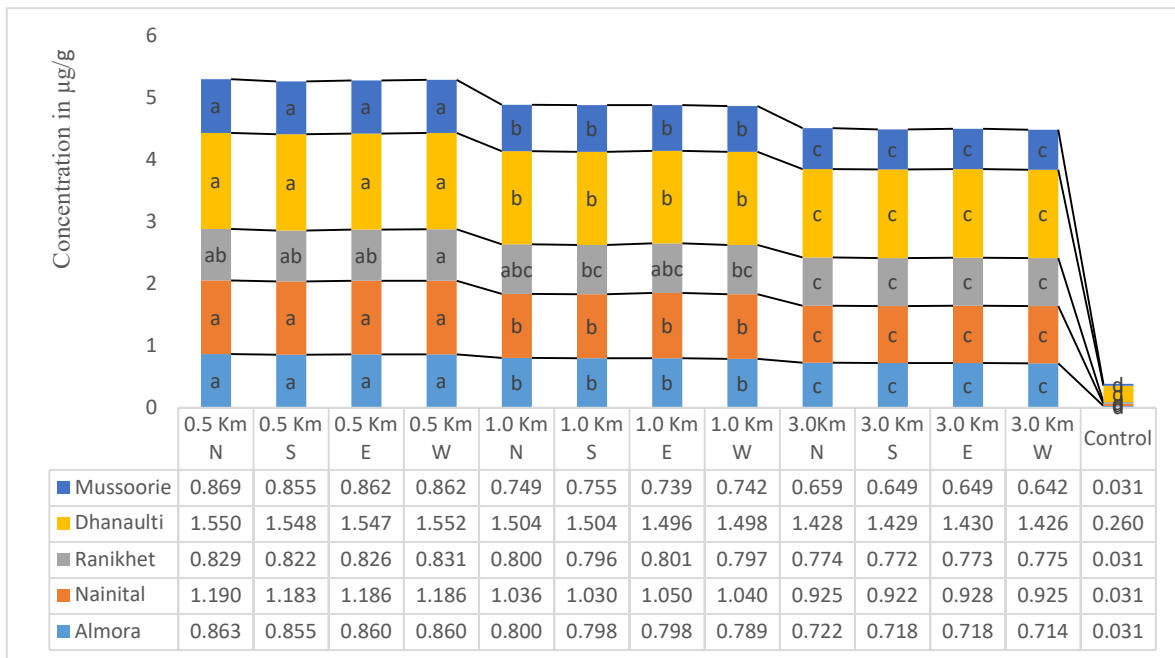


Fig. 3a: The concentration of arsenic during Monsoon 2021 in Mussoorie, Dhanaulti, Ranikhet, Nainital, and Almora.

