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ORIGINAL ARTICLE

Delineating biomonitoring potential of two crustose lichens *Bacidia convexula* and *B. submedialis* through elemental accumulation and microstructural parameters

Vartika Gupta[1] · **Namita Gupta**[2] · **Sanjeeva Nayaka**[3] · **Seshu Lavania**[1]

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Abstract

Lichens possess unique characteristics, relying on a protective cortex and a filtration mechanism for absorption in the absence of roots, enabling efficient mineral nutrition. However, this distinctive feature also renders them susceptible to accumulating airborne pollutants, particularly metal, beyond optimal levels. The current investigation focuses on elemental accumulation in two crustose lichen species, namely *Bacidia convexula* and *B. submedialis,* aiming to highlight their potential as biomonitoring tools and assess the impact of anthropogenic activities, disturbed environment surrounding brick-kilns as an illustrative example. Microstructural changes, surface sorption and fibrous deposition of elemental ions were scrutinized through the application of SEM–EDX microscopy and advanced analytical techniques such as ICP-MS and FTIR. The SEM images unveiled alterations in the lichen's microstructure, entrapment of gas bubbles as well as fibrous deposition and surface sorption of elemental ions. The highest mean concentration of Ag (0.36 ± 0.01), Al (1194.87 ± 67.6), As (0.6 ± 0.02), Cd (0.29 ± 0.01), Cr (107.79 ± 0.39), Cu (17.12 ± 0.07), Fe (1722.73 ± 8.48), Mg (1995.13 ± 31.28), Mn (235.06 ± 0.67), Ni (9.09 ± 0.05), and Zn (87.63 ± 0.84 mg kg^{-1}) were estimated in the thalli of *B. submedialis*, whereas *B. convexula* accumulated highest concentration of Co (1.34 ± 0.02), Li (3.67 ± 0.35), Pb (11.92 ± 0.13), and Se (0.27 ± 0.01 mg kg^{-1}). In both the lichens, FTIR analysis identified the functional groups such as, alcohol (O–H), alkenes (C–H), alkyl halides (C–Br), aromatic (C = C), methoxy ($O–CH_3$) and octahedral groups (AlO_8). The analytical results of EDX showed a higher weight of O with 46.11%, Mg (0.43%), Al (0.9%), Fe (0.38%), Si (1.62%), Zr (1.67%) in *B. submedialis.* Whereas, *B. convexula* revealed higher weight of C with 62.36% and Ca (0.62%). As such differential response was observed to metal ion stress, showing *B. submedialis* to be more tolerant than *B. convexula*. The information generated could be used as biomonitoring indicator of air quality and pollution.

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✉ Vartika Gupta
vartika1292@gmail.com

[1] Department of Botany, University of Lucknow, Lucknow 226007, India

[2] Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow 226025, India

[3] Lichenology Laboratory, CSIR-National Botanical Research Institute, Lucknow 226001, India

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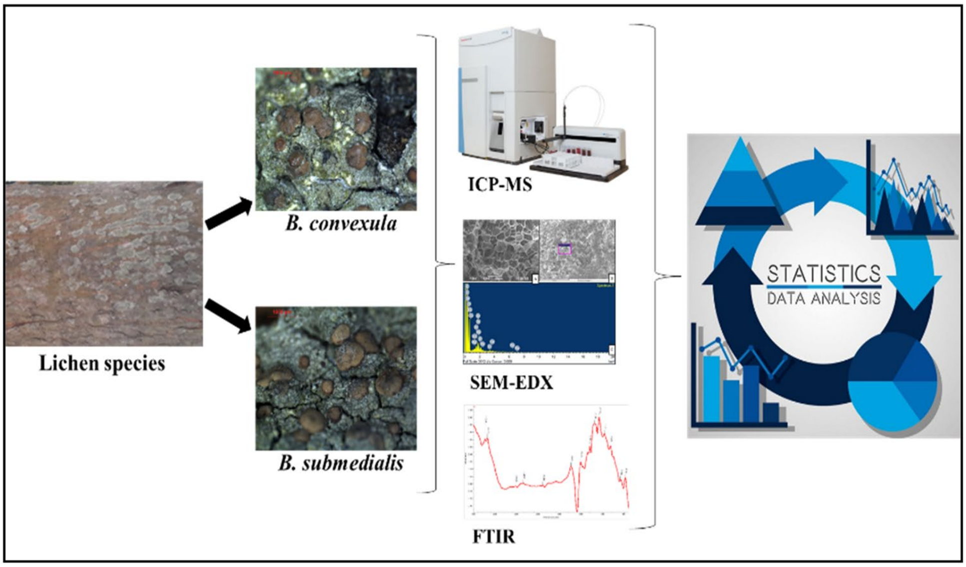
Graphical Abstract



Keywords Air pollution · Lichen biomonitoring · Ecosystem health · Heavy metal · Lichenized fungi

Introduction

In recent decades, metal pollution has emerged out as a significant global environmental concern [19]. There is a growing body of evidence pointing to the harmful effects of metals on living organisms. Anthropogenic sources have now surpassed natural sources as the primary contributors to metallic contaminants (Fig. 1). The mobility, dispersion and toxic effects associated with heavy metals pose a significant threat to human and environmental health, causing growth reduction and a decline in crop yield [3, 54]. The accumulation of metal-laden particulate matter in the human body is linked to chronic health issues such as neurological degeneration, multiple sclerosis, muscular dystrophy [57], and even lung cancer [66]. Consequently, addressing the issue has become imperative for the protection of public health and the environment.

Lichens are commonly used in biomonitoring studies of air pollution as they serve as bioindicators of air quality or bioaccumulators of atmospheric deposition [13, 22, 30]. These organisms can be easily sampled, the analysis is cost-effective, and allow monitoring of large areas. Lichens are perennial life forms that lack roots; rely on protective cortex and filtration mechanism. The waxy cortex makes them highly dependent on wet and dry deposition for mineral nutrients.

These unique characteristics offers lichens a capacity to readily accumulate airborne pollutants, especially metals, beyond their physiological needs [22, 48]. Therefore, lichens are frequently employed in air quality and biomonitoring studies that provide the information about the air quality of the regions [52, 63]. Being toxi-tolerant and pioneer, crustose lichens, such as *Bacidia convexula* and *B. submedialis*, possess unique characteristics that make them ideal for monitoring air quality and pollution. Their reliance on a protective cortex and filtration mechanism for nutrient absorption, in the absence of roots, enables them to efficiently accumulate airborne pollutants, particularly metals. This distinctive trait, which allows lichens to readily exceed their physiological requirements, makes them excellent bioindicators of air quality and bio-accumulators of atmospheric deposition. Lichens are extensively employed worldwide in biomonitoring studies, enabling a holistic assessment of atmospheric metal pollutants across construction sites, factories, forests, industries, thermal power plants, urban and rural areas [7, 11, 12, 24, 42, 44, 55].

