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Biodiversity, bioaccumulation and physiological changes in lichens growing in the vicinity of coal-based thermal power plant of Raebareli district, north India

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ABSTRACT

The lichen diversity assessment carried out around a coal-based thermal power plant indicated the increase in lichen abundance with the increase in distance from power plant in general. The photosynthetic pigments, protein and heavy metals were estimated in *Pyxine cocoes* (Sw.) Nyl., a common lichen growing around thermal power plant for further inference. Distributions of heavy metals from power plant showed positive correlation with distance for all directions, however western direction has received better dispersion as indicated by the concentration coefficient-R^2. Least significant difference analysis showed that speed of wind and its direction plays a major role in dispersion of heavy metals. Accumulation of Al, Cr, Fe, Pb and Zn in the thallus suppressed the concentrations of pigments like chlorophyll a, chlorophyll b and total chlorophyll, however, enhanced the level of protein. Further, the concentrations of chlorophyll contents in *P. cocoes* increased with the decreasing the distance from the power plant, while protein, carotenoid and phaeophytisation exhibited significant decrease.

1. Introduction

Coal is recognized as the primary source of energy in India, and its utilization in power generation is emerging as the biggest environmental problem as it emits fly ash, acid precursors, green house gases, non-combustible hydrocarbons, heavy metals and particulates. These pollutants can be carried to a long distance by wind and ultimately have a negative impact on both biotic and abiotic environment [1].

A large number of pollution studies are available in which lichens are used as bioindicators [2–4]. Due to their peculiar anatomical, morphological and physiological characteristics lichens are one of the most valuable biomonitors of atmospheric pollution. They can be used as sensitive indicators to estimate the biological effects of pollutants by recording changes at the community and as accumulative monitors of persistent pollutants, which can be estimated by assaying their trace element contents [4].

The epiphytic lichens have been used extensively to monitor air quality around urban areas, industrial sites and to document spatial distribution and accumulation of air borne pollutants [5–7]. Lichens are used as passive pollution monitors because they accumulate a variety of pollutants in their thalli at levels well above environmental concentrations and their own physiological needs. They lack root system and therefore intercept only allogenic atmospheric matter included in wet precipitations, dry depositions and gaseous emission [7]. They record an integrated signal over a few years of atmospheric fallout and thus minimize any signals due to variable (seasonal) atmospheric circulation patterns [6]. The use of lichens as biomonitors of geothermal air pollution was initiated by Bargagli-Petrucci [8] who reported the absolute absence of lichens in the geothermal area of Italy around 5 km vicinity.

Recent reports have shown deterioration in air quality around thermal power plants in India [9,10]. As a result destabilization of the ecosystem has occurred with loss of several sensitive plant species [10]. A lot of passive as well as active (transplant) biomonitoring studies using lichen have been carried out in India by several workers in different climatic regions of the country against various pollution sources [11–18]; but such studies around thermal power plant area are lacking.

The main objective of the present study is to assess the impact of thermal power plant emission on lichen community within 15 km of its radius. The parameters considered for the study include distribution pattern of heavy metals and abundance of lichens around thermal power plant, accumulation of heavy metals and changes in pigment concentrations in a commonly occuring lichen *Pyxine cocoes.*

2. Materials and methods

2.1. Study area

Firoz Gandhi Unchahar National Thermal Power Plant Corporation (FGUNTPC) is situated in the Unchahar town of Raebareli

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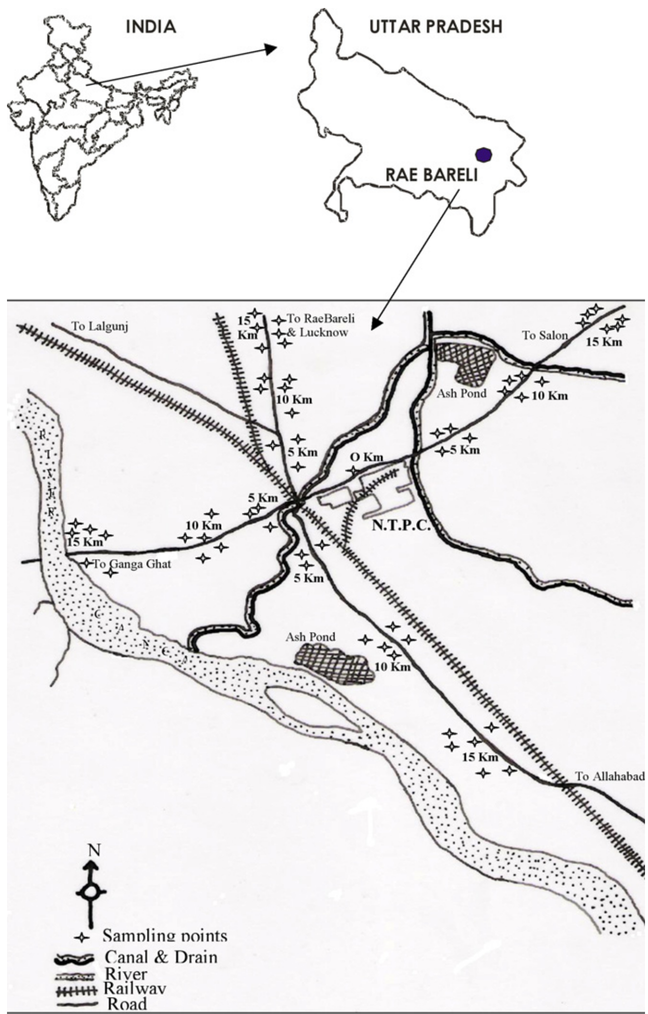

Fig. 1. Map of the study area with location of sampling sites.

district (Fig. 1), between coordinates 25°49′N to 26°36′N and 100°41′E to 81°34′E at an altitude of 120.4 m. FGUNTPC has the stack height of 130 m with electricity production capacity of 5 × 210 MW. The climate of the region is tropical, with eight months of dry period and four months of rain that ranges from 110 to 485 mm distributed between June to September, the temperature range that the area experiences is between 13.2 °C in winter and 45.2 °C in summer (Fig. 2A–C).

