ISSN 0376-5561
Geophytology 43(1): 17-28
May 2013

An assessment of atmospheric metal deposition in Garhwal Hills, India by moss *Rhodobryum giganteum* (Schwaegr.) Par.

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Manuscript received: 13 April 2012
Accepted for publication: 27 February 2013

ABSTRACT

Saxena D. K., Hooda P. S., Singh S., Srivastava K., Kalaji H. M. & Gahtori D. 2013. An assessment of atmospheric metal deposition in Garhwal Hills, India by moss *Rhodobryum giganteum* (Schwaegr.) Par. Geophytology 43(1): 17-28.

Moss *Rhodobryum giganteum* (Schwaegr.) Par. was validated to evaluate the intensity and trend of atmospheric deposition of metals (Zn, Cu, Cd and Pb) in the Uttarakhand region over a three year period, 2004–2006. Before inducting the moss, commonly available mosses were validated for their metal tolerance, and the tolerant species identified. *Rhodobryum giganteum* (Schwaegr.) Par. was inducted for biomonitoring of atmospheric metals. The biomonitoring experiments were performed by transplanting moss bags, which were prepared from fresh moss patches and exposed seasonally throughout the study period. The analysis of metals absorbed by this moss species confirmed its suitability for atmospheric metal biomonitoring. The findings showed significant variations in metal deposition over the seasons. The highest values of metals were in the moss exposed during summer; this was followed by winter and the lowest values were recorded in the monsoon seasons. These findings are consistent with the variations in local/seasonal anthropogenic activities and atmospheric conditions. The results further showed that the moss species used was capable of successfully delineating the expected differences in atmospheric metal deposition within the study area. The metal deposition loads in the study area were recorded in the order Zn>Pb>Cu>Cd during the 3-years study period. A general increasing metal deposition trend was also observed, i.e. annual average (over seasons) metal loads in 2006 increased by 7%, 19% and 11% for Zn, Pb and Cu, respectively, when compared with their loads in 2004. For rural sites, these increases were somewhat different, 12%, 11% and 14% for Zn, Pb and Cu, respectively.

Key-words: Atmospheric metals, biomonitoring, moss, seasonal deposition trend, validation of tolerant species.

INTRODUCTION

Atmospheric deposition of pollutants, such as metals, is extremely variable in space and time, and it is difficult and expensive to obtain detailed information over a vast area using traditional instrumental recording. Biomonitors or bioindicators are used in environmental research, particularly for the purpose of assessing impacts of pollution on terrestrial and aquatic environments (Szezepaniak & Biziuk 2003). Their use in environmental studies is also desirable as they are capable of providing an integrated response to pollution stress, rather than just a snapshot in time. The relevant information can be commonly deduced either from changes in the behaviour of the monitor species or by analysing the concentration of specific substances (e.g. metals).

Trace metals (e.g. Zn, Cu, Pb and Cd) are emitted from various anthropogenic activities, including transport activities and waste disposal. Their presence in the atmosphere as major air pollutants is not uncommon and therefore taken for present study (Saxena 2001).

Of all biological groups used in biomonitoring, mosses have several advantages over conventional techniques of monitoring atmospheric deposition of metals (Fernandez et al. 2004). Firstly, mosses have the most common occurrence and their morphology does not vary with the seasons, thus metal deposition can occur throughout the year on mosses, and they are capable of accumulating metals in relatively high concentrations due to active absorption (Saxena & Arfeen1995). Secondly, the low cost and ease of its application, i.e. biomonitoring allows simultaneous monitoring and sampling at multiple sites (Saxena 2001). Mosses absorb nutrients and moisture directly from the ambient air and retain them. Therefore, analysis of moss metal content reflects the atmospheric metal deposition. Bryomonitoring, i.e. biomonitoring using bryophytes (mosses), is simple, relatively inexpensive, employing both passive and active accumulators.

An advantage of active biomonitoring technique, i.e. where bryophytes are transplanted at desired space and time intervals, is that it provides considerable flexibility in choosing sampling sites. Also, it excludes the effect of different lifetime metal history of test plants (a common limitation in passive biomonitoring) in different areas. A further benefit of bryophyte species, which can accumulate metals without toxicity, is that they can be used to assess changes in metal uptake/deposition over a certain period (Saxena et al. 2008a). In passive monitoring (using naturally exiting mosses at a given location), age of the plant is not known; therefore, there are chances of variation in metal concentration in the moss (Kovács 1992). A number of plant groups, e.g. lichens (Sloof et al. 1998), mosses (Glime & Saxena 1991), ferns (Ho & Tai 1985) and trees (Kovács 1992, Streit & Stumm 1993), have been used for biomonitoring purposes but mosses have the following major advantages over other groups of plants: i. they are capable of accumulating relatively large amount of metals, e.g. 3,200 mg/kg Pb and 9,400 mg/kg Zn (Dietz 1972); ii. they have unique capability of absorbing nutrients/contaminants directly from the ambient air and of storing them for extended periods of time without impairing their physiological processes (Szezepaniak & Biziuk 2003); and iii. their high surface area to mass ratio is effective in trapping airborne nutrients and chemicals (Bargagli et al. 2002).

The general advantages of the biomonitoring approach are primarily due to the common occurrence of the organism in the field (Saxena et al. 2008b), the ease of sampling and no requirement of expansive field/technical equipments. Nonetheless, it is important that the common available mosses are screened for metal tolerance and a metal tolerant species be identified before undertaking a large scale biomonitoring of atmospheric metal deposition. In this study, we screened two widely distributed mosses of region (*Rhodobryum* and *Pohlia* spp.) for metal tolerance by measuring photosynthetic efficiency, indirectly via chlorophyll-fluorescence measurements (Papageorgiou & Govindjee 2004), before inducting metal-tolerant moss species for biomonitoring purposes.

