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# Estimation of physiological responses using two growth forms of lichens around coal-based Tanda Thermal Power Plant, Ambedkar Nagar district of Uttar Pradesh, India

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**Abstract**

Present study has been promulgated to study pigment profile and chlorophyll degradation of lichen communities around thermal power plant. Chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, chlorophyll degradation was measured to evaluate the possible damage caused by pollutants of the area in lichens. In order to determine the impact of power plant, two morphologically diverse lichen species viz., *Pyxine cocoes* and *Bacidia incongruens* have been used for conducting biomonitoring studies around Tanda thermal power plant, Ambedkar Nagar district of Uttar Pradesh. Correlations of various physiological parameters in lichen *P. cocoes* showed significant correlation (at $p<0.05$) of chlorophyll a with chlorophyll b (0.985) and negatively correlated with protein content (-0.958); while total chlorophyll content also negatively correlated with protein (-0.956). Besides that total chlorophyll showed highly significant correlations (at $p<0.01$ level) with chlorophyll a (0.998) and chlorophyll b (0.994); protein also showed negative correlation with all physiological parameters in *P. cocoes*. While in *B. incongruens* carotenoid showed negative significant correlation with chlorophyll a (-0.973), chlorophyll b (-0.598) as well as total chlorophyll (-0.858) at $p<0.05$ level. Total chlorophyll showed significant correlation at $p<0.05$ level (0.987) while carotenoid had negatively correlated with chlorophyll degradation (-0.927). Protein content showed negative correlation with chlorophyll b (-0.065) and carotenoid (-0.175). The study revealed that chlorophyll and protein content are an efficient physiological parameter can be correlated with quality of air.

Overall pattern evident that both *P. cocoes* while *B. incongruens* showed more or less similar concentration of photosynthetic pigment and protein content. Both the lichen species showed increasing trend with the increasing distance from the thermal power plant.

**Keywords** – Biomonitoring – Environment – Lichen – Pollution – Thermal power plants

## Introduction

The primary sources of energy in India are renewable resources such as coal, wood etc. and its utilization in power generation is emerging as the biggest environmental problem. Because it emits fly ash, acid precursors, green house gases, non-combustible hydrocarbons, heavy metals and

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particulates matters. These pollutants can be carried to a long distance by wind and ultimately have a negative impact on both biotic and abiotic environment (Cicek et al. 2001). The thermal power plants in the country are the major source of power generation which not only deteriorate the natural resources but also affects the human health upto a greater extends. Air pollution alters community structure which is produced by changes in the community composition. According to Wirth (1988), the advantages and disadvantages of phyto-sociological approaches to monitoring temporal and spatial changes in air quality.

Lichens are excellent organisms for environmental pollution monitoring due to their peculiar morphology, anatomy and chemistry. Lichens are being used as an effective biomonitors of air pollution. Several lichen species have been employed worldwide for monitoring of air quality (Conti & Cecchetti 2001, Garty et al. 2003, Upreti et al. 2009, Shukla & Upreti 2007a, b, 2009). Lichens are best organism to study the effect of air pollution on lichen communities, lichen physiology and/ or growth and for the study of pollutant distribution. Lichens are peculiar plants in having strange impulsivity for acidic gases, but collect several organic and inorganic even radiouclide contaminants in huge amount beyond their physiological level and thus can be used as biological indicators of air quality.

Lichens can be used as an important tool to assess the level of air borne pollutants arising from anthropogenic activities, mining, construction, agriculture, automobiles, smelters (Sawidis et al. 1995, Garty 2001). Lichens are very sensitive to air pollution and alteration in air quality directly affects the lichen diversity, therefore, they are generally considered as good indicators of air quality (Seaward 1993, Cislaghi & Nimis 1997, van Dobben & ter Braak 1999, Crespo et al. 2004). Lichens have certain characteristics viz., perennial nature, absence of root and lack of cuticles which meet several requirements of the ideal biological monitors. The high capability of lichens to accumulate air pollutants resistance to environmental stress and longevity are the other features that make them most suitable for biomonitoring studies (Garty 2001). A number of parameters are used to estimate the effect of air pollution on lichens as reported by Ronrn & Galun (1984).

