

Pollutant-specific Responses of Epiphytic Lichens at Selected Air Pollution Monitoring Stations in Peninsular Malaysia

Nur Syafiqah Abd Hakim ¹, Asmida Ismail ^{1,2,*}, Mohd Talib Latif ³, Faezah Pardi ^{1,2}, Rosla Zamzuri ⁴, Sofia Khalid ⁵, and Faeiza Buyong ⁶

¹ School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia

² Institute for Biodiversity & Sustainable Development, Universiti Teknologi MARA, Shah Alam, Malaysia

³ Department of Earth Sciences & Environment, Faculty of Science & Technology, Universiti Kebangsaan Malaysia, Bangi, Malaysia

⁴ Department of Environment, Ministry of Natural Resources and Environmental Sustainability, Putrajaya, Malaysia

⁵ Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi, Pakistan

⁶ School of Chemistry & Environment, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia

Email: syafiqahhakim2406@gmail.com (N.S.A.H.); asmida@uitm.edu.my (A.I.); talib@ukm.edu.my (M.T.L.); faezahpardi@uitm.edu.my (F.P.); roslah@doe.gov.my (R.Z.); sofia.khalid@aiou.edu.pk (S.K.); faeiza@uitm.edu.my (F.B.)

*Corresponding author

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Abstract—Lichens are vital bioindicators of air quality due to their sensitivity to pollutants. This study investigates lichen species composition and responses to atmospheric contaminants in selected industrial, urban, suburban, and rural areas across northern to central-eastern Peninsular Malaysia. Monthly air quality data from July 2017 to December 2023, including PM₁₀, PM_{2.5}, NO₂, SO₂, O₃, and CO, were obtained from the Department of Environment Malaysia. Lichen samples were collected from ten mature trees at each site using 20 cm × 30 cm quadrats and identified using morphological and chemical characteristics. A total of 19 lichen species were recorded, with crustose lichens dominating across all land-use types. Species richness peaked at the industrial site (12 species), followed by suburban (11 species), urban (10 species), and rural (7 species) sites. The urban site had the highest mean concentrations of PM₁₀ (26.56 ± 0.71 µg/m³), PM_{2.5} (18.96 ± 0.60 µg/m³), NO₂, SO₂, and CO, with significant differences among sites ($p < 0.001$). Canonical Correspondence Analysis (CCA) indicated that *Lepraria incana* and *Amandinea punctata* were associated with environments characterized by elevated particulate matter, whereas *Parmotrema tinctorum*, *Lecidea lithophila*, and *Chrysothrix candelaris* were associated with less disturbed environments. Several crustose taxa, particularly within the genera *Graphis* and *Lecanora*, were more prevalent in urban and industrial environments, suggesting greater tolerance to anthropogenic disturbance and nutrient-enriched conditions. These findings highlight the pollutant-specific and ecological responses of tropical lichen communities and support the application of lichen-based biomonitoring for long-term air quality assessment in rapidly developing regions.

Keywords—lichen, bioindicator, air pollution, species composition, canonical correspondence analysis

I. INTRODUCTION

Lichens are composite organisms arising from a mutualistic relationship between fungi (mycobiont) and photosynthetic partners (photobionts), typically green algae or cyanobacteria [1]. They colonize a wide range of terrestrial substrates, including bark, rocks, and soil, and are especially notable for their sensitivity to atmospheric conditions. Due to their reliance on atmospheric deposition for nutrients and the absence of protective cuticles or stomata, lichens are particularly vulnerable to air pollution [2, 3]. This unique physiology renders them effective bioindicators for assessing air quality and ecological health across various landscapes.

Land-use changes across Peninsular Malaysia have

produced a clear gradient in air pollution levels, ranging from heavily industrialized zones to suburban and rural regions with relatively minimal disturbance [4, 5]. In recent decades, increasing anthropogenic activities such as industrial development, urbanization, and vehicular emissions have intensified air pollution in many developing regions, including Malaysia [6–8].

To address the need for effective environmental monitoring, this study investigates epiphytic lichen diversity across four distinct land-use types in Peninsular Malaysia: industrial, urban, suburban, and rural areas. Field-based species composition surveys are combined with long-term atmospheric data (2017–2023) obtained from Continuous Air Quality Monitoring (CAQM) stations. Diversity metrics such as the Shannon-Wiener Index are used to evaluate patterns of species richness and evenness across sites. In addition, Canonical Correspondence Analysis (CCA) is applied to explore the relationships between lichen species distributions and pollutant gradients, with particular focus on identifying taxa that may serve as indicators of specific pollutants such as particulate matter (PM₁₀ and PM_{2.5}).

The findings of this study aim to enhance understanding of species–pollution relationships in tropical environments and support the use of lichen-based biomonitoring as a cost-effective approach for assessing air quality and ecological impacts in Malaysia.

II. LITERATURE REVIEW

Airborne pollutants such as particulate matter (PM₁₀ and PM_{2.5}), sulfur dioxide (SO₂), nitrogen dioxide (NO₂), ozone (O₃), and carbon monoxide (CO) are known to have detrimental effects on vegetation and biota, especially on non-vascular organisms like lichens [9, 10]. These pollutants can disrupt the physiological functioning of lichen thalli, often resulting in bleaching, reduced growth rates, decreased reproductive capacity, and, in severe cases, the local disappearance of sensitive species [11, 12].

Numerous international studies have demonstrated shifts in lichen community composition in response to specific pollutants, with certain taxa serving as reliable indicators of pollution types. For example, nitrophilous and pollution-tolerant species tend to dominate in urban areas, while sensitive species are often restricted to undisturbed

habitats [13, 14].

Despite the growing body of research on lichen biomonitoring, studies examining the responses of lichen communities to specific air pollutants remain limited in tropical regions such as Malaysia. Most existing bioindication research has been conducted in temperate regions, where environmental conditions, emission sources, and pollutant composition differ considerably from those found in Southeast Asia. Consequently, there is a need for region-specific studies to better understand how tropical lichen communities respond to air pollution gradients.

Identifying pollution-tolerant and sensitive species within tropical ecosystems can provide valuable insights for long-term environmental monitoring. Lichen-based biomonitoring offers a cost-effective and reliable method for detecting ecological impacts of air pollution, particularly in urban and peri-urban landscapes where anthropogenic emissions continue to increase.

III. MATERIALS AND METHODS

A. Study Area

This study was conducted across four selected locations in Peninsular Malaysia, ranging from the northern region to the central-eastern part of the peninsula. Each location represents a different level of urbanization, as classified by the Department of Environment Malaysia (DOE) from 2017 to the present (see Table 1). The industrial area is represented by Kulim in Kedah; the urban area by Seberang Jaya, Pulau Pinang; the suburban area by Jerantut in Pahang; and the rural area by Seri Manjung in Perak.

The selection of sites was based on their proximity to nearby Continuous Air Quality Monitoring (CAQM) stations (Fig. 1(a)), which ensures access to reliable pollutant data. These CAQM stations are part of a nationwide network of 51 stations managed by the DOE (Fig. 1(b)), strategically

located in residential, traffic, and industrial zones to monitor air quality trends and calculate the Air Pollutant Index (API) to detect harmful fluctuations that could impact public health and the environment [15].

