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Passive monitoring of atmospheric heavy metals in a historical city of central India by *Lepraria lobificans* Nyl.

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Abstract Using an organism living in situ for monitoring is referred as passive monitoring. *Lepraria lobificans* Nyl., a leprose lichen growing naturally on monuments and buildings in the city Mandav in central India is used for passive monitoring of atmospheric metals. Seven metals (Cd, Cr, Ni, Al, Fe, Cu, and Zn) were analyzed. Samples collected from road site exhibit the maximum concentration of Fe, Cd, Cr, Ni, and Zn. Iron exhibit maximum accumulation both in lichen thallus and the substratum with mean values of 2,195.63 $\mu g\ g^{-1}$ dry weight. As compared with other growth form of lichens, *L. lobificans* exhibits the higher accumulation of Fe than foliose and fruticose lichens. On the basis of these results, it can be hypothesized that *L. lobificans* is an excellent accumulator of different metals. The statistical analysis applied to the element concentration between the metals as well as between the sites by analysis of variance found the difference to be significant at 1% and 5%, respectively. Student–Newman–Keuls test also shows significant difference for iron between the different metals.

Keywords Heavy metals · *Lepraria lobificans* · Passive monitoring · SNK (Student–Newman–Keuls test)

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Introduction

Atmospheric particulates have attracted great environmental concern over the last few decades because of evidence that they are associated with respiratory and cardiovascular diseases in humans (Dockery and Pope 1994). During the last 30 years, many studies have underlined the relevance of using lichens as biomonitors of air quality. In the last two decades, many European cities clearly exhibit a decreeing trend in the SO_2 and particulate air pollution level, which resulted in the recolonization of many lichen taxa due to species returning (Crespo et al. 2004; Crespo and Bueno 1982; Hawksworth and McManus 1989). The change in lichen distribution could be a useful tool to assess bioclimatic features of a territory. As such, they have been widely used to assess trace element contaminants in the atmosphere. The advantages of using lichens over conventional air sampling techniques are that lichens are perennial and can be found in most terrestrial habitats (trees, soil, rock, as well as monuments). They also present easy sampling, low cost, and the possibility of monitoring wide areas. Besides, lichens do not have root system and thus are able to uptake elements and accumulate them in their tissues (Garty 2001). According to Witting (1993), the differentiation between airborne and soilborne substances is easier using physical instruments than passive biomonitors, and biomonitoring can never reach the same high quality as direct measurement. However, for economical and practical reasons, biomonitoring is absolutely necessary to establish and maintain regionwide monitoring systems and for retrospective studies. Jeran et al. (2002), passive (natural occurring) as well as active (transplant) biomonitoring using epiphytic lichens can be used on a national scale as well as around particular pollution sources to obtain information about the levels of particular elements in the atmosphere.

Consequently, various authors had studied on monitoring of heavy metals using lichens in different geographic areas (Godinho et al. 2008; Bergamaschi et al. 2004; Carreras and Pignata 2002; Conti and Cecchetti 2001; Upreti and Pandey 2000; Loppi and Bonini 2000) by different growth form of lichens in various climatic conditions. However, in India, also studies on the use of lichens as biomonitoring was adopted in different climatic zones by several workers (Dubey et al. 1999; Nayaka et al. 2003; Bajpai et al. 2004, 2009; Shukla and Upreti 2007).

Biomonitoring is of great interest in cities having fast pace of urbanization and industrialization as well as to encountered the serious problems of pollution, especially in places having heavy tourist activity. The central region in India has a rich heritage of monuments and historical buildings, and more than 25 to 30 thousand tourists visit this region each year to see the architect pattern as well as historical values of fourteenth- and fifteenth-century monuments. Vehicular emission, coal burning, and past mining activities are the major source of pollution in the area.

