RESEARCH ARTICLE

Heavy Metals Accumulation and Physiological Changes in the Lichens Growing in the Vicinity of Coal-Based Thermal Power of Kanti (Muzaffarpur), Bihar, India

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Abstract

The present study was designed with an aim to observe the effect of increasing pollution level on native lichen diversity, metal accumulation and physiological changes around a coal-based thermal power plant of Kanti, Muzaffarpur districts of Bihar, India. Three lichen species namely *Phaeophyscia hispidula* (Ach.) Essl, *Physcia dilatata* Nyl., and *Pyxine cocoes* (Sw.) Nyl., were found growing in their natural habitat in vicinity of fly ash. Among these, *Physcia dilatata* Nyl., a common foliose lichen, was growing as a most dominant species at highly polluted sites. During present study the species was analyzed for six heavy metals (Fe, Pb, Cr, Zn, Ni and Cu) and further physiological changes at five different sampling sites. The test species accumulated maximum levels of Fe (10,923), Pb (389), Cr (151), Zn (142), Ni (73.5), and Cu (39.5) at highly polluted dumping sites. However, there was high spatial variability in total metal accumulation in different species indicated by coefficient of variation (CV %) and showed higher values for Fe, Pb and Cr but lower for Cu and Ni. The concentration of most of the metals at different sites was statistically significant as compared to control site. Further six physiological parameters i.e., Chl *a*, Chl *b*, total pigment, chlorophyll degradation, carotenoid and total protein content were also measured and found maximum at least polluted control sites (residential sites). Total chlorophyll and protein content are most useful and efficient parameter to assess air pollution level of a region. Total chlorophyll was significantly higher at control sites (0.62) as compared to highly polluted dumping site (0.22) and similarly protein content was also higher at control sites (42.53) as compared to polluted sites (12.87). The results of the present study indicated that *P. diltata* is pollution tolerant (adaptation) and able to withstand local emissions from thermal power plants.

Keywords: Adaptation, Bioaccumulation, Fly ash, Heavy metals, India, Lichens, Physiological response.

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Introduction

Coal is recognized as the primary source of energy in India, and its utilization in power generation is emerging as the biggest environmental problem as it emits fly ash, acid precursors, green house gases, non-combustible hydrocarbons, heavy metals and particulates. These pollutants can be carried to a long distance by wind and ultimately have a negative impact on both biotic and abiotic environment (Cicek *et al*., 2001).

A large number of studies on pollution are available in which lichens are used as bioindicators (Conti and Cecchetti, 2001; Kircher and Daillant, 2002; Printsos and Loppi, 2008). Due to their peculiar anatomical, morphological and physiological characteristics lichens are one of the most valuable biomonitors of atmospheric pollution. Lichens are known to be sensitive to many types of pollution and are suitable to assess damage caused by air pollution. 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 in ATP content (Kardish *et al*., 1987), photosynthesis (Showman, 1972), chlorophyll degradation (Ronen and Galun, 1984; Garty *et al*., 1985), production of stress-ethylene (Garty *et al*., 1997), and malondialdehyde (MDA) content (Gonzalez and Pignata, 1994). They can be used as sensitive indicators to estimate the biological effects of pollutants by recording changes at the community and monitors of persistent pollutants, which can be estimated by assaying their trace element contents (Printsos and Loppi, 2008). The epiphytic lichens have been used extensively to monitor air quality around urban areas, industrial sites and to document spatial distribution and accumulation of air borne pollutants (Garty, 2001; Carignan *et al*., 2002; Purvis *et al*., 2004). Lichens are used as passive pollution monitors because they accumulate a variety of pollutants in their thalli at levels well above environmental concentrations and their own physiological needs.

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They lack root system and therefore intercept only allogenic atmospheric matter included in wet precipitations, dry depositions and gaseous emission (Purvis *et al*., 2004). They record an integrated signal over a few years of atmospheric fallout and thus minimize any signals due to variable (seasonal) atmospheric circulation patterns (Carignan *et al*., 2002). The use of lichens as biomonitors of geothermal air pollution was initiated by Bargagli-Pertrucci (1915), who reported the absolute absence of lichens in the geothermal area of Italy around 5 km vicinity. Recent reports have shown deterioration in air quality around thermal power plants in India (Bajpai *et al*., 2010a,b, 2011) As a result destabilization of the ecosystem has occurred with loss of several sensitive plant species (Rao *et al*., 1990). A lot of passive as well as active (transplant) biomonitoring studies using lichen have been carried out in India by several workers in different climatic regions of the country against various pollution sources (Upreti and Pandey, 2000, Bajpai *et al*., 2011, 2015, 2016 and Shukla *et al*., 2014), but such studies around thermal power plant area are scanty (Bajpai *et al*. 2010a,b). The main objective of the present study is to assess the impact of thermal power plant emission on lichen community within surrounding areas of its radius (5 km). The parameters considered for the study include distribution pattern of heavy metals and abundance of lichens around thermal power plant, accumulation of heavy metals and physiological changes like changes in pigment concentrations and total protein content in a commonly occuring foliose lichen *Physcia dilatata* Nyl.

