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Moss bag technique for monitoring of metal precipitation

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Abstract

Moss *Barbula vinealis* and *Rhodobryum roseum* used as biomonitor of metal precipitation at Nainital during 2004 - 2005 to examine deposition of Zn, Cu, Cd, Pb at Nainital area, around point sources in all the four directions. Moss bags were transplanted at 8 sites for fixed exposure time in sampling seasons (summer, monsoon and winter) and were harvested periodically after 4 months of exposure. An increase in amount of metals in 2004 - 2005 reflects an increase in metals in air. High metallic load was observed in locations in proximity of higher traffic density. From the result, it is concluded that local sources in Nainital, especially due to enhanced tourism during summer, contributes to elevated metal deposition in comparison to winter and monsoon season. Active monitoring by 4 months of exposure of mosses gives reliable results on metal contamination. Study also aim at assessing the suitability of both mosses as a biomonitor for metal deposition. Study confirms that in Nainital, where due to higher vehicular traffic, wear and tear of vehicular parts and beside it increasing tourist activity, high level of Zn, Pb was measured in moss transplant bags.Bioaccumulation ability in these two mosses was evaluated statistically using Dunkun's Multiple Range Test and was presented on contour maps obtained from SURFER program.

Key Words: *Element concentration, Barbula vinealis, Rhodobryum roseum, biomonitoring*

Introduction

Activities of man and the uncontrolled development of large and small cities and urbanization during the recent past and especially on hills resulted in the contamination of ecosystem. Combustion of fuel, increasing tourist activity and heavy traffic load contribute a considerable amount of pollutants to air. In recent decades the number and intensity of anthropogenic sources, such as waste burning, fertilizers, vehicle emissions, agricultural and sewage sludge, have increased the overall environmental element concentration (Bargagli, 1998). This fact seems to be true for the city of Nainital where there are large numbers of vehicles and other human activities increased with time. Therefore, monitoring of air contaminants of study area is necessary to determine impact upon ecosystem and control measure requires for abatement of their sources in investigated region.

Bryophytes have been used as terrestrial biomonitors and bioindicators of air pollution worldwide as well as by this laboratory and are recognized as more sensitive to pollution than other plants (Fernandez *et al.*, 2000). Among them mosses have tremendous ability to absorb metal as dry fallout from the atmosphere (Saxena, 2001). They have capability to receive and accumulate chemical substances predominantly from surrounding atmosphere (Fernandez *et al.*, 2004). Due to this accumulation potential they have been preferred in present study.

In present work, active monitoring (moss bags) has been used to determine total level of atmospheric deposition of heavy metals in Nainital city. This technique is very useful especially in such polluted areas where wild growing mosses are lacking (Makholm and Miadenoff, 2005).

Study is an attempt to use moss *Barbula vinealis* and *Rhodobryum roseum* to investigate the spatial distribution of the metals (Cu, Zn, Pb and Cd) contamination in Nainital city during Monsoon, summer and winter seasons with the help of moss bag (transplant) technique during different seasons of 2004 to 2005.

Material and Methods

Study area

Nainital is located at an altitude of 5900 ft. on Kumaon hills and connected by road only. It is situated in area of 11.7 Sq. Km. and surrounded by hills from all sides except east, which has only entry in the city (fig.1). The climate was quite cold between October - April and mild warm through May - June followed by monsoon rain till September. The average rainfall measured 80" and relative humidity range from 85 to 90% in the months of July and August. The maximum and minimum temperatures were recorded 27°C to 10°C in summer and 15°C to 3°C in winter respectively.

Sampling

Moss *Barbula vinealis* and *Rhodobryum roseum* were collected from the forest cover of Mukteshwar (unpolluted area), situated at an altitude of 2300 meters. A complete green patch of moss was transplanted in nylon bags at 8 study sites of investigated areas and sufficient amount of the same moss was taken for digestion to determine the baseline concentration of each season. Each moss bag was suspended 6 feets above the ground in triplicate. These moss bags were transplanted cross section wise in all the four directions at the distance of 100m and 300m and were harvested after an exposure period of four months i.e. first week of November (for monsoon monitoring), March (for winter monitoring) and July (for summer monitoring).