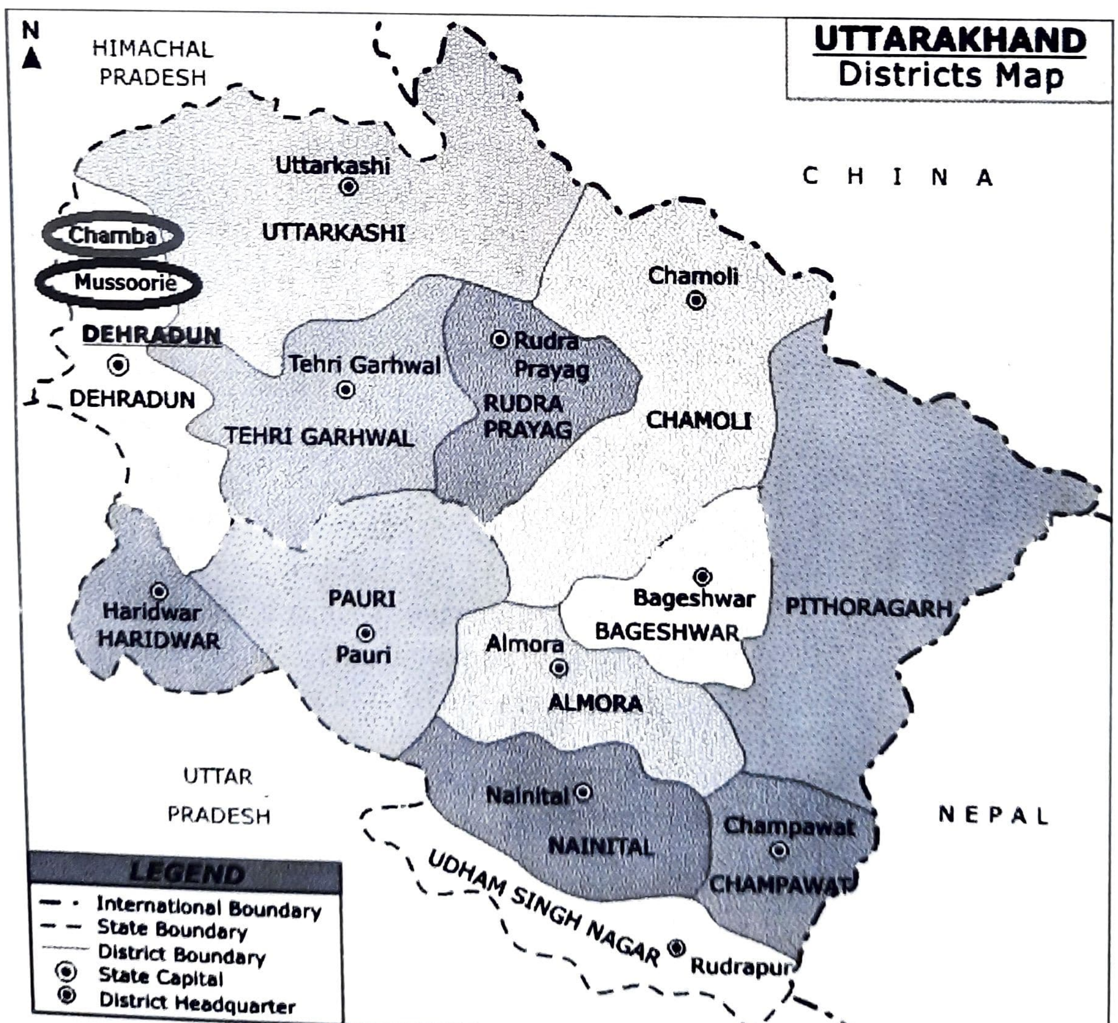
The main aim of this study was to measure atmospheric deposition of trace metals (Cu, Cd, Pb and Zn) as well as to assess their seasonal variability in the Garhwal Hill region (India). The specific research objectives were: a) to identify a metal tolerant moss species from the commonly available local mosses; b) to assess the suitability of moss as a biomonitor for atmospheric deposition of trace elements; c) to assess the degree and extent of metal deposition in relation to important metal emission sources and to map regional metal deposition pattern; and d) to assess seasonal and annual variability in metal deposition loads during the study period. Criteria for the sensitivity of the species towards the metal toxicity were based on short term response.

MATERIAL AND METHOD

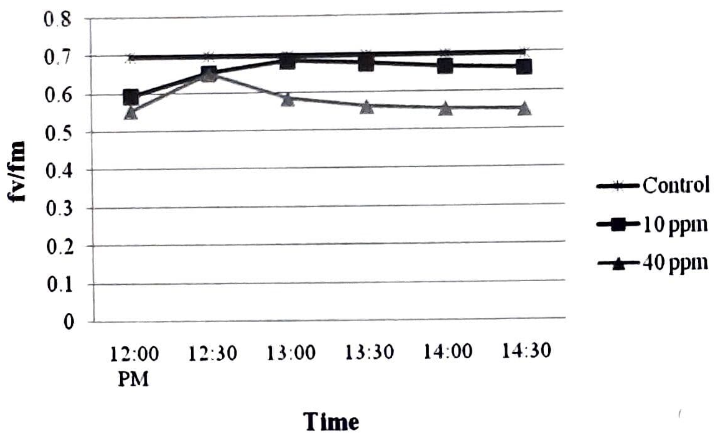
Samples of *Pohlia elongata* Hedw. and *Rhodobryum giganteum* (Schwaegr.) Par. were collected from the least polluted site close to the study area, i.e. Chamba forest-a rural site (Text-figure 1), situated at an altitude of 1542 m and about 35 km away from Mussoorie city of Garhwal Hills. Samples from this site are considered as control and were used for both metal tolerance testing and transplanting purposes at the sites to be monitored for metal deposition. The specimens were identified on the basis of taxonomical examination and with the help of information available in the literature (Gangulee 1969, Chopra 1975, Smith 1978).

Before selecting moss for biomonitoring purposes, a handy Photosynthetic efficiency analyser (Handy PEA, Hansatech Instruments Model No. 1011) was used to validate the metal tolerance potential of the two mosses (*Pohlia elongata* Hedw. and *Rhodobryum giganteum* (Schwaegr.) Par.) by measuring their chlorophyll-a fluorescence activity in the field. The moss beds were covered by plastic bel jar (30 cm^2 size) after spraying known concentration of the solution.

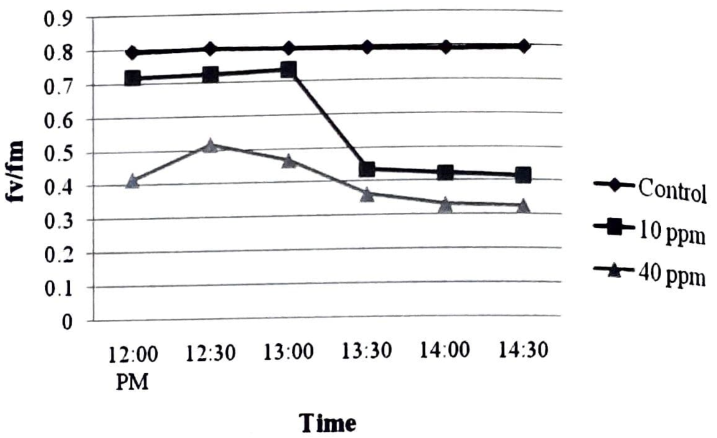
The ratio of variable (Fv) to maximal fluorescence (Fm), where Fv = Fm - Fo, and Fo stands for minimal initial fluorescence, was measured at different intervals to indirectly evaluate the photosynthetic activity response to the metal treatment (Text-figure 2). As noted above, Fm is the maximum chlorophyll-a fluorescence at the light intensity used, but Fv is related to the maximum capacity for photochemistry of Photosystem



Text-figure 1. The Uttarakhand region (North India), showing the study area. The control site was at Chamba (forest/rural) and the main study sites were in Mussoorie.



Text-figure 2a. *Rhodobryum giganteum* (Schwaegr.) Par. species validated for Pb tolerance. Photosynthetic efficiency (Chlorophyll fluorescence measurements) remains unaffected at two concentrations even after 4 hours of treatment



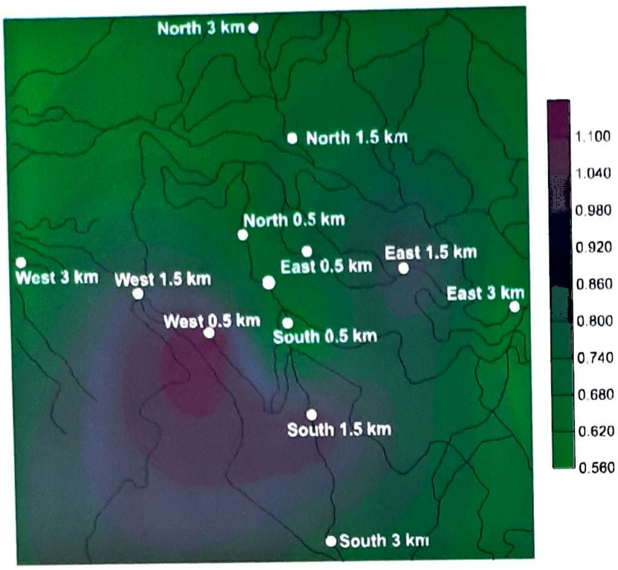
Text-figure 2b. Chlorophyll fluorescence measurements after the Pb treatment. Drop in photosynthetic activity can be seen at both Pb concentrations in moss *Pohlia* sp.