According to Gilbert (1968), the crustose and leprose lichens are more tolerant to air pollution followed by foliose and fruticose forms. *Lepraria lobificans* Nyl. is a common species of leprose lichen forming extensive patches both on vertical and horizontal surface of the monuments in both moist and shady places. The thallus forms a powdery mat-like structure on the substratum. The passage between granules helps both in absorption and adsorption of the metals in the thallus. The powdery (leprose) crust forming a fairly coherent but fragile membrane with poorly delimited granules at the center, irregular, usually continuous, sorediate, fluffy with long projecting hyphae may provide bigger space for entrapment of metals in the tissue. Most of the studies related with metal accumulation of lichen in India were conducted mostly with foliose and crustose lichens. Because of the luxuriant growth of *L. lobificans* in the study area, the present study aimed to explore the source of metals through this taxon and to compare the concentration of metals accumulated in lichen and its substratum. The samples were collected in three different sites so that source specific quantities of metals can be detected.

Materials and methods

Study area

Mandav, the historical city of Madhya Pradesh in central India, with a population of approximately 9,545, is situated in the Vindhyachal range at 2,000 ft above sea level. It is picturesquely situated among lakes, trees surrounded by barren hills, and above, more than 150 small and large (fourteenth and fifteenth centuries) monuments. In most of the monuments, sandstone is used as the major construction material. The area has a more or less dry and warm climate as the temperature varies from 6°C to 20°C in winter and 21°C to 48°C in summer; with a rainfall of 75–85 cm per annum. The three sites were selected on the basis of having low, moderate, and heavy vehicular activities (Table 1).

Sample collection

Specimens of *L. lobificans* were collected from 15 to 20 localities under three major sites in a good

Table 1 Site descriptions

Sites	Locations	Height from ground (Km.)	Distance from road (Km.)	Distance from mid city (Km.)	Remarks
Site I	Jehangir Mahal Jami Masjid Jahaz Mahal	0.75–1.0	0.1–0.5	0.0–0.5	Semi exposed areas with vehicular activity, coal combustion, paint and steel work (city center)
Site II	Delhi Gate I Alamgir Gate Delhi Gate I	1.0–2.0	0.01–0.04	1.5–2.0	These sites un exposed and having mainly vehicular activities (road side)
Site III	Sun set Point Lohani cave Main piller	0.5–1.0	3.0–4.0	10.0–11.5	These areas are fully exposed, no vehicular activity, but sites having past mining activity records and agricultural activities (away from city)

amount, growing on the horizontal plane of sandstone walls. The collection was performed from abandoned walls of monuments with the help of knife and forceps without any harm to the monuments, and samples were taken into polythene bags. Approximately 3–4 g of the thallus (of similar size up to 4 cm) was taken from each site, samples were collected in triplicate, and the thallus size varied from site to site. The lichen samples were identified, and voucher specimens are deposited in the herbarium of National Botanical Research Institute, Lucknow (LWG).

Analytical procedures

After collection, lichen samples were firstly cleaned from dust, leaf debris, fungus contamination, and degraded material. Lichen thallus as well as a piece of substrates were oven-dried for 24 h at 60°C temperature. The dried lichen samples and substrates (three replicates) were separately grinded to powder, and 0.5 g of this grinded sample was digested in a mixture of concentrated $HNO_3/HClO_4$ (v/v 9:1) for 2 h. Residues were filtered through Whatman filter paper no. 42 and diluted to 20.0 ml with double-distilled water. Concentration of heavy metals in the digested samples were analyzed by using flame atomic absorption spectrophotometer (Perkin Elmer, model A Analyst 300). Stock standards were from Merck India and traceable to National Institute of Standards Technology. Working standards were prepared from the stock using deionized water from dilution.

Results and discussion

Since the city of Mandav has no other source of pollution, except heavy vehicular and coal burning activities, the high level of metals in the lichen indicate higher traffic activity in this area. Table 2 presents the mean, standard deviation, standard error, and analysis of variance (ANOVA) of each of the seven metals analyzed in thallus as well as substratum and was found significant at 1% and 5% between sites as well as metals. The Student–Newman–Keuls (SNK) test also exhibit significant variation between the each metal.

