G- Journal of Environmental Science and Technology

(An International Peer Reviewed Research Journal)

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

# Quantitative assessment of atmospheric elements and their interaction with transplanted lichen *Pyxine cocoes* (Sw.) Nyl

**Manju Rani[1], Rajesh Bajpai, Neha Karakoti and D. K. Upreti***

Lichenology Laboratory, Plant Diversity Systematics and Herbarium Division
CSIR-National Botanical Research Institute, Lucknow-226001, INDIA
[1]Department of Botany, Govt. (P.G.) College, Rishikesh, Uttarakhand, INDIA

## ARTICLE INFO

***Received :*** **10 Apr 2013**
***Revised :*** **02 Jun 2013**
***Accepted :*** **03 Aug 2013**

***Key words*:**
Anthropogenic Elements, Carbon/Nitrogen, Biomonitoring, Transplant, Lichen

## ABSTRACT

A common foliose lichen *Pyxine cocoes* (Sw.) Nyl. was transplanted at 12 different sites of Dehradun City. The thalli of *P. cocoes* have been exposed for three months to monitor the accumulation of Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn along with C and N and their impact on photosynthetic pigments (Chl a, Chl b, Total Chl) were also estimated. The results showed that thalli transplanted close to the city centre (0 km) exhibit early stress as revealed by decreasing photosynthetic pigments as compared to control site. The qualitative and quantitative results of elements showed negative correlations with distance from the city centre. Among the metals analysed, Fe (3396.71µg $g^{-1}$ DW) at Haridwar road followed by Zn (279.52µg $g^{-1}$ DW) at Rajpur road found in maximum concentration. Similarly, the maximum carbon (C) concentration (52.05%) was observed at Haridwar road whereas the nitrogen (N) concentration (1.73%) was observed maximum at Rajpur road. The lichen *P. cocoes* possess good accumulation capacity for most of the atmospheric elements. It is well evident from this study that selected lichen species could be used to detect low to higher atmospheric elemental emissions from vehicular activity in the ambient air and the biomonitoring procedure could be further standardized and used as part of an environmental monitoring programme.

## 1) INTRODUCTION

Air emission and fugitive dust resulting from vehicular and industrial activities and the potential effects of these pollutants on surrounding ecosystem are of increasing scientific and environmental interest. The air pollution is an important environmental problem in modern cities for both humans and environmental health. A number of factors are causing air pollution in urban areas and also many techniques have been developed to determine the quality and quantity of the pollution load. Out of the different methods utilizing organisms for monitoring, the lichens are frequently used in order to determine and monitor the air pollution in large area [1, 2]. Lichens, symbiotic associations of fungi and green/blue-green algae, have no roots or waxy cuticles and depend largely on the atmospheric input of mineral nutrients. The unique morphological features together with good bioaccumulation capacity make lichen potential bioindicators to study air pollution [3, 4, 5, 6]. Since the year 1860, the lichens were recognized as potential indicators of air pollution in Europe [7, 8, 9]. In last few decades, the usage of lichens in urban areas as an indicator of air quality has been increased tremendously. As the amount of pollutants reaches a threshold level in lichens, their physiological, morphological and chemical structures changes and resulted decrease in species numbers. If the pollution continues to increase, they may be eliminated from the polluted area [9].

In order to monitor air pollution in urban areas, the passive (natural) and active monitoring (transplant) are the most popular techniques that have been used by several workers most frequently in different regions of the world as well as in India [10, 11]. The emissions and atmospheric dispersal of elements and the pollution caused by them have received much attention due to the fact that metals are unsafe for both human and environmental health [12]. Numerous studies have been carried out about accumulation and biomonitoring of atmospheric elements by lichens through passive monitoring but restricted to active monitoring [13, 10, 14, 15, 16, 17].

* Corresponding Author: Dr. D. K. Uperti
*Email addres :* upretidk@gmail.com

The elemental concentrations in lichen tissues are considered to reflect present atmospheric concentrations or deposition and shows possible toxic effects. Very few pollution monitoring studies with lichen were carried out in India [11] in the past, however, the active monitoring has not been carried out in this area. Thus the present study is aimed to analyse the concentration of atmospheric elements such as Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn together with carbon (C), nitrogen (N) contents and their interaction with Chl a, b and Total Chl, in transplanted thalli of *P. cocoes*. The sensitivity of this lichen was also tested for biomonitoring.

