



Unraveling the ameliorative potentials of native lichen *Pyxine cocolos* (Sw.) Nyl., during COVID 19 phase

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Abstract

Due to the rapid increase in the novel coronavirus virulence, the entire world implemented the practice of lockdown along with the constraint of human movement. The obligation of quarantine halted most of the commercial and industrial movement that prominently disturbed the distinct key environmental parameters directly associated with the plant's and animal's health conditions. In this regard, the research aims to study the sudden shut-off of vehicular activity impact on the naturally growing lichen of the genus *Pyxine cocolos*. The results showed an increase in the pigments, Fv/Fm ratio, and phytohormones during the lockdown and concurrently the decreasing levels in the post-lockdown period. Interestingly, modulations in the phytohormones occur in the lockdown period as compared to the post-lockdown period. The metals Al, Cr, and Fe show the highest increasing trends in the unlocking period, whereas As, Cd, Pb, Cu, Hg, Mn, and Zn show very little variation during the running and post-lockdown phases. The lichen photosynthetic activity justifies further examination as initial biological indicators of the abrupt environmental variations prompted by such types of atmospheric situations and, to a greater extent, for the risk assessment in the near future. In conclusion, stress-phytohormone and amino acids play a significant role as stress reducers. Although lichens are well known for long environmental assessment, the present study will provide qualitative and quantitative variation in physicochemical changes in the short term and sudden environmental fluctuations.

Highlights

- Qualitative and quantitative variation in biochemical parameters in lichen during and post-lockdown period was analyzed.
- Stress-phytohormone and amino acids play a significant role as stress reducers.
- Selectivity sequence reflection in heavy metal accumulation may be used in future studies.

Keywords COVID 19 phase · Lichen pigments · Metals · Physiology · Pollutants

Introduction

COVID-19 (CO stands for “corona”; VI stands for “virus”; D stands for “disease”; “19” stands for introduction in the year 2019) was declared a pandemic disease by WHO on

11th March 2020, and 114 countries suffering from several million positive cases with more than 5 million deaths until to date (<https://covid19.who.int/>). The simple mode to governing the virus spreading at that instant was advocated to be “social distancing” and lockdown, which is being experienced by several nations during the pandemic that still needs to be implemented in the present time. As a result, humans have suffered several negative consequences, while the natural environment has gained the advantages of the activities. Interestingly, the pandemic scenario reduces the environmental impact in many cities worldwide, decreases the emissions of greenhouse gas, reduces water contamination and noise, and removes demand on tourist attractions, some of which can aid in the ecological system's regeneration (Zambrano-Monserrate et al. 2020; Vadiati et al. 2021; Soga et al. 2021). However, as per the WHO report,

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approximately 7 million people die each year from contact with fine particles due to air pollution (WHO 2018). During the past two decades, India has observed a prompt growth in industry and vehicular activities on roads, which has undoubtedly enhanced the standards of the human population (Solanki et al. 2016).

Air pollution has a variety of negative health consequences (Mannucci and Franchini 2017). Due to high fluctuation in the levels of carbon dioxide (CO₂), sulfur dioxide (SO₂), nitrogen dioxide (NO₂), PM_{2.5}, PM₁₀, NH₃, lead, ozone (O₃), and several other pollutants in the environment causing a negative influence on human health as well as plant development. State of India's Environment (SoE) report 2019 specified that more than 12% of all deaths or mortalities in India were due to the curse of polluted air (India Today 2020). In India, the environmental conditions are tremendously damaged due to all air quality indexes and the pollution levels left the parameters lingering way behind. A broad-spectrum assessment among the key air pollutants and the effect of transportation, industrial development, and other anthropogenic events have already been scrutinized frequently in the past (Padula et al. 2020; Manisalidis et al. 2020; Singh et al. 2007). Particulate matter (PM) originates from automobiles and industries, enters the circulatory tract by inhalation, and causes cardiorespiratory disorders, reproductive and nervous system dysfunctions, and cancer. Growing reports suggest that the influence of atmospheric pollution on the Indian population and their health issues has been widely studied and examined (Gulia et al. 2015; Singh et al. 2007; Jat and Gurjar 2021; Manisalidis et al. 2020).

Conversely, with the increasing rate of corona patients in the present times and consequently impending crisis, Govt. of India (GOI) affirmed an entire lockdown for 21 days in the country, which was further prolonged for 19 days, 14 days, and 14 days in II, III, and IV periods, respectively (Supplementary Table S1). Various restrictions posed by GOI as well as state government and consequent lockdowns, anthropogenic events like several industrial schemes, construction developments, vehicular movement and tourism, and other general transportation happenings were instantaneously blocked. Accumulating evidence suggests that the results of the lockdown led to a better quality of our environment (Arora et al. 2020; Bera et al. 2020; Lokhandwala and Gautam 2020). However, the impact of the abrupt breaking of anthropogenic activities on cryptogams such as lichens is not studied well; therefore, it is a prerequisite to monitor how unexpected and sudden modifications in the atmosphere cause influence lichens development. Will lichens development during abrupt change conditions help us to biomonitor the environmental condition affected by pollution?

