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Monitoring of Metal Dispersion in Kumaon Hills (INDIA) Through Active Monitoring Using Moss

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Abstract
Bryophytes have been used as a terrestrial bio-monitors and bio-indicators of air pollution and are recognized as more sensitive to pollution than other plants. In present study common moss species, i.e. *Rhodobryumgiganteum* (Schwaegr) Par., *Rhynchostegielladivaricatifolia* (Renauld&Cardot), *PohliaelongataHedw*, *Racomitriumcrispulum* (Hook. f. et Wils.), *Pleuroziumshcreberi, Hylocomiumsplendens, Hypnumcupressiforme, Brachytheciumrutabulum, Homalotheciumsericeum, Racomitriumcrispulum* were collected from monitoring sites and were validated for their tolerance potential by measuring chlorophyll fluorescence signals. Amongst above species validated for tolerance, moss *Racomitriumcrispulum* (Hook. f. et Wils.) is inducted for monitoring program. Collected samples were air dried, then cleaned and digested. The levels of Zn, Cu, Cd and Pb have been determined in transplants of the moss *Racomitriumcrispulum* (Hook. f. et Wils.) from the area of Almora, Nainital and Pithoragarh of Kumaon hills. High metallic load was measured in moss harvested from locations in proximity of higher traffic density areas, which is attributed to the enhanced tourism during summer and monsoon season. In rural areas high value of Zn and, Cu attributes to their use in fertilizers. Positive significant correlations were obtained between Pb-Zn and Zn-Cu that suggest a common origin of these metals. Elemental concentration in moss *Racomitriumcrispulum* was in order Zn>Pb ~ Cu> Cd in summer while, same was Zn> Cu >Pb> Cd in winter season and in rain Zn> Cu >Pb> Cd that reflects atmospheric trace elemental load. Bioaccumulation ability of this moss was evaluated statistically using DMR test and cartographically presented on contour maps obtained from SURFER program.

Key words: Metal Emission, Kumaon hills, moss *Racomitriumcrispulum* (Hook. f. etWils.), active monitoring.

Introduction

Even in the advanced civilizations of the ancient world, the use of metals reached an order of magnitude that can be detected as metal deposition on global scale [1] [2]. Environmental pollution is a problem, both in developed and developing countries [3]. Factors such as population growth and urbanization invariably place greater demand on the planet and stretch the use of natural resources to the maximum.

Rapid industrialization and urbanization in the last century has greatly increased both quantity and quality of metal production worldwide. Air pollution comes from many different sources: stationary sources such as factories, power plants, smelters and smaller sources such as dry cleaners and degreasing operations; mobile sources such as cars, buses, planes, trucks and trains; and naturally occurring sources such as windblown dust and volcanic eruptions. Air pollution has many disastrous effects that need to be curbed. In order to accomplish this, governments, scientists and environmentalists are using or testing a variety of methods aimed at reducing pollution.

Anthropogenic source of metals can have a serious impact on environment; therefore, continuous or regular assessment of parameters depicting the state of the environment is pre-requisite for abatement of their sources in their respective region [4, 5]. The metals As, Cr, Be, Cu, Cd, Zn, Hg and Pb are closely associated with air precipitation and are derived from a wide spectrum of natural and anthropogenic sources [6]. In recent decades the number and intensity of anthropogenic sources have increased the overall environmental element concentration [7, 8]. This fact seems to be true for the Kumaon hills (India). High concentration of metals is a major source of environmental pollution in the ecosystem may lead to an excessive accumulation, which may be toxic to plants and cause possible health problem to animals and human. Determining environmental concentrations is an important part of understanding biogeochemical processes and gauging ecosystem's health.

Use of mosses as bio-monitor is a suitable method for determining the total levels of atmospheric deposition of metals and its trend due to their accumulation capability [9, 10]. The method is generally simple and relatively inexpensive when compared to direct measurement techniques without

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requiring electrical power or specialized equipment [11]. Additionally, the data provide an integration of deposition over the exposure period. It provides an integrated picture of air pollution levels of around the clock and it can also be used to monitor and control the success of environmental policies.

The specific biology of mosses enables them to receive and accumulate chemical substances predominantly from surrounding atmosphere without any selectivity parameter [12, 13]. They do not have conductive strand and root system, means directly uptake air pollutants and represent it by each cell's individually [14]. Due to their accumulation potential they have been preferred in present study.

The advantages of the biological monitoring and bryophyte as compared with instrumental and chemical methods can be summarized as follows:

- Inexpensive compared to the instrumental monitoring. As the former needs number of instruments for deployment on each site to obtain trends in atmospheric metal deposition. This includes, uninterrupted power supply, operating manpower additional to maintenance.
- Involved large number of monitoring instruments making it inconvenient and cumbersome.
- Risk of environment hazardous for chemical monitoring.

While Mosses and liverworts do not take anything from substratum; on the contrary they take nutrient and moisture from air, fungi and algae cannot be inducted in monitoring as they lack the chlorophyll some times and algae strive in water hence not fit for ambient air monitoring. Additonally, pteridophytes, gymnosperm and angiosperm are rooted plants and therefore in their analysis represent the metals absorbed by root.

Why validations of metal tolerant moss essential?

It is desirable to know the tolerance potentials of mosses against metals before inducting in a monitoring programme. Since metal toxicity in sensitive moss species may impair its physiology and therefore will effect on accumulation potentials. To omit out sensitive moss species, it is desirable to validate the tolerant species against metals to induct in ambient metal monitoring program.

Approach to validate the tolerant species

Chlorophyll fluorescence measurements are best, *non-invasive* tool to test the status of health of plant during the stress in the field and laboratory. It can be performed quickly without the use of any chemicals. For inducting any moss species in bio-mapping program, measurement of status of health is desired to validate the tolerance against metals by measuring their chlorophyll fluorescence. Although the total amount of chlorophyll fluorescence is very small (only 1 or 2% of total light absorbed), the measurement can be accurately acquired. The spectrum of fluorescence is different to that of absorbed light, with the peak of fluorescence emission being of longer wavelength than that of absorption. Therefore, fluorescence yield can be quantified by exposing a leaf to light of defined wavelength and measuring the amount of light re-emitted at longer wavelengths. For the first time PEA (photosynthetic efficiency analyzer) was applied to validate the tolerant species without in a non-invasive approach.

In present study, moss *Racomitriumcrispulum* (Hook. f. et Wils.) is attempted for the first time as an active bio-monitor to determine total and seasonal atmospheric deposition of metals in Kumaon hills during year 2004. Transplant technique is useful especially in such areas where identified or inducted moss is lacking in monitoring areas [15, 3].

Materials and Methods

Study area

The Kumaon region is spread over 21,073 Km^2 and had extensive track of natural forests until a few centuries back. The species is of wide range of distribution in Western Himalaya geographically ranging from 29º 5′-31º 25′ N in latitude to 79º 43′-81º E in longitude (IG, 1931). The Kumaon hill is extensively divided into three major zones for the bio-mapping study namely- Almora (E 79º 26'E to 80º 15'E and N 29º 15'N to 30º 29'N), Nainital (E 79º 54'E to 80º 18'E and N 28º 45'N to 29º 38'N) and Pithoragarh (longitudes 79º 45'E- 81º 21'E; latitudes 29º 32' N- 30º 47' N).. It is characterized with a quite cold weather between October – April and mild warm through May – June followed by monsoon rain until September. The average measured rainfall is 80in and the relative humidity range from 85 to 90% in the months of July and August. The maximum and minimum recorded temperatures were 27°C to 10°C in summer and 15°C to 3°C in winter seasons.