2.2. Sample collection

The area around FGUNTPC was surveyed for collection of lichens in the month of February–March 2008. Lichen, especially *P. cocoes* (Sw.) Nyl., growing abundantly on trees of *Mangifera indica* collected from 12 different sites at 5, 10 and 15 km distance on north, south, east and west directions from the FGUNTPC. *P. cocoes* is widely distributed in the area thus used for physicochemical and metal accumulation studies from each sites. The sites at 15 km away from the thermal power plant are considered as control sites. Approximately 3–4 g of the thallus of similar sizes were taken from each site in triplicate for further analysis.

2.3. Pigment analysis

Photosynthetic pigments (chlorophyll a, chlorophyll b, Total chlorophyll, Carotenoid) were extracted in 80% acetone (Merck, Analytical grade) and their concentrations determined using standard spectrophotometric procedures. 1.0 g of the sample was grinded with acid washed sand 50 mg calcium carbonate and 10 ml acetone (80%) on ice in dim light. The slurry was transferred to a 10 ml centrifuge tube, vigorously shaken and centrifuged at 10,000 rpm for 10 min. The supernatant was then decanted, kept in the cold and pellet resuspended in 1.5 ml chilled acetone (80%) and centrifuged as above. The supernatant were then combined, made to known volume and analyzed using Genesys 10 UV scanning spectrophotometer.

The chlorophyll content was calculated from absorbance values at 663 and 645 nm according to the equation of Arnon [19]. The total carotenoid content was calculated according to Parsons et al. [20] from absorbance values at 480 and 510 nm.

2.4. Chlorophyll degradation

The method developed by Ronrn and Galun [21] was used to measure intensity of the photobiont chlorophyll. The chlorophyll was extracted overnight in the dark in 5 ml dimethyl sulfoxide (DMSO, Merck, analytical grade). The ratio of chlorophyll a to phaeophytin a (OD 435/415 nm ratio) was determined.

2.5. Protein estimation

The protein content was measured using Folin phenol as reagents with bovine serum albumin (BSA) as standard and calculations were made from absorbance values at 700 nm [22].

2.6. Metal analysis

The lichen thalli (approximately 2–3 g) were removed from the bark with sharp knife. The samples were oven dried for 12 h to a constant weight at 90 °C. The dried lichen samples (three replicates) were grinded to powder (0.5 g) and digested in mixture of concentrated HNO_3 and $HClO_4$ (v/v, 9:1) for 1 h. Residues were filtered through Whatman Filter paper no. 42 and diluted to 20 ml with double distilled water. Analysis was done with 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.

2.7. Statistical analysis

Differences in chlorophyll response to air pollution and elemental content were compared using one-way analysis of variance and least significant difference (LSD) was calculated with significance correlated at $p < 0.05\%$.

3. Results and discussion

3.1. Passive monitoring by lichens

The diversity of epiphytic lichen at 12 monitoring sites around FGUNTPC is presented in Table 1. Eight different genera of lichens were found growing upto 15 km in all directions of the FGUNTPC. Within 5 km radius in all directions only two species i.e. *Phaeophyscia hispidula* and *P. cocoes,* showed their survivance, of which the former exhibits its occurrence with 1–3 thalli growing on bricks of canal arch. At distance of 10 km, the western direction showed maximum diversity of lichens represented by eight species followed by four species in east, three in north and two in south. At 15 km distance from FGUNTPC west and south directions had maximum number of species followed by north and east. However, in all direction *P. cocoes* showed its dominance over other species. It

