G- Journal of Environmental Science and Technology

(An International Peer Reviewed Research Journal)

Available online at http://www.gjestenv.com

Comparative bioaccumulation potential of *Pyxine cocoes* and *Bacidia submedialis* in and around Faizabad city, Uttar Pradesh, India

Namita Gupta[1*], Vartika Gupta[3], S. K. Dwivedi[1] and D. K. Upreti[2]

[1] Department of Environmental Science, Babasaheb Bhimrao Ambedkar (A Central) University, Lucknow, U.P., INDIA
[2] Lichenology Laboratory, CSIR- National Botanical Research Institute, Rana Pratap Marg, Lucknow U.P., INDIA
[3] Department of Environmental Science, Dr. Ram Manohar Lohia Avadh University, Faizabad U.P., INDIA

ARTICLE INFO

Received: **28 Apr 2015**
Revised : **15 May 2015**
Accepted: **09 Jul 2015**

***Key words*:**
Lichen, biomonitoring, accumulation, pollution, diversity, distribution

ABSTRACT

Spatial trend in lichens diversity pattern with respect to pollution source provides vital information regarding environmental condition of the study area. In order to assess lichen diversity changes in one of the cultural centre of Uttar Pradesh, Faizabad city, lichen zone mapping technique was employed to observe spatial trends of lichen diversity in a grid of 1x1 km within 0-5,6-12 and 13-20 km distance from city centre in all the four directions. Overall 15 species were recorded from different directions. Among the different lichen species, *Pyxine cocoes* and *Bacidia submedialis* were common in most of the grids. Changes in physiological parameters and metal profile with respect to distance from city centre to the outskirts of the city in both *P. cocoes* and *B. submedialis* were analyzed.

It was observed that the physiological parameters varied from site to site and in different directions, but the metal profile clearly indicates a decreasing trend of metal concentration with increasing distance from the city centre. The present study provides baseline data for future biomonitoring studies and further confirms the lichen biomonitoring study as an effective tool to monitor changes in environmental condition.

1) INTRODUCTION

Increasing population, anthropogenic activities including construction, unplanned developmental processes along with vehicular emission and rapid growth of industrialization results in higher pollution level which ultimately has deleterious impact on the biodiversity and human health. Atmospheric pollutant causes irreversible damage to living organisms. Damage can occur at all levels of biological organization, from the components of individual cells to ecosystems [1]. The places which are exposed to heavy tourist and vehicular activities are more susceptible to the increased metallic pollutant as vehicular and anthropogenic activity result in emission of pollutant in the atmosphere [2].

Lichens are being used as an ideal biomonitors of air pollution. Several lichen species have been effectively employed worldwide for monitoring of air quality [3, 4, 5, 6 and 7]. A large number of studies with reference to the pollution monitoring with plants are available in India however few cities in different geographical regions of the country have been systematically mapped utilizing lichens for their pollutant load [2].

Lichens can be utilized as the monitor of pollution by three different ways: (a) by identifying and mapping of lichen species present in the area; (b) by transplant the healthy lichen from non- polluted area to the polluted area and measuring the decrement in the structure of thallus; and (c) sampling of an individual species and measuring contaminants collected in the thallus [8]. Thus, the monitoring alteration in the distribution of lichens could be a serviceable tool to examine bioclimatic feature of an area.

In the last two-three decades, few studies utilizing lichens for monitoring in Indian cities are available. The city of Bangalore, Pune and Kolkata and some major towns in Himalaya are studied through lichen biomonitoring and metal load of lichens in the area are available [2]. Earlier, the accumulation of lead (Pb) in lichens at different sites in and around Faizabad city of Uttar Pradesh was studied and it was recorded that concentration of lead (Pb) in lichens decreased with distance from the source of pollution [9]. No records of lichen diversity in relation with air pollution and monitoring were available from the area, thus the present study is carried out with an aim to provide a detail distributional account of lichens in and around the Faizabad city followed by changes in physiological parameters and metal profile in *P. cocoes* and *B. submedialis* the two most common lichen species of the area.

2) MATERIALS AND METHODS

2.1 Study area: The city of Faizabad is situated in East of the state of Uttar Pradesh, spread over an area of 2,764 km² at an altitude of 26.90 m above sea level. Faizabad is placed at the

* Corresponding Author: Namita Gupta
Email address: namitag09@gmail.com

latitude 26°47' N and longitude 82º 12' E. The district had a population of 2,468,371 based on Census of the year 2011.