II (Govindjee 2004). The Fv/Fm ratio has thus been considered as a sensitive indicator of plant photosynthetic efficiency/performance with its maximum value being about 0.8-0.85. It should however be stressed that this is an indirect measure of photosynthesis efficiency; further, changes in Fo, due to several other reasons, may affect the photosynthesis. However, this measurement was used because it is a non-invasive, rapid and sensitive method. From our pilot study, it was evident that *Rhodobryum giganteum* (Schwaegr.) Par. is a metal tolerant species as its photosynthetic efficiency was considered not impaired, since the Fv/Fm values of this moss species measured were close to 0.8, i.e. unaffected compared to the other moss species (Text-figure 2). *Rhodobryum giganteum* (Schwaegr.) Par. was thus used for the purpose of biomonitoring atmospheric metal deposition. Ideally, two moss species should have been tested for all four metals (Cd, Cu, Pb and Zn) under consideration. Given Cu and Zn are essential trace elements and Cd is relatively less abundant in the atmosphere (Hooda 2010), it is not unreasonable to assume that a Pb-tolerant moss species will also tolerate Cd, Cu and Zn.

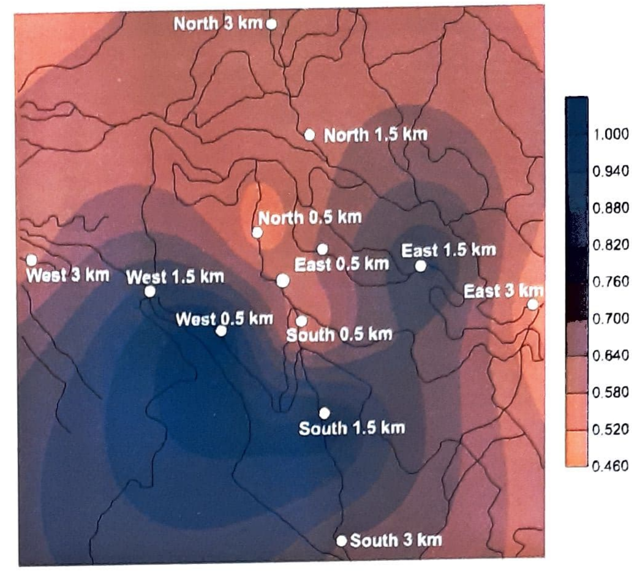
For the purpose of assessing metal deposition at the study sites, moss bags (Harmens et al. 2011a) containing 6 g moss per bag were prepared and transplanted at different catchment (16.42 km^2) sites within the Mussoorie city situated at an altitude of 2005 m (Text-figure 1).

Four transplants at each site were made in triplicate at nearly an equal distance and height in all the four directions (north, south, east and west). The distance of transplants was chosen at 0.5, 1.0 and 3.0 km away from the Mussoorie city centre. At the end of each exposure period of 4 months (representing one season), moss transplants were harvested for metal analysis and, in place, fresh transplants were made, as described above. This protocol was repeated in every season (summer, monsoon and winter) over the 3 years of study period. November to February represents winter due to low temperature, March to June is summer and July to October represents rainy season due to heavy rain. Harvested transplants were thoroughly washed with deionised water to remove adhere foreign bodies and soil contents before being dried at 70°C for 24 hours. The dried moss samples (0.5 g) were digested in a mixture of analytical grade concentrated 5 ml HNO_3 and 2 ml $HClO_4$. The digested samples were filtered through Whatman No. 42 filter paper. Metals in the acid mixture were analysed using atomic absorption spectroscopy (AAS, Electronic Corporation of India Ltd.). The sample preparation and analysis included the usual quality assurance protocols, including repeated measurements of a sample during the analysis runs, reagent blanks and an in-house certified reference plant material. The analysis was highly reliable with variability between replicates and between measurements of the same sample generally being <5%. The percentage recoveries of the analysed elements were generally consistent with the test used.

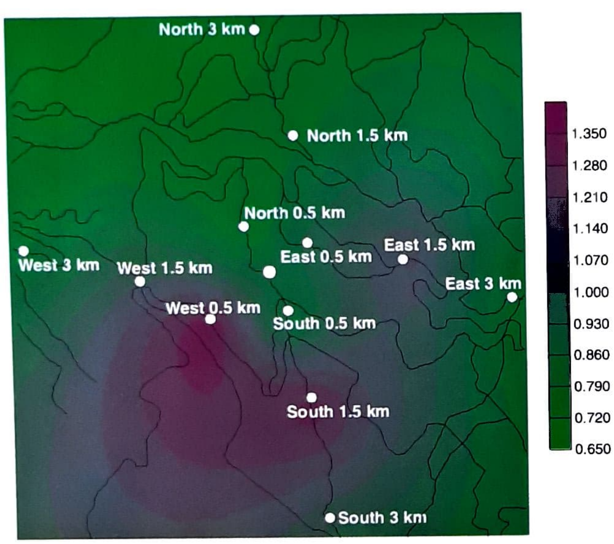
The analysis of variance, ANOVA test ($p< 0.01$, 0.05) was performed to compare the metal



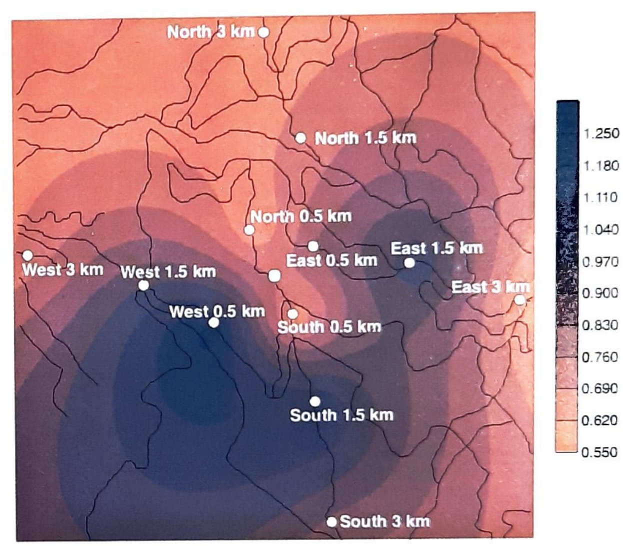
1a. Average zinc (mg g^{-1} DW) during summer at Mussoorie in *Rhodobryum giganteum*.



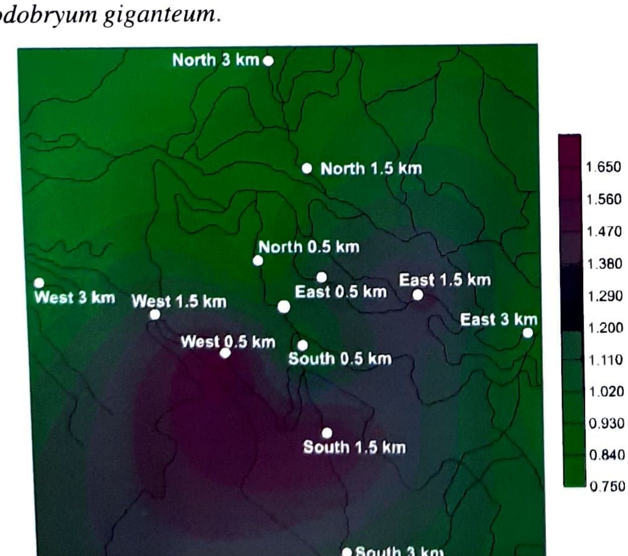
2a. Average lead (mg g^{-1} DW) during summer at Mussoorie in *Rhodobryum giganteum*.