Sampling of moss species

The collection of moss samples was carried out according to the guidelines of the UNECE ICP vegetation. The most dominant moss species in this study are *Rhodobryumgiganteum* (Schwaegr) Par., *Rhynchostegielladivaricatifolia* (Renauld&Cardot)

Broth., *PohliaelongataHedw*, *Racomitriumcrispulum* (Hook. f. et Wils.), *Pleuroziumshcreberi*, *Hylocomiumsplendens*, *Hypnumcupressiforme*, *Brachytheciumrutabulum*, *Homalotheciumsericeum*, *Racomitriumcrispulum.* Before any species inducted, each was validated for metal tolerance. For collection of each moss sample a separate set of disposable polyethylene gloves was used. Collected samples were stored in hygienic paper bags.

Validation of metal tolerance species

There are a number of physiological variables that can be used as vitality indicators. They may be based on: *(1)* photosynthetic pigment composition, i.e. chlorophyll concentration, chlorophyll a/b ratio, the quotient between absorbance at 430 and 410 nm, and several others; *(2)* photosynthesis rates; and *(3)* chlorophyll fluorescence. Chlorophyll fluorescence is probably the most direct, *non-invasive* and fastest method. Out of available ten moss species only six widely distributed mosses were short listed because the other four species were not commonly available at all sites.

A handy photosynthetic efficiency analyzer (Handy PEA, Hansatech Instruments Model No. 1011) was used to validate the best metal tolerance moss amongst the considerec six mosses, including i.e. *Rhodobryumgiganteum* (Schwaegr.) Par, *Pohliaelongata*Hedw., *Racomitriumcrispulum*, *Rhynchostegielladivaricatifolia* (Renauld&Cardot) Broth, *Hylocomiumsplendens* and *Pleuroziumshcreberi* by measuring their chlorophyll a fluorescence in the field. The moss beds were covered by plastic beljar stretching over 1ft^2 after spraying known concentration of the solution. The ratio of variable (F_v) to maximal fluorescence (F_m) was measured at different intervals to indirectly evaluate the photosynthetic activity response to the metal treatment. This is written as:

$$F_v = F_m - F_o \qquad (1)$$

Where F_m and F_o, are the maximum and initial fluorescence, respectively. In other words, F_m is the maximum chlorophyll *a* fluorescence at the used light intensity whereas F_v is related to the maximum capacity for photochemistry of Photosystem II (Govindjee 2004). The F_v/F_m ratio has thus been considered as a sensitive indicator of plant photosynthetic efficiency/performance, with its maximum value being about 0.80 - 0.85. A lower Value indicates impaired photosynthesis or it is an indication of stress. From our pilot study, it was evident that *Racomitriumcrispulum* is one of the metal tolerant species, thereby it is inducted for the purpose of bio-monitoring atmospheric metal as its photosynthetic efficiency was not impaired deeply as evident in the F_v/F_m values compared to the other mosses as depicted in Fig.1.

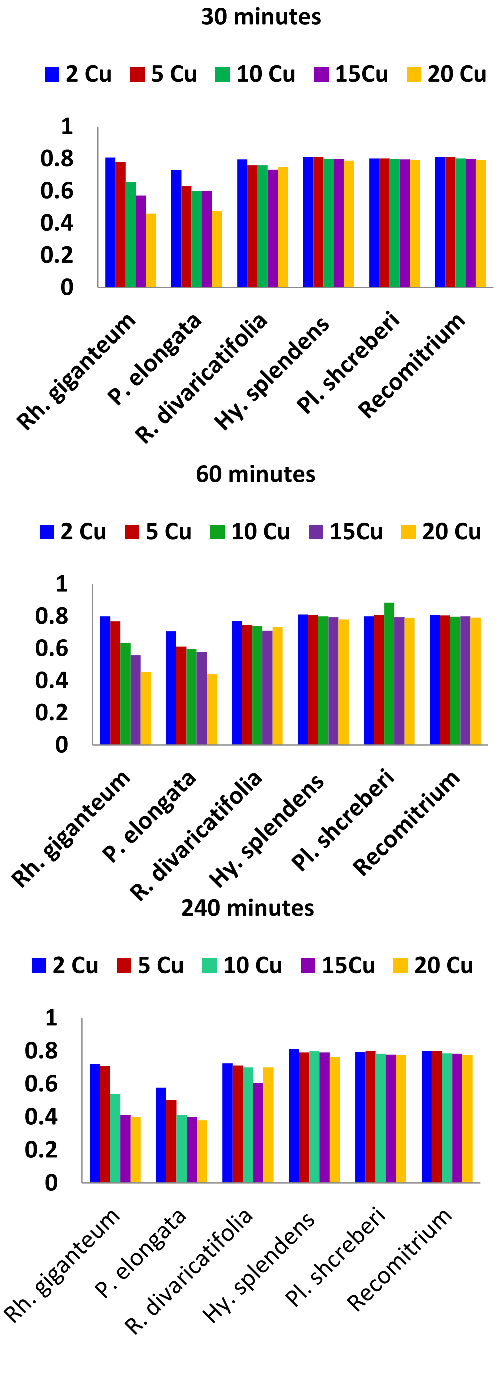

Fig. 1: Chlorophyll fluorescence value of different lead concentration tested against 5 moss species after 30, 60, and 120 minutes and

Ideally all six undertaken moss species should have been validated for all four metals (Cu, Pb Cd, and Zn) under consideration. Given Cu and Zn are essential trace elements and Cd is relatively less abundant in the atmosphere [16], it is not unreasonable to assume that a Pb and Cu tolerant moss species will be also tolerate Cd and Zn.

Moss transplants

Validated tolerant moss *Racomitriumcrispulum* (Hook. f. et Wils.). was transplanted at a distance at least 1000m from main roads. Each sample was composed of 5 to 10 sub-samples collected within an area of 50 × 50 m, in order to make the moss samples representative for a reasonably large area. A complete green patch of moss was transplanted in nylon bags at 19 study sites of the investigated area.

Seasonal Meta Data

Each moss bag was suspended at height of 8 feet above the ground in triplicate. These moss bags were transplanted in cross direction wise in all the four directions for a duration of four months (representing one season) and were harvested after an exposure in baseline metal load from Mukteswar site treated as control.

Reagents and standards

All of the reagents used for this study were with analytical grade of Nitric acid (Merck) (HNO_3), Perchloric acid (Merck) ($HClO_4$) and triple distilled water. Solutes used for preparing standard solutions was with concentration of 1000 mg L^{-1}.