2.2 Survey and Sample collection: Lichen species were collected, during March, 2014, in four directions North, West, South and East from 54 localities in and around Faizabad district **(Fig- 1)**. Mostly crustose and foliose lichens were collected on the bark of *Mangifera indica (Mango), Madhuca longifilia (Mahua), Pongamia pinnata (Karanj), Artocarpus heterophyllus (Kathal), Pithecellobium dulce (Jungle Jalebi), Ficus racemosa (Gular), Litchi chinesis (Litchi)* and *Azadirachta indica (Neem)* and cultivated Palm trees. The centre of the city is considered as '0' km. From city centre (0 km) the collection was performed towards each side of the district. Each side was further divided into 1x1 km^2 grid. The distribution of lichen taxa in all direction of area and within the grid was plotted. Approximately 0.2 g of the thallus of similar sizes was taken from sites from all directions in triplicate for further physiological and metal analysis.

The collected samples were kept at low temperature and wrapped in aluminum foil for the analysis. The collected specimens were identified by their morphological, anatomical and chemical characters [10, 11] and voucher specimens were preserved in the Lichen Herbarium of National Botanical Research Institute, Lucknow (LWG), India.

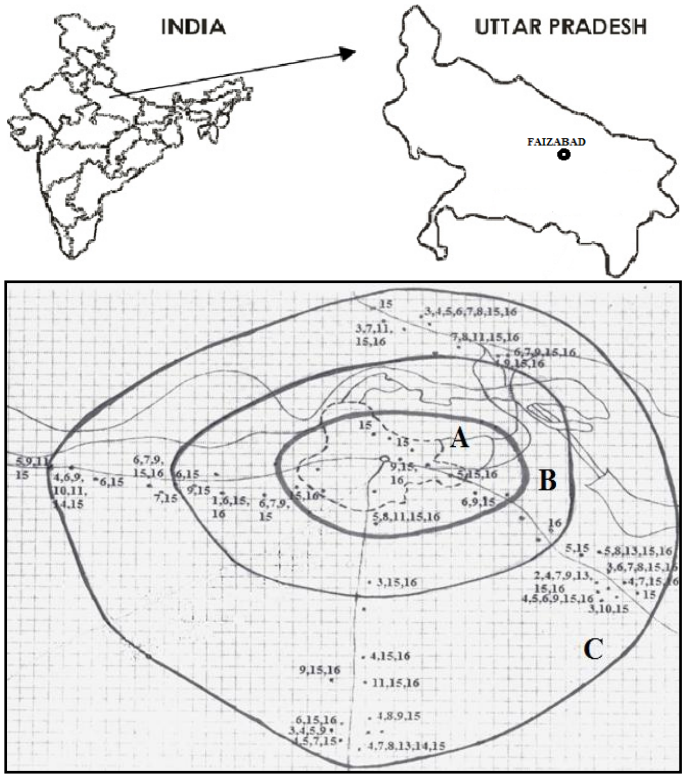

Fig- 1: Map showing collection sites in and around Faizabad City, Uttar Pradesh, India

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. 0.2 g of the sample was grinded with acid washed sand 10 mg calcium carbonate and 5 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 re-suspended in 0.5 ml chilled acetone (80%) and centrifuged. 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 [12] and total carotenoid content was calculated [13] from absorbance values at 480 and 510 nm.

2.4. Chlorophyll degradation: Chlorophyll content and its degradation are often used as one of the most accurate methods of biomonitoring. The method was used to measure intensity of the photobiont chlorophyll [14]. 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 435/415 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 [15].

2.6. Metal analysis: The lichen thalli (0.2g) were removed from the bark with sharp knife. The samples were oven dried for 12 h at 90°C. The dried lichen samples (triplicates) 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 Whatmann 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 standard were from Merck India and traceable to NIST (National Institute of Standards Technology). Working standards were prepared from the stock using deionized water.

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

3) RESULTS AND DISCUSSION

The identification of more than 250 specimens collected from 54 localities of Faizabad city revealed the occurrence of 15 species belonging to 11 genera from the area. The area showed dominance of crustose lichen represented by 12 species followed by 2 species of foliose lichen and single species of squamulose lichen. The *Mangifera indica* tree bears the maximum growth of 15 species on their trunks and branches followed by 3, 4 and 3 species on *Artocarpous heterophyllus*, *Azadirachta indica* and cultivated Palm trees respectively.

The total number of epiphytic lichen species increased with increasing distance from the centre of the city. The localities situated on outskirts of the city exhibited normal growth of most of the lichen species. Few sites within the city center with dense patches of trees also provides favourable habitat for normal growth of lichens. The *Azadirachta indica* (Neem) tree in such sites bear *P. cocoes, B.submedialis* and *Rinodina sophodes* on the trunk near the base. The *Mangifera indica* trees planted on the outskirts of the city exhibited good growth of differentlichen species.