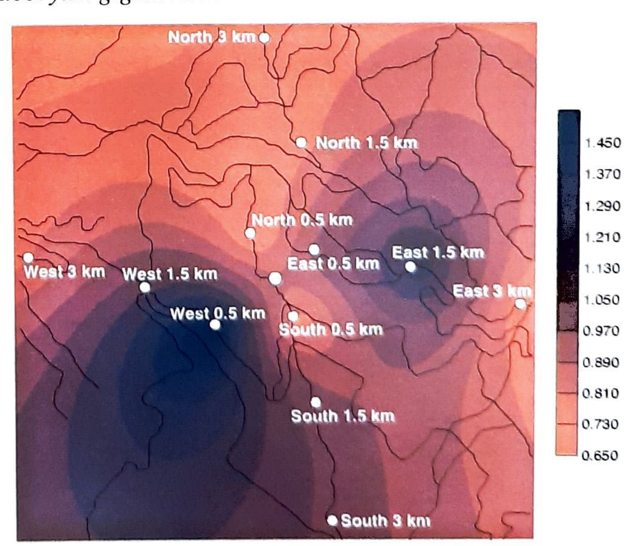
1b. Average zinc (mg g^{-1} DW) during monsoon at Mussoorie in *Rhodobryum giganteum*.



2b. Average lead (mg g^{-1} DW) during monsoon at Mussoorie in *Rhodobryum giganteum*.

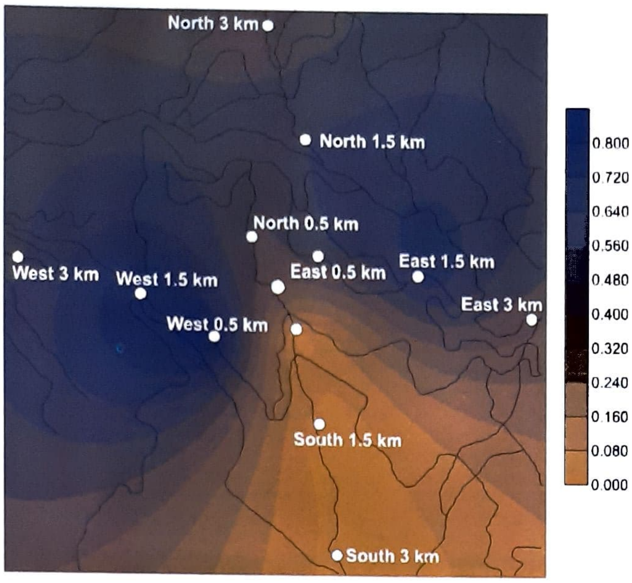


1c. Average zinc (mg g^{-1} DW) during winter at Mussoorie in *Rhodobryum giganteum*.

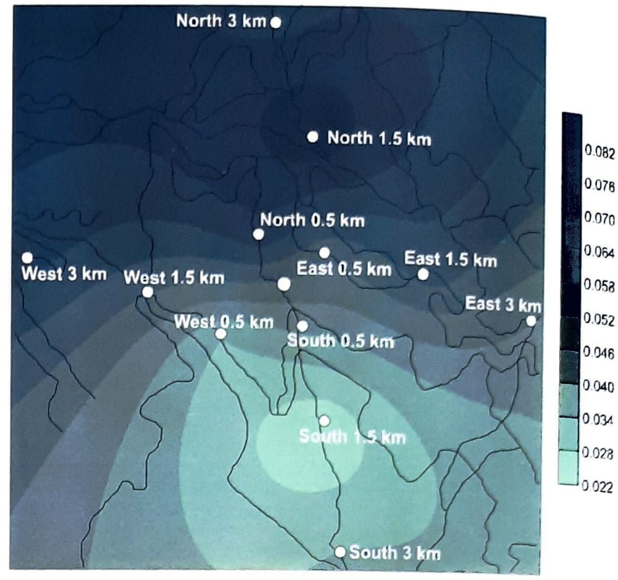


2c. Average lead (mg g^{-1} DW) during winter at Mussoorie in *Rhodobryum giganteum*.

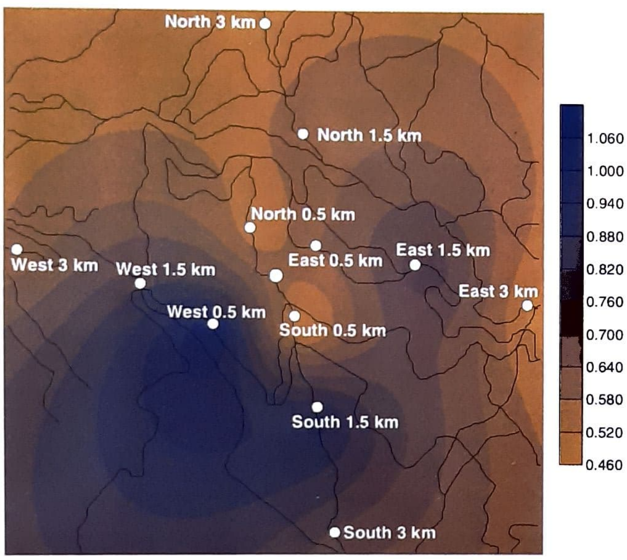
Plate 1



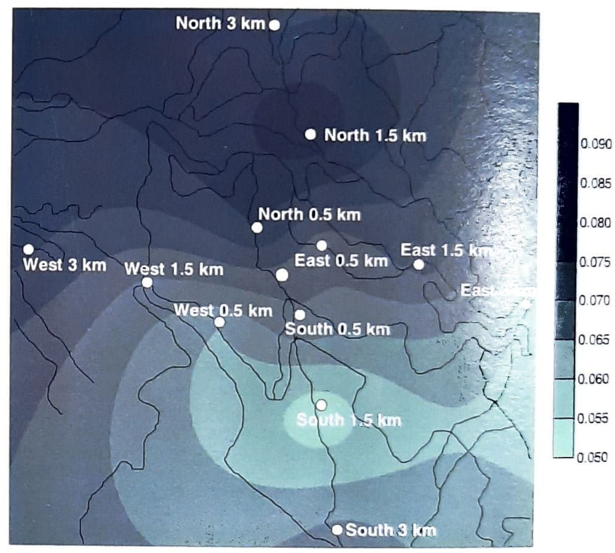
1a. Average copper (mg g^{-1} DW) during summer at Mussoorie in *Rhodobryum giganteum*.



