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### PHYTOCHEMICAL SCREENING BY FTIR SPECTROSCOPIC ANALYSIS OF LICHEN *RINODINA SOPHODES* COMMONLY GROWING AROUND PANKI AND FEROZE GANDHI UNCHAHR THERMAL POWER PLANTS OF UTTAR PRADESH, INDIA

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**Abstract:** Lichen *Rinodina sophodes*, a commonly growing around both thermal power plants of Uttar Pradesh, India are validated by Fourier- Transform Infrared (FTIR) Spectroscopy. FTIR analysis used to identify and determine spectral features of secondary metabolites in *Rinodina sophodes* growing around Panki and Feroze Gandhi Unchahar TPPs, to find specific spectroscopic biomarkers for rapid identification and discrimination. Identification of chemical nature of phytochemical compounds present in lichens will provide information on functional groups responsible for the particular properties. FTIR spectra showed structural peculiarities of metabolites along with variations in functional groups i.e. N-H stretching Amide-A, O-H stretching of hydroxyl groups and carboxylic acid, SO<sub>3</sub> asymmetric, C-O bonding due to polysaccharides and C-Br stretching (alkyl halides). However, secondary metabolites play an important role in chelation, therefore, functional group bands in IR region showed effect of pollutant on chemistry of functional groups in lichen species. The spectra of *R. sophodes* showed presence of metabolites which enhances the probability of detection of IR bands in 3500- 3300 cm<sup>-1</sup> (due to presence of more N-H and O-H groups), 3300-2500 cm<sup>-1</sup> (O-H stretching; carboxylic acid), 1450-1400 cm<sup>-1</sup> (C=O symmetric stretching –COO<sup>-</sup>), 1360-1290 cm<sup>-1</sup> (components of α- helix proteins), 1080-1010 cm<sup>-1</sup> (SO<sub>3</sub> asymmetric; C-O bonding due to polysaccharides) and 530-400 cm<sup>-1</sup> (Al-O stretching (AlO<sub>6</sub> Octahedral, isolated) were dominant spectral bands respectively. FTIR analysis provides spectroscopic evidence of the role of chemicals in metal absorption as the variation in the frequency is affected by bond dissociation and bond formation which plays important role in metal chelation.

**Keywords:** FTIR spectroscopy, Metabolites, Pollution, Lichens, Thermal power plants.

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## INTRODUCTION

Lichens are regarded as potential bio-monitors as well as sensitive indicators of air pollution to evaluate the effect of air pollutants by measuring changes at different level (Loppi and Bonini, 2000). Lichens are also sensitive to SO<sub>2</sub> due to their capability of absorption process which results in rapid sulphur accumulation when exposed to maximum level of SO<sub>2</sub> pollution (Satya *et al.*, 2015). According to Pirintsos and Loppi (2008), lichens are peculiar

in nature, having a special sensitivity for acidic gases but accumulate several organic as well as inorganic compounds in a large amount beyond their physiological needs and thus can be utilized to monitor pollutant load of a particular environment. Lichens are studied worldwide for assessment and determination of metals, polyaromatic hydrocarbons (Conti and Cecchetti, 2001; Kircher and Darllant, 2002; Protano *et al.*, 2014). The variations of concentration in lichens correlated with

increasing distance from the source of pollution i.e. thermal power plant. According to Conti and Cecchetti (2001); Wolterbeek (2002); Sczapaniak and Biziuk (2003); Bermudez et al. (2009); Giardona et al. (2005); Adamo et al. (2003) reported that the importance of distance from the source of pollution. Recently studies on pollution monitoring utilizing lichens as bioindicator have been carried out in India in different climatic regions of the country to monitor various pollution sources (Dubey et al., 1999; Upreti and Pandey, 2000; Pandey et al., 2002; Mishra et al., 2003; Bajpai et al., 2004; Saxena et al., 2007; Shukla and Upreti, 2007, 2008; Bajpai et al., 2010 a, b; 2013a, b; Gupta, 2014; Gupta et al., 2015; Gupta et al., 2017 a, b).

