Uptake of Metals in *Plagiochasma* and Their Use In Pollution Monitoring

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Plagiochasma had been exposed for one complete year during 1992-1993 on Kumaon hills at fifteen bio-monitoring sites. The amount of Pb, Cu, Ni, Mn & Cr were measured from transplants harvested from different pollution catchment sites at the end of exposed period. The data obtained were compared with new transplants of *Plagiochasma* exposed for same length and same study area at same height during the period 1993-94.

The metal contents measured are presented and discussed with the intention to expose the utility of *Plagiochasma* as biomonitoring agent for the estimation of air quality. The differences between the amount of metal detected during the two periods in *Plagiochasma* are discussed. An increase in amount of some of the metals in 1993-94 transplants reflects an increase in metals in air.

This increase in Pb and Cr was concomitant with increased number of vehicles, is suggestive that source of Pb and Cr in air may be from automobile exhaust spewed by vehicles and other sources. Significant positive correlation were found between concentration of zinc and in rural transplants. Zinc here used as an element for orchards, suggests that *Plagiochasma* species may be useful as monitors of zinc contamination due to bio-accumulation properties. Mn is pervasive air-borne of natural built and despite their presence on hills, it is difficult to enable their value as monitor to be assessed. Indeed, it was present in high quantity at Pithoragarh and Almora. Cu and Zn was not sufficiently wide spread, however, both were found in fairly high quantity in *Plagiochasma* from rural sites suggesting the occurrence of intermittent source of this metal in rural areas.

Key-words–Metals; bryophytes; *Plagiochasma*; biomonitor; bio-accumulation; Kumaon hills.

INTRODUCTION

MONITORING of air contaminants is necessary to determine impacts upon ecosystem and control measures require for abatement. Bryophytes have a long history of use as biological indicators or air quality. Bryophytes are out-standingly a successful group of cryptogams and biologists have given much attention to use them as air quality monitor paritcularly metal concentration on account of their known sensitivity to pollutants. There are known evidences that bryophytes may tolerate elevated levels of toxic elements (Url 1956). Some of them have tremendous ability to absorb metals as dry fall out from the atmosphere (Pickering & Pauia 1969; Brown & Bates 1972; Brown & House 1978).

Bryophytes can be used as pollution monitor in three ways (Saxena & Glime 1991): (i) by identifying and mapping all bryophytic species in an area, (ii) by transplanting bags of identical age into polluted area for fixed time of one year and measuring an increase in metal content in 2nd year transplants and (iii) by measuring deterioration (chlorophyll) and by sampling of an individual species and measuring contaminants accumulated within the thallus. Terrestrial mosses have been used by various workers extensively to monitor air borne metal contamination (Ward *et al*. 1977, Baddeley *et al.* 1994 Ruhling 1994, Steinnes 1994), but very little is known on biomonitoring potentials of liverworts (Briggs 1972; Saxena 1998b,c). The use in recent years of bryophytes as monitor of pollution (Martin & Coughtrey 1982; Brown 1984; Ruhling & Tyler 1984) has emphasized the ability of these plants to accumulate potentially toxic elements. Air pollutants are removed from atmosphere by wet and dry deposition process (Proctor 1981). The capacity of bryophytes to accumulate potentially toxic elements has led to their use as pollution monitor and when aerial deposition occurs, much of the accumulated metal is initially trapped in the form of particulate matter (Rinne & Barclay-Estrup 1980; Rao 1982; Brown & Beckett 1985). These metals uptaken by bryophytes, is characterised by large part played by ion-exchange site in the cell wall. Absorption band of carboxyl group in purified cell wall of *Sphagnum* and cation exchanger was identified as polygalacturonic acid in cellulose matrix (Bell 1959, Sachwarzmaier & Brehm 1975). This property has been made use of, in studies of deposition and uptake of metal ions from the environment. (Ferguson *et al.*1984). Indeed due to this characteristic, the localization of various elements in moss have been found to be more predictable (Malmer 1988; Watkinson & Watt 1992) but little is known in liverworts. Mosses are used after in the assessment of heavy metal pollution because of their metal bioaccumulation and tolerance potential (Ruhling *et al.* 1987; Grodzinska *et al.* 1990; Tyler 1990; Kovacs 1992; Godzik & Szarek 1993; Steinnes 1993; Saxena 1995, 1998a). However, little is known about liverworts and their metal tolerance. Therefore, the aim of the present study is to confirm the potential of *Plagiochasma* as biomonitor and to compare the amounts of some metals detected in transplanted *Plagiochasma*, after it had been exposed during 1992-93, with the amounts of the same metals detected in new transplants exposed to same length of exposure during 1993-94.