Materials and Methods

Study area

The study area is located in district of Muzaffarpur, Bihar which is situated in northern part of Indian sub continent, between 250°27′-26°13′N latitude and 85°27′-86°10′E longitude. KTPC has the stack height of 140 m with electricity production capacity of 5×210 MW. The climate of the region is tropical, with eight months of dry period and four months of rain that ranges from 110 to 485 mm distributed between June to September, the temperature range that the area experiences is between 13.2°C in winter and 45.2°C in summer.

Sample collection

The area around KTPC was surveyed for collection of lichens in the month of February-March 2008. Lichen, especially *Physcia dilatata* Nyl., growing abundantly on trees of *Mangifera indica* collected from 12 different sites at 2, 3 and 5 km distance on north, south, east and west directions. *P. dilatata* is widely distributed in the area thus used for physicochemical and metal accumulation studies from each sites. The sites at 5km away from the thermal power plant are considered as control sites. Approximately 3-4 g of the thallus of similar sizes was taken from each site in triplicate for further analysis.

Metal analysis

The lichen thalli (approximately 2-3 g) were removed from the bark with sharp knife. The samples were oven dried for 12 h to a constant weight at 90°C. The dried lichen samples (three replicates) were grinded to powder (0.5 g) and digested in mixture of concentrated HNO_3 and $HClO_4$ (v/v, 9:1) for 1 h. Residues were filtered through whatman Filter paper no. 42 and diluted to 20 ml with double distilled water. Analysis was done with Flame Atomic Absorption Spectrophotometer (Perkin Elmer, model A Analyst 300). Stock standards were from Merck India and traceable to NIST (National Institute of Standards Technology). Working standards were prepared from the stock using deionised water.

Pigment analysis

Photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoid) were extracted in 80 % chilled acetone (Merck, Analytical grade) and their concentrations determined using standard spectrophotometric procedures. 1.0 g of the sample was grinded with acid washed sand 50mg calcium carbonate and 10 ml acetone (80%) on ice in dim light. The slurry was transferred to a 10 ml centrifuge tube, vigorously shaken and centrifuged at 10,000 rpm for 10 min. The supernatant was then decanted, kept in the cold and pellet resuspended in 1.5 ml chilled acetone (80%) and centrifuged as above. The supernatant were then combined, made to known volume and analyzed using Genesys 10 UV scanning spectrophotometer. The chlorophyll content was calculated from absorbance values at 663 and 645 nm according to the equation of Arnon (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 Ronen and Galun (1984) was used to measure intensity of the photobiont chlorophyll. The chlorophyll was extracted overnight in the dark in 5ml dimethyl sulfoxide (DMSO, Merck, analytical grade). The ratio of chlorophyll *a* to phaeophytin a (OD 435/415 nm 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.

Statistical analysis

The results of the elemental analysis were evaluated with a one way analysis of variance (ANOVA). Results are presented as *f* values and f probability (Table 2-3).

Results

Extensive surveys were carried out around different directions of target area to monitor lichen diversity in vicinity of fly ash as well as pollution level monitoring of five selected sites in surrounding areas were also done (Table 1). Since the Kanti tahsil has no other industrial source of pollution except KTPC, the high levels of metal in the lichens are due to fly ash pollution. The maximum accumulation of Fe was found at dumping site, followed by village Kusi. Copper and nickel showed least variation in concentration between the different sites. Lead accumulation was highest at village Veriyahi, and lowest at residential campus on Motipur road. Laskaripur village has minimum accumulation of chromium while dumping sites had the maximum concentration of chromium 9,148 µg g^{-1}. From Table 2 it is evident that the accumulation of most metals by lichens is maximum at dumping site followed by Kusi village. The Fe content at these two sites is significantly different (1% level) from that at residential campus, the control site. The maximum concentration

Table 1: Sources of Lichen material of *Physcia dilatata* Nyl. for heavy metal estimation in KTPC, Muzaffarpur (Bihar), India.