FTIR Spectroscopy is most versatile analytical technique for identifying the types of chemical bonds (functional groups) present in lichens and can provide elemental information on the molecular structure of organic and inorganic compounds (Lis et al., 2005; Rossman, 2006; D'Angelo and Zodrow, 2011; Von Aulock et al., 2014). This technique is associated with transition between quantized vibrational energy states (Griffiths and de Haseth, 2007). FTIR spectroscopic studies revealed different characteristic peak values with a number of functional compounds in the extract. The wavelength of light absorbed is the characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the IR spectrum, the chemical bonds in a molecule can be determined. The excited states result in the vibrations of molecular bonds (i.e., stretching, bending, rocking, twisting, wagging, deformation) occurring at various frequencies (or wavelengths) in the IR bands of the spectrum. Molecular bonds with an electric dipole moment that can change by atomic displacement owing to natural vibrations are IR active. These vibrational modes are quantitatively measurable by IR spectroscopy providing a unique, label free tool for molecular composition and dynamics studies without perturbing the sample (Chen et al., 2015).

According to Purvis et al. (2004), lichens have been used extensively to

monitor air quality around urban areas, industrial sites and to assess spatial distribution and accumulation of airborne pollutants. But due to atmospheric decline any integrated signals can be minimized because of seasonal atmospheric circulation patterns as reported by Carignan et al. (2002). Very few studies have been carried out on the effect of thermal power plants on vegetation and air quality deterioration in localities around thermal power plants in India by Singh et al. (1994); Rao et al. (1990), though, studies regarding effect of thermal power plants emission on lichens done by Bajpai et al. (2010a, b) on bioaccumulation of metals, its spatial distribution and effect on the physiology of lichens in Feroze Gandhi Unchahar Thermal power plant, Raebareli, Uttar pradesh is known from India; Gupta et al. (2017a) on Uptake and Localization of metals in lichens growing around Thermal Power Plant through application of SEM and FTIR techniques and Gupta et al. (2017b) on physiological variation and accumulation of metals in two growth forms of lichens growing around Panki Thermal Power Plant of Uttar Pradesh are known from India. In the present study, an attempt has been made to reveal the accumulation mechanism and their localization in lichen species *Rinodina sophodes* in lichen thalli (Figure 1) with the application of FTIR analysis .

## EXPERIMENTAL

**Study Area:** Kanpur city, with a population of about 3 million, is situated in North central part of India at 88°22'E and 26°26'N in Gangetic plain, is the second largest and most populated industrial city in the state of Uttar Pradesh, India. The area around Panki Thermal Power Plant, Kanpur is situated in North Central part of India, lies between coordinate 26°28'35" N and 80°14'31" E in Gangetic Plain in the state of Uttar Pradesh (Figure 2 a, b & c). The area was surveyed for the collection of lichen species from 34 localities in all four directions i.e., east, west north and south. It is second largest as well as most populated industrial city (about 3 million) with installed capacity of 210MW (2x 105). The coal to all units is fed