Upon return to the laboratory, harvested moss samples was oven dried at 40°C for 24 hours. Prior to analysis, adhering substrate and dirt was removed by hand and great care being taken to avoid metal contamination. For analysis sufficient amount of the exposed moss species harvested and was taken for digestion to determine the metals in each season. For the baseline metal load, moss from Mukteswar site was treated as control. Triplicate samples were digested with the concentrated HNO_3 and $HClO_4$ solutes in a ratio of 4:1 v/v on a hot plate. The digestion was completed after all organic material had disappeared. The extract obtained was filtered and the filtrate was made up to a final volume of 50 ml by double distilled water and fraction was quantitatively analyzed using an atomic absorption spectrophotometer (flame type), the equipment having been previously calibrated (Model no. 4039). Suitable blank was used to check for possible contamination during extraction.

Data analysis

Samples were collected in triplicate to conduct the statistical analysis. Values were represented as mean ± standard error [17]. The significance in metal concentration at different distances and seasons were calculated utilizing Dunkun's Multiple Range test [18].

A cartographic representation of the results was performed with the program package Surfer (Golden Software Inc., U. S. A.). Pollution index (PI) of the study area is calculated on the basis of each metal, total number of metals, total amounts of metals and number of samples collected from a predefined area of the district [18, 19]. The formula of PI value is location specific and is defined as:

$$P_j = \sum_{j=1}^{i=j} \frac{x_{ij} - \bar{x}_i}{\bar{x}_i} \quad (1)$$

Where x_{ij} is the content of i^{th} metal at j^{th} location and $\bar{x}_i$ is the average of i^{th} metal at all locations. The percentage of the metal load (ML) was calculated according to:

$$ML\,(\%) = \left(\sum_{i=1}^{N} M_T - M_{Ti}\right) / \, N \sum_{i=1}^{N} M_{Ti} \quad (2)$$

Where $M_{T1}+M_{T2}\ldots+M_{Tj}$ is the total metal M at all sites starting from 1st to N site.

Results

Metal tolerant moss

Amongst 10 widely distributed mosses, six mosses (*Racomitriumcrispulum*, *Rhynchostegielladivaricatifolia* (Renauld&Cardot) Broth, *Hylocomiumsplendens, Pleuroziumshcreberi, Rhodobryumgiganteum* (Schwaegr.) Par, *Pohliaelongata*Hedw) were shortlisted on the basis of their wide distribution. Mosses were validated for metals tolerance using chlorophyll fluorescence signal, as these measurements gave more detailed insight into the function of the photosynthetic apparatus during metal treatment. This approach is noninvasive and was preferred. Results of the chlorophyll fluorescence measurements taken for these six mosses against different concentrations of metals suggests that *Racomitriumcrispulum* is a tolerant moss, as F_v / F_m values were minimally affected as depicted in Figs. 1 and 2. Thereby, this specific moss was inducted in a monitoring program.

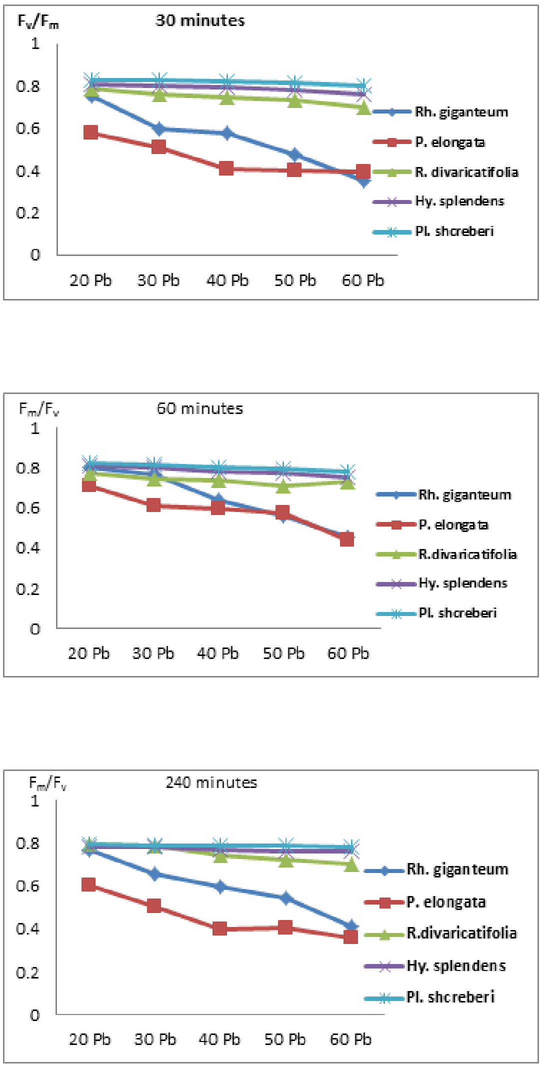

Fig. 2: Chlorophyll fluorescence value against metal lead tested against 5 moss species after 30, 60, and 120 minutes

Metal Lead

The mean value of each metal and its significance (ANOVA at $P \leq 0.05$ and $P \leq 0.01$) was calculated, along with its corresponding mean value at the Mukteswar control site . Amongst 19 catchments sites, the moss harvested from Nainital (bus stand) exhibited the maximum value of lead (56.32 µg g^{-1}) while the minimum value was measured from moss from Mukteswar (8.39µg g^{-1}) during 2004 (see Table 1 at the end).

Seasonal Pattern of metal Precipitation

The seasonally exposed moss samples were analyzed for metals at different regions during the year 2004, as listed in Table 1 and depicted Figs. 3 to 5 at the end. High values were measured during summer and winter from the Nainital site, whereas during the monsoon high values were recorded from Almora. Pb concentration in moss *Racomitriumcrispulum* in experimental area also measured seasonally (Table 1). Seasonal trends for Pb for maximum values were in order for summer, winter and rain measured as 56.32, 42.11 and 18.06 µg g^{-1}. Another important crowded hill station, Almora, led to values in order of 31.57, 36.49, and 18.06 µg g^{-1}during winter, summer and monsoon, respectively. The lowest Pb value was measured in moss *Racomitriumcrispulum*species harvested from Mukteswar forest of 12.34, 14.93 and 8.39 µg g^{-1} during winter, summer and monsoon seasons, respectively (Table 1). Both sites are heavily crowded during summer because of tourist inflow from all over the country. The overall percent metal Pb load did not exceed 8%. At Nainital bus stand, Almora petrol pump and Ranikhet golf court had maximum percentage of metal Pb as depicted in fig. 6. It also shows that the Tallital and the bus stand area of Nainital characterized with maximum value of Pb deposition in *Racomitriumcrispulum*. During the monsoon, a decrease in Pb of approximately 61% was noticed at Almora (Mall road). Moss from Pithoragarh area also exhibited high values for the lead, but were within the range of the 21.6, 27.95 and 14.08 µg g^{-1} during the summer, winter and in rain.