A number of studies have assessed the damage in transplanted lichens by using physiological parameters such as rate of respiration (Baddeley et al. 1972), decrease of ATP content, variations in respiration levels (Kardish et al. 1987), photosynthesis (Showman 1972, Ronrn & Galun 1984, Calatayud et al. 1999), chlorophyll content and degradation (Kardish et al. 1987, Ronrn & Galun 1984, Garty et al. 1988, Balaguer & Manrique, 1991, Zaharopoulou et al. 1993), production of stress ethylene (Garty et al. 1997) and Malondialdehyde (MDA) content (González & Pignata 1994).

Also studies on pollution monitoring utilizing lichens as bioindicator are available from different regions of the world (Conti & Cecchetti 2001, Kircher & Darllant 2002). Recently few passive as well as active (transplant) biomonitoring studies using lichens have been carried out in different cities of India in having varied climatic conditions (Dubey et al. 1999, Upreti & Pandey 2000, Pandey et al. 2002, Mishra et al. 2003, Bajpai et al. 2004, Saxena et al. 2007, Shukla & Upreti 2007a, b, 2008, Bajpai et al. 2010a, b, 2013a, b, Gupta 2014, Gupta et al. 2015, 2017a, b). Few reports on the effect on vegetation and decline of air quality are available around thermal power plants from India (Singh et al. 1994, Rao et al. 1990). But, studies done by Bajpai et al. (2010a, b) on physiological variations, metal accumulation and its spatial distribution around Feroze Gandhi Unchahar Thermal power plant, Raebareli, Uttar pradesh is known from India.

In the present study, an attempt has been made to elucidate the impact of thermal power plant emission in two growth forms of lichen species *Pyxine cocoes* and *Bacidia incongruens*, to determine the changes in their pigment profile, chlorophyll degradation and protein content.

## Materials & Methods

### Study area

Tanda is situated in Ambedkar Nagar district, located on North-Eastern part of Uttar Pradesh, India; lies between 26°09" N to 26°40" N latitudes and 82°12" E to 83°05" E longitudes. Total cover area of the district is 2520 km²; the total length (from east to west) of the district is approximately 75 km and the breadth (from north to south) is about 42 km. Tanda thermal power plant coordinates between 26º33'00"N and 82º39'00"E (Fig. 1A, B) and is surrounded by agriculture fields, mango orchards along with two National Highways 233A & 232 (India) also passes through the town. The coal to all units is fed from North Karnpura Coal Fields, while source of water for the power plant is from Tanda Pump Canal of Saryu River. It has electricity production capacity of 440 MWs (4 x 110MW=440 MW) for Stage-I; For Stage-II, it has started installation of two new units of 660 MWs each (2 x 660MW=1320 MW) from October, 2019; which convert heat energy into electric power. So, the total power generation is 1760 MWs from Tanda thermal power plant. The regional climate of study area is classified into three distinct seasons- Hot weather season (March to mid-June), monsoon season or rainy season (mid-June to October) and Cold weather season (November to February). The reversal of winds takes place twice a year. The prevailing winds blow from west to east, which are influenced by pressure distribution pattern of the Himalayas. In November last year, the high-pressure belt extends from north-west India and covers the whole of the Uttar Pradesh. The fast pace of industrialization, urbanization together with the destruction of forest resulted in few scattered, open canopy deciduous forests in the district.

**Sample collection**

The area around thermal power plant was randomly surveyed for collection of lichens from 42 localities in all four directions i.e. east, west, north and south of the power plant (Fig. 1C). Lichens especially, *P. cocoes* and *B. incongruens* were growing abundantly in all four directions on *Mangifera indica* bark, tree trunk, branches. These species were widely distributed in the area thus both the species were sampled for photosynthetic pigment and protein analysis.

The collected samples were dried and kept inside the paper packet. The lichen samples were determined by their morphological, anatomical and chemical characters by using LABOMED dissecting microscope for external morphology, while LEICA ATC 2000 compound microscope was used for microscopic anatomical details of the samples. The chemical substances present in the lichen thallus were identified through TLC in solvent system A (Toulene,1-4 Dioxane and Acetic acid, 180ml: 60ml: 8ml) (Orange et al. 2001, Culberson 1972, Walker & James 1980). The voucher specimens were preserved in Lichen Herbarium (LWG) of CSIR- National Botanical Research Institute, Lucknow, India.

**Pigment Analysis**

The chlorophyll content was calculated from absorbance values at 663 and 645nm according to the equation given by Arnon (1949). The total carotenoid content was calculated according to Parsons et al. (1984) from absorbance values at 480 and 510 nm.