from coal mines of BCCL and ECL by means of railways. The study area has the zone of humid subtropical climate and the year is divided into three seasons i.e. Cold season (November to February), Hot season (March to June) and Monsoon season (July to October). Heavy rainfall (generally 70-80% of the total rainfall) occurs during the monsoon season in the months of July, August and September. The fast pace of industrialization, urbanization together with the destruction of forest resulted in few scattered, open canopy deciduous forests in the district. Feroze Gandhi Unchahar National Thermal Power Plant Corporation (FGUNTPC) is situated at Unchahar in Raebareli district, located on Southern central part of Uttar Pradesh, India; lies between coordinates 25°49' to 26°36'N and 100°41' to 81°34'E at an altitude of 120.4 m (Fig- II a, b, d) and is surrounded by agriculture fields and mango orchards along with two National Highways 330A. This city is situated on the bank of Sai River. The coal for the power plant is sourced from North Karpura Coal Fields, while source of water for the power plant is from Sarda Sahayak Canal. It has the stack height of 130m with installed capacity of generation of electricity i.e., 1050 MW (5 x 210 MW) capacity of. The climate of the region is tropical, with eight months of dry period and four months of rain that ranges from 110 to 485mm 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 both the thermal power plants were randomly surveyed for collection of lichen samples from 114 localities in all four directions i.e., east, west, north and south of the power plant (Figure 2) around 21 km area. Lichens especially *Rinodina sophodes* was growing abundantly on *Mangifera indica* (Mango), *Acacia nilotica* (babool), *Azadirachta indica* (Neem) tree trunk, barks and branches around both the thermal power plants (Figure 2). Thus, *Rinodina sophodes* commonly occurring lichen species was sampled for FTIR analysis. Collected samples were dried and kept inside the paper packet. The lichen samples were determined by

their morphological, anatomical and chemical characters by using LABOMED dissecting microscope for external morphology, while LEICA ATC 2000 compound microscope was used for microscopic anatomical details of the samples. The chemical substance present in the lichen thallus were identified through TLC in solvent system A (Toluene: 1-4 Dioxane and Acetic acid; 180:60:4) (Orange et al., 2001; Culberson, 1972; Walker and James, 1980). The voucher specimens were preserved in the Lichen Herbarium (LWG) of CSIR-National Botanical Research Institute, Lucknow, India.

**Fourier Transform Infrared Spectrometer (FTIR) Analysis:** The pellets of lichen samples were dried for 4 to 5 hrs in an oven at 40-50°C to remove moisture content. Dried powder of lichen samples were used for FTIR analysis. 5 mg of dried extract powder of lichen samples was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The KBr based pellet was compressed into a thin disk using a hydraulic press (CAP-15T) by establishing ten tons pressure. The powdered sample of each lichen specimen was loaded in FTIR spectroscope (Thermo-Nicolet 6700), with a Scan range from 400 to 4000 cm<sup>-1</sup> against KBr background.

## RESULTS AND DISCUSSION

The study area revealed that lichen species *Rinodina sophodes* was found growing luxuriantly on barks, trunks and branches of different trees in all four directions (east, west, north and south). Common lichen species *R. sophodes* was examined by FTIR analysis to find specific spectroscopic biomarkers for rapid identification and discrimination around both the thermal power plants. FTIR analysis provides spectroscopic evidence of the role of chemicals in metal absorption as the variation in the frequency is affected by bond dissociation and bond formation which plays important role in metal chelation. Secondary metabolites play important role in chelation. So, variation in the functional group bands in IR region shows the effect of pollutant on the functional group chemistry of the species. This phenomenon is principally involved in metal absorption by formation of chelates or bond dissociation due to the presence of phytotoxic

gases mainly SO<sub>2</sub>. The wave numbers of prominent peaks obtained from the absorbance spectra showed in all three lichen species (Figure 3 a & b). Developing specific biomarkers by FTIR microscopy could be highly important for future rapid and reliable detection and identification of functional groups present in the species.

