

RESEARCH ARTICLE

Identification and characterization of functional groups in epiphytic lichen *Pyxine cocoes* (Sw.) Nyl. in proximity to coal based thermal power plants in Uttar Pradesh, India

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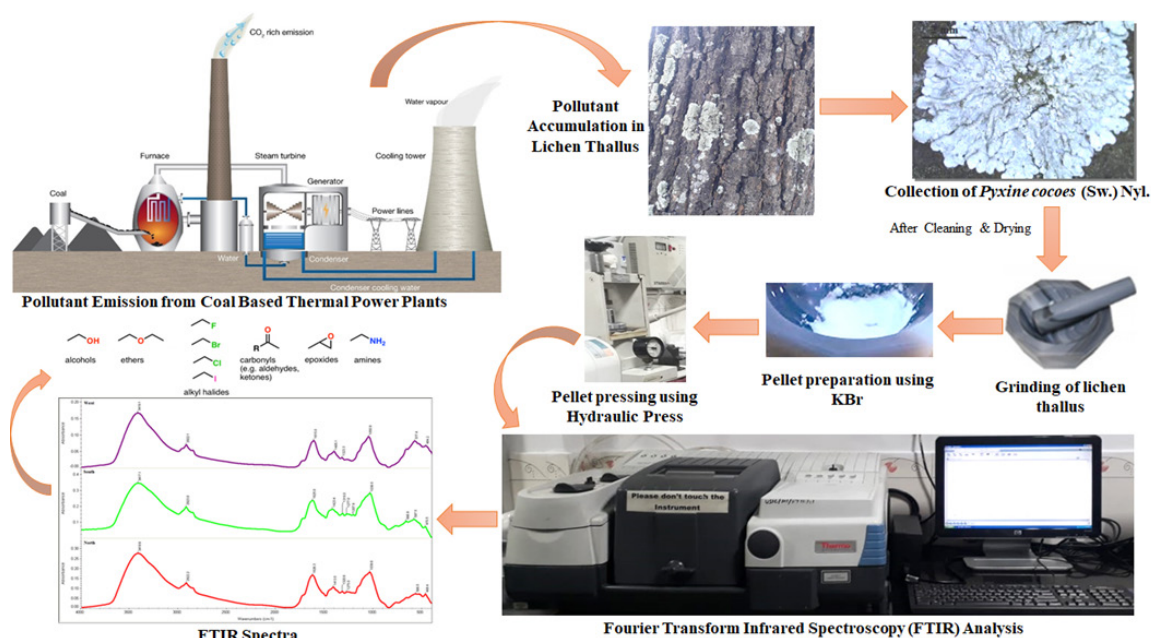
Abstract

Thermal power plants rely on fossil fuels such as coal, oil, or natural gas to produce heat, but they are significant contributors to environmental pollution, emitting metals and other harmful substances. However, the utilization of Fourier Transform Infrared Spectroscopy (FTIR) methods to assess atmospheric pollution in disturbed tropical environments using the spectral properties of lichens has been underexplored. In this study, toxictolerant foliose lichen, *Pyxine cocoes* (Sw.) Nyl. was collected from various disturbed sites around Panki and Feroze Gandhi Unchahar National Thermal Power Plants (TPPs) in Uttar Pradesh, India and subjected to FTIR analysis. The objective was to investigate and compare the functional groups associated with metal accumulation in lichen thalli.

Results of the FTIR analysis revealed similar absorption bands in appearance but varying in intensity, indicating that both TPPs produce comparable chemical components with varying metabolite quantities. The identified specific functional groups responsible for distinct characteristics within the chemical composition of phytochemical compounds in lichens. The presence of metabolites in *P. cocoes* spectra enhanced the detection probability of IR bands in various regions, including 3500-3300 cm^{-1} (N-H and O-H groups), 3300-2500 cm^{-1} (O-H stretching; carboxylic acid), 1450-1400 cm^{-1} (C=O symmetric stretching -COO-). FTIR offers a faster and more reliable approach, particularly with the cost-effectiveness and wide distribution of lichens. This study highlights the valuable information obtained through FTIR analysis, which enhances understanding of chemical involvement in polluted environments.

Keywords: Bioaccumulation, FTIR Spectroscopy, Lichens, Metabolites, Pollution, Thermal Power Plants

Graphical Abstract



Introduction

Coal is widely recognized as one of the foremost energy sources globally, widely utilized for electricity generation and remains the most abundant availability among fossil fuels. However, the emissions arising from coal-fired power plants worldwide contribute significantly to a host of illnesses and premature deaths. Despite its significant impact on human health and its role as a major source of toxic pollutants, coal has remained a staple in global energy production for decades. The United States (US) reportedly possesses a quarter of the world's total fossil fuel reserves, with significant reserves also found in countries like Russia, China, Australia, South Africa and India (Vig *et al.* 2023). In India particularly, atmospheric emissions from coal-fired power plants pose a significant threat to human health. In the period of 2010-2011, 111 plants with a collective generation capacity of 121 gigawatt consumed 503 million tons of coal. This consumption led to emissions of approximately 580 kilotons of particulates with a diameter less than 2.5 μm ($\text{PM}_{2.5}$), 2100 kilotons of sulfur dioxide (SO_2), 2000 kilotons of nitrogen oxides, 1100 kilotons of carbon monoxide (CO), 100 kilotons of volatile organic compounds and 665 million tons of carbon dioxide (CO_2). These emissions were linked to an estimated 80,000 to 115,000 premature deaths and 20 million asthma cases due to $\text{PM}_{2.5}$ pollution exposure. The resultant healthcare costs, borne by both the public and the government, were estimated at Rs. 16,000 to 23,000 crores (USD 3.2 to 4.6 billion) (Guttikunda and Jawahar 2014).

The environmental impact of TPPs, particularly concerning the emission of heavy metals, raises significant concerns. Metals such as arsenic (As), cadmium (Ca), lead (Pb) and mercury (Hg) emitted into the atmosphere, pose threats to both ecosystems and human health (Zhang *et al.* 2020). It is imperative to monitor the presence and concentration

of these metals to assess environmental damage and implement remedial measures. Biological indicators such as lichens, offer an effective, cheap and reliable method for monitoring heavy metal pollution.

