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Report on the bioaccumulation of heavy metals by foliose lichen (*Pyxine cocoes*) from air polluted area near Nagaon Paper Mill in Marigaon, Assam, North-East India

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ABSTRACT

This study is aims to report heavy metal accumulation by foliose lichen [*Pyxine cocoes* (Sw.) Nyl.] grown on the substrate *i.e.*, trees (*Artocarpus chaplasa* Lam., *Bombax ceiba* L. *and Areca catechu* L.) near the area of Nagaon Paper Mill (NPM), located in the Marigaon district of Assam. Lichen sampling was done from different locations, L1:Paschim Nagaon village area (acts as control), L2: NPM gate (passage of numerous loaded and unloaded vehicles) and L3: NPM nursery area (opposite side of the NPM). Results indicated that maximum concentration of Cr, Zn, Pb and Ni was observed at gate side while Fe concentration was found maximum in the nursery area (L3). But Cu concentration was observed maximum in control location (L1). Although Fe, Cr and Zn were increased significantly, but Pb and Ni were increased non significantly on decreasing the distance from paper mill except Cu which was significantly decreased. Fe showed positive high correlation with Cr, Zn and Ni while negative correlation with Cu. Zn had high positive correlation with Pb and Ni. Likewise, Cr was observed with high positive correlation with Zn, Pb and Ni but Cu showed high negative correlation. Dendrogram classified three different groups divided in to two clusters. L2 and L3 grouped in to cluster 1, while control sample grouped into cluster 2. The Dendrogram justifies that the *Pyxine cocoes* collected from L2 and L3 locations accumulated significant amount of the heavy metals (Fe, Cr, Cu, Zn, Pb, and Ni) as compared to control location (L3) which was less air polluted.

Key words: Bioaccumulation**,** lichen, *Pyxine cocoes,* Nagaon Paper Mill (NPM). Heavy metal.

INTRODUCTION

Lichens have been successfully used as bioindicators of environmental pollution and ecosystem health as they provide a relevant, sensitive and measurable indicator for long-term monitoring of the environment (Giordani 2007; Geiser et al. 2010; Giordani et al. 2012; Das et al. 2013). Biological monitoring by means of lichens as accumulators of trace elements is a very suitable tool to assess and monitor air pollution in different parts of the world as lichens show higher sensitivity to air quality. Absence of a protective cuticle has lead to the direct exposure of lichen thalli to atmosphere rather unspecific uptake of mineral nutrients from the surrounding environment.

Bioaccumulation of both essential and nonessential elements in lichens take place through various mechanisms including surface complexation, biomineralisation and physical trapping of dust and soil particulates in the intercellular spaces of the medulla (Richardson 1995; Wilson 1995; Nash III 2008). Surface of the thallus is involved in the absorption, so that elements present in the atmosphere as well as those present in the substrate can penetrate into the lichen thallus (Tyler 1989; Basile et al. 2008; Nash 2008). Atmospheric deposition is the main source of elements in the thalli due to lack of root system in lichens. However, if the concentrations of elements in the substrate are much greater than the deposition of elements from the atmosphere, accumulation in lichen thalli could be substrate-dependent (de Bruin & Hackenitz 1986; Loppi et al. 1999; Bajpai et al. 2009). Recently, Singh et al. (2018) have found that *Pyxine subcineria* was a tolerant species which accumulated significantly different level of heavy metals as per exposure of pollution level.