Table 1. Selected study sites based on the type of land use

Station	Land-Use	Sampling Site	Latitude	Longitude
Kulim	Industrial	Kulim Hitech Park	05°24'06" N	100°35'23" E
Seberang Jaya	Urban	SK Seberang Jaya II, Seberang Jaya	05°23'53" N	100°24'14" E
Jerantut	Suburban	SMK Jerantut	03°56'54" N	102°22'00" E
Seri Manjung	Rural	Pentadbiran Daerah Manjung, Seri Manjung	04°12'01" N	100°39'48" E

Fig. 2 displays the map of the selected sampling sites for this study. Geographic Information System (GIS) mapping (QGIS Desktop version 3.34.10) was used to visualize the spatial distribution of the selected sampling sites.

Seberang Jaya is classified as an urban township in Penang, Malaysia. According to the Department of Statistics Malaysia (DOSM)'s *Kawasanku* open data dashboard, Seberang Jaya has a population of 93,836 and covers approximately 17.6 km² [16]. The township is characterized by dense residential and commercial development, extensive road networks, and high traffic density. It is located near major highways including the Butterworth–Kulim Expressway (BKE), which facilitates continuous vehicular movement between industrial and urban centers.

Kulim Hi-Tech Park (KHTP) is an industrial zone located in Kulim District, Kedah, with a population of approximately 75,798 and an area of 19.16 km² [16]. It is a planned industrial hub specializing in high-technology manufacturing, including semiconductors, electronics, solar energy, and medical device industries [17]. The area hosts multiple multinational companies and is characterized by structured zoning, industrial facilities, and supporting transport infrastructure.

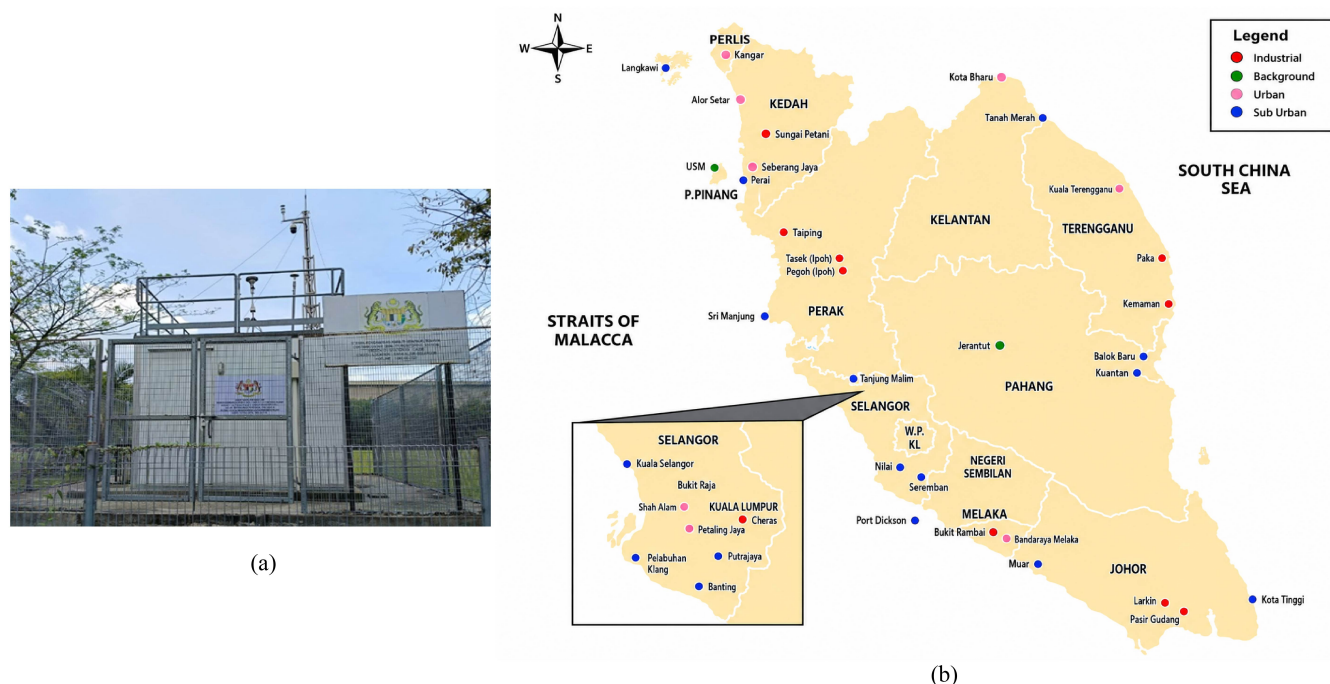


Fig. 1. Continuous Air Quality Monitoring (CAQM) network in Peninsular Malaysia: (a) CAQM machine and (b) Distribution of CAQM stations in Peninsular Malaysia (DOE, 2010).

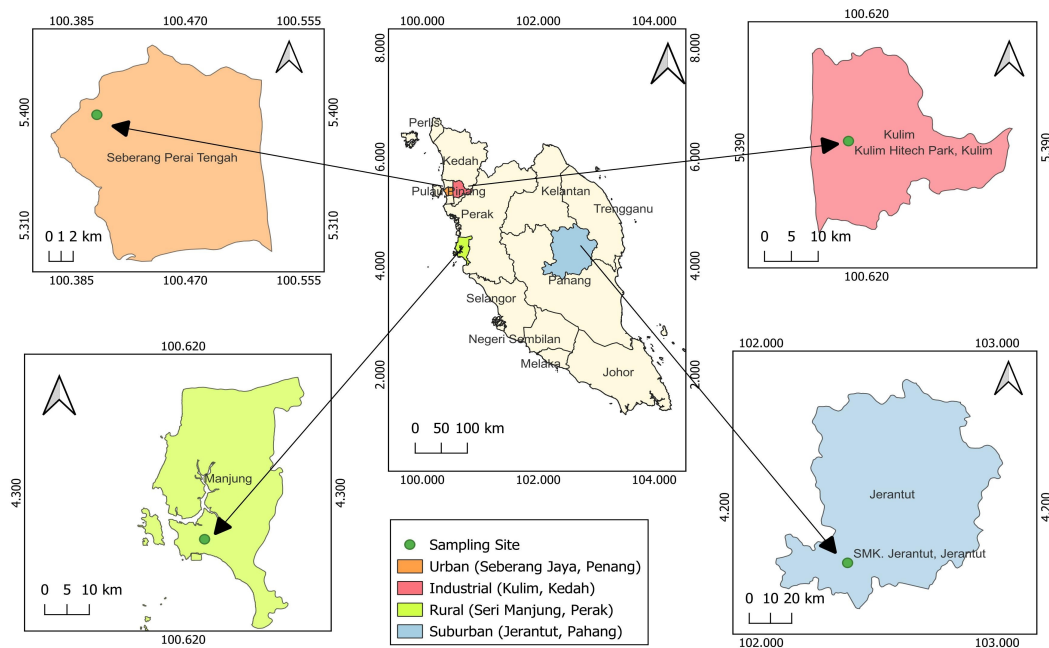


Fig. 2. Map of sampling locations generated using QGIS Desktop 3.34.10 (2024), showing (a) Peninsular Malaysia, (b) Seberang Jaya (urban), (c) Kulim (industrial), (d) Seri Manjung (rural), and (e) Jerantut (suburban).

