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Effect of coal mining and oil refinery on lichen community and heavy metal accumulation in Lichen Thallus in North East India

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Abstract
The present study was carried out in coal mining area and oil refinery in Margherita and Digboi respectively located in Assam and coal mining area in Khileriariat and Sutnga located in Meghalaya. The study was focused on the effect of coal mining and oil refinery on lichen community and heavy metal accumulation in lichen thallus. The investigation revealed that highest number of lichen species was reported from Digboi with 10 species and lowest in Khileriariat with 4 species. The distribution pattern of lichen varied across the sites. In Margherita, the dominat species were *Graphis scripta*, *Parmelia saxatilis* and *Parmelia sulcata*. In Digboi the dominant species were *Cryptothecia scripta*, *Cryptothecia striata* and *Phlyctis argena*. In Sutnga *Parmelia saxatilis* and *Parmelia sulcata* were dominant. In Khileriariat *Graphis scripta* and *Parmelia sulcata* were dominant. The pH of lichen substrata was analysed and was observed that pH was acidic to slightly acidic in all the substrata of coal mining and oil refinery. The heavy metal accumulation in lichen thallus varies significantly across the two coal mining area and oil refinery. The accumulation of heavy metals like lead, Zinc, Cadmium, Copper and Nickel in lichen thallus were found maximums in coal mining area as compared to oil refinery. The *Parmelia sulcata* was observed to have the high cadmium and Nickel content. The lead content was high in *Dirinaria purpurascens*. The Zinc content was high in lichen *Parmelia sulcata* and copper content was high in *Physcia caesia*.

Keywords: Lichen, coal mining, oil refinery, heavy metals

1. Introduction

Lichens are symbiosis of fungus, Cyanobacteria and Algae. The algal or the cyanobacterial part is called photobiont that responsible for photosynthesis and fungal part is called Mycobiont responsible for structural support. Lichen diversity comprises of approximately 20,000 to 30,000 species worldwide (Hawksworth and Grube, 2020) [19]. The Indian subcontinent has 2,450 species of lichens with India alone contributing to 2040 species (Kumar *et al*., 2011) [21]. Lichen has the ability to colonize to a wide range of habitat from terrestrial, desert to polar region. The excellent ability of the lichen to adapt to wide diversity of habitat is due to the symbiotic nature of lichens (Shukla *et al*., 2014) [32]. Development activities always lead to the disturbance of the natural habitat with series of changes in the environment. Mining makes huge impact on native plant communities and environmental degradation (Bell *et al*., 2001) [9]. Due to the mining activities the sulphuric acid is form which lead to acidic pH of the soil and water. In addition to this the chemical release from the coal mining are loaded with high concentration of heavy metals like Copper, Cadmium, Iron, Zinc and Arsenic which also affect the organism living in that area. Coal mining is not only degraded the environment but also has huge impact on agricultural land (Swer and Singh, 2004) [34]. Both nutrients and toxic elements can be absorbed directly through the surface of Lichen thallus (Wells *et al*., 1995, Agnan *et al*., 2017; Das *et al*., 2021) [38, 1, 15]. Heavy metal accumulation level of each lichen species determined the abilities of lichen in colonizing the polluted sites (Richardon, 1995; Nash, 2008) [28, 23]. On the other hand some lichen species are sensitive to various pollutants occurring in their environment (Nimis *et al*., 2002) [25]. Their unique morphology, sensitivity and tolerant level make them highly responsive to spatial temporal variation in atmospheric pollution. Hence they have been widely used as bioindicators of anthropogenic changes (Garty, 2001) [17] and for monitoring heavy metal pollution levels in the environment (Sujetoviene, 2015) [33]. Lichens are widely used for biomonitoring atmospheric heavy metals and progress in this research has been reported by many researchers. (Bargagli and Mikhailova, 2002; Vantova *et al*., 2013; Yang *et al*., 2023) [7, 37, 40]. This paper focus on effect of coal mining and oil refinery on lichen community and heavy metal accumulation in lichen thallus in North East India.