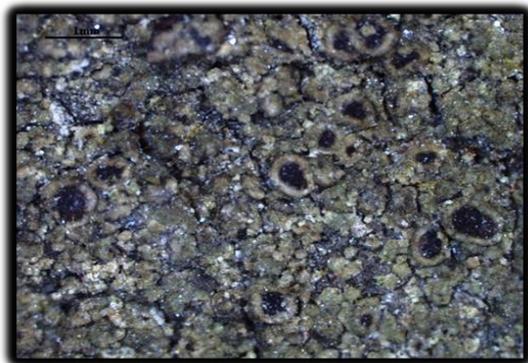


Figure 1. Thallus of Lichen Species *Rinodina sophodes* (Ach.) A. Massal

#### FTIR spectra measurement of *R. sophodes* around Panki Thermal Power Plant, Kanpur:

For lichen *R. sophodes* (Table- 1; Figure 3a), the strong absorbance bands ranged between 3500-3300 cm<sup>-1</sup> and observed at 3417.1 cm<sup>-1</sup> (north), 3416.1 cm<sup>-1</sup> (west) and 3411.9 cm<sup>-1</sup> (south) showed the stretching of bonded N-H/O-H and the presence of alcohol and phenol groups (Ramrakhiani et al., 2011). The spectral band observed between 3300-2500 cm<sup>-1</sup> due to O-H stretching in the presence of carboxylic acid. The absorbance bands observed at due to bending of N-H with the presence of primary amines at 1634.4 cm<sup>-1</sup> (north), 1618.3 cm<sup>-1</sup> (west) and 1627.5 cm<sup>-1</sup> (south). The range of wave numbers 1560-1530 cm<sup>-1</sup> showed the presence of C-N stretching, N-H bending with the presence of secondary amines only at 1514.0 cm<sup>-1</sup> in south direction (Jilie and Shaoning, 2007). The bonded C=O symmetric stretching (Naumann, 2000) absorbed infra-red at 1419.5 cm<sup>-1</sup>, 1421.1 cm<sup>-1</sup> and 1421.7 cm<sup>-1</sup>. The absorbance bands observed at 1317.9 cm<sup>-1</sup> in west and 1320.0 cm<sup>-1</sup> in south direction showed components of  $\alpha$ -helix (Adriana and Gabi, 2011). The absorbance bands ranged between 1250- 1220 cm<sup>-1</sup> were attributed to P=O asymmetric stretching and phosphodiester in all three directions

(Naumann, 2000). The range of wave number 1080-1010 cm<sup>-1</sup> showed SO<sub>3</sub> asymmetric stretching (Cirik et al., 2012); and C-O bonding due to polysaccharides (Das and Guha, 2007) at 1034.6 cm<sup>-1</sup> (north), 1050.8 cm<sup>-1</sup> (west) and 1046.0 cm<sup>-1</sup> (south) respectively. The range of wave number 900-600 cm<sup>-1</sup> showed O-CH<sub>2</sub> stretching of methoxy groups (Movasaghi et al., 2008) only in west direction at 779.6 cm<sup>-1</sup>. The peaks range between 690-515 cm<sup>-1</sup> resulted in absorption due the vibration modes of C-Br stretching coupled to alkyl halides. The observations are useful to detect rapidly and characterized the occurrence of lichen *R. sophodes* with different level of metal accumulation around thermal power plant. The results are presented in Table- 1; Figure 3 a, showed the spectra of *Rinodina sophodes* and can be also seen, presence of both primary and secondary metabolites enhances the probability of detection of IR bands in 3500-3300 cm<sup>-1</sup> and in 3300-2500 cm<sup>-1</sup> region due to the presence of more N-H and O-H groups. FTIR analysis provides spectroscopic evidence of the role of chemicals in metal absorption as the variation in the frequency is affected by bond dissociation and bond formation which plays important role in metal chelation. The variation in the functional group bands in IR region shows the effect of pollutant on the functional group chemistry of the species. This phenomenon is principally involved in metal absorption by formation of chelates or bond dissociation due to the presence of phytotoxic gases mainly SO<sub>2</sub>. The biodeterioration of granite monuments was done by Prieto et al., 1999, 2000) are available using FT Raman Spectroscopic studies on a number of lichens.