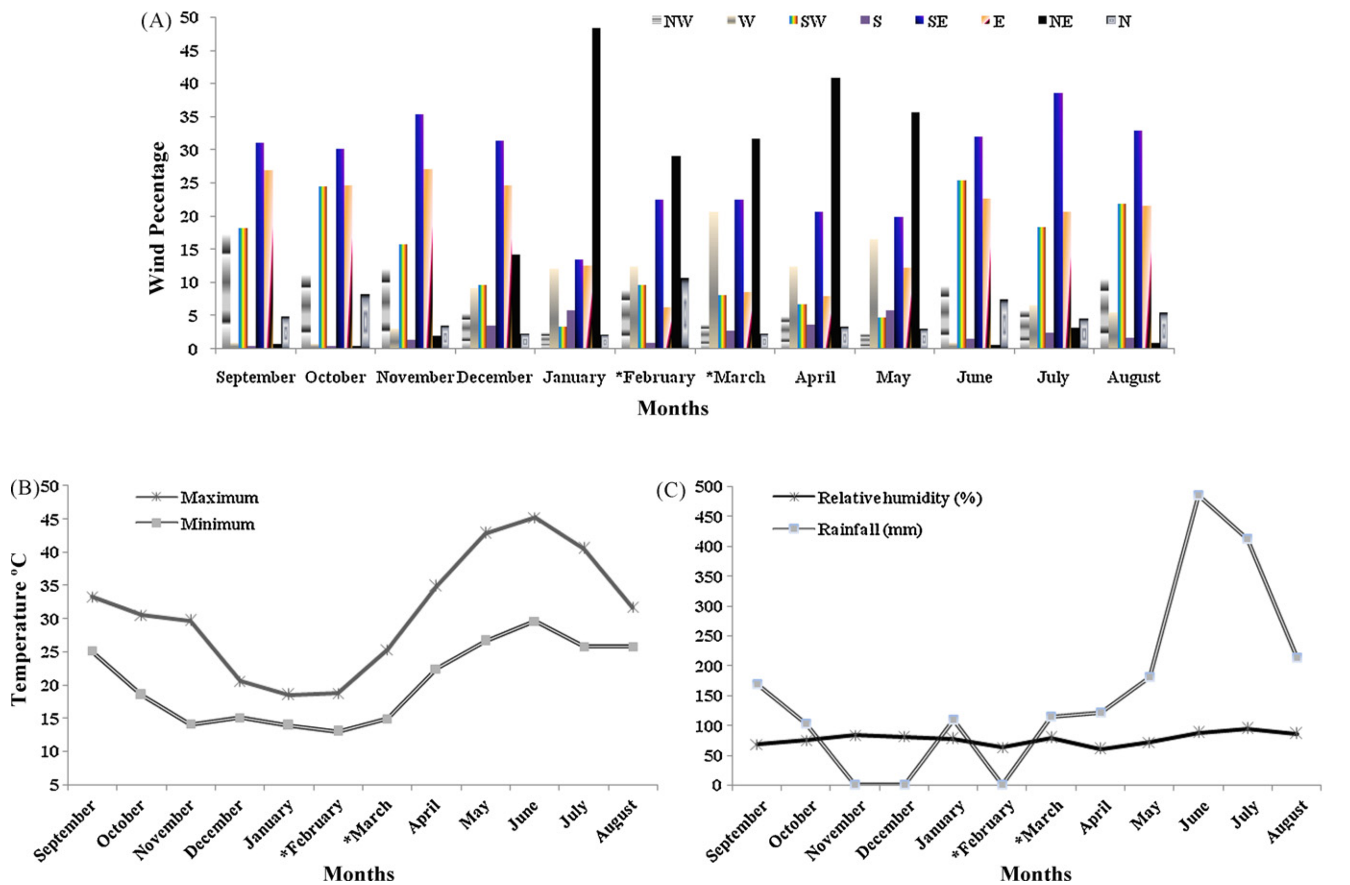

Fig. 2. Weather data measured by the, Environmental Division of FGUNTPC Raebareli: (A) mean monthly wind percentage; (B) mean monthly temperature; (C) mean monthly relative humidity and rainfall (*: sampling month).

is clear from the observations that the diversity of lichens increases with the increasing distance from the source of pollution.

The FGUNTPC is located in the centre of agricultural land that has many water canals and a small river all around, which provide humidity to the air thus support growth of many lichens. According to Bruteig [23], local differences in climate, topography and growing condition can affect the diversity of lichens. *P. cocoes* exhibits its presence in all the sampling sites and the number of thalli increases with the increasing distance from the power plant in all directions. *Anisomeredium*, a crustose lichen exhibits its occurrence on seven sites. *Lecanora*, *P. hispidula*, *Strigula* were recorded from six sites. Both the sites at 10 and 15 km in the west exhibit the maximum number of lichens.

3.2. Distribution of heavy metal at different sites

Accumulation of seven heavy metals Aluminium (Al), Chromium (Cr), Copper (Cu), Iron (Fe), Nickel (Ni), Lead (Pb) and Zinc (Zn) in thalli of *P. cocoes* were estimated for all directions at distances of 5, 10 and 15 km (Table 2). Among seven metals, Fe was accumulated in highest amount followed by the sequence of Al > Zn > Ni > Cr > Cu > Pb. Maximum Fe accumulation of $1498.4 \pm 2.0\,\mu g\,g^{-1}$ dry weight (DW) was observed at 5 km north and lowest of $206.7 \pm 2.4\,\mu g\,g^{-1}$ DW at 5 km west, that indicates settling of this metal in the north direction. Accumulation of Fe in the thalli of *P. cocoes* for all sides are significantly different from each site based on LSD analysis (ref. Table 2).

Table 1
The composition of epiphytic lichen communities around FGUNTPC (+ = presence; – = absence; abubance estimated on a scale 1–5 thalli (A); 6–10 thalli (B); 11–20 thalli (C); 21–30 thalli (D); 31–40 thalli (E); 41–50 thalli (F); more than 51 thalli (G); for 10 investigated trees).

Frequency	North (km)			0 km	South (km)			East (km)			0 km	West			Name of taxa
	15	10	5		5	10	15	15	10	5		5	10	15	
7	+ (E)	+ (D)	–	–	–	+ (D)	+ (F)	+ (F)	–	–	–	–	+ (E)	+ (G)	*Anisomeridium*
3	–	–	–	–	–	–	+ (E)	–	–	–	–	–	+ (B)	+ (D)	*Bacidia*
5	+ (B)	–	–	–	–	–	+ (B)	–	+ (A)	–	–	–	+ (C)	+ (B)	*Caloplaca*
6	+ (G)	+ (G)	–	–	–	–	+ (F)	–	+ (E)	–	–	–	+ (D)	+ (G)	*Lecanora*
6	–	–	+ (B)	+ (A)	–	–	–	–	+ (B)	+ (A)	–	+ (A)	+ (B)	–	*Phaeophyscia hispidula* (Ach.) Moberg
12	+ (G)	+ (F)	+ (E)	–	+ (A)	+ (D)	+ (G)	+ (G)	+ (F)	+ (D)	–	+ (D)	+ (G)	+ (G)	*Pyxine cocoes* (Sw.) Nyl.
5	+ (E)	–	–	–	–	–	+ (G)	+ (E)	–	–	–	–	+ (B)	+ (D)	*Rinodina sophodes* (Ach.) Massal.
6	+ (G)	–	–	–	–	–	+ (G)	+ (F)	–	–	–	+ (A)	+ (C)	+ (F)	*Strigula*
No. of taxa	6	3	2	1	1	2	7	4	4	2	0	3	8	7	

Table 2
Heavy metal analysis of *Pyxine cocoes* at 12 sites around FGUNTPC (values in each vertical column followed by the same alphabetic letter shows there is no significant difference between them at $p < 0.05\%$ level by least significant difference (LSD) analysis).