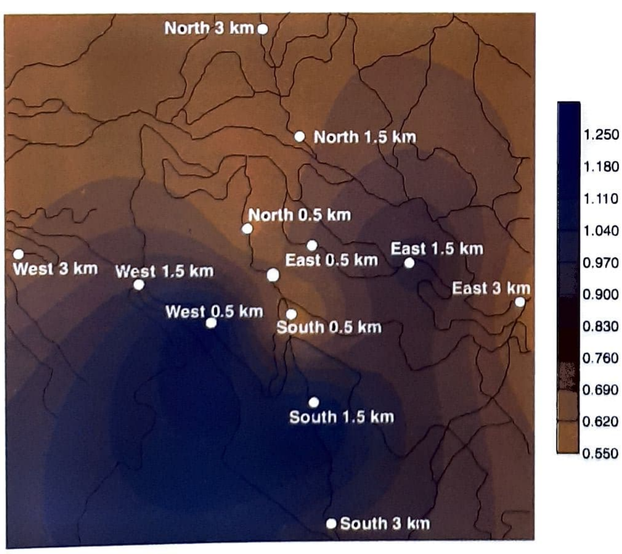
2a. Average cadmium (mg g^{-1} DW) during summer at Mussoorie in *Rhodobryum giganteum*.



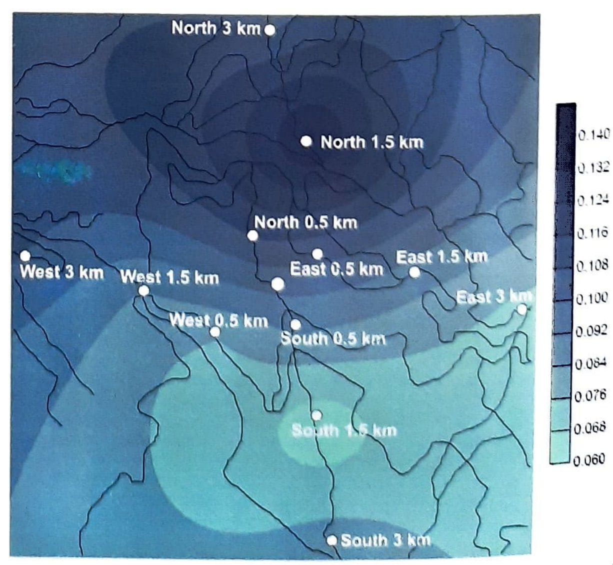
1b. Average copper (mg g^{-1} DW) during monsoon at Mussoorie in *Rhodobryum giganteum*.



2b. Average cadmium (mg g^{-1} DW) during monsoon at Mussoorie in *Rhodobryum giganteum*.



1c. Average copper (mg g^{-1} DW) during winter at Mussoorie in *Rhodobryum giganteum*.



2c. Average cadmium (mg g^{-1} DW) during winter at Mussoorie in *Rhodobryum giganteum*.

Plate 2

concentrations at different seasons and distances by utilizing Duncan's Multiple Range Test (Karmer 1956). Summary statistics were used to obtain the mean and standard error values (Snedecor & Cochran 1967). Cartographic representation of the results was performed with the program package Surfer (Golden Software Inc., U.S.A).

RESULTS

The Zn, Pb and Cu moss concentrations from the Chamba forest site, treated as control, do not show significant difference (SD < 0.01) between the seasons in all the three consecutive years (2004-2006). However, moss Cd concentration was either below its detection limit or showed no significant variations between the seasons and years at the control site (Tables 1-4). This confirms that the control site has little or no atmospheric metal (Zn, Pb, Cu and Cd) deposition as measured by their accumulation in the moss. This site is thus a reliable benchmark for evaluating metal deposition at other experimental sites in the region (Text-figure 1).

Zinc: In the Mussoorie catchment sites, moss Zn concentrations were generally highest at the west 0.5 km location during the study period, whereas, with some exceptions, its concentrations measured in the north at the 3 km distance location tended to be lowest (Table 1). All the study sites exhibited seasonally significant differences (SD < 0.01 or 0.05) in Zn concentration when compared with its baseline concentration, i.e. the control site (Table 1). It is plausible that high Zn concentration at 0.5 km west could be due to dumping of municipal wastes (trash from automobiles, construction material, paints and pigments, other street garbage, packaging and general household waste) in the town proximity; as such wastes are burnt frequently once or twice a week. We note that burning waste in the open is not appropriate as it can be a significant source of particulate material, other combustion by-products and metals (Hooda 2010); the practice, however, is not uncommon in Indian towns and cities. The generally lower Zn concentration at 3 km distance (in all directions) could be due to its rural/semi-rural nature or the outer areas being forested, away from transport and waste-burning emissions.

The maximum annual average (over 3 seasons) of Zn distribution pattern was observed in the west direction at 0.5 km distance, with levels of 1.117 mg Zn g^{-1}, 1.349 mg Zn g^{-1} and 1.622 mg Zn g^{-1} moss dry weight (DW), respectively for 2004, 2005 and 2006 (Table 1). This is largely due to the location being closer to the city centre and east to west wind. Compared to its baseline concentration, minimum Zn concentrations were recorded in the transplants of moss harvested from

Table 1. Seasonal variations in zinc (mg Zn g^{-1} DW) in the moss *Rhodobryum giganteum* (Schwaegr.) Par. at study sites in Mussoorie city during 2004-2006

Sampling Sites	Summer			Monsoon			Winter		
	2004	2005	2006	2004	2005	2006	2004	2005	2006
Baseline	0.145±0.010	0.203±0.058	0.298±0.057	0.018±0.005^{a}	0.028±0.005^{b}	0.051±0.006^{c}	0.034±0.015^{a}	0.042±0.012^{b}	0.072±0.012^{c}
North (0.5 km)	0.829±0.028	0.904±0.058^{a}	1.055±0.044^{b}	0.516±0.028^{c}	0.509±0.015	0.614±0.028	0.635±0.044^{c}	0.724±0.053^{a}	0.989±0.102^{b}
North (1.0 km)	0.886±0.043	*1.011±0.051	1.131±0.056	0.504±0.028	0.546±0.056	*0.708±0.044	0.693±0.032	*0.764±0.074	*0.932±0.088
North (3.0 km)	*0.760±0.039	0.839±0.048^{a}	0.957±0.045^{bc}	0.534±0.019	0.614±0.048	0.754±0.072^{bd}	*0.606±0.057	0.658±0.086^{a}	0.806±0.029^{d}
South (0.5 km)	0.783±0.015	1.012±0.071	1.183±0.086^{a}	0.463±0.045^{b}	0.655±0.058^{c}	*0.832±0.040	0.604±0.042b	0.805±0.056^{c}	*1.039±0.026^{a}
South (1.0 km)	*1.259±0.070	1.705±0.100	2.183±0.087	0.757±0.032	0.935±0.071	1.109±0.027	*1.112±0.047	1.307±0.057	1.457±0.028
South (3.0 km)	0.955±0.043	*1.184±0.089	*1.374±0.036	0.640±0.028^{a}	0.807±0.016	0.991±0.100	0.730±0.029^{a}	*0.912±0.054	*1.131±0.058
East (0.5 km)	1.056±0.086^{a}	*1.261±0.082	1.609±0.061	0.334±0.043	0.662±0.057	0.851±0.072	0.918±0.024^{a}	*1.047±0.048	1.313±0.061
East (1.0 km)	1.219±0.028	1.491±0.113	1.779±0.069	*0.739±0.014	*0.890±0.046	1.030±0.019	*0.892±0.058	*1.110±0.063	1.456±0.101
East (3.0 km)	*0.793±0.044	*0.962±0.056	1.149±0.068	0.315±0.026	0.461±0.032^{a}	*0.563±0.031	*0.593±0.029	*0.691±0.015^{a}	*0.840±0.043
West (0.5 km)	1.381±0.037	1.734±0.118	2.053±0.029	*0.911±0.027	1.078±0.057^{a}	1.256±0.028	*1.059±0.010	1.237±0.026^{a}	1.559±0.012
West (1.0 km)	1.181±0.073	1.411±0.089	1.742±0.097	0.715±0.059	0.808±0.031^{a}	0.907±0.053	0.918±0.030	1.016±0.057^{a}	1.211±0.104
West (3.0 km)	0.785±0.048	*0.909±0.054^{a}	1.016±0.028^{b}	0.516±0.024^{c}	*0.612±0.063^{d}	0.693±0.030^{e}	0.585±0.033^{c}	0.737±0.073^{ad}	0.863±0.059^{be}