2. Materials and Methods

2.1 Study sites

North East is one of the biodiversity hotspot in India and also holds natural resources like coal, oil, minerals, and different types of forests (tropical, temperate, and montane forests), many lake, waterfall and river. The study sites are located in the state of Assam and Meghalaya and selection were made based on activities like coal mining area and oil refinery. Margherita and Digboi are small towns located in Tinsukia district of Assam. Margherita is situated with an average elevation of 162 metres above the sea level and with a geographical coordination of 27°17’5.35’’ N latitudes and 95°40’4.66’’ E longitude. It is well known by the name Coal Queen. North Eastern Coalfields is a unit of Coal India Limited, which has its headquarters in Margherita in Assam. There are 5 coal mining in Margherita (three underground and two opencast). Digboi is situated with an average elevation of 165 metres above sea level and with a geographical coordination of 27°39’34’’ N latitude and 95°62’13’’ E longitude. It is one of the oldest Asia refineries and has oldest running oil well in the world. Khilerariat and Sutnga are located in East Jaintia Hills district of Meghalaya. Both of these sites are active coal mining area in Meghalaya. Khilerariat is situated with an average elevation of 1365 metres above sea level and with geographical coordination 25°35’98’’ N latitude and 92°21’59’’ E longitude. Sutnga is situated with an average elevation of 1631 metres above sea level and with geographical coordination 25°22’0’’N latitude and 92°27’0’’ E longitude (Table 1).

Table 1: Geographical coordination and activities of the study sites Margherita and Digboi (Assam) Khilerariat and Sutnga (Meghalaya).

SL. No.	Study sites	Altitude	Geographical coordination	Activities
1	Margherita	162 msl	27°17’5.35’’ N latitudes 95°40’4.66’’ E longitude	Coal mining
2	Digboi	165msl	27°39’34’’ N latitude 95°62’13’’ E longitude	Oil refinery
3	Khilerariat	1365 msl	25°35’98’’ N latitude 92°21’59’’ E longitude	Coal mining
4	Sutnga	1631 msl	25°22’0’’N latitude 92°27’0’’ E longitude	Coal mining

2.2 Collection of lichen samples

The lichen samples were collected following a standard procedure (Yadav *et al*., 2018) [39] from the selected sites during the period of March 2023 to April 2024. Lichen samples were carefully removed from their substrata, such as rocks or trees by hand using a knife, spatula, or by gently pulling them off. Once collected, the lichens were placed in paper bags or envelopes for transport to the laboratory. They are usually stored in paper bags or envelopes in order to avoid moisture buildup.

2.3 Identification

Identification of lichens often include-examining their growth pattern, form, colour and texture. The specimens were identified following the standard monographs (Coppins and James, 1984; Awasthi, 1991, 2000 and 2007) [13, 3, 4, 2]. Taxonomy was updated using The British Lichen Society website (britishlichensociety.org.uk) and the Lichen Portal (lichenportal.org).

2.3 Estimation of pH and Conductivity from Lichen substrata: Lichens substrata (tree bark) were taken and grind them into fine powder and added into distilled water and kept for 24 hours (Schmidt *et al*., 2001) [30]. The pH and conductivity were measured by using Systronics digital pH meter (Model-335) and a Systronics digital conductivity meter (Model-304) respectively.

2.4 Heavy metal analysis from Lichen thallus

Macroscopic foreign materials adhering thalli were carefully removed with a soft bristle brush. Then the samples were rinsed with deionised water to remove fine particular matter on the Lichen thallus (Pawlik-Skowronska and Backor, 2011) [20]. The samples were dried at 90°C for 24 hours to a constant weight. Subsequently, all powdered lichen samples were digested in 70% $HClO_4$ and 65% HNO_3 (1:4) and diluted with double distilled water. Concentration of Zn, Pb, Cd, Cu and Ni were determined by means of atomic absorption spectrometry (Model iCE 3500 AA system VP100 Thermo Scientific) was used to measure the heavy metals.

