Integrating Lichen Community Structure and Lead (Pb) Bioaccumulation to Assess Atmospheric Pollution Across Anthropogenic Landscape in Sleman, Indonesia

Wahida Aulia Rahma
wahida.a.r@mail.ugm.ac.id
Gadjah Mada University
Andhika Puspito Nugroho
Gadjah Mada University

Research Article

Keywords: Air quality, Bioindicators, ICP-MS, Epiphytic lichen, Heavy metals, Urban ecology

Posted Date: June 26th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9812254/v1

Additional Declarations: No competing interests reported.

Abstract

Anthropogenic activities have the potential to alter air quality, necessitating efficient biological monitoring systems. Lichens serve as biomonitors for atmospheric pollution due to their structural properties and sensitivity to environmental stressors. However, integrative studies combining community structure and heavy metal bioaccumulation in tropical regions are still very limited. This study aimed to evaluate the impact of anthropogenic landscape gradients on epiphytic lichen community structure and determine the lead (Pb) bioaccumulation capacity of local lichen species in Sleman Regency, Indonesia.

A stratified random sampling design was applied in three anthropogenic zones: Station 1 (Urban Traffic), Station 2 (High Elevation), and Station 3 (Industrial Zone), using three host tree species (*Polyalthia longifolia* , *Mangifera indica* , and *Roystonea regia*). Lichen community structure was analyzed using ecological indices and *Canonical Correspondence Analysis* (CCA). Pb concentrations in lichen thallus were measured using *Inductively Coupled Plasma–Mass Spectrometry* (ICP-MS), and pollution severity was evaluated through *Contamination Factor* (CF). Twelve lichen species from eleven genera were identified. Traffic pressure acted as an ecological filter, resulting in a very simple community dominated exclusively by tolerant forms of leprose (*Lepraria finkii* and *L. lobificans*), while the sensitive species (*Phlyctis argena*) was restricted to the high elevation area. Host tree architecture significantly modulated lichen diversity, with *P. longifolia* supporting the highest diversity (H' = 2.211). CCA confirmed that particulate matter (PM2.5 and PM10) and ambient temperature were the main driving forces shaping community distribution ($p < 0.01$). Chemical analysis showed a strong positive correlation between internal Pb concentration and CF values ($r = 0.89$; $p < 0.001$). *Flavoparmelia caperata* (7.27 mg/kg) and *Graphis scriptaa* (6.96 mg/kg) exhibited the highest bioaccumulation capacity and were categorized as "High Contamination" (CF > 0.3). Anthropogenic landscape gradients significantly disrupted lichen communities and accelerated heavy metal accumulation. The integration of ecological community metrics and CF indices serves as a robust and low-cost *nature-based solution* for high-resolution urban air quality monitoring in tropical ecosystems.

Introduction

Lichens are symbiotic organisms between fungi (mycobionts) and algae or cyanobacteria (photobionts) that are highly sensitive to environmental changes, particularly atmospheric quality. The anatomical characteristics of lichens, lacking cuticles and stomata, allow for direct element exchange through the thallus surface, enabling them to capture and accumulate various atmospheric pollutant compounds. These characteristics make lichens a widely used bioindicator for monitoring air quality and pollutant deposition in various ecosystems (Thomas, 2001; Yang et al., 2023). The use of lichens in modern biomonitoring is rapidly expanding because these organisms are capable of reflecting long-term pollutant exposure that cannot always be captured through instantaneous instrumental measurements. Recent studies have also shown that lichen-based biomonitoring can be used to map the spatial distribution of pollutants and identify pollution sources in urban environments and protected areas (Çobanoğlu & Kaan, 2024; Yenisoy et al., 2025).

The ability of lichens as biomonitors is not only related to their existence as passive accumulators of pollutants, but also through changes in their community structure. Lichen community structure, reflected through species composition, species richness, dominance, distribution, and evenness, is known to be highly responsive to variations in environmental conditions. Increased anthropogenic pressures such as vehicle emissions, industrial activities, and land-use changes can affect lichen communities through microclimate changes and increased atmospheric pollutant deposition. These community responses make lichens not only function as indicators of pollutant presence but also as biological indicators of broader changes in ecosystem conditions (Niepsch et al., 2024; Harikrishna & Mukherjee, 2024).