All values are represented as mean SE. NS= value is non-significant (SD > 0.05) as compared to control site. * = values in horizontal row are seasonally significantly different at 5% significance level during the same year. Values superscripted same alphabets in horizontal row are seasonally not significantly different (SD > 0.05) during the same year.

the east 3 km locations (0.315 mg Zn g^{-1}DW, 0.461 mg Zn g^{-1} DW, 0.563 mg Zn g^{-1} DW, respectively for 2004, 2005 and 2006). All these were observed in the monsoon season (Plate 1, figures 1a-c); the low Zn concentrations at 3 km east location are due to a combination of factors: a) 3 km the farthest distance from the centre, b) this being the rainy season, and c) a general wind direction being east to west.

Lead: Results of moss Pb concentrations show significantly greater values over its baseline concentration at nearly all the sites during all the three consecutive years (Table 2). Significant differences (SD < 0.01) were also found at a few transplant sites between the summer and winter months, and during the monsoon and the winter seasons. The highest annual average distribution pattern of Pb was 1.061 mg Pb g^{-1} DW (dry weight) (2004), 1.212 mg Pb g^{-1} DW (2005) and 1.493 mg Pb g^{-1} DW (2006) at the 0.5 km west moss sampling distance, as with Zn. Lead values towards the east 3 km location declined to 0.474 mg Pb g^{-1} DW, 0.571 mg Pb g^{-1} DW and 0.693 mg Pb g^{-1} DW during 2004, 2005 and 2006 respectively (Plate 1, figures 2 a-c), showing the diminishing trend of Pb emissions away from the urban centre, and the influence of generally east to west wind direction, as noted for Zn.

Copper: Compared to its baseline concentration, Cu showed significant variations at all sampling sites in all the three consecutive years (Table 3). Statistically, significant variations (SD < 0.05) in moss Cu contractions were detected over seasons (summer, monsoon and winter) and years (2004, 2005 and 2006) at most sampling locations (Table 3). The average annual concentrations measured were 0.828 mg Cu g^{-1} DW in 2004, 1.066 mg Cu g^{-1} DW in 2005 and 1.136 mg Cu g^{-1} DW in 2006. The average maximum Cu concentrations in moss are generally consistent with that observed for Zn and Pb at 0.5 km west location, apart from 2006 when it was recorded at 1 km south (Plate 2, figures 1a-c). The minimum Cu concentration did not show a particular direction/distance trend, perhaps due to its more variable emission sources, particularly during low atmospheric deposition season (monsoon), as measured by its uptake by the transplanted moss.

Cadmium: Cadmium results exhibited non-significant values (SD < 0.01) at some places during the winter of 2004 as compared to its baseline concentration (Table 4). Whilst significant differences in moss Cd levels (SD < 0.05) were observed over the seasons and sampling locations, there was no clear trend with respect to the sampling distance or the direction, possibly due to more variable sources of Cd emission. The annual average (over seasons) maximum Cd levels were recorded at 1 km north sampling location (0.081 mg Cd g^{-1} DW, 0.094 mg Cd g^{-1} DW and 0.142 mg Cd g^{-1} DW), and lowest levels were observed at 1 km south (0.023 mg Cd g^{-1} DW, 0.051 mg Cd g^{-1} DW and 0.063 mg Cd g^{-1} DW) during 2004, 2005 and 2006, respectively (Plate 2, figures 2a-c). Clearly, Cd uptake by moss has not followed its generally expected atmospheric deposition directional trend, the wind direction being from east to west. Nonetheless, maximum concentrations were generally around the central areas, as expected due to high traffic and population densities.

The results from this simple and inexpensive technique show that moss, *Rhodobryum giganteum* species is capable of reliably monitoring changes in atmospheric metal inputs.

DISCUSSION

The analysis of transplanted moss exhibited significant seasonal variation in metal concentrations as detected by *R. giganteum*, which is expected to be due to seasonal and/or climate-mediated variations in their local emission sources. Considerably higher concentration of metals (Zn, Pb, Cu and Cd) in moss during the summers (Tables 1-4) at different catchment sites of Mussoorie could be due to many fold increase in tourist activity and concomitant much increase in fuel consumption in summer (Saxena et al. 2007). These findings are consistent with other work (e.g. Huang et al. 1994, Gerdol et al. 2002), which suggested that emissions from automobiles have significant bearing on metal deposition.

Significant decline in moss metal concentrations during the monsoon season (compared to summer and winter seasons) could be due to a general decline in

Table 2. Seasonal variations in lead (mg Pb g^{-1} DW) in the moss *Rhodobryum giganteum* (Schwaegr.) Par. at study sites in Mussoorie city during 2004-2006

Sampling Sites	Summer			Monsoon			Winter		
	2004	2005	2006	2004	2005	2006	2004	2005	2006
Baseline	0.102±0.057	0.173±0.009	0.202±0.058	0.010±0.002^{a}	0.018±0.005^{b}	0.035±0.012^{c}	0.023±0.001^{a}	0.033±0.012^{b}	0.051±0.011^{c}
North (0.5 km)	0.747±0.021	0.833±0.032	0.956±0.101	*0.374±0.035	0.459±0.045	*0.509±0.045	*0.506±0.057	0.615±0.047	*0.712±0.059
North (1.0 km)	0.811±0.024	0.917±0.059	*1.039±0.013	0.459±0.020	0.519±0.068	0.562±0.029	0.581±0.045	0.691±0.028	*0.762±0.069
North (3.0 km)	0.754±0.057	0.809±0.042	0.883±0.060^{a}	0.505±0.028^{b}	0.563±0.041^{c}	0.734±0.054^{ad}	0.556±0.015^{b}	0.581±0.025^{c}	0.643±0.059^{bd}
South (0.5 km)	0.713±0.014	0.773±0.035^{a}	0.887±0.060^{b}	0.437±0.030^{c}	0.510±0.015^{d}	*0.585±0.029	0.558±0.031^{c}	0.633±0.041^{ad}	*0.785±0.042^{b}
South (1.0 km)	*1.242±0.103	1.434±0.070	1.806±0.057	0.687±0.029	0.779±0.013	0.959±0.072	*1.008±0.075	1.180±0.086	1.271±0.094
South (3.0 km)	0.892±0.073^{a}	*0.980±0.014	1.23±0.045	0.561±0.026^{b}	0.713±0.026^{c}	0.860±0.062^{d}	0.710±0.055^{ab}	*0.817±0.029^{c}	0.961±0.031^{d}
East (0.5 km)	0.943±0.070^{a}	*1.104±0.043	1.261±0.052^{b}	0.292±0.035	0.555±0.041	*0.753±0.101	0.780±0.043^{a}	*0.892±0.062	*1.051±0.086^{b}
East (1.0 km)	1.084±0.101^{a}	1.286±0.028	1.655±0.072	0.514±0.075	0.762±0.030	*0.907±0.015	0.936±0.071^{a}	1.035±0.091	*1.234±0.119
East (3.0 km)	0.684±0.029^{a}	*0.812±0.040	*0.986±0.115	0.232±0.013	0.313±0.017	0.410±0.032^{b}	0.507±0.025^{a}	*0.589±0.045	*0.685±0.087^{b}
West (0.5 km)	1.232±0.057	1.540±0.046	1.863±0.041	*0.808±0.017	0.987±0.056^{a}	*1.187±0.032	*1.009±0.075	1.111±0.042^{a}	*1.431±0.014
West (1.0 km)	1.081±0.013	1.261±0.046	1.410±0.075	0.709±0.059^{a}	*0.754±0.042	*0.858±0.045	0.761±0.039^{a}	*0.914±0.041	*1.085±0.031
West (3.0 km)	*0.756±0.042	*0.860±0.047	*0.983±0.116	0.511±0.045^{a}	0.556±0.014^{b}	0.631±0.057^{c}	*0.542±0.034^{a}	*0.657±0.072^{b}	*0.730±0.071^{c}

