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Research Article

Pollution monitoring and physiological changes of Lichens in and around National Thermal Power Corporation (NTPC) Unchahar, Raebareli, 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 and protein were estimated in Pyxinecocoes, Rinodinain, Lecanora and Anisomeredium which are common lichen growing around thermal power plant for further inference. Distributions of photosynthetic pigments from power plant showed positive correlation with distance for all directions. Least significant difference analysis showed that speed of wind and its direction plays a major role in dispersion of Chlorophyll content 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 and carotenoid exhibited significant decrease.

Keywords: Lichens, Pollution monitoring, Physiological changes.

1. Introduction

Air pollution is usually measured with instruments. However during last two decades lichens have received increasing attention as bio indicating organisms and their use as terrestrial bio monitors of environmental contamination in urban, industrial and rural areas has been a practice for quite some time. Weather we live in a city or way out in the country, each of us would like to have the assurance that the air in our neighborhoods is clean. We need a measuring device that is cheap, that can be used anywhere and that respond to many kind of pollutants. Lichens especially which grow on trees provide a partial but useful

substitute for the purpose. They are cheap and easily available.

Most of the work on the accumulation of heavy metals by lichens has focused on pollution from smelters, power plants and vehicular activities in both urban and rural areas. Lichens have certain characteristics, which meet several requirements of an ideal bio monitor. That includes their large geographical ranges, morphology that does not vary with seasons, lack of cuticles and well enveloped root structure, longevity, rapid uptake and accumulation of mineral nutrients from atmosphere. The lack of cuticles and stomata allows many contaminants to be absorbed over the whole lichen surface (Hale 1983; Puckett 1988). These plants are perennial, slow growing organisms that maintain a fairly uniform morphology in time (Nyangababo 1987; puckett 1988; garty 1993) and do not shed parts as readily as vascular plants. Lichen is not a single plant but is a result of symbiotic association of two different plants, algae and fungi. The lichens are widely distributed in almost all the phytogeographic regions of India.

They represent about 85 of the total terrestrial plants known from the world and out of the 20,000 lichen species so far known from the world only 2,450 species are present in the Indian subcontinent, of which India has got 2,150 species (Awasthi 2000). Lichen has a long history of use as biological indicator of air quality (Rao and LeBlanc 1967;Le Blanc and Rao 1975; Hawksworth and Rose 1976; Richardson and Nieboer 1981; Brown 1984; Foarmeret al., 1992; Riehardson 1993). Brunialti et al (2002) underwent quality control tests during five lichen bio monitoring workshops organized between 1999 and 2000 in central and north western Italy. Limbic et al (2005) used the zonation method based on average annual and short term concentration of pollutants in the air as well as on deposition loads of dust and calcium for characterization of landscapes in north-eastern Estonia affected by alkaline oil shale, fly ash and cement dust. In India, a large number of pollution monitoring studies with higher plants are available (Rao and Dubey 1992; Singh et al., 1997). However such studies utilizing lichens have started recently (Dubey et al., 1999; Upreti and Bajpai 2002). There has been comparatively few information available regarding use of lichen as bio indicator of pollution in India.in the present study it is proposed to evaluate lichens as bio monitor and bio indicator of pollution in and around National Thermal Power Corporation (NTPC),Unchahar, Raebareli district based

on the specimens collected from different site of Unchahar and zonal map study with the help of lichen distribution pattern. This study included survey and collection, distribution of lichens in the study area and physiological experiments and study also determine pollution tolerant and sensitive species in and around NTPC. The study also provides a complete list of different lichen species in and around NTPC, Unchahar district together with their distribution. No such bio monitoring studies using lichens was done earlier in this area. Henceforth, the present study reveals the level of pollution in that area by dividing the area in three zones. The study provides a base line data for carrying out future bio monitoring studies as well.

2. Methodology

2.1 Study area

NTPC, Unchahar is situated in Raebareli district, Uttar Pradesh (UP) which was established in 1975. It is a public sector corporation having governmental share of 80%. NTPC uses coal to generate electricity, because of which various gases and fly ash is released as major pollutants. The district Raebareli has an area of 1748 m^2. Figure.1 showing map of the study area.