Lichens are regarded as promising biomonitors and sensitive indicators of air pollution, capable of assessing changes at various levels to determine the impact of air pollutants (Malaspina *et al.* 2018). Lichens are particularly susceptible to SO_2 due to their ability to absorb it, resulting in fast sulphur accumulation when exposed to high levels of SO_2 pollution (Satya and Upreti 2015). Due to their high sensitivity to acidic gases and enormous ability to accumulate both organic and inorganic chemicals beyond their physiological needs, lichens serve as effective tools for quantifying pollution in specific environments (Sujetovienė 2015). Lichens are studied worldwide for assessing physiological parameters, metals and polyaromatic hydrocarbons (Protano *et al.* 2014, Chetia *et al.* 2021, Thakur *et al.* 2023). The changes in lichen concentration are often linked to the proximity to pollution sources i.e., TPPs (Conti and Cecchetti 2001, Wolterbeek 2002, Adamo *et al.* 2003, Sczapaniak and Bizuk 2003, Giardano *et al.* 2005, Bermudez *et al.* 2009). In India, several studies on pollution monitoring employing lichens as bioindicators have been conducted in various regions. These studies aimed to monitor diverse pollution sources, as reported by (Dubey *et al.* 1999, Upreti and Pandey 2000, Pandey *et al.* 2002, Mishra *et al.* 2003, Saxena *et al.* 2007, Shukla and Upreti 2007, 2008, Bajpai *et al.* 2004, 2010a, b, 2013 a, b, Gupta *et al.* 2017a, b, 2020, 2014, 2015, 2021, 2023, 2024).

FTIR Spectroscopy is the most adaptable analytical technique for determining the kinds of chemical bonds (functional groups) within lichens, offering information about the molecular structure of both organic and inorganic compounds (D' Angelo and Zodrow 2011, Von *et al.* 2014). This technique relies on the transition between quantized vibrational energy states (Coxon *et al.* 2020). Through FTIR spectroscopy analysis, a diverse array of functional chemicals in lichens reveal distinct characteristic peak values. In the spectrum's IR regions, excited states prompt the vibration of molecular bonds, encompassing stretching, bending, rocking, twisting, wagging and deformation. Molecular bonds that have an electric dipole moment susceptible to alteration due to natural vibrations are classified as IR active. IR spectroscopy quantifies these vibrational modes, offering a unique, label-free approach to examining molecular composition and dynamics without disrupting the sample (Chen *et al.* 2015).

Lichens have traditionally served as valuable indicators for studying contaminant distribution and monitoring air quality in urban and industrial environments (Lucadamo *et al.* 2021). However, combined signals can be affected by seasonal atmospheric circulation patterns, leading to

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a reduction in combined signals (Vigouroux *et al.* 2015). In India, research on the impact of TPPs on vegetation and air quality deterioration has been limited (Rao *et al.* 1990, Singh *et al.* 1994). Nonetheless, studies have explored the effect of TPPs emissions on bioaccumulation of metals, spatial distribution, and lichen physiology in Feroze Gandhi Unchahar Thermal Power Plant, Raebareli, Uttar Pradesh as reported by (Bajpai *et al.* 2010a,b); from Tanda Thermal Power Plant, Ambedkar Nagar, Uttar Pradesh by (Gupta *et al.* 2017a); from Panki Thermal Power Plant, Kanpur, Uttar

Pradesh by (Gupta *et al.* 2017b). Other studies have focused on physiological responses using two growth forms of lichens around coal-based Tanda Thermal Power Plant, Ambedkar Nagar, Uttar Pradesh, India (Gupta *et al.* 2020) and phytochemical screening by FTIR spectroscopic analysis of lichen *Rinodina sophodes* commonly growing around Panki and Feroze Gandhi Unchahar Thermal Power Plants of Uttar Pradesh, India as reported by (Gupta *et al.* 2021). Despite these research, only few research have utilized FTIR analysis with lichens summarized in Table 1. Hence,

Table 1: Utilization of lichen for FTIR analysis in the World

S. No.	Countries	State/Region	Locations	Species involved	Technique Used	References
1	Malaysia	Pahang	Gohtong Jaya, Genting Highlands,	<i>Parmotrema tinctorum</i>	FTIR Analysis	Fam <i>et al.</i> 2014
			Genting Highlands, Golf Course area, Taman Awana (non-polluted); JalamAwana, Semi polluted area, JalanGohtong (polluted area)	<i>Usnea baileyi</i>	FTIR Analysis	Abu <i>et al.</i> 2014
2	Southeast Asia	Thailand	KhaoYai National Park in NakornRachasima Province and Queen Sirikit Botanic Garden in Chiang Mai Province	<i>Parmotrema tinctorum</i>	ATR-FTIR Analysis	Srivibbon <i>et al.</i> 2014
3	India	Andhra Pradesh	Goolapalli, Ramakuppammandalam, Chittoor district	<i>Parmeliopsis ambigua</i> , <i>Punctelia subrudecta</i> , <i>Evernia mesomorpha</i> , <i>Xanthoparmelia plitti</i>	Biosynthesis of SNPs, Production of Mycelia Mat, Synthesis SNPs by Mycelia Mat, Characterization of SNPs, UV-VIS Spectra Analysis, FTIR Analysis, Antimicrobial Activity of SNPs, Determination of Biological Activities of the SNPs	Dasari <i>et al.</i> 2013
		Uttar Pradesh	Ambedkar Nagar, Tanda TPP	<i>Pyxine cocoes</i> and <i>Bacidia incongruens</i>	Metals analysis, SEM and FTIR	Gupta <i>et al.</i> 2017a
			Panki Thermal Power Plant, Kanpur and Feroze Gandhi Unchahr Thermal Power Plant, Raebareli	<i>Rinodina sophodes</i>	FTIR Analysis	Gupta <i>et al.</i> 2021
			Pharmacy Unit, Aligarh Muslim University, Aligarh	<i>Usnea longissima</i>	UV-VIS, FTIR, TEM and SEM Analysis, Evaluation of antibacterial activity	Siddqi <i>et al.</i> 2018
		Maharastra	Bahraich	<i>Bacidia convexula</i> and <i>B. submedialis</i>	Metals analysis, SEM and FTIR	Gupta <i>et al.</i> 2024
			Kohlapur	<i>Parmelia perlata</i> (Decolourization and biodegradable of Solvent Red 24 (SR24))	HPTLC, FTIR and GC-HRMS; Phytotoxicity and genotoxicity analysis	Kulkarni <i>et al.</i> 2014
		Tamil Nadu	Local Herbal Market	<i>Parmelia sulcata</i>	Estimation of free radicals, total phenols, total flavonoids; GC-MS, FTIR and NMR Analysis	Gandhi <i>et al.</i> 2022
4	Asia & Europe	Turkey	Middle Anatolian Region	<i>Cetraria isladica</i>	Synthesis and Characterization (by TEM, EDX, UV-VIS, FTIR Analysis) of Ag nanoparticles	Yildiz <i>et al.</i> 2014
			Textile Factory [Acid Red P-2BX (ARP-2BX) dye was supplied]	<i>Cladonia convolute</i> and <i>Evernia prunastri</i>	Dye biosorption analysis, FTIR Analysis	Bayazit <i>et al.</i> 2019

the present study aims to: (i) measure the identified wave numbers corresponding to functional groups, (ii) reveal the functional groups present in lichen thalli of *P. cocoes* using FTIR analysis, (iii) compare the identified FTIR spectra observed from both TPPs.

