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Physiological attributes of lichen, *Phaeophyscia hispidula* in heavy metal rich sites of Dehra Dun, India

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Abstract

P*haeophyscia hispidula* (Ach.) Moberg*,* foliose lichen is widespread in Garhwal Himalayas and grows luxuriantly on different substratum in polluted as well as non polluted sites, where no other macrolichens are able to colonize. The elemental concentration and biochemical parameters in *P. hispidula* collected from Dehra Dun city were analyzed to assess its tolerance to heavy metals. The major source of pollution in the city is automobiles. Among the biochemical parameters protein was significantly and negatively correlated with pigment concentrations (r= -0.3838 (Chl.b); -0.5809 (Carotenoid); -0.5034 (OD)) however it is significantly positively correlated with Cd (r = 0.6822: P<0.01). Among heavy metals, Cd was negatively correlated with Cu (r = -0.4639), Fe (r = -0.2676), and Zn (r = -0.0549). It was observed that the chlorophyll and protein content increased parallel to the level of metallic pollutants indicating the mechanism of stress tolerance in *P. hispidula*. The study shows that *P. hispidula* is tolerant to inorganic pollution and a useful tool for biomonitoring of air quality in the Himalayan region.

Key words

Biomonitoring, Metal tolerance, Stress physiology, Vehicular activity, Lichen

Introduction

Tolerance is an ability of individuals to withstand stresses caused due to pollution that are inhibitory or lethal to non-tolerant individuals to which they are exposed. Metallic pollutants are known to cause irreversible damage to the physiology of organism by inhibiting enzyme mediated vital metabolic pathways (Prasad, 1997). Most of the macrolichens are known to show high degree of sensitivity to metallic pollutants but some species like *Dirinaria pappillulifera*, *Hypogymnia physodes, Parmelia sulcata, Pseudevernia furfuracea,* and *Pyxine subcinerea* grow luxuriantly in metal rich environment and are known to be hyperaccumulator of various metals (Dubey *et al*., 1999; Shukla and Upreti, 2008).

P. hispidula, foliose lichen widespread in tropical and temperate areas of India grows luxuriantly in both polluted and non-polluted sites (Shukla and Upreti, 2007a). Abundance of P. hispidula in polluted sites indicates toxitolerant nature, but its tolerance to heavy metals has not been explored yet. In the present study, *P. hispidula* was collected from different sites, with varying degree of pollution, were analyzed for metal concentration and its probable physiological response.

Materials and Methods

Study area: The present study was conducted in Dehra Dun city, one of the densely populated cities, situated at 30º19' N and 78º 04' E with an elevation of 673 m asl. The city has a typical topography as it is situated in a valley. The vehicular and other human activities are quite high in the area. The SO_2 and NOx levels have increased since year 2000. Nine monitoring sites (Fig 1) were selected for collection of *P. hispidula* lichens including Old Mussorrie Road as control site. In each site, samples were taken from nine isolated mango trees (*Mangifera indica*), at each site having 10 trees (trunk diameter >35 cm at height 1.5 to 2 m) were surveyed to collect lichen sample (5-10 whole thalli of 3 cm).

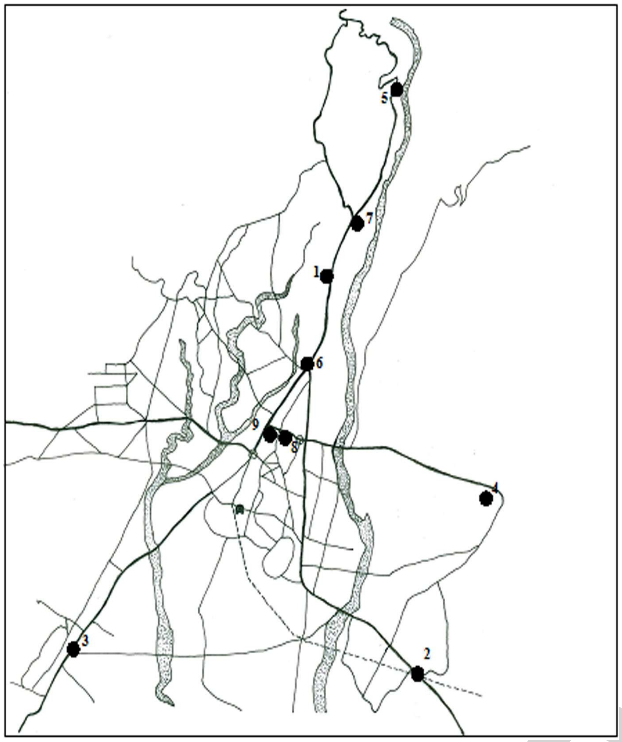

Fig. 1: Map showing the locations for lichen collection in DehraDun, India 1. Hathibarkala Estate 2. Hardwar Crossing 3. Interstate Bus Terminal 4. Ordinace Factory 5. Old Mussorrie Road 6. Dilaram Chowk 7. Sai Temple, Rajpur Road 8. Gandhi Chowk near Kanak Cinema 9. Gandhi Chowk near Parade Ground