Sites	Al	Cr	Cu	Fe	Ni	Pb	Zn
5 km north	937.6 ± 0.8^{g}	6.3 ± 0.2^{e}	3.0 ± 0.1^{f}	1498.4 ± 2.0^{a}	2.0 ± 0.3^{g}	7.8 ± 0.2^{c}	55.3 ± 0.1^{b}
5 km south	1307.5 ± 2.1^{e}	6.9 ± 0.6^{d}	2.0 ± 0.8^{h}	1205.0 ± 3.1^{f}	1.7 ± 0.0^{gh}	8.1 ± 0.1^{b}	50.2 ± 0.1^{c}
5 km east	1511.5 ± 4.1^{c}	7.8 ± 0.8^{c}	7.8 ± 0.8^{d}	1310.7 ± 5.6^{d}	1.0 ± 0.2^{ij}	9.3 ± 0.7^{a}	59.6 ± 0.5^{a}
5 km west	1074.5 ± 4.3^{f}	3.1 ± 0.2^{f}	3.1 ± 0.9^{f}	206.7 ± 2.4^{j}	0.6 ± 0.1^{j}	2.2 ± 0.3^{h}	19.8 ± 0.4^{i}
10 km north	1437.3 ± 5.9^{d}	12.1 ± 0.4^{a}	12.1 ± 0.1^{a}	1338.6 ± 3.0^{c}	8.3 ± 0.2^{f}	5.0 ± 0.1^{f}	46.9 ± 0.1^{d}
10 km south	1578.6 ± 5.0^{b}	9.6 ± 0.5^{b}	4.2 ± 0.9^{e}	1288.7 ± 6.5^{e}	9.8 ± 0.9^{e}	5.6 ± 0.1^{e}	38.8 ± 0.8^{f}
10 km east	1631.3 ± 4.7^{a}	10.8 ± 0.4^{b}	7.8 ± 0.2^{d}	1440.3 ± 0.9^{b}	10.6 ± 0.3^{d}	6.9 ± 0.1^{d}	41.3 ± 0.1^{e}
10 km west	533.8 ± 5.1^{k}	1.1 ± 0.0^{hi}	1.3 ± 0.0^{i}	128.3 ± 1.3^{k}	1.0 ± 0.0^{ij}	1.1 ± 0.0^{j}	12.7 ± 0.2^{j}
15 km north	678.0 ± 6.2^{j}	1.8 ± 0.0^{g}	8.9 ± 0.6^{c}	281.8 ± 3.9^{i}	12.2 ± 0.4^{c}	1.6 ± 0.0^{i}	11.2 ± 0.1^{k}
15 km south	736.5 ± 4.2^{i}	1.3 ± 0.0^{h}	7.8 ± 0.1^{d}	345.6 ± 4.5^{h}	16.8 ± 0.3^{b}	2.2 ± 0.0^{h}	21.2 ± 0.1^{h}
15 km east	807.9 ± 4.0^{h}	3.2 ± 0.2^{f}	9.9 ± 0.8^{b}	910.4 ± 2.4^{g}	18.3 ± 0.1^{a}	3.2 ± 0.1^{g}	32.8 ± 0.0^{g}
15 km west	297.2 ± 2.8^{l}	0.9 ± 0.0^{i}	2.5 ± 0.0^{g}	58.6 ± 0.8^{l}	1.3 ± 0.1^{hi}	0.5 ± 0.3^{k}	7.8 ± 0.2^{l}
One-way ANOVA							
f	4154.5**	484.9**	872.8**	16163.3**	1080.9**	1629.2**	15636.9**
LSD ($p < 0.05\%$)	17.1	0.3	0.2	10.9	0.4	0.1	0.3

Mean ± S.D., $n = 3$ in $\mu g\,g^{-1}$ dry weight.
** Significance at the level of 0.01%.

Accumulation of Al ranges from 297.2 ± 2.8 to 1631.3 ± 4.7 $\mu g\,g^{-1}$ DW. Maximum Al was reported at 5 and 10 km distance in all the directions. In lichens, Al has limited metabolic significance and is commonly used as an indicator of sample contamination by wind borne soil, rock dust. Al and Fe two principal elements in the earth's crust are strongly correlated in lichens and environmental contamination [24]. In this study Al was maximum reported at the vicinity of power plant (Table 3).

Among all the metals chromium was reported in lowest concentration in all directions. The maximum level of Cr was reported at 10 km north (12.1 ± 0.4) and minimum (0.93 ± 0.07 $\mu g\,g^{-1}$ DW) at 15 km west. According to Fernandez et al. [25], Cr and Fe are normally associated with the coarsest fraction of fly ash, which tends to fallout close to the source. In the present study the sample site at 10 km of both east and north direction has fly ash dumping sites thus shows higher accumulation of Cr.

Thalli of *P. cocoes* around FGUNTPC accumulated Cu in the ranges of 0.9 ± 0.2 (at 15 km west) to 9.9 ± 0.8 $\mu g\,g^{-1}$ DW (5 km east). Wind direction may be a probable reason for dumping of this metal from out side the source. Maximum concentration of the Cu was reported at 15 km followed by 10 and 5 km. According to Garty [5], dispersion of metals depends on the gravity of a particular metal along with speed and direction of wind.

The sites at 5 km west and 15 km east exhibit accumulation of nickel ranging from 0.6 ± 0.1 to 18.3 ± 0.1 $\mu g\,g^{-1}$ DW, respectively. Both the metals Ni and Cu were accumulated maximum in western site at 15 km and minimum at 5 km. Ni and Cu both are large particle metals emitted in the immediate vicinity of the station are incapable of long-range dispersion [26].

The site 5 km east has maximum accumulation of both Pb and Zn as 9.3 ± 0.7 and 59.6 ± 0.5 $\mu g\,g^{-1}$ DW, respectively and minimum accumulation was reported at 15 km as 0.5 ± 0.1 and 7.8 ± 0.2 $\mu g\,g^{-1}$ DW Pb and Zn, respectively. Accumulation of Pb and Zn at different sites exhibits sequence of accumulation as 5 km > 10 km > 15 km i.e. decreasing concentration with increasing distance from source. The reason for higher concentration of Pb and Zn around the thermal power plant may be due to heavy vehicular activity involved in disposal of coal waste and other activities. Apart from engine emission, Ni, Pb, Zn and Cr enter the surrounding environment due to abrasion of metallic vehicle parts. Pb indicates its origin from automobile exhaust [27], whereas Zn may be emitted by automobile tires and brake pads [28].