Numerous studies conducted by various researchers have delved into distinct lichen communities thriving on rocks and soil in globally distributed heavy metal-polluted areas, predominantly linked to metal mining activities. Some lichens thriving in environments rich in heavy metals are resilient, common species capable of withstanding metal exposure in both polluted and unpolluted regions. Conversely, certain species are exclusively found in areas abundant in heavy metals, exhibiting a fragmented distribution dependent on the availability of suitable sites. The majority of lichens with an affinity for metal-rich substrates are categorized within

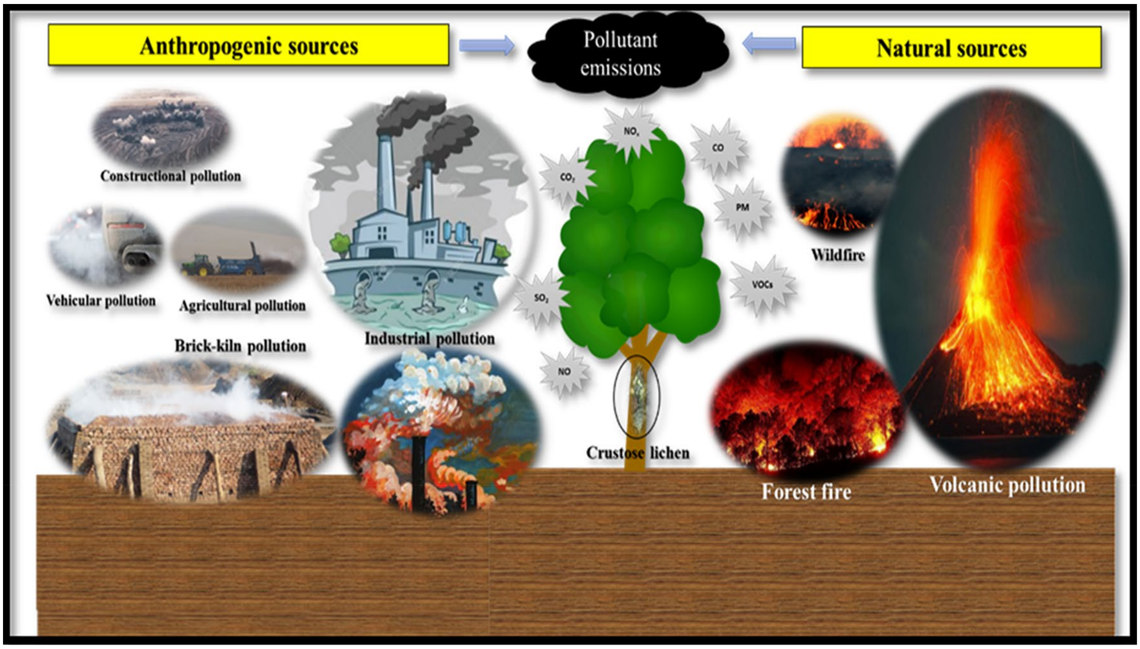


Fig. 1 Sources, emissions, dispersal of pollutants and its interplay with lichens present in the environment

the genera *Acarospora, Aspicilia, Lecanora, Lecidea, Porpidia, Rhizocarpon*, or *Tremolecia* [6, 45, 53]. Seaward [61], examined *Lecanora muralis*, an epilithic crustose lichen, in urban environments failed to identify patterns of lead pollution zoning.

Conversely, comprehensive air pollution study conducted in Israel using the epilithic lichen *Caloplaca aurantica* revealed higher levels of heavy metal pollution in urban areas compared to rural control areas [31, 32]. Some of the species such as *Diploschistes muscorum* [23, 59], *Acarospora rugulosa* [21], and *Lecanora polytropa* [50] demonstrated an impressive capacity to accumulate exceptionally high levels of metals, earning them the designation of hyperaccumulators. The surface-dwelling lichens *Cladonia rei* and *Diploschiste muscorum* have proven to be resilient colonizers of severely polluted and disrupted environments, showcasing significant tolerance to heavy metals. Contrasting abilities in terms of bioaccumulation, accumulation trends, and responses to heavy-metal-induced stress, particularly in relation to cell membrane damage are known to be reported. Notably, in the thalli of *Cladonia rei*, over half of the loads of Zn, Pb, Cd, and As are accumulated externally, while *Diploschistes muscorum* tends to accumulate the same elements intracellularly [49]. *Acarospora rugulosa* exhibited an accumulation of up to 16% of Cu on a dry mass basis [21]. Pawlik-Skowronska et al. [50] observed that apothecia of *Lecanora polytropa* accumulated Cu up to 1.3% (dry matter), with approximately 50% of it being in an exchangeable form. As such, there exists substantial disparities between the total and intracellular contents of these heavy metals. Numerous studies have utilized three distinct species of epilithic-crustose lichen viz., *Candelariella* sp., *Lecanora* sp., and *Caloplaca* sp. as bioindicators for various trace elements, including V, Cr, Mn, Co, Ni, Cu, Zn, As, Rb, Sr, Mo, Cd, Sb, Ba, Pb, Bi, and U. The study suggested that *Candelariella* sp., *Lecanora* sp., and *Caloplaca* sp. are proficient accumulators of airborne heavy metals [18]. Bajpai et al. [9] conducted a biomonitoring study in India, employing *Lapraria lobifican* crustose lichens, which exhibited greater Fe accumulation compared to other species of foliose and fruticose lichens. In Brazil, epilithic crustose lichens have been employed for biomonitoring arsenic in the environment [28].

In India, several studies have been conducted to assess the multi-elemental composition of the environment using lichens as biomonitors by employing various techniques [10, 24, 35, 39, 62]. Comparisons between different lichen species of different growth forms have been performed to determine the most suitable biomonitor for specific environmental conditions [4, 14, 33, 34]. However, being pioneer coloniser and pollution tolerant behaviour, comparisons of crustose lichens are limited. The choice to investigate the lichen genus *Bacidia* in the study was made after careful consideration and evaluation of various factors. While *Artocarpus heterophyllus* and *Mangifera indica* serve as recognized habitats for a diverse range of lichen species, including *Pyxine, Dirinara, Caloplaca, Lecanora, Phaeophyscia, Hyperphyscia,* and *Bacidia,* the specific emphasis on *Bacidia* is substantiated by multiple reasons.

Firstly, several lichen species, such as *Pyxine* [11, 25, 34, 37], *Dirinara* [1, 58, 67], *Caloplaca* [8, 29, 65], *Flavoparmelia* [43], *Lecanora* [2, 40, 51], *Hyperphyscia* [15, 26], and *Phaeophyscia* [33, 36, 41, 56, 60, 62, 64] have been extensively studied in global biomonitoring efforts. These lichens, documented in numerous research studies, have significantly contributed to our understanding of ecological indicators and environmental health. In contrast, the selection of *Bacidia*, a crustose lichen, introduces a unique perspective to the study, as it has been comparatively utilized in biomonitoring studies [33, 34]. *Bacidia* possesses distinctive ecological characteristics and secondary metabolites, including atranorin and triterpenes, which play a vital role in conferring tolerance to environmental stresses.