Composite samples from each site were removed from the bark with sharp knife and sorted to remove extraneous material in the laboratory. Samples were then divided into two groups, one for biochemical analysis and the other for heavy metal analysis. Samples were air dried for biochemical analysis while samples for metal estimation were oven dried for 12 hr to a constant weight at 90° C.

Biochemical analysis

Chlorophyll degradation ratio : Analysis of the integrity of the chlorophyll in the lichen thallus was carried by method of Ronen and Galun (1984). The ratio of Chl *a* to Phaeophytin a (OD 435 nm/ OD 415 nm) was determined using a Genesys 10 UV scanning spectrophotometer.

Pigment analysis : Pigments (Chl *a*, Chl *b*, Total Chl and Carotenoid) were extracted in 80% acetone (Merck, Analytical grade) and their concentrations were determined using standard. (Brown and Hooker, 1977). The Chl content was calculated according to the equation of Arnon (1949) at absorbance values of 663 and 645 nm. The total carotenoid content was calculated from absorbance values at 480 and 510 nm using Genesys 10 UV scanning spectrophotometer Parsons *et al*. (1984).

Protein estimation : Protein was estimated using Folin phenol reagent and calculations were made from absorbance values at 600 nm by the method of Lowrey *et al*. (1951).

Metal analysis : The dried lichen samples (3 replicates) were grounded to powder (1.0 g each) and digested in mixture of concentrated HNO_3 and $HClO_4$ (v/v 9:1) for 1 hr. Residues were filtered through Whatman Filter paper No. 42 and diluted to 15 ml with double distilled water. The samples were analyzed for copper (Cu), lead (Pb), Nickel (Ni), Zinc (Zn), Iron (Fe), cadmium (Cd) and chromium (Cr) by Flame Atomic Absorption Spectrophotometer (Perkin Elmer, model A Analyst 300). Stock standards were from Merck India and traceable to NIST (National Institute of Standards Technology). Working standards were prepared from the stock using deionised water for dilution.

Statistical analysis : The data obtained were subjected to one way analysis of variance (ANOVA) to evaluate the probable correlation between the biochemical parameters and metal content using statistical program INDOSTAT (Hyderabad, India).

Results and Discussion

Physiological parameters determined in lichen samples collected from nine localities of Dehra Dun city (Table 1), showed significant variation in the biochemical parameters. The total chlorophyll content varied between 0.483 – 0.090 µg g^{-1} and chlorophyll degradation ratio ranged between 0.995 – 0.579. The total chlorophyll content increased significantly with the increase in the metallic content, especially Zn and Fe (Table 5) but at the same time, there was an increase in the degradation of Chl *a*. These results indicate that chlorophylls were affected by air pollution and as an adaptation mechanism the lichen would increase their synthesis. Chlorophyll degradation value is considered to be an appropriate index for evaluating the effects of heavy metal pollution in lichens (Ronen and Galun, 1984). It was interesting to note that total chlorophyll content and chlorophyll degradation ratio in lichen samples collected from Dilaram Chowk (Critically polluted site) was 0.343 µg g^{-1} and 0.948 that showed stress mechanism to be operational to slow down the conversion of Chl *a* to phaeophytin and maintain the integrity of chlorophyll, whereas total Chl content,

Table- 1: Physiological data (µg g^{-1}) of *P. hispidula* collected from different sites of Dehra Dun city

