Retrospective metal data of the last 100 years deduced by moss, *Barbula* sp. from Mussoorie city, Garhwal Hills, India

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This communication is the first attempt at contributing to monitoring and systematic gathering of information on the heavy metal profile of the environment, i.e. retrospective study of the environmental metal data (Pb, Fe, Zn, Cd and Ni) of the past 100 years, i.e. 1895–1999 from Mussoorie city, Garhwal Hills, India by analysing herbarium voucher specimens of moss, *Barbula* species as a tool. Our laboratory has been using this tool successfully to delineate the past metal profile by analysing moss samples. A significant increase in metal content was observed by *non-invasive* analysis of herbarium specimens of *Barbula* sp. belonging to Mussoorie city, which seems to be a reflection of atmospheric metal load. The lowest concentrations were found in the voucher specimens of the early period, i.e. 1895 and the level increased over a period of time. However, difference in trend was also observed. The maximum significant increase observed for metals Zn, Ni and Pb was 5743.369, 3195.238 and 1362.207% respectively, with respect to first metal data of the year 1895 in *Barbula* sp. The same could be due to an increase in metal load resulting from progressive industrialization in the early period. The ratios between the content of the various metals in bryophyte tissue increased nearly identically for all metals examined. The novel aspect of this study is that it provides information on retrospective metal data of the past 100 year from Mussorie hills (India) as well as Asia by analysis of bryophytes, where no information was earlier available.

Keywords: *Barbula* species, biomonitoring, retrospective metal data, voucher specimens.

THE increasing trend in concentration of metals in the environment has created considerable attention amongst ecologists globally during the last decades. Measurements have been made of atmospheric metallic precipitation in Europe and America. However, no such studies have been carried out in India and there are no past metal load data available. Therefore, we initiated such a study. There is need for extensive monitoring efforts over long periods of time in order to describe average metal precipitation[1] and its trend, which is an essential component of any pollution control management. Use of classical instruments cannot retrieve the past metal profile. Monitoring of metal precipitation using biomonitors like bryophytes is emerging as a potentially effective, more economical and reliable alternative biological tool. Biological monitoring for metals has several advantages over other methods of obtaining an integrated picture of metal precipitation levels. By this method it is possible, in a less exacting way, to reveal the area of high metal burden to assess the level of metal input at different tropical levels and to obtain some relative estimates of the total aerial burden of metals[2].

The usefulness of mosses in determining metal concentration in different geographical areas has been discussed and demonstrated in several studies[3–6]. This is because bryophytes are precise and sensitive bioindicators as well as bioaccumulators of metal deposition in the environment. They have been used for long-term biomonitoring of metals in many European countries since the beginning of 1970s, for both local and regional metal sources[7–14]. Mosses preserved in the herbarium are suitable for determining environmental pollution of the past. Since mosses are largely dependent on the atmosphere for nutrient and moisture, it is safe to assume that the metal concentrations in the samples correlate with atmospheric input as well as the corresponding periods[15,16]. However, little has been done with regard to retrospective studies of metal content by bryophytes from India, despite luxuriant bryophytic flora. Literature survey shows that there is no record till date on metal precipitation profile of Mussoorie and its adjoining areas, as well as on retrospective metal data from any part of India.

Retrospective monitoring cannot be done using instruments. Bryophytes can become tool, using which it is possible to get the past metal load[17], because of their slow death rate, decomposition and active absorption, which make them ideal for retrospective studies[18]. Metals are efficiently retained by the bryophytes for a long period even in a damaged plant, as they do not release them quickly after death. Since bryophytes absorb moisture and nutrients from the air, their analysis provides the atmospheric metal load. Thus, their potential of being efficient active accumulators has been exploited in the present study, based on metal analysis of a portion of herbarium voucher specimens borrowed from different herbaria.

To reconstruct the past metal trend, an extensive literature review was done and a deposition scenario was developed. However, there are some uncertainties about exact location of voucher specimens, associated with the deposition scenario. Hence voucher specimens were procured on loan for temporal records of historical metal deposition. An attempt was made to borrow as many voucher samples as possible from different institutes and herbaria, and the same were analysed for metals.