The sites within the range of 0-5 km in the study area all around the centre of the city had scarce growth of epiphytic lichen growth with only occurrence of few toxitolerant species. It is interesting to note that, out of 54 sites, *P. cocoes*

and *B. submedialis* exhibited their presence within localities of all the three zones.

The localities situated between the distances of 6-12 km from the city centre exhibit increasing number of species indicate a more or less moderate pollution. The localities between distances of 13-20 km around of the city have luxuriant and normal growth of lichens. The normal and rich lichen diversity of both sensitive and tolerant species in the outskirts of the city clearly indicates a more or less pollution free zone of the city. The probable reason for luxuriant growth of lichen in the outskirts of the city may be attributed due to the large number of orchards with dense canopy having moist and shady condition which supports good growth of varied lichen taxa.

Urbanization and human activities seems to affect the lichen diversity of an area upto a greater extends. Similar, to most of the Indian cities, the city of Faizabad too has undergone fast pace of urbanization resulting in loss of substratum and suitable microclimatic condition which ultimately leads to decline in epiphytic lichens of the area. Faizabad district is devoid of major factories and industries thus indicated that vehicular activity is the major source of air pollution of the study area. Vehicular activity together with machines operated for generating electricity and growth of urban areas are the main sources of pollution and responsible for the loss of substratum for lichen colonization in the area.

It is clear from the study that air pollution is not equally spread throughout the district. Distribution data of lichen taxa collected from five major areas of the city indicates the separation of the city area into three distinct zones. Zone A (0-5 km) having poor lichen growth indicates a more polluted zone. Zone B (6-12 km) is characterized by moderate growth of fewcrustose lichens on some scattered trees of mango and it is transitional zone of the city and corresponding to the area of the moderate pollution. Zone C (13-20 km) of the city exhibits normal growth of different epiphytic lichen taxa, situated on the boundary of the district and experiences almost no air pollution **(Fig- 1)**.

The zone mapping technique provided circumstantial evidence about the prevalent lichen diversity of the area, but physiological parameters and pollutant (metal) profile provide direct evidence about the deteriorated air quality in the area.

3.1 Comparative physiological response of *Pyxine cocoes* and *Bacidia submedialis:* *P. cocoes* (foliose) and *B. submedialis* (crustose) lichen commonly occurring within the polluted and non-polluted area were selected for estimation of their pigment and accumulated metal concentration.

The concentration values for chlorophyll a in *P. cocoes* were similar in north and west directions, but showed dissimilar pattern in south and east directions and ranges from 0.19±0.01 μgg^{-1} at 13-20 km to 0.63±0.03 μgg^{-1} at 13-20 km west and east respectively. Chlorophyll b content also varied in different sites from different directions and it ranges from 0.08±0.02 μgg^{-1} to 0.41±0.31 μgg^{-1} at 0-5 km in south and east directions respectively.

The total chlorophyll (chlorophyll a + chlorophyll b) ranges from 0.27±0.01 μgg^{-1} at 13-20 km in west to 1.00±0.33 μgg^{-1} at 0-5 km in east direction. The concentration of chlorophyll a is known to get affected in stress condition [16]. Concentration of total chlorophyll is governed by the ambient environment, anthropogenic sources, vehicular traffic pollution and urban emission [17]. Photosynthetic parameters (chl. a, chl. b and total chlorophyll) showed similar pattern of variation in concentration in north, west and south directions, but different in east direction in the study area which may be due to pollution load in different directions and effect of wind direction.

The highest values of carotenoid were detected in samples of *P. cocoes* in east (0.35±0.04 μgg^{-1}) while lowest detected in the west (0.14±0.01 μgg^{-1}) at 13-20 km also affirms that east direction has suitable condition for lichen growth. The concentration of chlorophyll degradation was highest at 6-12 km and it decreased with increasing distance in north, east and west direction from city centre and it ranges from 0.99±0.00 μgg^{-1} at 13-20 km in west to 1.10±0.02 μgg^{-1} to 0-5 km in south direction.

Protein concentration ranged from 0.04±0.03 μgg^{-1} at 13-20 km in west to 0.24±0.09 μgg^{-1} at 6-12 km in north direction. The values of chlorophyll degradation and protein content were maximum at 13-20 km in west and minimum at 6-12 km in north (**Table- 1**). The increased level of protein in present study, at most polluted sites corresponds with the findings for the *Ramalina ecklonii* [18]. LSD analysis at $p< 0.01\%$ level showed significant difference in chl. a, total chlorophyll and carotenoid at different direction, while chl. b and protein may vary significantly at the level 0.05%. But there is no significant difference recorded in case of chl. degradation (**Table- 1**).