One group of pollutants of concern in air quality studies is heavy metals. Heavy metals are persistent, difficult to biodegrade, and can persist in the environment for long periods. Certain heavy metals can accumulate in organismal tissues and move through the food chain, potentially causing ecological and health impacts. Lead (Pb) is one such heavy metal often associated with anthropogenic activities such as transportation, fossil fuel combustion, industrial activity, and atmospheric particle deposition. Although the use of leaded fuel has declined, Pb remains a significant contaminant in urban areas due to its origins from various secondary emission sources and other human activities (Mitra et al., 2022; Işik & Yildiz, 2022).

Various studies have shown that heavy metal accumulation in lichens is influenced by a combination of biological and environmental factors. Biological factors include thallus shape, anatomical characteristics, surface structure, and growth type, while environmental factors include climate conditions, topography, substrate type, and the intensity of pollution sources. Foliose and fruticose lichens are known to have a higher ability to capture atmospheric particles than other types due to their larger contact surface area. Furthermore, metal content in lichens tends to increase in locations closer to anthropogenic emission sources, such as highways or industrial areas (Przych et al., 2016; Yenisoy et al., 2025).

Sleman Regency, a region in the Special Region of Yogyakarta, is experiencing intensive anthropogenic activity. Increased vehicle mobility, residential growth, educational development, and industrial activity have resulted in a heterogeneous environmental character in this region. Data from the Sleman Regency Environmental Agency from 2018 to 2023 shows a change in the Air Quality Index from 78.73 to 89.51. This change in conditions demonstrates the importance of developing an air quality monitoring system that relies not only on conventional ambient measurements but also on a biomonitoring approach capable of recording cumulative pollutant deposition over a longer period. Modern biomonitoring studies have demonstrated that organism-based approaches can complement instrumental monitoring systems in understanding spatial variations in atmospheric pollution (Niepsch et al., 2024; Bahinskyi et al., 2025).

Research related to air quality in Sleman Regency has focused primarily on measuring ambient parameters and their implications for human health, while studies integrating lichen community structure with Pb bioaccumulation as an indicator of anthropogenic pressure are still very limited. Most lichen-based biomonitoring studies tend to focus on measuring heavy metals without linking them to the ecological responses of the communities, or conversely, only assess community diversity without evaluating pollutant content. However, an integrative approach between community structure and bioaccumulation can provide a more comprehensive picture of organisms' ecological responses to environmental pressures (Niepsch et al., 2024; Taurozzi et al., 2024).

Based on these conditions, three main research gaps remain. First, information on lichen community structure in landscapes with varying levels of anthropogenic pressure in Sleman Regency is still limited. Second, the relationship between community structure variation and Pb accumulation capacity among local lichen species is not yet clearly understood. Third, biomonitoring approaches that integrate ecological indicators and atmospheric contamination indicators in tropical urban-peri-urban areas are still relatively rare. These limitations hamper the development of a local biomonitoring framework that can serve as a basis for long-term environmental quality evaluation (Çobanoğlu & Kaan, 2024; Niepsch et al., 2024).

The novelty of this study lies in its integrative approach, combining lichen community structure and Pb bioaccumulation to evaluate atmospheric pollution along an anthropogenic gradient in Sleman Regency. This study not only documents lichen species composition but also evaluates the distribution, abundance, and patterns of Pb accumulation within an environmental biomonitoring framework. Integrating community responses and metal accumulation is expected to yield more comprehensive information on environmental stress than either approach alone. This approach aligns with recent developments in biomonitoring studies that emphasize the importance of combining biological and chemical indicators to improve the interpretation of environmental quality (Yenisoy et al., 2025; Niepsch et al., 2024).

This study aims to: (1) identify the community structure and diversity of lichens in areas with different anthropogenic characteristics in Sleman Regency; (2) analyze the distribution and abundance of lichens along the anthropogenic gradient; and (3) evaluate the Pb bioaccumulation capacity of the lichen species found. The results of this study are expected to provide basic data for the development of lichen-based air quality biomonitoring in Sleman Regency, while also supporting the development of a more integrated and sustainable environmental monitoring system.