**Chlorophyll Degradation**

The method developed by Ronrn & Galun (1984) was used to measure intensity of the photobiont chlorophyll. The chlorophyll was extracted overnight in the dark in 5.0 ml dimethyl sulfoxide (DMSO, Merck, analytical grade). The ratio of chlorophyll a to phaeophytin a (OD 435/415nm ratio) was determined.

**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 700nm (Lowry et al. 1951).

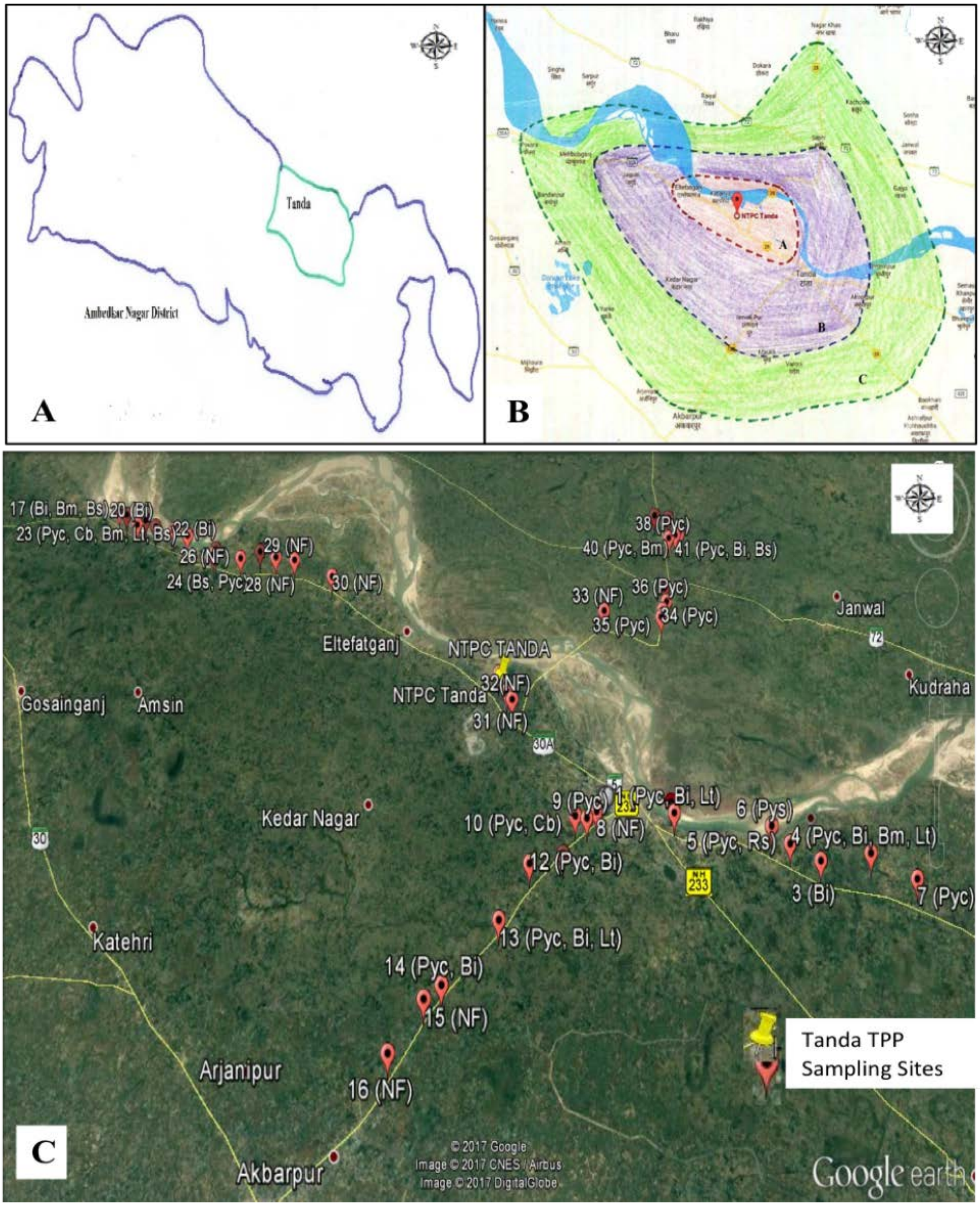


**Fig. 1** – Study area: A Uttar Pradesh Region Showing Ambedkar Nagar district. B Zone Map showing distribution pattern of lichen taxa around Tanda Thermal Power Plant, Ambedkar Nagar: Zone A: Poor lichen growth- Polluted; Zone B: Moderate lichen growth- Moderate Pollution; Zone C: Normal lichen growth- More or less Pollution free area. C Sampling sites around Tanda Thermal Power Plant, Ambedkar Nagar (Source: Google Earth).

