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Biomonitoring of metal deposition by using moss transplant method through *Hypnum cupressiforme* (Hedw.) in Mussoorie

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Abstract*: Metals Cu, Zn, Cd and Pb were surveyed at 14 sampling sites by using moss Hypnum cupressiforme through active monitoring technique. Samples were transplanted in all four directions of Mussoorie city and were harvested after exposure of four months (representing each season) to analyze metal precipitation and its trend at different sites during 2005. Bioaccumulation ability for metals was evaluated seasonally, exhibiting maximum in summer followed by winter and minimum in rainy season. However, at some places Cu shows highly significant values in rainy season in comparison to winter. In case of Zn and Pb significantly different ($p \leq 0.05$) values were observed between summer and rainy season. Baseline concentration of Cu, Pb and Zn was significantly different at 5% in comparison to other transplant sites. Result indicates Dhanaulti as most polluted location might be due to higher tourist activity and vehicular load, whereas, same was found low at Chamba might be due to place was free from pollution sources or away from in proximity to road and have low human interference. The present study allows us to determine the extent of the area affected by metal precipitation load in different rural and urban areas and abundance of metals in order of Zn>Pb>Cu>Cd.*

Key words: *Hypnum cupressiforme, Biomapping, Metals, Precipitation, Garhwal region, Himalayan hills*
PDF of full length paper is available with author ***(*kajals101@rediffmail.com)***

Introduction

Human population, man made activities and industrial activity are principally responsible for change in environment globally. Problem of air pollution especially has become worldwide. Mitigating the air pollution is a very complex problem and for that sources of emissions have to be identified (Borut *et al.,* 2002; Giordano *et al.*, 2005; Tripathi and Gautam, 2007). The necessary information on air pollutants can be obtained by field measurements but it requires equipments, electricity and manpower, which is generally associated with high cost. However, biomonitoring includes a broad array of environmental study and examines the relationship between biological variables and pollutants. The tools of biomonitoring are bioaccumulators, species or organisms that accumulate metals and bioindicator, organism that react to specific pollutants in a consistent, measurable fashion.

Biomonitoring methods are used to measure metal precipitation levels in the environment or to examine the trend of pollution (Thoni *et al.*, 1996). Amongst plant kingdom bryophytes are excellent biomonitors of pollution because of their differential ability to accumulate a wide range of metals (Fernandez *et al*., 2000; Saxena, 2006). They have also been reported as very precise, cheaper and sensitive biomonitors of heavy metals. (Markert, 1993). As a result they are preferred over other plants and have been used for short and long term ecological monitoring in many countries (Ruhling, 1994; Sawidis *et al.*, 1993). They have great significance for biomonitoring due to following reasons.

1. Bryophytes react quickly to changes in the environment due to thin one layered epidermis.
2. They have a wide range of geographical distribution, therefore, large areas even continents can be examined.
3. Need no or minimum care during transplantation.
4. Small size makes them suitable for all sorts of experiments.
5. They can be stored for long period and can be sent for inter and intra species examination as they are not attacked by microorganisms.

The data from several moss survey provide, at best, good relative values for the deposition of the elements of concern. The moss bag technique is very useful, especially in polluted areas for measuring the intensity and trend of metal precipitations.

Present study is a first report on comparison of element concentration in common biomonitor moss species *Hypnum cupressiforme* from Garhwal. It is common and widely distributed moss on Garhwal hills and used in present work to make a clear picture of metal deposition over the study sites of Garhwal hills. Other workers like Faus-Kessler *et al.* (1999) and Fernandez *et al.* (2004) used moss bags of *H. cupressiforme* to measure the temporal and spatial trends of metal content, level and source of atmospheric deposition of heavy metals. Objective of present work is to summarize the result of heavy metal trend of different seasons (*i.e.* winter, summer and rains) during year 2005. Since no data is available on Garhwal hills on metal precipitation load therefore, present study is first report on metal precipitation data by moss.