Jerantut is classified as a suburban site in Pahang, with a population of 104,771 and an area of approximately 7563 km² [16]. The district comprises mixed land uses, including residential areas, small-scale commercial activities, and extensive forested regions. Human activity and traffic levels are moderate, and the area serves as a transitional zone between urban development and natural forest landscapes.

Seri Manjung is classified as a rural site and serves as the administrative centre of Manjung District, Perak. The district has a population of 246,977 and covers approximately 1171 km² [16]. The area is dominated by agricultural activities, aquaculture (particularly prawn farming), and fishing-related livelihoods. Although classified as rural based on land-use structure and lower urban density, the district is also influenced by nearby coastal industrial zones, Lumut Port, and associated maritime activities.

A. Lichen Sampling and Identification

At each study site, lichen samples were collected from 10 trees, specifically from the sides facing the presumed direction of pollutant exposure. Trees were chosen based on accessibility and maturity, with only those exhibiting a Diameter at Breast Height (DBH) greater than 80 cm included to provide adequate substrate for lichen colonization.

To minimize variability in bark characteristics, each study site was represented by a single dominant phorophyte species. Sampling at the suburban site was conducted on *Mimusops elengi*; at the rural site, on *Terminalia catappa*; at the industrial site, on *Carallia brachiata*; and at the urban site, on *Hopea odorata*. Site selection was primarily determined by proximity to CAQM stations, resulting in differing vegetation composition across land-use types. Therefore, the same phorophyte species was not consistently available at all sites. The dominant and most locally abundant mature tree species at each site were selected to ensure standardized sampling.

To maintain internal consistency, sampling within each site was standardized using mature trees with stable, non-exfoliating, and relatively rough bark surfaces, as well as

comparable trunk diameter (DBH > 80 cm), to reduce variability in substrate texture and lichen colonization suitability. Nevertheless, differences in bark chemical properties among phorophyte species, particularly bark pH, may still influence lichen colonization patterns and therefore represent a potential source of variation across sites.

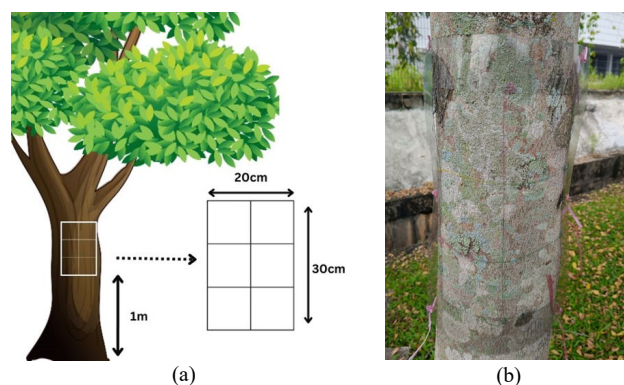


Fig. 3. Method of sample collection using a 20 × 30 cm quadrat on tree bark. (a) Schematic showing quadrat placement at 1 meter above ground level. (b) Actual photograph of the quadrat used during fieldwork.

To standardize the sampling procedure, a 20 × 30 cm flexible plastic quadrat with internal 5 × 5 cm grid subdivisions was used (Fig. 3). The grid lines provided visual guides for estimating the coverage of each lichen species within the defined sampling area, thereby ensuring uniform assessment of the bark-sampling area.

Lichen thalli were carefully detached using a chisel or ceramic knife, and the samples were placed into labeled paper envelopes for preservation. Sampling was conducted 1 to 3 m above ground level.

The collected specimens were air-dried and subsequently examined using stereo and light microscopes to assess their morphological features. Chemical spot tests were conducted to facilitate accurate species identification and detect the presence of secondary metabolites based on the characteristic color reactions of the lichen tissue [18, 19]. The K test involved applying a 10%–25% potassium hydroxide (KOH)

solution to the cortex, medulla, and apothecial parts. The C test utilized a freshly prepared calcium hypochlorite solution, while the KC test consisted of sequential applications of both the K and C reagents. The P test employed a 1%–5% solution of paraphenylenediamine in ethanol. These diagnostic tests were essential for determining specific chemical profiles critical for distinguishing between morphologically similar lichen species.

Species identification was conducted using standard dichotomous keys and taxonomic references based on morphological, anatomical, and chemical characteristics, following established lichen identification literature [20, 21].

B. Air Pollutant Data

Air quality data for the four selected sites were obtained from the Department of Environment (DOE) through their Continuous Air Quality Monitoring (CAQM) stations. This dataset includes key atmospheric pollutants that are crucial for ecological assessments and contribute to the Air Pollution Index (API). The recorded pollutants are particulate matter (PM₁₀ and PM_{2.5}), nitrogen dioxide (NO₂), sulfur dioxide (SO₂), ozone (O₃), and carbon monoxide (CO).

Monthly pollutant records spanning from 2017 to 2023 were utilized. 2017 aligns with the DOE's current urbanization categories, while 2023 represented the most recent complete and verified dataset available at the time of the study.

The availability of pollutant data varies by site, depending on each station's monitoring capacity and local emission profiles. Both the urban site (Seberang Jaya) and the suburban site (Jerantut) have complete datasets for all six pollutants (PM₁₀, PM_{2.5}, NO₂, SO₂, O₃, and CO).

In contrast, the rural site (Seri Manjung) only reports levels of PM₁₀ and PM_{2.5}, as gaseous pollutants in that area are generally minimal and fall below detection thresholds. The industrial site (Kulim) provides data on PM₁₀, PM_{2.5}, NO₂, and SO₂ but does not monitor O₃ and CO due to their lower readings compared to other dominant pollutants. These differences reflect the DOE's prioritization of site-specific pollutant profiles and the main contributors to air quality degradation in each region.

C. Determination of Lichen Diversity Indices

To evaluate the diversity of lichen communities, a combination of complementary indices was employed, including species richness (S), the Shannon–Wiener diversity index (H'), evenness (E), and dominance (D). These indices collectively describe different aspects of community structure.

Species richness (S) represents the total number of lichen species recorded at each site and provides a direct measure of taxonomic diversity. However, it does not account for the relative abundance of each species. The Shannon–Wiener index (H') captures both species richness (the number of species) and evenness (how evenly individuals are distributed among species). It is calculated as:

$$H' = - \sum_{i=1}^S p_i \ln(p_i)$$

where p_i is the proportion of individuals belonging to species i relative to the total number of individuals.

Higher H' values indicate greater diversity, suggesting a

more balanced community where no single species dominates. Table 2 shows the classification of the Shannon diversity index (H') used in this study to interpret lichen community diversity. The classification was adapted from [22].