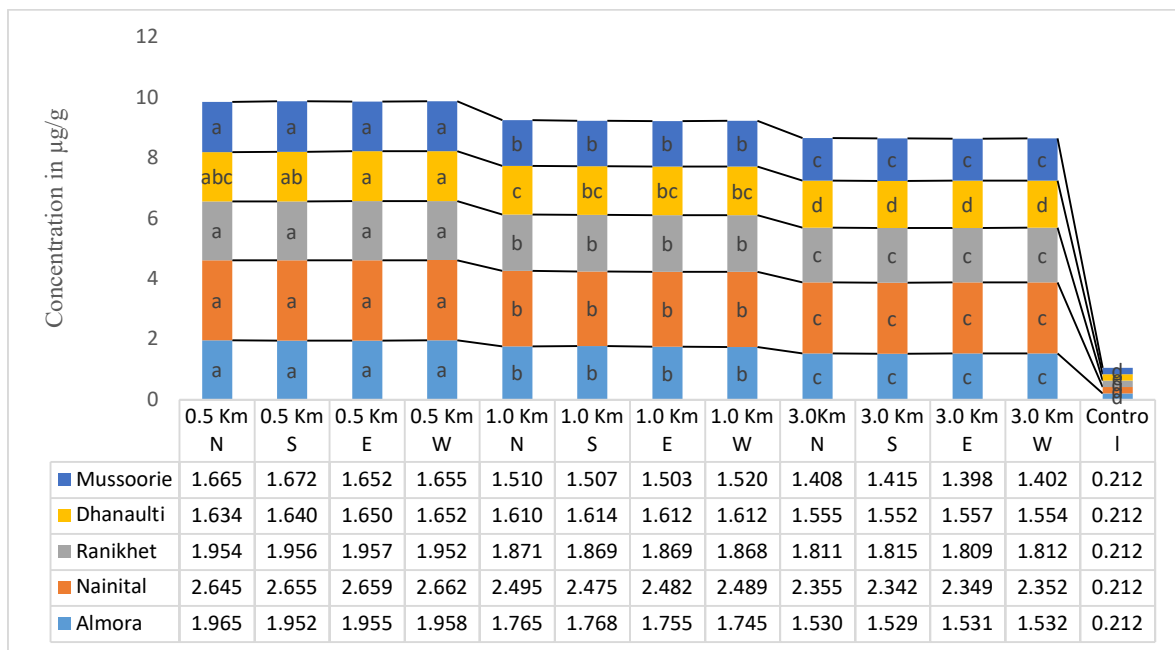


Fig. 3b: The concentration of arsenic during Winter 2021 in Mussoorie, Dhanaulti, Ranikhet, Nainital, and Almora.

The highest concentration exhibited during winter 2021 was observed in Mussoorie of Garhwal region at 0.5 km south (106.400 $\mu\text{g.g}^{-1}$). Significant values were also observed at the same Km of north, east, and west. The maximum load of zinc was found in the Mussoorie region, which might be caused due to high traffic density and a major road component if the asphalt surface contains cobalt, nickel, zinc, etc. (Saxena et al. 2013). And lowest concentration was found in Dhanaulti at 3 Km south (61.198 $\mu\text{g.g}^{-1}$), and non-significant different values were observed at the same km of east (61.565 $\mu\text{g.g}^{-1}$) and west (61.532 $\mu\text{g.g}^{-1}$) (Fig. 2b). Zinc deposition in lichens was also discovered, which is frequently linked to increases in traffic along the roads that serve inner-city urban regions. Additionally, multiple studies discovered greater zinc concentrations in *Parmotrema* species found in metropolitan areas with high levels of automobile traffic (R Team 2015).

Arsenic: Compared to the baseline concentration, Arsenic (As) showed non-significant values in monsoon and winter 2021. The maximum concentration of As during monsoon 2021 was found in Mussoorie of Garhwal region at 0.5 km west (1.552 $\mu\text{g.g}^{-1}$), and non-significant values were also observed at the same distance north (1.550 $\mu\text{g.g}^{-1}$), south (1.548 $\mu\text{g.g}^{-1}$) and east (1.547 $\mu\text{g.g}^{-1}$). Minimum concentration was found in Almora of Kumaon region at 3 km west (0.714 $\mu\text{g.g}^{-1}$), and non-significantly different values were observed at the same distance in the north (0.722 $\mu\text{g.g}^{-1}$), south (0.718 $\mu\text{g.g}^{-1}$), and east (0.718 $\mu\text{g.g}^{-1}$) (Fig. 3a). Pollutant load decreased during monsoon could be attributed to two possible reasons, firstly, the low tourism load, and second, the less settlement time for atmospheric heavy metals.

The highest concentration of arsenic during winter 2021 was measured in Nainital of Kumaon region at 0.5 km west (2.662 $\mu\text{g.g}^{-1}$), and non-significant values were also observed at the same km in the north (2.645 $\mu\text{g.g}^{-1}$), south (2.655 $\mu\text{g.g}^{-1}$), east (2.662 $\mu\text{g.g}^{-1}$) respectively. The lowest concentration was found in Mussoorie of Garhwal region at 3 km west (0.714 $\mu\text{g.g}^{-1}$). Non-significantly different values were observed at the same distance of north (1.408 $\mu\text{g.g}^{-1}$), south (1.415 $\mu\text{g.g}^{-1}$), and west (1.402 $\mu\text{g.g}^{-1}$), respectively. Arsenic is mainly emitted from the mining industry, especially lead and zinc mining (Islam et al. 2015) (Fig. 3b). This could be because moss is frequently exposed to dust with airborne dust, which is rich in heavy metals (Markert & Weckert 2008).