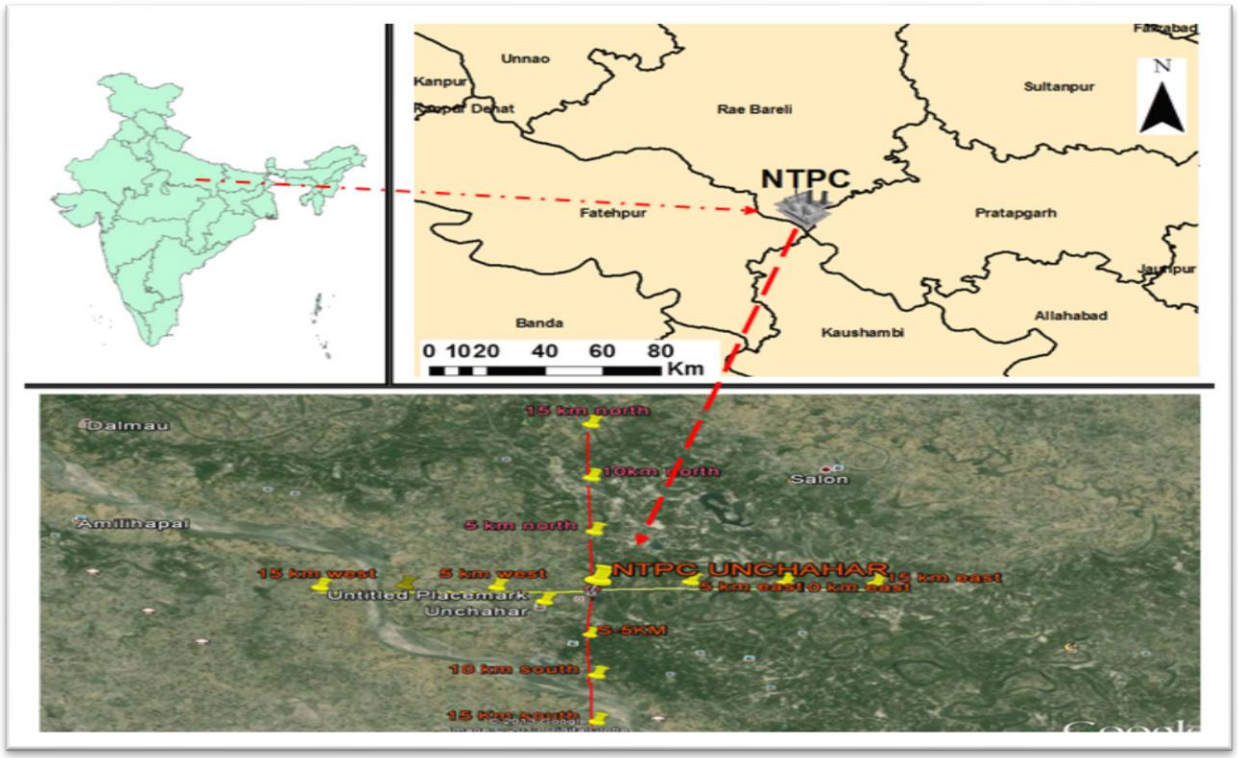

Figure.1 Map of sampling site

2.2Survey and collection

The lichens were collected during February to April 2008. They were collected from all the four directions i.e. North, West, South and East around NTPC. Mostly epiphytic and foliose lichens were collected from the bark of mongo, banyan, jackfruit and lasoda trees. Epiphytic lichens respond better to air pollution. All lichens were scored that was able to determine to the genera level in the field and collect rest of the species for the laboratory determination. Only well-known and easily recognized species can be determined and mapped in the field. Lichens were collected from far away from the roadside from above the chest height. The specimens were usually removed along with bark of the substratum. The lichens on the twigs and trunks were collected using a sheath knife; whereas geological hammer and chisels were used for those hard pressed. Collected specimens were placed in paper bags together with details of locality, substrate, ecological notes and date of collection. All the collected specimens of the lichen in the present study were identified and preserved in the herbarium of CSIR (National Botanical Research Institute), Lucknow.