Various interactions are known to occur when plants are exposed to unfavorable concentrations of more than one trace metal. Such combinations effects were categorized by Berry and Wallace [28] as independent, additive, syner-

Table 3
Pigment analysis (Chlorophyll a, Chlorophyll b, Total chlorophyll, Chlorophyll degradation, Carotenoid) and protein content of *Pyxine cocoes* at twelve sites around FGUNTPC (values in each vertical column followed by the same alphabetic letter show there is no significant difference between them at $p < 0.05\%$ level by least significant difference (LSD) analysis).

Sites	Chlorophyll A	Chlorophyll B	Total chlorophyll	Chlorophyll degradation	Carotenoid	Protein
5 kmkm north	0.6 ± 0.0^{h}	0.3 ± 0.0^{f}	1.0 ± 0.0^{i}	0.5 ± 0.1^{h}	1.8 ± 0.1^{e}	18.6 ± 2.0^{b}
5 km south	0.7 ± 0.0^{g}	0.4 ± 0.0^{e}	1.1 ± 0.0^{h}	0.4 ± 0.0^{j}	2.1 ± 0.8^{b}	14.3 ± 1.8^{c}
5 km east	0.5 ± 0.0^{i}	0.6 ± 0.0^{i}	0.9 ± 0.0^{j}	0.6 ± 0.0^{g}	1.9 ± 0.9^{c}	19.3 ± 2.1^{a}
5 km west	0.2 ± 0.0^{j}	0.8 ± 0.0^{i}	0.1 ± 0.0^{k}	0.4 ± 0.0^{i}	1.8 ± 0.4^{d}	8.2 ± 1.4^{f}
10 km north	1.2 ± 0.5^{d}	0.6 ± 0.1^{d}	1.8 ± 0.5^{e}	0.6 ± 0.0^{g}	1.0 ± 0.2^{g}	12.8 ± 2.0^{d}
10 km south	1.0 ± 0.8^{f}	0.3 ± 0.2^{g}	1.4 ± 0.4^{f}	0.6 ± 0.2^{g}	1.0 ± 0.5^{g}	7.7 ± 1.9^{g}
10 km east	0.5 ± 0.0^{i}	0.2 ± 0.0^{j}	0.5 ± 0.2^{j}	0.9 ± 0.2^{f}	2.3 ± 0.9^{a}	8.8 ± 2.3^{e}
10 km west	1.1 ± 0.6^{e}	0.6 ± 0.0^{h}	1.2 ± 0. 5^{g}	1.1 ± 0.3^{d}	0.7 ± 0.2^{j}	4.8 ± 0.9^{e}
15 km north	1.8 ± 0.2^{a}	0.8 ± 0.3^{a}	2.6 ± 0. 5^{a}	1.8 ± 0.8^{a}	0.9 ± 0.4^{h}	5.4 ± 1.0^{h}
15 km south	1.4 ± 0.7^{c}	0.7 ± 0.0^{c}	2.1 ± 0.7^{d}	1.0 ± 0.0^{e}	1.4 ± 0.6^{f}	4.9 ± 1.4^{i}
15 km east	1.6 ± 0.0^{b}	0.8 ± 0.0^{b}	2.4 ± 0.9^{c}	1.4 ± 0.5^{c}	0.9 ± 0.8^{h}	4.9 ± 1.0^{i}
15 km west	1.8 ± 0.6^{a}	0.7 ± 0.0^{b}	2.5 ± 1.0^{b}	1.7 ± 0.5^{b}	0.8 ± 0.6^{i}	3.2 ± 1.1^{k}
One-way ANOVA						
f	577.1**	982.6**	1444.1**	2282.3**	1468.1**	21326.9**
LSD ($p < 0.05\%$)	0.05	0.02	0.05	0.02	0.03	0.09

Mean ± S.D., $n = 3$ in $\mu g\,g^{-1}$ fresh weight.
** Significance at the level of 0.01%.

gistic or antagonistic. In the present study the selectivity sequence are shown as mean values of each metals at 5 km Fe > Al > Zn > Pb > Cr > Cu > Ni; 10 km Al > Fe > Zn > Ni > Cr > Cu > Pb and at 15 km Al > Fe > Zn > Ni > Cu > Pb > Cr. It is clear from the sequences that Fe, Al and Zn exhibit least dispersion as compared to Pb, Cr, Cu and Ni. According to Garty [5], the pattern of increase near the source of metal/ash content and of decrease away from its relevant to the particulate nature of metals accumulated in lichen thalli. A significant decrease that correlates with distance from point of emission source refers to coarse particles that carry to a limited extent. Other studies detected a decrease of metal content at a distance of a few kilometers from the source of pollution [29,30] or even at a greater distance from emission points [31]. In the present study lichen samples showed accumulation phenomena for Fe, Al, Zn, Pb and Cr but not for Cu and Ni. The Cu, Ni levels in stack emission are low or that it is much better dispersed than the other once released from the power plant stack. In fact, during the combustion process Ni and Cu are known to undergo volatilization but not condensation, thus remaining in the gas phase; on the contrary, after volatilization elements such as Fe, Al, Zn, Pb and Cr condense on fly ash particles in increasing concentration as fly ash particle size diminishes [25].

3.3. Correlation of metals with distance at four directions

The distribution pattern of some metals and their exponential relationship with distance from power plant, showed that the combustion facility is a local source of atmospheric pollution like Cu, Ni, Pb and Zn. This result is in good agreement with other studies of environmental levels of heavy metals in the vicinity of power plants [3,32]. Dispersion of Cu, Ni, Pb and Zn exhibits better correlation with distance from FGUNTPC and its correlation (R^2) was found more than 0.8 for each direction. In case of Al, Cr and Fe this value was more than 0.8 for west direction only. Dispersion of Al is lowest in south followed by north and east, whereas Cr dispersed least in north followed by east and south. Fe showed its least dispersion in east followed by south and north on the bases of R^2 value (Fig. 3).