## 2) MATERIALS AND METHODS

### *2.1 Site and transplantion*

The study is carried out in the city of Dehradun, capital of Uttrakhand. Dehradun City is located at 30$^o$32'34.0" N to 78$^o$04'21.0" E at an elevation of 673 meter with a population of 5,89,420 (according to 2011 census) is bounded in the north by higher range of Himalaya and in south by the younger Shivalik mountain ranges. The city has a typical topography as it is situated in a valley. Being the capital of the state, the vehicular and other human activities are quite high, therefore, this area was selected for study. Except few avenue trees mostly the city centre areas are devoid of trees. The outskirts of the city has thick forest patches with good growth of the lichens. *Pyxine cocoes* (Sw.) Nyl., an epiphytic foliose lichen found to be dominated in the surrounding of the city is selected for transplantation. The healthy thallus of the lichen was collected from the forest near northeast site of the city. This region is far from the pollution sources and thought to be clean with respect to air pollution.

The specimens were rinsed with distilled water and about 3-4 cm diameter of 10 thalli were sticked on the 45 x 45 cm cardboard with the help of Araldite glue. At each monitoring site **(Table 1)**, the lichen thalli with board were hanged on road side trees/old building, above 4 m away from the ground. The boards were removed after three month from the localities and carried out in the laboratory for further analysis.

**Table1: Lichen transplant sites in and around City**

| Site | Localities | Elevations | Altitude | Remark |
|---|---|---|---|---|
| Site 1 | North eastern forest | 30$^o$19′12.58″N and 78$^o$02′40.25″E | 695 mt | Control site |
| Site 2 | Toward Rajpur 5 km | 30$^o$19′31.23″N and 78$^o$02′34.65″E | 675 mt | Heavy vehicular traffic activity area |
| Site 3 | Toward Rajpur 10 km | 30$^o$19′37.28″N and 78$^o$02′42.93″E | 680 mt | Heavy vehicular traffic activity area |
| Site 4 | Toward Chakrata 5 km | 30$^o$19′29.59″N and 78$^o$02′29.22″E | 673 mt | Heavy vehicular traffic activity area |
| Site 5 | Toward Chakrata 10 km | 30$^o$19′33.24″N and 78$^o$02′14.05″E | 667 mt | Heavy vehicular traffic activity area |
| Site 6 | City centre 0 km toward Rajpur | 30$^o$19′31.20″N and 78$^o$02′32.78″E | 670 mt | Cross road area |
| Site 7 | City centre 0 km toward Haridwar | 30$^o$19′31.20″N and 78$^o$02′32.78″E | 670 mt | Cross road area |
| Site 8 | City centre 0 km toward Chakrata | 30$^o$19′31.20″N and 78$^o$02′32.78″E | 670 mt | Cross road area |
| Site 9 | Toward Saharanpur 5 km | 30$^o$19′15.36″N and 78$^o$02′28.65″E | 668 mt | Low vehicular activity area |
| Site 10 | Toward Saharanpur 10 km | 30$^o$19′20.24″N and 78$^o$02′30.50″E | 672 mt | Low vehicular activity area |
| Site 11 | Toward Haridwar 5 km | 30$^o$19′20.98″N and 78$^o$02′23.42″E | 669 mt | Higher vehicular and industrial site |
| Site 12 | Toward Haridwar 10 km | 30$^o$19′21.12″N and 78$^o$02′23.48″E | 665 mt | Highway area |

### *2.2 Sample preparation for heavy metal analysis*

After three months, the transplanted lichen specimens were harvested and washed with deionized water to remove unwanted substances. The samples were further subdivided into subsamples for measurement of photosynthetic parameters and for analysis of elemental content. For determination of elemental content the dried lichen samples (3 replicates) were ground to powder (weight ≈1.0 g each) and digested in mixture of concentrated $HNO_3$ and $HClO_4$ (v/v 9:1) for 1 hour. Residues were filtered through Whatman Filter paper No. 42 and diluted upto 25 ml with double distilled water. Analysis was done by using ICP – MS (Perkin Elmer SCIEX ELAN DRCe). Stock standards were procured from Merck, India.

### *2.3 Pigment analysis*

Photosynthetic pigments (chlorophyll a "Chl a", chlorophyll b "Chl b", Total chlorophyll "Total Chl") were extracted in 80% chilled acetone (Merck, Analytical grade) and their concentrations were 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 kept in the cold and pellet was resuspended in 1.5 ml chilled acetone (80%) and centrifuged again 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 645nm, according to the equation of Arnon [18].

### *2.4 Chlorophyll degradation*

The method developed by Ronen and Galun [19] 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 Chl a to phaeophytin a (OD 435/415nm ratio) was determined.

### *2.5 Carbon Nitrogen estimation*

The carbon (C) and nitrogen (N) content were estimated by isotopic ratio mass spectrometry (IRMS) (Thermo Fisher Scientific Delta Plus) coupled to an elemental analyzer (CE Instruments FLASH EA 1112 NA 1500), Conflo II and GC box to analyze the composition of either solid or gaseous samples. This coupling allows the determination of the nitrogen and carbon content of the sample.