Shannon Diversity Index (H')	Community Structure
<0.60	Not stable
0.61–1.2	Less stable
1.21–1.8	Moderately stable
1.81–2.4	Stable
>2.41	Very stable

Evenness (E) describes how uniformly individuals are distributed across the recorded species. Values closer to 1 indicate a more even community, whereas lower values suggest dominance by certain species.

Dominance (D), on the other hand, reflects the extent to which a community is dominated by one or a few species. Higher dominance values indicate that a small number of species account for a large proportion of the total abundance.

Together, these indices provide a comprehensive assessment of lichen community structure by capturing species richness, distribution balance, and dominance patterns across different land-use types.

D. Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics version 30.0. Data were first tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. For datasets that did not meet parametric assumptions, non-parametric tests such as the Kruskal–Wallis test were applied to compare multiple groups. Where significant differences were identified, post hoc pairwise comparisons were conducted using the Mann–Whitney U test. For variables that followed a normal distribution, the independent-samples *t*-test was used to compare the means of two groups. Lichen diversity indices were also calculated using PAST. Results are presented as mean ± Standard Error of the Mean (SEM).

Canonical Correspondence Analysis (CCA) was performed using PAST version 4.03 (University of Oslo, Norway) to explore the relationships between species composition and environmental variables. For CCA, only PM₁₀ and PM_{2.5}, which were consistently measured across all sites, were included to avoid bias arising from incomplete pollutant datasets. Other pollutants with site-specific missing values were excluded from the CCA and were instead interpreted separately using site-based statistical comparisons and ecological interpretation of species distribution patterns.

IV. RESULT AND DISCUSSION

A. Air Pollution Levels across Sites

Concentrations of key atmospheric pollutants, including PM₁₀, PM_{2.5}, NO₂, SO₂, O₃, and CO, were compared across four land-use types: industrial, urban, suburban, and rural, using monthly data from July 2017 to December 2023. However, not all pollutants were consistently measured at every site, as certain parameters are not prioritized in routine monitoring when their contribution to the Air Pollution Index (API) is minimal. For instance, CO and O₃ data were only available for the urban (Seberang Jaya) and suburban

(Jerantut) sites, while SO_2 and NO_2 were not recorded at the rural site (Seri Manjung). Statistical analyses were therefore restricted to comparisons among sites with complete datasets.

1) Particulate matter (PM_{10} and $\text{PM}_{2.5}$)

According to DOE, the Malaysian Ambient Air Quality Standard for PM_{10} is $45 \mu\text{g}/\text{m}^3$, while for $\text{PM}_{2.5}$ is $25 \mu\text{g}/\text{m}^3$. Monthly average PM_{10} and $\text{PM}_{2.5}$ concentrations differed significantly among sites (Fig. 4).

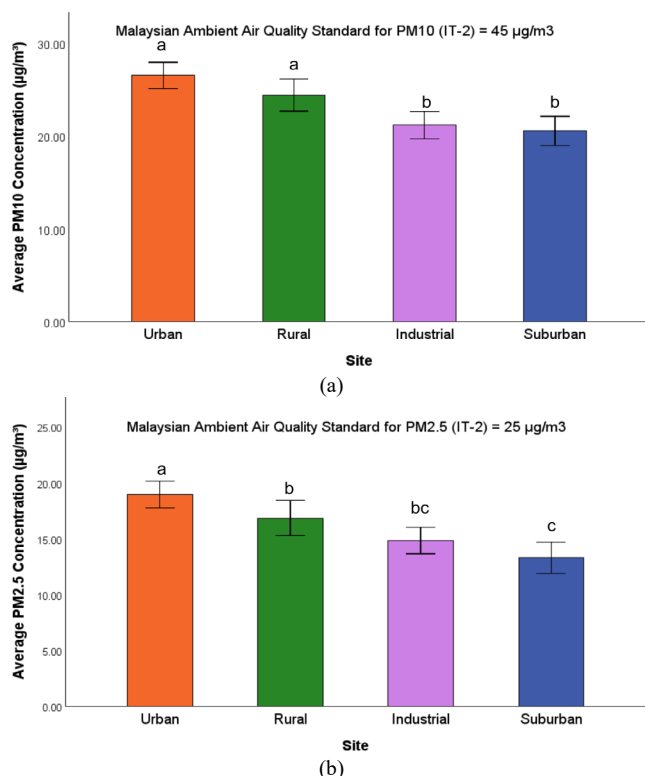


Fig. 4. Mean concentration of particulate matter: (a) PM_{10} and (b) $\text{PM}_{2.5}$ between sites.

For PM_{10} , the urban site recorded the highest mean concentration ($26.56 \pm 0.71 \mu\text{g}/\text{m}^3$), followed by rural (24.46 ± 0.87), industrial (21.20 ± 0.73), and suburban sites (20.57 ± 0.79). The Kruskal–Wallis test confirmed significant differences among sites ($H = 51.119$, $\text{df} = 3$, $p < 0.001$). Pairwise comparisons indicated significantly higher PM_{10} in urban versus suburban ($U = 1262$, $p < 0.001$) and industrial sites ($U = 1561$, $p < 0.001$), while the urban–rural difference was not significant after Bonferroni correction ($U = 2352$, $p = 0.01$). The rural site had higher PM_{10} than suburban ($U = 1883.5$, $p < 0.001$) and industrial sites ($U = 2202$, $p = 0.002$).

$\text{PM}_{2.5}$ showed a similar pattern, with the urban site highest ($18.96 \pm 0.60 \mu\text{g}/\text{m}^3$), followed by rural (16.86 ± 0.80), industrial (14.85 ± 0.60), and suburban sites (13.31 ± 0.71). The Kruskal–Wallis test also indicated significant differences among sites ($H = 60.374$, $\text{df} = 3$, $p < 0.001$). Pairwise comparisons revealed significantly elevated $\text{PM}_{2.5}$ in urban areas relative to all other sites. Rural $\text{PM}_{2.5}$ was higher than suburban ($U = 1850$, $p < 0.001$) but not significantly different from industrial ($U = 2447$, $p = 0.026$).

Anthropogenic emissions, particularly those originating from vehicular traffic and intensive human activities, significantly affect urban environments and constitute a primary source of fine particulate matter [23, 24]. In this

study, elevated particulate concentrations observed at the Seberang Jaya urban site are attributed to persistent traffic emissions and the resuspension of road dust. These impacts are intensified by the site's extensive urban road network and its proximity to the Butterworth–Kulim Expressway (BKE), a major transportation corridor connecting residential, commercial, and industrial areas.

In contrast, the industrial site at Kulim Hi-Tech Park recorded lower PM concentrations than urban site, reflecting its planned spatial layout and regulated distribution of industrial activities, which minimize localized particulate accumulation [25]. This observation is consistent with findings that effective spatial planning contributes to reductions in $\text{PM}_{2.5}$ and PM_{10} concentrations [26]. The suburban site at Jerantut exhibited the lowest PM concentrations among all sites, which correspond to its lower traffic intensity, dispersed human activities, and greater vegetation cover, which facilitate particulate deposition and atmospheric dispersion.