All values are represented as mean SE. NS= value is non-significant (SD > 0.05) as compared to control site. * = values in horizontal row are seasonally significantly different at 5% significance level during the same year. Values superscripted same alphabets in horizontal row are seasonally not significantly different (SD > 0.05) during the same year.

Table 3. Seasonal variations in copper (mg Cu g^{-1} DW) in the moss *Rhodobryum giganteum* (Schwaegr.) Par. at study sites in Mussoorie city during 2004-2006

Sampling Sites	Summer			Monsoon			Winter		
	2004	2005	2006	2004	2005	2006	2004	2005	2006
Baseline	0.092±0.001	0.118±0.005	0.135±0.019	0.007±0.001^{a}	0.011±0.005^{b}	0.025±0.011^{c}	0.015±0.005^{a}	0.021±0.006^{b}	0.034±0.018^{c}
North (0.5 km)	0.635±0.056	0.734±0.014	*0.790±0.027	*0.309±0.016	0.363±0.032	*0.417±0.018	*0.433±0.016	0.518±0.026	*0.593±0.031
North (1.0 km)	0.734±0.054	0.788±0.036	*0.881±0.056	*0.408±0.029	0.435±0.028	0.477±0.050	*0.530±0.015	0.582±0.043	*0.660±0.073
North (3.0 km)	*0.309±0.036^{a}	0.734±0.056	*0.806±0.042	0.407±0.026^{ab}	0.435±0.059^{c}	0.580±0.028^{d}	*0.464±0.015^{b}	0.505±0.029^{c}	*0.630±0.071^{d}
South (0.5 km)	0.092±0.019	*0.587±0.032	0.656±0.071^{a}	0.016±0.005^{b}	*0.337±0.033^{c}	*0.406±0.014	0.034±0.002^{b}	0.471±0.049^{c}	*0.582±0.029^{a}
South (1.0 km)	*0.051±0.001	1.232±0.116	1.489±0.060	0.011±0.001^{a}	*0.707±0.042	0.829±0.043	*0.023±0.001^{a}	*0.932±0.056	1.092±0.029
South (3.0 km)	0.092±0.005	*0.861±0.047	1.041±0.028	0.016±0.000	0.584±0.027^{a}	0.743±0.028^{b}	0.038±0.001	*0.661±0.053^{a}	0.815±0.031^{b}
East (0.5 km)	0.754±0.028^{a}	0.831±0.056^{b}	0.987±0.072^{c}	0.231±0.014	0.363±0.017	0.556±0.058	0.636±0.057^{a}	0.760±0.028^{b}	0.839±0.036^{c}
East (1.0 km)	0.903±0.056^{a}	*1.028±0.015	*1.230±0.072	0.381±0.043	0.504±0.028	0.660±0.043	0.784±0.043^{a}	*0.904±0.044	*1.006±0.054
East (3.0 km)	*0.580±0.044	0.685±0.044	*0.762±0.028	0.182±0.013	0.263±0.027	0.310±0.029	*0.436±0.015	0.471±0.043	*0.569±0.054
West (0.5 km)	0.958±0.059^{a}	1.335±0.019	1.535±0.076	0.689±0.016^{b}	0.861±0.063^{c}	0.982±0.054^{d}	0.837±0.045^{ab}	1.003±0.028^{c}	1.162±0.077^{d}
West (1.0 km)	*0.881±0.059	1.056±0.026	1.137±0.062	0.610±0.044^{a}	0.661±0.075^{b}	0.739±0.057^{c}	*0.678±0.042^{a}	0.736±0.057^{b}	0.857±0.045^{c}
West (3.0 km)	*0.657±0.056	*0.735±0.059	*0.836±0.032	0.433±0.015^{a}	0.486±0.015^{b}	0.561±0.033^{c}	*0.484±0.060^{a}	*0.535±0.030^{b}	*0.637±0.062^{c}

All values are represented as mean SE. NS= value is non-significant (SD > 0.05) as compared to control site. * = values in horizontal row are seasonally significantly different at 5% significance level during the same year. Values superscripted same alphabets in horizontal row are seasonally not significantly different (SD > 0.05) during the same year.

Table 4. Seasonal variations in cadmium (mg Cd g^{-1} DW) in the moss *Rhodobryum giganteum* (Schwaegr.) Par. at study sites in Mussoorie city during 2004-2006

Sampling Sites	Summer			Monsoon			Winter		
	2004	2005	2006	2004	2005	2006	2004	2005	2006
Baseline	0.006±0.000^{a}	0.016±0.004	0.021±0.005	ND	ND	0.004±0.001^{ab}	0.004±0.002	0.009±0.001	0.012±0.003^{b}
North (0.5 km)	0.110±0.014	0.139±0.013	0.184±0.029	0.037±0.003^{a}	0.057±0.014^{b}	0.061±0.015^{c}	0.059±0.011^{a}	0.060±0.017^{b}	0.080±0.013^{c}
North (1.0 km)	0.130±0.014	0.140±0.012	0.210±0.011	0.055±0.002^{a}	0.063±0.002^{b}	0.082±0.002^{c}	0.060±0.006^{a}	0.079±0.001^{b}	0.134±0.013^{c}
North (3.0 km)	0.111±0.015	0.134±0.014	0.167±0.019	0.034±0.006^{a}	0.055±0.014^{b}	0.061±0.006	0.055±0.014^{a}	0.061±0.015^{b}	0.085±0.016
South (0.5 km)	*0.053±0.031^{a}	0.106±0.014	0.117±0.011	*0.022±0.005^{b}	0.043±0.006^{c}	0.066±0.003^{d}	0.045±0.004^{ab}	0.058±0.002^{c}	0.069±0.004^{d}
South (1.0 km)	0.031±0.072^{a}	0.088±0.017	0.109±0.001	*0.011±0.007	0.027±0.002^{b}	0.036±0.002^{c}	*0.028±0.006^{a}	0.038±0.004^{b}	0.046±0.003^{c}
South (3.0 km)	0.047±0.067^{a}	0.114±0.001	0.137±0.002	0.027±0.009	0.035±0.003	0.045±0.002	0.034±0.016^{a}	0.058±0.003	0.067±0.004
East (0.5 km)	0.106±0.014	0.136±0.011	0.185±0.014	0.021±0.001	0.024±0.000	0.034±0.002	0.034±0.003	0.054±0.014	0.085±0.013
East (1.0 km)	0.114±0.013	0.136±0.015	0.160±0.019	0.028±0.001^{a}	0.038±0.004^{b}	0.042±0.005^{c}	0.043±0.004^{a}	0.059±0.016^{b}	0.060±0.015^{c}
East (3.0 km)	0.088±0.016	0.106±0.014	*0.117±0.018	0.022±0.000^{a}	0.032±0.003^{b}	0.041±0.004^{c}	0.027±0.001^{aNS}	0.037±0.006^{b}	*0.059±0.014^{c}
West (0.5 km)	0.062±0.015	0.089±0.016^{a}	0.129±0.015	0.016±0.001^{b}	0.035±0.004^{c}	0.040±0.005^{d}	0.026±0.001^{bNS}	0.056±0.001^{ac}	0.061±0.014^{d}
West (1.0 km)	0.085±0.014	0.111±0.001	0.137±0.017	0.035±0.005^{a}	0.038±0.003^{b}	0.055±0.014^{c}	0.037±0.004^{a}	0.058±0.014^{b}	0.059±0.014^{c}
West (3.0 km)	0.108±0.017	0.113±0.016^{a}	0.155±0.014	0.039±0.001^{b}	0.054±0.002^{c}	0.063±0.002^{d}	0.058±0.014^{b}	0.081±0.012^{ac}	0.086±0.014^{d}