In *B. submedialis*, chlorophyll a concentration ranges from 0.16±0.05 μgg^{-1}in east to 0.54±0.02 μgg^{-1} in west at 6-12 km. The similar pattern of chlorophyll a content was recorded in north as well as west direction and decreasing trend with increasing distance was observed in south direction. The value of chlorophyll b ranges from 0.07±0.01 μgg^{-1} at 13-20 km in south to 0.23±0.02 μgg^{-1} at 6-12 km in west direction. The total chlorophyll ranged from 0.25±0.01 μgg^{-1} at 13-20 km in south to 0.77±0.05 μgg^{-1} at 6-12 km in west direction. Total chlorophyll showed similar variation in concentration in north and west direction.

Carotenoid content of *B. submedialis* was maximum (0.58±0.02 μgg^{-1}) at 6-12 km and minimum (0.13±0.03 μgg^{-1}) at 13-20 km in west. Carotenoid content analyzed from the east and south direction of the study area decreased with increasing distance from the city centre while samples from west as well as north direction have same carotenoid concentration. Chlorophyll degradation content ranged from 0.67±0.01 μgg^{-1} at 13-20 km in north to 1.10±0.01 μgg^{-1} at 6-12 km in west.

Protein content of *B. submedialis* ranged from 0.13±0.06 μgg^{-1} at 13-20 km in east to 0.53±0.13 μgg^{-1} at 6-12 km in west direction. The trend of protein was similar for north and south as well as for east and west.

The west part of the study area at distance of 6-12 km showed maximum level of concentration in each parameter, but the trend of chlorophyll degradation was different from other photosynthetic pigment analysis. Overall pattern for south showed decreased level of physiological parameters with increasing distance with respect to chl. a, chl. b, total chlorophyll and carotenoid. The north area of the Faizabad city have similar trend for chl. a, total chlorophyll, carotenoid and chlorophyll degradation. Particularly in east direction carotenoid decreases with increasing distance from the city centre but the level of chl. degradation in the present study increases with increasing distance. The east direction at 0-5 km showed maximum value for chlorophyll a, chl.b, total chl.

and carotenoid (**Table- 2**). Statistical correlation carried out using LSD analysisshowed that directions and distance from the Faizabad city play an important role in pigment content of lichens and mostly exhibited significant differences at 0.01% level. LSD analysis at p< 0.01% level showed significant difference in chl. a, chl. b, total chlorophyll, carotenoid and chl. degradation at different direction, while only non-significant difference was recorded in case of protein (Table-2).

It is clear from the observation that *P. cocoes* and *B. submedialis* showed similar trend of physiological parameters, but the variation in values may be attributed to the difference in morphology of the two species. The overall physiological attributes are able to represent the negative effect of air pollution on physiological status of both the lichen species.

3.2 Metal accumulation in *P. cocoes* and *B. submedialis* at different sites of Faizabad city: Total metal concentration was reported higher at 0-5 km north (3082.25 μgg^{-1}) and lower at 13-20 km west (750.31 μgg^{-1}). Accumulation of seven heavy metals Aluminium (Al), Iron (Fe), Cadmium (Cd), Chromium (Cr), Manganese (Mn), Lead (Pb) and Zinc (Zn) were estimated in thalli of *P. cocoes* in all directions at distance of 0-5, 6-12 and 13-20 km from the city centre (Table- 3). Among seven metals, Al was accumulated in maximum concentration followed by the sequence of Fe > Zn >Mn> Cr >Pb> Cd. Highest Al accumulation of 1609.3±0.87 $\mu gg^{-1}$was observed at 0-5 km south and minimum 551.95±0.80 μgg^{-1} at 13-20 km west, that indicates settling of this metal in the south direction. Accumulation of Al in the thalli of *P. cocoes* at all the sides is significantly different from each site based on LSD analysis at level 0.01% (Table-3). Accumulation of Fe ranged from 181.78±0.85 μgg^{-1} at 13-20 km west to 1403.0±0.12 μgg^{-1} at 0-5 west. The two most important metals i.e. Al and Fe in the earth's crust are strongly correlated in lichens and environmental contamination [20].

Among all the metals, Cd was reported as the lowest concentrated heavy metal in the present study area which ranged from 0.36±0.07 μgg^{-1} at 13-20 km south to 1.96±0.17 μgg^{-1} at 0-5 km north. The maximum level of Cr was recorded at 0-5 km west (12.38±0.53 μgg^{-1}) and minimum of 1.39±0.38 μgg^{-1} at 13-20 km west. The site at 0-5 km south and at 13-20 km north exhibited Mn accumulation ranged from 52.63±0.45 μgg^{-1} to 1.29±0.21 μgg^{-1} respectively. Thalli of *P. cocoes* in and around Faizabad city accumulated Pb in the ranges of 0.95±0.12 μgg^{-1} at 13-20 km east to 9.45±0.20 μgg^{-1} at 0-5 km north. Zn had maximum accumulation (60.23±0.73 μgg^{-1}) at 0-5 km west and minimum at 13-20 km north (11.05±0.22 μgg^{-1}). Dispersion and distribution of metals depends on wind speed and direction as well as density of the element under consideration [16].