Materials and Methods

Study Area

Kanpur, the second largest and most populated industrial city in Uttar Pradesh, India, accommodates over 3 million residents, situated within the Gangetic plain, its coordinates ranges from 26°57'11.86" N to 25°55'25.12" N and 80°00'53.79" E to 80°14'08.95" E. The Panki Thermal Power Plant, Kanpur is located at 26°28'31.41" N and 80°14'25.39" E within the Gangetic Plain of Uttar Pradesh (Figure 1a, b, c). During the investigation, the area surveyed lichen species from 34 different locations in all four directions (i.e., east, west, north, and south). It has an installed capacity of 210 MW (2 x 105 MW), with all units being supplied with coal via railways from BCCL and ECL coal mines. The study area has a humid subtropical climate characterized by three distinct seasons: the Cold season (November to February), the Hot season (March to June) and the Monsoon season (July

to October). The monsoon typically brings heavy rainfall (usually 70–80% of the total rainfall) occurs. Despite this, the district has few dispersed, open-canopy deciduous forests due to the fast pace of industrialization, urbanization, and forest degradation.

Raebareli, the district's population is 3,405,559 (as per the census 2011), making it the 27th largest in the state, situated within the coordinates of 26°35'39.76" N to 25°48'36.00" N and 81°09'59.41" E to 81°20'18.00" E. The Feroze Gandhi Unchahar National Thermal Power Corporation (FGUNTPC) is located in Unchahar, Raebareli, Uttar Pradesh, India, between 25°54'58.18" N and 81°19'45.15" E at an elevation of 120.4 m (Figure 1a, b, c) and situated along on the banks of the Sai River. It is surrounded by agricultural fields and mango orchards. This power plant sources its coal from the North Karnpura Coal Fields and its water from the Sarda Sahayak Canal. It has a stack height of 130 m with an installed capacity of 1050 MW (5 x 210 MW) of installed energy output. The climate of the region is tropical, with eight months of dry weather and four months of rainfall ranging from 110 to 485 mm from June to September. The temperature ranging from 13.2°C in winter to 45.2°C in summer.

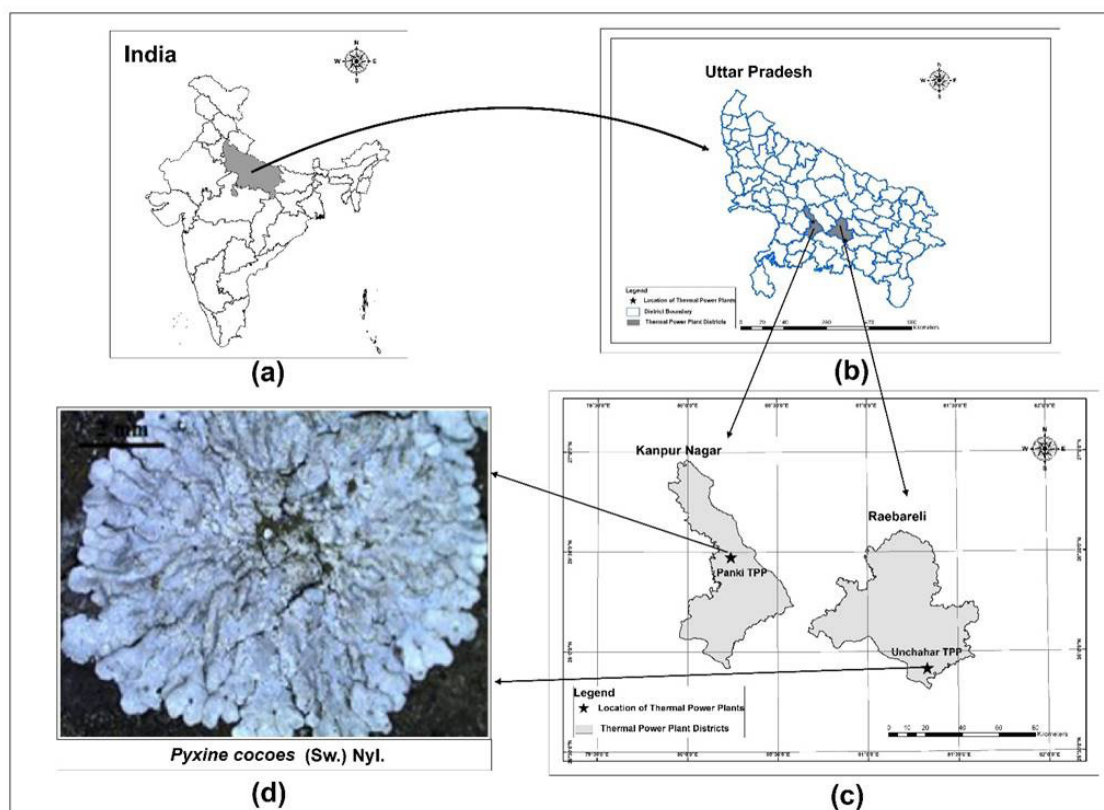


Figure 1: Sampling Locations: (a) Map of India showing Uttar Pradesh; (b) Uttar Pradesh Region showing Kanpur and Raebareli districts; (c) Panki TPP, Kanpur and Feroze Gandhi Unchahar NTPC, Raebareli; (d) Lichen species *Pyxine cocoes* (Sw.) Nyl. collected from the locations around TPPs

Sample Collection

The area around both TPPs was randomly surveyed for lichen sample collection from 114 locations around the thermal power plants. The lichen *P. coccodes* exhibited abundantly growth on the trunks, barks, and branches of *Mangifera indica* (Mango), *Acacia nilotica* (Babool) and *Azadirachta indica* (Neem) trees around both the thermal power plants and further taken for FTIR analysis (Figure 2).

Collected samples were dried and stored in the paper packet. The voucher specimens were preserved in the Lichen Herbarium (LWG) of CSIR- National Botanical Research Institute, Lucknow, India. The morphological, anatomical and chemical characteristics of the lichen samples were evaluated using a LABOMED dissecting microscope for external morphology and LEICA ATC 2000 compound microscope for microscopic anatomical features of the samples. The macro lichen key outlined by (Awasthi 2007) was utilized as the main literature reference for the identification process. TLC in solvent system A (Toluene: 1-4 Dioxane and Acetic acid; 180:60:4) was used to identify the chemical component contained in the lichen thallus (Orange *et al.* 2001).

Fourier Transform Infrared (FTIR) Analysis

The FTIR analysis was done by an FTIR spectroscope (Thermo-Nicolet 6700). To eliminate moisture content, the pellets of lichen samples were dried in an oven at 40-50°C for 4 to 5 hrs. Dried powders of lichen samples were utilized for FTIR analysis. The sample (5 mg) was mixed with KBr pulverized and formed into a disk-shaped pellet. The dried biomass of lichens was ground into powders (blended with dry spectroscopic grade powders) and then hydraulic pressure (CAP-15T) was used to compress the KBr-based pellet into a thin disc under ten tons of pressure. Samples were scanned at the frequency region of 400- 4000 cm^{-1} against the KBr background.