2.3Zone mapping

The major approach to the study of lichens In the areas affected by air pollution has been based on distributional studies. These have resulted in the construction of zonal maps, in which the distribution of one or more species correlates well with prevailing levels of pollution.

NTPC was chosen as the study area and the whole district was divided into four major areas from the center of the NTPC. The center of the area is considered as 0 km. from this starting point (0km) the collection was performed towards all the four sides of the district. The survey was started from the center of the NTPC where pollution levels were known to be high. Magniferaindica is relatively abundant plant species in the area. Most of the sites surveyed consisted of mango orchard in patches and exposed to more or less similar conditions of light, temperature and humidity. Being situated in and around NTPC the area experience heavy gaseous pollution due to emission from NTPC, like SOX, NOX and other gaseous species.

2.4 Identification of the Thallus

In the laboratory the specimens were investigated morphologically, anatomically and chemically. Morphological identification of lichen is done by Stereo zoom microscopy

and anatomically by thin hand section cutting and chemically by the color test and TLC. The color test for identification of lichens was performed with the usual reagent that is K (5% potassium hydroxide), C(aqueous solution of calcium hypochlorite) and PD (para-phenylenediamine).

2.5 Morphological test

The external morphology of thalluswas studied under a dissecting binocular microscope with magnification of 10x to 100x. The surface of the thallus, areoles, margin of lobes, presence or absence of isidia, soredia, external cephalodia, cyphellae, pseudocyphellae, cilia, rhizinewere examined.

2.6 Pigment analysis

The method developed by 'Ronen and Galun' (1984) was used to measure integrity of the photobiont chlorophyll. The ratio of chlorophyll-a tophaeophytin (OD 663 nm/OD 645nm) was determined using a Genesys 10 UV scanning spectrophotometer.Photosynthetic pigments (chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids) were extracted into 80% acetone and their concentrations were determined using spectrophotometer.

The chlorophyll content was calculated from absorbance values at 663 and 645 nm according to the equation of Arnon (1949). The total carotenoid content was calculated

2.6.1 Procedure

First of all, the lichen material was identifiedwith the help of steriozoom microscope on genera level, then a sample (0.5 g) was takenand kept in a refrigerator in a folded tissue paper. Next day, scraped material was kept in a foil in a petridish and again kept in a fridge. Then, materialwas weighed and taken with 80% acetone (5ml) and sodium carbonate (25 mg) in mortar pestle and crushed. The material was poured into tubes and kept in the freezer for a few minutes and centrifuged (10,000 rpm/ 10min/4^0C), prepare NaOH of 1N (250 ml distle water + 10gm NaOH). After centrifuge, the supernatant was pouredin the test tube and the reading was taken with the help of spectrophotometer. The pellet of chlorophyll left in the centrifuge tube was filled with 1.5 ml NaOH. It was kept at room temperature and centrifuged again, so that the protein settled in the pellet came above in the supernatant. 0.5 ml supernatant and 0.5 ml reagent (C) was added to it and kept for 10 minutes. After 10 minute, 0.5 ml reagent (D) was added and kept it for 5 minutes and optimum density read at 700 nm.

from absorbance value at 480 and 510 nm using Genesys 10 UV scanning spectrophotometer.

2.7 Protein estimation

Protein was estimated using Lowery method (Lowery et al., 1951) using phenol as reagent and calculations were made at absorbance values at 700 nm.