The lichens as a bioindicator can be utilized to evaluate the situation of the neighboring atmosphere, which provides initial warning signals in the environmental fluctuations (Abas 2021;

Conti and Cecchetti 2001; Anderson et al. 2022; Boonpeng et al. 2018, 2017; Loppi 2019). The unique symbiotic association empowers lichens to settle on a wide range of habitations, for instance, temperate, tropical, arctic tundra, and hot deserts. Lichens, as symbiotic organisms, are capable of recuperating active metabolism at any stage because the hydration amount is high enough based on their endurance to the water and nutrients absorption from the air, indicating distinct features against higher plants, which is mostly dependent on the soil for their water and nutrition (Gasulla et al. 2021; Lange 2002). With the lack of a vascular system, lichens are sensitive to environmental factors such as availability of water, temperature changes, and air impurities (Garty 2001; Gasulla et al. 2021; Loppi 2019; Purvis et al. 2004; Anderson et al. 2022). Several previous studies are available in relation to lichens and pollutants, indicating that lichens may act as a tool for determining the environmental conditions of a particular area. Different techniques for evaluating environmental quality have been used notably, biomonitoring using lichens such as pigments estimation and degradation, hormone analysis, and heavy metal estimation (Abas 2021; Boonpeng et al. 2018, 2017; Loppi 2019). The present study aimed to examine the biological interaction of the sensitive organism showing alongside a local climatic gradient during the lockdown and post-lockdown phases in the specific or selective area. The hypothesis was to know the behavior of naturally growing lichen in this abrupt lockdown and to further identify physico-chemical parameters toward specifying the sensitive one due to an increase or decrease in air pollution.

Materials and methods

Sample collection

The samples of lichen *Pyxine cocolos* (Sw.) Nyl., naturally growing on the trunk of a date palm, at the area of Lucknow Cantonment Board near Garrison Church (N 26°48'59.29", E 80°56'41.78" alt 125 m; Supplementary Fig. S1), a low pollutant area was selected. The fresh lichen samples ($n = 5$) were collected on the same date in the months from March to September 2020.

Pigments analysis

The lichen samples (0.25 g) were kept in ice and crushed to a powder in the dark conditions using acid-washed silica beads, 0.12 g calcium carbonate, and 2.5 ml of 80% ice-cold acetone. The mixture was transferred in a 5.0 ml tube, mixed vigorously, and centrifuged for 10 min at 10,000 rpm. The supernatant was dispensed and kept in the cold, whereas the pellet was re-mixed in 1.5 ml 80% of ice-cold acetone and spun at 10,000 rpm for 10 min. After spin, again, the supernatant was taken, then mixed to a fixed amount, and examined with a UV

scanning spectrophotometer. The quantity of pigments (carotenoid, Chl *a*, Chl *b*, and total chlorophyll) was observed at 645 nm and 663 nm absorbance values (Sarker and Oba 2019). The total amount of carotenoid was measured at 480 nm and 510 nm absorbance values. The chlorophyll degradation was calculated according to Ronen and Galun (1984).

Chlorophyll fluorescence (Fv/Fm) analysis

The chlorophyll fluorescence was measured in lichens by PAM-2000 Chlorophyll Fluorometer (Walz Effeltrich, Germany). The lichen samples were placed in the dark for 5 min, and then, the values of fluorescence were measured. The F_0 was observed with a minimum level of fluorescence and open PSII (P680) reaction points by a low red wavelength, and for the determination of the maximum level of fluorescence (F_m) with closed PSII reaction centers, a saturation light pulse was given. The F_v variable fluorescence was calculated by the difference between F_m and F_0 , whereas the ratio F_v/F_m represents the chlorophyll fluorescence.

Electrolyte conductivity (EC) analysis

The permeability of cell membranes and leakage of the electrolyte occurred due to damage and K^+ ions predominantly. To determine EC analysis, the samples were absorbed in 50 mL of deionized H_2O for 1 h, then EC of the water (represented as $\mu S\text{cm}^{-1}$) at 25 °C was computed earlier and later lichen absorption employing a conductivity meter (Basic 30 EC-meter, Crison), (Marques et al. 2005).

Chlorophyll stability index

The CSI was considered by mixing Chl *a* and Chl *b* amount in control and evaluated lichen samples according to the formula mentioned in early published work (Bajpai and Upreti 2012).

$$\text{CSI}(\%) = \frac{\text{Chl in control} - \text{Chl in contaminated}}{\text{Chl in control}} \times 100$$

Protein and amino acids analysis

The concentration of protein in the homogenates was calculated at 700 nm, and bovine serum albumin (BSA) was used as a standard (Lowry et al. 1951). For the estimation of the amino acids (AAs), the Pico-tag method was used using the HPLC system (Bidlingmeyer et al. 1984). Homogenized lichens samples (300 mg) were hydrolyzed in 10 mL of 6 N HCl at 150 °C for 1 h in an oven and then cleared up for the following study. Lichen samples (10 μl) and standard (2.5 $\mu\text{mol mL}^{-1}$ in 0.1 N HCl) were dried out at 55 °C for 30 min at 75 m Torr in a vacuum oven. Both lichen samples and standard were again dried by mixing in 20 μL of