All values are represented as mean SE. NS= value is non-significant (SD < 0.05) as compared to control site. * = values in horizontal row are seasonally significantly different at 5% significance level during the same year. Values superscripted same alphabets in horizontal row are seasonally not significantly different (SD > 0.05) during the same year.

tourist activity, resulting in low vehicular emissions, and also leaching out of pollutant deposits is expected during the rainy season (Saxena et al. 2007). Another important factor related to the rains is washing-out of ambient particles entered in the atmosphere from different sources, which could result in losses of up to 20%.

The analysis of moss samples exhibited unambiguous seasonal variations in metal concentrations (Tables 1-4). These results do not agree with those of Thoni et al. (1996) and Berg & Steinnes (1997) who did not find any seasonal variations in metal content whilst monitoring their atmospheric inputs using mosses in Switzerland and Norway, respectively. This could be because of no significant seasonal difference in metal emission sources, for example no major change in transport or other industrial activities in those regions. It is also important to note that rain in North European countries is more uniformly distributed unlike the Indian sub-continent. In the present study, it is quite safe to say that seasonal variations in moss metal concentrations are driven by tourist activities (increased transport and waste burning, leading to increased emissions and thus greater uptake by moss) and prolonged/intense rainy season, which also coincides with much reduced tourist activities. Nonetheless, seasonal variations in atmospheric metal deposition are not uncommon, and considerable seasonal changes in moss metal concentrations have been observed by other researchers (Markert & Weckert 1989). The findings, thus clearly demonstrate that biomonitoring using moss is capable of detecting seasonal changes in atmospheric metal deposition.

Despite absence of industries in the area, there is a general increase in Zn concentration over the 3 years study period (Table 1). This increasing trend in moss Zn concentration is clearly an indicator of elevated anthropogenic activity in the proximity of the study sites. In particular, a steady growth in tourism (and its impact on emission via transport and waste generation, which is usually burned) and car ownership are likely factors responsible for increased Zn emissions in and around urban sites (Asthana & Asthana 2005).

The mean moss Pb concentrations show trends similar to Zn, i.e. higher levels in summers than winters, low levels during the rainy season, and a general increase over time (Table 2), possibly due to similar reasons, i.e. a steady growth in tourist activity, waste generation (and its burning) and increased number of vehicles. Such increases in moss Pb concentrations have been correlated with growing number of automobiles (Saxena & Saxena 2000, Saxena et al. 2007). Despite low levels of Pb in unleaded petrol, an increase in its emission is expected with increase in the number of automobiles.

The observed increasing moss Cu levels (Table 3) are likely to be due to the aforementioned factors for Zn and Pb; however, additional sources of Cu are also possible, such as the use of kerosene oil (Loppi & Bonini 2000). Furthermore, fungicide spraying in horticulture which is common in the area may also have contributed, as Cu is an active ingredient in many fungicides, and its aerosol can travel long distances due to high wind conditions in the hills (Gerdol et al. 2000, Otvos et al. 2003). Burning of municipal waste (Markert et al. 1996, Scharova & Suchara 1998, Grodzinska & Szarek-Lukaszewska 2001) and transport activities may have contributed to the increased Cd emission trend as recorded by its accumulation in the moss. Abrasion of automobile parts is a known source of Cd (Stefano & Bonini 2000); thus, the general increase in transport activities seen all over India in recent years may have been a contributory factor.

Metal loads in 2006 increased by 7%, 19% and 11% for Zn, Pb and Cu, respectively, when compared with their loads in 2004. However, different trends were found for rural sites (12%, 11% and 14%) for Zn, Pb and Cu, respectively. The metal deposition loads in the study area were in the order Zn>Pb>Cu>Cd during the 3 years study period.

It seems plausible that increased number of vehicles and significant growth in tourism and waste generation have contributed to increased metal emissions and thus their atmospheric deposition. Furthermore, the increased industrial production in India may also be partly responsible for the increasing metal deposition trend observed in this study, as metals emitted from industrial activities in the northern Indian plains can be deposited in the study area through their long-range transport (Hooda 2010). Moreover, it is important to recognise that trace metals in the atmosphere can also arise from land in the form of dust (Steinnes 1995, Santelmann & Gorham 1988), which can be transported to long distances. The metal concentrations measured in this study were much higher than those obtained from European moss survey (Harmens et al. 2011b). This is hardly surprising as many-fold reduction in the atmospheric emission of metals have been achieved in Europe as compared to their peaks in the early 1970s (Hooda 2010).

It is concluded that this study tested two locally available moss species (*Pohlia elongata* and *Rhodobryum giganteum*) for metal tolerance. The findings of photosynthetic efficiency measurements, obtained indirectly via chlorophyll-a fluorescence measurements, following treatment with lead (0, 10, 40 mg/kg) showed that *Rhodobryum giganteum* (Schwaegr.) Par. moss species was found suitable for biomonitoring of atmospheric metal deposition purposes, as its photosynthetic efficiency remained unaffected.

The findings further confirm the suitability of *Rhodobryum giganteum* (Schwaegr.) Par. as a useful technique for monitoring of atmospheric metal deposition, as this moss species was able to detect successfully the expected seasonal and annual changes in metal emissions/depositions. Whilst biomonitoring using moss, *Rhodobryum giganteum,* cannot provide absolute atmospheric metal deposition rates (e.g. metal mass per unit area), it is particularly suitable for assessing temporal and spatial changes in metal deposition, as is seen in the findings. The principal advantage of biomonitoring is that it is relatively inexpensive. This technique is capable of monitoring atmospheric metal deposition on a regional scale by transplanting a given moss species on a large scale.

ACKNOWLEDGEMENT

The authors are thankful to the Ministry of Environment and Forest (India) for the financial support. Authors thank Professor Govindjee, Professor Emeritus of Biophysics and Plant Biology, University of Illinois for his critique and suggestions on chlorophyll fluorescence measurements. Thanks are also extended to the Department of Biotechnology for funding the purchase of an atomic absorption spectrophotometer. We express our gratitude to Dr. D. Swaroop (I.V.R.I., Izzatnagar) and Dr. R. D. Sharma (C.A.R.I., Izzatnagar, Bareilly) for their support. Help rendered by Bareilly College, Bareilly is gratefully acknowledged.

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