The Seri Manjung site, although classified as rural based on land-use characteristics and low built-up density, exhibited relatively high particulate concentrations. This pattern is likely influenced by the site's surrounding environmental setting, including agricultural activities, aquaculture operations, coastal transportation, and its proximity to Lumut Port and associated maritime-industrial zones. Port-related activities such as cargo handling, transportation, fuel combustion, and shipping emissions contribute to elevated particulate levels within the surrounding coastal environment [27, 28]. In addition, regional biomass burning and agricultural emissions may further contribute to particulate accumulation [29, 30]. Although no major point sources such as power plants or quarries were located within the immediate sampling vicinity, the site represents a rural–agriculturally influenced coastal environment subjected to mixed anthropogenic inputs rather than a pristine rural baseline.

2) Gaseous pollutants (SO_2 , NO_2 , CO , O_3)

Fig. 5(a)–(b) show the mean concentrations of SO_2 and NO_2 across industrial, urban, and suburban sites, while Fig. 5(c)–(d) show the mean concentrations of CO and NO_2 between urban and suburban sites.

SO_2 concentrations were generally low but slightly elevated in urban (0.001074 ± 0.000026 ppm) and industrial sites (0.001018 ± 0.000032 ppm) compared to suburban (0.000937 ± 0.000021 ppm). Differences were significant among sites ($H = 13.803$, $\text{df} = 2$, $p = 0.001$). Post hoc tests showed that SO_2 was significantly higher in the urban site relative to the suburban site ($U = 1970.5$, $p < 0.001$), whereas the difference between urban and industrial sites was not statistically significant ($U = 2533.5$, $p = 0.069$). No significant difference was observed between the suburban and industrial sites.

NO_2 exhibited pronounced spatial variation, with the highest concentrations recorded in urban (0.013577 ± 0.000221 ppm), followed by industrial (0.007072 ± 0.000170 ppm) and suburban (0.002563 ± 0.000072 ppm) sites. Significant differences were observed among sites ($H = 204.799$, $\text{df} = 2$, $p < 0.001$). Urban NO_2 concentrations were significantly higher than both suburban ($U = 0$,

$p < 0.001$) and industrial sites ($U = 70.5$, $p < 0.001$). Additionally, industrial sites exhibited significantly higher

NO_2 concentrations than suburban sites ($U = 0$, $p < 0.001$).

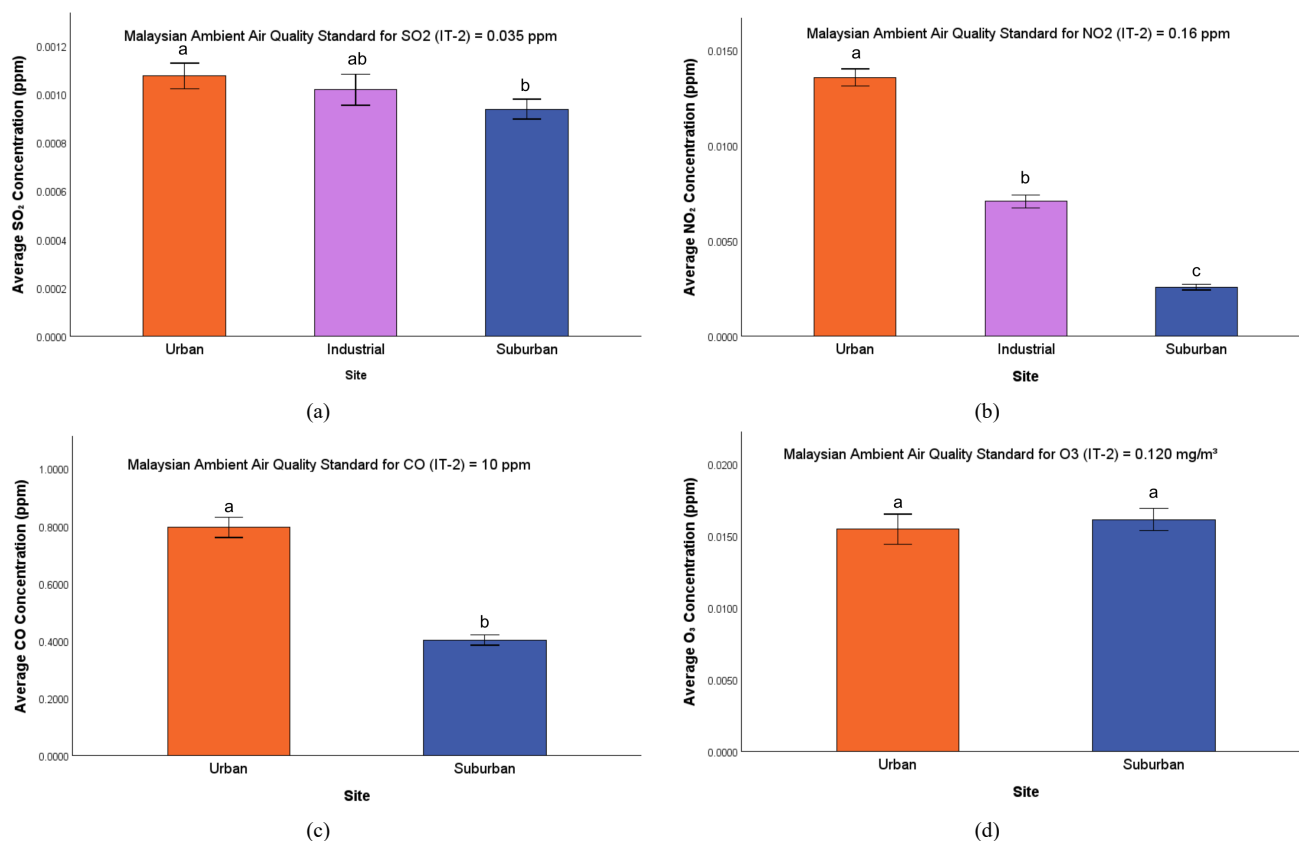


Fig. 5. Mean concentrations of (a) SO_2 and (b) NO_2 across industrial, urban, and suburban sites, (c) CO and (d) O_3 between urban and suburban sites.

CO was substantially higher in urban (0.7948 ± 0.1745 ppm) than suburban (0.4022 ± 0.0088 ppm; $U = 82.5$, $p < 0.001$). O_3 showed the reverse pattern, being slightly higher in suburban (0.016142 ± 0.000389 ppm) versus urban (0.015479 ± 0.000527 ppm), but the difference was not significant ($t = -1.012$, $p = 0.313$).

The elevated concentrations of NO_2 and CO in urban areas are consistent with their primary sources from vehicular emissions and fuel combustion, reflecting higher traffic intensity and population density [31]. Slightly elevated SO_2 levels in both urban and industrial sites may be linked to fossil fuel combustion and industrial processes [32]. In contrast, higher O_3 levels in suburban areas likely result from photochemical reactions involving precursor gases (NO_x and volatile organic compounds) under lower NO titration conditions, which are typically reduced in less congested environments [33].