Cadmium: During the monsoon of 2021, the highest concentration was found in the Dhanaulti catchment site of the Garhwal region at 0.5 km east (0.234 $\mu\text{g.g}^{-1}$), and non-significant values were also observed at the same distance in the north (0.233 $\mu\text{g.g}^{-1}$), south (0.230 $\mu\text{g.g}^{-1}$) and west (0.231 $\mu\text{g.g}^{-1}$). Cd is a substance that is primarily of anthropogenic origin and is released by the residential, industrial, and transportation sectors (Cuniasse & Glass 2020). Cd is probably also found in gasoline from the manufacture of cadmium compounds and colored plastic bags. An increase in vehicular pollution can be another factor for the rise in Cd level (Saxena et al. 2013). Declined concentration was observed in Mussoorie city at 3 km north (0.136 $\mu\text{g.g}^{-1}$), and non-significant values were observed at the same distance of south (0.138 $\mu\text{g.g}^{-1}$), east ((0.140 $\mu\text{g.g}^{-1}$), and west (0.141 $\mu\text{g.g}^{-1}$) (Fig. 4a). In addition, the transboundary pollution load of Cd-containing particles may travel far through wet and

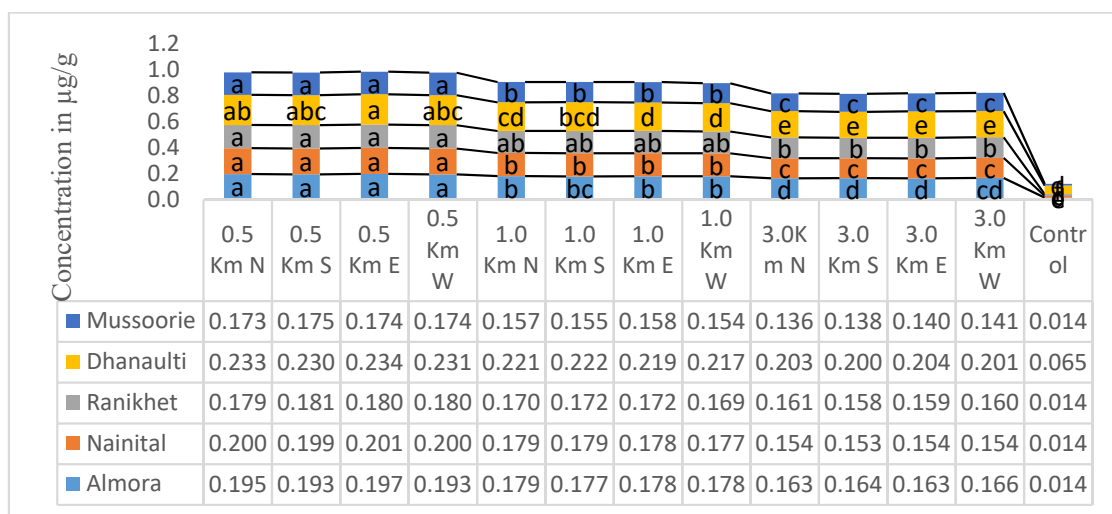


Fig. 4a: The concentration of cadmium during Monsoon 2021 in Mussoorie, Dhanaulti, Ranikhet, Nainital, and Almora.