The high consumption of fuel during the tourist inflow could be the reason for the high lead value in all seasons except during rainy season, particularly along the roads and near the Nainital bus stand as depicted in Figs. 3a, 4a and 5a. This observation is in line with others, who have suggested that tourist places intensify vehicle pollution [20, 21]. The overall maximum percent metal Pb load in moss harvested from the locations of Nainital bus stand, Almora petrol pump and Ranikhetgolf court is in line with their maximum value, as depicted in Fig. 6. Another reason is due to mushroom growth in hotels and the unwise construction of houses that resulted in deforestation. Intensity further amplifies because of the increase in transportation mean to meet their daily needs. However, decrease in Pb load during monsoon at Mall road of Almora could be due to a low turnout in tourist activities and the wash out during rain (see Fig. 5a). Combustion of leaded gasoline continues to be the major source of atmospheric Pb emissions, and the present finding is in agreement with the report of Pacyna and Pacyna [22]. Even though low lead gasoline has been utilized, the high congestion and staggering emission still outweighs this factor[23].

Metal Zinc

The site measurement of Zn was high at Nainital during summer 91.83µg g^{-1} followed by 56.34µg g^{-1} in winters. During monsoon, however, and at Ranikhet (Golf court) and Ranikhet, the measured Zn was 66.32, and 49.97 µg g^{-1}, respectively. Measured Zn values in moss from the control side (Mukteswar) was 9.35 and 15.03 µg g^{-1} in winters and summer, respectively. Farming sites i.e. Dinapani, Kosi and Ranikhet (golf court) showed a significant increase in Zn (34 %) during summer compared to winter, as shown in Fig. 6. The monsoon season exhibits completely different precipitation pictures for Zn in moss *Racomitriumcrispulum*. The sites of Almora (Mall road), Nainital (Bus stand) observed 31-43 % reduction in Zn concentration with respect to summer during monsoon in *Racomitriumcrispulum* (Fig. 6). The control Mukteswar site showed a decrease of 18% in Zn during the rainy season compared to summer, as depicted in Fig. 6.

The increase in metal Zn measured in moss harvested from the same sites have a similar trend to that of Pb, with regression correlation of R^2 being 0.8781 in Almora and Pithoragarh. Locations close to orchards sites, such as Kosi and the Ranikhet golf court, are used to growing fruits, i.e. apples, peach, plums, pear and oranges, during summer, in which Zn is applied as a micronutrient. An increase in Zn in this area is amplified due to the fact that zinc is applied through foliar or aerial spray growth, promoting element [24].

The enrichment ratios for Zn in moss was not constant throughout the year, therefore, an interpretation of enrichment of Zn is complicated. A seasonal trend for metal Zn suggests that summers have high value of Zn followed by winters and rain, as depicted in Figs. 4b and 5b. Heavy rain fall could be another reason to explain the decrease in values. Aside from being a nutrient, farmers also implicate Zinc as Zn – based insecticides or fungicides [25]. Moderately high concentration of metal Zn was measured in moss harvested from tourist places i.e. Nainital (Tallital& bus stand) and Almora (Mall road) during winter 2004, as listed Table 1. Here one cannot overrule the effect of traffic to increase Zn along the road, as it is the part of automobile stratum [26] additional to the wear and tear of automobile parts, as well as abrasion of tire.

Metal Copper

In comparison to Pb and Zn, a moderate deposition rate of metal copper was observed from different catchment areas. Measured values of Cu in moss *Racomitriumcrispulum* was maximum (25.64μg g^{-1}) at the Nainital (Tallital) site and same was lowest at Pithoragarh in winters. On the contrary, values of copper were 37.04 μg g^{-1} in transplant moss harvested from the Nainital Bus stand during summer and low again from Pithoragarh. Almost the same trend was seen in monsoon (see Table 1 at the end).

The comparative study on the seasonal basis revealed that summer is the season for high Cu load in moss and lowest in monsoon. A total decrease of 24% was measured in Cu content from moss *Racomitriumcrispulum* from catchment areas of Nainital during winter with respect to 2004 summer. Present findings deployed that Ranikhet golf court and Almora (petrol pump) exhibited a decrease of 94% and 81% in metal Cu during winter season with reference to summer in the year 2004. The catchment area of Almora (mall road) exhibited a decrease in Cu of 43% in the rainy season in reference to summer (Fig. 6). A decrease in Cu in winter with respect to summer could be explained by considering that dry deposition increases on moving from arid to humid climates [27] (Couto*et al.*, 2004). A maximum value of copper was in Almora during summer and in Nainital in winter seasons, as listed in table 1.

Metal Cu was high in rural areas (see Figs. 3c, 4c and 5c) in comparison to urban areas, which could be due to its applications as copper based insecticides and pesticides. Present findings are also further supported by the presence of Cu mosses in these areas [26].

Concentration and distribution patterns of both metals Zn and Cu in moss *Racomitriumcrispulum* were quite similar and both metals were high in moss of rural transplants located in vicinity of orchards. Cu contamination mainly originates from fertilizers, fungicides and pesticides used in agricultural areas [26, 28]. A significant correlation was found between Zn and Cu content (R^2 = 0.8804) in *Racomitriumcrispulum* species of Kumaon hill as listed in table 2. Cu pollution may also originate from domestic waste disposal. The use of $CuSO_4$ mixed kerosene oil could also be one of the facts of increase of Cu concentration in domestic areas.

Table 2: Correlation coefficients amongst metal concentration in Kumaon Hill (India) through active monitoring in year 2004 using *Racomitrium crispulum* as a biomonitoring species.

	Pb	Zn	Cu	Cd
Pb	1.0000			
Zn	0.8781	1.0000		
Cu	0.6113	0.8804	1.0000	
Cd	0.4618	0.5801	0.4236	1.0000

Metal cadmium

On the basis of indirect method of metal distribution study, analyzed moss samples were found to have a somewhat higher amount of Cd from Jageshwar and from the agricultural areas of Kosi. During winter, a high value of Cd was measured in transplants of moss harvested from Ranikhet (petrol pump). In winter season there was around 83.31 % increase in Cd at the Ranikhet golf court in winter with respect to summer, which a striking outcome (Fig. 6).

Cadmium metal is very easily leached out from surface and the similar results were observed in monsoon season in moss *Racomitrium*c*rispulum*. All catchment areas show significant (P≤ 0.01) decrease in metal Cd value in rainy season with respect to summer (Table 1). Easily leaching property could be the reason for their low value even in the forest areas, [29] but a different experiment of Cd adsorption on surface reports that kinetics study for the rate of adsorption is also an important factor to consider before commenting on leaching property [30, 31]. There is frequent use of breaks in the hilly region compare to the land areas. This could be the reason for high value of Cd at Ranikhet (petrol pump) in winter 2004 (Fig. 4d). An increase in the Cd on such places could be from abrasion of clutch, breaks of the vehicles. Higher concentrations in moss from an agricultural land might be due to the use of phosphate fertilizers [25, 26 and 31].

Percent metal load and pollution index value

Nainital (Tallital) had maximum percent metal loading i.e. 6.33 % during year 2004 (Fig. 6). This could be explained by taking into consideration the internal environment factors, such as meta-data influencing the metal deposition. In addition, converting the value measured in bio-monitor into deposition value is problematic, because there is not enough information available about the factor affecting the concentration in mosses [2, 31].