The inclusion of *Bacidia* in the study design also considers its dispersion and distribution in the selected areas. While different lichen genera may exhibit varying sensitivities to pollutants, the utilization of *Bacidia* in this study allows for the collection of bulk samples, facilitating multiple analyses and providing a more comprehensive understanding of environmental conditions. Furthermore, the decision to focus on *Bacidia* addresses existing knowledge gaps, as limited research has been conducted on this genus compared to others. This approach aims to contribute to a more holistic understanding of lichen diversity, distribution, and ecological roles within the ecosystem. The selection of *Bacidia* for this study is not arbitrary, rather, it is grounded in a thoughtful evaluation of its unique ecological attributes, potential contributions to biomonitoring efforts, specific responses to environmental factors, and considerations of existing knowledge gaps in lichen research. Through this focused investigation, the study endeavours to offer a nuanced and comprehensive understanding of the lichen communities associated with *Artocarpus heterophyllus* and *Mangifera indica*. Thus, the present study is aimed (i) to conduct the comprehensive assessment of atmospheric heavy metal contamination, (ii) to assess the impact of anthropogenic activities on the environment, (iii) to assess the elemental load in two crustose lichen species viz., *Bacidia convexula* and *B. submedialis* by employing ICP-MS (Inductively Couple Plasma-Mass Spectrometry), (iv) to confirm the effectiveness of lichen as a reliable biomonitoring species for detecting a wide range of airborne metals by employing some advanced technique such as SEM–EDX (Scanning Electron Microscopy with Energy Dispersive X-Ray Spectroscopy) and FTIR (Fourier Transform Infrared Spectroscopy).

Materials and methods

Study area and collection of samples

Lichen samples were collected from the Bahraich district of Uttar Pradesh, India within the geographical coordinates of 28.24 to 27.40° N and 81.65 to 81.30° E (Fig. 2). The district's landscape comprises a combination of plains and hills. The sites are characterized by the presence of several brick kiln units, small-scale industries, construction areas, waste burning, forest fires and agricultural practices surrounded by an individual tree and densely clustered tree population also. The samples were collected from the bark of two phorophytes viz., *Artocarpus heterophyllus* and *Mangifera indica*. Thalli of lichen species growing on the tree trunk at 0.5–2 m from the ground were estimated. Thalli of lichen species were collected randomly from the trees in each sampling site, within the year 2020–2021. The samples were collected separately in winter seasons.

Identification of lichen samples

Lichen samples collected from sites underwent identification based on morphological, anatomical and chemical characteristics. The micro lichen key [5] served as the primary literature reference for the identification process. A set of collected specimens is preserved in the herbarium (LWG) of the CSIR-National Botanical Research Institute, Lucknow, India. Morphological examinations of the lichens were conducted using a stereo-zoom LEICA S8APO microscope, while anatomical details were scrutinized with DM2500 optical microscopes equipped with a camera and image analysis software. Thin Layer Chromatography (TLC) was done according to standard method [46]. The analysis was conducted with *B. convexula* and *B. submedialis* in triplicates ($n = 12$) collected from both the regions of Bahraich district, Uttar Pradesh, India.

Sample preparation and elemental analysis

The lichen samples were removed carefully from the bark and placed in small petri dishes. Lichens thalli were cleaned with distilled water to eliminate dust and residue. Further, lichen samples were dried in an oven at 80°C for a minimum of 48 h. After drying, they were crushed, powdered and then passed through a mesh with a pore size of 2 mm. Samples were digested through a microwave-digestion (BERGHOF Speedwave-MWS-3 +) using HNO_3 (69%, ACS quality, Germany). The total elemental content in the digested sample was determined by using ICP-MS (Thermo iCAP TQ).

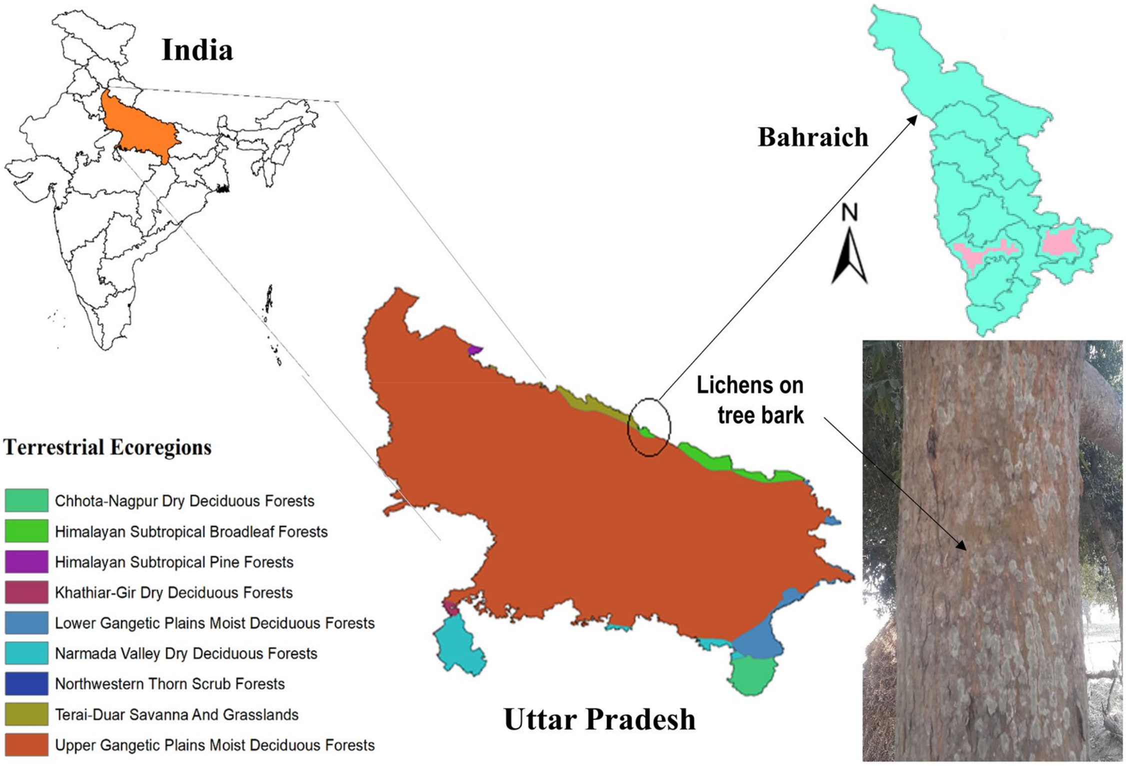


Fig. 2 Map showing the lichen collection sites in Bahraich district of Uttar Pradesh, India

Calculation of environmental indexes of pollution

Contamination Factor (CF): The contamination factor was used to assess the atmospheric contamination levels of each element at each monitoring site. The CF was calculated as follows:

$$\mathrm{CF} = \mathrm{C_s}/\mathrm{C_c}$$

where $\mathrm{C_s}$ represents the mean concentration of each element in the lichens, and $\mathrm{C_c}$ is the corresponding average minimum concentration of element in the lichen.

The CF values were categorized into four contamination classes based on the ranges defined by Boamponsem et al. [16] as follows: < 1.2, none, 1.2–2, light, 2–3, medium, and ≥ 3, heavy.

Pollution Load Index (PLI): The pollution load index was estimated as follows:

$$\mathrm{PLI} = \left(\mathrm{CF_1 x\, CF_2 x\, CF_3 x\, CF_4 x \ldots x\, CF_n}\right)^{1/n}$$

The CF represents the contamination level of each selected element, and ‘n’ denotes the total number of elements studied.