**Statistical analysis**

The data obtained were subjected to one-way analysis of variance (ANOVA) to evaluate Differences in chlorophyll response to air pollution and the probable correlation between pigments and protein content using statistical SPSS 16.0 program.

## Results & Discussion

The study area revealed the occurrence of 11 lichen species belonging to 7 genera and 6 families from 42 monitoring sites upto a distance of 21 km in all directions (i.e. east, west, north and south) (Fig. 2). It is observed that lichen thalli of *P. cocoes* and *B. incongruens* increases with increasing distance from thermal power plant and showed more or less similar trend of concentration. Among all the collected lichen species, *Pyxine cocoes* and *Bacidia incongruens* were showed their dominance over other species in the area surveyed. The results of the physiological analysis were evaluated by one way ANOVA, thus evaluating the effects of the sampling sites on the bioaccumulation and physiological status of lichens (Tables 1, 2).

**Table 1** Photosynthetic pigment and protein content analysis of lichens in all directions around Tanda Thermal Power Plant, Ambedkar Nagar

| Directions | *P. cocoes* (concentration in $\mu gg^{-1}$ Fresh weight) | | | | | |
|---|---|---|---|---|---|---|
| | **Chl. a** | **Chl. b** | **Total Chl.** | **Carotenoid** | **Chl. deg.** | **Protein** |
| **East** | 0.40±0.05 | 0.16±0.04 | 0.56±0.09 | 0.40±0.04 | 1.08±0.04 | 1.54±0.06 |
| **West** | 0.77±0.07 | 0.37±0.11 | 1.13±0.18 | 0.58±0.10 | 1.02±0.02 | 1.28±0.17 |
| **North** | 0.59±0.02 | 0.23±0.01 | 0.82±0.03 | 0.22±0.14 | 0.70±0.02 | 1.36±0.16 |
| **South** | 0.79±0.05 | 0.38±0.08 | 1.17±0.13 | 0.53±0.09 | 1.06±0.01 | 1.15±0.13 |
| **CV%** | 7.814 | 24.614 | 12.85 | 22.70 | 2.640 | 10.179 |
| **LSD ($p$< 0.05)** | 0.099** | 0.141* | 0.237** | 0.197** | 0.051** | 0.271* |
| **Directions** | ***B. incongruens* (concentration in $\mu gg^{-1}$ Fresh weight)** | | | | | |
| | **Chl. a** | **Chl. b** | **Total Chl.** | **Carotenoid** | **Chl. deg.** | **Protein** |
| **East** | 0.59±0.31 | 0.31±0.07 | 1.06±0.10 | 0.46±0.11 | 1.13±0.09 | 1.54±0.47 |
| **West** | 0.68±0.10 | 0.27±0.06 | 0.95±0.15 | 0.42±0.01 | 1.12±0.01 | 1.34±0.09 |
| **North** | 0.27±0.05 | 0.22±0.01 | 0.48±0.06 | 0.53±0.07 | 0.56±0.01 | 1.40±0.31 |
| **South** | 0.66±0.01 | 0.25±0.03 | 0.91±0.04 | 0.45±0.01 | 1.01±0.06 | 2.10±0.29 |
| **CV%** | 29.693 | 17.283 | 11.553 | 13.63 | 5.595 | 20.097 |
| **LSD ($p$< 0.05)** | 0.327* | 0.091 NS | 0.197** | 0.127 NS | 0.107** | 0.640NS |

Mean ± S.D., n = 3 in $\mu gg^{-1}$ Fresh weight; S. D. = Standard Deviation; NS = Non-Significant
* Significance at the level of 0.05
** Significance at the level of 0.01