Pulp and paper mills are amongst some major industries in India that are causing great ecological concerns due to disposal of large quantity of toxic waste into the environment causing both land and water pollution. Besides, gases are also emitted from the pulp and paper mills in the form of SO_2 and NO_x, along with some other pollutants causing air pollution. Nagaon Paper Mill, one of the units of Hindustan Paper Corporation Ltd., (A Govt. of India Enterprise), has the capacity of manufacturing 100000 MT/yr of super quality of writing and printing paper. The mill is adopting alkaline sulphate (kraft) process for pulping bamboo. It requires approximately 680 MT/day of bamboo, 650 MT/day of coal, 180 MT/day of lime and other chemicals to run the mill. This mill is regarded as one of the largest paper mills in Asia. The mill discharges about 90,000 m^3 of effluent water daily excluding its huge volume of semi-solid lime and bleacher waste products causing heavy pollution load in the land, air as well as in the water bodies of downstream area (Haque et al. 2010; Das & Nath 2013; Sen & Baruah, 2014). The entire effluent products are discharged through an open canal into Elenga Beel System which has linkage with the mighty river Brahmaputra.

Since lichens show high degree of sensitivity to air quality, the availability of *Pyxine cocoes* in the vicinity of Nagaon paper mill area bears a great significance. *Pyxine* – a foliose genus of lichenized fungi under the family Caliciaceae (Lücking et al. 2017) has a widespread distribution in tropical regions and consists of about 70 species (Jaklitsch et al. 2016). The present study throws some light on the heavy metal accumulation by the lichen species more particularly, *Pyxine cocoes* exposed at different distances from the Nagaon paper mill, located in the Morigaon district of Assam and thereby to find out the level of atmospheric pollution in and around paper mill area. The lichenology in the state of Assam was concentrated primarily on the works related to diversity (Das 2008; Singh & Bujarbarua 2002; Sinha et al. 2013; Rout et al. 2010; Gupta & Sinha 2018). However, Das et al. (2012 & 2013) had initiated a few bio monitoring studies using lichens in the paper mill area of Barak valley region, in the state of Assam. Therefore, the present study is being carried out to assess the level of heavy metal accumulation by lichen *Pyxine cocoes* exposed at different distances from Nagaon Paper Mill. It is also imperative from toxicological perspective to study the accumulation of heavy metals present in the particulate matter by using the species of lichen.

STUDY SITE

Nagaon Paper Mill (NPM): NPM located at Jagiroad, in the Marigaon district of Assam state, about 60 km of Guwahati, on the National Highway(NH) 37 at latitude 26°4$^{/}$ N and longitude 92°5$^{/}$ E is one of the major industrial units of the region in northeast region of India. NPM site covering an area of 240 hectre is 3 km away from Jagiroad railway station and 90 km from Guwahati air port. The mean monthly rainfall around the region ranges between 9.6 cm in December and 296 cm in June. The annual average rainfall is 195 cm and the monsoon lasts for more than 8 months period starting from middle of the March. The maximum and minimum temperature of the region is 35°C in summer (June) and 12°C in winter season (December). Nearly three sides of NPM plant areas are covered by small hills and forests and other side is NH 37. Kopili and Kolong rivers, both tributaries of mighty river Brahamaputra are flowing nearby the NPM plant site. The main occupations of the inhabitants are cultivation, fishing, small business and service

MATERIALS AND METHODS

Lichen samples of *Pyxine cocoes* were collected as per availability of desired amount of material (Table 1) from three different locations i.e., Paschim Nagaon village area at an altitude of 58m from sea level (L1): 7.70 km distance before reaching the NPM that acted as control, NPM gate at an altitude of 65m (L2): 20m distance from the main gate from where numerous loaded and unloaded vehicles pass carrying raw and processed materials of NPM and NPM nursery area at an altitude of 65m (L3): opposite side of the NPM and about 300m distance from L2 location. In all three localities, this species of lichen occurred on the trunks of various trees. It has been observed that the lichen *Pyxine cocoes* is the only available resistant foliose lichen species growing in this environment although some small developing thalli of *Dirinaria* species were also observed in L1 locality. The host trees in L1 was *Artocarpus heterophyllus* Lam., in L2 *Bombax ceiba* L. and in L3 *Areca catechu* L. Lichen samples thus collected were cleaned and all foreign matter including the bark of tree was removed. The lichen samples were then oven-dried at 70ºC for 48 hrs and powdered by grinding. Powdered sample measuring 0.5g was digested in a diacid mixture (HNO_3 + $HClO_3$) in 3:1 v/v ratio on hot plate under controlled temperature for estimation of Fe, Cr, Zn, Cu, Pb and Ni (Piper, 1967). Residues were filtered through filter paper Whatmann no. 42. The analysis of heavy metals concentration in digested sample of lichens was done by Atomic Absorption Spectroscopy (AAS, Model GBC Avanta-Sigma, Australia). Hollow cathode lamps (Varian) for respective metals were used at a working current ranging from 5-30 m A with 213.9- 357.9 nm spectral line.