Metal analysis

Upon return to the laboratory, harvested moss samples were oven dried at 40°C for 24 hours. Prior to analysis, adhering substrate and litter was removed by hand, great care being taken to avoid metal contamination. Triplicate samples were digested with concentrated HNO_3 and $HClO_4$ in ratio of 5:2 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 and fraction was quantitatively analyzed by atomic absorption spectrophotometer. Suitable blank were used to check for possible contamination during extraction.

Data analysis

Samples were collected in triplicate to conduct the statistical analysis. Value represented as mean $\pm$ standard error (Snedecor and Cochran, 1967). ANOVA revealed significant differences in the metal concentration at different distances and seasons (for $p \leq 0.01$, $p \leq 0.05$) utilizing Dunkun's Multiple Range test (Karmer, 1956). Catographic representation of the results was performed with the program package Surfer (Golden Software Inc., U. S. A.).

Results

Mean concentration (mg g^{-1} dry wt.) of metal ions were detected in *Rhodobryum roseum* and *Barbula vinealis* exposed during 2004-2005 transplant period at 8 monitoring stations of Nainital city (tables: 1 and 2). From analysis of moss transplant, it appears that different concentration of metal accumulation was observed which was related to different pollution sources, its level in the study sites and seasonal variations. A positive correlation was observed in all four metals in each season.

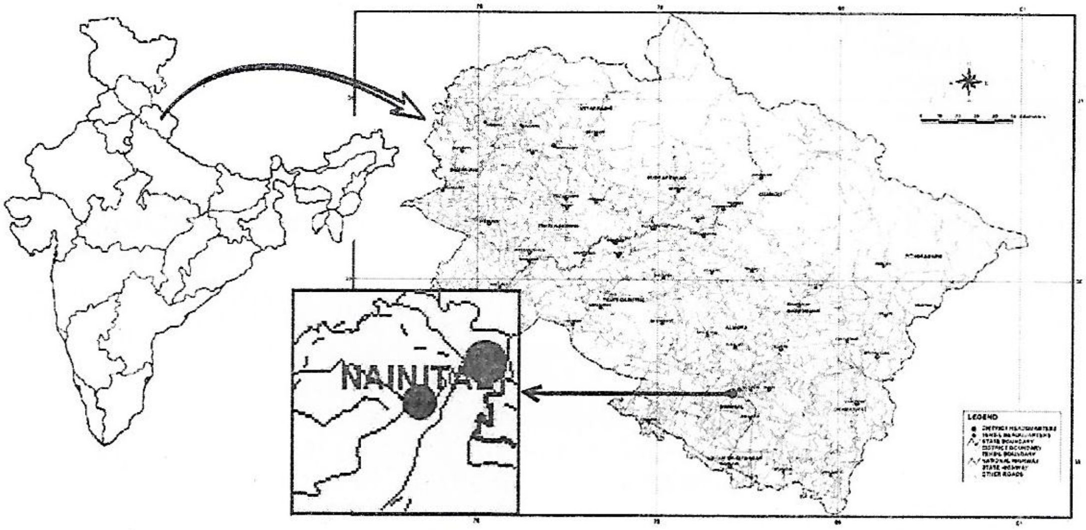

Fig 1. Map of monitoring sites (Nainital) of Kumoan hills, India.

Rhodobryum roseum

In view of seasonal affect maximum concentration of Zn, Pb, Cu and Cd were measured 2.056, 1.585, 0.785 and 0.064 mg g^{-1} dry wt. respectively at 100 m in south direction. However, minimum concentration was measured in west direction of Nainital at 300 m for Zn (0.709 mg g^{-1} dry wt.), Pb (0.477 mg g^{-1} dry wt.), Cd (0.008 mg g^{-1} dry wt.), whereas, in Cu (0.325 mg g^{-1} dry wt.) was lowest in north (300 m). Results were highly significant different for values ($p \leq 0.05$) of Zn, Pb and Cu in each site and for all metals undertaken, while, Cd value was non-significant at north direction between winter and summer season. An identical trend was observed in both transplant belongs to west direction (monsoon and winter season).