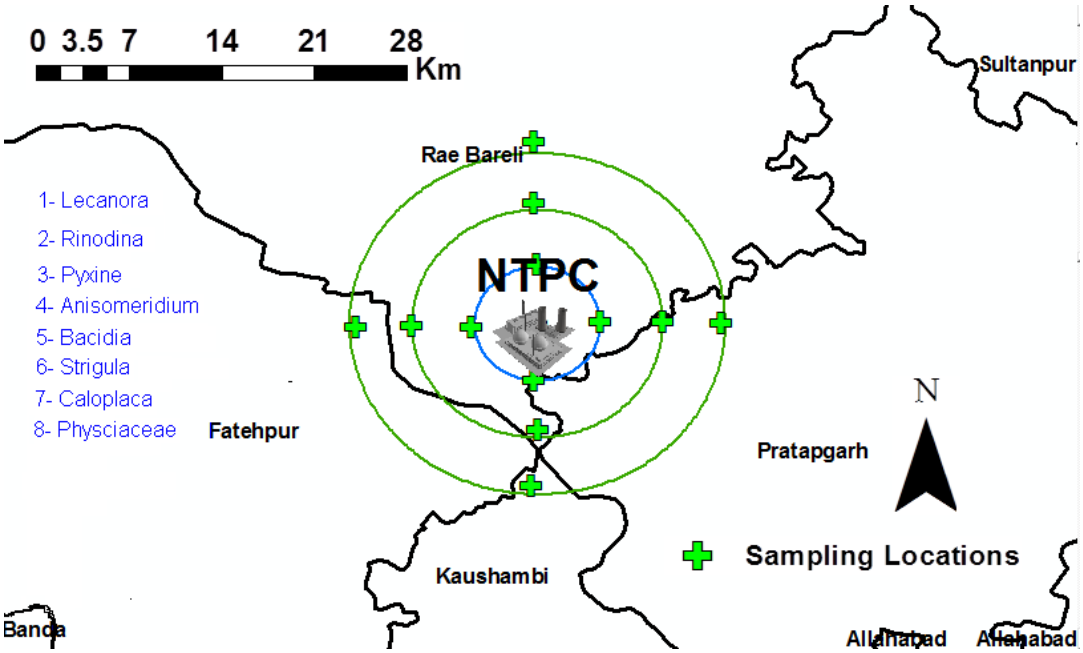

Figure 2. Zonal distribution of lichens growing in and around NTPC (symbols indicating various lichen genera)

3. Results and discussion

3.1 Zone mapping

Based on the distribution pattern i.e. absence and presence of the species, the study area can be divided into three zones - inner, transition and outer zone. Some of the species e.g. *Pyxine* were found growing in open canopy area of orchard, and some species like *Rinodina, Lecanora, Bacidia, Pyrenocarpus*and*Chrysothrix* were found growing on the trees under dense canopy. Lichen genera like *Rinodina* and *Pyxine* were found growing luxuriantly in all the three zones, indicating that these are pollution tolerant species. *Strigula* a pollution sensitive lichen species were found growing on the leaves of *Mangiferaindica*in outer zoneindicating the purity of air.

Inner zone

It is the most polluted zone as it is closest to the sourceof pollution. It showed poor occurrence of lichen species belonging to the genera like *Rinodina, pyrenocarpus, Pyxine* and *Bacidia*.

Transition zone

The localities under this zone showed good growth of *Pyxine, Rinodina, Lecanora, Pyrenocarpus, Bacidia*and*Caloplaca.*

Outer normal zone

This zone was located away from the source of pollution and showed luxuriant growth of both crustose and foliose lichens. *Pyxine, Lecanora, Bacidia*and*Rinodina*are the main lichen species which were found in this zone.

The lichens were not evenly distributed in the study area. In orchard having young tree exhibit almost absence of lichens and a few trees exhibited indistinct white patches of lichens, as lichens requires a lot of time to colonize on a particular substratum. Orchard having mature trees sometimes showed poor lichen growth because of the nature of bark. The tree bark may be sometime infected by termites or covered with mud which inhibits the lichen growth. The trees having more or less smooth bark and of middle age mostly bear good growth of lichens.

In Pyxine the highest concentration (1.4) of chlorophyll-a was found at a distance 10 kmaway from NTPC in South direction and chlorophyll b also found higher (0.7) in the same direction. Concentration of total chlorophyll was found highest at 10 km away in the South direction and at 15 km in West direction of same amount (2.1). The concentration of total chlorophyll increased with the increasing distance from the thermal power plant in East-West direction. The highest concentration of carotenoid was measured at 10 km in South (1.5) and at 15 km in West direction (1.5) followed by at the center of NTPC in North-South direction and concentration of protein was recorded highest (26) at 10 km away from NTPC in South direction.