Due to their specific biology, bryophytes are most remarkable for pollution monitoring and have proved to be efficient collectors of metal from environment (Richardson 1981). Main objectives of present study is to summarize the results of heavy metal concentration of two periods with the help of bag transplant technique at Kumaon hills through *Plagiochasma*.

Further, the aim of the present study is to assess and present the regional deposition pattern of heavy metal through liverwort i.e. *Plagiochasma*.

MATERIAL AND METHOD

Kumaon hill is approximately 680 Kms in length. Climate on it varies, between October to April which is cold and mild warm throughout May, June followed by monsoon rains till October. The average rainfall is 80" during the monsoon month from July to September. The maximum and minimum temperature are 4°C to 30°C. The relative humidity is 85-90%, highest in the months of July and August and is 60%, lowest in the month of January. Kumaon hill represents an ideal condition for luxuriant growth of bryophytes. The *Plagiochasma* is specially well suited for use in biomonitoring as it is known to be tolerant of pollutants, therefore being available for sampling in close proximity to point source.

Sampling of *Plagiochasma* started in 1992, a month before starting *Plagiochasma* experiments. Forty eight bags were prepared. Each bag was of 1.5"x1.5" quadrangular, made up of wire net. It had two quadrangular wire nets of 1.5"x1.5" connected by wire at one end. Free distal part had a handle to fold these two nets close to each other. Inside the wire net there *Plagiochasma* pad of 1.0"x1.0". Each pad had .15 kg of *Plagiochasma* spread over area at 1.0"x1.0". *Plagiochasma* of same age and size were collected from Mukteswar at the height of 7500' for experiments. These were brought to the lab and surface washed for several times and only gametophyte was sorted.

Samples were transplanted in triplicate at 15 sites of Kumaon and Garhwal hills at different locations (Figure 1). This was done to obtain data on accumulation of metallic elements which are spewed by automobile in heavily traffic sites. The study sites were : (1) Golf Court, (2) Ramnagar, (3) Nainital bus-station, (4) Rudraprayag, (5) Kapkot, (6) Mukteswar, (7) Garampạni, (8) Garuna Temple, (9) Jeoli Kot, (10) Bhimtal. Besides this, samples were exposed at Almora, Ranikhet, Pithoragarh, Chamoli and Haldwani (Table1). For control, pine forest site of Mukteswar I.V.R.I., research complex at Almora has been selected as there were no motor roads around this spot and may be presumed as free from pollutants.

Plagiochasma samples were thawed and carefully cleaned. Then oven dried at 100°C for 24 hours. From the dried material, 1 gm of moss was weighed and placed in a 30 ml acid-washed porcelain crucible. The 1gm sample was ashed at 600°C for 4 hr. the ash was digested in 3:1 concentrated $HCl:HNO_3$ over moderate heat. The solid residue was centrifuged

Table 1 : Details of transplants site used during 1992-93 & 1993-94 periods.