According to Boonpeng et al. [17], a Pollution Load Index (PLI) value less than 0.9 indicates an unpolluted area. PLI values greater than 1 were categorized into different pollution levels as follows: $1.1 < \mathrm{PLI} < 1.5$, low pollution; $1.5 \le \mathrm{PLI} < 2.0$, moderate pollution; $2.0 \le \mathrm{PLI} < 2.5$, high pollution; and $\mathrm{PLI} \ge 2.5$, very high pollution.

Scanning Electron Microscopy with Energy Dispersive X-Ray Spectroscopy (SEM–EDX) Analysis

For SEM observation, the dehydrated lichen samples were mounted onto aluminum stubs using double-sided carbon tape method [33]. These stubs were then coated with gold–palladium in a sputter coater (JFC 1600; JEOL, Tokyo, Japan) at 20 mA. The samples were equipped with an EDX, EV Dry Detector and viewed under a Scanning Electron Microscope (JFC 1600; JEOL, Tokyo, Japan). To analyze the composition of the lichen samples, EDX (Energy Dispersive Spectroscopy) was performed on the same SEM instrument by scanning an electron beam with an accelerating voltage of 15 kV. This process allowed for the determination of the elemental composition of each particle.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

For FTIR analysis, lichen samples were dried in an oven at a temperature of 40-50ºC for 4 to 5 h to eliminate any moisture content. The pellet was prepared by combining dried lichen samples with potassium bromide (KBr) method [33]. After homogenization, the KBr-based mixture was then compressed into a thin disk shape using a hydraulic press (CAP-15 T) under a pressure of ten tons. Subsequently, the prepared disks were fixed into an FTIR spectrophotometer (Thermo-Nicolet 6700) for analysis. The FTIR scan range used for this investigation spanned from 400 to 4000 cm^{-1}, allowing for the detailed examination of the chemical bonds and functional groups in the lichen.

Statistics and Data Analysis

Pearson's correlation was performed with SPSS 26.0, and the level of statistical significance was set at $p \leq 0.05$ (2-tailed).

Results and Discussion

Morphological and anatomical study of lichen samples

a. *Bacidia convexula* (Müll. Arg.) Zahlbr.: Thallus corticolous, crustose, granular to furfuraceous, greyish green. Apothecia frequent sessile, biaorine, 0.1–0.6 mm in diam., disc reddish brown, plane to convex. Exciple hyaline to yellowish; epihymenium yellowish; hymenium hyaline; paraphyses simple to branched, apical cell swollen. Ascus 8-spored, cylindrical to clavate, ascospores transversely 3–5 septate, fusiform to acicular, 18.1–30.9 × 1.9–4.9 µm (2.5x), Chemistry: Thallus K-, C-, KC-, P-. TLC: No substances tested (Fig. 3A).
b. *Bacidia submedialis* (Nyl.) Zahlbr.: Thallus corticolous, crustose, granulose to verruculose, whitish grey to grey. Apothecia numerous, round, sessile, 0.2–0.8 mm in diam., margin biatorine, thin, disc pale brown, plane to slightly convex. Exciple brown, Epihymenium pale brown, hymenium hyaline; paraphyses branched and anastamosing. Asci 8-spored, ascospores acicular, hyaline, transversely (-3) 6-(-7) 9 septate, 31.4–40.9 × 3.0–3.8 µm (2.5x), Chemistry: Thallus K ± yellow, C-, KC-, P-. TLC: Triterpenes (Fig. 3B).

Elemental accumulation in lichen thalli and its substrate

The elements that were estimated in the lichen samples are Ag (silver), Al (aluminium), As (arsenic), Cd (cadmium), Co (cobalt), Cr (chromium), Cu (copper), Fe (iron), Li (lithium), Mg(magnesium), Mn (manganese), Ni (nickel), Pb (lead), Se (selenium), and Zn (zinc) (Fig. 4). Among all elements, Ag ranged from 0.27 ± 0.003 to 0.36 ± 0.01 mg kg^{-1} followed by Al (502.59 ± 3.86 to 1194.87 ± 67.57), As (0.43 ± 0.04 to 0.6 ± 0.02), Cd (0.23 ± 0.003 to 0.29 ± 0.01), Co (1.1 ± 0.003 to 1.34 ± 0.02), Cr (21.21 ± 0.1 to 107.79 ± 0.39), Cu (11.21 ± 0.06 to 17.12 ± 0.06), Fe (1119.95 ± 3.27 to 1722.73 ± 8.48), Li (2.19 ± 0.12 to 3.67 ± 0.35), Mg (1405.19 ± 7.81 to 1995.13 ± 31.28), Mn (72.47 ± 0.2 to 235.06 ± 0.66), Ni (1.07 ± 0.01 to 9.09 ± 0.05), Pb (8.56 ± 0.03 to 11.92 ± 0.13), Se (0.16 ± 0.01 to 0.27 ± 0.01), and Zn (48.66 ± 0.31 to 87.63 ± 0.84 mg kg^{-1}). Whereas, Ag concentration in the bark of both the lichen species

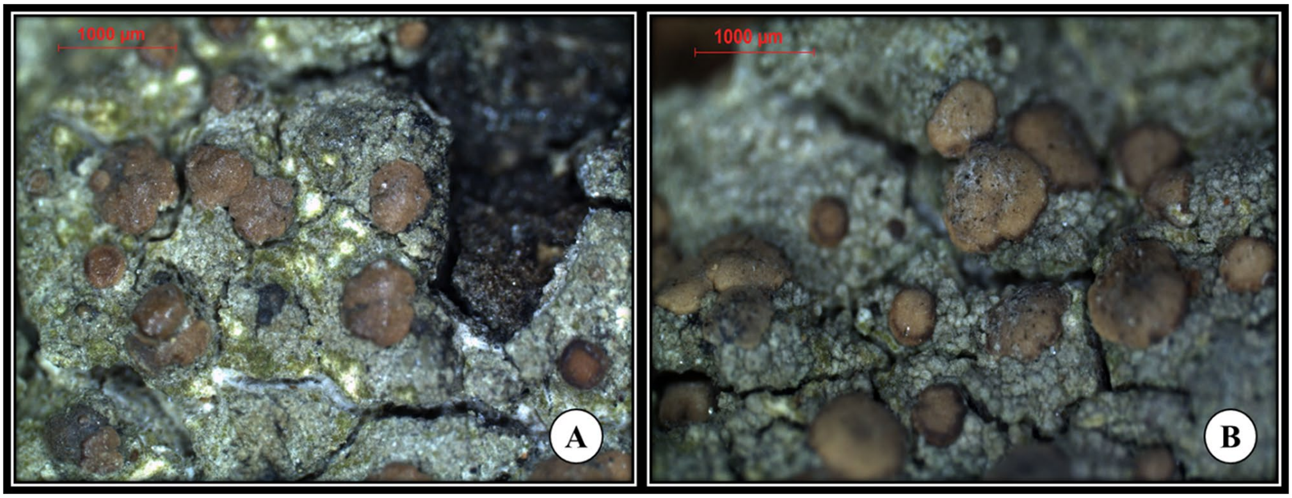


Fig. 3 Thallus and ascomata of **A:** *B. convexula*, and **B:** *B. submedialis*