re-drying ethanol:triethylamine:water (ratio 2:1:2) mixture. Then, these specimens were derivatized by mixing 20 μL of derivatization solution comprising ethanol:water:triethylamine:phenylisothiocyanate (7:1:1:1), and once more vacuum dehydrated. These specimens were diluted in 1 mL of Pico-tag diluent and cleaned with 0.22 μm syringe filters. Using a C18 column Pico-tag amino acid (5 μm ; 3.9 \times 15 cm), the separation was performed at 40 °C. The 20 μL of the extract of each sample was introduced, and elution of the column was done by using solvent A (0.14 M sodium acetate, comprising 6% acetonitrile and 0.05% triethylamine, pH 6.4) and solvent B (60% acetonitrile in water) at 1 mL min^{-1} . A gradual gradient was performed with a rise in solvent B quantity up to 46% in 10 min, followed by a rise equal to 100% in 5 min at 1 mL min^{-1} of flux speed. Then, the column was cleaned and raised to 100% solvent A for 8 min at 1 mL min^{-1} . Chromatograms were incorporated with Empower-2 HPLC (V 6.0). The amino acids were evaluated by this process and represented in mg Kg^{-1} fresh weight.

Stress hormones analysis

The stress hormones, especially Abscisic acid (ABA), Indole-3-acetic acid (IAA), and ethylene, were measured (Epstein et al. 1986; Ergün et al. 2002). Using UltraTurrax² homogenizer, lichen samples (0.5 g) were homogenized and isolated by 70% acetone comprising of 100 mg/L butylated hydroxytoluene. After mixing, 10 mM phosphate buffer (pH 6.5) containing 200 μCi of [¹⁴C] IAA (59 mCi/mmol, Amersham) was added to each sample and kept overnight at 4 °C to determine the endogenous auxin activity. The homogenates were centrifuged for 10 min at 12,000 g on a rotary evaporator at 50 °C, which leads to a reduction of the aqueous phase. The supernatant was used further for measuring hormones in GC–MS.

Metals analysis

The lichen samples were oven-dried (70 °C) and cursed to a powder, and 0.25 g of processed in HNO_3 : H_2O_2 (3:1 v/v), and the final amount was adjusted in 5 ml by Milli Q water. Furthermore, samples were diluted 10 times; then, element concentration was examined in Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer. The metals/metalloids (E-Merck, Germany) standard reference materials were taken for calibration. For each analytical batch, standard reference materials of metals/metalloids (E-Merck, Germany) were utilized for calibration and quality assurance. The analytical data quality of metals/metalloids was confirmed by repeated analysis ($n = 3$) of quality control samples, and the findings were determined to be within (± 1.50) of the certified values. Fe, Zn, Mn, Cu, Cr, Al, Cd, Hg, Pb, and As recovery from samples was shown to be greater than 98%, as

evaluated by spiking samples with a known number of elements. Each element's detection limit was $1 \mu\text{gL}^{-1}$ (Bajpai et al. 2016; Dwivedi et al. 2010).

Pollution load

The data of pollution load was taken from the website of the central pollution control board, on a daily 24-h basis from the CPCB station situated approximately 1 km from the study site (www.https://app.cpcbcr.com/AQI_India; NAQI-CPCB 2020). Whereas the number of vehicles was counted manually based on physical appearance and the number of vehicles moved per hour.

Results and discussion

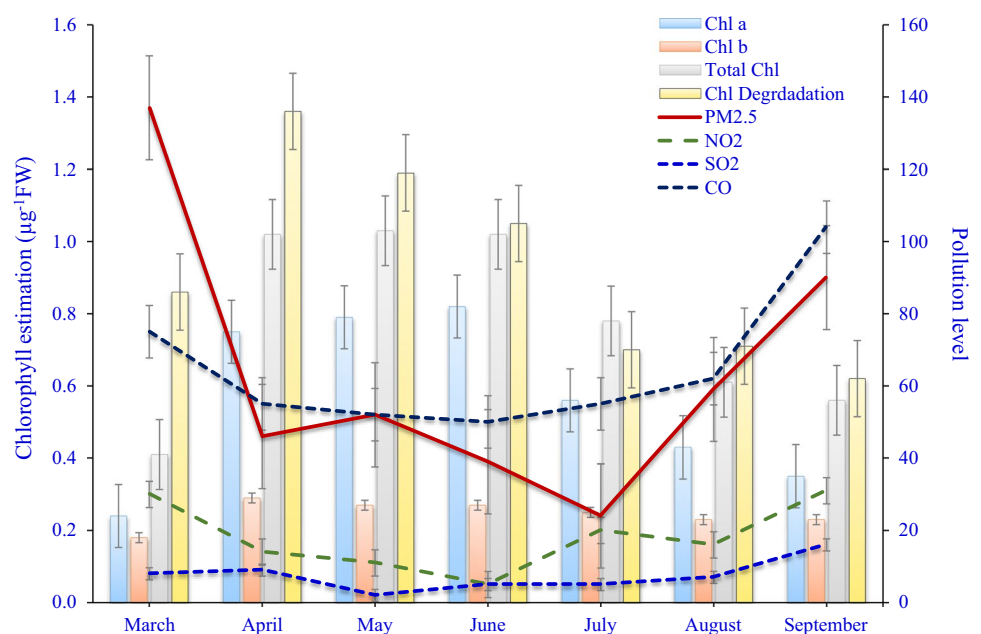
Changing trend of pigments, chlorophyll fluorescence during, and post-lockdown phase