S.No.	Transplant Sites	Description of Sites
1.	Golf Court	Pine deep forest
2.	Ramnagar	City
3.	Nainital	Busy bus terminal/road intersection
4.	Rudraprayag	Village
5.	Kapkot	Village
6.	Mukteswar	Pine trees close to village (control)
7.	Garampani	Close to village
8.	Garuna Temple	Busy road
9.	Jeoli kot	Road close to orchards
10.	Bhimtal	Road close to agricultural village
11.	Almora	Close to bus station, close to agricultural village
12.	Ranikhet	Close to bus station, Pine trees
13.	Pithoragrah	Close to city, road intersection
14.	Chamoli	Close to village
15.	Haldwani	Road intersection in thickly populated city

off and further digested using 3:1 concentrated HF: HNO_3. The combined solid and liquid digests were diluted to 100ml. The method of Shimwell and Laruie (1972) was used for metal determination. The amount of Mn, Pb, Cu, Ni, & Zn were measured by Atomic Absorption Spectroscopy (Perkin-Elmer Model). Statistical analysis were performed to study local differences between the amount of metal accumulated in *Plagiochasma* at different transplants sites.

RESULTS

The present results are based on exposure periods of 1992-1993 & 1993-1994 in same catchment areas. A different performance of metal accumulation was observed which was related to different pollution sources and levels in the study areas. Mean concentration (ppm dry wt. basis) of metal ions detected in *Plagiochasma* exposed during 1992-1993 &1993-1994 transplants periods at 15 biomonitoring stations of Kumaon hills are given in Tables 2 & 3. At the end of 1993-1994 period of exposure few *Plagiochasma* plants exposed to Haldwani & Nainital showed chlorosis. It may be likely that accumulated pollutants or particultates get entry resulting some morphological changes and degeneration leading to dead. This possibly suggests the alarming increase in ambient metal concentration in this area and transplants.

A remarkable increase in Pb along with Cr at all stations except control were observed. Zn and Cu was high in rural transplants in both the periods while Mn was observed high at Pithoragarh and Almora and its value further increased in 1993-1994.

After one year exposure during 1992-1993 lead concentrations was highest in transplants area at Nainital. High concentration of lead were detected in some other catchment areas located at Haldwani, Garuna Temple and Garampani. An increase in lead was detectable further during 1993-1994 transplants in same catchment areas. Indeed in the present investigation high level of lead were found at all sites. The level of lead in the transplants collected from Kapkot,