Barbula vinealis

The maximum concentration of Zinc was measured as 2.055 mg g^{-1}dry wt. at 100m (south) and minimum as 1.408 mg g^{-1} dry wt.at 300m (west), whereas, in case of lead, higher value was analyzed 1.846 mg g^{-1} dry wt. at 300m (east) and lowest at 0.753 mg g^{-1} dry wt. at 300m (west). In copper concentration was as high as 1.229 mg g^{-1} dry wt. in south direction (100m) and decline up to 0.416 mg g^{-1} dry wt. in west (300m). Cadmium was detected quite low in all samples, the maximum and minimum values were measured as 0.070 mg g^{-1} dry wt. at 300m (south) and 0.013 mg g^{-1} dry wt. at 100 m in north direction respectively.

After calculating ANOVA Zn, Pb and Cu show almost significant different values in different seasons. Cd shows almost non-significant results. However, results of eight study sites show significant different values for other metals Zn, Pb and Cu in all three seasons in comparison to control.

Metal distribution pattern

The distribution mapping pattern of undertaken metals (Cu, Zn, Cd, Pb) show maximum concentration in south and south east direction, whereas, minimum concentration was observed in west direction of transplant sites (in most of the cases)at Nainital during different seasons in both the mosses (figs: 2-25).

The reason could be due to higher vehicular load as bus station and petrol pump is located in south side of Nainital city. In addition, at some places samples get direct exposure while in other areas not. We cannot ignore even meteorological factor and direction of prevailing wind, which may be the other cause for variation in distribution pattern.

Discussion

The metal concentration of the analyzed samples varied greatly, depending on the environmental precipitation and correlated well with the distribution of emission sources. In different seasons, metal precipitation was not constant and recorded maximum during summer followed by winter and lowest in monsoon season. The more significant seasonal trend is reflected by the fact that consumption of gasoline peaks during summer due to many fold increased in tourist activity and also due to decrease in growth during dry summer period (Gerdol *et al.*, 2002). However, in monsoon tourist activity decreases and pollutants leach out. Further more, increase in growth and biomass occurs more rapidly during rains and thus reduces the metal percentage in leaves in proportion to biomass. Present observation are ample documented by tables 1 and 2 confirm that statistically only Pb, Zn and Cu showed any degree of positive correlation for the whole data set.

The study confirms that there are different levels of contaminants accumulation in the transplants. It is evident from analytical parameters that high amount of metals (Pb, Cu, Cd and Zn) was detected in south direction in moss *Rhodobryum roseum* and *Barbula vinealis* due to the reason that site is near bus station and petrol pump, dry deposition of metals spewed out from automobile (Imperato *et al.*, 2003), the only source of transportation connecting the foot hill (Haldwani) to Nainital. On the contrary, Pb was measured maximum at 100m (north) in *Barbula vinealis* may be due to traffic density, proximity to other roads, precipitation, meteorological factors, and finally by direction of prevailing wind. The lowest ratios of metal were found in the transplants, west direction along with north site during monsoon season. This reveals that mosses exposed to traffic are relatively high (south) compared with those having low traffic volume and human interference (west).

This is further confirmed for Pb and Zn both elements were found high in concentration. This may be due to busy roads, motorways, traffic density, use of fertilizers for growth of crops and orchards (Saxena and Saxena, 2000). Besides, it is an integral constituent of lead as steel and automobile industries. The likeliest source of the Zn from vehicles is engine and particularly tires wear, along with very little from exhaust emission (Pearson *et al.*, 2000). The main source of Pb is vehicular emission. It is also found associated with anthropogenic variables of the environment. Since, Cu is an integral part of discarded metallic waste and used in fertilizers etc.On the contrary it is also used as fungicides and pesticides, practices in agriculture (Gerdol *et al.*, 2000; Otvos *et al.*, 2003). Higher values in domestic waste were reported due to their improper dispersal in atmosphere. In addition, higher concentration of Cd was due to automobile exhausts (Stefano and Bononi, 2000). The contamination may also come from use of metallic or plastic pipes, sewage sludge, abrasion of automobile tires and from domestic wastes in urban areas (Grodzinska and Szarek-Lukaszewska, 2001).

Study successfully demonstrated the regional gradients of metals. Mosses *Rhodobryum roseum* and *Barbula vinealis* were very common in urban areas and were often the only species near busy main road, an indication of their general tolerance to atmospheric pollution in Nainital.