#### FTIR spectra measurement of *R. sophodes* around Feroze Gandhi Unchahar National Thermal Power Plant Corporation (FGUNTPC), Raebareli:

For lichen *R. sophodes* (Table 1; Figure 3b), the major absorbance bands ranged between 3500- 3300 cm<sup>-1</sup> and 3000- 2800 cm<sup>-1</sup> in all directions of the study area (north, east, south, west) 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 absorbance bands observed at 2036.1 cm<sup>-1</sup> in south and

2037.2 cm<sup>-1</sup> in west direction of study area due to NH<sub>3</sub><sup>+</sup> torsional vibration (Mohan, 2005).

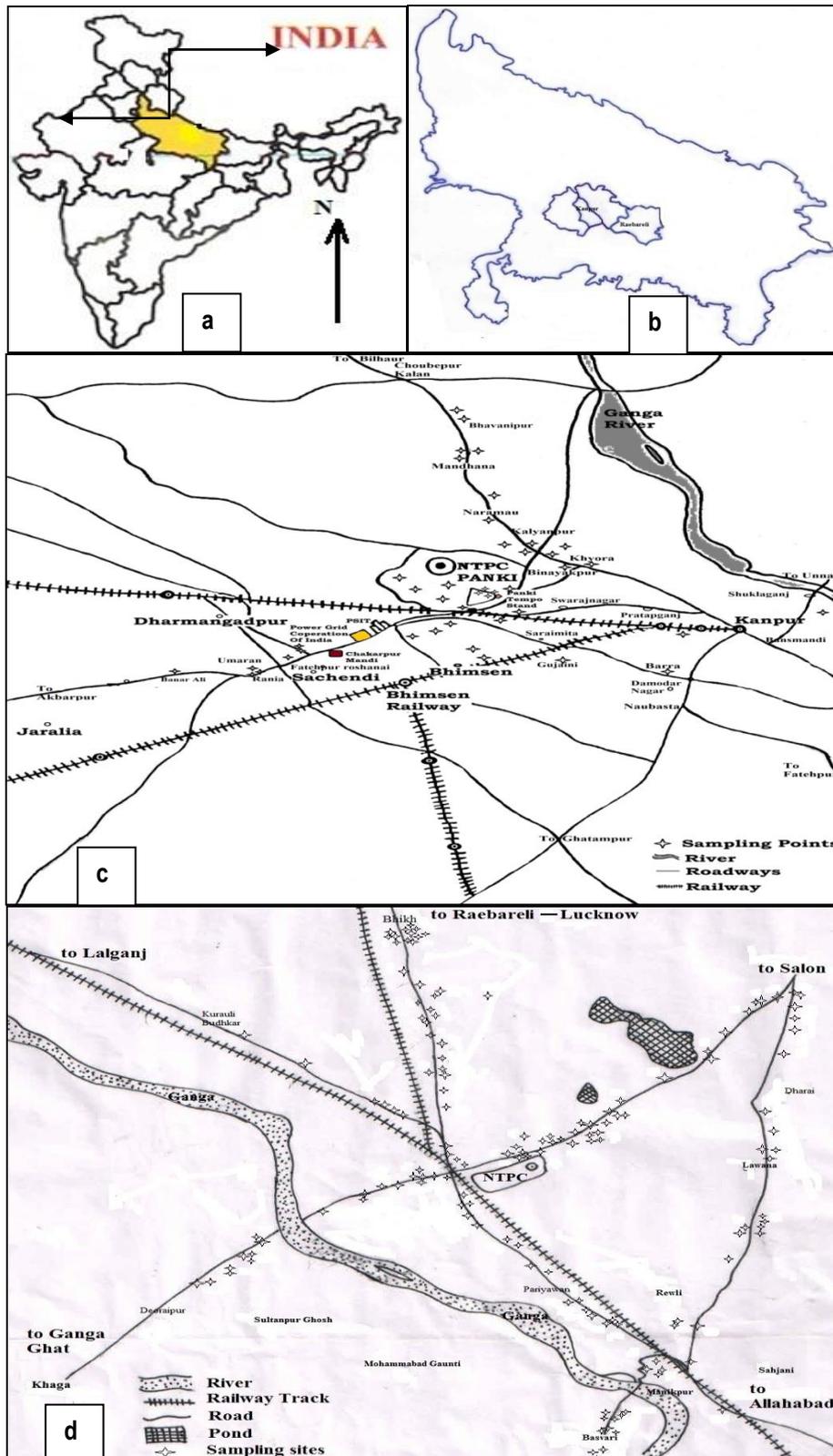


Figure 2. Sampling Locations: (a) Map of India showing Uttar Pradesh Region; (b) Uttar Pradesh region showing Kanpur and Raebareli district; (c) Sampling site around Panki Thermal Power Plant, Kanpur; (d) Sampling site around Feroze Gandhi Unchahar Thermal Power Plant, Raebareli