### *2.6 Statistical analysis*

One way analysis of variance was applied to determine the significance of differences ($P<0.005$) in all measured parameters (pigments, carbon, nitrogen content etc.) across all the sites by SigmaPlot 11.0 (Systat Software, Inc, Richmond, CA, USA).

## 3) RESULTS AND DISCUSSION

The heavy metals such as Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Zn and Chlorophyll a, Chlorophyll b contents of *P. cocoes* samples, transplanted at 11 sites in Dehradun city and 1 site in forest as a control were determined **(Table 2 and 3)**. Most of the metals were found in higher concentration in samples transplanted in the city centre and concentrations get decreased with increasing distance from the city centre. Among the city centre, the area towards Rajpur road has the maximum load of metals .The sites situated within 5 km radius of the city showed moderate pollution load followed by sites situated 10 km from the city centre **(Fig. 1)**. Besides the city centre, the maximum accumulation of almost all the metals were reported at Haridwar Road (on high way to New Delhi) may be attributed to the high traffic density, because of its location on railway crossing, which restricts traffic movement quite often and causes traffic jam for hours. The selectivity sequence of the metals according to their concentration found in the city that expressed as mean concentration was Fe>Zn>Cr>Mn>Cu>Ni>Pb>Cd>Hg. Most of the metals represent their vehicular origin such as Mn, Cr, Pb, Hg, and Zn.

The concentration of the Cd, Cr, Hg, Ni and Pb metals showed a significant difference between the control site and the polluted site. Most of the samples transplanted in city centre showed high level of Cd, Cr, Hg, Ni and Pb followed by highway sites **(Fig 2)**. The probable reason for difference in metal concentration in two areas may be due to the difference in frequency and type of vehicles along with variation in micro-climatic conditions. Sites around 10 km distance from city centre also showed higher concentration of metals probably due to their location in the proximity of main roads and highways. Garty et al. [12] stated that apart from engine emissions, some metals enter the surrounding environment