The site having coal burning, automobile, and small industries (site I) exhibit maximum accumulation of Al, Fe, and Cu in lichen thallus, followed by area having only vehicular (site II) and have past mining and agricultural activities (site III). Cd, Cr, Ni, and Zn were maximum in the lichen thallus collected from the roadside (site II) followed by center of the city (site I) and away from the city (site III). Quantity of metals in substratum samples shows significance at 0.1% between the sites but not between each metal. Cd, Cr, and Ni were not detected in the substratum, which may indicate their origin from vehicular activity in the area.

Distribution of some elements allowed the detection of their emission sources and their range of dispersion within the study area (Garty 2001). Leading to these contexts, aluminum metal is widely spread through air dust but is found in small quantities in the substratum. Similarly, in the study area, the samples collected from the

Table 2 Metal accumulation in thallus and substratum of *Lepraria lobificans* (mean ± standard deviation, in μg g^{-1} dw)

Metals	Site1	Site2	Site 3	Total	Mean	SD	SE	*f* value
Thallus								
Al	124.82 ± 1.11	112.31 ± 2.48	2.15 ± 0.45	239.28	79.76	67.50	38.9726	4.666[a]
Cd	1.17 ± 0.12	3.05 ± 0.12	N.D.	4.22	1.41	1.54	0.88	
Cr	370.06 ± 2.50	978.99 ± 2.41	17.12 ± 1.63	1366.17	455.39	486.58	280.92	
Cu	41.4 ± 1.12	30.83 ± 0.68	12.46 ± 0.62	84.69	28.23	14.64	8.45	
Fe	3196 ± 2.40	3071.11 ± 2.43	319.8 ± 2.01	6586.91	2195.63	1625.72	938.61	
Ni	4.63 ± 0.41	17.05 ± 0.49	1.53 ± 0.35	23.21	7.74	8.21	4.74	
Zn	145.24 ± 1.24	165.67 ± 2.52	110.49 ± 1.08	421.4	140.47	27.89	16.11	
Total	3883.32	4379.01	463.55					
Mean	554.76	625.57	66.22					
SD	1171.57	1132.25	118.48					
SE	442.81	427.95	44.78					
One-way ANOVA								
f value	0.729							
Substratum								
Al	0.99 ± 0.07	0.25 ± 0.07	N. D.	1.24	0.41	0.514814	0.29	37.348[b]
Cd	N. D.	N. D.	N. D.	0	0	0	0	
Cr	N. D.	N. D.	N. D.	0	0	0	0	
Cu	1.07 ± 0.05	0.94 ± 0.04	0.8 ± 0.07	2.81	0.94	0.13	0.07	
Fe	11.37 ± 0.72	8.85 ± 0.87	6.65 ± 0.86	26.87	8.96	2.36	1.36	
Ni	N. D.	N. D.	N. D.	0	0	0	0	
Zn	0.94 ± 0.03	0.27 ± 0.04	N. D.	1.21	0.40	0.48	0.28	
Total	14.37	10.31	7.45					
Mean	2.05	1.47	1.06					
SD	4.14	3.27	2.48					
Se	1.56	1.24	0.93					
One-way ANOVA								
f value	0.152508							

Mean ± SD; $n = 3$

N.D. not detectable

[a]Significant at 5%

[b]Significant at 1%

center of the city having coal burning, electroplating, paint, and pesticide application exhibit higher concentration of Al within the range of 2.12 ± 0.45 to 124.82 ± 1.11 μg g^{-1} dry weight in thallus, while the substratum contain <0.99 ± 0.07 μg g^{-1} dry weight of Al.

Cadmium content was very less in thallus, i.e., <3.05 ± 0.12μg g^{-1} dry weight and was not detected in the substratum. According to Nimis et al. (1990), the burning of fossil fuel, incineration of solid waste, Ni and Cd batteries, and paint phosphate fertilizers are the main sources of Cd in air. In the present study, most of the Cd was detected at roadside samples, clearly indicating its origin from vehicular as well as agricultural applications.