**Table 2** Values of correlation matrix between the physiological parameters around Tanda Thermal Power Plant, Ambedkar Nagar

| ***P. cocoes*** | **Chl. a** | **Chl. b** | **Total Chl.** | **Carotenoid** | **Chl. deg.** | **Protein** |
|---|---|---|---|---|---|---|
| **Chl. a** | 1 | .985* | .998** | 0.595 | 0.072 | -.958* |
| **Chl. b** | | 1 | .994** | 0.722 | 0.241 | -0.933 |
| **Total Chl.** | | | 1 | 0.639 | 0.133 | -.956* |
| **Carotenoid** | | | | 1 | 0.813 | -0.485 |
| **Chl. deg.** | | | | | 1 | -0.042 |
| **Protein** | | | | | | 1 |
| ***B. incongruens*** | **Chl. a** | **Chl. b** | **Total Chl.** | **Carotenoid** | **Chl. deg.** | **Protein** |
| **Chl. a** | 1 | 0.621 | 0.904 | -.973* | 0.944 | 0.377 |
| **Chl. b** | | 1 | 0.891 | -0.598 | 0.842 | -0.065 |
| **Total Chl.** | | | 1 | -0.858 | .987* | 0.247 |
| **Carotenoid** | | | | 1 | -0.927 | -0.175 |
| **Chl. deg.** | | | | | 1 | 0.19 |
| **Protein** | | | | | | 1 |

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

**Physiological response of *P. cocoes* around Tanda Thermal Power Plant, Ambedkar Nagar**

*P. cocoes* (foliose) and *B. incongruens* (crustose) lichen commonly occurring within the contaminated and non-contaminated area were selected for photosynthetic pigment analysis and protein estimation. Concentration values for chlorophyll a in *P. cocoes* showed minimum concentration (0.40±0.05 $\mu gg^{-1}$ Fresh weight) in east direction whereas maximum concentration (0.79±0.05 $\mu gg^{-1}$) in south direction. Chlorophyll b content also varied in different directions and it ranged from 0.16±0.04 $\mu gg^{-1}$ (east) to 0.38±0.08 $\mu gg^{-1}$ (south). According to Ronrn & Galun (1984) and Chapin et al. 1987), concentration of chl. a + b is altered by vehicular traffic pollution. Total chlorophyll (chlorophyll a + b) ranged from 0.56±0.09 $\mu gg^{-1}$ (in east) to 1.17±0.13 $\mu gg^{-1}$ (in south direction). Photosynthetic parameters (chl. a, chl. b and total chlorophyll) showed the same pattern of variations in higher and lower concentration which might be due to effect of wind direction and pollution loads. Chlorophyll contents are often utilized as one of the most accurate methods of biomonitoring in the study.

In *P. cocoes,* carotenoid showed its higher concentration of in north direction (0.22±0.14 $\mu gg^{-1}$), while lower concentration in west direction (0.58±0.10 $\mu gg^{-1}$) respectively. Carotenoid and protein contents in *P. cocoes* have significantly decreased with the increasing distance from thermal power plant. Chlorophyll degradation showed maximum concentration in east direction and decreases with increasing distance in west, south and north directions from thermal power plant. Chlorophyll degradation ranged from 0.70±0.02 $\mu gg^{-1}$ (in north) to 1.08±0.04 $\mu gg^{-1}$ (in east direction). According to Carreras et al. (1998), the concentration of total chlorophyll is governed by the ambient environment, anthropogenic sources, vehicular emission, urban emission and pollution loads. Protein concentration ranged from 1.15±0.13 $\mu gg^{-1}$ (in north) to 1.54±0.06 $\mu gg^{-1}$ (in east direction). The values of chlorophyll degradation and protein content were minimum in north and maximum in east direction as shown in Table-1. The increased level of protein in the present study, at most contaminated sites corresponds with the findings for the *Ramalina ecklonii* (González et al. 1996). LSD analysis at $p< 0.01$ level showed significant difference in chl. a, total chlorophyll, carotenoid and chlorophyll degradation at different directions, while chl. b and protein may vary significantly at the $p< 0.05$ level (Table 1, 2, Fig. 2A, 3).