The pollution index (PI) value at the Nainital bus stand, Almora mall road, Kosi, Almora petrol pump, Ranikhet petrol pump and Pithoragarh market area was +1.102393, +1.012493, +1.075294, +1.109318, + 1.013296 and +1.000281, respectively during year 2004 as listed in Table 3. In present study, Mukteswar forest was considered a control site. The least value of percent metal load at Mukteswar deploys that our consideration of taking Mukteswar as control site is significant (Fig. 6). The pollution index (PI) value at Nainital bus stand, Almora mall road, Kosi, Almora petrol pump, Ranikhet petrol pump and Pithoragarh market area was +1.102393, +1.012493, +1.075294, +1.109318, + 1.013296 and +1.000281, respectively during year 2004 and is tabulated in table 3. At Mukteshwar the PI value was recorded as -1.73241.

Table 3: Comparison PI value of by using moss *Racomitrium* c*rispulum* as a biomonitoring species exposed in Kumaon hills.

Catchment Sites	Year (2004)
Mukteswar forest	-1.73241
Deenapnai	0.13038
Jageswar	-0.59865
Hwalbagh	0.34087
Almora (Mall road)	1.012493
Kosi (G.B. Pant inst.)	1.075294
Almora (Petrol pump)	1.109318
Ranikhet (Golf court)	0.020039
Ranikhet(Bus stand)	0.100131
Ranikhet (Petrol pump)	1.013296
Nainital (Bus stand)	1.102393
Nainital(Mallital)	0.723167
Nainital (Tallital)	0.209319
Nainital (Capitol cinema)	0.912817
Bhowali (Bus stand)	0.931823
Ramgarh (Ramgarh malla)	0.052352
Pithoragarh (Siltham)	0.101013
Pithoragarh (Market area)	1.000281
Pithoragarh (Bus stand)	0.142591

The major anthropogenic pollutant released is Cd and Cu from phosphate fertilizers [25, 32] Amundsen et al., 2000. Phosphate fertilizers contain 5-100 mg Cd Kg^{-1} and excessive use of this metal will decrease the natural fertility rate of soil. Zn is chemically similar to Cd; it is also readily taken up by plants and enters edible portions which is a major concern for the edibility of foods such as grains and vegetables. Although Pb is not easily accumulated by plants, their presence in excessive in environments like as organo-lead compounds e.g., alkyl lead (from automobiles) are highly toxic, even when they occur at very low concentrations [33]. However, excessive concentrations analyzed in transplants of mosses indicates that these hills stations are also victims of the elevated atmospheric metal concentrations due to poor policies, unwise constructions and heavy vehicular traffic which are the only mode of transportation for the public and supply daily needs [6, 34].

The pollution index (PI) value was highly positive at the areas near the bus stand and at petrol pumps. This was further supported by percent metal loading in respective locations during these years. The maximum positive values were measured in proximity to the city area. Negative value at Mukteshwar forest reveals that it is a relatively cleaner site. The effect of intensity of traffic compared to the influence of other factors such as farming on sampling points are much higher, as the spots were nearer to the roads (except control site). Besides this, contamination was high and reduces with distance [23].

In conclusion, the present research gives supportive evidence of metal pollution on Kumaon hills. Elemental concentration in *Racomitriumcrispulum* was in order Zn >Pb ~ Cu> Cd in summer, while the same was Zn> Cu >Pb> Cd in winter season and in rain Zn> Cu >Pb> Cd indeed reflects atmospheric trace elemental load. Amongst season summer deployed maximum metal load followed by winter and monsoon. Present findings describe that Nainital is most polluted for heavy metal (Pb, Zn, Cu and Cd), followed by Almora and Pithoragarh. Present funded study of ministry of Environment, Government of India, is very useful for a policy maker to take the necessary steps for control measure on Himalayan belt, which directly reflects the weather condition of north India.

Conclusion

A moss bio-monitoring technique was used for quantification of under taken metals in the air from Kumaon regions. Higher values of the element were measured in moss transplants harvested from the study regions in proximity to the road and from the center of tourist cities. Inter-seasonal variability in metal value depicts that for active monitoring, tolerant and widely distributed moss can be used as bio monitor. Value of percent metal load at Nainital (Tallital) during experimental year 2004 was very significant. Values measured were high in moss during summer, followed by the winter samples. Higher contents of this element were found in moss samples collected in the regions of central city sites. By comparing median values from this and previous study (2004), it was observed that the content of metals had an increasing trend. Two types of petroleum distillates are used in gasoline-powered automotive vehicles. One contains 0.63 g l^{-1} of lead while 'Regular' contains 0.42 g l^{-1} of lead. The consumption of super graded petrol is higher than that of regular grade. It could be concluded from the above figures that burning of gasoline with high lead content is the main cause of the high levels of lead in particulate deposits collected from different designated sites in the study area.

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Table 1. Metals Pb concentration (µg g^{-1}D.wt.) ± SE in seasonally exposed moss samples of *Racomitrium crispulum* at Kumaon hills (2004). Significance differ from Control (Muktes27.95, war forest) (^{a}) P ≤ 0.05 and (^{b}) P ≤ 0.01 significance level.