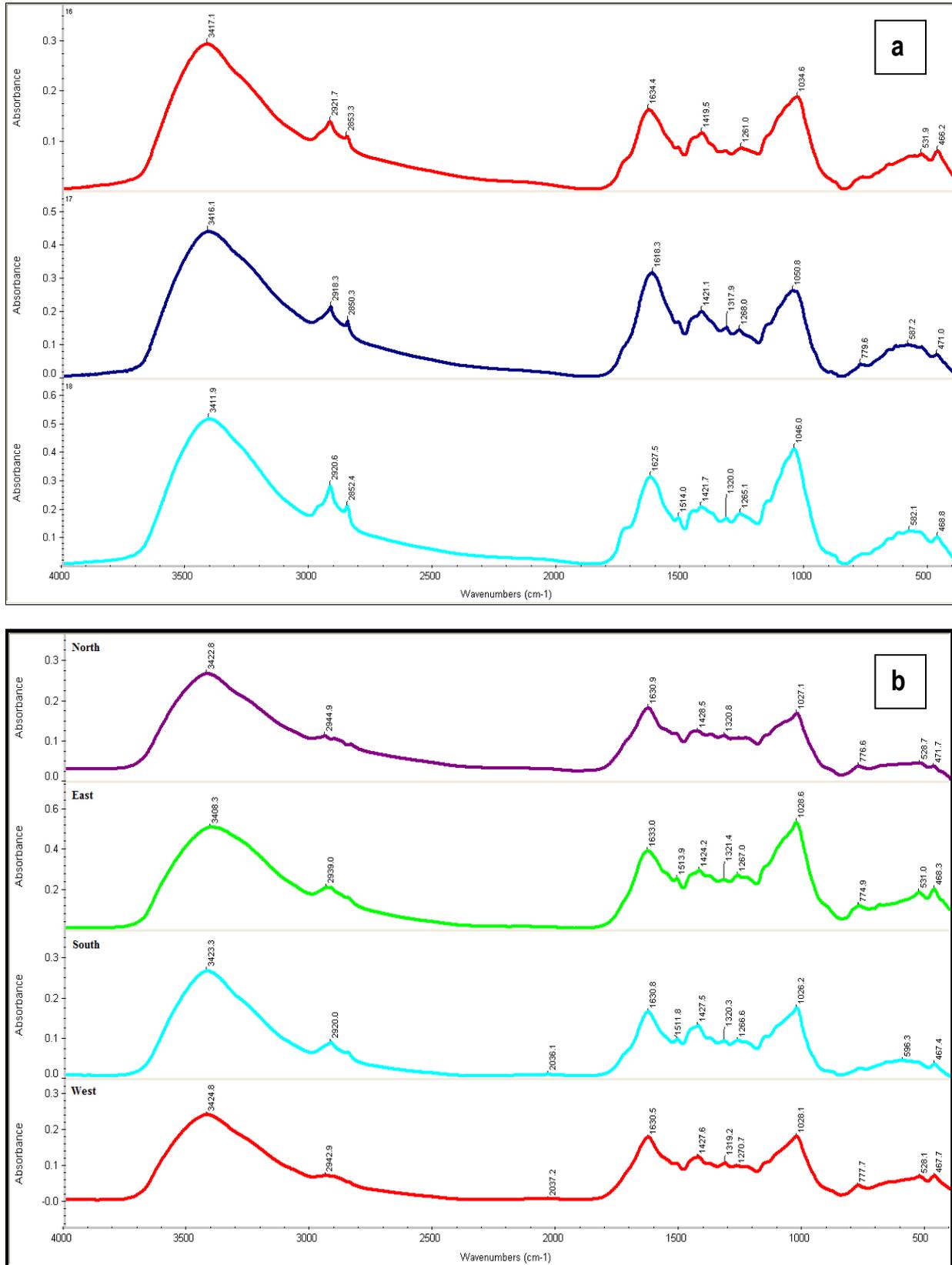


Figure 3. Direction-wise FTIR spectra of lichen species *R. sophodes* (a) Panki Thermal Power Plant, Kanpur; and (b) Feroze Gandhi Unchahar thermal power corporation, Raebareli.

**Table 1. Absorption frequencies of FTIR spectra of lichen species *R. sophodes***

S.No.	Range of wave number (cm <sup>-1</sup> )	Panki Thermal Power Plant			Unchahar Thermal Power Plant				Band Assignment
		Band Position (in cm <sup>-1</sup> )							
		North	West	South	North	East	South	West	
1	3500-3300	3417.1	3416.1	3411.9	3422.8	3408.3	3423.3	3424.8	N-H stretching Amide- A, O-H stretching of hydroxyl groups (primary, secondary, amines and amides) (Ramrakhiani et al., 2011)
2	3300-2500	2921.7	2918.3	2920.6					O-H stretching (Carboxylic acid)
		2853.3	2850.3	2852.4					
3	3000-2800				2944.9	2939.0	2920.0	2942.9	Asymmetric vibration C-H of CH <sub>2</sub> , protein and phospholipids (Ramrakhiani et al., 2011)
4	2200-2000				-	-	2036.1	2037.2	NH <sub>2</sub> <sup>+</sup> torsional vibration (Mohan, 2005)
5	1650-1580	1634.4	1618.3	1627.5					C=O stretching (Carbonyls group)