In the present study, accumulation of all heavy metals at different sites exhibited sequence of accumulation as 0-5 km> 6-12 km> 13-20 km i.e. decreasing concentration with increasing distance from the city centre of the study area. The reason for higher concentration of Al and Fe around Faizabad city may be due to air pollution as well as natural origin.

At all the zones *P. cocoes* showed more or less similar selectivity sequence of metals such as Al> Fe>Mn> Zn> Cr> Pb> Cd (0-5 km); Al> Fe> Zn> Mn> Cr> Pb> Cd (6-12 km) and Al > Fe > Zn> Mn> Cr> Pb> Cd (13-20 km) (**Table- 3**).

B. submedialis also exhibited similar sequence of concentration of heavy metal (**Table- 4**). Samples from 0-5 km south has maximum concentration of both Al and Fe as 1337.4±0.52 μgg^{-1} and 1506.4±0.22 μgg^{-1}respectively and minimum accumulation was recorded at 13-20 km west as 108.62±0.28 $\mu gg^{-1}$and 55.45±0.11 μgg^{-1} respectively.

Thalli of *B. submedialis* in and around Faizabad city concentrated higher accumulation of metals, Cd (1.85±0.75 μgg^{-1}), Cr (12.04±0.25 μgg^{-1}), Mn (49.61±0.21 μgg^{-1}), Pb (8.31±0.38 μgg^{-1}) and Zn (59.62±0.30 μgg^{-1}) at 6-12 km south sites, while lower amount of Cd (0.14±0.08 μgg^{-1}), Cr (0.55±0.19 μgg^{-1}), Mn (0.67±0.11 μgg^{-1}), Pb (0.94±0.07 μgg^{-1}) and Zn (4.55±0.38 μgg^{-1}) at 13-20 km west.

The accumulation of Cr, Pb, and Cd on lichens in localities situated at outskirts of the city clearly indicates wide dispersion of the metals. *B. submedialis* also exhibited concentration of the most of the metals with increasing distance from the city centre of the study area (0-5 km> 6-12 km> 13-20 km). Total metal concentration in the species was recorded higher at 0-5 km south (2970.93 μgg^{-1}) and lower at 13-20 km west (170.92 μgg^{-1}).

B. submedialis also have the similar concentration of metal sequence to *P. cocoes* as Al> Fe> Zn> Mn> Cr> Pb> Cd (0-5 km); Al> Fe> Zn> Mn> Cr> Pb> Cd (6-12 km) and Al> Fe> Zn> Cr> Mn> Pb> Cd (13-20 km). Accumulation of Fe in the thalli of *P. cocoes* and *B. submedialis* are significantly different (LSD analysis at p< 0.01%; ref. Table 4) in all directions.

It is clear from the observation of the metal content that both *P. cocoes* and *B. submedialis* showed higher accumulation of Al and Fe and lower accumulation of Pb and Cd in all directions. Thus, the result showed that the vehicular and anthropogenic activities are the major cause of metal load in the study area.

4) CONCLUSION

The present study establishes the role of lichen biomonitoring studies in correlating the loss of lichen diversity with deterioration in air quality. Lichen zone mapping is a standardized technique to observe the impact of microclimatic changes on lichen diversity. Further assessment of physiological response and accumulation of metals in samples provides direct evidence about the air quality status. Lichen biomonitoring studies not only helps to know the status of pollutants in the environment but also provides an account the damage caused to the biological elements. In India, the use of lichen biomonitoring data has been so far not incorporated in air regulatory practices.

The present study provides the lichen diversity along with species distribution within each zone. The metal variation observed in the two toxitolerant species in Faizabad district will act as a baseline record for carrying out future biomonitoring studies in the area. Any further increase in metal load of the area in future may be better monitored observing changes in the lichen community composition and metal content comparing with the baseline data.

Acknowledgements

The authors are thankful to the Head, Department of Environmental Science, BBAU, Lucknow and Director, CSIR-National Botanical Research Institute, Lucknow for providing laboratory facilities. One of the authors (Namita Gupta) is grateful to University Grant Commission, New Delhi for financial support by providing Junior Research Fellowship.