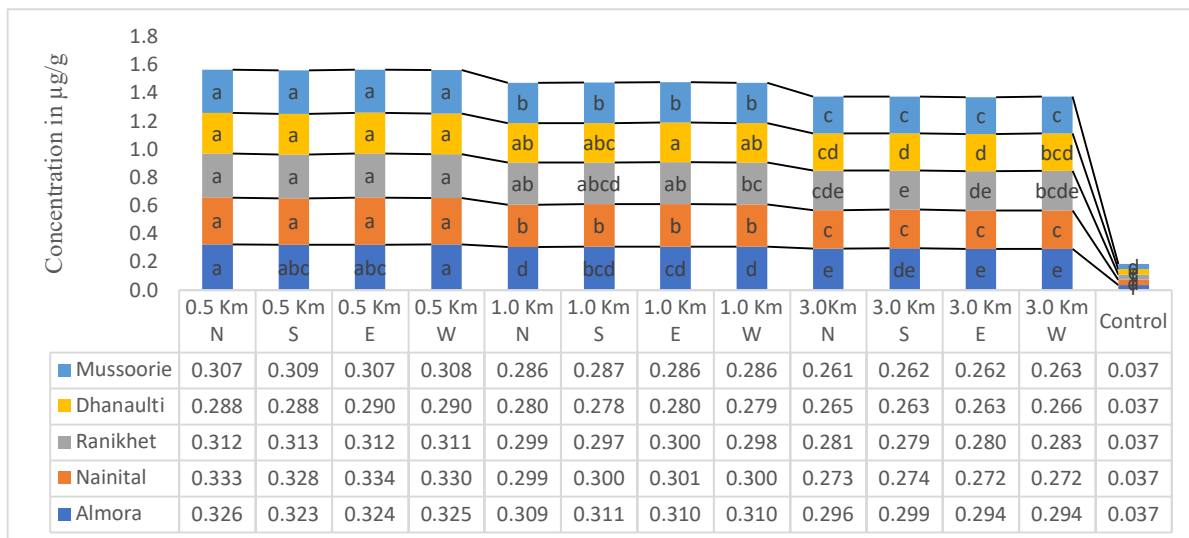


Fig. 4b: The concentration of cadmium during Winter 2021 in Mussoorie, Dhanaulti, Ranikhet, Nainital, and Almora.

dry deposition before settling on soil and aquatic systems (Sakata et al. 2008).

The maximum concentration loading of cadmium during winter 2021 was observed in the Nainital catchment of the Kumaon region at 0.5 km east (0.334 $\mu\text{g}\cdot\text{g}^{-1}$), and non-significant values were also observed at distance to the north (0.333 $\mu\text{g}\cdot\text{g}^{-1}$), south (0.328) and west (0.330). The concentration of cadmium was maximum in Dhanaulti during monsoon, possibly due to the influx of tourist visits to Tehri dam. Other sources include colored polyethylene bags, household waste, discarded plastics, utensils, etc., and the lowest concentration was found in Mussoorie of Garhwal

region at 3 km north (0.261 $\mu\text{g}\cdot\text{g}^{-1}$). Non-significant different values were observed at the same distance in of south (0.261 $\mu\text{g}\cdot\text{g}^{-1}$) and east (0.262 $\mu\text{g}\cdot\text{g}^{-1}$) and (0.263 $\mu\text{g}\cdot\text{g}^{-1}$), respectively (Fig. 4b). It is crucial to note that the emissions resulting from incinerated materials and their components, for example the quantity of dry batteries with varying Cd contents, will significantly differ based on how these materials are processed, whether they are segregated or burned.

Lead: The maximum concentration of lead during monsoon 2021 was found in Dhanaulti of Garhwal region at 0.5 km north (3.350 $\mu\text{g}\cdot\text{g}^{-1}$), and non-significant values were also observed at the same distance south (3.347