3. Results

A total 17 lichen species were recorded from all the study sites spreading over 10 families and 13 genera belonging to 3 growth form (Table 2). The 3 growth forms were Foliose, Crustose and Leprose. The lichen species *Dirinaria purpurascens, Cryptothecia scripta, Cryptothecia striata, Graphis scripta, Graphis* sp, *Lecanora thysanophora, Lecidella scabra, Permalia perlata, Parmelia saxatilis, Parmelia sulcata, Parmotrema tinctorum, Pertusaria* sp., *Pyxine cocoes, Phyllopsora* sp., *Phlyctis argena, Physcia caesia* and *Ochrolechia subviridis* were recorded from the study sites. Among the 4 study sites, highest number of lichen species was reported from Digboi with 10 species followed by Margherita with 8 species, Sutnga with 7 species and Khilerariat with 4 species. The distribution pattern of lichen varied across the sites. In Margherita, the dominat species were *Graphis scripta*, *Parmelia saxatilis* and *Parmelia sulcata*. In Digboi the dominant species were *Cryptothecia scripta, Cryptothecia striata* and *Phlyctis argena*. In Sutnga *Parmelia saxatilis* and *Parmelia sulcata* were dominant. In Khilerariat, it was dominated by *Graphis scripta* and *Parmelia sulcata*. *Graphis scripta* was recorded from all the study sites whereas *Parmelia saxatilis* and *Parmelia sulcata* were recorded only from coal mining area. *Lecanora thysanophora* was recorded only from Oil refinery (Digboi).

Table 2: Distributions of Lichen species from Coal mining area (Margherita, Khilerariat, Sutnga) and oil refinery (Digboi)

SL. No.	Lichen taxa	Growth form	Margherita	Digboi	Khilerariat	Sutnga
	Caliciaceae					
1.	*Pyxine cocoes*	Foliose	-	-	-	+
2.	*Dirinaria purpurascens*	Foliose	+	+	-	
	Arthoniaceae					
3.	*Cryptothecia scripta*	Crustose	+	+	-	
4.	*Cryptothecia striata*	Crustose	+	+	-	
	Graphidaceae					
5.	*Graphis scripta*	Crustose	+	+	+	+
6.	*Graphis* sp.	Crustose	-	+	-	
	Physciaceae					
7.	*Physcia caesia*	Foliose	-	-	+	+
	Parmeliaceae					
8.	*Parmotrema tinctorum*	Foliose	+	+	-	-
9.	*Permalia perlata*	Foliose	-	-	-	+
10.	*Parmelia saxatilis*	Foliose	+	-	+	+
11.	*Parmelia sulcata*	Foliose	+	-	+	+
	Pertusariaceae					
12.	*Pertusaria* sp.	Crustose	+	-	-	-
	Lecanoraceae					
13.	*Lecanora thysanophora*	Leprose	-	+	-	-
14.	*Lecidella scabra*	Crustose				+
	Ramalinaceae					
15.	*Phyllopsora* sp.	Crustose	-	+	-	-
	Phlyctidaceae					
16.	*Phlyctis argena*	Crustose	-	+	-	-
	Ochrolwchiaceae					
17.	*Ochrolechia subviridis*	Crustose		+	-	-

+ indicate present and - indicate absent

The pH and conductivity of lichen substrata from all the 4 study sites were recorded (Table 3). It was observed that pH was acidic to slightly acidic in all the substrata of coal mining and oil refinery. In Margherita the pH ranges from 3.56 to 6.28 and acidic pH favour the growth of *Pertusaria* sp and slightly acidic pH favour the growth of *Graphis scripta*. In Digboi the pH ranges from 4.39 to 6.71 and acidic pH favour the growth of *Parmotrema tinctorum*, *Lecanora thysanophora* and *Ochrolechia subviridis* were found growing in slightly acidic condition. In Khilerariat the pH ranges from 3.72 to 6.56 and acidic pH promotes the growth of *Parmelia sulcata* and in slightly acidic pH *Physcia caesia* was found growing. In Sutnga the pH ranges from 4.56 to 6.80 and acidic pH favour the growth of *Lecidella scabra* and slightly acidic condition favour the growth of *Graphis scripta*. The conductivity was highest in Digboi with 0.80 mS/cm and lowest in Sutnga with 0.03 mS/cm.

Table 3: The pH and conductivity of Lichen substrata (Tree bark) from the study sites (Margherita = M, Digboi = D, Khilerariat = K, Sutnga = S).