Results and Discussion

Morphological, anatomical and chemical analysis

Pyxine coccodes (Sw.) Nyl.

Thallus corticolous, foliose, whitish grey, orbicular, 1.8-6.0 cm across, UV+ yellow, maculate, sorediate; lobes rotund, pruinose, 0.4-1.3 mm wide; medulla white to off-white; lower surface pale yellow to brown, rhizines brownish. Ascomata apothecia, rare, 0.3-1.2 mm diam.; margin con colours with the thallus, gradually darkening and becoming lecidine; disc black, plane to concave, epruinose of slightly white pruinose. Exciple hyaline, with algal cell when young, brownish at maturity; epihymenium brown, K+ purple; hymenium hyaline to slightly yellowish; hypothecium brown; paraphyses simple to branching, apical cell swollen and brown pigmented. Ascus 8 spored, clavate; ascospores brown, transversely 1 septate, mischoblastiomorphic,

oblong, 15.3-22.1 \times 7.6-9.1 μm (2.5x). Chemistry: Thallus K+ yellow, C-, KC-, P-. TLC: Triterpenes between Rf 4-5, a yellow spot of lichenoxanthane between Rf 6-7 were detected.

Fourier Transform Infrared (FTIR) Analysis

FTIR analysis was employed to find specific spectroscopic biomarkers in *P. coccodes*, facilitating rapid and reliable identification and discrimination of functional groups around both thermal power plants in Uttar Pradesh.

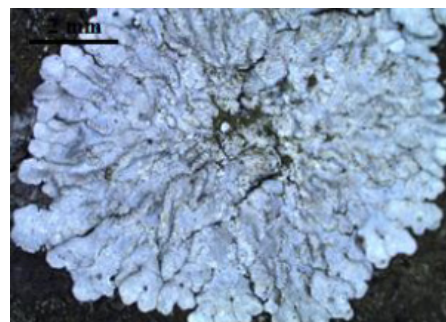


Figure 2: Thallus of Lichen Species *Pyxine coccodes* (Sw.) Nyl.

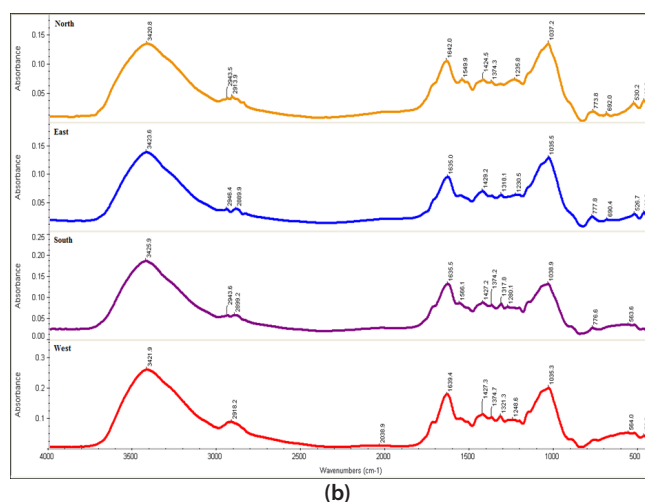
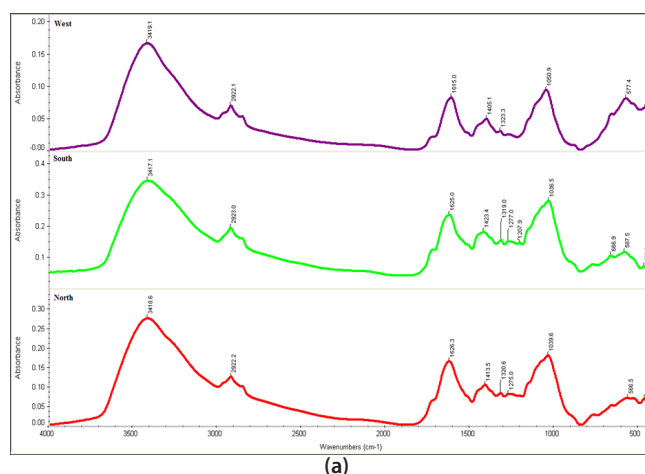


Figure 3: Direction-wise FTIR spectra of lichen species *P. coccodes* (a) Panki Thermal Power Plant, Kanpur; and (b) Feroze Gandhi Unchahar National Thermal Power Corporation, Raebareli

Table 2: Band assignments for FTIR spectra of lichen species *P. coccis* around Thermal Power Plants

S. No.	Range of wave number (cm ⁻¹)	Panki Thermal Power Plant, Kanpur			Feroze Gandhi Unchahar Thermal Power Corporation, Raebareli				Band Assignment
		Band Position (in cm ⁻¹)			Band Position (in cm ⁻¹)				
		North	South	West	North	East	South	West	
1	3500-3300	3418.6	3417.1	3419.1	3420.8	3423.6	3425.9	3421.9	N-H stretching (primary, secondary, amines and amides), O-H stretching (alcohols, phenols groups) (Ramrakhiani <i>et al.</i> 2011)
2	3300-2500	2922.2	2923.0	2922.1	2943.5	2946.4	2943.6	-	O-H stretching (carboxylic acids)
3	2200-2000				2913.9	2889.9	2899.2	2918.2	C≡N stretch (nitriles), -C≡C- stretch (alkynes), NH ₃ ⁺ torsional vibration (Mohan 2005)
4	1760-1665	1626.3	1625.0	1615.0				-	α, β-unsaturated aldehydes, ketones; (Singh <i>et al.</i> 2020) C=O stretching (Carbonyls group)
5	1680-1640				1642.0	1635.0	1635.5	1639.4	-C≡C- stretch (alkenes), Amide- I, C=O stretching (Jilie and Shaoning 2007)
6	1560-1530				1549.9	-	1566.1	-	Amide- II, CN stretching, NH bending (Jilie and Shaoning 2007)
7	1450-1400	1413.5	1423.4	1405.1	1424.5	1429.2	1427.2	1427.3	C=O symmetric stretching –COO ⁻ (Naumann 2000)
8	1370-1350				1374.3	-	1374.2	1374.7	C-H rock (alkanes)
9	1350-1300	1320.6	1319.0	1323.3	-	1318.1	1317.8	1321.3	components of α- helix proteins (Adriana and Gabi 2011)
10	1250-1220	1275.0	1277.0	-	1235.8	1230.5	1280.1	1248.6	P=O stretching (asym.) of PO ₂ ;phosphodiesters (Naumann 2000)
11	1080-1010	1039.6	1036.5	1050.9	1037.2	1035.5	1038.9	1035.3	SO ₃ asymmetric (Cirik <i>et al.</i> 2012); C-O bonding due to polysaccharides (Das and Guha 2007)
12	900-600	-	666.9	-					O-CH ₃ stretching of methoxy groups (Movasaghi <i>et al.</i> 2008)
13	800-640				773.8	777.8	776.6	-	C-H out of plane bending vibrations (Jilie and Shaoning 2007)
14	770-620				692.0	690.4	-	-	Amide- V, Out of plane NH bending (Jilie and Shaoning 2007)
15	690-515	566.5	587.5	577.4	530.2	526.7	563.6	564.0	C-Br stretching (alkyl halides)
16	530-400	468.4	470.5	464.2	469.2	469.2	-	470.0	Al-O stretching (AlO ₈ Octahedral, isolated) (Tarte 1962, 1964)

Variation in frequency, which govern both bond dissociation and bond formation crucial for metal chelation, were monitored through FTIR analysis (Clark *et al.* 2019). This spectroscopic technique furnishes evidence of compounds involved in metal absorption, with alterations in the IR region unveiling the pollutant's influence on the functional groups of lichen species (Figure 3 a, b).