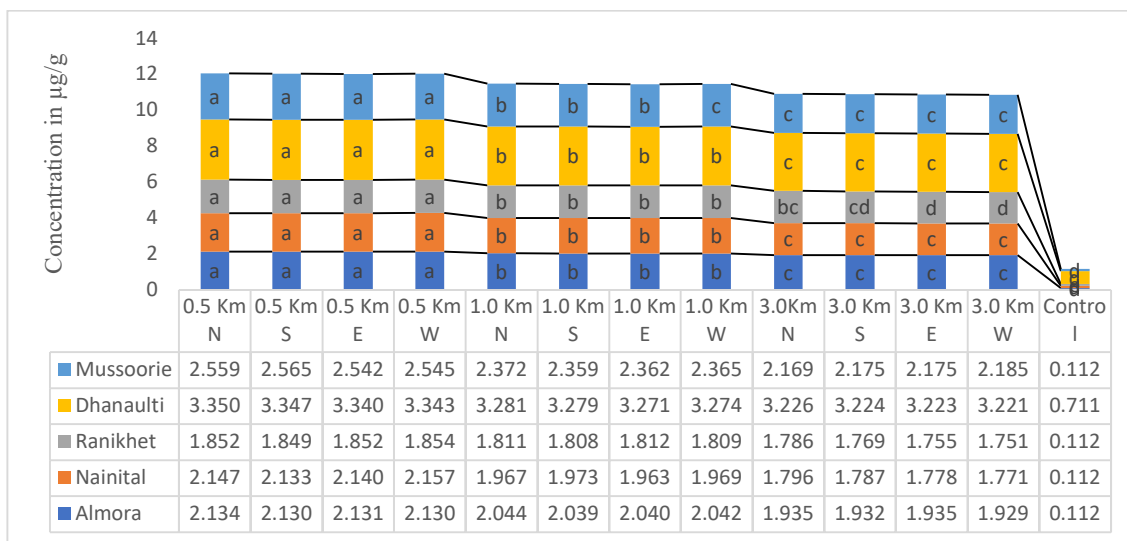


Fig. 5a: The concentration of lead during Monsoon 2021 in Mussoorie, Dhanaulti, Ranikhet, Nainital, and Almora.

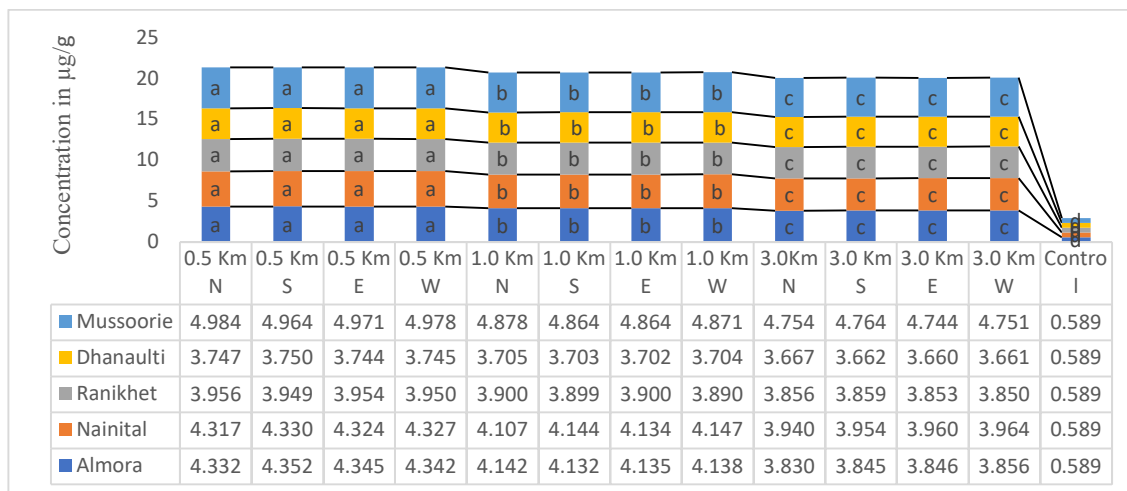


Fig. 5b: The concentration of lead during Winter 2021 in Mussoorie, Dhanaulti, Ranikhet, Nainital, and Almora.

µg.g⁻¹), east (3.340 µg.g⁻¹) and west (3.343 µg.g⁻¹). The minimum concentration was found in Ranikhet of Kumaon region at 3 km west (1.751 µg.g⁻¹), and non-significantly different values were observed at the same distance in the north (1.786 µg.g⁻¹), south (1.769 µg.g⁻¹) and east (1.755 µg.g⁻¹) (Fig. 5a). Lead is derived from zinc, silver, and copper ores (MMSD 2010).

The highest concentration of lead during winter 2021 was found in Nainital of the Kumaon region at 0.5 km north (4.984 µg.g⁻¹), and non-significant values were also observed at the same km of the north (4.984 µg.g⁻¹), south (4.964 µg.g⁻¹) and east (4.971 µg.g⁻¹). The concentration loading of Lead was found to be maximum in Mussoorie due to industries located in Haridwar and Dehradun as well as emission of lead-based batteries (Sun et al. 2017b). The lowest concentration was found in Mussoorie of Garhwal region at 3 km east (3.660 µg.g⁻¹), and non-significantly different values were observed at the same distance in of north (3.667 µg.g⁻¹), south (3.662 µg.g⁻¹) and west (3.661 µg.g⁻¹) (Fig. 5b). Mining and other human activities discharge lead into the atmosphere, polluting soils and water (Makwe & Okobia 2020).

Principal Component Analysis

The relationship between heavy metals and their association with winter (Dry season) and monsoon (Wet season) 2021 was done through a principal component with two components explaining maximum variation in data and followed by factor rotation through the varimax method.