SL. No.	Lichen taxa	Substrata	pH				Conductivity (mS/cm)			
			M	D	K	S	M	D	K	S
1	*Pyxine cocoes*	Bark	-	-	-	6.10	-	-	-	0.43
2	*Dirinaria purpurascens*	Bark	5.2	5.0	-	-	0.07	0.08	-	-
3	*Cryptothecia scripta*	Bark	5.59	5.61	-	-	0.10	0.08	-	-
4	*Cryptothecia striata*	Bark	5.14	6.15	-	-	0.13	0.14	-	-
5	*Graphis scripta*	Bark	6.28	6.00	6.10	6.80	0.19	0.12	0.15	0.12
6	*Graphis* sp.	Bark	-	6.58	-	-	-	0.10	-	-
7	*Physcia caesia*	Bark	-	-	6.56	6.2	-	-	0.06	0.03
8	*Parmotrema tinctorum*	Bark	5.03	4.39	-	-	0.22	0.10	-	--
9	*Permalia perlata*	Bark	-	-	-	5.20	-	-	-	0.10
10	*Parmelia saxatilis*	Bark	5.80	-	5.21	5.02	0.17	-	0.21	0.23
11	*Parmelia sulcata*	Bark	5.30	-	3.72	5.02	0.23	-	0.06	0.11
12	*Pertusaria* sp.	Bark	3.56	-	-	-	0.05	-	-	-
13	*Lecanora thysanophora*	Bark	-	6.71	-	-	-	0.80	-	-
14	*Lecidella scabra*	Bark	-	-	-	4.56	-	-	-	0.04
15	*Phyllopsora* sp.	Bark	-	6.21	-	-	-	0.10	-	-
16	*Phlyctis argena*	Bark	-	6.25	-	-	-	0.18	-	-
17	*Ochrolechia subviridis*	Bark	-	6.71	-	-	-	0.19	-	-

The heavy metal accumulation in lichen thallus varies significantly across the different coal mining area and oil refinery (Table 4). The accumulation of heavy metals like lead, Zinc, Cadmium, Copper and Nickel in lichen thallus were found maximums in coal mining area as compared to oil refinery. The *Parmelia sulcata* was observed to have the high cadmium and Nickel content with 0.64 ppm and 0.07 ppm respectively from Margherita. The lead content was high with 0.74 ppm in *Dirinaria purpurascens* from Margherita. The Zinc content was high in lichen *Parmelia sulcata* with 0.98 ppm and copper content was high in *Physcia caesia* with 1.26 ppm in Khilerariat.

Table 4: Heavy metals accumulation in Lichen thallus from different coal mining area (M=Margherita; K= Khilerariat; S=Sutnga) and oil refinery (D= Digboi) of North East India.

SL.No.	Lichen taxa	Site	Pb (ppm)	Zn (ppm)	Cd (ppm)	Cu (ppm)	Ni (ppm)
1	*Cryptothecia scripta*	M	0.47	0.001	0.21	1.11	0.001
2	*Cryptothecia striata*	M	0.51	0.004	0.02	1.19	0.004
3	*Graphis scripta*	M	0.13	0.20	0.02	0.35	0.02
4	*Parmotrema tinctorum*	M	0.28	0.76	0.23	0.04	0.01
5	*Parmelia saxatilis*	M	0.18	0.47	0.41	0.10	0.03
6	*Parmelia sulcata*	M	0.26	0.46	0.64	0.09	0.07
7	*Dirinaria purpurascens*	M	0.74	0.50	0.11	1.20	0.002
1	*Dirinaria purpurascens*	D	0.17	0.04	0.01	0.05	0.006
2	*Cryptothecia scripta*	D	0.06	0.002	0.001	0.01	0.001
3	*Cryptothecia striata*	D	0.04	0.002	0.003	0.02	0.002
4	*Graphis scripta*	D	0.03	0.21	0.004	0.03	0.0001
5	*Graphis* sp.	D	0.31	0.02	0.002	0.02	0.0002
6	*Parmotrema tinctorum*	D	0.12	0.24	0.06	0.06	0.004
7	*Lecanora thysanophora*	D	0.04	0.33	0.006	0.05	-
8	*Phyllopsora* sp.	D	0.02	0.32	0.002	0.02	-
9	*Phlyctis argena*	D	0.05	0.001	0.002	0.03	0.0001
10	*Ochrolechia subviridis*	D	0.03	0.002	0.005	0.07	0.0002
1	*Graphis scripta*	K	0.04	0.30	0.04	0.03	0.001
2	*Parmelia saxatilis*	K	0.15	0.57	0.50	0.07	0.04
3	*Parmelia sulcata*	K	0.21	0.98	0.46	0.05	0.05
4	*Physcia caesia*	K	0.61	0.52	0.32	1.26	0.003
1	*Pyxine cocoes*	S	0.12	0.24	0.36	0.08	0.002
2	*Graphis scripta*	S	0.05	0.32	0.03	0.03	0.02
3	*Physcia caesia*	S	0.11	0.34	0.06	0.06	0.03
4	*Permalia perlata*	S	0.58	0.45	0.34	0.04	0.04
5	*Parmelia saxatilis*	S	0.42	0.41	0.36	0.03	0.02
6	*Parmelia sulcata*	S	0.23	0.38	0.21	0.03	0.001
7	*Lecidella scabra*	S	0.001	0.02	0.01	0.002	0.002