The alterations in photosynthetic steps are a precise index of lichens for stress responses, which is correlated with the variations in environmental states and endogenous properties of the lichen thalli. All the lichen thalli samples were collected at the site of high-traffic polluted sites during lockdown times and post-lockdown periods (Supplementary Table 1). In the present study, changes in photosynthetic pigments were observed during lockdown times and post-lockdown periods. The Chl *a*, Chl *b*, and total Chl were reported between 0.24 and $0.75 \mu\text{g}^{-1}$, 0.18 and $0.29 \mu\text{g}^{-1}$, 0.41 and $1.02 \mu\text{g}^{-1}$ fresh weight (FW), respectively, during the lockdown, while 0.35 to $0.82 \mu\text{g}^{-1}$, 0.23 to $0.27 \mu\text{g}^{-1}$, 0.56 to

$1.03 \mu\text{g}^{-1}$ FW correspondingly after the lockdown phase. The Chl *a* and total Chl were more than two times higher in the lockdown period, whereas Chl *b* changes every minute. The decrease in Chl *a* and total Chl during the unlocking period indicated the disturbances in the surrounding environment. As per pigment data, compared with pollution load in the area, clearly indicate the increase of pollutant gasses SO_2 , NO_2 , and CO in the area. Various studies have shown different findings about whether automobile traffic or urban pollution enhances or decreases chlorophyll concentrations (Sujetoviene and Sliumpaite 2013; Lackovičová et al. 2013; Conti and Cecchetti 2001; Bajpai et al. 2010; Wakefield and Bhattacharjee 2011; Carreras et al. 1998). Interestingly, chlorophyll pigment modulations in the *Pyxine cocoloes* were directly correlated with heavy changes in the number of vehicles and pollution load in the area (Figs. 1 and 2). The comparison of the chlorophyll content and chlorophyll degradation monitored with pollutant gasses or with the numbers of vehicles by regression analysis showed a significant correlation R^2 (Table 1). However, it is also reported that the changes in the level of chlorophyll concentrations are largely dependent on distance or directions from the source of pollution or nitrogen compounds (NO_3^- , NH_4^+) availability during pollution conditions (Carreras et al. 1998; Boonpragob and Nash III 1991).

The carotenoid content was found to be between 0.92 and 0.96 in lockdown and 0.93 to 1.82 g^{-1} FW in the post-lockdown phase, which indicated the organism was under stress due to a change in the amount of carotenoids. The levels of carotenoids were generally found higher in samples from contaminated sites than in samples from clean locations. However, similar to the changes in chlorophyll

Fig. 1 Correlations between pigments and pollutants



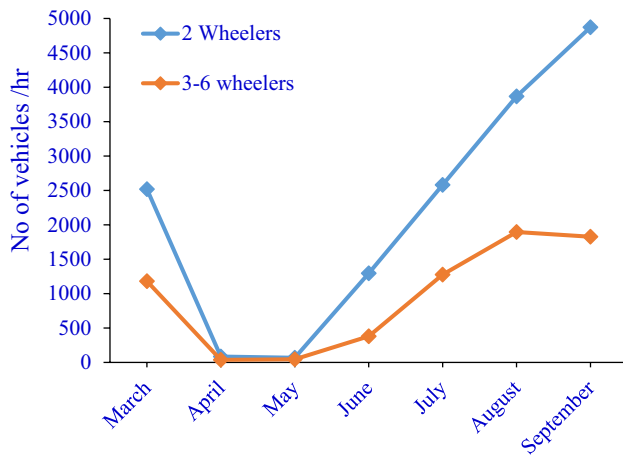


Fig. 2 Number of vehicles passing in the area during the study period

mentioned above, various fluctuations in carotenoids levels in lichen have been reported during pollution stress (Shukla and Upreti 2008; Guvenc et al. 2018; Czczuga and Krukowska 2001; Ibarrondo et al. 2016). The maximal quantum yield of photosystem II Fv/Fm ratio is routinely done to assess the operation of photosynthetic machinery (Genty et al. 1989; Murchie and Lawson 2013). The Fv/Fm values were found to be high during the locked period and lower during the unlock phase (Fig. 3).

Interestingly, during the observation, the correlations of chlorophyll and Fv/Fm showed opposing patterns with carotenoids (Figs. 1 and 3). The length/time of pollution exposure also played a significant role for pigments since they fell down over the unlock period and reached their lowest values during stress conditions. During the lockdown period, temporal changes in overall pigment levels were visible due to the combined effect of pollution level and duration in the area. Furthermore, any variations in environmental conditions (including air–water availability, temperature, light intensity, and duration) that affect lichen physiology cannot be ignored. However, our findings suggest that sudden variations in pollution levels alter the regulation of chlorophyll pigments and fluorescence in *Pyxine coxae*.