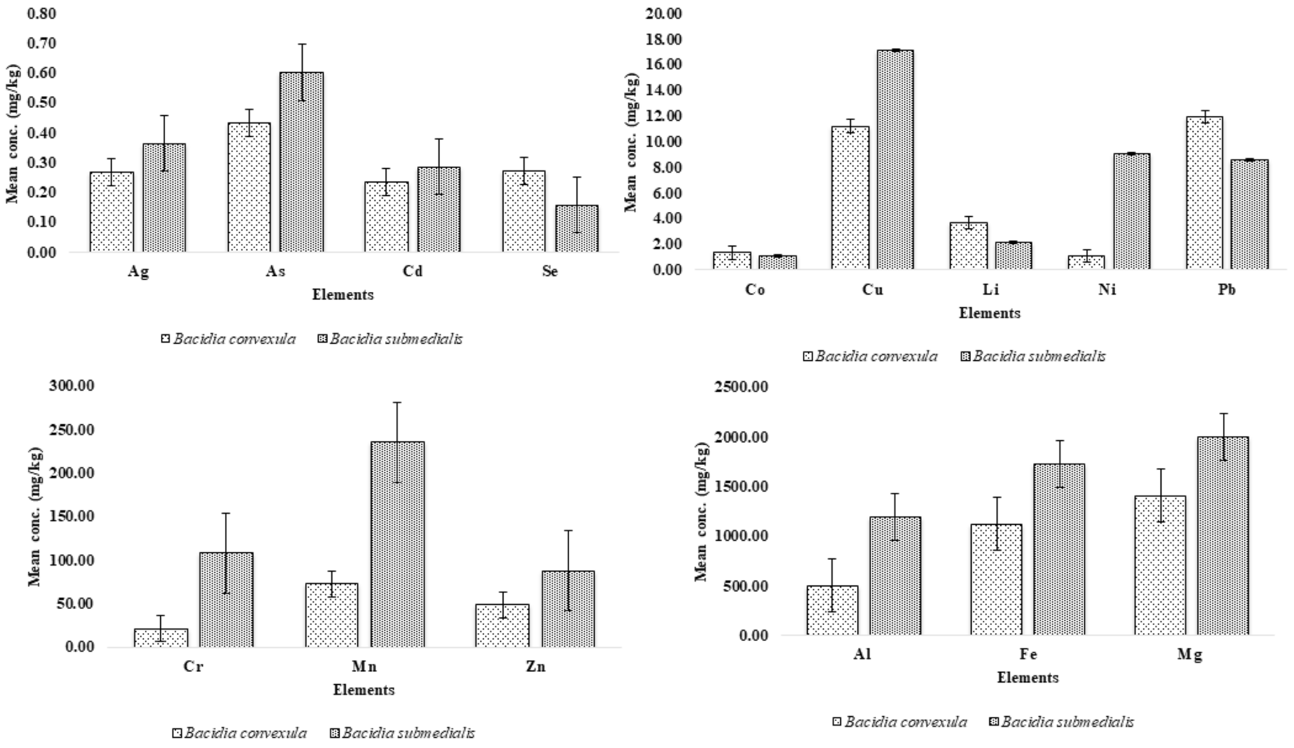


Fig. 4 Mean elemental concentration (mg kg^{-1}) in lichen thalli of *B. convexula* and *B. submedialis*

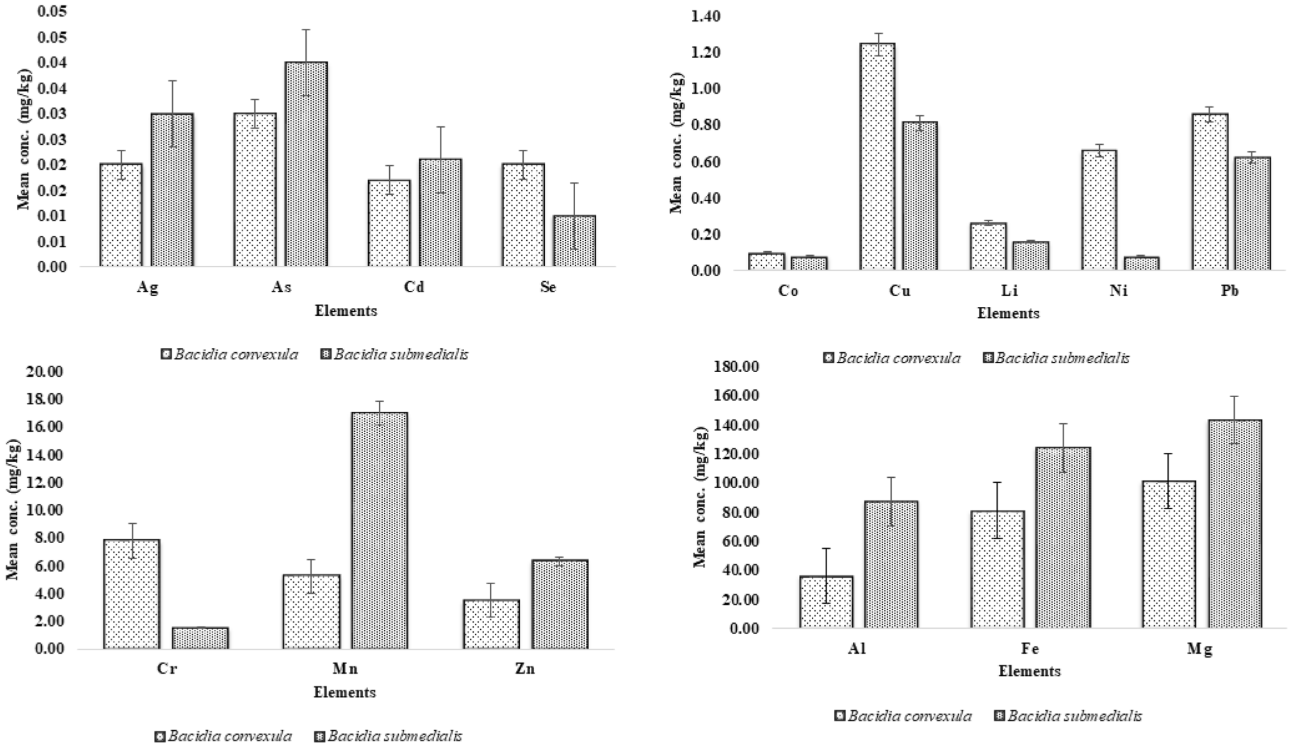


Fig. 5 Mean elemental concentration (mg kg^{-1}) in the substrate of *B. convexula* and *B. submedialis*