3.2 Pigment analysis

Table-1 Photosynthetic pigments along with protein content (µg/g FW) in lichen genera *Pyxine*in North-South direction.

Components	A	B	C	D	E	F	G
Chlorophyll a	nd	0.6	nd	1.1	0.4	1.4	0.8
Chlorophyll b	nd	0.4	nd	0.6	0.4	0.7	0.4
Total Chl.	nd	1.0	nd	1.7	1.3	2.1	1.2
Carotenoid	nd	1.0	nd	1.4	1.0	1.5	1.2
Protein	nd	9.6	nd	12.2	13.8	26.0	10.7

Whereas A>B>C>D<E<F<G 15>10>5>0<5<10<15 km

Table-2 Photosynthetic pigments along with protein content (µg/g FW) in lichen genera *Pyxine*inEast-West direction.

Components	A	B	C	D	E	F	G
Chlorophyll a	0.5	0.5	nd	0.1	0.3	nd	0.5
Chlorophyll b	0.2	0.6	nd	0.3	0.2	nd	0.3
Total Chl.	0.5	0.6	nd	0.1	0.2	nd	2.1
Carotenoid	0.3	0.5	nd	0.4	0.1	nd	1.5
Protein	6.8	8.5	nd	15.1	23	nd	11.8

Whereas A>B>C>D<E<F<G 15>10>5>0<5<10<15 km

Table-3 Photosynthetic pigments along with protein content (µg/g FW) in lichen genera *Rinodina*inNorth-South direction.

Components	A	B	C	D	E	F	G
Chlorophyll a	1.3	1.7	nd	1.5	1	0.9	1.2
Chlorophyll b	0.7	1.1	nd	0.7	0.6	0.6	06
Total Chl.	2.0	2.8	nd	2.3	1.6	0.9	1.9
Carotenoid	1.1	2.0	nd	1.8	1.6	1.6	1.6
Protein	21.4	4.5	nd	20	12.6	21.5	20.5

Whereas A>B>C>D<E<F<G 15>10>5>0<5<10<15 km

Table-4 Photosynthetic pigments along with protein content (µg/g FW) in lichen genera *Rinodina*inEast-West direction.

Components	A	B	C	D	E	F	G
Chlorophyll a	1.6	2.3	1.1	0.8	1.4	0.9	0.4
Chlorophyll b	0.7	2.7	0.2	0.1	1.0	0.1	0.2
Total Chl.	2.4	5.0	1.2	1.0	2.5	1.0	1.1
Carotenoid	1.3	2.0	0.8	0.5	1.2	1.2	0.2
Protein	24.5	43	9.2	22.6	47	14.6	12.7

Whereas A>B>C>D<E<F<G 15>10>5>0<5<10<15 km

Table-5 Photosynthetic pigments along with protein content (µg/g FW) in lichen genera *Lecanora*in North-South direction.

Components	A	B	C	D	E	F	G
Chlorophyll a	nd	0.8	nd	1.2	0.8	nd	0.1
Chlorophyll b	nd	0.3	nd	0.6	0.4	nd	0.1
Total Chl.	nd	1.2	nd	2.0	1.3	nd	0.2
Carotenoid	nd	0.7	nd	1.6	1.0	nd	0.5
Protein	nd	10.2	nd	28.9	13.8	nd	11.6

Whereas A>B>C>D<E<F<G 15>10>5>0<5<10<15 km

Table-6 Photosynthetic pigments along with protein content (µg/g FW) in lichen genera *Lecanora*in*East-West* direction.

Components	A	B	C	D	E	F	G
Chlorophyll a	0.4	0.5	nd	nd	nd	nd	0.2
Chlorophyll b	0.0	nd	nd	nd	nd	nd	0.1
Total Chl.	0.4	0.4	nd	nd	nd	nd	0.4
Carotenoid	0.3	0.4	nd	nd	nd	nd	0.4
Protein	3.6	6.1	nd	nd	nd	nd	54

Whereas A>B>C>D<E<F<G 15>10>5>0<5<10<15 km

Table-7 Photosynthetic pigments along with protein content (µg/g FW) in lichen genera *Anisomeredium*inNorth-South direction.