The aim of the present study is to interpret the metal burden of Mussoorie and its adjoining areas from the period of 1895–1999 using a modern and promising emission technique, i.e. Induced Coupled Plasma Emission Spectroscopy (ICPES) and Proton Induced X-ray Emission (PIXE). The novel aspect of this study is that it

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provides information on retrospective metal data of the past 100 years from India and Asia, where no information was earlier available.

Mussoorie is an easily accessible hill station in Garhwal, northern India, located at an altitude of 2005.5 m asl and having an area of 6.425 sq. km. It is renowned for its salubrious atmosphere that helps revive ailing visitors. The city remained uncrowded till the late 1950, but with the growing population coupled with the ever-increasing tourist influx, the number of vehicles has also increased many folds, leading to the heavy metal pollution in the area. To conduct the present study, the area was identified on the basis of availability of herbarium voucher specimens.

The area has cool climate from April to October and monsoon showers between June and August. Conditions are moderate from September to November. However, snowfall is seldom observed in January. The maximum and minimum temperature ranges from 25.6°C to 31.7°C and 7.2°C to 12.8°C respectively, during summer. Maximum temperature in winter ranges between 7.2°C and 2.2°C, and the minimum between 1.0°C and 4.4°C. Variation was observed during monsoon season due to humidity. High relative humidity (90%) was measured in July and August, whereas it was lowest (60%) in January.

Herbarium specimens of *Barbula* sp. for the period 1895–1999 were borrowed on loan from different herbaria around the world. These species obtain nutrients and metals predominantly from the atmosphere and are, therefore, particularly useful for the study of atmospheric metal deposition as their analysis reflects the atmospheric metal load of the period to which they belong. Duplicate herbarium specimens from different regions of the study area were obtained on loan from New York Botanical Garden and University of Michigan, vide reference number 7627, where they have sufficient periodical collections of *Barbula* sp. belonging to this region.

Permission was taken to use the specimens obtained on loan for analysis by *non-invasive* method. On careful removal, 0.1 g of each sample was collected and stored in polythene bags for analysis.

Voucher specimens were analysed in triplicate for metals like Pb, Zn, Ni, Fe and Cd using ICPES and PIXE multi element analyser. Metal concentration was expressed as μg per g dry wt and the value represented as mean ± standard error[19]. ANOVA revealed significant differences in metal concentration depending upon distance from the source and season (for $P < 0.01$, $P < 0.05$), utilizing Duncan's Multiple Range Test (DMRT)[20].

Since the herbarium samples were not collected at regular intervals, it was not possible to analyse time periods of equal length. However, a plan was worked out from 1895 to 1999, according to the availability of vouchers of loan samples.

The metal concentration of different *Barbula* specimens obtained from analysis of voucher specimens from 1895 to 1999 using ICEPS revealed different trends for the various elements by linear regression analysis and significant increase was found between metals and year of collection (Figure 1). The observed increase for Pb, Zn and Fe in the specimens can be ascribed to the increasing anthropogenic emission.

The present study reveals that Zn and Pb show maximum percentage fluctuation during the past 100 years. The percentage increase for Zn and Pb was 609.438 and 167.878 respectively, during 1903–36 (33 years). Whereas percentage increase in metal data of moss specimens was 596.295 in Zn and 402.750 in Pb during 1936–94 (58 years). Nearly the same trend was observed for Fe, Cd and Ni during 1903–36 and 1936–94.

Significant increase was observed in the case of Zn, Ni and Pb, i.e. 5743.369, 3195.238 and 1362.207% respectively, with respect to the first metal data of 1895. The same trend was also found when compared to previous year's data and high values were obtained, i.e. 663.712% for Ni, 596.295% for Zn and 402.750% for Pb, between 1936 and 1994 (Table 1). Thus the increase in concentration of metals in the moss could be due to the increase in anthropogenic activities.

Concentration of these elements in the moss voucher specimens exhibited the atmospheric trend of the metal load. Metal data of the first 40 years (1895–1936) exhibited an increase in concentration of Zn and Ni in atmosphere approximately five times and that of Pb three times compared to the initial concentration. Contamination level of Fe was doubled during the same period. In the next 63 years (1936–99), similar increasing trend was observed for Fe and Ni, while Pb content rose by up to five times and Zn nearly six times (Table 1). The moss showed this trend precisely because it can accumulate elevated levels of metal deposition.