Measurement by FTIR spectra of *P. coccis* around Panki Thermal Power Plant, Kanpur

The strong absorbance bands 3500- 3300 cm⁻¹ were found for the lichen *P. coccis* (Table 2; Figure 3a) at 3418.6 cm⁻¹ (north), 3419.1 cm⁻¹ (west), 3417.1 cm⁻¹ (south), which indicated the stretching of bonded N-H/ O-H and the presence of alcohol

and phenol groups (Ramrakhiani *et al.* 2011).

The spectral band ranged between 3300-2500 cm⁻¹ due to O-H stretching in the presence of carboxylic acid at 2922.2 cm⁻¹ (north), 2922.1 cm⁻¹ (west), 2923.0 cm⁻¹ (south). The absorbance bands observed ranged between 1760-1665 cm⁻¹ due to C=O stretching (carbonyl group) and α, β-unsaturated aldehydes, ketones (55) and observed at 1626.3 cm⁻¹ (north), 1625.0 cm⁻¹ (south), 1615.0 cm⁻¹ (west). The bonded C=O symmetric stretching absorbed infrared at 1423.4 cm⁻¹ (south), 1413.5 cm⁻¹ (north), 1405.1 cm⁻¹ (west) respectively (Naumann 2000). The absorbance bands observed at 1323.3 cm⁻¹ (west), 1320.6 cm⁻¹ (north) and 1319.0 cm⁻¹ (south) showed components of α- helix protein (Adriana and Gabi 2011). The absorbance bands

ranged between 1250-1220 cm^{-1} were attributed to P=O asymmetric stretching and phosphodiester at 1275.0 cm^{-1} (north), 1277.0 cm^{-1} , and 1207.9 cm^{-1} (south) respectively (Naumann 2000). The range of wave number 1080-1010 cm^{-1} showed SO_3 asymmetric stretching (Cirik *et al.* 2012); and C-O bonding due to polysaccharides at 1036.5 cm^{-1} (south), 1039.6 cm^{-1} (north) and 1050.9 cm^{-1} (west) respectively (Das and Guha 2007). The range of wave number 900-600 cm^{-1} showed O-CH₂ stretching of methoxy groups only in the south direction at 666.9 cm^{-1} (Movasaghi *et al.* 2008). The peaks range between 690-515 cm^{-1} resulted in absorption due to the vibration modes of C-Br stretching coupled to alkyl halides at 566.5 cm^{-1} (north), 577.4 cm^{-1} (west) and 587.5 cm^{-1} (south) respectively. This phenomenon is principally involved in metal absorption by the formation of chelates or bond dissociation due to the presence of phytotoxic gases mainly SO_2 . The biodeterioration of granite monuments is available using FT Raman Spectroscopic studies on several lichens (Prieto *et al.* 2000). The peaks observed at 468.4 cm^{-1} (north), 470.5 cm^{-1} (south) and 464.2 cm^{-1} (west) showed the presence of vibrational frequencies of Al-O stretching (AlO_6 Octahedral; isolated) (Tarte 1962, 1964). The observations are useful to detect rapidly and characterize the occurrence of lichen *P. coccinea* with different levels of metal accumulation around the thermal power plants.

Measurement by FTIR spectra of P. coccinea around Feroze Gandhi Unchahar National Thermal Power Corporation (FGUNTPC), Raebareli

For lichen *P. coccinea* around Feroze Gandhi Unchahar National Thermal Power Corporation (FGUNTPC), Raebareli (Table 2; Figure 3b), the major absorbance bands ranged between 3500- 3300 cm^{-1} at 3420.8 cm^{-1} (north), 3421.9 cm^{-1} (west), 3423.6 cm^{-1} (east), 3425.9 cm^{-1} (south) and showed the stretching of N-H/ O-H groups and due to asymmetrical vibration of C-H showed the presence of protein and phospholipids (Ramrakhiani *et al.* 2011). The wave numbers observed at 2923.5 & 2913.9 cm^{-1} (north), 2946.4 & 2889.9 cm^{-1} (east), 2943.6 & 2899.2 cm^{-1} (south) and 2918.2 cm^{-1} (west) ranged between absorbance bands 3300-2500 cm^{-1} due to O-H stretching (carboxylic acids). The absorbance bands observed ranged between 2200- 2000 cm^{-1} due to NH_3^+ torsional vibration and C \equiv N stretch (nitriles), -C \equiv C- stretch (alkynes), at absorbance value 2038.9 cm^{-1} only in west direction of the study area (Mohan 2005). The wave numbers observed at 1635.0 cm^{-1} (east), 1635.5 cm^{-1} (south), 1639.4 cm^{-1} (west), and 1642.0 cm^{-1} (north) ranged between absorbance bands 1680- 1640 cm^{-1} due to Amine-I, C=O stretching and -C \equiv C- stretch (alkenes) (Jilie and Shaoning 2007). The wave numbers observed 1560-1530 cm^{-1} due to Amide- II, CN stretching, NH bending at 1549.9 cm^{-1} (north) and 1566.1 cm^{-1} (south) respectively (Jilie and Shaoning 2007). The bonded C=O symmetric stretching absorbed infra-red at wave number 1450-1400 cm^{-1} at all at 1429.2 cm^{-1} (east),

1427.3 cm^{-1} (west), 1427.2 cm^{-1} (south) and 1424.5 cm^{-1} (north) (Naumann 2000). The spectra observed at 1374.7 cm^{-1} (west), 1374.3 cm^{-1} (north) and 1374.2 cm^{-1} (south) showed stretching of C-H rock (alkanes) in the bands ranged from 1370-1350 cm^{-1} . The absorbance bands ranged 1350-1300 cm^{-1} showed components of α - helix protein at 1317.8 cm^{-1} (south), 1318.1 cm^{-1} (east) and 1321.3 cm^{-1} (west) (Adriana and Gabi 2011). The absorbance bands ranged between 1250-1220 cm^{-1} at 1280.1 cm^{-1} (south), 1248.6 cm^{-1} (west), 1230.5 cm^{-1} (east) and 1235.8 cm^{-1} (north) and 1080-1010 cm^{-1} were attributed to P=O asymmetric stretching and phosphodiester at 1038.9 cm^{-1} (south), 1037.2 cm^{-1} (north) 1035.5 cm^{-1} (east), 1035.3 cm^{-1} (west) (Naumann 2000) and asymmetric stretching of SO_3 (14) as well as C-O bonding due to polysaccharides in all directions respectively (Das and Guha 2007).