The relationship between the cities and their correlation to heavy metal concentration. In the case of zinc (Zn), the first two components explained 99.9% of the total variance.

The factor loading for PC1, PC2, and PC3 indicated positive correlations with Nainital, Dhanaulti, and Mussoorie, and negative correlations with Almora and Ranikhet. The total variance of As in the cities explained by the first two components was 100.00%. The factor loading in PC1, PC2, and PC3 shows positive loading with Nainital and Ranikhet and negative loading with Almora, Dhanaulti, and Mussoorie. The total variance of Cd in cities explained by the first two components was 99.90%. The factor loading of Cd in PC1, PC2, and PC3 Almora and Mussoorie shows positive loading and negative loading with Nainital, Ranikhet, and Dhanaulti. The total variance of Pb between cities explained by the first two components was 100.00%. Factor loading of Pb for PC1, PC2, and PC3 varies greatly. All the components show positive loading with PC1 and Negative loading with PC2; in PC3, Almora, Dhanaulti, and Mussoorie show negative loading (Tables 2a and 2b). High vehicle traffic, the soil in the area, and the abrasion of brake pads, brake linings, car tires, paints, and other materials were all identified by the PCA as dust contributors' vehicle component varnishes and fuel combustion processes (Skorbiłowicz et al. 2020).

The relationship between the city with their correlation to heavy metal concentration. The total variance of Zn in the cities, explained by two components, was 100.00%. The factor loading of Zn for PC1, PC2, and PC3 was showed negative values for Nainital, Dhanaulti, and Mussoorie, while Almora and Ranikhet showed a positive loading. The total variance of arsenic between cities explained by the first two components was 100.00%. Factor loading of Pb for PC1, PC2 and PC3 varied significantly. All the components showed positive loading with PC1 and Negative loading with PC2; in PC3, Nainital and Ranikhet displayed negative loading, and Almora, Dhanaulti, and Mussoorie exhibited positive

Table 2a: The total variance of the Almora, Nainital, Ranikhet, Dhanaulti, and Mussoorie analyzed Liverwort during Monsoon 2021.

Components		Initial eigenvalues			Extraction sum of squared loadings			Rotation sums of squared loadings		
		Total	% of variance	Cumulative %	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
Zn	1	4.841	96.800	96.800	4.841	96.800	96.800	2.618	52.400	52.400
	2	0.153	3.100	99.900	0.153	3.100	99.900	2.374	47.500	99.900
	3	0.004	0.100	100.000						
	4	0.001	0.000	100.000						
	5	0.000	0.000	100.000						
As	1	4.927	98.500	98.500	4.927	98.500	98.500	2.518	50.400	50.400
	2	0.071	1.400	100.000	0.071	0.014	1.000	2.478	49.600	100.000
	3	0.001	0.000	100.000						
	4	0.000	0.000	100.000						
	5	0.000	0.000	100.000						
Cd	1	4.972	99.400	99.400	4.972	99.400	99.400	2.518	50.400	50.400
	2	0.024	0.500	99.900	0.024	0.500	99.900	2.478	49.600	100.000
	3	0.001	0.000	100.000						
	4	0.000	0.000	100.000						
	5	0.000	0.000	100.000						
Pb	1	4.963	99.300	99.300	4.963	99.300	99.300	2.536	50.700	50.700
	2	0.036	0.700	100.000	0.036	0.700	100.000	2.463	49.300	100.000
	3	0.000	0.000	100.000						
	4	0.000	0.000	100.000						
	5	0.000	0.000	100.000						

Table 2b: Factor loadings for the Liverwort using (varimax rotation).