Materials and Methods

The moss *Hypnum cupressiforme* was used as test plant in present study. The specimens were collected from wet calciferous soil at Chamba forest cover, situated at an altitude of 1921 meter. The climate is cool from April to October, whereas, heavy rainfall

was observed during the month of July and August. Conditions were moderate from September to November, however, snowfall observed in the months of December or in January. Maximum and minimum temperature measured during summer were 25.6°C to 31.7°C and 7.2°C to 12.8°C respectively, where as maximum temperature in winter season was 2.2°C to 7.2°C and minimum 1.0°C to 4.4°C. Great variation was observed in temperature during rainy season. Highest average relative humidity was measured in month of July and August *i.e.* 85-90% where as same observed lowest in month of January (60%).

The study area comprises of Mussoorie, Dhanolti and Chamba sites of Garhwal hills. The topography of the study area varies because the area is surrounded by rocky hills. Mussoorie consist of great density of floating population and have very high traffic density. Study was conducted on 14 study sites (Table 1) of Garhwal hills in year 2005. Fresh moss samples were collected from the deep forest cover of Chamba (avoided from the tree canopy), treated as control site, and same moss (6 g) were placed in a nylon moss bag (20 cm^2) and were transplanted at different sites in triplicate, at nearly equal height (away from the buildings). After the end of exposure period one season (nearly after 122 days or 4 months) these moss transplants were harvested and single sample was taken from each transplant bag for the metal analysis and in its place fresh moss transplants (in triplicate) were made for the same duration for the next season (four months). Same was repeated for another season.

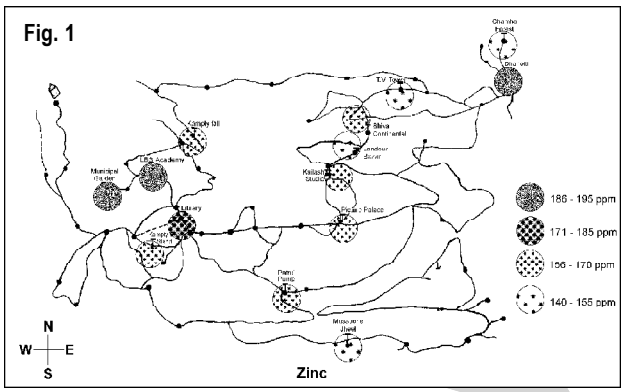
Harvested moss bags were brought to the laboratory, cleaned to removal of all foreign particles and dataries by digestion method. In each case 0.5 g of the homogenized dried samples of moss *H. cupressiforme* were digested with concentrated HNO_3 and $HClO_4$ in ratio of 4:1 respectively at 150°C for 2-3 hr (Sergio *et al.*, 1993). The residue was made up to a final volume of 25 ml and filtered. The digested homogenized samples were filtered and measurements were carried out by atomic absorption spectrophotometer.

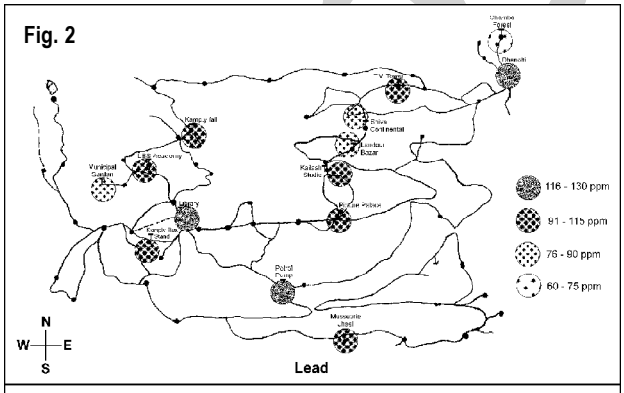
Concentrations of heavy metals were determined in transplants of *H. cupresssiforme* after exposure in study sites of Garhwal hills during the year 2005. Fresh *H. cupressiforme* was also collected from Chamba forest cover to determine base line level of the elements.

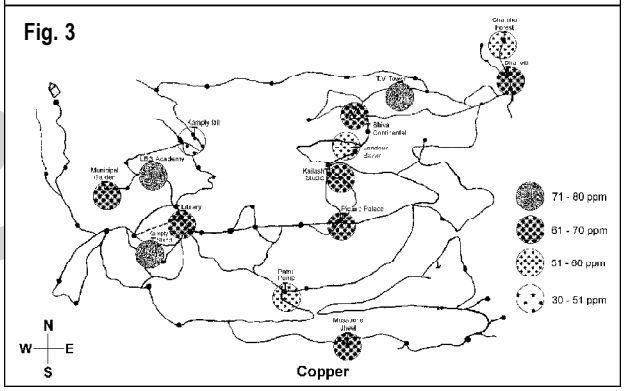
Samples were collected in triplicates to conduct the statistical analysis. Values are represented as mean ± standard error (Snedecor and Cochran, 1967). ANOVA was carried out to compare the metal concentration of different seasons. Significant differences were determined using the method of critical difference (CD) calculated at the 5% level.