The wave numbers 777.8 cm^{-1} (east), 776.6 cm^{-1} (south), and 773.8 cm^{-1} (north) showed C-H out-of-plane bending vibrations. The bands range 770-620 cm^{-1} represents N-H bending with the presence of amine-V at absorbance values 692.0 cm^{-1} (north) and 690.4 cm^{-1} (east) respectively (Jilie and Shaoning 2007). The peaks ranged between 690-515 cm^{-1} resulted in absorption due to the vibration of C-Br stretching (alkyl halides) at 564.0 cm^{-1} (west), 563.6 cm^{-1} (south), 530.2 cm^{-1} (north) and 526.7 cm^{-1} (east) respectively. The peaks observed at 470.0 cm^{-1} (west) and 469.2 cm^{-1} in each (i.e., north and east) direction showed Al-O stretching (AlO_6 Octahedral; isolated) (Tarte 1962, 1964).

The absence of secondary metabolites may explain the large number of bands seen in *P. coccinea*. Due to the presence of more -COOH and -OH groups with varying levels of absorption throughout the study area. The presence of both metabolites in *P. coccinea* increases the chance of detecting the IR band in the 3500-2800 cm^{-1} range. The effect of pollutants on the functional group chemistry of the species is shown by changes in the functional group bands in the IR region of FTIR analysis (Table 2). This mechanism is mostly involved in metal absorption via chelation or bond dissociation caused by the presence of phytotoxic gases primarily SO_2 .

Comparative assessment of FTIR spectral analysis of both TPPs

Comparing the FTIR spectra data of *P. coccinea* collected around the Panki Thermal Power Plant in Kanpur and the Feroze Gandhi Unchahar National Thermal Power Corporation (FGUNTPC) in Raebareli reveals distinct variations in absorbance bands and chemical composition within the lichens. In the vicinity of the Panki Thermal Power Plant, strong absorbance bands were observed in the range of 3500-3300 cm^{-1} , indicating the stretching of bonded N-H/O-H groups and the presence of alcohol and phenol groups. Spectral bands between 3300-2500 cm^{-1} were linked to O-H stretching associated with carboxylic acid, while

absorbance bands in the range of 1760-1665 cm^{-1} indicated C=O stretching and the presence of α , β -unsaturated aldehydes and ketones. Moreover, various absorbance bands corresponding to protein and phospholipids were evident across different spectral regions. Vibrational frequencies linked to metal absorption, such as Al-O stretching, were also detected.

Conversely, around the FGUNTPC in Raebareli, major absorbance bands were observed primarily within the 3500-3300 cm^{-1} range, indicative of N-H/O-H groups stretching. Similar to the findings at the Panki site, absorbance bands between 3300-2500 cm^{-1} were associated with O-H stretching in carboxylic acids. Additionally, various absorbance bands corresponding to protein and phospholipids were observed, indicating the presence of similar functional groups as observed in the Panki site. Notably, differences in absorbance bands related to metal absorption and structural vibrations were observed between the two study sites.

Overall, these differences in absorbance bands and chemical composition highlight variations in metal accumulation levels and environmental conditions between the two study sites, providing valuable insights into the impact of thermal power plants on lichen species. Table 2 represents the comparison of band assignments for FTIR spectra of *P. cocoes* around TPPs.

Conclusion

The present study investigated the presence of metabolites within *P. cocoes*, a toxitolerant lichen species collected from areas surrounding two thermal power plants in Uttar Pradesh, India. The study reveals the presence of metabolites in *P. cocoes* spectra, with distinct IR bands observed within specific wavelength ranges. These bands, ranging from 3500-3300 cm^{-1} (related to N-H and O-H functional groups) to 530-400 cm^{-1} (linked with Al-O stretching), signify variations in functional groups potentially influenced by environmental stressors such as pollution. The observed alterations in functional groups, encompassing changes in carbonyl, hydroxyl and aliphatic groups, suggest a responsive mechanism of *P. cocoes* to pollution-induced stress. These alterations, controlled by bond dissociation and formation, indicate the lichen's adaptability to environmental disturbances, highlighting its resilience in a polluted environment. The findings underscore the significance of FTIR spectroscopy as an effective tool for assessing the impacts of environmental pollution on biological systems. By establishing baseline data through FTIR analysis of *P. cocoes* in the vicinity of thermal power plants, this study sets the stages for future investigations into the biochemical mechanisms underlying stress responses in lichen species. Such insights are crucial for guiding conservation strategies aimed at mitigating the detrimental effects of pollution on ecosystems.

Declarations

- All authors are members.
- The manuscript contains unpublished data in the related field of research.
- There is no conflict of interest among authors or with any publisher concerned.

References

- Abu Bakar SZ Latip J Din LB and Sansuddin MW (2014). Metabolic Fingerprinting of Lichen *Usnea baileyi* by Fourier Transform Infrared Spectroscopy. *AIP Conference Proceedings* **1614**: 452-455.
- Adamo P Giardona S Vingiani S Castaldo Cobianchi and Violante P (2003). Trace element accumulation by moss and lichen bags in the city of Naples (Italy). *Environ. Pollut.* **122**: 91-103.
- Adriana A and Gabi D (2011). Rapid Characterization of peptide secondary structure by FT-IR spectroscopy. *Revue Roumaine de Chimie* **56**: 783-791.
- Awasthi DD (2007). A Compendium of the macrolichen from India, Nepal and Sri Lanka. Bishen Singh MahendraRai Singh, Dehra Dun, India.
- Bajpai R Karakoti N and Upreti DK (2013b). Performance of a naturally growing Parmeloid lichen *Remototrachyna awasthii* against organic and inorganic pollutants. *Environ. Sci. and Pollution Research* **20(8)**: 5577-5592.
- Bajpai R Upreti DK and Mishra SK (2004). Pollution monitoring with the help of lichen transplant technique at some residential sites of Lucknow city, Uttar Pradesh. *Journal of Environmental Biology* **25(2)**: 191-195.
- Bajpai R Upreti DK Nayaka S and Kumari B (2010a). Biodiversity, bioaccumulation and physiological changes in lichens growing in the vicinity of the coal-based thermal power plant of Raebareli district, north India. *Journal of Hazardous Materials* **174**: 429-436.
- Bajpai R Upreti DK Nayaka S and Kumari B (2010b). Accumulation of Arsenic and Fluoride in Lichen *Pyxine cocoes* (Sw.) Nyl., Growing in the vicinity of Coal-based thermal power plant of Raebareli district, North India. *Journal of Experimental Sciences* **1(4)**: 37-40.
- Bajpai R Upreti DK and Shukla V (2013a). Impact assessment of anthropogenic activities on air quality, using lichen *Remototrachyna awasthii* as biomonitors. *Int. J. Environ. Sci. Technol.* **10(6)**: 1287-1294.
- Bayazit G Gül UD and Unal D (2019). Biosorption of Acid Red P-2BX by lichens as low-cost biosorbents. *Int. J. Env. Studies* **76(4)**: 608-615.
- Bermudez GMA Rodriguez JH and Pignata ML (2009). Comparison of air pollution monitoring ability of three Tillandsia species and the lichen *Ramalinacelestri* in Argentina. *Environ. Res.* **109**: 6-14.
- Chen Y Zou C Mastalerz M Hu S Gasaway C and Tao X (2015). Application of Micro-Fourier Transform Infrared Spectroscopy (FTIR) in the Geological Sciences – A Review. *Int. J. Mol. Sci.* **16**: 30223-30250.
- Chetia J Gogoi N Gogoi R and Yasmin F (2021). Impact of heavy metals on physiological health of lichens growing in differently polluted areas of central Assam, North East India. *Plant Physiology Reports* **26**: 210-219.
- CirikY Bekci ZM BuyukatesY IlknurA and Merdivan M (2012). Heavy