**Physiological response of *B. incongruens* around Tanda Thermal Power Plant, Ambedkar Nagar**

In case of *B. incongruens*, chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll degradation showed similar variations in lower concentration in north direction; whereas carotenoid and protein content showed settling of lower concentration in west direction of the study area. Chlorophyll a ranged between (0.27±0.05 $\mu gg^{-1}$ to 0.68±0.10 $\mu gg^{-1}$) while chlorophyll b and total chlorophyll showed minimum concentration in north direction having values 0.22±0.01 $\mu gg^{-1}$ and 0.48±0.06 $\mu gg^{-1}$ respectively. But maximum concentration of chlorophyll b (0.31±0.07 $\mu gg^{-1}$) and total chlorophyll (1.06±0.10 $\mu gg^{-1}$) have settled in east direction of the study area. Carotenoid content of *B. incongruens* showed maximum concentration (0.53±0.07 $\mu gg^{-1}$) in north and minimum concentration (0.42±0.01 $\mu gg^{-1}$) in west direction of the thermal power plant. Chlorophyll degradation ranged from 0.56±0.01 $\mu gg^{-1}$ (in north) to 1.13±0.09 $\mu gg^{-1}$ (in east direction). Chlorophyll content and its degradation are cheapest and most accurate methods of biomonitoring (Garty 2001, Bajpai et al. 2010a). According to Garty et al. (2000), optical density values of pigment samples examine at 435 and 415 nm, is valuable parameter for chlorophyll degradation. Protein content in *B. incongruens* ranged from 1.34±0.09 $\mu gg^{-1}$ (in west) to 2.10±0.29 $\mu gg^{-1}$ (in south direction). LSD studies showed that directions from sources of pollution (i.e. thermal power plant) play an important role in pigment concentration of lichen thalii as well as exhibit significant difference at 0.01 levels in total chlorophyll and chlorophyll degradation in particular. While, only chl. a showed significant difference at $p< 0.05$ level but Chl. b, carotenoid and protein showed non-significant differences (Table 1, 2, Fig. 2B, 4).

Overall pattern of photosynthetic pigments and protein content showed more or less similar concentration trend in both lichen species *P. cocoes* (foiliose lichen) and *B. incongruens* (crustose lichen); but most of the variations in depicted from Zone b and c from the study area. Photosynthetic pigment analysis and protein content varied with the direction and distance from source of pollution. The study also revealed the increasing trend with the increasing distance from the thermal power plant. Levin & Pignata (1995), Silberstein et al. (1996), reported a ratio of 1:4 indicates that chlorophyll is unchanged; any reduction in this value indicates chlorophyll degradation with ensuring stress to the organism. Babula et al. (2008) concluded the tolerance mechanism adapted by plant to with still air pollution, which includes synthesis of stress metabolite and protein. In the area with fly ash dumping sites, vehicular exhausts are the main source of metals that can alter the biosynthesis of protein (Tangahu et al. 2011).