The data thus generated were subjected to one way Analysis of Variance (ANOVA) using statistical program Sigma State 3.5, followed by Fisher LSD method for all pair wise multiple comparisons. The difference in mean values among the different groups were found to be significant (P= <0.001) and represented in the form of mean ± SEM (Standard Error Mean).

RESULTS AND DISCUSSION

Pyxine cocoes, growing in three different localities i.e. L1, L2 and L3 (Map1) near Nagaon Paper Mill (NPM) showed interesting level of heavy metal accumulation. The results of heavy metal analysis indicated maximum Fe accumulation in *Pyxine cocoes and ranged from 1878.6* µg g^{-1} to 1910 µg g^{-1} (fig.1) at different distances from the paper mill and increased significantly on decreasing the distance (Table 2). The accumulation of Fe was significantly variable as per exposure of pollutants in different locations from NPM. Similar observation regarding Fe accumulation in lichen was also reported earlier by some other workers (Kinalioglu *et al*. 2006; Shukla & Upreti 2007b; Saxena *et al.* 2007and Singh *et al.*2018) in different areas. Increased accumulation of Fe in the thallus of lichen may be one of the tolerant mechanisms that directly affect the photosynthetic pigments. Fe showed positively high correlation with Cr, Zn and Ni while negative correlation with Cu (Table 3).

Plate 1. Sampling sites of *Pyxine cocoes* (SW.) Nyl. on National High Way 37 at L1, L2 and L3 locations in and around Nagaon Paper Mill area, Assam.

Accumulation of Zn and Cr were observed maximum in the lichen samples collected from the trees of *Bombax ceiba* growing near the NPM gate side (L2) which was significantly higher than the control (L1) and in nursery area (L3) collected samples. Accumulation of Zn was ranged from 82.32 µg g^{-1} to 131.62 µg g^{-1} while Cr ranged from 0.63 µg g^{-1} to 4.55 µg g^{-1} (table 2). These finding were in agreement with Saxena *et al*. (2007), Shukla & Upreti (2007b), Aslan *et al.*(2011) and Singh *et el.* (2018). Zn had high positive correlation with Pb and Ni while Cr had observed high positive correlation with Zn, Pb and Ni, but Cu showed high negative correlation (Table 3). One of the sources of emission of Pb and Zn is motor vehicles. Zn exists as alloys in accumulators of motor vehicles or in carburetors and released as combustion product. The body of motor vehicle is galvanized and Zn oxides are also released by wear and tear on car tyres (Bloemen *et al*., 1995). Elevated concentrations of these elements may therefore have been caused by effects of high traffic density (Kutbay & Kilinc 1991). In the present study also maximum concentration of Zn and Cr in the lichen samples collected from the trees of *Bombax ceiba* growing near the NPM gate side were observed from where many heavy vehicles pass through the gate for unloading and loading of raw and processed materials. Reports of high metal contents in lichen hyper accumulators are mainly due to the trapping of particulate matter and extracellular accumulation (Purvis and Pawlik-Skowrońska 2008; Bačkor and Loppi 2009). Functional groups of mycobiont cell walls (Sarret et al., 1998), metal oxalates (Chisholm et al., 1987), and metal-lichenic acid complexes (Pawlik-Skowrońska et al., 2006;

Pawlik-Skowrońska & Bačkor 2011) may be involved in extracellular metal accumulation. Leaded petrol and diesel contain high level of lead (Pb) while the unleaded petrol emission contains lead in a lesser level (Aslan et al., 2011). The combustion of leaded petrol released Pb into the atmosphere where it could cause lead poisoning and thus, Pb is the main pollutant from the traffic activities.