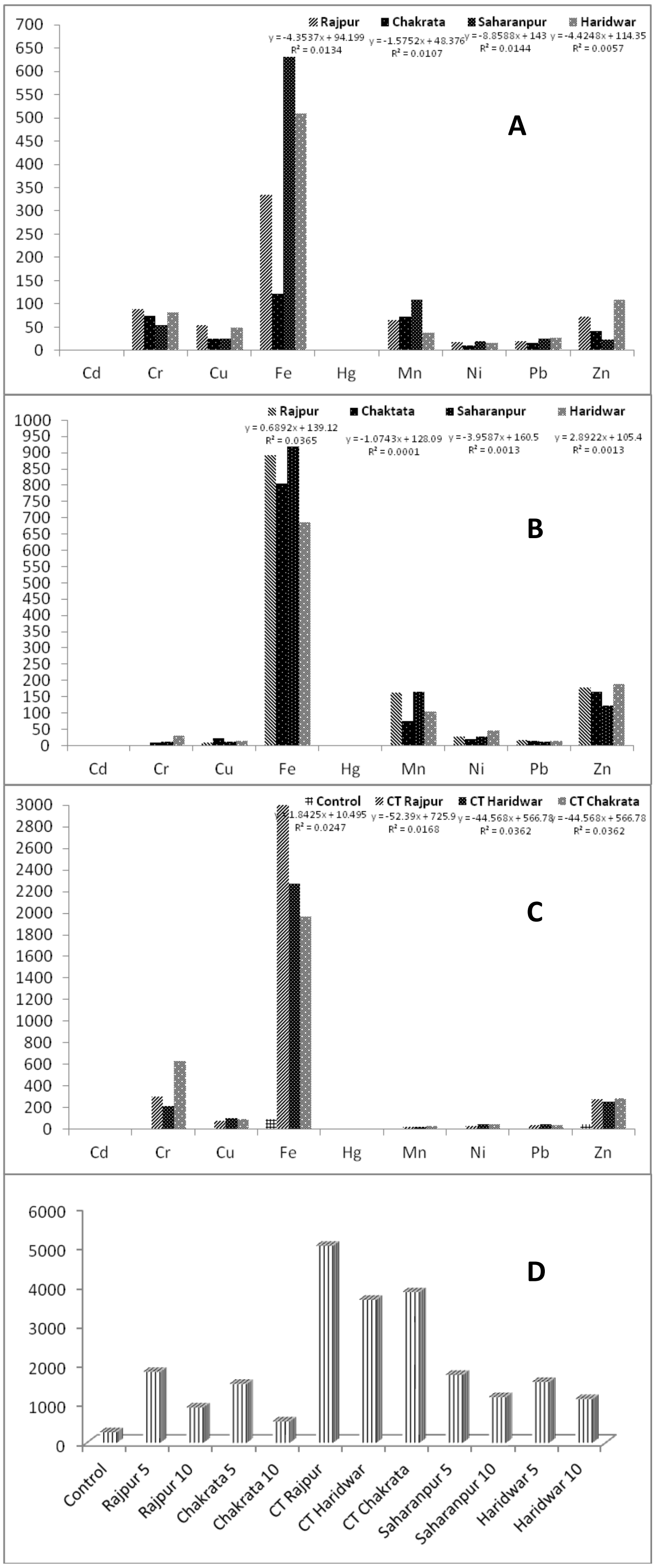



**Figure 1:** shows the pollution load in Dehradun city, **A.** at city centre, **B.** 5 km around the city, **C.** 10 km around the city, **D.** total metal load at different sites in the city.

**Table 2: Heavy metals content in lichen thallus (μg $g^{-1}$ dry weight) in and around City**

| Metals | Control site | Toward Rajpur | | Toward Chakarata | | City centre 0 km (Clock Tower) | | | Towards Saharanpur | | Towards Haridwar | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 Km | 10 Km | 5 km | 10 Km | Towards Rajpur | Towards Haridwar | Towards Chakarata | 5 km | 10 Km | 5 Km | 10 km |
| | | | | | | | | | | | | |
| **Cd** | 0.18±0.02 | 0.26±0.05 | 0.25±0.05 | 0.29±0.04 | 0.47±0.07 | 1.12±0.09 | 1.26±0.12 | 1.01±0.08 | 0.27±0.04 | 0.57±0.03 | 0.46±0.06 | 0.18±0.02 |
| **Cr** | 1.49±0.08 | 633.80±3.74 | 88.92±1.21 | 8.78±0.91 | 74.96±1.08 | 307.61±2.07 | 209.73±1.94 | 5.1±0.80 | 11.66±0.74 | 54.82±0.97 | 30.34±1.18 | 81.49±1.07 |
| **Cu** | 7.09±0.14 | 8.27±0.12 | 53.79±1.56 | 20.81±1.71 | 26.01±1.02 | 84.02±1.69 | 99.21±1.57 | 93.93±2.04 | 11.11±1.14 | 24.74±1.08 | 14.48±0.98 | 48.35±1.49 |
| **Fe** | 95.79±1.57 | 891.49±3.47 | 333.67±4.67 | 803.42±3.42 | 122.36±2.81 | 3396.71±4.27 | 2267.61±3.43 | 1965.31±2.46 | 918.33±3.57 | 631.36±2.95 | 684.85±2.55 | 509.62±2.76 |
| **Hg** | 0.17±0.03 | 0.89±0.09 | 0.25±0.07 | 0.91±0.02 | 0.35±0.01 | 2.37±0.08 | 2.31±0.14 | 1.27±0.09 | 0.97±0.05 | 0.43±0.06 | 0.46±0.08 | 0.37±0.04 |
| **Mn** | 12.52±1.05 | 161.39±1.57 | 65.81±1.91 | 74.32±1.42 | 72.85±2.08 | 22.5±0.94 | 19.94±0.86 | 27.47±1.02 | 163.33±2.11 | 109.20±2.58 | 103.09±1.97 | 38.78±1.06 |
| **Ni** | 8.71±0.95 | 25.25±1.54 | 17.27±1.08 | 18.17±0.85 | 10.22±0.96 | 37.16±1.26 | 46.66±1.75 | 46.19±2.04 | 27.63±1.14 | 18.92±1.07 | 45.07±2.09 | 15.42±0.86 |
| **Pb** | 2.83±0.09 | 15.05±0.13 | 20.24±0.28 | 13.64±0.18 | 15.78±0.21 | 44.59±1.09 | 44.76±1.12 | 41.31±0.99 | 11.66±0.18 | 24.31±0.37 | 13.19±0.55 | 26.72±0.89 |
| **Zn** | 47.41±0.98 | 176.58±1.46 | 71.68±1.14 | 164.12±2.07 | 41.5±1.11 | 279.52±2.53 | 256.75±3.07 | 285.19±2.78 | 121.38±1.09 | 24.02±0.97 | 186.85±1.82 | 109.06±1.05 |

(Mean ± S. D., n=3)