The presence of Cr only in thallus (17.12 ± 1.63 to 978 ± 0.99 μg g^{-1} dry weight) exhibit its airborne origin. Maximum Cr was reported in samples collected from the gate side (site II) area having only vehicular activities. According to Nriagu and Pacyana (1988), Cr is emitted in the atmosphere due to coal and oil combustion, especially diesel-fed vehicles, refuse incineration, etc.

Diesel engines do not emit lead but are the main source of atmospheric Cu (Seaward and Richardson 1989), which also occurs in unleaded gasoline (Monaci and Bargagli 1997). According to Baptista et al. (2008), copper mainly originates from Cu-containing fungicides, metal working factories, and welding and electroplating materials.

The mean concentration of Cu in thallus of *L. lobificans* ranged between 12.46 ± 0.62 to 41.4 ± 1.12 μg $g^{-1}$and 0.8 ± 0.07 to 1.07 ± 0.05 μg g^{-1} in substratum. Higher accumulation of Cu were found in samples collected from the area having coal burning, automobile, and electroplating records (site I).

Apart from fuel combustion, vehicular activities pollute roadside environment by tire wear that release Zn (Christensen and Guinn 1979); Cd (Ward and Sampson 1989) and Cu, Fe, Mn, and Ni (Sadiqu et al. 1989). Certain metals, such as Cd, Cu, and Zn, are used in lubricating oils, whereas Cr, and Ni are used for car painting and Mn, Cu, and Zn for brake pad (Ward 1989; Sloof 1991).

Concentration of Fe in thallus significantly differs from all other metals $p < 1\%$, except for Cd where significance was observed only at $p < 5\%$ (Table 3). Fe concentration in substratum was significantly different from all metals at $p < 1\%$; Cu concentration only in the case of substratum significantly differed from Cd, Cr, and Ni. Iron

Table 3 SNK test on the different metal concentration and sites

Comparison	q_{cal}	$q_{tab}(0.01)$	$q_{tab}(0.05)$	p value
Thallus				
Fe∼Cd	5.920[a]	6.085	4.829	7
Fe∼Ni	5.903[b]	5.881	4.639	6
Fe∼Cu	5.847[b]	5.634	4.407	5
Fe∼Al	5.708[b]	5.322	4.11	4
Fe∼Zn	5.544[b]	4.895	3.702	3
Fe∼Cr	4.695[b]	4.21	3.033	2
Cr∼Cd	1.225	5.881	4.639	6
Cr∼Ni	1.208	5.634	4.407	5
Cr∼Cu	1.152	5.322	4.11	4
Cr∼Al	1.013	4.895	3.702	3
Cr∼Zn	0.85	4.21	3.033	2
Zn∼Cd	0.375	5.634	4.407	5
Zn∼Ni	0.358	5.322	4.11	4
Zn∼Cu	0.303	4.895	3.702	3
Zn∼Al	0.164	4.21	3.033	2
Al∼Cd	0.21	5.322	4.11	4
Al∼Ni	0.19	4.895	3.702	3
Al∼Cu	0.13	4.21	3.033	2
Cu∼Cd	0.07	4.895	3.702	3
Cu∼Ni	0.05	4.21	3.033	2
Ni∼Cd	0.02	4.21	3.033	2
Site 2∼Site 3	1.57	4.703	3.609	3
Site 2∼Site 1	0.19	4.071	2.971	2
Site 1∼ Site 3	1.37	4.21	3.033	2
Substratum				
Fe∼Cd/Cr/Ni	49.87[b]	5.634	4.407	5
Fe∼Zn	47.62[b]	5.322	4.11	4
Fe∼Al	47.57[b]	4.895	3.702	3
Fe∼Cu	44.65[b]	4.21	3.033	2
Cu∼Cd/Cr/Ni	5.21[a]	5.322	4.11	4
Cu∼Zn	2.96	4.895	3.702	3
Cu∼Al	2.91	4.21	3.033	2
Al∼Cd/Cr/Ni	2.3	4.895	3.702	3
Al∼Cu	0.05	4.21	3.033	2
Zn∼Cd/Cr/Ni	2.24	4.21	3.033	2
Site 1∼Site 3	0.51	4.895	3.702	3
Site 1∼Site 2	0.29	4.21	3.033	2
Site 2∼Site 3	0.21	4.21	3.033	2

q_{cal} value calculated, q_{tab} tabulate value according to degree of freedom and p, p step between two values, (∼) versus