6	1680-1640				1630.9	1633.0	1630.8	1630.5	Amide- I, C=O stretching (Jilie and Shaoning, 2007)
7	1560-1530	-	-	1514.0	-	1513.9	1511.8	-	Amide- II, CN stretching, NH bending (Jilie and Shaoning, 2007)
8	1450-1400	1419.5	1421.1	1421.7	1428.5	1424.2	1427.5	1427.6	C=O symmetric stretching -COO <sup>-</sup> (Naumann, 2000)
9	1360-1290	-	1317.9	1320.0	1320.8	1321.4	1320.3	1319.2	components of $\alpha$ -helix proteins (Adriana and Gabi, 2011)
					-	1267.0	1266.6	1270.7	
10	1250-1220	1261.0	1268.0	1265.1					P=O stretching (asym.) of PO <sub>2</sub> phosphodiester (Naumann, 2000)
11	1080-1010	1034.6	1050.8	1046.0	1027.4	1028.6	1026.2	1028.1	SO <sub>3</sub> asymmetric stretching (Cirik et al., 2012); C-O bonding due to polysaccharides (Das and Guha, 2007)
12	900-600	-	779.6	-	776.6	774.9	-	777.7	O-CH <sub>3</sub> stretching of methoxy groups (Movasaghi et al., 2008)
13	690-515	-	587.2	582.1	-	-	596.3	-	C-Br stretching (alkyl halides)
		531.9	-	-					
14	530-400	466.2	471.0	468.8	528.7	531.0	-	528.1	Al-O stretching (AlO <sub>8</sub> Octahedral, isolated) (Tarte, 1962; 1964)
					421.7	468.3	467.4	467.7	