**Table 3: Photosynthetic pigments, carbon and nitrogen content in lichen thallus (μg $g^{-1}$ fresh weight) in and around City**

| Pigments | Control site | Toward Rajpur | | Toward Chakarata | | City centre 0 km (Clock Tower) | | | Towards Saharanpur | | Towards Haridwar | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5km | 10Km | 5km | 10km | Towards Rajpur | Towards Haridwar | Towards Chakrata | 5km | 10km | 5km | 10km |
| **Chl a** | 2.10±0.85 | 1.80±0.13 | 1.65±0.25 | 1.77±0.96 | 1.21±0.79 | 1.45±0.81 | 1.39±0.67 | 1.17±0.96 | 1.11±0.85 | 1.09±0.76 | 1.79±0.85 | 1.52±0.93 |
| **Chl b** | 1.46±0.07 | 0.82±0.08 | 0.72±0.09 | 1.23±0.84 | 1.09±0.92 | 1.29±0.09 | 1.26±0.08 | 1.08±0.12 | 0.65±0.07 | 0.94±0.08 | 1.19±0.67 | 1.13±0.85 |
| **Total Chl** | 3.58±0.06 | 2.62±0.52 | 2.53±0.63 | 2.46±0.14 | 2.17±0.98 | 2.65±0.75 | 2.78±0.85 | 2.23±0.89 | 1.76±0.61 | 1.78±0.52 | 2.97±1.08 | 2.67±0.94 |
| **Chll degradation** | 1.06±0.09 | 0.73±0.76 | 0.62±0.08 | 0.67±0.07 | 0.54±0.04 | 0.59±0.08 | 0.61±0.09 | 0.78±0.06 | 0.80±0.08 | 0.97±0.08 | 0.59±0.06 | 0.65±0.08 |
| **Carbon (C)** | 36.49±1.81 | 41.94±1.45 | 52.05±2.04 | 34.56±1.08 | 39.78±2.01 | 40.89±1.97 | 45.68±2.10 | 41.51±2.64 | 40.79±1.86 | 46.59±1.89 | 47.29±1.67 | 47.21±2.08 |
| **Nitrogen (N)** | 0.45±0.83 | 1.33±0.13 | 0.68±0.8 | 1.81±0.07 | 0.67±0.05 | 1.73±0.0.89 | 1.62±0.98 | 1.59±0.94 | 2.07±0.73 | 0.51±0.32 | 1.51±0.81 | 0.80±0.12 |

(Mean ± S. D., n=3)

due to abrasion of metallic vehicles parts. Among these Fe, Cr and Cu are main metals. High anthropogenic activities (combustion of coal), automobile activities all around a distance of 5 km within the city centre is also responsible for higher levels of most of the metals **(Fig 1)**. It is clear from **Table 2** and figure 1D that Cr showed a peculiar accumulation pattern being exceedingly high at Rajpur road (633.8µg $g^{-1}$ DW) at 5km distance and minimum at control site 1.49 µg $g^{-1}$ DW) it shows its origin from vehicular emission.

as a by product of industrial and automobile exhaust. The sites in Saharanpur road area are (moderately polluted) situated near agriculture fields showed higher accumulation of nitrogen (2.07%) followed by Rajpur road and Haridwar road.

The morphological and physiological properties of lichen species plays vital role in entrapment of nutrients. Carbon monoxide is one of the major pollutants present in the exhaust gases of petrol-driven engines. Maximum C content (52.05%) was found at Haridwar road area having higher number of automobile garage and heavy traffic density. Satya and Upreti [22] reported higher C & N concentration in lichens growing near the agriculture field and area having higher industrial activities in Kanpur city.

**Table 4: Comparison between previous studies**

| **Metals** | **Shukla et al. [17] (Passive monitoring)** | | **Rani et al. [20] (Passive monitoring)** | | **Current study (Active/transplant)#** | |
|---|---|---|---|---|---|---|
| | *Phaeophyscia hispidula* (Ach.) Moberg. | | *Phaeophyscia hispidula* (Ach.) Moberg. | | *Pyxine cocoes* (Sw.) Nyl. | |
| | **Clock tower** | **Rajpur road** | **D R Chowk** | **Rajpur road** | **Clock tower** | **Rajpur road** |
| **Cr** | 320.56 | 260.42 | 3700.00 | 1950.00 | 174.12 | 361.36 |
| **Fe** | 11759.98 | 12543.40 | 12612.00 | 11820.00 | 2543.21 | 612.58 |
| **Ni** | 14.81 | 14.76 | 566.60 | 487.50 | 43.33 | 21.26 |
| **Pb** | 17.42 | 17.36 | 12433.00 | 700.00 | 43.55 | 17.64 |
| **Zn** | 198.78 | 158.25 | 1833.30 | 1650.00 | 273.82 | 124.13 |

# mean of 0, 5 and 10 km

Lichens accumulate Fe more readily than other metals. The Fe concentration in all samples was higher, ranging between 95.79 -3396.71 µg $g^{-1}$ DW. The sample from Rajpur road area showed highest accumulation of Fe (3396.71 µg $g^{-1}$ DW). Shukla et al. [17] also reported higher Cr and Fe concentration (1189.56 µg $g^{-1}$ DW and 12543.40 µg $g^{-1}$ DW) in Rajpur road.