Components	Zn			As			Cd			Pb		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Almora	0.851	0.524	-0.023	0.715	0.699	-0.023	0.689	0.724	0.025	0.721	-0.693	-0.004
Nainital	0.798	0.602	0.038	0.779	0.627	0.016	0.774	0.633	-0.009	0.626	-0.780	0.008
Ranikhet	0.551	0.834	-0.026	0.592	0.806	0.010	0.631	0.776	-0.001	0.771	-0.636	0.007
Dhanaulti	0.577	0.815	0.042	0.623	0.782	-0.014	0.699	0.714	-0.033	0.775	-0.632	-0.009
Mussoorie	0.788	0.614	0.026	0.808	0.588	-0.010	0.747	0.664	0.020	0.655	-0.755	-0.015

loading. The total variance of Cd in the cities, explained by two components, was 100.00%. The factor loading of Cd for PC1 showed positive loading while in PC2, Nainital and Mussoorie show negative loading, and Almora, Dhanaulti, and Ranikhet showed positive loading. In PC3, Almora and Nainital, along with Ranikhet, Dhanaulti, and Mussoorie, showed negative loading. The total variance of Pb in the cities, explained by two components, was 100.00%. The factor loading of Pb is positive for all component in PC1 and negative for PC2 in PC3 Almora shows negative loading, and Nainital, Ranikhet, Dhanaulti, and Mussoorie shows positive loading (Table 3a and 3b). Source identification relies on correlation, which can confirm and explain PCA results (Jiang et al. 2019). The result of the PCA table, along with factor loading, indicates

the source of metals and their association with other elements, and factor loading denotes their relation to other elements up to three components using varimax rotation.

CONCLUSION

The study was designed to acquire information about the atmospheric heavy metals in the western Himalayan region. Mountains are a great site of attraction for tourists. Every year large population visits Uttarakhand for its natural beauty. The results imply that their simplicity, totipotency, high multiplication rate, and cost-effective tool make them excellent for pollution research. Research showed wet and dry metal contamination.

Table 3a: The total variance of the Almora, Nainital, Ranikhet, Dhanaulti, and Mussoorie for analyzed Liverwort during Winter 2021.

Components		Initial eigenvalues			Extraction sum of squared loadings			Rotation sums of squared loadings		
		Total	% of variance	Cumulative %	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
Zn	1	4.880	97.600	97.600	4.880	97.600	97.600	2.649	53.000	53.000
	2	0.117	2.300	100.000	0.117	2.300	100.000	2.347	47.000	100.000
	3	0.001	0.000	100.000						
	4	0.001	0.000	100.000						
	5	0.000	0.000	100.000						
AS	1	4.941	98.900	98.900	4.941	98.900	98.900	2.621	52.400	52.400
	2	0.056	0.100	100.000	0.056	0.100	100.000	2.375	47.500	100.000
	3	0.001	0.000	100.000						
	4	0.000	0.000	100.000						
	5	0.000	0.000	100.000						
Cd	1	4.976	99.500	99.500	4.976	99.500	99.500	4.976	99.500	99.500
	2	0.022	0.400	100.000	0.022	0.400	100.000	0.022	0.400	100.000
	3	0.000	0.000	100.000						
	4	0.000	0.000	100.000						
	5	0.000	0.000	100.000						
Pb	1	4.978	99.600	99.600	4.978	99.600	99.600	2.583	51.700	51.700
	2	0.021	0.400	100.000	0.021	0.400	100.000	2.415	48.300	100.000
	3	0.000	0.000	100.000						
	4	0.000	0.000	100.000						
	5	0.000	0.000	100.000						

Table 3b: Factor loadings for the Liverwort using (varimax rotation).

Components	Zn			As			Cd			Pb		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Almora	0.828	0.561	0.003	0.595	-0.803	0.006	0.998	0.057	0.021	0.643	-0.766	-0.005
Nainital	0.789	0.614	-0.006	0.739	-0.673	-0.021	0.994	-0.106	0.009	0.684	-0.729	0.017
Ranikhet	0.566	0.824	0.018	0.784	-0.621	-0.023	0.999	0.038	-0.009	0.760	-0.650	0.003
Dhanaulti	0.612	0.790	-0.03	0.803	-0.596	0.007	0.998	0.062	-0.008	0.762	-0.648	0.001
Mussoorie	0.804	0.594	-0.008	0.679	-0.732	-0.044	0.999	-0.051	-0.012	0.738	-0.675	0.000

Additionally, bryophytes (mosses) are supportive and functional monitoring tools that could aid biomonitoring research around similar metal pollution sources and alert local inhabitants of potential metal deposition threats. A continuous monitoring program to understand pollution trends and protect the ecosystem and human health. Apart from it, future prospects reduce the pollution load and minimize the exposure of tourist sites. Public transport should be used, and the use of CNG and electrical vehicles should be encouraged.

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