- metals uptake from aqueous solutions using marine algae (*Colpomenia sinuosa*): kinetics and isotherms. *Chemistry and Ecology* **28**(5): 469-480.
- Clark AA Yang B Rodgers MT and Armentrout PB (2019). Experimental and Computational Study of the Group 1 Metal Cation Chelates with Lysine: Bond Dissociation Energies, Structures, and Structural Trends. *The J. Physical Chem. B* **123**(9): 1983-1997.
- Conti ME and Cecchetti G (2001). Biological Monitoring: lichens as bioindicators of air pollution assessment: a review. *Environ. Pollut.* **114**(3): 471-492.
- Coxon DJ Staniforth M Breeze BG Greenough SE Goss JP Monti M, ..., Newton ME (2020). An ultrafast shakedown reveals the energy landscape, relaxation dynamics, and concentration of the N3VH0 defect in diamond. *The J. Physical Chem. Letters* **11**(16): 6677-6683.
- D'Angelo JA and Zodrow EL (2011). Chemometric study of functional groups in different layers of *Trigonocarpus grandis* ovules (Pennsylvanian seed fern, Canada). *Org. Geochem.* **42**: 1039-1054.
- Das SK and Guha AK (2007). Biosorption of chromium by *Termitomyces sclypeatus*. *Colloids and Surfaces B: Biointerfaces* **60**: 46-54.
- Dasari S Suresh KA Rajesh M Reddy CSS Hemalatha CS Wudayagiri R and Valluru L (2013). Biosynthesis, Characterization, Antibacterial and Antioxidant Activity of Silver Nanoparticles Produced by Lichens. *Journal of Bionanoscience* **7**: 1-8.
- Dubey AN Pandey V Upreti DK and Singh J (1999). Accumulation of lead by lichens growing in and around Faizabad, U.P., India. *J. of Environment Biology* **2**: 223-225.
- Fam WH Latip J Din LB and Samsuddin MW (2014). Direct discrimination of *Parmotrema tinctorum* from various location by Fourier transform Infrared Spectroscopy. *AIP Conference Proceedings* **1614**: 347-350.
- Gandhi AD Umamahesh K Sathiyaraj S Suriyakala G Velmurugan R Al Farraj DA Gawwad MRA Murugan K Babujanarthanam R and Saranya R (2022). Isolation of bioactive compounds from lichen *Parmeliasulcata* and evaluation of antimicrobial property. *Journal of Infection and Public Health* **15**: 491-497.
- Giardona S Adamo P Sorbo S and Vingiani S (2005). Atmospheric trace metal pollution in the Naples urban area based on results from moss and lichen bags. *Environ. Pollut.* **136**: 431-432.
- Gupta N Dwivedi SK and Upreti DK (2017a). Studies on Uptake and Localization of metals in lichens growing around Thermal Power Plant through the application of SEM and FTIR techniques. *Journal of Cryptogam Biodiversity and Assessment* **2**(1): 37-52.
- Gupta N Dwivedi SK and Upreti DK (2017b). Physiological variation and accumulation of metals in two growth forms of lichens growing around Panki Thermal Power Plant of Uttar Pradesh, India. *International Journal of Advanced Research* **5**(7): 1554-1564.
- Gupta N Dwivedi SK and Upreti DK (2020). Estimation of physiological responses using two growth forms of lichens around coal-based Tanda Thermal Power Plant, Ambedkar Nagar district of Uttar Pradesh, India. *Current Research in Environmental & Applied Mycology* **10**(1): 131-141.
- Gupta N Gupta V Dwivedi SK and Upreti DK (2015). Comparative bioaccumulation potential of *Pyxine coccinea* and *Bacidia submedialis* in and around Faizabad city, Uttar Pradesh, India. *G- Journal of Environmental Science and Technology* **2**(6): 86-92.
- Gupta N Gupta V Dwivedi SK and Upreti DK (2021). Phytochemical Screening by FTIR spectroscopic analysis of lichen *Rinodina sophodes* commonly growing around Panki and Feroze Gandhi Unchahar Thermal Power Plants of Uttar Pradesh, India. *Octa Journal of Environmental Research* **9**(2): 052-061.
- Gupta V (2014). Air Pollution Monitoring in and around Faizabad city utilizing lichen distribution pattern, M. Sc. Dissertation Thesis, Dr. R.M.L. Avadh University, Faizabad.
- Gupta V Gupta N Nayaka S Lavania S and Srivastava PK (2023). *Pyxine coccinea* (Sw.) Nyl. as an ideal lichen species for biomonitoring studies: A systematic review. *J. Indian bot. Soc.* **103**(4): 245-256.
- Gupta V Gupta N Nayaka S and Lavania S (2024). Delineating biomonitoring potential of two crustose lichens *Bacidia convexula* and *B. submedialis* through elemental accumulation and microstructural parameters. *The Nucleus* 1-15.
- Guttikunda SK and Jawahar P (2014). Atmospheric emissions and pollution from the coal-fired thermal power plants in India. *Atmospheric Environment* **92**: 449-460.
- Jilie K and Shaoning YU (2007). Fourier Transform Infrared Spectroscopic Analysis of Protein Secondary Structures. *Acta Biochimica et Biophysica Sinica* **39**(8): 549-559.
- Kulkarni AN Kadam AA Kachole MS and Govindwar SP (2014). Lichen *Permelia perlata*: A novel system for biodegradation and detoxification of disperse dye Solvent Red 24. *Journal of Hazardous Materials* **276**: 461-468.
- Lucadamo L Gallo L and Corapi A (2021). PAHs in an urban-industrial area: The role of lichen transplants in the detection of local and study area scale patterns. *Environmental Pollution* **284**: 117136.
- Malaspina P Casale M Malegori C Hooshyari M Di Carro M Magi E and Giordani P (2018). Combining spectroscopic techniques and chemometrics for the interpretation of lichen biomonitoring of air pollution. *Chemosphere* **198**: 417-424.
- Mishra SK Upreti DK Pandey V and Bajpai R (2003). Pollution monitoring with the help of lichen transplant technique in some commercial and industrial areas of Lucknow city. *Pollution Research* **22**(2): 221-225.
- Mohan J (2005). *Organic Spectroscopy Principle and Application*. 2nd Edition, Narosa Publishing House, New Delhi.
- Movasaghi Z Rehman S and Rehman IU (2008). Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues. *Applied Spectroscopy Reviews* **43**(2): 134-179.
- Naumann D (2000). Infrared Spectroscopy in Microbiology In: *Encyclopedia of Analytical Chemistry*. R. A. Meyers (Ed.). John Wiley & Sons Ltd, Chichester 102-131.
- Orange A James PW and White FJ (2001). *Microchemical Methods for the Identification of Lichens*. British Lichen Society, London.
- Pandey V Upreti DK Pathak R and Pal A (2002). Heavy metal accumulation in Lichens from the Hetauda Industrial Area Narayani Zone Makwanpur District, Nepal. *Environmental Monitoring and Assessment* **73**(3): 221-228.
- Prieto B Edwards HGM and Seaward MRD (2000). A Fourier Transform-Raman Spectroscopic study of lichen strategies on granite monuments. *Geomicrobiology Journal* **17**: 55-60.
- Prieto B Seaward MRD and Edwards HGM (1999). Biodeterioration of granite monuments by *Ochrolechia parella* (L.) Mass: An FT Raman Spectroscopic Study. *Biospectroscopy* **5**: 53-59.