4. Discussion

Lichen is symbiotic organism having the ability to responds and adapt to a vast range of ecological conditions. They are valuable indicators of health of an ecosystem because of their sensitivity and tolerant to different environmental factors. The present study was carried out to investigate the effect of coal mining and oil refinery on lichen distribution and accumulation of heavy metal by the lichen thallus. The potential impact of coal mining and oil refinery in lichen thallus in Assam and Meghalaya were observed. It was observed that a very low number of lichen species were recorded in coal mining and oil refinery area. Mining activities reduced the lichen communities because of harsh and disturbed environmental conditions (Liu *et al*., 2001) [22]. Many researchers reported the decrease in diversity in industrial and quarries as well as the road traffic (Gombert *et al*., 2003; Das *et al*., 2014; Sett and Kundu 2016; Khastini *et al*., 2019) [18, 14, 31, 20]. The pH is an important factor affecting the species composition on tree bark and regulates the lichen composition (Van Herk, 2001) [36]. The present study also revealed that all the tree barks were acidic to slightly acidic in nature. In Margherita and Khilerariat the lowest pH were recorded. The pH influences the absorption of elements from soil and can obstruct the heavy metal or impurities adsorption (Catinon *et al*., 2009; 2012) [11, 10]. The heavy metal on tree bark can disturb the development of spores and the growth of protonema and hinder the colonization of habitats in polluted sites (Basile *et al*., 1995) [8].

Many environmental factors as well as habitat conditions influence the uptake of heavy metals by lichen (Nieboer, 1976; Rola 2020) [24, 29]. The lichens are particularly sensitive to heavy metals and presence of these elements in the environment may produce changes at community level (Backor and Loppi, 2009) [5]. The study revealed that all the 17 lichen species has been accumulated significant amount of heavy metal like Zinc, lead, cadmium, copper and nickel collected from the coal mining area and oil refinery. Maximum amount of heavy metals were found in lichen species collected from coal mining area that is Margherita, Khilerariat and Sutnga as compared to Digboi (oil refinery). Many lichen species found in coal mining area were pollution tolerant, resistant to pollution and can accumulate heavy metals. These species are *Graphis scripta*, *Parmelia saxatilis*, *Parmelia sulcata*, *Physcia caesia*, *Lecanora thysanophora* and *Ochrolechia subviridis*.

Lichen species associated with substrata rich in heavy metal are common pollution tolerant species and grow well in polluted area. Lichen growing in metal rich substrates belong to the genera *Lecanora*, *Acarospora*, *Aspicilia*, *Lecidea*, *Ochrolechia*, *Parmeila*, *Physcia*, *Phlyctis*, *Flavoparmelia* and *Melanohalea* (Backor and Fahselt, 2004; Fatima *et al*.,2019) [6,16]. The present study revealed that *Parmelia sulcata* was observed to be the strong accumulators of cadmium and Nickel. *Dirinaria purpurascens* has been found to be the strong accumulators of lead. *Parmelia sulcata* and *Physcia caesia* were the strong accumulators of zinc and copper respectively. Heavy metal accumulation level fundamentally determined the success of lichens in the colonization of polluted sites (Pawlik-Skowronska *et al*., 2006) [27].

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Author Contributions

Conceptualization, methodology, writing and editing by PH sampling and analysis by AT and BB All the authors have read and agreed to the published version of the manuscript.

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