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Table 1: Metals concentration (mg g-1 dry wt.) in transplants of *Rhodobryum roseum* at different sites of Nainital city during monsoon, winter and summer season (2004-2005).

Sites	*Rhodobryum roseum*											
	Monsoon 2004				Winter 2005				Summer 2005			
	Zn	Pb	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb	Cu	Cd
Control	0.408± .004	0.268±0.005	0.207±0.004	0.005±0.002^{ABC*}	0.582±0.001	0.399±0.006	0.312±0.006^{DEF*}	0.024±0.052^{GHIJa*}	0.758±0.052	0.488±0.046	0.403±0.002	0.026±0.035^{KLa}
South,100m	1.030±0.008	0.668±0.004	0.412±0.006	0.026±0.005^{A*}	1.205±0.003	0.776±0.003	0.525±0.013	0.034±0.026	2.056±0.018	1.585±0.003	0.785±0.003	0.064±0.003
South,300m	1.354±0.002	0.925±0.014	0.456±0.003	0.023±0.002	1.575±0.006	1.125±0.014	0.577±0.004	0.044±0.029	1.659±0.005	0.954±0.023	0.762±0.015	0.054±0.001
North,100m	1.183±0.007	0.844±0.012	0.378±0.004	0.009±0.006^{B}	1.277±0.009	0.958±0.004	0.484±0.006^{D}	0.024±0.027^{Gb}	1.728±0.045	1.286±0.035	0.623±0.024	0.027±0.013^{Kb}
North,300m	0.880±0.002	0.723±0.013	0.325±0.009	0.022±0.002	1.010±0.008	0.804±0.027	0.454±0.002^{E}	0.039±0.043^{c}	1.277±0.038	1.023±0.002	0.529±0.004	0.055±0.021^{c}
East,100m	0.980±0.003	0.771±0.002	0.453±0.008	0.020±0.003*	1.134±0.003	0.950±0.014	0.604±0.002	0.034±0.021*	1.428±0.026	1.123±0.021	0.756±0.035	0.046±0.033*
East,300m	1.207±0.004	0.903±0.026	0.449±0.011	0.009±0.001^{B}	1.476±0.009	1.003±0.002	0.506±0.003*	0.029±0.038^{Hf}	1.627±0.002	1.295±0.026	0.668±0.041	0.030±0.036^{Lf}
West,100m	0.876±0.011	0.706±0.003	0.348±0.004	0.017±0.021^{g}	1.156±0.004	0.837±0.064	0.518±0.011	0.026±0.032^{gI}	1.390±0.003	1.125±0.003	0.556±0.015	0.046±0.031
West,300m	0.709±0.005	0.477±0.004	0.404±0.003^{de}	0.008±0.001^{h}	0.828±0.046	0.704±0.004	0.221±0.166^{Fd*}	0.033±0.038^{Jh*}	1.228±0.004	1.025±0.024	0.678±0.047^{e*}	0.050±0.055*

- * $p \leq 0.05$
- Values are represented as mean ± standard error.
- With in vertical columns of different seasons, values superscripted with same small alphabets are not significantly different at $P<0.01$.
- With in horizontal rows, values with same Capital alphabets are not significantly different at $P<0.01$ in comparison to control.

Table 2: Metals concentration (mg g-1 dry wt.) in transplants of *Barbula vinealis* at different sites of Nainital city during monsoon, winter and summer season (2004-2005).