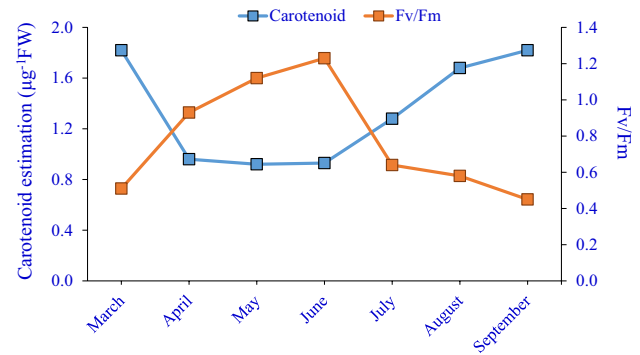


Fig. 3 Carotenoid contents and Fv/Fm ratio

Chlorophyll degradation is commonly used as one of the most effective and precise biomonitoring measures (Das et al. 2021; Massimo 2011). The chlorophyll degradation ratio was higher during the lockdown period, and it ranges between 1.05 and 1.36, and the minimum ratio was observed in unlock period between 0.62 and 0.86 ratio. The optical density (OD) ratio of chlorophyll samples taken at 435 nm and 415 nm is most commonly used to assess chlorophyll degradation. A chlorophyll degradation ratio of 1.4 indicates that chlorophyll is unaltered, but any deviation in the ratio indicates chlorophyll degradation during any sort of stress (Garty et al. 2000; Silberstein et al. 1996). It is reported in *Ramalina duriaei* Jatta that chlorophyll degradation has a 0.88 ratio value at the polluted site due to high vehicular traffic conditions (Kardish et al. 1987). In the present study, a lower value between 0.62 and 0.86 was detected during the unlock time, indicating that the variation of this ratio implies pollution stress conditions. Previous research revealed that when lichens were exposed to copper metal in a laboratory setting, their chlorophyll degradation ratio decreased (Bačkor and Zetíková 2003). According to another study, the chlorophyll degradation ratio decreases significantly after 6 weeks of exposure in areas near the tanning and metallurgical industries (González et al. 1996).

The chlorophyll stability index (CSI) is a key tool for selecting plants during environmental stress (Hussein et al. 2007), which was observed during lockdown between 25.18

Table 1 Regression analysis between chlorophyll content, no of vehicular and pollutants

	Chl a		Chl b		Chl degradation	
	R ²	p-value	R ²	p-value	R ²	p-value
2 wheelers	0.8976	0.0015	0.9016	0.0026	0.8821	0.0020
3–6 wheelers	0.9146	0.0010	0.9280	0.0014	0.8998	0.0014
PM _{2.5}	0.5978	0.0010	0.7035	0.0001	0.2616	0.0061
NO ₂	0.8197	0.0001	0.5902	0.0004	0.6190	0.0017
SO ₂	0.3269	0.0047	0.3067	0.0030	0.4133	0.0036
CO	0.5502	0.0053	0.3219	0.0011	0.3577	0.0080

R², correlation coefficient

and 35.10%, whereas in post-lockdown, it ranges between 53.12 and 63.25% (Fig. 4). The increase in CSI % suggested that these lichens were under more stress. The current study also demonstrated that the CSI score would be a simple, quick, and reliable indication for identifying lichen under stress during abrupt changes in the environment. Electrical conductivity is another simple tool to examine the rigidity of the lichen cell's plasma membrane. It is reported that the cell permeability is altered and leakage of electrolytes (generally K^+ ions) occurs in the damaged cell membranes (Marques et al. 2005). Our result for electrical conductivity also displayed the same trends as CSI in the lockdown period (10.92 to 14.17) as well as unlock period (20.65 to 28.37) (Fig. 4), suggesting that pollution stress wounds the cell permeability due to an increase in automobile activity.

Furthermore, we cannot overlook the fact that environmental variables (abiotic or biotic stress) might affect lichen activity in ways other than pollution during the study. Furthermore, even up to 1 km from the sample sites, there is no industry or activity related to coal in that area. Altogether, our findings suggest that sudden changes in motor traffic and urban pollutants have an influence on lichen pigments and chlorophyll fluorescence during and after lockdown.