Table-1: Photosynthetic pigment analysis and protein content of *Pyxine cocoes* in all directions in and around Faizabad city

Localities	*Pyxine cocoes* (concentration in µgg^{-1})					
	Chl. a	Chl. b	Total Chl.	Carotenoid	Chl. deg.	Protein
0-5 kms north	0.57±0.07	0.33±0.08	0.90±0.15	0.31±0.06	1.06±0.01	0.16±0.06
6- 12 kms north	0.56±0.09	0.25±0.09	0.80±0.18	0.30±0.05	1.07±0.05	0.24±0.09
13-20 kms north	0.47±0.08	0.17±0.05	0.63±0.13	0.28±0.09	1.03±0.04	0.22±0.07
0- 5 kms east	0.58±0.02	0.41±0.31	1.00±0.33	0.33±0.05	1.03±0.04	0.19±0.11
6- 12 kms east	0.38±0.10	0.19±0.06	0.57±0.10	0.20±0.04	1.06±0.03	0.08±0.10
13-20 kms east	0.63±0.03	0.31±0.06	0.94±0.09	0.35±0.04	1.00±0.00	0.18±0.07
0- 5 kms south	0.24±0.07	0.08±0.02	0.32±0.09	0.15±0.04	1.10±0.02	0.08±0.02
6- 12 kms south	0.40±0.09	0.15±0.03	0.55±0.12	0.31±0.01	1.00±0.01	0.18±0.12
13-20 kms south	0.50±0.14	0.20±0.06	0.70±0.20	0.31±0.03	1.02±0.01	0.13±0.03
0- 5 kms west	0.47±0.10	0.18±0.04	0.65±0.14	0.31±0.04	1.02±0.09	0.08±0.08
6- 12 kms west	0.45±0.14	0.18±0.08	0.63±0.22	0.28±0.09	1.04±0.09	0.10±0.01
13- 20 kms west	0.19±0.01	0.09±0.00	0.27±0.01	0.14±0.01	0.99±0.00	0.04±0.03
F	7.19**	2.49*	5.51**	5.22**	1.60 ^{NS}	2.33*
CV%	19.34	50.77	25.05	19.54	4.25	52.54
LSD (p< 0.05%)	0.148	0.180	0.280	0.090	0.074	0.123

(at p < 0.05% level by least significant difference (LSD) analysis) NS= Non- Significant
Mean±S.D., n=3 in µgg^{-1}
** Significance at the level of 0.01%.
* Significance at the level of 0.05%.

Table-2: Photosynthetic pigment analysis and protein content of *Bacidia submedialis* in all directions in and around Faizabad city

Localities	*Bacidia submedialis* (concentration in µgg^{-1})					
	Chl. a	Chl. b	Total Chl.	Carotenoid	Chl. deg.	Protein
0-5 kms north	0.38±0.03	0.20±0.02	0.58±0.05	0.34±0.04	0.82±0.08	0.23±0.06
6- 12 kms north	0.44±0.12	0.22±0.05	0.66±0.17	0.44±0.04	0.98±0.03	0.20±0.06
13-20 kms north	0.18±0.02	0.11±0.01	0.28±0.03	0.23±0.02	0.67±0.01	0.21±0.05
0- 5 kms east	0.25±0.08	0.18±0.06	0.43±0.14	0.44±0.13	0.76±0.10	0.30±0.08
6- 12 kms east	0.16±0.05	0.10±0.03	0.27±0.08	0.34±0.08	0.84±0.07	0.37±0.14
13-20 kms east	0.18±0.18	0.11±0.09	0.29±0.27	0.33±0.04	0.90±0.13	0.13±0.06
0- 5 kms south	0.28±0.10	0.15±0.05	0.42±0.16	0.44±0.16	0.92±0.03	0.46±0.23
6- 12 kms south	0.24±0.05	0.11±0.02	0.35±0.07	0.26±0.07	1.08±0.01	0.21±0.14
13-20 kms south	0.18±0.00	0.07±0.01	0.25±0.01	0.24±0.03	0.91±0.03	0.32±0.15
0- 5 kms west	0.46±0.06	0.19±0.03	0.65±0.09	0.40±0.03	1.01±0.03	0.41±0.09
6- 12 kms west	0.54±0.02	0.23±0.02	0.77±0.05	0.58±0.02	1.10±0.01	0.53±0.13
13- 20 kms west	0.20±0.06	0.11±0.02	0.30±0.08	0.13±0.03	1.08±0.01	0.38±0.56
F	7.91**	5.12**	6.79**	8.37**	15.03**	1.11^{NS}
CV%	27.90	27.68	27.56	20.69	6.54	63.34
LSD (p< 0.05%)	0.136	0.069	0.204	0.122	0.102	0.333

(at p < 0.05% level by least significant difference (LSD) analysis) NS= Non- Significant; Mean±S.D., n=3 in µgg^{-1}
** Significance at the level of 0.01%.
* Significance at the level of 0.05%.