Catchment sites	WINTER 2004				SUMMER 2004				MONSOON 2004			
	Pb	**Zn**	**Cu**	**Cd**	**Pb**	**Zn**	**Cu**	**Cd**	**Pb**	**Zn**	**Cu**	**Cd**
Mukteswar forest	12.34±0.92	09.35±1.05	11.34±0.91	2.36±0.25	14.93±0.94	15.03±1.55	13.83±0.91	3.02±0.34	8.39±0.81	07.90±0.97	09.86±0.97	1.65±0.15
Deenapnai	20.34^{a}±1.04	32.06^{a} ±2.22	8.37±0.86	6.5^{a} ±0.64	27.45^{a} ±1.76	43.60^{a} ±3.62	11.38^{b} ±0.91	8.32^{a} ±0.87	13.83±0.91	28.21^{a} ±1.89	7.28^{b} ±0.72	4.55^{a} ±0.42
Jageswar	14.95^{b}±0.94	36.29±2.65	9.34^{b} ±0.94	1.23^{b} ±0.15	18.68±0.98	46.08^{a} ±3.62	11.76^{b} ±0.91	1.57±0.15	10.16^{b} ±0.91	31.93^{a} ±2.12	8.12^{b} ±0.83	0.86^{b} ±0.03
Hwalbagh	15.37±0.95	23.34±1.34	11.67^{b} ±0.91	0.12±0.05	18.44±0.98	28.47±1.81	14.12^{b} ±0.94	0.15±0.05	10.45^{b} ±0.90	20.52±1.03	10.15^{b} ±0.90	0.08±0.01
Almora (Mall road)	16.24^{a} ±0.96	30.67^{a} ±2.02	11.24±0.91	2.77±0.22	25.82±1.51	49.37^{a} ±3.94	18.20^{a} ±0.92	3.54^{b} ±0.34	11.04±0.91	26.98^{a} ±1.61	9.77^{b} ±0.92	1.93±0.15
Kosi (G.B. Pant institute)	15.33±0.95	40.37^{a} ±3.05	12.31^{b} ±0.92	6.28^{a} ±0.61	20.23^{a} ±1.02	53.69^{a} ±4.35	16.44±0.96	8.03^{a} ±0.85	10.42^{b}±0.910	35.52^{a} ±2.55	10.70^{b} ±0.90	4.39±0.42
Almora (Petrol pump)	31.57^{a} ±1.16	45.26^{a} ±3.50	12.38^{b} ±0.92	3.61±0.35	36.49^{a} ±2.62	62.91^{a} ±5.25	17.04±0.91	4.62^{b} ±0.45	18.06^{a} ±0.98	39.82^{a} ±2.93	10.77^{b} ±0.90	2.52±0.20
Ranikhet (Golf court)	21.64^{a} ±1.11	60.94^{a} ±5.02	8.34±0.81	3.23±0.32	30.07^{a} ±2.01	86.53^{a} ±7.64	11.74^{b} ±0.91	4.26^{b} ±0.44	15.14^{a} ±0.91	49.97^{a} ±3.94	6.58±0.63	2.16±0.21
Ranikhet(Bus stand)	16.34^{a} ±0.96	54.36^{a} ±4.44	8.67±0.82	2.12^{b} ±0.24	21.24±1.91	71.75^{a} ±6.12	11.68^{b} ±0.91	2.79±0.25	11.43^{b} ±0.91	44.57^{a} ±3.46	6.84±0.64	1.42±0.14
Ranikhet (Petrol pump)	22.34^{a} ±1.94	66.32^{a} ±5.52	10.38^{b} ±0.90	4.14±0.45	31.05±2.12	86.87^{a} ±7.69	14.03^{b} ±0.94	5.46^{a} ±0.57	15.63^{a} ±0.95	54.38^{a} ±4.54	8.20 ±0.85	2.77±0.22
Nainital (Bus stand)	42.11^{a} ±1.62	56.34^{a} ±4.62	22.87^{a} ±1.21	1.24^{b} ±0.14	56.32±3.31	91.83^{a} ±8.14	37.04^{a} ±2.74	1.64±0.15	17.03^{a} ±1.90	50.70^{a} ±4.03	18.29^{a} ±0.98	0.79^{b} ±0.04
Nainital(Mallital)	16.38^{a} ±0.96	45.67^{a} ±3.54	16.39^{a} ±0.96	0.96±0.02	23.42^{a} ±1.93	66.22^{a} ±5.62	23.60^{a} ±1.36	1.27±0.12	12.44±0.92	41.10^{a} ±3.11	13.11±0.93	0.61^{b} ±0.02
Nainital (Tallital)	12.37^{b} ±0.92	80.34^{a} ±7.01	25.64^{a} ±1.52	1.23^{b} ±0.14	17.31±0.97	114.08^{a}±11.45	36.66^{a} ±2.62	1.63±0.14	9.40^{b} ±0.91	72.30^{a} ±6.23	20.51^{a} ±1.04	0.78^{b} ±0.04
Nainital (Capitol cinema)	14.22^{b} ±0.92	34.95^{a} ±2.44	10.99^{b} ±0.90	4.22±0.42	18.77±0.81	46.48^{a} ±3.63	14.72^{b} ±0.94	5.61±0.55	10.80^{b} ±0.91	31.45^{a} ±2.15	8.79^{b} ±0.83	2.70±1.72
Bhowali (Bus stand)	15.38±0.95	34.12^{a} ±2.41	12.87±0.92	3.18±0.34	22.60^{a} ±1.22	50.83^{a} ±4.03	19.04^{a} ±0.96	4.22±0.45	11.68 ±0.91	30.70^{a} ±2.05	10.29±0.90	2.03±0.24
Ramgarh malla	15.64±0.95	40.29^{a} ±3.05	12.64±0.92	1.25^{b} ±0.12	20.33^{a} ±1.01	53.18^{a} ±4.35	16.82±0.96	1.66±0.15	11.88±0.91	36.26^{a} ±2.66	10.11±0.90	0.80^{b} ±0.82
Pithoragarh (Siltham)	14.27^{b} ±0.94	34.6^{a} ±2.41	12.37±0.92	2.56±2.15	17.14±0.97	42.29^{a} ±3.26	15.21±0.95	3.07^{b} ±0.34	9.27^{b} ±0.91	28.08^{a} ±1.81	09.27^{b} ±0.93	1.99±0.14
Pithoragarh (Market area)	21.6^{a} ±1.22	60.25^{a} ±5.04	07.98±0.74	5.17^{a} ±0.51	27.95^{a} ±1.72	79.53^{a} ±6.94	10.48^{b} ±0.90	6.20^{a} ±0.65	14.08±0.94	48.80^{a} ±3.82	05.98±0.52	4.03^{a} ±0.35
Pithoragarh (Bus stand)	16.97±1.61	20.35^{b} ±1.05	07.64±0.72	2.36±0.23	22.40^{a} ±1.21	25.03^{b} ±1.55	10.12^{b} ±0.90	2.83^{b} ±0.25	11.03^{b} ±0.91	27.90±0.97	05.73±4.31	1.84±0.96