Results and Discussion

The metal values of all metals (*i.e.* Cd, Pb, Cu and Zn) analyzed from moss transplants exposed four month were significantly higher than those of baseline concentration. Significant differences were found amongst the urban and rural transplants for each site of Mussoorie, Dhanolti, Chamba, for each metal besides







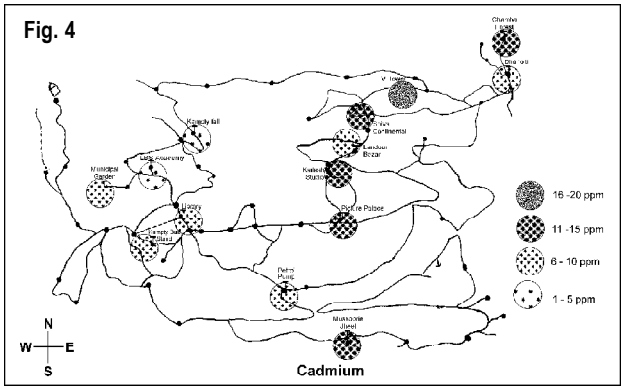


Fig. 1-4: Annual metal concentration (ppm) in transplants of *Hypnum cupressiforme* (Hedw.) sp Musc. at different sites of Garhwal hills during the year 2005-2006

Table- 1: Zn metal concentration (ppm) in transplants of *Hypnum cupressiforme* at different sites of Garhwal hills during winter, summer and rainy season (2005)

Transplant sites	Winter-2005	Summer-2005	Rains-2005
Baseline concentration	15.927 ± 1.526	18.198± 2.421	11.265 ± 2.243
Picture palace	40.251 ± 1.236	85.326 ± 1.851	32.145 ± 1.254
Kailash studio	45.265 ± 2.321	92.364 ±1.205	29.241 ± 2.014
Shiva continental	43.235 ± 1.353	79.357 ± 1.521	36.254 ± 1.245
Landour bazar	53.254 ± 1.325	68.245 ± 1.024	29.245 ± 2.036
Dhanolti	56.365 ± 2.325	94.254 ± 0.325	40.325 ± 1.024
T.V. Tower	48.325 ± 1.556	82.254 ± 1.451	25.245 ± 2.145
Chamba (rural area)	41.345 ± 1.346	72.324 ± 1.325	32.254 ± 1.245
Kampty bus stand	53.215 ± 2.354	81.325 ± 1.451	23.125 ± 1.364
LBS academy	49.325 ± 1.256	93.284 ± 1.364	46.124 ± 1.645
Library	53.265 ± 2.352	100.325 ± 0.145	25.314 ± 1.364
Municipal garden	62.325 ± 2.636	89.254 ± 2.012	36.214 ± 3.210
Kampty fall	38.321 ±1.362	92.325 ± 1.024	29.154 ± 1.643
Jheel	52.325 ± 1.235	76.214 ± 1.421	26.145 ± 1.346
Petrol pump	52.365 ± 1.325	69.215 ± 1.612	44.315 ± 2.145

- Values are represented as mean ± standard error
- Values superscripted with same alphabets in horizontal row are seasonally not significant different at $p < 0.05$ significant level
- With in vertical column, values with same capital alphabets are not significantly different at $p<0.05$ in comparison to baseline concentration

Table - 2: Pb metal concentration (ppm) in transplants of *Hypnum cupressiforme* at different sites of Garhwal hills during winter, summer and rainy season (2005)