B. Lichen Species Composition

A total of 19 lichen species representing 13 genera and 12 families were recorded across the four study sites (see Appendix Table A1). Among these, 16 species were crustose, and three were foliose. The family Graphidaceae was the most represented, contributing five species within the genus *Graphis*. Other families recorded included Caliciaceae, Phlyctidaceae, Parmeliaceae, Arthoniaceae, and several others. Overall, crustose lichens dominated the lichen assemblage across all sites.

A summary of chemical spot test reactions (K, C, KC, and P) for all recorded lichen species is provided in Appendix Table A2 to support species identification and enhance

methodological transparency. These reactions were interpreted alongside morphological characteristics as supporting diagnostic evidence. Several crustose taxa, particularly within the *Graphis* complex, did not exhibit diagnostic chemical reactions, which is consistent with their known chemotaxonomic characteristics. Consequently, species within the *Graphis* complex were differentiated primarily based on morphological characters, including lirellae morphology, disc structure, and ascospore features, following standard taxonomic references in lichen systematics [20, 21].

In terms of growth form composition, crustose lichens dominated across all land-use types, whereas foliose lichens formed a relatively larger proportion of the community in suburban and rural environments. The predominance of crustose forms in urban and industrial sites may be associated with their closely adhering thalli, which provide greater resistance to desiccation, substrate disturbance, and airborne pollutant deposition [34].

Beyond growth form differences, the observed community structure may also reflect broader ecological strategies related to environmental tolerance. Several crustose taxa recorded in this study, including *Lepraria incana*, *Amandinea punctata*, and members of the genus *Graphis*, are commonly associated with disturbed or anthropogenically influenced environments and may exhibit relatively broad ecological tolerance. Previous studies have shown that urban lichen community composition can be strongly influenced by NO_2 concentrations, with nitrophytic or pollution-tolerant taxa becoming increasingly dominant under nitrogen-enriched

conditions and atmospheric deposition [35]. The persistence of these taxa within urban environments characterized by elevated NO₂ concentrations may therefore suggest potential nitrophytic tendencies and adaptive tolerance to nitrogen-related pollution stress.

In contrast, foliose taxa such as *Parmotrema tinctorum* are generally considered more sensitive to atmospheric disturbance and nitrogen enrichment due to their broader thallus morphology and greater physiological exposure to airborne pollutants [36, 37]. Such taxa are often linked to comparatively oligotrophic or acidophyte-associated ecological conditions, which are known to decline under elevated nitrogen deposition and urban atmospheric enrichment [38, 39]. The restriction of *Parmotrema tinctorum* to the suburban and rural sites in the present study is consistent with this ecological pattern and may indicate lower levels of anthropogenic stress relative to the urban environment.

Several species also exhibited distinct distribution patterns across land-use types. *Graphis scripta*, *Protoblastenia rupestris*, *Dirinaria applanata*, and *Dirinaria picta* occurred across all study sites, indicating broad ecological amplitude and tolerance to varying environmental conditions. Previous studies have similarly reported *Dirinaria* species from both polluted and relatively unpolluted environments, reflecting their generalist ecological behaviour [40].

In contrast, other *Graphis* species such as *G. librata* and *G. furcata* were restricted to the industrial site, while *G. intricans* and *G. casiella* exhibited more limited distributions. These differences support previous observations that species within the genus *Graphis* may differ in ecological preference and environmental sensitivity [41].

C. Lichen Diversity across Sites

Lichen diversity varied across the four land-use types, as reflected by species richness, Shannon diversity, evenness, and dominance indices. The industrial site recorded the highest species richness (12 species), followed by suburban sites (11 species), urban sites (10 species), and the rural site had the lowest richness (7 species). Table 3 shows the comparison of diversity indices between the sites.

Table 3. Diversity indices between the sites

Index	Urban	Suburban	Rural	Industrial
Species richness	10	11	7	12
Shannon diversity (H')	1.409	1.895	1.622	2.048
Evenness (E)	0.4090	0.6655	0.7321	0.5965
Dominance (D)	0.3198	0.1832	0.2214	0.1704

Shannon diversity (H') showed a similar pattern, with the highest value observed at the industrial site (2.048), followed by suburban (1.895), rural (1.622), and urban sites (1.409). These results indicate that the industrial and suburban sites supported relatively more diverse lichen communities with more balanced species distributions [22], whereas the lower Shannon value at the urban site suggests greater dominance by a limited number of tolerant taxa.

The diversity values recorded in the present study are comparable to those reported from other tropical lichen biomonitoring studies conducted under moderate anthropogenic influence. For example, a study in Indonesia reported a Shannon diversity index of 1.8233, classified as moderate diversity [42]. Similarly, a study on corticolous

lichen communities in a tropical urban and peri-urban ecosystem in Colombia reported Shannon diversity values ranging from 1.60 to 1.76 [43].

Evenness values further supported these patterns. The rural (0.7231) and suburban sites (0.6655) exhibited relatively higher evenness, indicating more uniform distributions of individuals among species. The industrial site showed moderate evenness (0.5965), while the urban site recorded the lowest value (0.409), reflecting stronger dominance by a few tolerant species. Consistently, the dominance index (D) was highest at urban sites (0.3198) and lowest at the industrial (0.1704) and suburban (0.1832) sites.

The relatively high diversity observed at the industrial site may reflect the coexistence of multiple pollution-tolerant taxa under moderate pollutant exposure and favorable habitat conditions. In contrast, the urban site exhibited lower diversity and higher dominance, likely associated with stronger environmental filtering caused by vehicular emissions, habitat disturbance, and reduced substrate suitability.

Although the rural site recorded lower species richness, its relatively high evenness suggests a more balanced community composition with reduced competitive dominance among taxa. The suburban site demonstrated a balance of relatively high diversity and evenness, indicating favorable environmental conditions that support both species richness and equitable distribution.

In a broader biogeographical context, the patterns observed in this study differ somewhat from those commonly reported in temperate biomonitoring systems. Studies from temperate regions frequently report pronounced reductions in lichen diversity, strong species turnover, and clear spatial zonation along pollution gradients, particularly in heavily urbanized or industrialized environments [44, 45]. Sensitive species in these systems are often rapidly replaced by a limited number of pollution-tolerant taxa under elevated anthropogenic pressure.

In contrast, the tropical lichen communities examined in the present study retained moderate diversity across all land-use types, with several taxa occurring across multiple environmental settings. Although reduced diversity and increased dominance were still evident at the urban site, the overall compositional shifts appeared less abrupt than those commonly documented in temperate regions. These findings suggest that tropical lichen communities may exhibit different ecological responses to environmental gradients, potentially influenced by climatic stability, habitat heterogeneity, and differences in pollutant dynamics.

D. Species–Pollutant Relationships

Fig. 6 shows the CCA ordination of all species and PM₁₀ and PM_{2.5} variables.