ranged from 0.02 ± 0.01 to 0.03 ± 0.01 mg kg^{-1} followed by Al (36.23 ± 12.5 to 87.39 ± 35.9), As (0.03 ± 0.01 to 0.04 ± 0.01), Cd (0.017 ± 0.01 to 0.021 ± 0.01), Co (0.08 ± 0.03 to 0.1 ± 0.03), Cr (7.79 ± 2.75 to 1.53 ± 0.54), Cu (0.81 ± 0.28 to 1.24 ± 0.43), Fe (80.9 ± 28.64 to 124.28 ± 43.24), Li (0.16 ± 0.06 to 0.26 ± 0.09), Mg (101.38 ± 35.21 to 143.58 ± 48.27), Mn (5.23 ± 1.84 to 16.97 ± 5.93), Ni (0.08 ± 0.03 to 0.66 ± 0.23), Pb (0.62 ± 0.22 to 0.86 ± 0.3), Se (0.01 ± 0.004 to 0.02 ± 0.01), and Zn (3.51 ± 1.21 to 6.32 ± 2.17 mg kg^{-1}) (Fig. 5). The highest mean concentration of Co (1.34 ± 0.02), Li (3.67 ± 0.35), Pb (11.92 ± 0.13), and Se (0.27 ± 0.01 mg kg^{-1}) were estimated in the thalli of *B. convexula,* whereas *B. submedialis* accumulated highest concentration of Ag (0.36 ± 0.01), Al (1194.87 ± 67.6), As (0.6 ± 0.02), Cd (0.29 ± 0.01), Cr (107.79 ± 0.39), Cu (17.12 ± 0.07), Fe (1722.73 ± 8.48), Mg (1995.13 ± 31.28), Mn (235.06 ± 0.67), Ni (9.09 ± 0.05), and Zn (87.63 ± 0.84 mg kg^{-1}) respectively. Thus, it is clear that in comparison to thalli of lichen, substrate (tree bark) analysis showed less accumulation of metals.

In the present study, lichen thalli showed higher concentrations of all elements compared to tree bark. Notably, elements like Ag, Al, As, Cd, Cr, Cu, Fe, Li, Mg, Mn, Ni, Pb, Se, and Zn exhibited higher elemental contents in lichen thalli than in tree bark, indicating the capacity of lichens to accumulate metals from the environment. The data emphasizes the potential role of lichens as bioindicators for environmental metal pollution. In the previous study, Gupta et al. [34] observed metal accumulation in *B. submedialis* and revealed accumulation of Al (108.62 ± 0.28 to 1337.4 ± 0.52), Cd (0.14 ± 0.08 to 1.85 ± 0.75), Cr (0.55 ± 0.19 to 12.04 ± 0.25), Fe (55.45 ± 0.11 to 1506.4 ± 0.22), Mn (0.67 ± 0.11 to 49.61 ± 0.21), Pb (0.94 ± 0.07 to 8.31 ± 0.38) and Zn (4.55 ± 0.38 to 59.62 ± 0.3) respectively. It was also concluded that metal accumulated in thalli of *B. submedialis* might be attributed to the vehicular and anthropogenic activities in the study area. Gupta et al. [33] also showed the accumulation of Al (12.59 ± 0.23 to 16.56 ± 0.49), Fe (137.96 ± 0.12 to 293.46 ± 0.42), Cd (0.39 ± 0.01 to 2.05 ± 0.06), Cr (0.87 ± 0.09 to 12.76 ± 0.43), Cu (16.5 ± 0.09 to 41.83 ± 0.13), Pb (9.44 ± 0.10 to 12.74 ± 0.24) and Zn (94.94 ± 0.08 to 147.73 ± 0.03 μg g^{-1}) in all directions around Tanda Thermal Power Plant, U.P., highlighting the accumulation potential of crustose lichens. Thus, lichen thalli act as effective accumulators of various elements, showcasing higher concentrations compared to their substrate (tree bark).

Correlation analysis between *B. convexula* and *B. submedialis*

Correlation analysis was conducted on eight elements Ag, Al, As, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Ni, Pb, Se, and Zn in both the lichen samples (Table 1). The lichen samples collected from various sampling sites exhibits a remarkably strong positive correlation between the elements Co–Cu, Co–Ni, Cu-Ni, Fe–Mn, Fe–Zn, and M–Zn ($r^2 = 1$). This correlation suggests that all the pollutants likely originate from the same pollution source. According to [38], these elements are recognized as indicators of anthropogenic emissions, such as vehicular traffic, power generation plants, fossil fuel combustion, and burning of wastes, metal works, and the application of anti-corrosion paints. Mg-Zn, and Ni-Pb have also showed a positive correlation between each other ($r^2 = 0.999$). Ag–Fe, Cr–Co, Cr–Cu, Cr–Ni, Cr–Pb, and Pb–Se ($r^2 = 0.997$) also established a positive significant relation. Apart from the significant correlations, there is a strong negative correlation between Co–Fe, Co–Mn, and Co–Zn ($r^2 = -1$) suggesting the different pollution source.

The association between Cu and Zn in lichens is of common occurrence, attributed to anthropogenic sources or metabolic interactions [47]. This author suggested that two elements are essential for lichen metabolism. But in the present study, there is a negative correlation between Cu and Zn which reflects the decline in survivorship of lichen species in polluted areas.

Contamination Factor (CF) and Pollution Load Index (PLI)

The results showed that in *B. convexula*, the CF of Ag, Al, As, Cd, Cr, Cu, Fe, Mg, Mn, Ni, and Zn was 1–1.1 showed that it has not contributed for contamination, whereas Co, Li, Pb, and Se was 1.2–1.8 which play role for light contamination. In *B. submedialis,* the CF value of Co, Li, Pb, and Se was 1.0 which falls in the category of no contamination, whereas Ag, As, Cd, Cu, Fe, Mg, and Zn was 1.2–1.8 that are the responsible factor for light contamination and Al was 2.4 for medium contamination. It was revealed that heavy contamination was created by Cr, Mn, and Ni with 3.3–8.6. PLIs were deliberated from the CFs for further understanding the implication of air pollutants accumulation. The result revealed that thalli of *B. convexula* carries low pollution load (1.1) in comparison to *B. submedialis* (1.8). Thus, it is clear from the results that *B. submedialis* has more capability to thrive in a polluted area and accumulate pollutants in their intracellular spaces (Table 2).

Analysis of structural morphology through SEM & EDX

The SEM–EDX analysis was performed on both the lichen's surface (Fig. 6). Figure 6i depicts SEM images of *B. convexula* reveal significant damage to the internal structure of

Table 1 Pearson correlation matrix between elements accumulated in lichen thalli

Elements	Ag	Al	As	Cd	Cr	Co	Cu	Fe	Li	Mg	Mn	Ni	Pb	Se	Zn
Ag	1														
Al	0.985**	1													
As	0.959**	0.947**	1												
Cd	0.989**	0.964**	0.977**	1											
Cr	−0.997**	−0.992**	−0.967**	−0.989**	1										
Co	−0.996**	−0.994**	−0.963**	−0.985**	0.997**	1									
Cu	−0.995**	−0.995**	−0.966**	−0.984**	0.997**	1.000**	1								
Fe	0.997**	0.992**	0.965**	0.987**	−0.997**	−1.000**	−1.000**	1							
Li	−0.975**	−0.948**	−0.908*	−0.974**	0.972**	0.963**	0.959**	−0.964**	1						
Mg	0.995**	0.985**	0.965**	0.988**	−0.993**	−0.998**	−0.997**	0.999**	−0.961**	1					
Mn	0.996**	0.993**	0.964**	0.985**	−0.997**	−1.000**	−1.000**	1.000**	−0.962**	0.998**	1				
Ni	−0.996**	−0.994**	−0.963**	−0.985**	0.997**	1.000**	1.000**	−1.000**	0.964**	−0.998**	−1.000**	1			
Pb	−0.997**	−0.992**	−0.955**	−0.985**	0.997**	0.999**	0.998**	−0.999**	0.973**	−0.997**	−0.999**	0.999**	1		
Se	−0.992**	−0.997**	−0.941**	−0.970**	0.993**	0.997**	0.996**	−0.996**	0.961**	−0.991**	−0.996**	0.997**	0.997**	1	
Zn	0.996**	0.991**	0.963**	0.986**	−0.995**	−1.000**	−0.999**	1.000**	−0.963**	0.999**	1.000**	−1.000**	−0.999**	−0.995**	1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Table 2 Comparison of Contamination Factor (CF) and Pollution Load Index (PLI) calculated from the metal concentration accumulated in *B. convexula* and *B. submedialis*