Materials and Methods

Study Location and Research Design

This study was conducted in Sleman Regency, Yogyakarta Special Region, Indonesia, using a lichen-based environmental biomonitoring approach to evaluate lichen community structure and lead (Pb) bioaccumulation in landscapes with different levels of anthropogenic stress. The lichen biomonitoring approach has been widely applied in environmental quality studies because lichens have the ability to passively accumulate atmospheric deposition and reflect pollutant exposure over a relatively long period of time, so they can be used to describe spatial variations in atmospheric pollution in a region (Niepsch et al. 2024; Çobanoğlu & Kaan 2024).

The study design used a stratified random sampling method with location grouping based on the dominant characteristics of anthropogenic activity. Sleman Regency was selected because of its high land use heterogeneity, including areas with high motorized vehicle traffic intensity, areas with higher elevation characteristics, and areas influenced by industrial activity and built-up development. Anthropogenic gradient-based approaches are widely used in modern biomonitoring studies to evaluate variations in the biological responses of indicator organisms to environmental stress and atmospheric pollutant deposition (Taurozzi et al. 2024). Furthermore, spatial gradient approaches are known to be effective in explaining changes in community structure and contaminant accumulation patterns in urban and peri-urban environments (Niepsch et al. 2024). Sampling was conducted in April–May 2025 at several research locations representing the anthropogenic gradient categories. The research locations were grouped into three categories: areas dominated by traffic activity, areas with relatively higher elevations, and areas influenced by industrial activity. This grouping was carried out to obtain a representation of different environmental conditions, thus allowing a comparative evaluation of the lichen community structure and Pb bioaccumulation patterns in each landscape category (Çobanoğlu & Kaan 2024).

The research was conducted in three main stages: lichen sampling in the field, lichen species identification, and Pb content analysis in the lichen thallus. Species identification was conducted from June to August 2025 at the Plant Systematics Laboratory, Faculty of Biology, Gadjah Mada University, while Pb content analysis was conducted at the PT Saraswanti Indo Genetech (GIS) Laboratory. The separation of the research stages was carried out to ensure the traceability of the procedure and increase the repeatability of the method, which is an important aspect in biomonitoring and environmental assessment studies (Niepsch et al. 2024). The research location and distribution of sampling points are presented in Fig. 1.

Determination of sampling points and lichen sampling

Lichen sampling was conducted on three host tree species, namely *Mangifera indica* L., *Roystonea regia* (Kunth) OFCook, and *Polyalthia longifolia* (Sonn.) Thwaites. The selection of these three tree species was based on differences in stem architecture and substrate surface properties that could potentially influence lichen colonization, distribution, and community structure. Host tree characteristics, such as bark texture, water retention capacity, surface pH, and microhabitat stability, are known to contribute to variations in epiphytic lichen community composition and atmospheric pollutant accumulation patterns (Ellis 2024; Harikrishna & Mukherjee 2024).

A total of 90 sampling points were used in this study, with 30 observations per host tree species. Sampling points were determined using a stratified random sampling approach based on predetermined anthropogenic gradient categories. This approach was used to increase the spatial representation of the study site and reduce potential bias due to environmental heterogeneity. In atmospheric biomonitoring studies, the use of spatial replication and stratified design is known to improve the ability to detect biological variation and contaminant distribution patterns at the landscape scale (Niepsch et al. 2024).

Lichen community observations were conducted using 10 × 10 cm quadrat plots placed on the surface of host tree trunks at a position of 100–150 cm above the ground. The use of standardized plot sizes is necessary to achieve uniformity of observation units and facilitate comparison of community structure between locations. The quadrat approach to lichen biomonitoring has been widely applied in studies of epiphyte communities and evaluation of atmospheric pollutant deposition (Asta et al. 2002; Niepsch et al. 2024).