Transplant sites	Winter-2005	Summer-2005	Rains-2005
Baseline concentration	13.168 ± 2.257	15.106 ± 1.024	09.326 ± 2.612
Picture palace	25.326 ± 1.255	36.132 ± 0.234	32.625 ± 1.364
Kailash studio	36.215 ± 1.265	42.326 ± 0.212	21.316 ± 2.034
Shiva continental	35.235 ± 1.265	22.314 ± 0.542	19.325 ± 2.451
Landour bazar	26.325 ± 1.235[a]	36.214 ± 0.024	24.124 ± 1.241[a]
Dhanolti	36.325 ± 1.256	61.245 ± 0.024	30.145 ± 1.614
T.V. Tower	34.256 ± 1.263	42.124 ± 0.214	27.324 ± 1.345
Chamba (rural area)	16.369 ±1.325[b]	19.145 ±1.245[b]	30.124 ± 1.024
Kampty bus stand	26.365 ± 2.366	52.214 ± 0.541	19.125 ± 1.254
LBS academy	36.326 ± 1.365[c]	35.214 ± 0.614[c]	26.145 ± 1.314
Library	46.362 ± 1.025	52.124 ± 0.654	19.345 ± 2.014
Municipal garden	23.125 ± 1.025[d]	44.214 ±1.231	23.145 ± 1.024[d]
Kampty fall	37.145 ± 0.365	48.214 ± 0.014	19.214 ± 2.014
Jheel	36.326 ± 3.265	43.124 ± 0.514	23.145 ± 1.514
Petrol pump	35.145 ± 2.136	56.325 ± 0.541	25.145 ± 1.325

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- Values superscripted with same alphabets in horizontal row are seasonally not significant different at $p < 0.05$ significant level
- With in vertical column, values with same capital alphabets are not significantly different at $p<0.05$ in comparison to baseline concentration

difference in each season. Concentration and trend for each metal has been described.

Zinc: The baseline level of Zn in different seasons was measured 15.927 ppm in winter, 18.198 ppm in summer and 11.265 ppm in rains in moss samples collected from interior (non polluted) areas of Chamba forest cover. Zinc in moss was measured maximum at Municipal Garden (62.325 ppm), Library (100.325 ppm) and L. B. S. Academy (46.124 ppm) during exposure of winter, summer and rainy seasons respectively in year 2005. Where as minimum concentration of Zn was measured as 38.321 ppm at Kampty fall in winter season, 68.245 ppm at Landour bazar in summer and 23.125 ppm in rains at Kampty bus stand. The annual accumulation of Zn in moss ranges from 190.944 ppm at Dhanolti as highest value and 145.923 ppm as zinc in moss was harvested from Chamba as lowest value (Fig. 1). ANOVA revealed significant difference in the metals concentration during different seasons (Table 1).

Higher concentration of zinc in urban could be associated with dry deposition of metal spewed out from automobiles, motor oil, wear and tear of vehicular parts and abrasion of tires (Imperato *et al.,* 2003). However, its high concentration in rural area could be attributed to its use as chemical to increase crop productivity. The present finding is an agreement with Makhol and Mladenoff (2005),

Table - 3: Cu metal concentration (ppm) in transplants of *Hypnum cupressiforme* at different sites of Garhwal hills during winter, summer and rainy season (2005)

Transplant sites	Winter-2005	Summer-2005	Rains-2005
Baseline concentration	06.672 ± 1.038	08.147 ± 1.678	05.747 ± 1.524
Picture palace	18.326 ± 1.325	24.124 ± 1.241^{a}	23.125 ± 1.235^{a}
Kailash studio	16.230 ± 2.036	28.314 ± 1.641	21.258 ± 1.326
Shiva continental	19.326 ± 5.032^{b}	19.214 ± 1.364^{b}	25.326 ± 1.235
Landour bazar	20.365 ± 2.036^{cd}	22.145 ± 1.345^{c}	17.325 ± 1.255^{d}
Dhanolti	22.036 ± 1.625	29.014 ±0.124	19.256 ± 1.365
T.V. Tower	19.326 ± 2.305	26.325 ± 1.236^{e}	26.257 ± 0.235^{e}
Chamba (rural area)	19.124 ± 2.134^{f}	22.145 ± 1.854	18.256 ± 0.891^{f}
Kampty bus stand	13.256 ± 3.036^{g}	45.215 ± 1.345	16.245 ± 2.536^{g}
LBS academy	26.325 ± 1.853	30.214 ± 1.451	23.556 ± 1.236
Library	24.021 ± 2.031	32.145 ± 1.642	20.354 ± 2.354
Municipal garden	32.315 ± 4.320	19.214 ± 1.645^{h}	18.254 ± 2.356^{h}
Kampty fall	12.326 ± 1.025^{i}	16.145 ± 1.647	13.215 ± 1.266^{i}
Jheel	30.325 ± 1.750	21.245 ± 1.364	15.236 ± 1.231
Petrol pump	19.125 ± 2.034 ^{jk}	20.754 ± 2.042^{j}	17.254 ± 2.356^{k}