The wind direction mostly remains east to west in and around FGUNTPC, thus the west site has maximum dispersion of metals followed by north, south and east. The higher moisture content due to presence of a river in the west also helps in dispersion of metals in the west site. According to Bari et al. [33], climatic factors most probably play an important role in the bioaccumulation of heavy metals. The directions in which pollutants are transported by the wind are most surely fundamental in determining their main fallout point. Loppi et al. [26], correlates pollution from an industrial pole (Italy) with that at a distant agricultural centre, situated in the predominant wind direction.

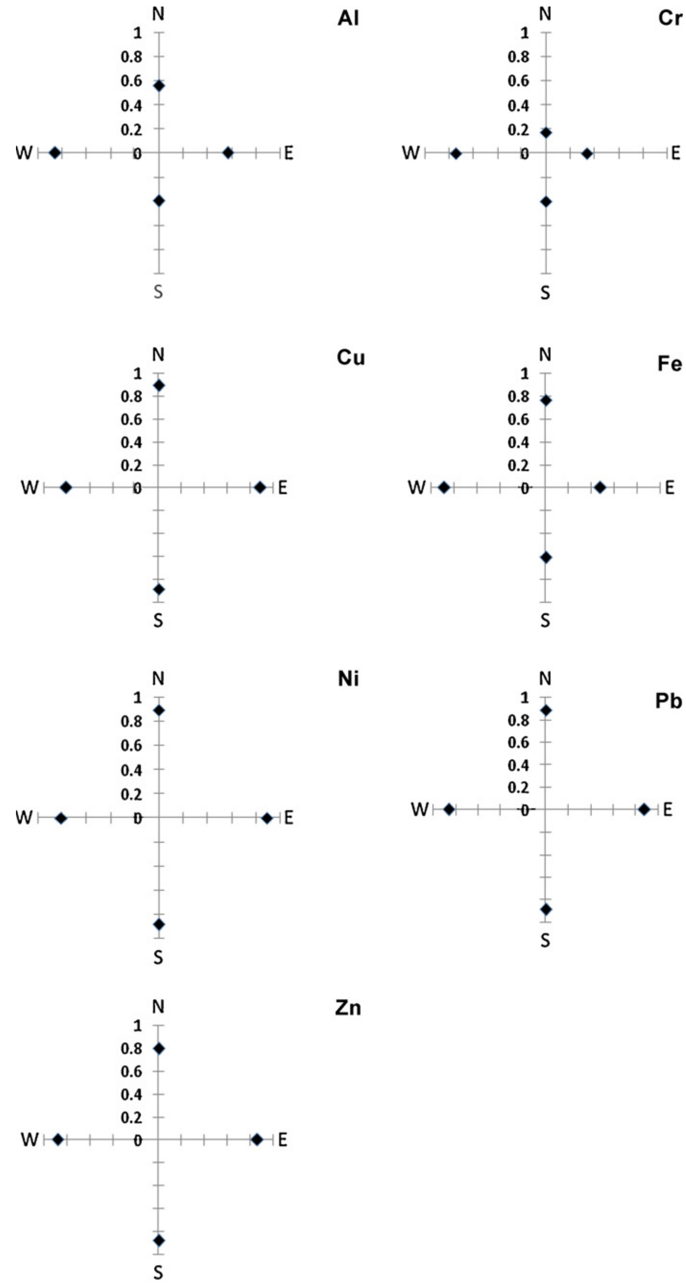

Fig. 3. Dispersion of heavy metal in the vicinity of FGUNTPC represented by R^2 values. (N = north, E = east, S = south, W = west).

3.4. Changes in lichen photobiont chlorophyll content

The highest values for chlorophyll a concentration were found at a distance 15 km away from FGUNTPC in all directions and ranges from 0.02 ± 0.0 to $1.8 \pm 0.2\ \mu g\ g^{-1}$ fresh weight (FW). The concentration of chlorophyll a increases with the increasing distance from the thermal power plant. Chlorophyll b content was less influenced as it ranges from 0.02 ± 0.0 to $0.8 \pm 0.2\ \mu g\ g^{-1}$ FW. The highest chlorophyll b was measured in the outskirts of thermal power plant i.e. 15 km followed by 10 and 5 km.

The total chlorophyll (chlorophyll a + chlorophyll b) ranges from 0.5 ± 0.2 to $2.6 \pm 0.5\ \mu g\ g^{-1}$ FW. Total chlorophyll also increases with increasing distance from the power plant. The concentration of total chlorophyll is altered by vehicular traffic pollution and urban emission [34]. Chlorophyll a, chlorophyll b concentrations as well as total chlorophyll concentrations were significantly affected by pollution in sites around power plant in accordance with distance and directions.

Chlorophyll degradation increased with increasing distance from FGUNTPC and it significantly differed in this parameter between peripheral parts of the FGUNTPC, it ranges from 0.4 ± 0.2 to $1.8 \pm 0.2\ \mu g\ g^{-1}$ FW. In this study maximum phaeophytisation was observed at the vicinity of 5 km followed by 10 and 15 km in all the directions.

Chlorophyll content and its degradation are often used as one of the cheapest and most accurate methods of biomonitoring. The ratio of optical density of chlorophyll samples read at 435 and 415 nm is the most frequently used parameter for chlorophyll degradation [35]. A ratio of 1.4 indicates that chlorophyll is unchanged, any reduction in this value indicates chlorophyll degradation with ensuring stress to the organism [36,37]. Kardish et al. [38], reported a value of 1.4 for chl/Ph ration of *Ramalina duriaei* in the control site, while for a polluted site with high levels of vehicular traffic, the value was 0.8. In the present study this ratio was highly affected at 5 and 10 km radius of FGUNTPC in *P. cocoes*, the highest amount of degradation was observed at close vicinity of sources.