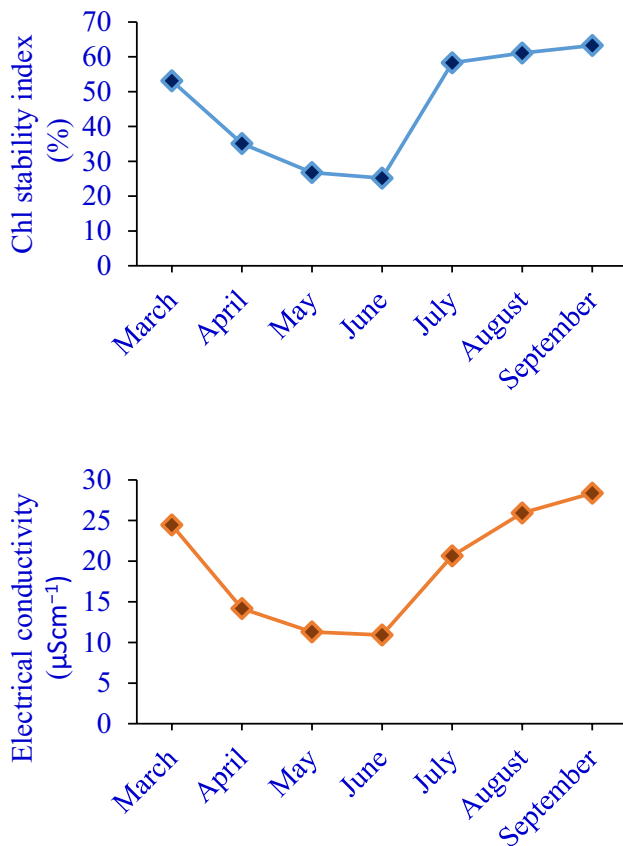


Fig. 4 Chlorophyll stability index and electric conductivity

Effect on the protein content and amino acids during and post-lockdown phase

The protein content was measured in the lockdown period between 10.85 and 12.09, and in the post-lockdown period, it ranges between 16.87 and 20.72 (μg^{-1} FW) (Fig. 5). The increased protein level in our study agrees with the outcomes in the *Ramalina ecklonii* (González et al. 1996). Babula et al. (2008) have reported that plants have adopted severe tolerance mechanisms, including high production of protein or other stress metabolites, to endure air pollution for their improvement in stress conditions (Babula et al. 2008). For example, molecular chaperones, like heat shock proteins, are induced and defended against toxicity due to heavy metals. Likewise, the lichens also implement this kind of tolerance mechanism under stress conditions, which dynamically associate with the metallic pollutant and therefore safeguards the photosynthetic machinery from the adverse consequence of contaminants.

Accumulating evidence suggests that lichens show a significant accumulation of proline and other amino acids during stress (Hayat et al. 2012). The amino acid content was found to be higher in the post-lockdown period, and it ranges between 0.61 and 0.94 (GLU), 0.18 and 0.28 (CYS), and 16.37 and 21.73 (PRO), whereas in the lockdown period, it ranges, between 0.26 and 0.29 (GLU), 0.11 and 0.13 (CYS), 5.27 and 9.06 (μg^{-1} FW) (Fig. 5). Increased amino acid content has also been seen in lichens exposure to pollution or toxic heavy metals (Silberstein et al. 1996; Bačkor et al. 2007; Bačkor and Loppi 2009). These findings show that amino acid buildup in lichens acts as a stress reliever and may be a signal of unexpected changes in air pollution.

Changing trend of stress hormones during and post-lockdown phase

Phytohormone ABA has been widely considered for its role in stress response. Several studies indicate that once plants are exposed to stressors, distinct modulations in the activity of auxins occur during metabolism, synthesis, and transport in addition to their major roles in development. The level of phytohormones ABA and IAA ranges between 28.18 to 39.825 and 147.36 to 172.12 in the lockdown phase, whereas between 30.95 to 43.29 and 38.12 to 98.25 in the post-lockdown phase, respectively (Fig. 6). The level of the auxin IAA was decreased post-lockdown as supported by findings of a few reports in other mycobionts (Battal et al. 2004; Pichler et al. 2020). However, the ABA level was not changed significantly, but the report suggests that ABA levels were increased due to pollution stresses (Battal et al. 2004).

Another endogenous phytohormone, ethylene, is released in slight quantities during normal physiological requirements but appears to enhance in different stress conditions (Srivastava et al. 2014). The ethylene level was estimated between 0.50 and 0.94 (μg^{-1} FW) in the lockdown period, whereas