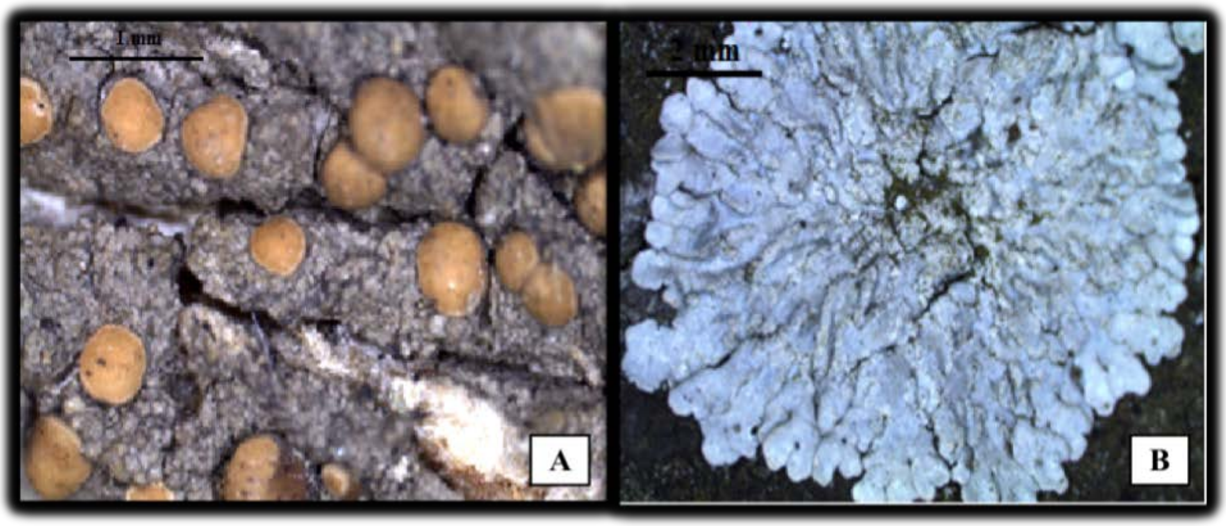


**Fig. 2** – Thallus of Lichen Species: A *Bacidia incongruens* (Stirton) Zahlbr. B *Pyxine cocoes* (Sw.) Nyl.

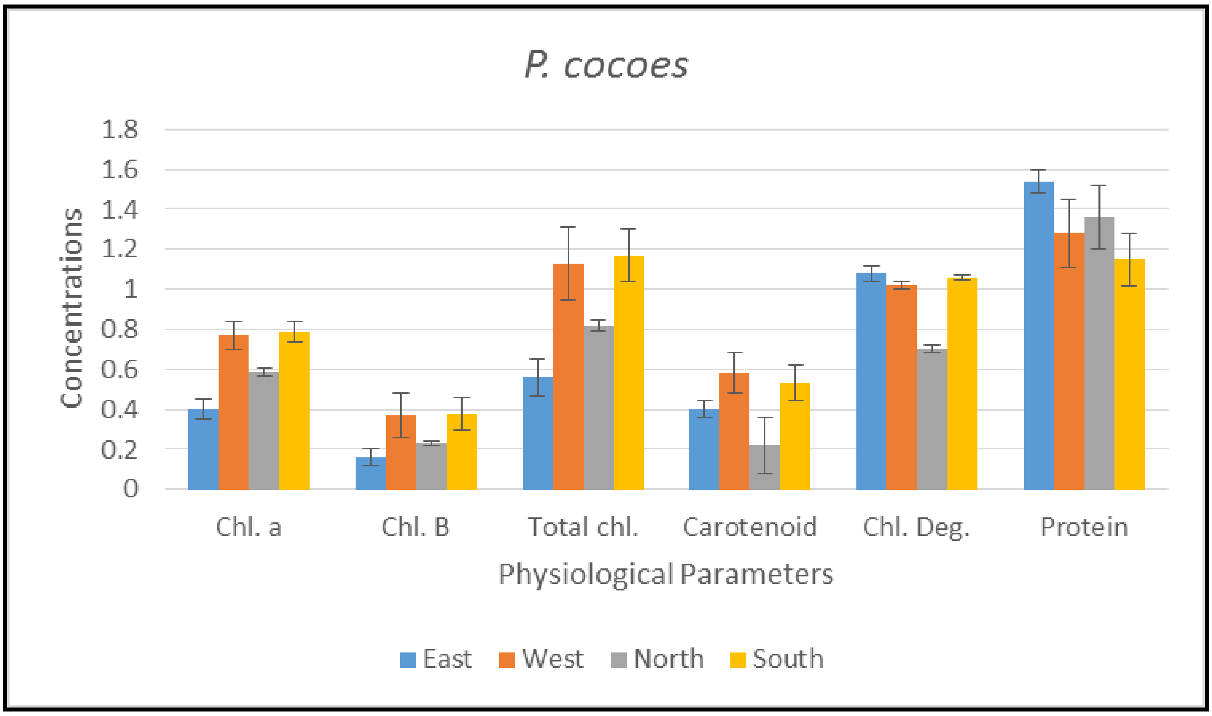


**Fig. 3** – Photosynthetic pigments in the thalli of *P. cocoes* in all directions around Tanda Thermal Power Plant, Ambedkar Nagar.