Canonical Correspondence Analysis (CCA) based on PM₁₀ and PM_{2.5} demonstrated weak separation among study sites and lichen taxa along the particulate matter gradient. Axis 1 accounted for the majority of the explained variation (eigenvalue = 0.2535), while Axis 2 contributed minimally to the ordination structure. Permutation tests further indicated that the relationships between particulate matter variables and species distribution were not statistically significant ($p > 0.05$). These findings suggest that PM₁₀ and PM_{2.5} alone

do not fully account for the observed variation in lichen community composition.

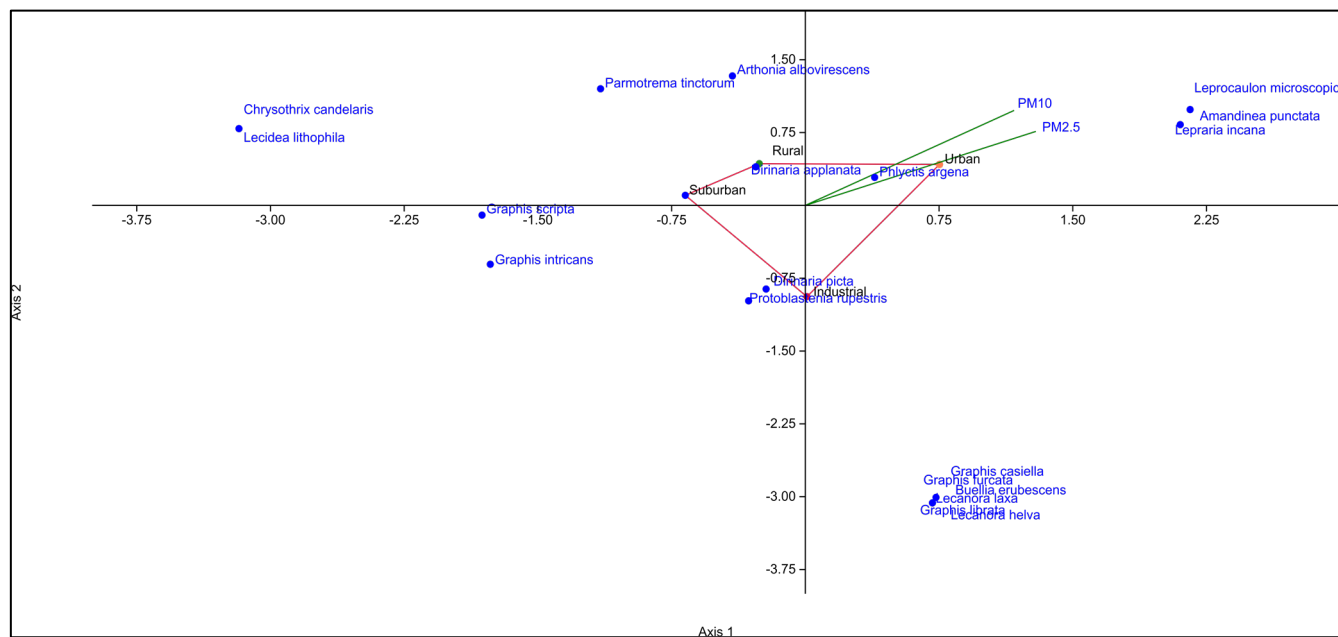


Fig. 6. CCA ordination of all species and pollutant variables.

Despite the weak ordination pattern, several taxa exhibited positional tendencies along the particulate matter gradient. *Leprocaulon microscopium*, *Amandinea punctata*, and *Lepraria incana* were positioned nearest to the PM_{10} and $PM_{2.5}$ vectors, indicating stronger association with particulate-rich environments. These taxa are frequently reported from disturbed or pollution-influenced habitats and are considered relatively tolerant to atmospheric deposition and urban environmental stress [46, 47]. Their predominant occurrence within urban and industrial environments in this study further corroborates this ecological tendency.

In contrast, *Parmotrema tinctorum*, *Chrysothrix candelaris*, and *Lecidea lithophila* were positioned further from the particulate matter vectors and occurred primarily in suburban and rural sites characterized by comparatively lower gaseous pollutant concentrations. The occurrence of these taxa in less disturbed environments may indicate preference for habitats experiencing lower atmospheric stress and reduced anthropogenic disturbance [36, 37, 48].

Several taxa restricted to the industrial site, including *Graphis librata*, *Graphis furcata*, *Lecanora helva*, and *Lecanora laxa*, did not exhibit strong associations with particulate matter vectors despite occurring within the most industrialized environment. Notably, the industrial site did not record the highest concentrations of particulate matter or gaseous pollutants than those observed at the urban site. This may have reduced severe pollution stress while still permitting the persistence of pollution-tolerant taxa, contributing to the relatively high species richness observed at the site.

In addition, the Kulim Hi-Tech Park represents a planned industrial landscape characterized by structured land-use planning, landscaped vegetation, and designated green buffer zones surrounding industrial facilities. These features may enhance habitat continuity, substrate availability, and microclimatic stability for corticolous lichens. The presence of established roadside vegetation and relatively stable host tree conditions may also have promoted lichen colonization and persistence despite the industrial setting.

The comparatively higher richness at the industrial site relative to the suburban and rural sites suggests that lichen diversity within the study area was not determined solely by pollution intensity. Instead, local habitat structure, vegetation management, substrate characteristics, and species-specific ecological tolerance likely interacted to shape observed distribution patterns across the urbanization gradient.

Although PM_{10} and $PM_{2.5}$ were the only pollutants consistently measured across all study sites and thus were suitable for multivariate ordination analysis, the broader pollutant profiles associated with each land-use type offer additional ecological context for interpreting community patterns. The urban site exhibited the highest concentrations of PM_{10} , $PM_{2.5}$, NO_2 , and CO , indicating a stronger influence from vehicular emissions and dense anthropogenic activity. Correspondingly, the urban lichen community showed lower diversity and greater dominance, suggesting that elevated traffic-related pollution may contribute to environmental filtering that favors a limited number of tolerant taxa.

The rural site exhibited a different pattern, with relatively high particulate matter concentrations but low levels of gaseous pollutants. Nevertheless, the rural lichen community maintained relatively high evenness and supported foliose taxa, suggesting that particulate matter alone may not exert the same ecological pressure as combined exposure to traffic-related gaseous pollutants and intensive urban disturbance.

Overall, the combined ordination and species distribution analyses indicate that lichen community composition within the present study is influenced by a complex interaction between atmospheric pollutants and local environmental conditions. While urban traffic-related emissions appear to contribute substantially to reduced diversity and increased dominance, habitat characteristics and substrate-related factors also play important roles in shaping lichen distribution across tropical land-use types.