[a]Significant at 5%

[b]Significant at 1%

Table 4 Iron accumulated in different growth forms of lichen species

Sample no.	Lichen species	Form	Accumulation (ppm)	Reference
1	*Lecanora muralis*	Crustose	35,760	Seaward (1973)
2	*L. polytropa*	Crustose	26,200	Alstrup and Hansen (1977)
3	*Hypogymnia physodes*	Foliose	19,000	Pilegaard (1979)
4	*Phaeophyscia hispidula*	Foliose	10,923	Shukla and Upreti (2007)
5	*Parmelia sulcata*	Foliose	4,613	Garty and Ammann (1987)
6	*Stereocaulon paschale*	Fruticose	5,200	Tomassini et al. (1976)
7	*Ramalina duriaei*	Fruticose	2,067	Garty et al. (1979)
8	*Cladonia alpestris*	Fruticose	1,615	Nieboer et al. (1972)

was the most common metal detected in samples collected from areas of mixed pollution (site I) followed by areas with heavy vehicular activities (site II). The site (III) 10–11 km away from the center of the city exhibit ten times less concentration of Fe than the city center. Site III, despite the absence of vehicular activity, exhibits accumulation of 319.82 ± 0.201 Fe in the samples, indicating a wide dispersion of this metal. Iron concentration varied from 319.82 ± 0.201 to 3196.0 ± 2.40 and 6.65 ± 0.86 to 11.37 ± 0.72 μg g^{-1} dry weight in thallus as well as in substratum, respectively. The data clearly indicates the origin of iron from vehicular and coal and oil burning activities. According to Baptista et al. (2008), the Fe content is evidently affected by iron originating from fuel and soil dust. The sites having soil dust and human activities may be a reason for higher accumulation of iron at site I.

Lichens demonstrate specific affinity for Fe (Puckett et al. 1973; Garty and Delarea 1991). The competitive uptake studies revealed the selectivity sequence of different metals as Fe > Cr > Cu > Zn > Ni > Co (Garty and Delarea 1991), while in the present study, *L. lobificans* shows the selectivity sequence as Fe > Cr > Zn > Al > Cu > Ni > Cd. According to Garty et al. (1979), Fe was absorbed and adsorbed in very high amount in various lichen species (Table 4), and comparatively, *L. lobificans* also accumulates significant amount of Fe (3196 ± 2.40 μg g^{-1} dry weight).

Nickel ranged between 1.53 ± 0.35 and 17.05 ± 0.49μg g^{-1}in thallus but not detected in substratum, thus clearly indicating its airborne origin. According to Nriagu and Pacyana (1988), the automobile, coal, and oil combustion is the main anthropogenic emission of Ni. All the three sites of the study area exhibit average accumulation of Ni, and it was reported at maximum in samples collected adjacent to the road side (site II) having heavy vehicular activity.

Zinc content varied from 110.49 ± 1.08 to 165.87 ± 2.52 in thallus and <0.94 ± 0.03μg g^{-1} in substratum. The higher concentration of Zn was recorded from the samples collected adjacent to the roadside (site II) having high vehicular activity. Higher Zn concentration was associated with automobile tire and incomplete combustion of fossils fuel (Garty 2001).

Out of the three sites in the study area, sites (I and II) having multisources of pollution exhibit similar selectivity sequence of metals as Fe > Cr > Zn > Al > Cu > Ni > Cd, while the site away from the city (III) has the selectivity sequence as Fe > Zn > Cr > Cu > Al > Ni.

Conclusion

It is clear from the study that *L. lobificans*, a single ubiquitous leprose lichen, can be used to determine the heavy metal pollution in the dry and warm climate of central India. The lichen accumulates higher concentration of almost all the metals in its thallus as compared to the substratum. The present level of metallic pollutants will be a useful baseline data for carrying future studies related on ambient air quality in the area.

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