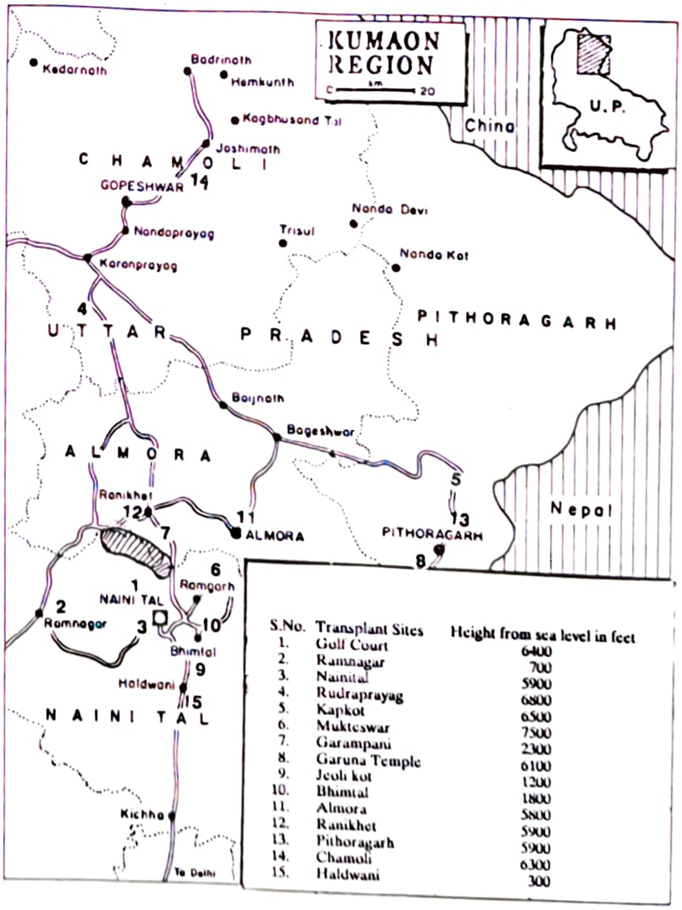

Fig. 1. Locations of sample transplant sites in Kumaon hills 1. Golf Court, 2. Ramnagar,3. Nainital, 4. Rudraprayag, 5. Kapkot, 6. Mukteswar, 7. Garampani, 8. Garuna Temple, 9. Jeoil kot, 10. Bhimtal, 11. Almora, 12. Ranikhet, 13. Pithoragarh, 14. Chamoli, 15. Haldwani.

Table 2 : Details of metals detected (in ppm on dry weight basis) in *Plagiochasma* exposed for 12 months (during 1992-93)

S.No.	STATIONS	Cu	Zn	Cr	Pb	Mn
1.	Mukteswar	5.1±3.2	0.52±10.1	0.01±0.01	16.7 ±	1.3 1.7±1.9

Mukteswar and Chamoli were found minimum, while the mean value of amount of lead detected in the transplants at Nainital bus station, Garuna Temple and Haldwani were higher. Marked increase in concentration of lead in *Plagiochasma* in these areas suggest that deposition pattern of metals were quite similar during two surveys, indeed metal concentration increased markedly during 1993-1994 transplants.

The results presented herein show chromium was relatively low in concentration in *Plagiochasma* analyzed from 1992-1993 transplants, but it was above the detection limit on those stations in which Pb was detected quite high. Among the analysis carried at the most significant correlation was found is deposition of lead and chromium together. It seems probable that fuel may be partly responsible for the increased chromium content of *Plagiochasma* at sites. In Tables 3 & 4 comparison between concentrations of Cr & Pb is given during two surveys. However, higher Pb concentration had more pronounced effect and therefore, Pb accumulated more in *Plagiochasma* while Cr is present at very low concentration could be because it was quite low in automobile exhaust. Same is amply documented by the fact that slight increase in Cr was observed again on those stations where Pb was detected high in 1993-1994 transplants. Chromium values was recorded maximum in *Plagiochasma* harvested from Almora and Pithoragarh bus-stations and same could not be detected from transplants of control site (Table 3).

Mn showed no obvious pattern except for marked increase in concentrations at two sites only and was not too much evident in other transplants of 1992-1993. The mean amount of Mn was quite lower in transplants analyzed from Haldwani and Ramnagar and was not detectable in comparison to control. High Mn was detectable in transplants of Almora and Pithoragarh only. Same trend was observed in 1993-1994 transplants except an increase was more pronounced at Pithoragarh in comparison to Almora.

The pattern in accumulation of copper and zinc were more complex and differed than Pb and Cr. In

Table 3 : Details of metals detected (in ppm on dry weight basis) in *Plagiochasma* exposed for 12 months (during 1992-93)

S.No.	STATIONS	Cu	Zn	Cr	Pb	Mn
1.	Golf court	5.9±0.3	7.6±3.9	0.4±0.3	38 ± 3.9	5.3±1.9
2.	Ramnagar	6.1±1.1	26.9±3.3	2.1±0.7	51±0.9	2.4±6.3
3.	Nainital	6.01±1.9	5.1±0.81	4.3±3.8	167±11.2	5.7±3.9
4.	Rudraprayag	11.3±0.9	4.1±7.3	0.5±1.0	23±2.6	2.8±6.6
5.	Kapkot	13.6±1.5	5.9±3.9	0.1±0.3	11.9±0.9	3.2±6.3
6.	Mukteswar	17.9±1.7	21.9±13.1	1.6±0.3	10.3±1.9	3.5±0.3
7.	Garampani	6.2±1.9	22.3±2.8	2.5±1.0	59±3.8	5.3±1.3
8.	Garuna Temple	17.2±4.3	26.1±9.0	4.3±0.1	166±0.1	12.1±0.5
9.	Jeolikot	7.1±6.9	41.9±7.4	1.9±1.1	103.±3.9	5.8±1.9
10.	Bhimtal	14.3±1.9	37.3±9.1	0.3±0.9	39.0±1.7	6.9±0.3
11.	Almora	8.1±5.3	39.1±9.3	3.9±2.1	188±3.9	17.1±1.9
12.	Ranikhet	6.9±0.1	22.3±3.1	0.1±0.3	68±4.1	5.1±2.0
13.	Pithoragarh	16.3±0.3	39.1±13.9	7.9±3.9	136.±0.3	26.7±1.1
14.	Chamoli	9.1±0.2	15.9±2.0	0.9±1.4	13.3±2.1	19.1±0.9
15.	Haldwani	3.1±0.5	2.1±4.1	2.26±0.1	198±1.3	1.1±0.1