Sites	*Barbula vinealis*											
	Monsoon 2004				Winter 2005				Summer 2005			
	Zn	Pb	Cu	Cd	Zn	Pb	Cu	Cd	Zn	Pb	Cu	Cd
Control	0.479±0.023	0.370±0.020	0.275±0.002	ND	0.675±0.014	0.470±0.012	0.395±0.003	0.005±0.014^{a*}	0.775±0.005	0.576±0.010	0.435±0.012	0.015±0.012^{aAB*}
South,100m	1.355±0.102^{b}	0.954±0.023^{cd}	0.505±0.003	0.018±0.052^{fg}	1.531±0.009^{b}	1.417±0.241^{ce}	0.666±0.015	0.038±0.005^{fh}	2.055±0.012	1.481±0.052^{de}	1.229±0.011	0.064±0.018^{gh}
South,300m	1.333±0.017	1.030±0.087	0.704±0.023	0.025±0.046^{i*}	1.653±0.007	1.346±0.021	0.884±0.006	0.047±0.011^{ij}	1.805±0.029	1.630±0.008	0.931±0.016	0.070±0.011^{j*}
North,100m	1.226±0.006	0.896±0.017	0.457±0.006	0.013±0.072^{kl}	1.378±0.121	1.052±0.016	0.585±0.072	0.025±0.002^{km}	1.714±0.008	1.421±0.011	0.829±0.058	0.032±0.007^{lmA}
North,300m	1.151±0.027	0.792±0.001	0.429±0.012	0.025±0.002^{n*}	1.278±0.052	0.977±0.012	0.766±0.013	0.040±0.011^{no}	1.501±0.007	1.204±0.002	0.955±0.104	0.062±0.069^{o*}
East,100m	1.300±0.028	0.846±0.035	0.568±0.179	0.017±0.069^{p}	1.578±0.012	1.260±0.017	0.636±0.023	0.030±0.005^{pq*}	1.774±0.013	1.519±0.058	0.746±0.010	0.060±0.077^{q}
East,300m	1.380±0.011	1.076±0.029	0.517±0.007	0.030±0.087^{rs}	1.704±0.023	1.430±0.008	0.629±0.011	0.045±0.011^{rt}	2.004±0.023	1.846±0.098	0.795±0.002	0.058±0.052st
West,100m	1.178±0.005	0.876±0.005	0.526±0.010	0.018±0.005^{t}	1.379±0.011	1.093±0.001	0.723±0.013	0.033±0.006^{t*}	1.618±0.052	1.307±0.024	0.954±0.053	0.053±0.053*
West,300m	1.048±0.003	0.753±0.075	0.466±0.015	0.014±0.055^{uv}	1.254±0.013	0.994±0.003	0.676±0.009	0.029±0.005^{uw}	1.453±0.075	1.126±0.041	0.873±0.088	0.036±0.075^{vwB}

- * $p \leq 0.05$
- Values are represented as mean ± standard error.
- With in vertical columns of different seasons, values superscripted with same small alphabets are not significantly different at $P<0.01$.
- With in horizontal rows, values with same Capital alphabets are not significantly different at $P<0.01$ in comparison to control.

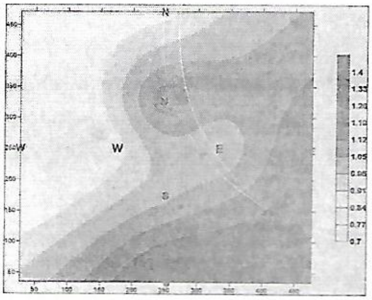

Fig.2.Distribution map of Zn content *R. roseum* (mg g^{-1}) in monsoon at Nainital

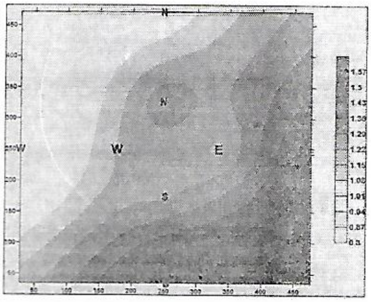

Fig.3.Distribution map of Zn content *R. roseum* (mg g^{-1}) in winter at Nainital

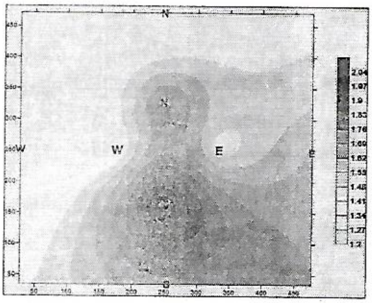

Fig.4.Distribution map of Zn content *R. roseum* (mg g^{-1}) in summer at Nainital

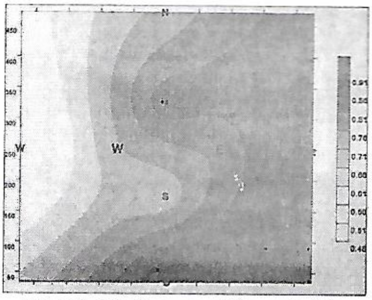

Fig.5.Distribution map of Pb content *R. roseum* (mg g^{-1}) in monsoon at Nainital