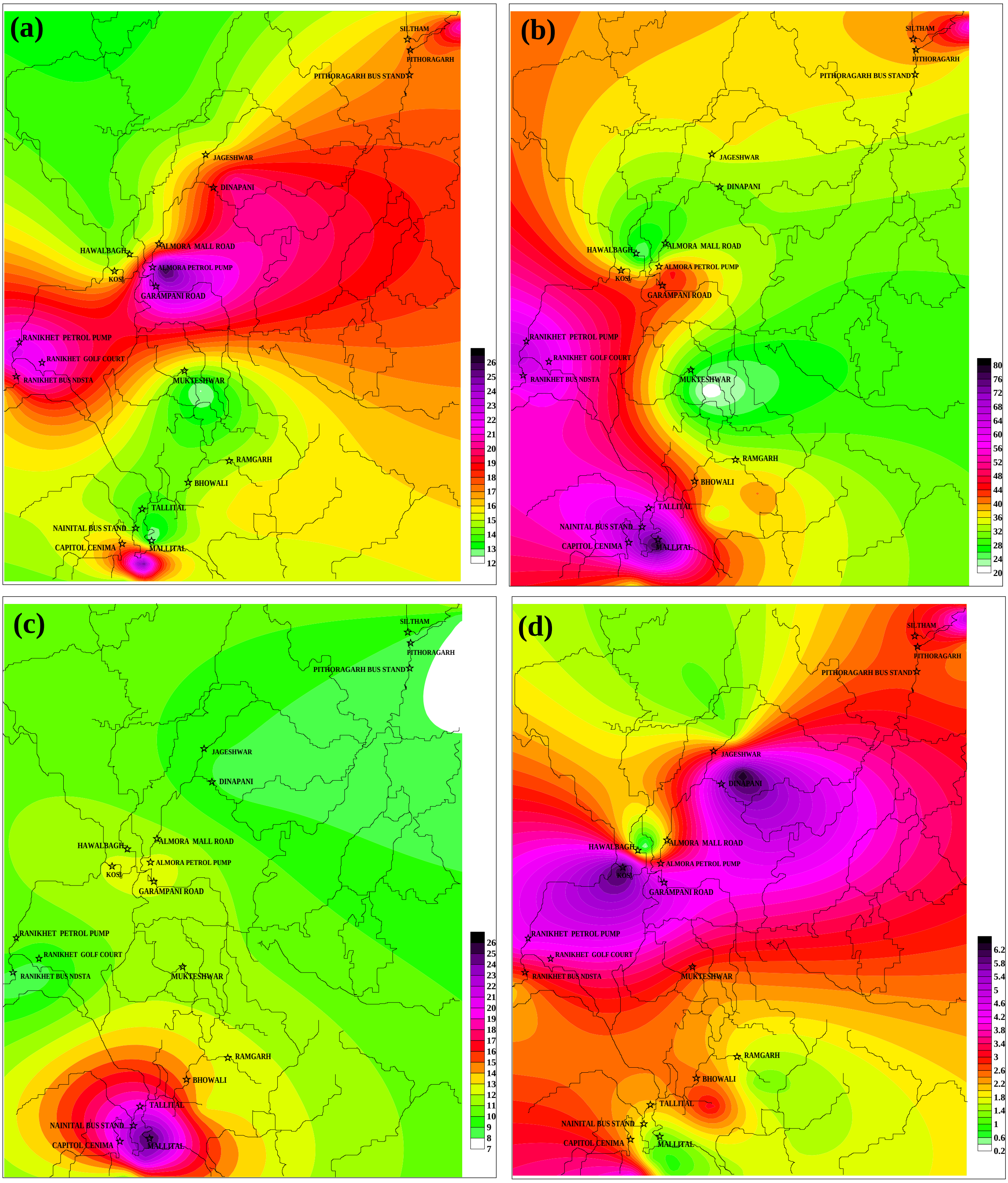

Fig. 3: Distribution map of metal content in *Racomitrium crispulum* rotherus (µg g-1 dw) during winter 2004: (a) Pb, (b) Zn, (c) Cu and (d) Cd.

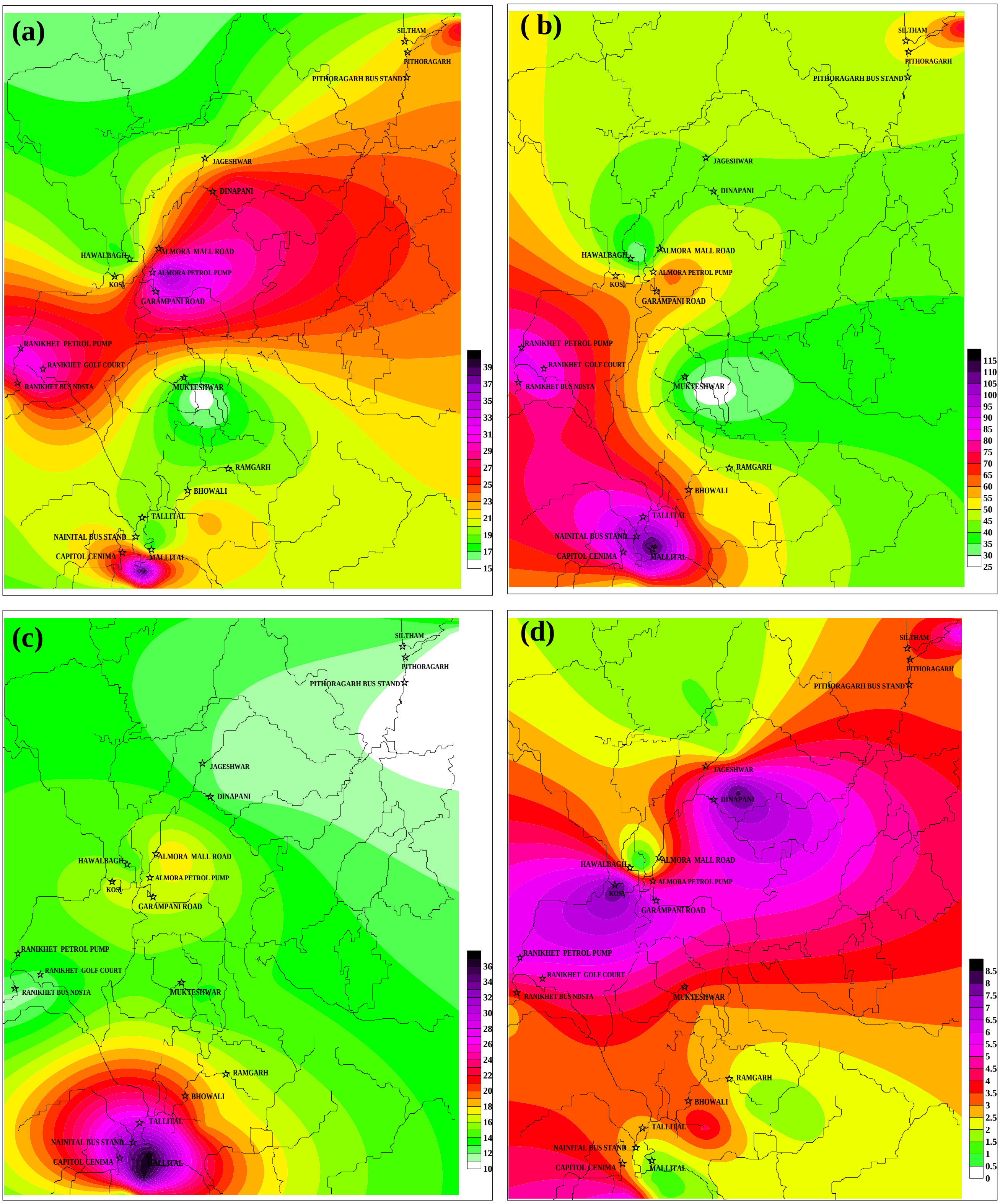

Fig. 4: Distribution map of metal content in *Racomitrium crispulum* (µg g-1 dw) during summer 2004: (a) Pb, (b) Zn, (c) Cu and (d) Cd.