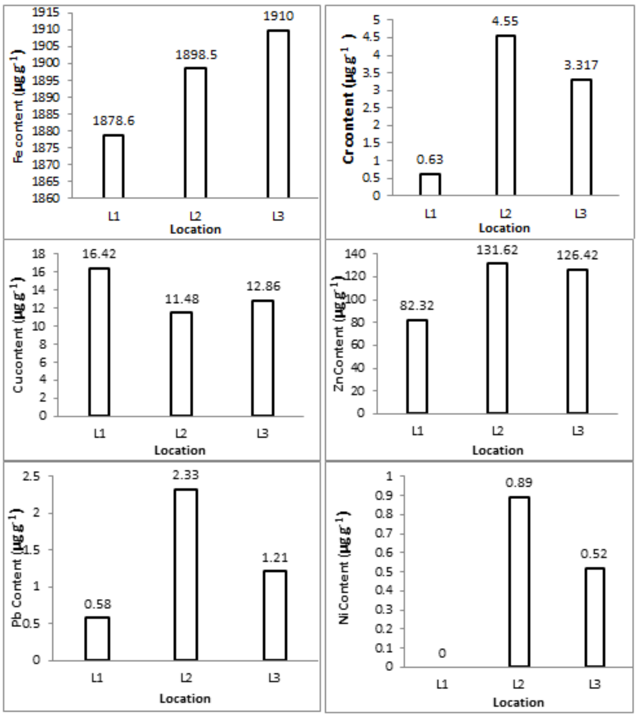

Fig 1. Heavy metals accumulation by *Pyxine cocoes* exposed at different distances from Nagaon Paper Mill (NPM) in the Marigaon district of Assam.

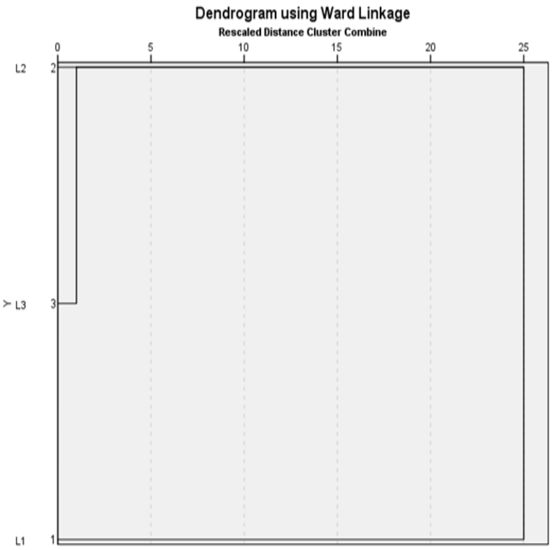

Fig 2. Hierarchical cluster analysis (HCA) of different locations of *Pyxine cocoes* exposed at different distances from Nagaon Paper Mill (NPM) in the Marigaon district of Assam.

Hierarchical cluster analysis (HCA)

Hierarchical cluster analysis is an advantageous multivariate statistical technique, assembling of objects, parameters and samples into clusters based on their similarities representing higher clusters step by step in dendrogram. The dendrogram can be fragmented at dissimilar levels to yield different clusters of the data set and provides a visual summary of the cluster through a picture of the groups and their proximity with a dramatic reduction in dimensionality of the original data. In this study the Ward's method with squared Euclidean distance uses the significant variance approach to evaluate distance between clusters.