The pattern of Cd was completely different. The results were beyond detection limit (ND) during the first 7 years. However, about eight times increment in Cd concentration was found during the period 1936–99. A strong positive correlation was found between all metal concentrations in *Barbula* sp. The change in metal precipitation in specimens is a reflection of atmospheric metal load (Table 2). The present investigation also shows that the whole study area is exposed to high emission levels, thus putting the functioning of the ecosystem of this area at great risk.

It is well known that environmental pollution is a product of urbanization, mechanization and technology that serve to provide the necessities of a growing population. Traffic jams are an everyday occurrence in this area and have reached a disturbing magnitude, resulting in high air pollution[21]. Till date, relatively little or no data are available on the extent of environmental metal pollution because there are no agencies for routine monitoring. The increased concentration of metals in the recent collection of mosses

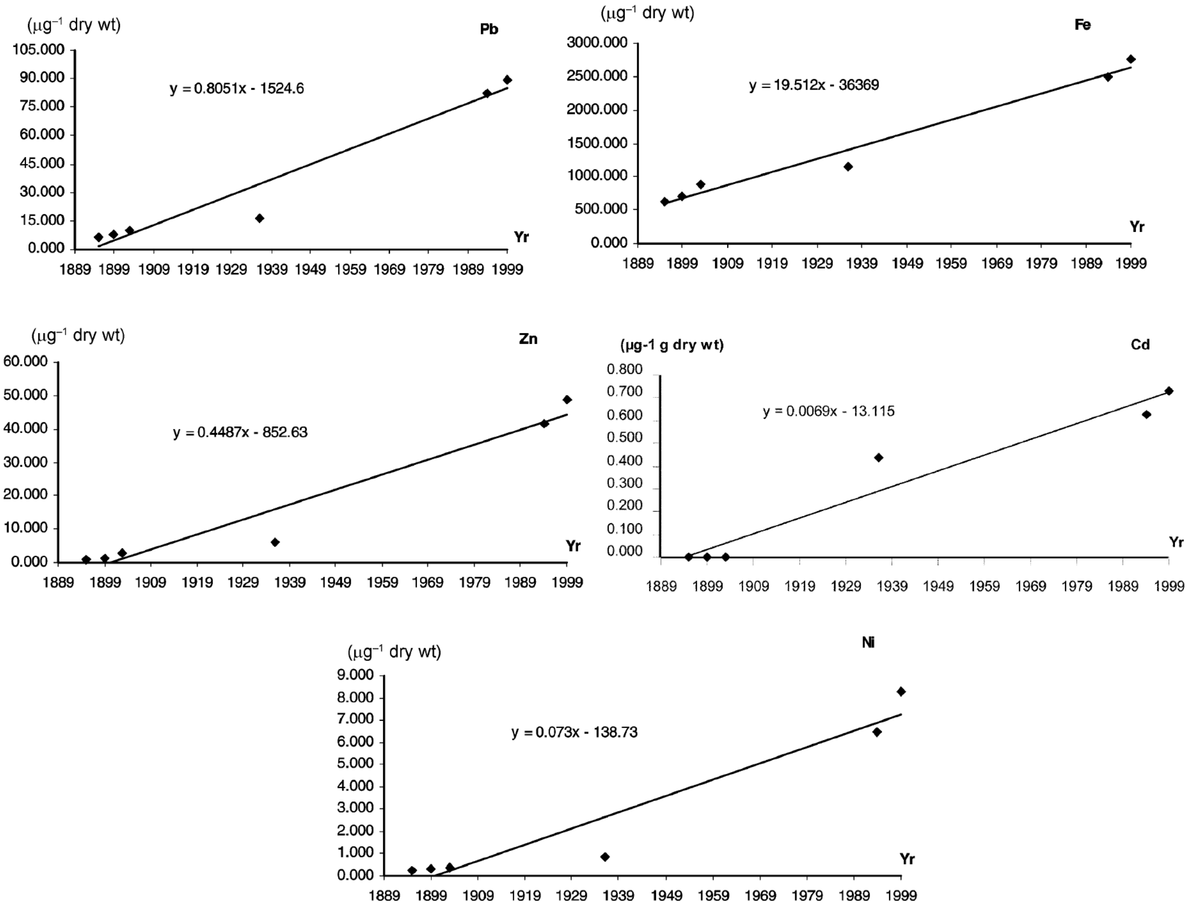

Figure 1. Element content (μg per g dry wt) in *Barbula* sp. related to year of collection.