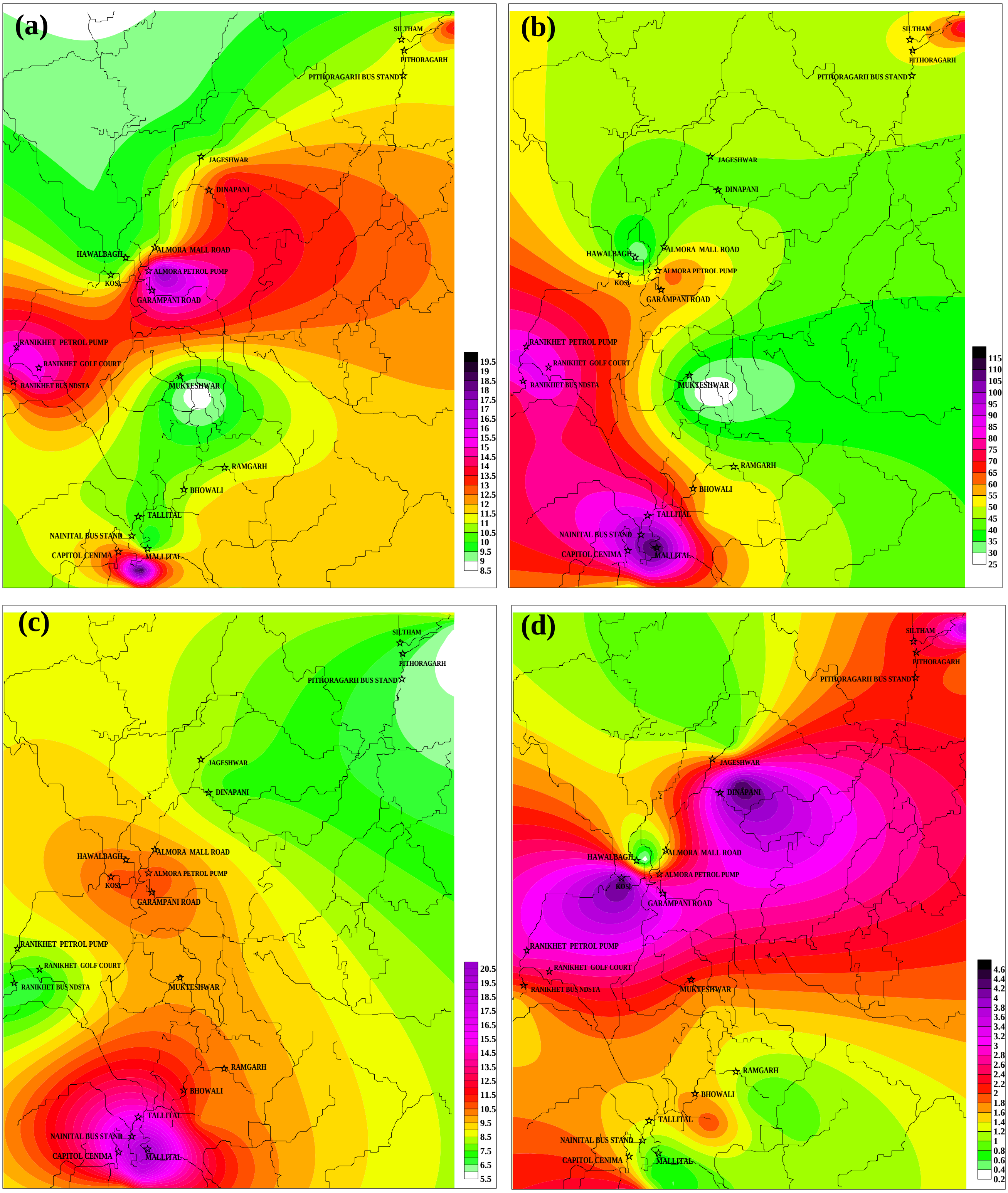

Fig. 5: Distribution map of metal content in *Racomitrium crispulum* (µg g-1 dw) during monsoon 2004: (a) Pb, (b) Zn, (c) Cu and (d) Cd.

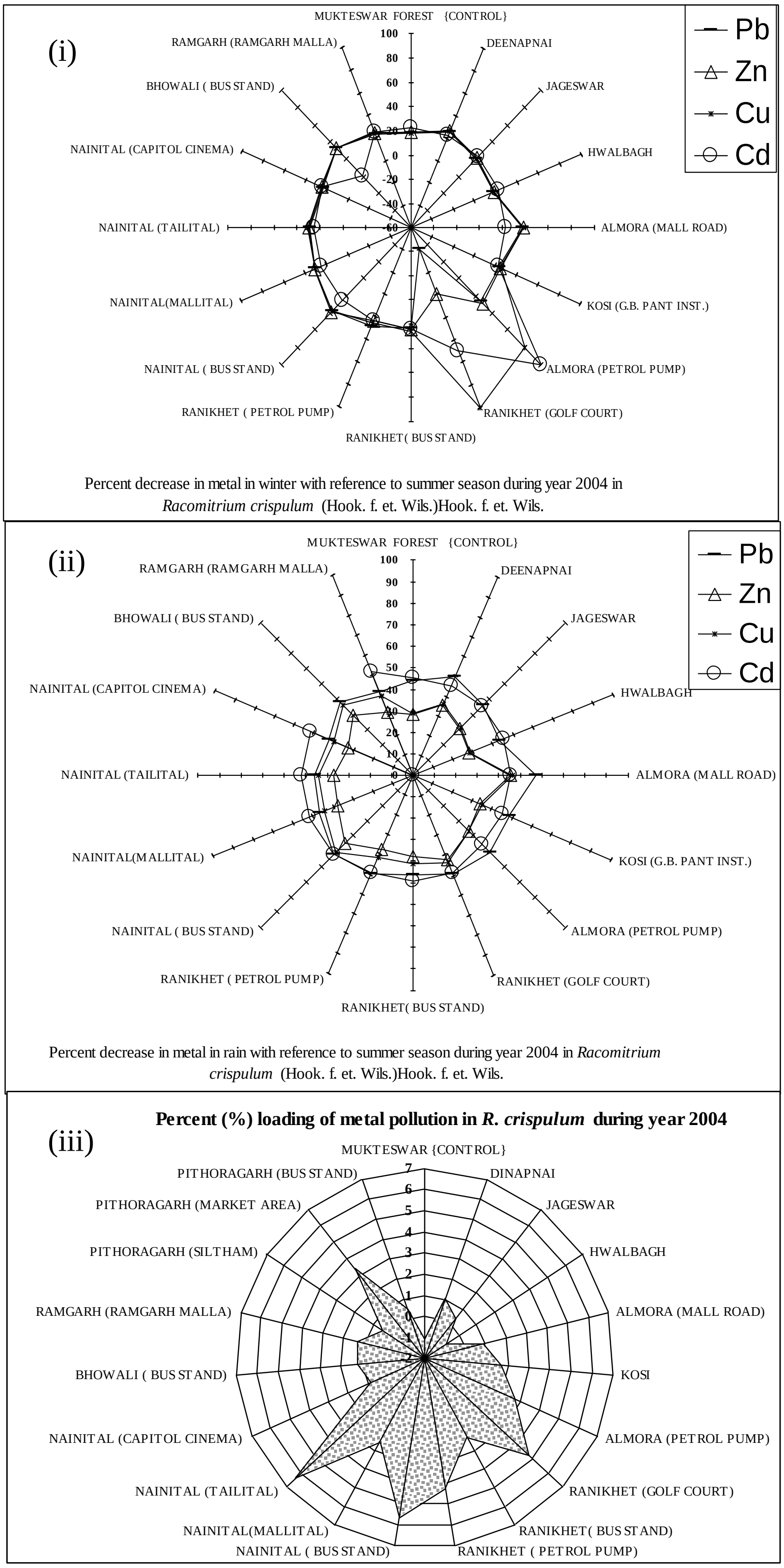

Fig. 6: Percent decrease in metal in (i) winter and (ii) monsoon with reference to summer season during year 2004 (iii) percent metal load (%) by using moss *Racomitrium crispulum* as a biomonitoring species exposed in Kumaon hills.