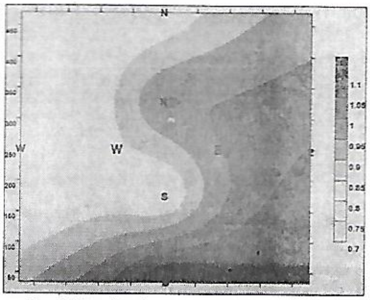

Fig.6.Distribution map of Pb content *R. roseum* (mg g^{-1}) in winter at Nainital

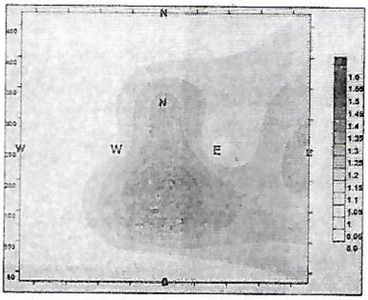

Fig.7.Distribution map of Pb content *R. roseum* (mg g^{-1}) in summer at Nainital

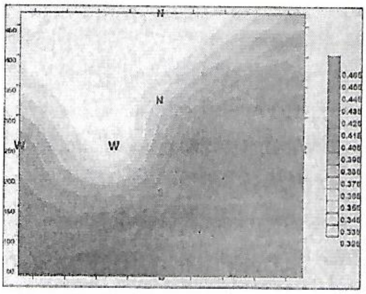

Fig.8.Distribution map of Cu content *R. roseum* (mg g^{-1}) in monsoon at Nainital

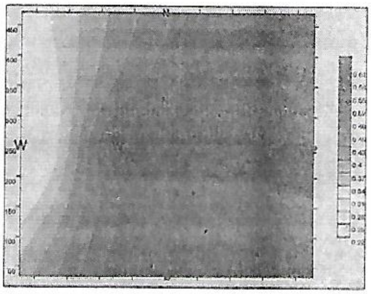

Fig.9.Distribution map of Cu content *R. roseum* (mg g^{-1}) in winter at Nainital

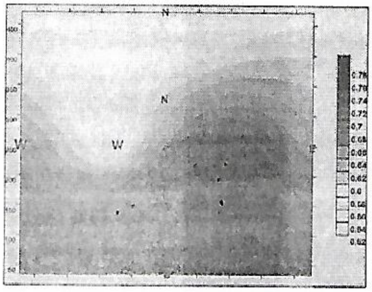

Fig.10.Distribution map of Cu content *R. roseum* (mg g^{-1}) in summer at Nainital

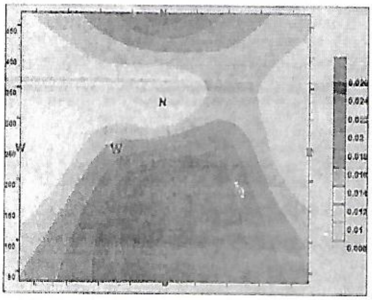

Fig.11.Distribution map of Cd content R. roseum (mg g^{-1}) in monsoon at Nainital

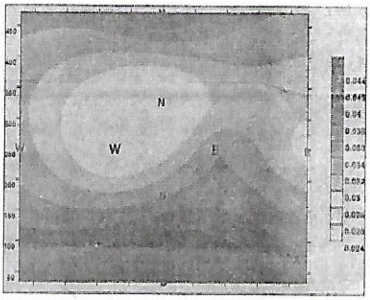

Fig.12.Distribution map of Cd content *R. roseum* (mg g^{-1}) in winter at Nainital

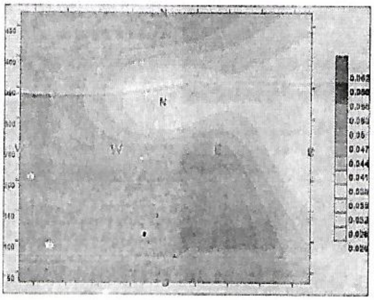

Fig.13.Distribution map of Cd content *R. roseum* (mg g^{-1}) in summer at Nainital