Elements	*B. convexula*	*B. submedialis*
	CF	CF
Ag	1.0	1.4
Al	1.0	2.4
As	1.1	1.5
Cd	1.0	1.2
Cr	1.0	5.1
Co	1.2	1.0
Cu	1.0	1.5
Fe	1.0	1.5
Li	1.8	1.1
Mg	1.0	1.4
Mn	1.0	3.3
Ni	1.0	8.6
Pb	1.4	1.0
Se	1.8	1.0
Zn	1.0	1.8
PLI	**1.1**	**1.8**

the lichen. Micrographs of lichen thalli exhibited a moderate degree of damage influenced by the varying levels of air pollution which is considered to be the primary cause of the substantial damage found in the lichen samples through the entrapment of gas bubbles. Additionally, spherules present distinct characteristics from the other particles, displaying an irregular surface with aggregates of submicron-sized particles. Upon observing the SEM microscopic images of the *B. submedialis* (Fig. 6ii), fibrous deposition, irregular morphology, distinct edges, and agglomerates of various sizes and shapes are evident.

SEM images reveal the existence of an array of particles on the lichen's surface. These particles exhibit diverse characteristics, appearing flat, irregular, rough or smooth, oval, and forming agglomerates of varying sizes. The edges of these particles generally appear rough or smooth and rounded, signifying their nature as atmospheric dust that has settled onto the surface of the lichens. According to Chaparro et al. [20], particles ranging from 2 upto 5 µm, as well as finer particles and smaller irregular ones, are likely derived from vehicles.

Based on the findings presented in Table 3, *B. convexula* revealed higher weight of C with 62.36%, O (32.97%), Pt (2.22%), Ca (0.62%), In (0.6%), S (0.49%), Si (0.37%), Mg (0.21%), Na (0.2%), Al (0.17%), Fe (0.03%), Cr and Ni (0.01%) whereas, the lichen surfaces in *B. submedialis* indicates the predominance of the percentage by weight of C with 48.48%, O (46.11%), Zr (1.67%), Si (1.62%), Al (0.9%), Mg (0.43%), Fe (0.38%), As (0.3%), K (0.22%), Ca (0.2%), Co (0.1%), P and Pb (0.01%) respectively. In comparison to the previous study conducted by Gupta et al. [33] utilizing *B. incongruens* (22.78% to 55.7%)*,* the present study reveals noteworthy higher percentage of elements in *B. convexula* and *B. submedialis*.

It is clear from the observations that, *B. submedialis* exhibits higher weight percentages of Al and Si, implying that the particles deposited on these surfaces are likely in the form of silicates or alumosilicates. Notably, in comparison to *B. convexula*, *B. submedialis* contains a substantial mass fraction of K, suggesting that the surface damage could be attributed to an elevation in cell membrane permeability that can be induced by stress. Furthermore, the significant mass percentage of C, Ca, and O raises the possibility that

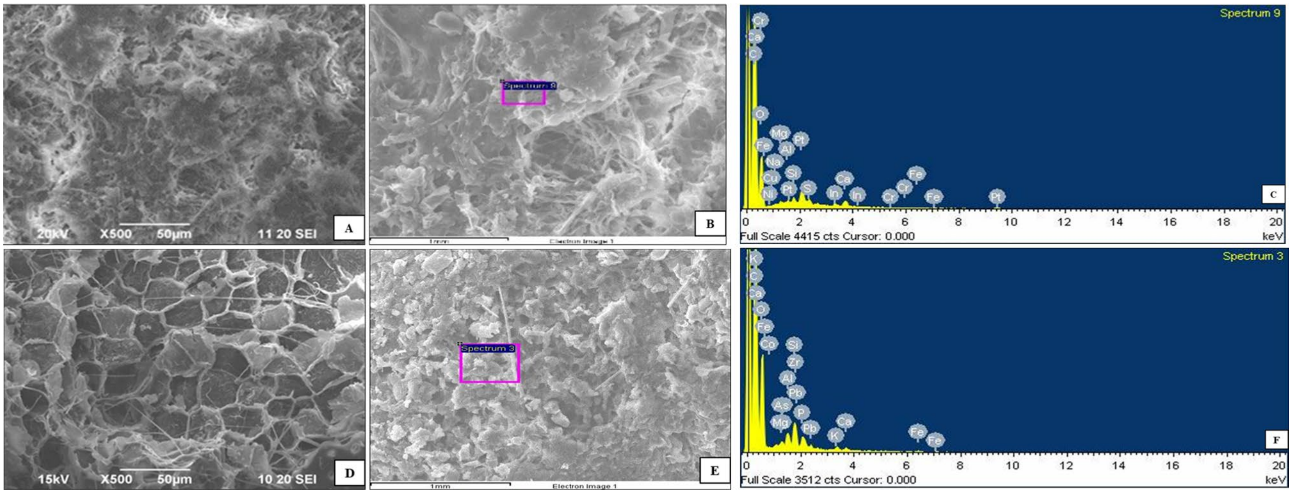


Fig. 6 Anatomical observations under SEM & EDX Analysis (**A**–**C**) *B. convexula*, and (**D**–**F**) *B. submedialis*

Table 3 Percentage weight of elements in lichen thalli through EDX analysis

B. convexula			*B. submedialis*		
Elements	Weight %	Atomic %	Elements	Weight %	Atomic %
C K	62.36	70.85	C K	48.48	57.15
O K	32.97	28.12	O K	46.11	40.81
Pt M	2.22	0.16	Zr L	1.67	0.26
Ca K	0.62	0.21	Si K	1.62	0.82
In L	0.6	0.07	Al K	0.9	0.47
S K	0.49	0.21	Mg K	0.43	0.25
Si K	0.37	0.18	Fe K	0.38	0.1
Mg K	0.21	0.12	As L	0.3	0.15
Na K	0.2	0.1	K K	0.22	0.08
Al K	0.17	0.08	Ca K	0.2	0.07
Fe K	0.03	0.015	Co K	0.1	0.05
Cr K	0.01	0.005	P K	0.01	0.005
Ni K	0.01	0.005	Pb M	0.01	0.005

the particles on both the lichen surface may be in the form of oxalate [68]. It is evident that *B. submedialis* has more potential to carry pollution load and accumulate metals in significant proportions. Thus, SEM & EDX analysis, revealing structural morphology and elemental composition, contributes significantly to understanding the impact of pollution on lichen species. This information aids in evaluating the damage caused by pollution and provides insights for effective environmental management strategies.