Site No.	Chl *a*	Chl *b*	Total Chl	Carotenoid	Chlorophyll degradation	Protein
1	0.167 ±0.03	0.086 ±0.02	0.253 ±0.04	0.187 ±0.02	0.717 ±0.02	72 ±6.6
2	0.292 ±0.03	0.192 ±0.02	0.483 ±0.02	0.591 ±0.05	0.964 ±0.06	50 ±12
3	0.137 ±0.01	0.096 ±0.03	0.233 ±0.03	0.215 ±0.01	0.995 ±0.19	52 ±9
4	0.114 ±0.01	0.087 ± 0.01	0.201 ±0.01	0.249 ±0.03	0.973 ±0.03	75 ±12
5 (control)	0.099 ±0.03	0.056 ±0.01	0.155 ±0.01	0.122 ±0.01	0.749 ±0.03	85 ±8
6	0.209 ±0.02	0.134 ±0.02	0.343 ±0.01	0.218 ±0.02	0.948 ±0.02	70±9
7	0.158 ±0.01	0.074 ±0.03	0.231 ±0.02	0.096 ±0.02	0.899 ±0.08	82 ±18
8	0.127 ±0.01	0.057 ±0.01	0.184 ±0.02	0.098 ±0.02	0.925 ±0.03	87±20
9	0.056 ±0.02	0.034 ±0.01	0.090 ±0.01	0.060±0.02	0.954 ±0.03	62 ±12
F value	**119****	**22****	**92****	**301****	**5.35***	**9.17****
F prob.	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

Values are mean of replicated ± SE; (** P< 0.01; * P<0.05)

Table- 2: Elemental concentrations (µg g^{-1} d.wt.) in thalli of *P. hispidula* collected from various sites of Dehra Dun City

S.No.	Cu	Pb	Ni	Zn	Fe	Cd	Cr
1	13.5±1	17.1±2.60	24.2±0.89	62.7±1.93	232±1.32	0.1±0.02	11.7±1.99
2	16.4±2.16	23.0±2.26	15.3±1.49	68.12±1.07	229.80±1.54	0.06±0.01	5.6±1.65
3	15.3±1.13	38.9±3.26	10.9±1.36	62.8±2.18	223.50±2.52	0.1±0.02	2.68±1.57
4	0.9±0.1	17.9±2	10.3±2	54.2±2	211±0.2	0.3±0..3	5.46±1
5 (control)	8.2±1.44	16.5±1.79	9.26±0.84	51.5±1.93	223±2.74	0.1±0.01	5.18±1.95
6	21.3±1.85	52.4±2.07	22.6±1.66	69.6±1.23	238±1.10	0.2±0.03	10.7±1.59
7	10.2±0.95	22.5±1.05	24.1±1.64	55.88±1.45	234±2.79	0.2±0.17	9±1.73
8	15.2±1.76	18.4±2.77	15.3±2.02	59.3±1.84	232.7±2.04	0.36±0.04	5.86±1.75
9	6.8±2.75	6.35±2.30	7.9±1.28	16.1±1.67	136±1.73	33.6±0.03	22±0.82
F value	**161****	**191****	**78****	**120****	**378****	**5.5***	**12****
F prob.	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**	**0.00**

values are mean of replicates ± SE and f values along with f probability ; ** P< 0.01; * P<0.05

(0.155 µg g^{-1}), carotenoid (0.122 µg g^{-1}) chlorophyll degradation ratio (0.749) and protein (85 µg g^{-1}) from Old Mussoorie Road (control site) reflects natural conditions prevailing at the site.

Table 2 shows variation in the metallic content between the nine sites studied. Total metal concentration was highest in lichen samples collected from Dela Ram Chowk (highly polluted site). Concentration of Pb, Zn and Fe was highest in samples from Dela Ram Chowk which may be attributed to the location of the site in the center of the city with narrow lanes. The site is exposed to heavy vehicular activities throughout the day. Samples from Gandhi Chowk Parade ground showed high concentrations of Cd (33.63 µg g^{-1}) and Cr (22 µg g^{-1}), which is due to location of high number of motor repairing garages in the vicinity of the collection site. Samples from Hathibarkala Estate (moderately polluted site) showed highest concentration of Ni as the site was close to electricity transformer, while Old Mussorrie Road (control site) had minimum concentration of Cd (0.1 µg g^{-1}) and Cr (5.18 µg g^{-1}).

Samples from Old Mussorrie Road (control site) also had lower concentration of Pb (16.5 µg g^{-1}) in comparison to samples from other polluted sites (Dilaram Chowk: 52.4 µg g^{-1}; Interstate Bus Terminal: 38.9 µg g^{-1} and Hardwar Crossing: 23.0 µg g^{-1}).

Coefficient of correlation between different physiological parameters (Table 3) showed that chlorophyll content was significantly correlated (P<0.01) with carotenoid content while protein content was negatively correlated with all other physiological parameters (with Chl *b*: r = -0.5491, Carotenoid: r = -0.5809, OD: r = -0.5034).