Components	A	B	C	D	E	F	G
Chlorophyll a	nd	0.7	nd	nd	0.6	nd	nd
Chlorophyll b	nd	0.5	nd	nd	0.4	nd	nd
Total Chl.	nd	1.2	nd	nd	1.0	nd	nd
Carotenoid	nd	1.1	nd	nd	0.8	nd	nd
Protein	nd	0.8	nd	nd	12.3	nd	nd

Whereas A>B>C>D<E<F<G 15>10>5>0<5<10<15 km

Table-8 Photosynthetic pigments along with protein content (µg/g FW) in lichen genera *Anisomeredium*inEast-West direction.

Components	A	B	C	D	E	F	G
Chlorophyll a	1.3	nd	nd	1.2	0.8	0.9	3.2
Chlorophyll b	0.3	nd	nd	0.3	0.4	0.1	0.1
Total Chl.	1.6	nd	nd	1.5	1.3	1.1	3.3
Carotenoid	2.8	nd	nd	1.1	1.0	0.6	2.1
Protein	27	nd	nd	32.1	13.8	9.4	19.2

Whereas A>B>C>D<E<F<G 15>10>5>0<5<10<15 km

Through eight lichen species were found during collection at 12 different sites, only four species were chosen for the physiological analysis as those species were collected in sufficient amount. The effect of pollution on lichen genera like *Rinodina, Pyxine, Lecanora,* and*Anisomeridium* were analyzed for their physiological parameters.

The *Anisomeridium* lichen had the highest level of Chlorophyll a (3.2 mg/gf.wt) at 10 km away from NTPC in East-West direction, while it was found lowest (0.1mg/gf.wt) inPyxine, collected from polluted site (0 km) in East-West direction. *Rinodina*had the maximum amount of (2.7 mg/gf.wt) of chlorophyll b at 10 km before NTPC in East-West direction followed by *Anisomeridium* (59mg/gf.wt) at 10 km before NTPC in North-South direction. Total chlorophyll found lowest (0.1mg/gf.wt) in Pyxine in East-West direction at the center of the study area while highest total chlorophyll was found in Rinodina (5.0mg/gf.wt) at 10 km before

NTPC in East –West direction. Chlorophyll in lichens is very sensitive to change in environmental stress including air pollution. Change in Chlorophyll and phaeophytin can be used to assess changes in air quality (Balanquer and Manrique 1991; Garty et al., 1985; Von Arb and Brunold 1990). Maximum concentration of carotenoid was found in an *Anisomeridium* (2.8mg/gf.wt) at 15km before NTPC in East-West direction and minimum (0.3mg/gf.wt) in Chrysothrix at 10 km after NTPC in the same direction. Carotenoid is thought to protect Chlorophyll from the absorption of excess energy which might otherwise photobleach the chlorophyll (Krinsky 1968). Maximum concentration of protein was recorded in *Rinodina* (47mg/gf.wt) at 5 km away from NTPC in East-West direction while *Anisomeridium* showed the lowest concentration (0.8 mg/gf.wt) at 10 km before NTPC in North-South direction. Variation in protein is due to the climatic conditions because the pollution not only affects the concentration of physiological pigment of lichens but also morphology of the thallus. The moisture condition and wind direction play an important role in physiological changes.

Conclusion

In the present study, the account of the toxitolerent and sensitive lichen species is provided in and around NTPC in Unchahar district. Most of the member of physciaceae family such as *Rinodina*and *Pyxine* belong to the toxitolerent group of species, while the species like *Strigula* found growing on leaves, belong to the sensitive group of lichen, the crustose member of physciaceae (*Rinodina*) is more tolerant than the foliose members. The lichen species, growing near the source of pollution exhibit more deterioration of pigments and higher levels of protein, while mostly the chlorophyll contents were higher in lichens, growing away from the source of pollution. Lichens shows decrease in chlorophyll content and related pigment when the pollution increases higher and some lichen species can adopt to (become tolerant) increasing level of pollution.

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