At each observation point, species composition, number of individuals, or percentage of thallus cover, growth type (growth form), and other visual characteristics necessary for identification were recorded. Lichen samples were then collected using a sterile knife, including a small portion of substrate if necessary to maintain thallus integrity. Samples were placed in collection envelopes and coded to include information on location, host tree species, collection date, and sampling point number. Recording detailed metadata is a crucial component of modern biomonitoring because variations in metal content and lichen community structure are influenced by a combination of biological, spatial, and environmental factors (Çobanoğlu & Kaan 2024).

All samples obtained were then taken to the laboratory for taxonomic identification and Pb analysis. Collection procedures were carried out consistently across all locations to minimize variation due to differences in sampling techniques and increase the repeatability of the research method (Niepsch et al. 2024).

Lichen identification

Lichen species identification is performed using a combination of morphological, anatomical, and chemical characters to improve the accuracy of taxonomic determination. This multiparameter approach is commonly used in lichen studies because some species have similar morphological characters, so identification based on a single character is often insufficient to accurately distinguish taxa (Singh et al. 2019; Lücking et al. 2021). The use of morphological and anatomical characters combined with chemical tests is also known to improve the consistency of identification in epiphytic lichen groups that exhibit high phenotypic variation (Brodo et al. 2016).

Morphological observations were conducted on thallus characteristics, including growth form, thallus color, lobe shape, reproductive structures, surface characteristics, and the presence of other diagnostic structures. Lichen growth types were categorized into crustose, foliose, leprose, and other forms found during observations. Morphological characteristics are known to be related to lichen ecological strategies and atmospheric pollutant accumulation capacity, so this information is also used as a basis for biomonitoring interpretation (Yenisoy et al. 2025).

Anatomical observations were made using a stereomicroscope on relevant thallus sections to observe the arrangement of the cortex, medulla, reproductive structures, and other microscopic characters necessary for the identification process. Anatomical analysis was used to verify diagnostic characters of species that could not be observed through macroscopic observation. The use of anatomical characters is known to be important in identifying certain lichen groups that share similar external morphological characters (Lücking et al. 2021).

A chemical approach was carried out using spot tests with reagents K, C, and P. The color reactions that appeared on the surface or medullary part of the thallus were recorded as additional characters to support species identification. Chemical spot tests remain a standard procedure in lichen taxonomy and are widely used in community studies and biomonitoring because they are able to detect secondary metabolites that are diagnostic (Orange et al. 2010; Singh et al. 2019).

Species determination was carried out by comparing observed characters with identification keys and relevant taxonomic literature, namely Brodo et al. (2016), Thomson (2019), Muvida (2020), and Singh et al. (2019). Validation of species nomenclature and classification was adjusted to the Consortium of Lichen Herbaria database to ensure the conformity of scientific names and current taxonomic status. The use of online taxonomic databases is essential in modern community studies to minimize the use of synonyms and nomenclature discrepancies (Lücking et al. 2021).

Pb bioaccumulation analysis

Identified lichen samples were selected for Pb analysis based on the presence of the species at the observation site and the adequacy of the sample biomass. Prior to analysis, samples were manually cleaned to remove visually visible foreign particles, such as coarse dust, bark debris, and other non-thallus material. Samples were then air-dried at room temperature (22°C–27°C) for three to seven days until they reached a constant weight and then homogenized using a sterile mortar to obtain a uniform particle size. This preparation step is commonly used in lichen-based metal biomonitoring studies to minimize variation due to external contamination and increase the consistency of analysis results (Çobanoğlu & Kaan 2024; Niepsch et al. 2024).

Pb content analysis was performed using the Inductively Coupled Plasma–Mass Spectrometry (ICP–MS) method at the PT Saraswanti Indo Genetech (SIG) Laboratory. The ICP–MS technique was chosen because it has high sensitivity for detecting trace metals at low concentrations and has been widely used in atmospheric biomonitoring analysis using lichen and other biological matrices (Correggia et al. 2024). Prior to instrumental analysis, the dried samples were digested using a 50 mL HNO3 acid mixture through a microwave digestion procedure to obtain a dissolved metal fraction ready for analysis. The acid digestion method is known to affect the efficiency of metal extraction and the accuracy of trace element measurements (US EPA 2014).