Table-3: Heavy metal content of *P. cocoes* at 12 sites around Faizabad city

Localities	*P. cocoes* (Concentration in µgg^{-1})						
	Al	Fe	Cd	Cr	Mn	Pb	Zn
0-5 km north	1565.7±0.98	1395.8±0.82	1.96±0.17	10.71±0.56	51.47±5.55	9.45±0.20	47.16±0.81
6- 12 km north	946.27±0.10	278.56±0.49	0.85±0.38	7.107±0.21	20.19±0.65	3.09±0.11	21.28±0.61
13-20 km north	586.56±0.57	196.32±0.66	0.44±0.23	1.63±0.40	1.29±0.21	1.59±0.34	11.05±0.22
0- 5 km east	1456.7±1.06	1340.7±0.50	1.95±0.57	10.92±0.14	50.23±0.66	9.31±0.49	46.79±0.43
6- 12 km east	928.55±0.72	339.98±0.69	0.79±0.22	6.017±0.15	26.51±0.31	2.28±0.48	28.89±0.71
13-20 km east	569.45±0.52	201.52±0.20	0.39±0.17	1.84±0.12	2.54±0.22	0.95±0.12	12.78±0.23
0- 5 km south	1609.3±0.87	1340.7±0.35	1.87±0.47	10.8±0.74	52.63±0.45	9.32±0.25	46.97±0.14
6- 12 km south	952.12±0.23	357.71±0.76	0.76±0.31	7.59±0.71	22.08±0.84	3.33±0.08	21.15±0.38
13-20 km south	601.63±0.23	195.41±0.66	0.36±0.07	1.95±0.22	2.86±0.08	1.08±0.19	12.95±0.27
0- 5 km west	1378.6±0.50	1403±0.12	1.39±0.28	12.38±0.53	46.85±0.59	8.05±0.36	60.23±0.73
6- 12 km west	937.66±0.92	285.4±0.79	0.95±0.19	7.95±0.10	23.48±0.43	5.61±0.26	32.92±0.58
13- 20 km west	551.95±0.80	181.78±0.85	0.47±0.20	1.39±0.38	1.56±0.47	1.78±0.41	11.38±0.38
F	996755.02**	2094332.43**	12.572**	291.127**	4776.19**	391.52**	3440.717**
CV %	0.069	0.101	29.856	6.239	2.065	6.537	1.717
LSD(p< 0.05%)	1.239	1.126	0.511	0.704	0.924	0.513	0.853

(at p < 0.05% level by least significant difference (LSD) analysis)
Mean±S.D., n=3 in µgg^{-1}
** Significance at the level of 0.01%.
* Significance at the level of 0.05%.

Table-4: Heavy metal analysis of *B. submedialis* at 12 sites around Faizabad city

Localities	*B. submedialis* (Concentration in µgg^{-1})						
	Al	Fe	Cd	Cr	Mn	Pb	Zn
0-5 km north	1120.2±0.30	216.45±0.43	0.79±0.14	7.89±0.31	23.12±0.36	5.42±0.49	32.82±0.17
6- 12 km north	910.83±0.07	356.25±0.11	0.89±0.16	6.36±0.11	24.63±0.19	2.31±0.12	28.95±0.22
13-20 km north	125.12±0.29	88.25±0.49	0.42±0.10	1.06±0.04	1.12±0.04	1.45±0.16	9.17±0.19
0- 5 km east	1007.9±0.60	247.6±0.22	0.84±0.05	7.79±0.12	24.07±0.36	5.81±0.14	32.81±0.14
6- 12 km east	894.45±0.20	299.67±0.23	0.86±0.12	6.58±0.35	25.07±0.03	2.27±0.13	27.79±0.36
13-20 km east	119.78±0.23	79.64±0.22	0.21±0.08	0.98±0.16	0.76±0.13	1.08±0.18	6.18±0.33
0- 5 km south	1337.4±0.52	1506.4±0.22	1.26±0.13	12.02±0.27	46.57±0.39	8.1±0.08	59.18±0.07
6- 12 km south	1408±0.13	1421.3±0.19	1.85±0.75	12.04±0.25	49.61±0.21	8.31±0.38	59.62±0.30
13-20 km south	206.23±0.29	98.2±0.24	0.48±0.11	1.35±0.22	1.37±0.15	1.65±0.22	11.21±0.07
0- 5 km west	985.65±0.34	370.55±0.24	0.94±0.09	6.24±0.07	21.51±0.30	3.21±0.11	21.41±0.11
6- 12 km west	619.6±0.27	184.6±0.27	0.45±0.10	1.8±0.28	3.05±0.20	1.21±0.04	12.41±0.04
13- 20 km west	108.62±0.28	55.45±0.11	0.14±0.08	0.55±0.19	0.67±0.11	0.94±0.07	4.55±0.38
F	6658164.52**	10482277.34**	11.786**	1102.656**	16177.24**	472.865**	20717.49**
CV %	0.044	0.065	31.496	4.447	1.186	6.234	0.887
LSD (p< 0.05%)	0.549	0.454	0.403	0.403	0.369	0.365	0.381

(at p < 0.05% level by least significant difference (LSD) analysis)
Mean±S.D., n=3 in µgg^{-1}
** Significance at the level of 0.01%.
* Significance at the level of 0.05%.