- Protano C Zinna L Giampaoli S Spica VR Chiavarini S and Vitali M (2014). Heavy Metal Pollution and Potential Ecological Risks in Rivers - A Case Study from Southern Italy. *Bull. Environ. Contam. Toxicol.* **92**: 75-80.
- Ramrakhiani L Majumder R and Khowala S (2011). Removal of hexavalent chromium by heat-inactivated fungal biomass of *Termitomyces clypeatus*: Surface characterization and mechanism of biosorption. *Journal of Chemical Engineering* **171**: 1060-1068.
- Rao DN Agrawal M and Singh J (1990). Study of pollution sink efficiency, growth response and productivity pattern of plants with respect to fly ash and SO₂: Final technical report submitted to MOEF India. DOE 14/266/85
- Satya Upreti DK (2015). Air Quality Assessment by *Pyxine cokes* concerning Seasonal Variation and Traffic Influence in India. *Int. J. of Curr. Microbiol. App. Sci.* **4(9)**: 549-559.
- Saxena S Upreti DK and Sharma N (2007). Heavy metal accumulation in lichens growing in North side of Lucknow city, India. *J. of Environ. Biol.* **28(1)**: 49-51.
- Sczapaniak K and Biziuk M (2003). Aspects of biomonitoring studies using mosses and lichens as indicators of metal pollution. *Environ. Res.* **93**: 221-230.
- Shukla V and Upreti DK (2007). Heavy Metal Accumulation in *Phaeophyscia hispidula* En Route to Badrinath, Uttaranchal, India. *Environmental Monitoring and Assessment* **131**: 365-369.
- Shukla V and Upreti DK (2008). Effect of metallic pollutants on the physiology of lichen *Pyxine subcinera* Stirton in Garhwal Himalayas. *Environmental Monitoring and Assessment* **141(1)**: 237-243.
- Siddqi KS Rshaid M Rahman A Tajuddin Husen A and Rehman S (2018). Biogenic fabrication and characterization of silver nanoparticles using aqueous-ethanolic extract of lichen (*Usnea longissima*) and their antimicrobial activity. *Biomaterials Research* **22**: 23-31.
- Singh J Agrawal M and Narayan D (1994). Effect of power plant emission on plant community structure. *Ecotoxicology* **3**: 110-122.
- Singh RK Ruj B Sadhukhan AK and Gupta P (2020). A TG-FTIR investigation on the co-pyrolysis of the waste HDPE, PP, PS and PET under high heating conditions. *Journal of the Energy Institute* **93(3)**: 1020-1035.
- Sriviboon T Sittiosod R Boonpragob K and Prombut T (2014). Sample preparation for analysis of chemical composition of lichen by ATR-FTIR Spectroscopy. Proceedings of the 40th Congress on Science and Technology of Thailand (STT40). Section H – Biology/ Biodiversity. Pp. 877-881.
- Sujetovienė G (2015). Monitoring lichen as indicators of atmospheric quality. Recent Advances in Lichenology: Modern Methods and Approaches in Biomonitoring and Bioprospection **1**: 87-118.
- Tarte P (1962). Etude infra-rouge des orthosilicates et des orthogermanates Une nouvelle méthode d'interprétation des spectres. *Spectrochimica Acta* **18(4)**: 467-483.
- Tarte P (1964). Infrared study of dicalcium ferrite, 2CaO.Fe₂O₃ and the solid solutions 2CaO.(Al,Fe)₂O₃. *Rev. Chim. Minérale* **1**: 425-438.
- Thakur MB Bhardwaj S Kumar V and Rodrigo-Comino J (2023). Lichens as Effective Bioindicators for Monitoring Environmental Changes: A Comprehensive Review. *Total Environment Advances* 200085.
- Upreti DK and Pandey V (2000). Determination of heavy metals in lichens growing on different ecological habitats in Schirmacher Oasis, East Antarctica. *Spectr. Lett* **33**: 435-444.
- Vig N Ravindra K and Mor S (2023). Environmental impacts of Indian coal thermal power plants and associated human health risk to the nearby residential communities: A potential review. *Chemosphere* 140103.
- Vigouroux C Blumenstock T Coffey M Errera Q García O Jones NB ...De Mazière M (2015). Trends of ozone total columns and vertical distribution from FTIR observations at eight NDACC stations around the globe. *Atmospheric Chemistry and Physics* **15(6)**: 2915-2933.
- Von Aulock FW Kennedy BM Schipper CI Castro JM Martin DE Watkins JM Wallace PJ Puskar L Behue F and Nichols ARL (2014). Advances in Fourier transform infrared spectroscopy of natural glasses: From sample preparation to data analysis. *Lithos* **206-207**: 52-64.
- Wolterbeek B (2002). Biomonitoring of trace element air pollution: Principles, possibilities and perspectives. *Environ. Pollut.* **120**: 11-21.
- Yildiz N Ates C Yilmaz M Demir D Yildiz A and Calmili A (2014). Investigation of lichen based green synthesis of silver nanoparticles with response surface methodology. *Green Process Synth.* **3**: 259-270.
- Zhang Y Wu D Wang C Fu X and Wu G (2020). Impact of coal power generation on the characteristics and risk of heavy metal pollution in nearby soil. *Ecosystem Health and Sustainability* **6(1)**: 1787092.