Tables 3 & 4, comparison between concentrations of Cu, Zn with Pb, Cr and Mn are given for *Plagiochasma* during two surveys. Concentrations and distribution pattern of both metals (zinc and copper) were quite similar and both metals were high in rural transplants located in or nearby villages. Indeed, both metals increased markedly during 1993-1994 in transplants of the same catchment areas of all rural sites.

A marked high concentration of copper was detected at rural site of Mukteswar, Jeolikot, Kapkot, Rudraprayag and Chamoli during 1992-1993, while same was high again in *Plagiochama* analyzed from Rudraprayag, Mukteswar, Kapkot, in the samples of 1993-1994. Copper was undectectable in transplants of urban sites (Nainital, Ramnagar and Haldwani). Zinc concentration was highest in collected transplants of Bhimtal, Jeoli kot, Almora and Ranikhet. All these locations are close to orchards sites which are used to grow fruits (apple, peach, plums, pear, oranges) in which Zn was applied as nutrient. Increase in zinc in this area is amplidocumented by fact that zinc is used as element to promote the growth of orchards.

DISCUSSION

Heavy metal concentration through transplant were monitored at fifteen sites and are summarized in Tables 3 and 4. This study shows that there are different levels of contaminant accumulation in transplants which was due to different pollution source and pollution level in the area. Study has revealed that *Marchantia* thallus has also bioaccumulation potential for metals as has been observed in mosses (Bell 1959; Brown 1982; Sergio *et al.* 1992).

The most obvious drop in Cu level occurs in transplants analyzed from Nainital. Cu levels decreased in other urban sites too and these areas also have very low levels of Zn in transplants. Present study shows level of Zn and Cu accumulation is lower in transplant of urban areas and further significant decrease was observed in 1993-1994 transplants. Rural transplants had the highest levels of Cu and Zn deposition. Same increased in second year transplants.

Discussing the changes in the Cu, it can be concluded that the lower concentration of Cu in transplanted *Plagiochasma* of city area reflects lower Cu concentration in the air and these lower value in the 1992-1993 period is due to restricted use of $CuSO_4$

Table 4 : Details of metals detected (in ppm on dry weight basis) in *Plagiochasma* exposed for 12 months (during 1993-1994).