REFERENCES

1) Weinstein, D.A. and Birk E.M. 1989. The effect of chemicals on the structure of terrestrial ecosystems: Mechanisms and patterns of change. In: Ecotoxicology: Problems and approaches. (Ed. S. A. Levin, M.A. Harwell, J.R. Kelly, and K.D. Kimball.) Springer, New York, 76-94.
2) Shukla, V., Upreti, D.K. and Bajpai, R. 2014. Lichens to biomonitors the environment. Springer, Heidelberg New York printed at Netherland.
3) Loppi, S. and Frati, L. 2006. Lichen diversity and lichen transplants as monitors of air pollution in a rural area of central Italy. Environmental Monitoring and Assessment, 114, 361-375.
4) Conti, M.E. and Cecchetti, G. 2001. Biological Monitoing: lichens as bioindicators of air pouution assessment- a review. Environ. Pollut., 114, 471-492.
5) Garty, J., Tomer, S., Levin, T. and Lehr, H. 2003. Lichens as biomonitors around a coal-fired power station in Israel. Environmental Research, 91(3), 186-198.
6) Shukla, V. and Upreti, D.K. 2007. Heavy metal accumulation in Phaeophysciahispidula en route to Badrinath, Uttaranchal, India. Environ. Monit. Assess., 131(1-3), 365-369.
7) Shukla, V. and Upreti, D.K. 2009. Polycyclic Aromatic Hydrocarbon (PAH) accumulation in lichen, Phaeophysciahispidula in Dehra Dun City, Garhwal Himalayas, Environ.Monit.Assess., 149, 1-7.
8) Pfeiffer, H.N. and Barclay-Estrup, P. 1992. The use of a single species, Hypogymniaphysodes, as an indicator of air quality in northwestern Ontario. Bryologist, 95(1), 38–41.
9) Dubey, A.K., Pandey, V., Upreti, D.K. and Singh, J. 1999. 'Accumulation of lead by lichens growing in and around Faizabad city, U.P., India', Journal of Environmental Biology, 20(3), 223-225.
10) Awasthi, D.D. (1988). A key to the macrolichens of India and Nepal.J Hatt. Bot. Lab., 65: 207-303.
11) Awasthi, D.D. (1991). A key to the microlichens of India, Nepal and Sri Lanka (Berlin: J Cramer). Bibl. Lichenol., 40: 1-337.
12) Arnon, D.I. 1949. Copper enzyme in isolated chloroplast polyphenoloxidases in Beta vulgaris, Plant Physiol., 24, 1-15.
13) Parsons, T.R., Maita, Y. and Lalli, C.M. 1984. A manual of chemical and biological methods for seawater analysis.Pergamon Press, Oxford.
14) Ronrn, R. and Galun, M. 1984. Pigment extraction from lichens with dimethyl sulfoxide (DMSO) and estimation of chlorophyll degradation.Environ. Exp. Bot., 24, 239–245.
15) Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent, J. Biol. Chem. 193, 265–275.
16) Garty, J. 2001. Biomonitoring atmospheric heavy metals with lichens: Theory and application. Critical Reviews in Plant Sciences, 20(4), 309-371.
17) Carreias, H.A., Gudino, D., Pignata, M.L. 1998. Comparative biomonitoring of atmospheric quality in five zones of Cordoba city (Argentia) employing the transplanted lichen Usnea sp. Environ. Pollut., 103, 317-325.
18) Gonzalez, C.M., Casanovas, S.S., Pignata, M.L. 1996. Biomonitoring of air pollution from traffic and industries employing Ramalinaecklonm (Spreng.)Mey and Flot. In Cordoba, Argentina, Environ. Pollut., 91, 269-277.
19) Garty, J. 2000. Trace metals, other chemical elements and lichen physiology: research in the nineties. In: Trace Element. Their Distribution and Effects in the Environment (Eds. B. Markert& K. Friese).Elesvier Science, UK, 277-322.
20) Loppi, S., Cenni, E. Bussotti, E., Ferreti, M. 1998. Biomonitoring of geothermal air pollution by epiphytic lichens and forest trees. Chemosphere, 36, 1079-1082.