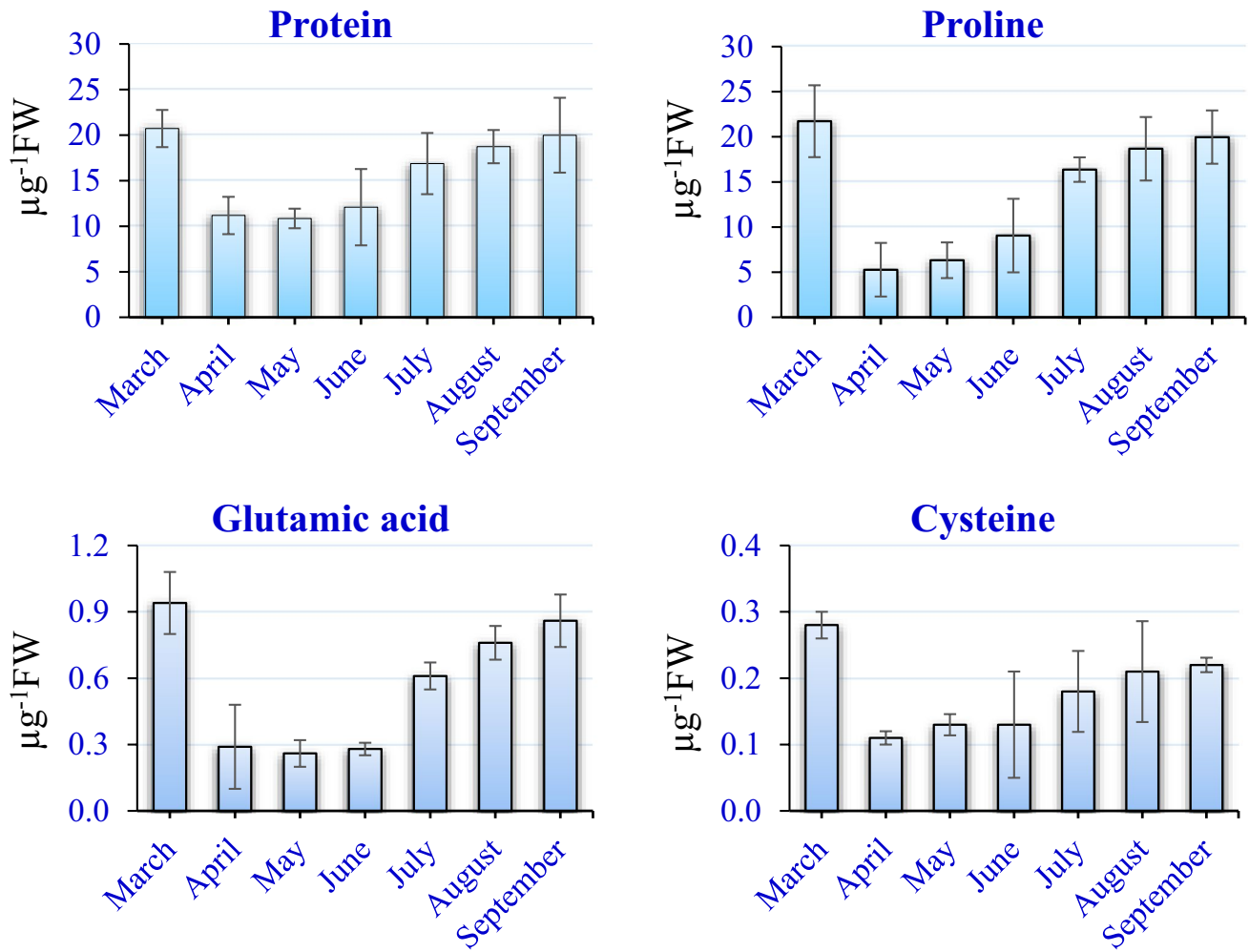


Fig. 5 Protein and amino acid contents in lichen

in post-lockdown, it ranges, between 1.26 and 1.89 (μg^{-1} FW), suggesting the level of ethylene upregulated during an increase in pollution stress (Fig. 6). There are several reports suggestive of the fact that air pollution exposure to lichens leads to upregulation of stress ethylene hormone (Garty et al. 1995, 2001). Altogether, lichens exposed to chemicals, heavy/toxic metals, or sudden fluctuations in air pollution regulate more endogenous stress hormone levels.

Changing trend of metals during and post-lockdown phase

The quantitative assessments of ten metals were observed during and the post-lockdown period. The cluster analysis revealed that the accumulation of ten metals in *P. coccoides* split into two major groups when the environmental circumstances rapidly changed (Fig. 7A). However, we categorized metals into three

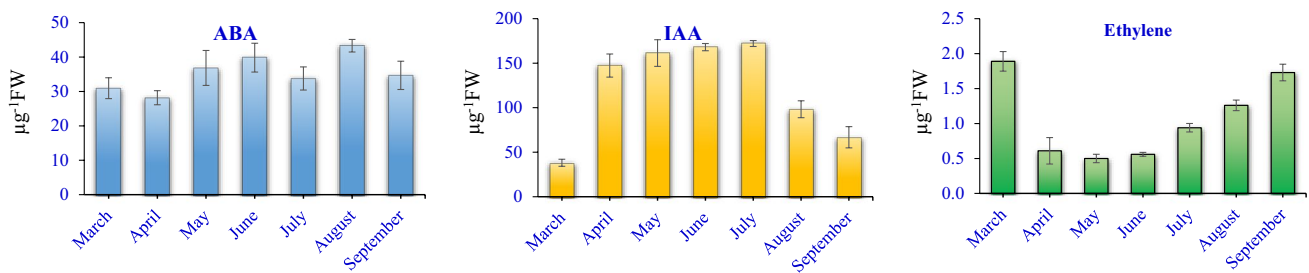


Fig. 6 Qualitative and quantitative variation in stress hormones

classes based on the number of metals accumulated in the thallus (Fig. 7B). Class I metals show the highest increasing trends in unlock period (Al, Cr, Fe) (Fig. 7A). Class II metals show moderate increasing trends with increasing unlock down period (As, Cd, Pb, Cu) (Fig. 7B); the class III metals were showing very minute changes during unlocking period (Hg, Mn, Zn) (Fig. 7B). The class I metals range between 806.7 and 1508.3 in the lockdown period and between 925.4 and 2936.79 μg^{-1} dry weight (DW) in unlock period. However, class II metals range between 0.97 and 8.79 in the lockdown period and between 1.10 and 21.25 μg^{-1} DW in unlock period. Class III metals show their quantity between 0.135 and 20.98 in lockdown and between 0.138 and 49.09 μg^{-1} DW in unlock period. The metals Cr, Al, and Fe are basically known to be associated with soil dust, and these metals were highly accumulated in the roadside samples. The neighborhoods residing nearby the road experience most of the vehicle emission-related problems directly. Interestingly, the foliose lichen particularly of bigger size thallus act as a metal reservoir, which facilitates the removal of the highest metals quantities from the atmosphere near the polluted roadside (Shukla and Upreti 2006). According to a previous report, Cr is released into the atmosphere as a result of coal and oil combustion, particularly in diesel-fed vehicles and waste ignition, whereas Al is widely distributed by air dust and present in lichen in notable amounts (Nriagu and Pacyna 1988). Loppi et al. (2000) indicated that the Al has a partial role in the metabolic association and is frequently used as an indicator of sample impurity for rock dust and air-borne soil (Loppi 2019; Loppi et al. 2000). Therefore, it might be a cause for a more concentration of Al found in lichen samples in this study. However, the amount of

Fe is significantly influenced by iron emitted from fuel combustion and soil dust and places exposed to heavy vehicular traffic (Lenka et al. 2017; Otnyukova 2007).