Table 1. Metal content (μg per g dry wt) in *Barbula* sp. during the period of 1895–1999 from Mussoorie hills

Moss	Year	Pb	Fe	Zn	Cd	Ni
Barbula flavesceus	1895	$6.136^{abc} \pm 0.035$	$630.526^{ade} \pm 0.154$	$0.837^{bdf} \pm 0.005$	ND	$0.252^{cef} \pm 0.002$
B. flavesceus	1899	7.906 ± 0.003	699.136 ± 0.145	1.296 ± 0.003	ND	0.329 ± 0.002
Comparison from 1895 in %		28.846	10.881	54.839		30.556
B. temista	1903	$9.816^{ghi} \pm 0.004$	$886.970^{gjk} \pm 0.130$	$2.683^{hjl} \pm 0.005$	ND	$0.344^{ikl} \pm 0.002$
Comparison from 1895 in %		59.974	40.671	220.550		36.508
Previous year in %		24.159	26.867	107.022		4.559
B. icamadophyll	1936	16.437 ± 0.006	1154.471 ± 0.200	5.938 ± 0.011	0.440 ± 0.003	0.846 ± 0.002
Comparison from 1895 in %		167.878	83.096	609.438		235.714
Previous year in %		67.451	30.159	121.319		145.930
B. gregaria	1994	$82.637^{mn} \pm 0.579$	2499.377 ± 0.574	$41.346^{mo} \pm 0.575$	$0.626^{n} \pm 0.016$	$6.461^{o} \pm 0.059$
Comparison from 1895 in %		1246.757	296.396	4839.785	42.273	2463.889
Previous year in %		402.750	116.495	596.295	42.273	663.712
B. constricta	1999	$89.721^{p} \pm 0.029$	2760.780 ± 0.301	$48.909^{pq*} \pm 0.003$	$0.730^{r*} \pm 0.002$	$8.304^{qr} \pm 0.003$
Comparison from 1895 in %		1362.207	337.853	5743.369	65.909	3195.238
Previous year in %		8.572	10.459	18.292	16.613	28.525

Values are represented as mean ± SE.
Significance test of ANOVA and DMRT has been done at 1 and 5% significance level.
Values superscripted with the same alphabets in horizontal row are not significantly different at 1 and 5% significance level for different metals.
Values in horizontal row are significantly different at 5% significance level for different metals.
ND, Not detectable.

Table 2. Correlation coefficient matrix between metals of *Barbula* sp. during the period 1895–1999

	Lead	Iron	Zinc	Cadmium	Nickel
Lead	1.000				
Iron	0.994	1.000			
Zinc	0.999	0.993	1.000		
Cadmium	0.963	0.978	0.981	1.000	
Nickel	0.994	0.988	0.998	0.993	1.000

in relation to the year of collection might be due to the following reasons.

1. High variability in values of these metals was observed due to variations in climatic conditions and dispersion could be due to many factors, including wind.
2. Recent increase in the past decade is associated with different variables like heavy traffic load, wear and tear of automobiles, tourist activity, fuel consumption and high population density.
3. Use of wood and coal for fire and energy in households may have significant influence as these were used as a primary source of energy during 1900–70.
4. Moreover, difference in macro- and micro-environmental conditions has influenced the concentration of pollutants.

Retrospective study of the past 100 years (1895–99) exhibited an overall significant increase of metals. This can be explained by the establishment of new localized emission sources. The study suggests and recommends the viability of using *Barbula* herbarium voucher specimens in retrieving retrospective metal data. The study also reports that the sources of atmospheric pollution are not due to industrial emission, but from combustion of fossil fuels. Simple burning of solid wastes could have also been a mode of pollution between 1950 and 1990, when coal combustion was the main source of pollution. There is need to take up such studies in other areas as well, to reveal the trend. In view of the lack of sufficient technical manpower for effective monitoring of environmental pollution using moss species as accumulator/indicator, other related centres should be encouraged for a coordinated study.

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