Identification of the presence of functional groups

FTIR spectroscopy was employed to identify the functional groups involved in metal biosorption for both lichen species (Fig. 7). The FTIR analysis of lichen thalli revealed peaks in the range of 400–4000 cm^{-1}, corresponding to various functional groups found in *B. convexula* and *B. submedialis*. The FTIR study of *B. convexula* exhibited stretching distinctive peaks at 3418.4, 2923.4, 2857.3, 1655.3, 1516.4, 1456.4, 1292.9, 1219, 1155.7, 1085.111, 811.7, 598.888, and 482.5 cm^{-1} associated with functional groups like –OH, =C–H, –C=O, C=C, P=O, C–O, O–CH_3, C–Br, and Al–O respectively (Fig. 7i), whereas *B. submedialis* demonstrated distinct characteristic asymmetric vibration and stretching frequencies, showing peaks at 3733.1, 3677.9, 3024.9, 2845.7, 2389.2, 1736.3, 1511.2, 1296.6, 1191.9, 1084.1, 961.9, 819.3, 589.2, and 476.5 cm^{-1}, corresponding to functional groups like –OH, =C–H, C–H, –C=C, –C=O, –C=C–, C–O, C–F,C–N, *trans* –CH=CH–, O–CH_3, C–Br, and Al-O (Fig. 7ii). The previous studies on *B. incongruens* [33] also showed the presence of strong absorbance bands ranged from 400 to 3500 cm^{-1}. Thus, it is clear from the study that in *B. submedialis* slight modifications in the peaks and emergence of diverse peaks were observed. These findings indicate that in *B. submedialis*, the majority of surface functional groups actively participate in the metal adsorption/absorption process. Particularly, carbonyl (–C=O), methoxy groups (O–CH_3), alkyl halides (C–Br), hydroxyl (− OH), aromatic (C=C) and isolated octahedral (AlO_8) groups were identified as the primary contributors to metal biosorption process. Recognizing these functional groups helps in understanding the chemical bonding mechanisms involved in the absorption of metals and contributes to environmental surveillance.

Conclusion

The present study represents the comprehensive analysis of metal pollution in crustose lichen species *B. convexula* and *B. submedialis*, which exhibits pollution tolerant behaviour. The results demonstrate the effectiveness of *B. submedialis* as biomonitoring tool with discriminant metal sorption process in the environment. In comparison to *B. convexula*, species *B. submedialis* revealed its exceptional ability to accumulate elements distributed in the environment. The study also demonstrates the pollutant deposition, degree of damage and presence of active sites for chemical bonding in *B. submedialis*, making it a potential and effective biomonitor. It is clear that this form of analysis holds the potential to introduce an innovative SEM–EDX based approach for evaluating lichen vitality and delineation of pollution monitoring. It could be concluded that *B. submedialis* could be a preferred species monitor and manage environmental risks in the area.

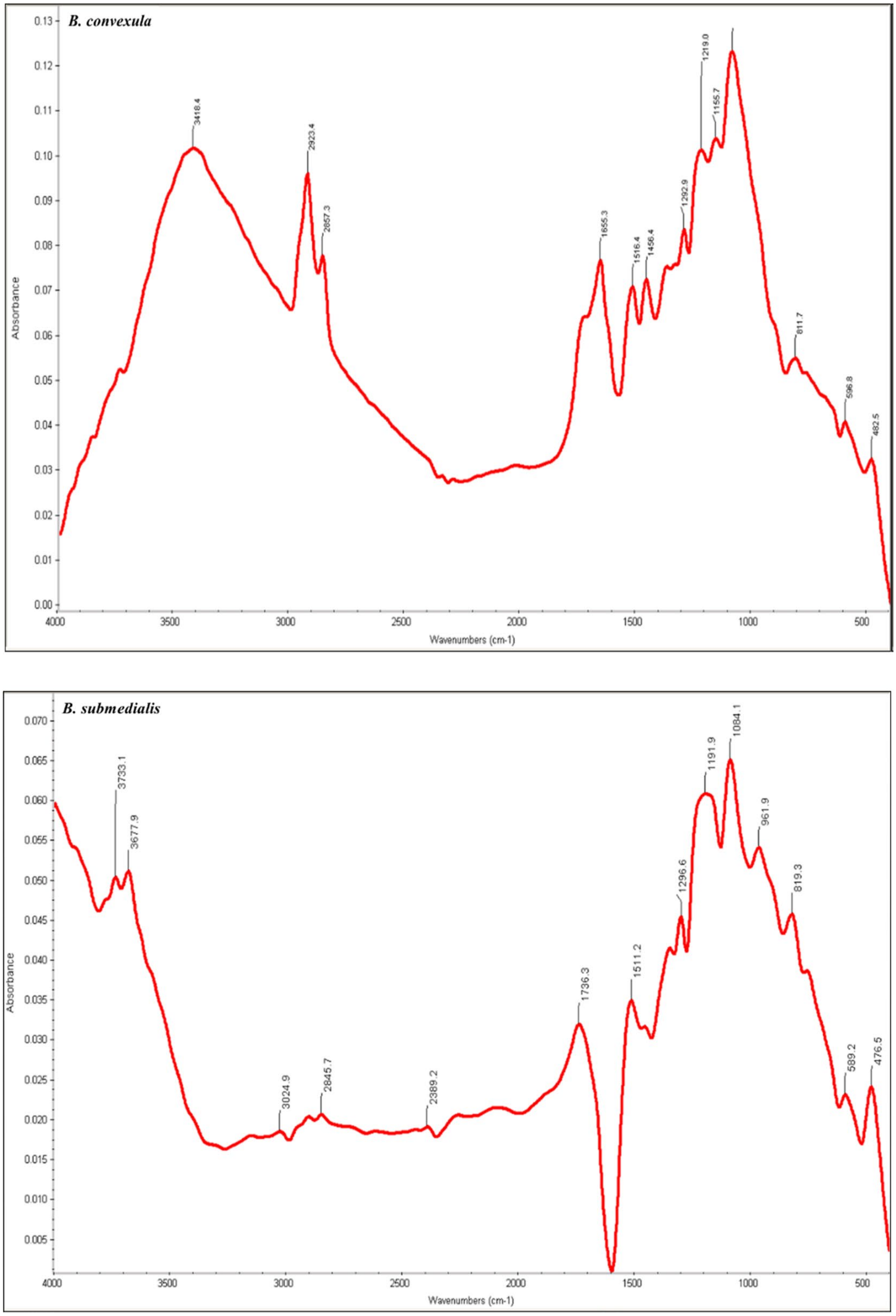


Fig. 7 FTIR analysis of lichen species showing different peaks (i) *B. convexula*, (ii) *B. submedialis*

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Author contributions VG: Formal analysis, Methodology, Writing-original draft. NG: Formal analysis, Methodology, Writing-original draft. SN: Writing-review & editing. SL: Writing-review & editing.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval Not applicable.

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