The Zn, Mn, Cu, and Pb are basically originated from vehicular activity only and reported in moderate concentrations. According to Ward (1990), Zn augments with the density of traffic, and the emission is mostly depending on the tire's activity, lubrication oil, and brake pads (Ward 1990). Generally, Mn and Cu accumulation comes from copper-comprising fungicides, metal operational industrial units, welding, and electroplating constituents (Baptista et al. 2008). There was no such direct source for Mn and Cu observed in the present study area; therefore, it could be a reason for the lowest concentration reported in that area. The site had only an automobile source of contamination and revealed a comparable selectivity order of metals ranging from $\text{Cr} > \text{Al} > \text{Fe} > \text{Zn} > \text{Mn} > \text{Cu} > \text{Pb} > \text{As} > \text{Cd} > \text{Hg}$. Altogether, our result revealed that the metal data specify that the air of the neighboring area is polluted extremely with Cr, Al, and Fe, followed by Zn, Mn, Cu, and Pb and least contaminated with As, Cd, and Hg.

Several interactions were identified to take place as soon as plants introduce to the adverse applications of more than one component (Kant et al. 2015; Rodriguez et al. 2019). The effect of these components is characterized as independent, additive, antagonistic, or synergistic, which accumulative leads to synchronized impacts on the plants. Although it is still challenging to emphasize the precise causes and source of metal accumulation by lichen in the studied area, but their distribution supports to explain their origin and mostly are emitted by traffic.

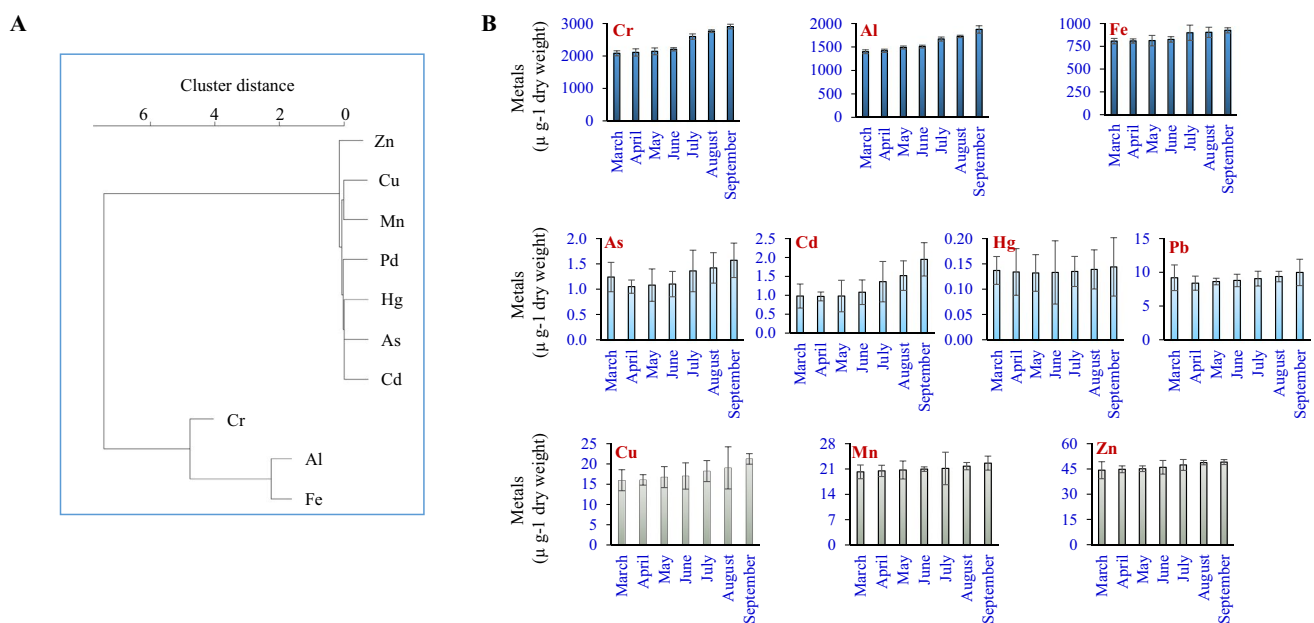


Fig. 7 Qualitative and quantitative estimation of metals observed in lichen thallus

Though, the lockdown within a short period may help to augment air quality predominantly in terms of heavy metals in the studied area.

Conclusion

The current study contributed that the photosynthetic performance of naturally developing lichens justifies future exploration as major markers of the biological issues of stress caused by an unexpected environmental disruption. Interestingly, phytohormones (particularly IAA and ethylene) and amino acids were modulated throughout the post-lockdown phase. The metals Al, Cr, and Fe show the highest increasing trends in the post-lockdown phase. Therefore, this study suggests novelty about that stress-phytohormones and amino acids can act as effective stress relievers and heavy metals accumulation provide tolerance to, which might be employed as a biomarker for pollution study in future investigations. However, it is necessary to investigate and correlate how these alterations impact the phenotypic development of *P. cocolos*. Finally, our findings suggest that *P. cocolos* may be utilized for biomonitoring in the future and will serve as a unique material for analyzing sudden changes in environmental health risk assessment.

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Author contribution Rajesh Bajpai – original concept, collection of samples, analysis of the material, manuscript preparation; Rakesh Srivastava – analysis of material and interpretation, and manuscript preparation; D K Upreti – review and editing manuscript.

Declarations

Ethics approval This article does not contain any studies with human or animal participants.

Conflict of interest The authors declare no competing interests.

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