S.No.	STATIONS	Cu	Zn	Cr	Pb	Mn
1.	Golf Court	6.8 ± 2.8	8.8±5.7	0.5±0.1	42±6.1	6.1±5.2
2.	Ramnagar	6.3±1.3	39.3±1.1	2.6±1.9	56±2.8	2.3±0.1
3.	Nainital	7.4±1.65	6.3±0.3	5.9±3.8	210±0.9	6.1±0.9
4.	Rudraprayag	24.8±9.1	7.1±0.1	1.1±1.3	36±9.1	3.2±9.1
5.	Kapkot	18.3±0.9	9.3±4.0	0.3±1.7	22±0.3	7.3±8.1
6.	Mukteswar	22.3±11.8	32.1±11.0	2.1±0.9	26±0.1	4.1±0.9
7.	Garampani	8.1±3.2	37.8±11.9	3.6±1.1	66±2.9	6.6±0.9
8.	Garuna Temple	21.3±6.1	30.3±2.9	6.9±0.4	180±1.8	18.3±2.9
9.	Jeolikot	8.9±3.8	50.8±0.1	2.8±1.7	140±11.2	`5.9±1.9
10.	Bhimtal	16.8±0.9	59.1±13.5	1.3±0.1	44±3.9	6.1±1.1
11.	Almora	11.1±1.8	40.1±8.8	8.8±1.9	210±11.6	18.9±2.4
12.	Ranikhet	8.2±5.7	42.3±1.8	0.5±0.9	80±3.6	11.3±3.9
13.	Pithoragarh	18.3±1.1	50.1±16.2	11.3±8.1	149±4.4	28.3±1.1
14.	Chamoli	10.5±10.1	18.3±7.9	1.4±1.4	28±3.6	10.2±1.1
15.	Haldwani	3.9±6.3	4m.2±1.1	3.3±0.1	210±11.9	1.0±0.1

mixed kerosene oil. Remarkable decrease in Cu in the Nainital, Almora, Pithoragarah, Ranikhet during 1993-1994 transplants of urban stations may be explained that inhabitants earlier used $CuSO_4$ mixed kerosene oil, now in place, gas is used for cooking and electricity for light. Concomitant, reduction in Cu at above stations, there was an increase in Cu in rural transplants. This increase is because rural inhabitants still used $CuSO_4$ mixed kerosene oil as source of light and enegry to cook food. This is in agreement with the finding of Lopez (1990) that such rise in levels of Cu could be considered as background values of the site.

An increase in Zn is observed in transplants of the year 1993-1994 in comparison to the 1992-1993 in rural sites only. It is concluded that the higher amounts of Zn in transplanted *Plagiochasma* reveals higher Zn value in area. These sites are being used as orchard to grow fruits. It is interesting to note that constant higher concentration of Zn was detected only in and nearby such stations, while same was absent or low in transplants close to the urban sites. The relatively high level of zinc at orchards site could be related to application of foliar or aerial spray of zinc. This change may be explained as high Zn amount in the air and Zn spraying in orchards and on other fruit plants is probably the main reasons of an increase. Radical change in some agricultural methods in country in past few years is among them to boost up the production by application of fertilizers and elements. Apricot, apples, oranges, plums, peaches, pear are the main fruits grown in Kumaon hills and these fruit-trees are used, to be sprayed with Zn because this element is used as a nutrient by soil application or spraying. The enrichment ratios for zinc do not remain constant throughout the year, therefore, interpretation of enrichment of zinc is complicated (Pickering & Pauia 1969).

A mild correlation between Pb and Cr was found in *Plagiochasma* exposed near by traffic, located to areas of Nainital, Haldwani, Rainkhet, Jeoli kot and Ramnagar. The value of Pb was much more high in transplants located to urban sites and chromium in comparison to lead was detected to be non significant. High values of lead could be related to the proximity of roads, but presence of detectable amount of Cr in above sites shows the strong relationship of deposition. This is because Cr considered as a constituents part of vehicle engines (as Ni) hence it is always present in detectable amount in all those transplants which had high Pb values (Nieboer *et al.*, 1976). Nevertheless, both lead and chromium showed a general increase in all the polluted sites sampled during 1993-1994 transplantes, which is not apparent for other metals (Ward *et.al.*, 1977). It seems that both has significant correlation. Contrary to the above, transplants analyzed from Almora and Pithoragarh had the highest levels of deposition of Cr and this can not be from vehicle engines. Therefore, other source, of an increase can not be ruled out on these two spots. The relatively very high level of Cr could be related to dust from Magnesite factory located nearby. Chromium may be emitted by combustion of coal in furnace along with ore in magnesite factory. The coal used by magnesite factory has content of Cr in the coal samples. There exists as possibility that fly ash containing Cr may reach many transplantation sites. The significant increase in Cr concentration at Almora and Pithoragarh transplants was related to regional magnesite industry located in this area, uses coal for burning of ore and therefore, its high concentration was due to regional pollution intensity (Empain, 1976b). Thus, in this area, Cr may be hazardous air borne element released by Magnesite factory.