The wave numbers ranged between 1680-1640 cm<sup>-1</sup>, 1450-1400 cm<sup>-1</sup> and 1360-1290 cm<sup>-1</sup> observed in all four directions of the study area. The minor absorption bands observed at 1513.9 cm<sup>-1</sup> in east and 1511.8 cm<sup>-1</sup> in south direction which showed presence of Amide-II, CN stretching, NH bending (Jilie and Shaoning, 2007). The range of wave number 1080-1010 cm<sup>-1</sup> showed SO<sub>3</sub> asymmetric stretching (Cirik et al., 2012); and C-O bonding due to polysaccharides (Das and Guha, 2007) in all directions of the area. The range of wave number 900-600 cm<sup>-1</sup> showed O-CH<sub>3</sub> stretching of methoxy groups at 776.6 cm<sup>-1</sup> (north), 774.9 cm<sup>-1</sup> (east) and 777.7 cm<sup>-1</sup> (west) (Movasaghi et al., 2008). The peaks ranged between 690-515 cm<sup>-1</sup> only observed at 596.3 cm<sup>-1</sup> in south direction due the vibration modes of C- Br stretching coupled to alkyl halides, whereas 530-400 cm<sup>-1</sup> resulted absorbance values in all directions of the study area due to the presence of vibrational frequencies of Al-O stretching (AlO<sub>8</sub> Octahedral; isolated) respectively (Tarte, 1962; 1964). The biodeterioration of granite monuments by *Ochrolechia parella* (L.) Mass (Prieto et al., 1999) and *Lecidea fuscoatra* (L.) Ach., *Porpidia cinereoatra* (Ach.) Hertel & Knoph and *P. macrocarpa* (DC.) Hertel & Schwab (Prieto et al., 2000) are available using FT Raman Spectroscopic studies.

In *R. sophodes* number of bands detected is less than band observed in other species which

may be due to absence of secondary metabolites in the species. Secondary metabolites play important role in chelation. So, variation in the functional group bands in IR region shows the effect of pollutant on the functional group chemistry of the particular lichen species. The results revealed that the presence of both metabolites in *R. sophodes* enhances the probability of detection of IR bands in 3500-2800 cm<sup>-1</sup> region due to the presence of more COOH and OH groups with different level of absorption around the study area. The FTIR analysis provides variations in the functional group bands in IR region shows the effect of pollutant on the functional group chemistry of the species (Table 1). This phenomenon is principally involved in metal absorption by formation of chelates or bond dissociation due to the presence of phytotoxic gases mainly SO<sub>2</sub>.

## CONCLUSION

Finally, in this study, we have compared the presence of metabolites in *R. sophodes* around two thermal power plants of Uttar Pradesh. The results indicate the spectra of *R. sophodes* showed presence of metabolites which enhances the probability of detection of IR bands in 3500- 3300 cm<sup>-1</sup> (due to presence of more N-H and O-H groups), 3300-2500 cm<sup>-1</sup> (O-H stretching; carboxylic acid), 1450-1400 cm<sup>-1</sup> (C=O symmetric stretching -COO<sup>-</sup>), 1360-

1290 cm<sup>-1</sup> (components of  $\alpha$ - helix proteins), 1080-1010 cm<sup>-1</sup> (SO<sub>3</sub> asymmetric; C-O bonding due to polysaccharides) and 530-400 cm<sup>-1</sup> (Al-O stretching (AlO<sub>8</sub> Octahedral, isolated) were dominant spectral bands around both the thermal power plants of Uttar Pradesh. The absorption mechanisms also provide spectroscopic indication of chemical role in absorption process as the variation in the frequency is affected by bond dissociation and bond formation which plays important role in metal chelation. The studies also provide baseline data on FTIR analysis of *R. sophodes* around both the thermal power plants which will be helpful for carrying out future studies in the area.

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