The carotenoid and protein contents of *P. cocoes* have significantly decreased with the increasing distance from FGUNTPC. The highest amount of carotenoid recorded was at 10 km east (2.3 ± 0.9) and lowest accumulated was at 10 km west (0.7 ± 0.5 $\mu g\,g^{-1}$ FW). The protein ranges from 3.2 ± 1.1 to 19.3 ± 2.1 $\mu g\,g^{-1}$ FW and the maximum content of protein was recorded at 5 km followed by 10 and 15 km. The increased level of protein in present study, at most polluted sites corresponds with the findings of Gonzalez et al. [39] for the *Ramalina ecklonii*. In higher plants role of heat shock proteins in heavy metal tolerance have been established. Babula et al. [40] have reported the tolerance mechanism adapted by plant to withstand air pollution, which includes synthesis of stress metabolite and or protein. In the area with fly ash dumping sites, vehicular exhausts are the main source of metals that can alter the biosynthesis of protein. LSD studies showed that directions and distance from sources of pollution (FGUNTPC) play an important role in metal as well as pigment content of lichen thalii and exhibit significant difference at 0.05% level.

3.5. Impact of heavy metal stress on lichen pigment

According to Garty et al. [35], the chlorophyll integrity is inversely correlated with concentration of Cr, Fe, Mn, Ni and Pb.

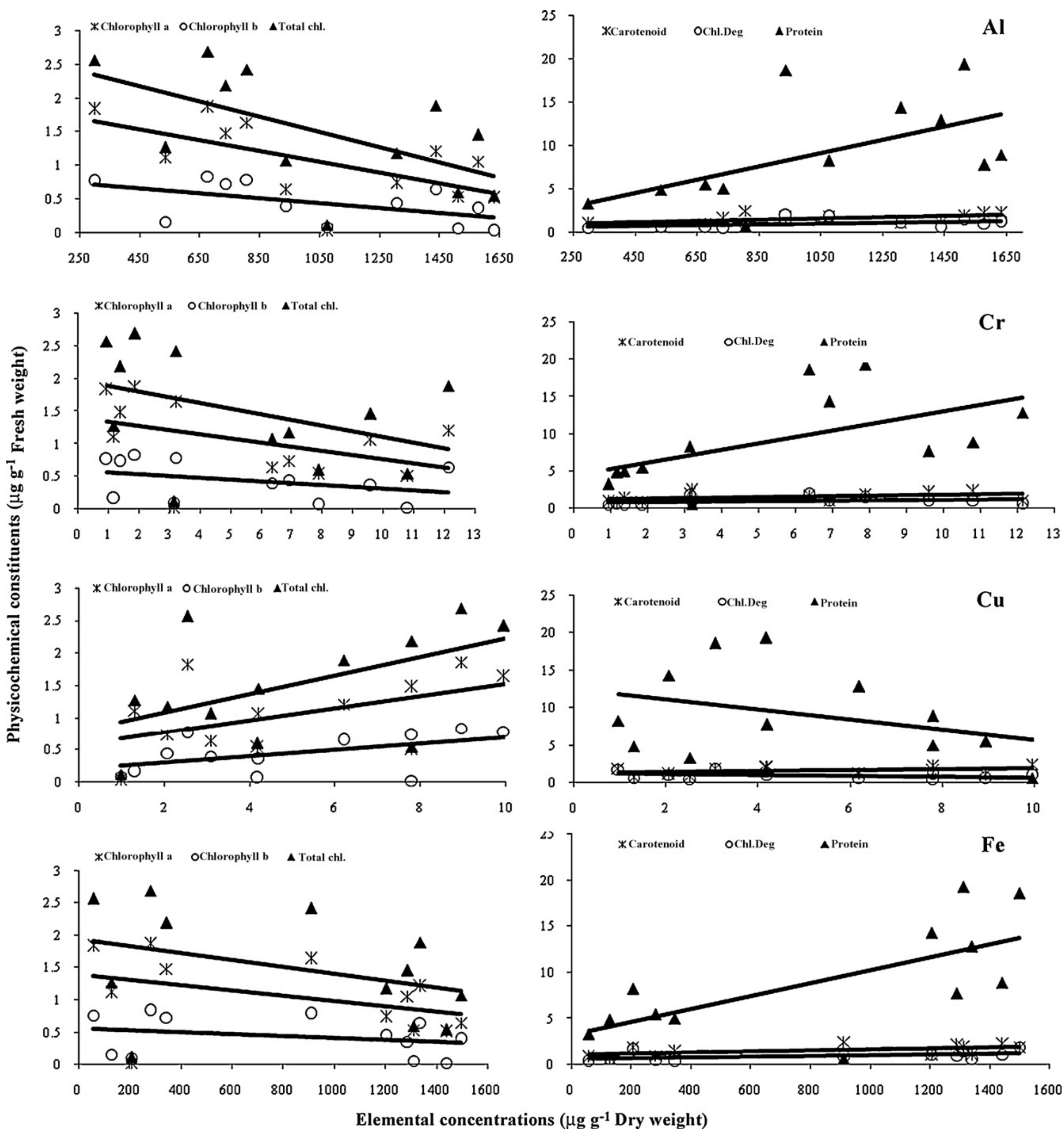

Fig. 4. Comparison between heavy metals stress and various physicochemical parameters, in a foliose lichen *P. cocoes* around FGUNTPC.