S.N.	*Site*	*Site direction*	*Pollution level*
1.	A. Dumping Sites	On *Mangifera indica* L, 1.5 m above ground facing fly ash dumping site, North direction of KTPC	Slurry disposal site in fly ash pond. Less tree plants and highly polluted site
2	B. Kusi Village	On Various trees, 1.5- 2 m above ground facing road, South direction of KTPC	Moderate polluted Site due to rich vegetation.
3.	C. Veriyahi Village	On Various trees, 1- 2 m above ground facing road , North-east corner of KTPC	Moderate polluted Site due to rich vegetation.
4.	D. Lashkaripur village	On *Bauhinia variegate* tree, 1 m above ground facing road side, East direction of KTPC	Open road side areas, low pollution
5.	E. Residential Campus (Control)	On *Bauhinia variegate* tree, 1.5-6.5 m above ground facing road side	Rich lichen diversity, least polluted.

Table 2: Sources of Lichen material of *Physcia dilatata* Nyl. for heavy metal estimation in KTPC, Muzaffarpur (Bihar), India.

	Metals (mg g^{-1} ^{fw})					
Selected sites	*Iron*	*Chromium*	*Copper*	*Zinc*	*Nickel*	*Lead*
A. Dumping Sites	10,923±38.9	151.89±5.6	39.54±5.6	142±5.6	73.57±6.5	389±11.5
B. Kusi Village	9,429±50.3	148.65±3.6	29.76±2.2	113±7.4	65.45±5.3	313±9.5
C. Veriyahi Village	6,937±45.6	89.5±0.6	33.45±3.6	94±3.5	49.97±3.7	278±8.5
D. Lashkaripur Village	4, 960±76.2	93.7±5.5	26.65±1.6	105±6.5	56.63±5.6	87±3.3
E. Residential Campus (Control)	4,505±55.6	69±0.6	19.94±0.6	23±1.7	37.85±2.6	27±1.5
CV%	39.7	30.9	16.79	18.7	8.9	22.5
One way ANOVA						
f value	3.793*	700*	43*	788*	86*	32,171*
f probablity	0.00	0.00	0.00	0.00	0.00	0.00

*Significantly different at 1% level

Table 3: Physiological data (in mg g^{-1} fw) of *P. diltata* Nyl. from various sites of FA polluted sites of KTPC, Muzaffarpur, India (Values ± SE).

Parameters	*f ratio*	*f probab.*	*Dumping site*	*Kusi village*	*Veriyahi village*	*Lashkaripur village*	*Residential sites (Control)*
Chl *a*	163*	0.00	0.151±0.1	0.24±0.1	0.36±0.2	0.55±0.3	0.38±0.02
Chl *b*	23*	0.00	0.072±0.3	0.21±0.1	0.33±0.1	0.30±0.1	0.29±0.01
Total Chl	181*	0.00	0.224±0.5	0.45±0.1	0.69±0.01	0.85±0.2	0.67±0.13
Carotenoid	39.5*	0.00	0.139±0.1	0.51±0.1	0.71±0.1	0.61±0.1	0.61±0.10
Chl degr.	10.7*	0.00	0.98±0.3	1.01±0.1	1.03±0.3	99±0.2	1.07±0.05
Protein	82.7*	0.00	12.87±1.1	16.8±0.1	21.5±1.4	15.6±0.5	42.53±1.3

*Significantly different at 1% level

of most of the metals at both the polluted sites can be attributed to the fact that the heavy deposition of fly ash due to frequent disposal of slurry (industrial waste) throughout the whole year at these localities. The low concentration of most of the metals in lichens from residential campus i.e. Control site.

From Table 3 it is clear that all the physiological parameters changes with the variation in the air quality of the area. Two separate ANOVA based on the data obtained from KTPC, Muzaffarpur shows significant variation in the *f* value at 1% level of significance (depicted by *f* value and *f* probability) for all the traits (physiological parameters). The highest concentration of total chlorophyll was found at residential campus area (control site) at 0.85 mg g^{-1} fw. The lowest value was at Kusi sites a moderately polluted site. The most polluted site among all the 5 sites i.e. dumping site (high metal deposition, highly polluted) had net chlorophyll content of 0.22 mg g^{-1} which is quite comparable with the data from the non-polluted site of residential campus (0.67 mg g^{-1} fw). The chlorophyll degradation ratio indicating integrity of chlorophyll ranged from 1.06 to 0.90. Acidic conditions results in low chlorophyll degradation ratio, as it favours conversion of chlorophyll *a* into Phaeophytin. The chlorophyll degradation ratio was highest at residential area (1.06) indicating high integrity of chlorophyll and non-acidic conditions while it was lowest with 0.90 at dumping site may be due to emission of fly ash by industrial wastes and other anthropogenic sources. Variation in the carotenoid content is dependent on the ecological conditions as it is recorded highest (0.90 mg g^{-1} fw) a Kusi and Veriyahi village site having dense tree canopy while minimum at dumping areas. (0.14 mg g^{-1} fw), a much polluted site.