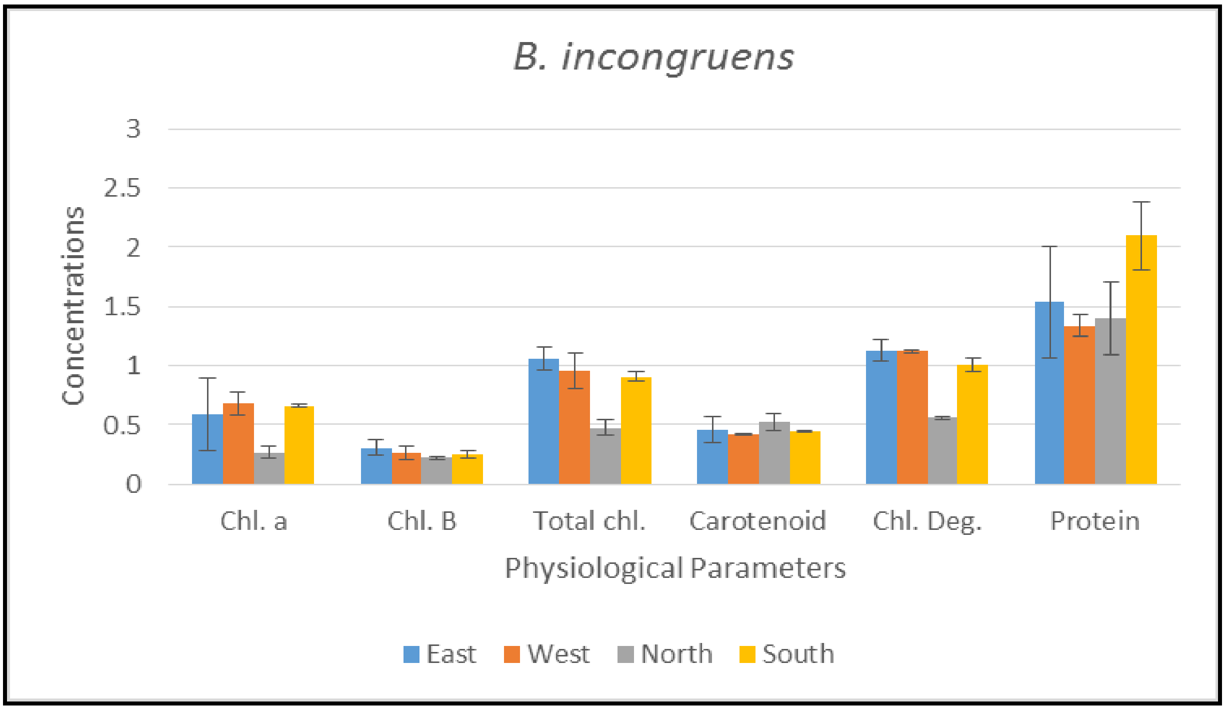


**Fig. 4** – Photosynthetic pigments in the thalli of *B. incongruens* in all directions around Tanda Thermal Power Plant, Ambedkar Nagar

Correlations of various physiological parameters in lichen *P. cocoes* (Tables 1, 2) showed significant correlation (at $p<0.05$) of chlorophyll a with chlorophyll b (0.985) and negatively correlated with protein content (-0.958) while total chlorophyll content also negatively correlated with protein (-0.956). Besides that, total chlorophyll showed highly significant correlations (at $p<0.01$ level) with chlorophyll a (0.998) and chlorophyll b (0.994); protein also showed the negative correlation with all physiological parameters in *P. cocoes*. Whereas *B. incongruens* (Table-1 & 2), the carotenoid showed negative significant correlation with chlorophyll a (-0.973) at $p<0.05$ level and negative significant with chlorophyll b (-0.598) as well as total chlorophyll (-0.858). Total chlorophyll showed significant correlation at $p<0.05$ level (0.987) while carotenoid had negatively correlated with chlorophyll degradation (-0.927). In *B. incongruens*, protein content showed negative correlation with chlorophyll b (-0.065) and carotenoid (-0.175).

## Conclusion

Based on the above observations, it is well evident that lichens are excellent indicator and the utility of lichen species as an environmental sensor for the monitoring of air quality. Present study revealed that both lichen species *P. cocoes* and *B. incongruens* at all sites showed more or less similar selectively sequence of physiological pattern and protein concentration around coal-based thermal power plant i.e. increases with the increasing distance towards the outskirts from study area. It also provides direct evidence about the air quality status of the area. The reason for the higher concentration levels of photosynthetic pigments and protein may be due to pollutants emerging from thermal power due to coal burning together with anthropogenic activities in the area.

The pigment and protein concentration in both the lichens helped in evaluation of risk to the population living in vicinity of coal-based thermal power plant together with long-term hazard due to photosynthetic concentration. The study also provides an understanding about the mechanisms adopted by different growth form of lichens for physiological response and baseline data on concentration of photosynthetic pigment and protein analysis around thermal power plant which will be helpful for carrying out future biomonitoring studies in the area. The physiological studies carried out on lichens with chlorophyll degradation, protein estimation and carotenoid will help to understand the damage caused by pollutants to living organisms around thermal power plant.

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