Discussing the change in lead amount, it is of interest to point out high Pb levels in all transplants of 1992-1993 and same was quite high during 1993-1994. This is an alarming increase in Pb in *Plagiochasma* in 1993-1994 transplants, except control site at Mukteswar. In general the results from analysis of *Plagiochasma* showed that a constant higher concentration of Pb was present in transplants. Apparent elevated amount of Pb present at Nainital, Pithoragarh, Almora, Haldwani, Garuna Temple could be related to high vehicular traffic. Another reason behind the increase in metal is that most of the tourist spots and hill resorts in India, at present are badly disturbed due to insurgency, as a result, tourists have diverted to most safe resorts on Kumaon hills and ultimately there is many fold increase in number of tourists (30 to 40 times) and so the vehicular traffic too. On the Kumaon hills the mode of transportation are roads only. Therefore, the contribution of vehicular traffic cannot be ignored. The emission rate of the pollutants get further magnified because vehicles carrying passengers or goods plying on the steep road of hilly region gain momentum with the application of second or first gear results into more smoke thus crossing the standards of tolerance limit (Calvert 1993; Xuolong *et.al.* 1994).

A different performance of Mn accumulation was observed during 1992-1993 transplants which was very high and restricted to Almora and Pithoragarh that could be due to Magnesite factory. After another period of exposure in 1993-1994 high concentration of Mn was again detected at these two sites only which was related to regional pollution intensity. Other transplants of both periods show levels of accumulation of Mn quite low except in Ramnagar site in which Mn could not be detected. High source of Mn at Almora and Pithoragarh is probably associated with magnesite dust which is emitted by Magnesite factory after burning coal and heating ore at quite high temperature.

CONCLUSION

An increase in Cu and Zn in transplants near village is attributed to the factor that the use of copper sulfate mixed with kerosene oil in absence of gas and electrcity to cook food and as source of energy. The apparent increase in prevalence of Zn was detected near agricultural lands bacause its use as elements to increase cultivation of apple, apricot, oranges, plums, pear, and peach which are grown on hills. Aerial and soil application of zinc is given for better cultivation. Cr at the proximity of road is low and is associated with exhaust spewed by automobiles while its higher value near Almora and Pithoragarh is because coal is used in furnace along with magnesite ore. Prevalence of Mn in high quantity at Almora and Pithoragarh is also attributed to Magnesite factory.

The result are in board agreement that liverwort species are also of potential value as general monitors of levels of metals in air. Secondly, at several sites Cr, Cu and Mn were detectable in transplants even when, on the same occasion, they were not detectable by other plants or sources. With the data available it is possible beyond doubt to establish the ability of the liverwort too to accumulate these metals. These results conform earlier reports on *Marchantia* and their role in biomonitoring (Briggs 1972; Martin & Coughtrey 1982; Brown 1984; Ferguson *et.al.* 1984 & Steinnes 1997). Data also illustrate the use of *Plagiochasma* as one of the means available of detecting pulse of metals after they have been spewed out in the environment from source.

The author recommends the continuation of air monitoring on Kumaon hills by use of the methods described in the present study since *Plagiochasma* has been found to be useful bioindicator of metals.

It is also pointed out that there may be some effects on respiratory health problems on the local inhabitants due to increased levels of metals as it is rather higher than expected. The prevalence of cough, phlegm, wheezing and several loss in pulmonary function may be associated with tenure. Study on this aspects is nill and needs attention.

Over all, an increasing trend of metallic pollution has been observed during course of study which is attributed to the urbanization, many fold increase in automobile, faulty planning i.e. haphazard construction of houses, hotels etc. Present study on *Plagiochasma* and its bioaccumulation potential suggests that this liverwort is well suited for biomonitoring of metallic precipitation over the hills.

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