The net protein content varied from 12.87 to 42.53 mg g^{-1} fw maximum concentration was recorded at the most polluted site at dumping site having heavy fly ash deposition. In residential sites, the lichen was collected from 4-6 m above the road on a *Bauhinia variegat*e tree. All the measured physiological parameters were at minimum among all the 5 sites studied i.e. 0.151, 0.072, 0.224, 0.139 and 12.866 mg g^{-1} fw for Chl *a*, Chl *b*, total pigment, carotenoid, and protein respectively. In dumping site, the most polluted sites of the area, the total chlorophyll content is quite comparable with the chlorophyll content at residential campus, a non-polluted site. The chlorophyll degradation ratio of 0.98 and reflects exceedingly higher protein content of 42.53 mg g^{-1} at highly polluted sites the adaptation by lichen *P. dilatata* towards stressed environmental condition.

Discussion

In the present study lichens taken from their natural habitat were investigated, which had been exposed to a particular level of pollution for years. In transplant experiments, lichens taken from relatively clean sites undergo drastic changes when transferred to more polluted places. These changes are so sudden and strong that the lichen thallus becomes damaged (decrease of NPS and chlorophyll content) and finally dies off (von Arb, 1987). The process of adaptation of the photosynthetic pigment in response to the environmental conditions and physiological response of plants to certain stress has been mentioned by Schreiber *et al*. (1994). von Arb (1987) reported that in lichens growing in their natural habitat the chlorophyll content was higher at the most polluted locations as an adaptation to air pollution. According to Prasad (1997), induced heat shock proteins (HSPs) act as molecular chaperones and protects against heavy metal toxicity in higher plants. NOx emitted by traffic activity is known to increase the chlorophyll content of lichens (von Arb *et al*., 1990). The increased level of protein in lichens, of the study area, at most polluted sites corresponds with the findings of Gonzalez *et al*. (1996) for the *Ramalina ecklonii*. In higher plants role of heat shock proteins in heavy metal tolerance has been established. Neumann *et al*. (1994) have reported the tolerance mechanism adapted by the plant to withstand air pollution, which include synthesis of stress metabolite and/or protein. In the area with heavy fly ash emission are the main source of metals that can alter the biosynthesis of protein. Various interactions are known to occur when plants are exposed to unfavourable concentration of more than one trace element. Such combination effects were categorized by Berry and Wallace (1981) as in independent, additive, synergistic or antagonistic. One of the heavy metal, Cadmium, is known to increase the gluthathione levels in plants (Ruegsegger and Brunold, 1992). The luxuriant growth of *P. diltata* on Mangifera *indica* tree near fly ash dumping site, a most polluted site indicates that lichen has adapted to the prevailing environmental condition. This observation has been further affirmed by the physiological parameters recorded which shows increase in the metabolic activity and high value of integrity of the chlorophyll content as a tolerance measure to overcome the stress condition.

Conclusion

This study, using ubiquitous epiphytic lichen, showed that a single species can be used to determine air pollution levels around KTPS, Muzaffarpur. The present data of metallic pollutants level will be useful baseline data for carrying out future studies related to ambient air quality in the area.

Chlorophyll content and protein level thus seems to be appropriate tool to monitor the air pollution of an area, though the elevation in protein level in lichens needs further investigation. Lichens shows decrease in chlorophyll content and related parameters but when the pollution increases some lichen species can adapt to (become tolerant) increasing level of pollution. According to Prasad (1997), induced heat shock proteins (HSPs) act as molecular chaperones and protects higher plants against heavy metal toxicity. Thus adaptation Mechanism in the case of lichens may be attributed to the fact that under stress condition biosynthesis of protein (heat shock proteins/stress proteins) increases which readily react with the pollutants especially metallic pollutant and thus shields the photosynthetic apparatus from adverse effect of pollutants.

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