HCA was applied in metals concentration (Fe, Cr, Cu, Zn, Pb, and Ni) in three different treatments in the thalli of *Pyxine cocoes.* The output of cluster analysis (dendrogram) was given in fig. 2. Dendrogram classified three different groups divided in to two clusters. Samples L2 and L3 are grouped in to Cluster 1, while control sample grouped in to cluster 2. The Dendrogram justifies that *Pyxine cocoes* lichen collected from both L2 and L3 locations accumulated significant amount of heavy metals i.e, Fe, Cr, Cu, Zn, Pb, and Ni as compared to control location.

Table 1. Sources of *Pyxine cocoes* (Sw.) Nyl. collected from three different sites.

Sl. No.	Sampling site	Altitude	Lichen species	Substrate	Site direction
1	Paschim Nagaon Village area (L1)	58m	*Pyxine cocoes* (Sw.) Nyl.	*Artocarpus heterophyllus* Lam.	7.70 km away from NPM on NH37
2	NPM main Gate (L2)	65m	*Pyxine cocoes* (Sw.) Nyl.	*Bombax ceiba* L.	20 m away from NPM main gate
3	NPM Nursery area (L3)	65m	*Pyxine cocoes* (Sw.) Nyl.	*Areca catechu* L.	300 m away from NPM (opposite site)

Table 2. Heavy metals concentration in *Pyxine cocoes* exposed at different distances from Nagaon Paper Mill (NPM) in the Marigaon district of Assam.

Location	Fe (μg g^{-1})	Cr (μg g^{-1})	Cu(μg g^{-1})	Zn(μg g^{-1})	Pb (μg g^{-1})	Ni (μg g^{-1})
L1	1878.6±3.35	0.63±00	16.42±0.26	82.32±2.59	0.58±0.021	0
L2	1898.5±4.34	4.55±0.323	11.48±0.60	131.62±4.96	2.33±0.85	0.89±0.13
L3	1910±3.75	3.317±0.16	12.86±0.32	126.42±3.12	1.21±0.64	0.52±0.08
$LSD_{(\alpha=0.05)}$	**13.27**	**0.722**	**1.468**	**12.81**	**NS**	**NS**

Table 3. Spearman correlation Coefficient within different heavy metals accumulated in *Pyxine cocoes* exposed at different distances from Nagaon Paper Mill (NPM) in the Marigaon district of Assam.

	Fe	Cr	Cu	Zn	Pb	Ni
Fe	1					
Cr	0.776**	1				
Cu	-0.799**	-.999**	1			
Zn	0.893**	0.977**	-0.984**	1		
Pb	0.494	0.932**	-0.917**	0.832**	1	
Ni	0.699*	0.994**	-0.988**	0.946**	0.967**	1

Correlation is significant at the 0.05 level; *Significant, **Highly Significant

CONCLUSION

It can be concluded from this study that there is a direct correlation between automobiles movement and heavy metals accumulation in studied samples. The study of heavy metals accumulation in lichens with the atmospheric deposition reflects the toxicity of our environment and gives evidence of air contamination at industrial sites with vehicular traffic. The ability of *Pyxine cocoes* to accumulate heavy metals and retain them for a long period in their thalli makes them a beneficial tool in biomonitoring of various aerial heavy metal pollutants. Singh *et al.*, (2018) found high tolerance in *Pyxine subcineria* that accumulated significantly different level of heavy metals as per exposure to pollution. Among all the lichen species used in India to study the bioaccumulation, *Pyxine cocoes* is found to be more toxi-tolerant and suitable for biomonitoring studies (Shukla & Upreti, 2007b; Bajpai et al, 2010; Danesh et al, 2013.). Rout et al (2010) found varied response of pigment profile and chlorophyll degradation of *Pyxine cocoes* lichen to air pollution scenario in Cachar district of Assam, India. Lichen genus *Pyxine* is considered to be tolerant to an unfavourable environment and exhibited luxuriant growth (Shukla and Upreti, 2011) and thus is a good indicator of air pollution. Global increase in members of families Caliciaceae and Physicaceae more particularly genera *Phaeophyscia* and *Pyxine* (van Herk et al, 2002) may also be linked with the changing climate around the globe resulted the dominance of climate resilient species

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