The level of Cu ranged between 7.09- 99.21 µg $g^{-1}$ DW. The Haridwar road area having maximum traffic activity also had higher concentration of Cu (99.21 µg $g^{-1}$ DW), whereas areas having less traffic as in Saharanpur area had less accumulation of Cu (11.11 µg $g^{-1}$ DW). Hence, the Cu accumulation was positively related with traffic activity.

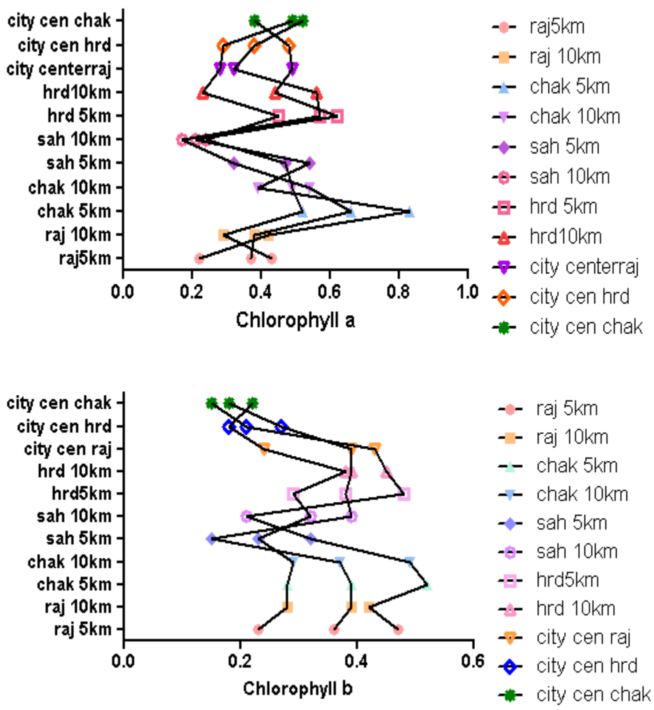
Nickel as a by-product of coal combustion, industrial processes and automobile exhaust, was higher (46.66 µg $g^{-1}$ DW) in the sites having higher vehicular activity while the control site having less traffic activity has lower level of Ni (8.71 µg $g^{-1}$ DW).

Lichens are efficient accumulators of Pb. The main source of Pb in cities is leaded gasoline whereas in rural areas or isolated industrial sites, the sources of Pb may be processes involving coal combustion. The Haridwar road area having heavy vehicular activity had maximum concentration of Pb (44.76 µg $g^{-1}$ DW) while it was recorded least at control site (2.83 µg $g^{-1}$ DW). Rani et al [20], recorded 930±8.5 µg $g^{-1}$DW of Pb in Haridwar road estimated in passive monitoring that was quite high with the present concentration **Table (4)**.

Emission of zinc occurs from zinc smelters and in automobile exhaust. Level of Zn in the studied sites, affirms its anthropogenic origin from automobile exhaust where its level was maximum (285.19 µg $g^{-1}$ DW) towards Chakrata road area due to higher traffic density. The comparison of heavy metal load from previous studies, presented in **Table (4)** clearly indicates the increase in concentration of some metals in the past years.

According to VanHerk [21], different forms of nitrogen are supplied in the atmosphere by two major sources i. e. ammonia in rural environment through exhaustive use of fertilizers and nitrous oxide present in the urban environments

The maximum *Chl* degradation was observed in the city centre area followed by sites situated at 10 km distance of the city **(Table 3)**. The decrease in Chlorophyll content is the sign of destruction in photosynthesis. Because of the small changes in the values of photosynthetic pigments it altered the *Chl b/a* ratio. Heavy metal accumulation in lichen tissues leads to degradation of chlorophylls. The increase in pollution results to decrease in chlorophyll a [12, 22, 15]. The environmental stress as a result of pollution may be explained with the changes in *Chl a* and *Chl b* contents **(Fig 2)**.