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- Values superscripted with same alphabets in horizontal row are seasonally not significant different at $p < 0.05$ significant level
- With in vertical column, values with same capital alphabets are not significantly different at $p<0.05$ in comparison to baseline concentration

Table - 4: Cd metal concentration (ppm) in transplants of *Hypnum cupressiforme* at different sites of Garhwal hills during winter, summer and rainy season (2005)

Transplant sites	Winter-2005	Summer-2005	Rains-2005
Baseline concentration	0.434±1.981^{NS}	1.918± 0.021^{NS}	ND
Picture palace	4.325 ±1.021^{a}	6.124 ± 1.304^{a}	1.254 ± 1.325
Kailash studio	3.236 ± 1.025	9.214 ± 1.614	0.254 ± 1.254
Shiva continental	1.364 ± 3.102^{NS}	8.214 ± 1.821	3.564 ± 1.254
Landour bazar	2.346 ± 2.100^{NS}	6.214 ± 1.362	0.298 ± 1.021
Dhanolti	1.124 ± 1.545^{NS}	6.512 ± 2.014	ND
T.V. Tower	2.364 ± 2.145	8.325 ± 1.424	6.894 ± 1.584
Chamba (rural area)	5.214 ± 0.254^{b}	7.451 ± 1.024^{b}	2.985 ± 1.365
Kampty bus stand	2.364 ± 1.254^{cd}	3.214 ± 1.245^{ceNS}	3.165 ± 2.356^{de}
LBS academy	1.254 ± 0.651^{fgNS}	2.314 ± 2.014^{fNS}	0.254 ± 1.621^{g}
Library	4.134 ± 1.254^{hi}	3.214 ± 1.124^{hjNS}	1.236 ± 1.00^{ij}
Municipal garden	1.845 ± 2.014^{kNS}	7.214 ± 1.254	1.205 ± 0.321^{k}
Kampty fall	3.021 ± 1.041	1.325 ± 1.024^{lNS}	0.120 ± 1.024^{l}
Jheel	5.047 ± 2.031^{m}	5.214 ± 1.325^{m}	2.013 ± 1.032
Petrol pump	2.451 ± 1.854	7.214 ± 1.351	ND

- Values are represented as mean ± standard error
- Values superscripted with same alphabets in horizontal row are seasonally not significant different at $p < 0.05$ significant level
- With in vertical column, values with same capital alphabets are not significantly different at $p<0.05$ in comparison to baseline concentration
- ND = Not detectable, NS = Non significant

who also described high concentration of Zn along the road as well as forest area and he attributed that fuel is the reason for this. The level of Zn in catchments sites ranges from 23 ppm to 95 ppm but in proximity to city center it was found often higher than 100 ppm due to heavy pollution load from tourist activity.

Lead: The baseline level on Pb in moss from control site *i.e.* Chamba forest cover, during, winter, summer and rainy seasons were measured 13.168 ppm, 15.106 ppm and 9.326 ppm respectively. Values for lead metal were measured highest at library during winter (46.362 ppm) and at Dhanaulti during summer (61.245 ppm) while at Picture palace (32.625 ppm) during rains. However low value for Pb concentration was measured at Chamba in winter (16.369 ppm) and in summer (19.145 ppm) and at Kampty bus stand (19.125 ppm) during rains. The annual metal precipitation (Fig. 2) of Pb in moss was observed maximum at Dhanolti (127.715 ppm) and minimum at same site of Chamba, rural area (65.638 ppm). Few sites do not have significant difference in metal concentration during rainy and winter seasons. However there is no significant difference between sites during summer and rainy periods (Table 2).