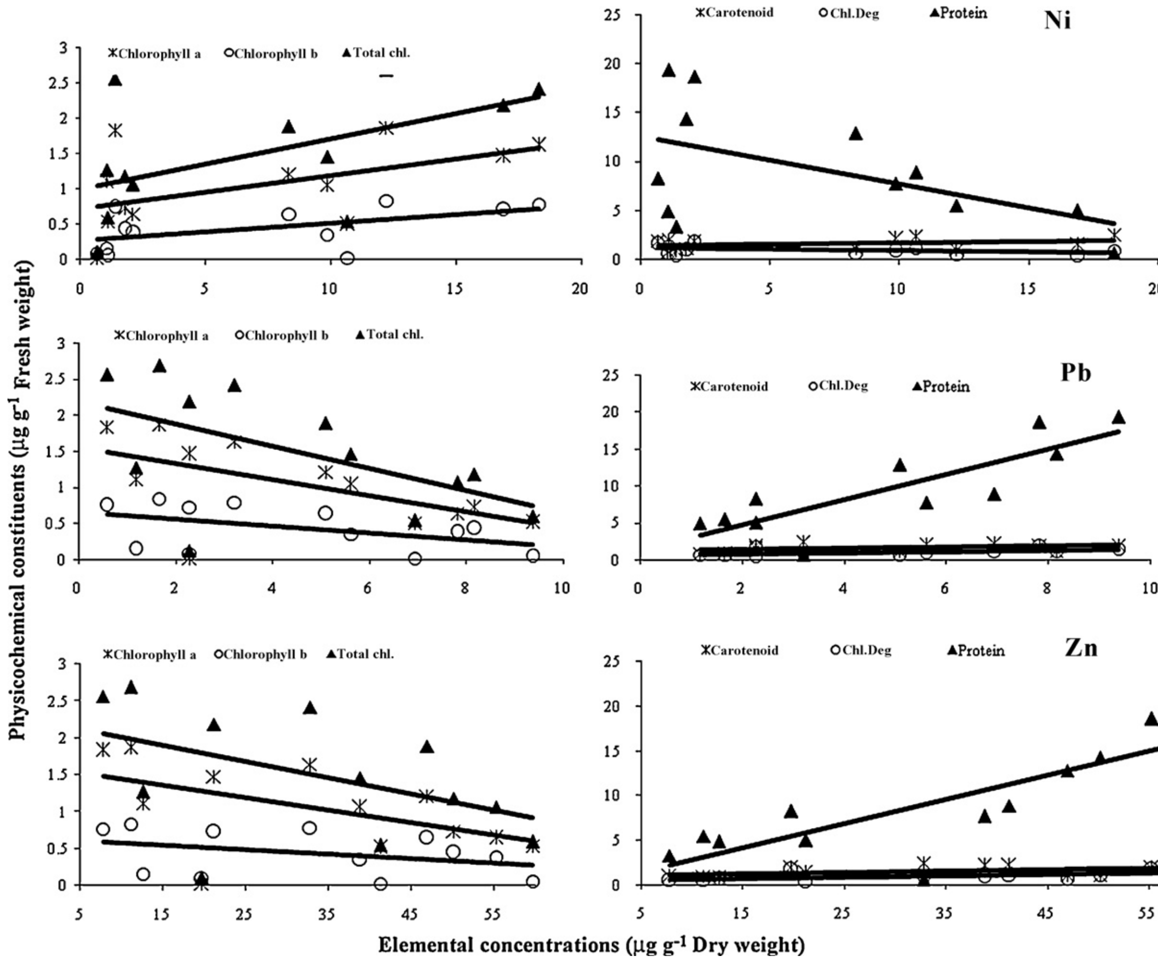

Fig. 4. (*Continued*)

In the present study due to accumulation of Al, Cr, Fe, Pb and Zn in the lichen thalli showed reduced amount of chlorophyll a, chlorophyll b and total chlorophyll, while the amount of protein was enhanced. Amount of carotenoid and chlorophyll degradation showed least correlation with the accumulation of metals. Trend of these pigments with Cu and Ni was opposite to other metals (Fig. 4). Brown and Beckett [41], studied the differential sensitivity of lichens to heavy metals and showed that Zn, Cd and Cu at substantially lower concentrations inhibit photosynthesis in lichens and Pb also decreased the total chlorophyll content and chlorophyll a/chlorophyll b ration in higher plant too.

Carotenoid has significant positive correlation with accumulation of Cu, Cr, Ni and Zn ($p < 0.05$) while it shows negative correlation with Al, Fe and Pb. Carotenoid content has maximum positive correlation with Cu, as its elevated concentration may supplement synthesis of carotenoid content [42]. Cu in high concentration can decrease total carotenoid in *Trebouxia* cell [43], the known photobiont of the family Physciaceae, tolerant species shows no alteration in the total carotenoid content.

Various interactions are known to occur when plants are exposed to unfavorable concentrations of more than one trace element. Such combination effects were categorized by Bery and Wallace [28] as independent, additive, synergistic or antagonistic. In the present study protein content showed positive correlation with all metals except Cu and Ni. The Cu and Ni have least effect on protein content or it may be due to synergistic or antagonistic effect by other metals [28]. *P. hispidula*, growing naturally in polluted areas showed increase in protein contents in order to combat the negative impact of increased elemental concentrations [15].

In the present study a direct relation between the metallic pollutants and some physiological parameters of *P. cocoes*, are evident. The higher correlation of chlorophyll degradation ratio with metallic pollutants is consistent similar to the studies of Garty and co-workers [5,35]; Levin and Pignata [36]. Among the seven metals analyzed Al, Cr, Fe, Pb and Zn seem to cause extensive damage to the photosynthetic pigments.

4. Conclusion

The present study illustrates the influence of prolonged exposure of a coal-based thermal power plant pollutants on diversity of lichen. *P. cocoes* a foliose, most common epiphytic lichen species was found to be an excellent accumulator of different metals, as the elements such as Fe, Al, Zn, Ni and Cr tend to concentrate in noticeable amounts in this lichen. *P. cocoes* as a biomonitor, indicates that metal pollution increases with decreasing distance from thermal power plant. The north direction of the study area is more polluted followed by west, east and south. The Pb, Cr, Ni and Zn significantly affect the physiology of *P. cocoes*. The function of *P. cocoes* as an environmental sensor is clearly displayed as physiological damage coincided with the accumulation of certain heavy metals. This study provides baseline data on metal concentration at different sites in and around the thermal power plant, which will be helpful for carrying out future biomonitoring studies in the area.

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