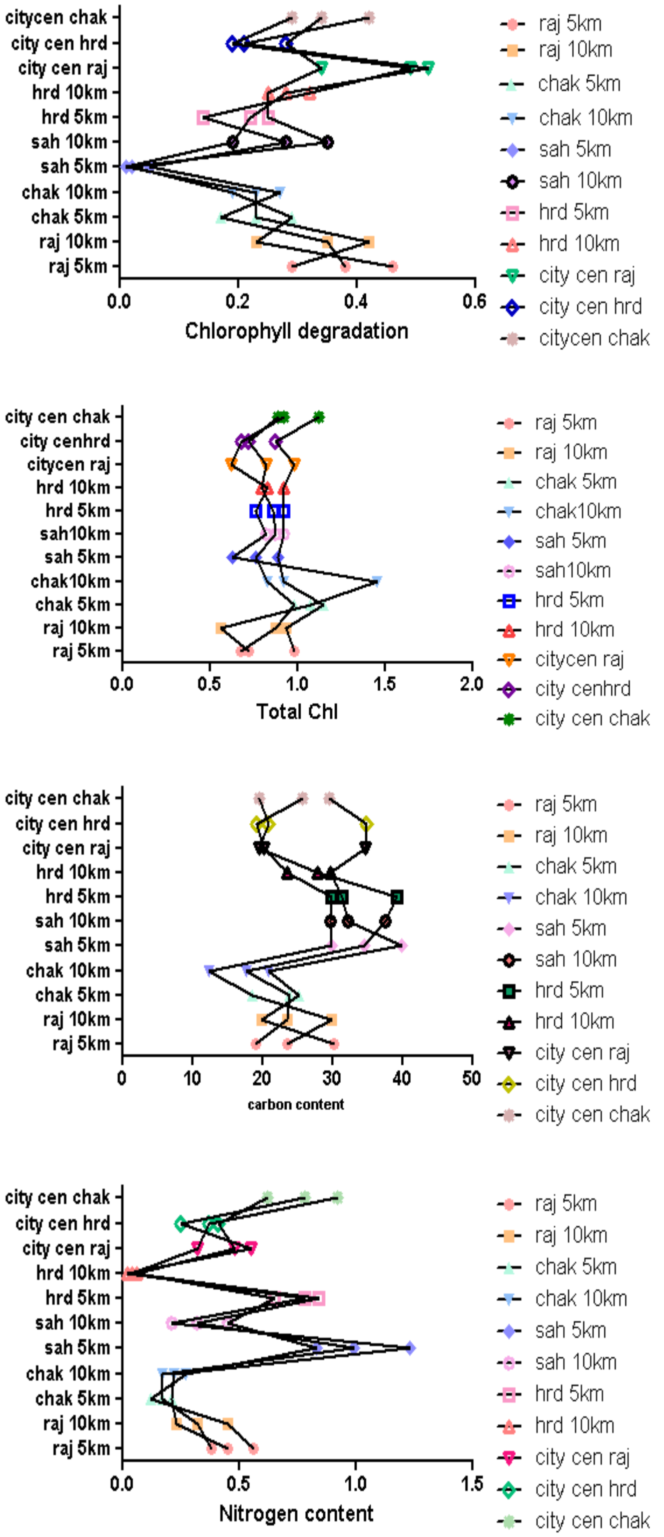



**Figure 2: Photosynthetic pigments, carbon and nitrogen content in transplanted lichen**

The chlorophyll content of the lichen decreases with increasing pollution due to physiological effect of pollution on lichens. This may be due to the inhibition of "the novo synthesis" and (or) an increase in amino acid degradation [23]. Air pollutants ultimately reduce both photosynthetic and respiration rates in lichens. Air pollution is not only a single reason to indicate the stress on organism, instead of these the quality and quantity of air pollution, climatic conditions, seasons, strength of the light and the substratum are also influence the stress over organism.

## 4) CONCLUSIONS

The results clearly indicate that the quality and quantity of atmospheric elements significantly affects the physiology of lichen *Pyxine cocoes*. The unplanned urbanization, increasing population, increase in vehicular activity, and deforestation along with location of city in a valley are the major factors responsible for pollution in the city. The reduction in the value of chlorophyll contents as well as its degradation ratio in lichen species growing in the area indicates the atmospheric pollutant stress for that area. The study, further affirms that the previous study [20] with *Phaeophyscia hispidula* (Ach.) Moberg, is similar to prove, another common tropical lichen *Pyxine cocoes* species has efficient accumulation potential of variable pollutants; therefore it can also be used as a model lichen species for pollution monitoring in urban and industrial areas. It is clear from this study that the transplant technique would certainly work as a stepping stone to lead the success in ecophysiology and pollution biomonitoring in the near future.

## ACKNOWLEDGEMENTS

The authors are thankful to the Director, CSIR-National Botanical Research Institute, Lucknow for providing laboratory facilities. One of the authors (RB) is grateful to Department of Science and Technology (DST-SERB), New Delhi (SR/FTP/ES-134/2010) for award of Young Scientist fellowship.