The main sources of lead include use of leaded petrol, mining, metal electro plating, waste incineration and industry. Background atmospheric deposition of lead in the study areas was relatively higher in summer but measured low in rainy season. On the basis of data it was found that nearly 25% of samples exceeding the concentration above 40 ppm. It could be due to intensive traffic (Wagela *et al.,* 2002) and proximity to the road and wear and tear of the automobile parts could be the reason (Pearson *et al.*, 2000).

Copper: The background concentration of Cu was 6.672 ppm during winter, 8.147 ppm in summer and 5.747 ppm for rains; in moss collected from deep forest cover. The high concentration of the Cu was measured in moss *Hypnum* transplants of Municipal garden (32.315 ppm) during winter, Library (32.145 ppm) during summer and near T.V. Tower (26.257 ppm) during rains. Its minimum concentration was measured in moss at Kampty fall in all the three seasons *i.e.* 12.326 ppm in winter, 16.145 ppm in summer and 13.215 ppm in rains (Fig. 3). ANOVA showed significance difference in the metal concentration during different seasons (Table 3). On doing annual grading of maximum and minimum concentrations 80.095 ppm was recorded in moss harvested from LBS Academy and 41.686 ppm at Kampty fall respectively.

In Garhwal hills probably sources having Bordeaux mixture and other copper containing fungicides, which were used in orchards. Elevated level of copper in city could be due to discharge metal scrap from factories, domestic waste, laundry and use of kerosene oil. Contamination by Cu was very identical to the pattern of Pb deposition (Otvos *et al*., 2003). The present finding is also in agreement with the finding of Loppi and Bonini (2000) that rise in levels of Cu could be due to automobile exhausts. It was found that its concentration did not exceed 30 ppm in about 90% of the samples.

Cadmium: The background level of Cd was beyond detection limit during monsoon, while the same was measured 1.918 ppm during summer and 0.434 ppm in winter season Increase cadmium concentration was found in moss transplanted at Chamba (5.214 ppm) in winter, at Kailash studio (9.214 ppm) in summer and at TV. Tower during rains (6.894 ppm) where as decrease in concentration was measured at Dhanolti (1.124 ppm) during winter, at Kampty fall during summer (1.325 ppm) and rains (0.120 ppm). The annual Cd content (Fig. 4) in moss *H. cupressiforme* used in study sites seems to be higher at TV Tower (17.583 ppm) and lower at LBS Academy (3.822 ppm). ANOVA showed significance difference in the metal concentration during different seasons (Table 4).

Elevated cadmium concentration was could be due to polythenes, domestic waste, sewage sludge, mining, plastic pipe, automobile tires and exhaust (Grodzinska and Szarek-Lukaszewska, 2001; Phetsombal *et al.,* 2006). Use of fertilizers and municipal refuses also contain cadmium, which results in Cd input at such sites. The lowest level of Cd concentration in moss was found close to the Dhanolti and Petrol pump during rainy season.

Present study is first report on biomapping of metal precipitations by moss *H. cupressiforme* from Garwhal hills of India. The accumulation values of Zn, Pb, Cu, Cd were calculated on transplanted moss. The emission sources were identified which are responsible for heavy load of metal precipitation. One of the most polluted locations with high concentration of all metals is Dhanolti, because it is a tourist spot and there are so many resorts where these tourist visit, where as high level of Zn, Pb and Cu was also found at LBS Academy. Concentration of all heavy metal was low near Chamba. However the concentration of Cd was found high at this site but the reason is not clear. It might be due to effect of long-range transport.

A source of high metal precipitation was found in some sites due to vehicular traffic (Saxena *et al.,* 2000). It is considered the main source of higher metal content in exposed biomonitors. Many fold increase in metal concentration could be due to high input of tourist activities and seasonal variations (Fernandez *et al.,* 2002).

Load of heavy metals present at the study sites of Garhwal hills could be readily detected on the basis of moss analysis. On doing over all study it was concluded that moss *H. cupressiforme* absorb metal from atmospheric deposition more effectively due to its very dense carpets. Present analysis of moss for heavy metal deposition in *H. cupressiforme* reflects the seasonal trend of metal. Present data serves as a reference database for the future to monitor the trend in heavy metal deposition in various study sites of Garhwal hills.

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