V. CONCLUSION

This study demonstrated clear variation in lichen species composition and atmospheric pollutant levels across industrial, urban, suburban, and rural sites in Peninsular Malaysia. A total of 19 lichen species were recorded, with crustose forms dominating across all land-use types. Species richness was highest in the industrial site, followed by suburban, urban, and rural sites, highlighting the complex interactions between species tolerance and local environmental conditions. Air quality analysis revealed significantly higher PM₁₀ and PM_{2.5} concentrations in urban and rural environments, while gaseous pollutants such as SO₂ and NO₂ were elevated in urban and industrial areas, reflecting the influence of vehicular emissions, combustion-related activities, and other anthropogenic sources associated with different land-use settings. Canonical Correspondence Analysis (CCA) based on PM₁₀ and PM_{2.5} demonstrated distinct species tendencies along the particulate matter gradient. Taxa such as *Lepraria incana* and *Amandinea punctata* were positioned closer to particulate matter vectors, suggesting greater tolerance to environments influenced by elevated particulate deposition and urban environmental stress. In contrast, more sensitive taxa such as *Parmotrema*

tinctorum and *Lecidea lithophila* were associated with less disturbed environments characterized by lower pollutant influence. Several crustose taxa, particularly within the genera *Graphis* and *Lecanora*, were more prevalent in urban and industrial environments, reflecting functional traits associated with tolerance to anthropogenic disturbance and nutrient-enriched conditions. These findings highlight the pollutant-specific and ecological responses of tropical lichen communities and reinforce the value of lichens as effective bioindicators for long-term air quality assessment. By integrating long-term atmospheric monitoring data with biological assessment, this study contributes to the growing application of lichen-based biomonitoring in tropical regions, where such studies remain comparatively limited. Future research should further investigate seasonal variability associated with Malaysia's monsoon climate, particularly during the Northeast Monsoon. Heavy rainfall during this period may enhance wash-out processes, reducing the accumulation of particulate and soluble pollutants on lichen thalli. In contrast, drier periods may permit greater atmospheric deposition and pollutant retention. Understanding these seasonal dynamics would improve the reliability and ecological interpretation of long-term lichen biomonitoring frameworks in tropical environments.

APPENDIX

Table A1. Lichen species in each site (Note: +, present; -, absent)

Thallus	Families	Genera	Species	Industrial	Urban	Suburban	Rural
Crustose	Graphidaceae	Graphis	<i>Graphis librata</i>	+	-	-	-
			<i>Graphis scripta</i>	+	+	+	+
			<i>Graphis intricans</i>	+	-	+	-
			<i>Graphis casiella</i>	+	+	-	-
			<i>Graphis furcata</i>	+	-	-	-
	Arthoniaceae	Arthonia	<i>Arthonia albovirescens</i>	-	+	+	+
	Phlyctidaceae	Phlyctis	<i>Phlyctis argena</i>	-	+	+	+
	Lecideaceae	Lecidea	<i>Lecidea lithophila</i>	-	-	+	-
	Psoraceae	Protoblastenia	<i>Protoblastenia rupestris</i>	+	+	+	+
	Caliciaceae	Amandinea	<i>Amandinea punctata</i>	-	+	-	-
		Buellia	<i>Buellia erubescens</i>	+	-	+	-
	Lecanoraceae	Lecanora	<i>Lecanora helva</i>	+	-	-	-
			<i>Lecanora laxa</i>	+	-	-	-
	Chrysothricaceae	Chrysothrix	<i>Chrysothrix candelaris</i>	-	-	+	-
	Leprariaceae	Lepraria	<i>Lepraria incana</i>	+	+	-	-
Foliose	Leprocaulaceae	Leprocaulon	<i>Leprocaulon microscopium</i>	-	+	-	-
	Parmeliaceae	Parmotrema	<i>Parmotrema tinctorum</i>	-	-	+	+
	Caliciaceae	Dirinaria	<i>Dirinaria applanata</i>	+	+	+	+
			<i>Dirinaria picta</i>	+	+	+	+

Table A2. Diagnostic morphological characters and chemical spot test reactions (K, C, KC, P) of lichen species recorded in the study

Species	Key Diagnostic Characters	K	C	KC	P	Chemical Notes
<i>Graphis librata</i>	Lirellae elongate, simple; immersed; slit-like disc	-	-	-	-	Chemically negative
<i>Graphis scripta</i>	Lirellae simple, linear; open disc; trans-septate spores	-	-	-	-	Chemically negative
<i>Graphis intricans</i>	Lirellae partly immersed; irregular branching	-	-	-	-	Chemically negative
<i>Graphis casiella</i>	Short lirellae; slightly raised; simple branching absent	-	-	-	-	Chemically negative
<i>Graphis furcata</i>	Distinctly furcate (branched) lirellae	-	-	-	-	Chemically negative
<i>Arthonia albovirescens</i>	Thin crustose thallus; minute ascomata; epiphytic	-	-	-	-	Typically unreactive
<i>Phlyctis argena</i>	Thick white thallus; granular surface	K+ yellow	-	-	P+ orange	Norstictic acid often reported
<i>Lecidea lithophila</i>	Crustose grey thallus; apothecioid structures	K+ faint yellow	-	-	P+ yellow	Weak secondary chemistry
<i>Protoblastenia rupestris</i>	Orange crust; apothecia immersed to sessile	K+ yellow	-	-	-	Xanthone-related compounds
<i>Amandinea punctata</i>	Small black apothecia; crustose grey thallus	K+ yellow	-	-	P+ orange	Atranorin-containing species

<i>Buellia erubescens</i>	Dark apothecia; crustose thallus	K+ yellow	-	-	P+ orange	Common cortical chemistry
<i>Lecanora helva</i>	Crustose yellow-grey thallus; lecanorine apothecia	K+ yellow	-	-	-	Atranorin group
<i>Lecanora laxa</i>	Thin grey crust; scattered apothecia	K+ yellow	-	-	-	Atranorin group
<i>Chrysothrix candelaris</i>	Bright yellow granular thallus	-	C+ yellow	KC+ yellow	-	Pulvinic acid derivatives
<i>Lepraria incana</i>	Powdery (leprose) grey-green thallus	K+ yellow	-	-	P+ yellow	Leprarin / atranorin complex
<i>Leprocaulon microscopicum</i>	Leprose, filamentous thallus; inconspicuous	-	-	-	-	Chemically inconspicuous
<i>Parmotrema tinctorum</i>	Foliose, lobate thallus; ciliate margins	K+ yellow	KC+ yellow	-	P+ orange	Atranorin + medullary acids
<i>Dirinaria applanata</i>	Foliose, lobate; grey underside	K+ yellow	-	-	P+ yellow	Atranorin-dominant
<i>Dirinaria picta</i>	Foliose; sorediate lobes; grey-green surface	K+ yellow	-	-	P+ yellow	Atranorin-dominant

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

N.S.A.H. was responsible for field sampling, data processing, and preparation of the manuscript; The study was conceptualized by A.I., who also oversaw the research progress and critically revised the manuscript; M.T.L. contributed specialized knowledge on atmospheric pollutants and supported the interpretation of results; F.P. provided guidance on statistical evaluation and ecological analysis; R.Z. assisted in obtaining environmental datasets and offered input on regulatory aspects; S.K. contributed to the interpretation of environmental implications, while F.B. supported the chemical analysis component and assisted in revising the manuscript; all authors had approved the final version.

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