## REFERENCES

1. Carreras, H. A., Gudino, D. and Pignata M. L. 1998. Comparative biomonitoring of atmospheric quality in five zones of Co´ rdoba city (Argentina) employing the transplanted lichen *Usnea* sp. Environmental Pollution, 103, 317–325.
2. Adamo, P., Giordano, S., Vingiani, S., Cobianchi, R. and Violante P. 2003. Trace element accumulation by moss and lichen exposed in bags in the city of Naples (Italy). Environmental Pollution, 122, 91–103.
3. Jahns, M. 1973. Anatomy, morphology and development. In: Ahmadjian V, Hale ME (eds.) The Lichens. Academic, New York
4. Herkvan, C.M. 2001. Bark pH and susceptibility to toxic air pollutants as independent causes of changes in epiphytic lichen composition in space and time. Lichenologist, 33, 441–519.
5. Carreras, H. B. and Pignata, M. L. 2002. Biomonitoring of heavy metals by *Pseudevernia furfuracea* (L) Zopf in Ankara (Turkey). Environmental Pollution, 117, 77–87.
6. Backor, M. and Zetikova, J. 2003. Effects of copper, cobalt and mercury on the chlorophyll content of lichen *Cetraria islandica* and *Flavocetraria cucullata*. Journal of Hattori Botany Laboratory, 93, 175-187.
7. Puckett, K. J., Nieboer, E., Flora, W. P. and Richardson, D. H. S. 1973. Sulphur di oxide: its effect on photosynthesis $^{14}C$ fixation in lichens and suggested mechanism of phytotoxicity. New Phytologist, 72, 141-154.

8. Garty, J., Karary, Y. and Harel, J. 1993. A Review of Lichen and Bryophyte Elemental content literature with reference to Pacific Northwest species. Archives of Environmental Contaminant and Toxicology, 24, 455–460.
9. Hawksworth, D. L. and Rose, F. 1996. Lichens as Pollution Monitors. Edward Arnold, London, UK
10. Garty, J. 2001. Biomonitoring atmospheric heavy metals with lichens: theory and application. Critical Review in Plant Science, 2(4), 309–371.
11. Shukla, V. and Upreti, D. K. 2012. Air quality monitoring with lichens in India: Heavy metals and polycyclic aromatic hydrocarbon. (In: Lichtfouse E, Schwarzbauer J, Robert D, Eds.) Environmental Chemistry for a sustainable world vol. 2 Remediation of air and water pollution, (pp 277-294), Springer Verlag, New York.
12. Garty, J., Sharon, T., Levin, T. and Lehr, H. 2003. The effect of heavy metals on some aspects of lichen physiology. Environmental Research 91,186–198.
13. Loppi, S., Nelli, L., Ancora, S. and Bargagli, R. 1997. Passive monitoring of trace elements by means of tree leaves, epiphytic lichens and barks substrate. Environmental Monitoring Assessment, 45(1), 81-88.
14. Bajpai, R., Upreti, D. K. and Mishra, S. K. 2004. Pollution monitoring with the help of lichen transplant technique at some residential sites of Lucknow City, Uttar Pradesh. Jouranl of Environmental Biology, 25 (2), 191-195.
15. Bajpai, R., Upreti, D. K., Nayaka, S. and Kumari, B. 2010. Biodiversity, bioaccumulation and physiological changes in lichens growing in the vicinity of coal-based thermal power plant of Raebareli district, north India. Journal of Hazardous Materials, 174, 429–436.
16. Bajpai, R., Pandey, A. K., Deeba, F., Upreti D. K., Nayaka, S. and Pandey, V. 2012. Physiological effects of arsenate on transplant thalli of the lichen *P. cocoes* (Sw.) Nyl. Environmental Science and Pollution Research, 19, 1494-1502
17. Shukla, V., Upreti, D. K. and Nayaka, S. 2006. Heavy metal accumulation in lichens of Dehradun City, Uttaranchal, India. Indian Journal of Environmental Science, 10(2), 165-169.
18. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts polyphenoloxidases in *Beta vulgaris*. Plant Physiology, 24, 1-15.
19. Ronen, R. and Galun, M. 1984. Pigment extraction from lichens with dimethyl sulfoxide (DMSO) and estimation of chlorophyll degradation. Environmental Experimental Botany, 24, 239-245.
20. Rani, M., Shukla, V., Upreti, D. K. and Rajwar, G. S. 2011. Periodical monitoring with lichen, *Phaeophyscia hispidula* (Ach.) Moberg in Dehradun city, Uttarakhand, India. Environmentalist, 31, 376-381.
21. VanHerk, C. M. 1999. Mapping of ammonia pollution with epiphytic lichens in the Netherland. Lichenologist, 31, 9-20.
22. Satya and Upreti, D. K. 2009. Correlation among carbon, nitrogen, sulphur and physiological parameters of *Rinodina sophodes* found at Kanpur city, India. Journal of Hardous Materials, 169, 1088-1092.
23. Godzik, S. and Linskens, H. F. 1974. Concentration changes of free amino acids in primary bean leaves after continuous and interrupted $SO_2$ fumigation. Environmental Pollution, 7, 25-30.