In many studies from different regions of the world concentration of total chlorophyll has been found to be affected by traffic level (Carreras *et al.*, 1998; Shukla and Upreti, 2007b). Specifically, lichen located at sampling sites with high traffic level showed increased chlorophyll concentration. It might be inferred

Table- 3: Values of correlation coefficient between the various physiological parameters

	Chl *b*	Total Chl	Carotenoid	OD	Protein
Chl *a*	0.9495**	0.9913 **	0.8383**	0.1269	-0.3838
Chl *b*		0.9825**	0.9288**	0.2889	-0.5419*
Total Chl			0.8863**	0.1955	-0.4545
Carotenoid				0.2735	-0.5809*
OD					-0.5034*

** P< 0.01; * P<0.05

Table- 4: Values of correlation coefficient between the amounts of some elements found in *P. hispidula*

	Pb	Ni	Zn	Fe	Cd	Cr
Cu	0.3857	0.1457	0.5985**	0.3271	-0.4639	0.0861
Pb		0.2903	0.6740**	0.4383	0.0602	0.1768
Ni			0.4458	0.8222**	0.1406	0.9058**
Zn				0.5688**	-0.2676	0.3201
Fe					-0.0549	0.6536**
Cd						0.2476

** P< 0.01; * P<0.05

Table- 5: Significant correlation matrix between pairs of elemental content and physiological parameters in *P.hispidula*

Parameter	Correlation coefficient
Protein; Cu	- 0.5561*
Protein; Zn	- 0.6190**
Protein; Cd	0.6822**
Chl degradation ratio; Cr	- 0.4855*
Carotenoid; Zn	0.5860*
Total Chl; Zn	0.7804**
Total Chl.; Fe	0.5119*
Chl *b*; Zn	0.7686**
Chl *a*; Ni	0.4976*
Chl *a*; Zn	0.7706**
Chl *a*; Fe	0.6019**

** P< 0.01; * P<0.05

that the content of chlorophylls increased parallel to the level of pollutant emitted by traffic.

Cd and Zn compete with one another for sites holding bivalent cations. It is consistent with the observation (Table 4) that the correlation coefficients between Cd and Zn, although not statistically significant exhibit negative trends. Usually Zn, Cu and Fe are supposed to act antagonistically against Cd. Zn, Fe, and Cu is negatively correlated with Cd. The significant positive correlation (Table 5) of protein with Cd indicates the synthesis of protein under Cd stressed condition similar to expression of stress protein 70 (hsp 70) in the lichen photobiont *Trebouxia erici* (Baèkor *et al*., 2006). Metal tolerance in animal and plants (vascular and non vascular) is known to be conferred by production of a special class of proteins called metallothioneins and phytochelatins (PCs). The PCs play a central role in the detoxification of several heavy metals, especially Cd (Prasad, 1997).

The higher resistance of chlorolichens to heavy metals compared with cyano lichens may be attributed to the phytochelatin synthesis in lichens with *Trebouxia* algae (Branquinho *et al*., 1997). Similarly in the present study, *P. hispidula,* a trebouxioid lichen may gain an ecological advantage from their ability to counter heavy metal with prompt phytochelatin synthesis. However, there are few researches on the biosynthesis and function of phytochelatins in lichens in response to heavy metal exposure (Branquinho, 2001; Pawlik-Skowrońska *et al*., 2002). The assessment of lichen diversity in Dehra Dun city clearly indicates that the members of lichen family Physciaceae, especially *P. hispidula* grows luxuriantly in both busy site in the center of the city and periurban rural areas (Shukla and Upreti, 2007b). Increase in Physciaceae in temperate regions has been associated with both increasing temperature and increasing availability of nutrients (Loppi and Pirintsos, 2000; Saipunkaew *et al*., 2007; Van Herk *et al*., 2002).

Though the sensitivity rating of *P. hispidula* is not known but as *P. hispidula* co-occurs with *P. orbicularis* a known intermediate nitrophillous species which occurs in SO_2 rich environment (upto 35 ppb) (Peterson *et al*., 1992) which is quite consistent with the SO_2 level in DehraDun city (30 ppb). Wide distribution of *P. hispidula* in pollutant enriched urban environment shows its toxitolerant characteristics as it is not only potential bioaccumulators of inorganic pollutants but also accumulates high proportion of organic pollutants too (Shukla and Upreti, 2009; Shukla *et al*., 2010).

The above observations affirm that the variation in the metal profile in lichen samples from different sites (control and polluted sites) is mainly due to vehicular activity and thus comparison of physiological attributes from control site and polluted site, supplemented by statistical correlation studies, establishes the role of stress mechanism to withstand higher levels of metal concentration.

The present study establishes *P. hispidula* as a poleotolerant species which survives/ tolerates high levels of pollutants in the urban settlements of India. It also provide evidence of probable role of protein to combat metal stress in particular, Cd in *P. hispidula.*

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