Pb concentration readings were performed using an Agilent Technologies 7900 Single Quadrupole ICP-MS instrument following established laboratory operating procedures. Measurement results are expressed in mg/kg dry weight. To improve traceability and reproducibility of the analysis, instrument parameters that need to be included in the final manuscript include the Pb isotope used, the calibration curve range, the limit of detection (LOD), the limit of quantification (LOQ), and the calibration linearity coefficient (Correggia et al. 2024).

Quality assurance/quality control (QA/QC) procedures were implemented throughout the analysis to ensure the validity of the metal data. Quality control was performed using reagent blanks, method blanks, multilevel standard solutions, duplicate sample analyses, and certified reference material (CRM) when available. Analytical accuracy was evaluated through percent recovery, while precision was determined based on the relative standard deviation (RSD) value. QA/QC implementation is a crucial component in trace metal studies because data quality is crucial for interpreting bioaccumulation patterns and evaluating environmental pollution (US EPA 2014; Correggia et al. 2024).

To assess the relative level of Pb accumulation in lichen samples, a Contamination Factor (CF) analysis was performed using the equation:

$$CF = \frac{C\ (heavy\,metal)}{C\ (background)}$$

where C (heavy metal) is the Pb concentration in lichen thallus and C (background) is the metal concentration value that is naturally present in the earth's crust, referring to Turekian and Wadepohl (1961).

Data analysis

The research data were grouped into two main categories: lichen community structure data and Pb bioaccumulation data. The analysis was conducted to evaluate the relationship between lichen community structure, environmental conditions, and Pb accumulation levels in landscapes with different anthropogenic characteristics. Integrating biological indicators and environmental contamination indicators is known to improve the interpretation of atmospheric biomonitoring and strengthen the evaluation of environmental conditions at spatial scales (Niepsch et al. 2024).

Lichen community structure was analyzed based on species composition, frequency, density, relative abundance, and importance index. Community diversity was calculated using the Shannon – Wiener index (H '), while species dominance was calculated using the Simpson index (D). Species richness and evenness indices were also calculated to describe the complexity and distribution of the community at each observation location. This combination of community indices is commonly used in lichen ecology studies and atmospheric biomonitoring because it is able to describe the community's response to environmental stress and habitat change (Asta et al. 2002; Ellis 2024).

Differences in community index values between growth forms were analyzed using Analysis of Variance (ANOVA) if the data met the assumptions of normality and homogeneity of variance. Normality was tested using the Shapiro–Wilk test, while homogeneity of variance was tested using Levene's test. If the data did not meet the parametric assumptions, the Kruskal–Wallis test was used, followed by the Dunn post hoc test to identify differences between groups. The use of both parametric and nonparametric approaches is recommended to improve the validity of statistical inferences in community and environmental biomonitoring data (Zuur et al. 2010).

The relationship between lichen community structure and environmental variables was analyzed using Canonical Correspondence Analysis (CCA) or Redundancy Analysis (RDA), depending on the characteristics of the data distribution. Ordination analysis was used to identify the dominant environmental factors influencing lichen community distribution and response patterns to anthropogenic gradients. Integrating community analysis and environmental variables can improve ecological interpretation in atmospheric biomonitoring studies (Borcard et al., 2018).

Pb concentration data are presented as mean ± standard deviation, minimum–maximum values, and number of replications for each species, location category, and host tree. Differences in Pb concentrations between locations, thallus types, and host trees were analyzed using ANOVA or Kruskal–Wallis analysis according to data distribution. The relationship between Pb content and environmental variables, such as $PM_{2.5}$, PM_{10}, temperature, humidity, light intensity, elevation, and distance to the emission source, was analyzed using Pearson or Spearman correlation (Niepsch et al. 2024).

Predictive models are used to identify the relative contribution of environmental factors to variations in Pb content in lichens. This type of statistical modeling approach is increasingly being used in modern biomonitoring studies to link pollutant deposition with environmental factors and habitat characteristics (Bahinskyi et al. 2025).

The level of Pb contamination was analyzed using the Contamination Factor (CF) based on the equation:

$$CF = \frac{C\ (heavy\, metal)}{C\ (background)}$$

where C (heavy metal) is the Pb concentration in the lichen thallus and C (background) is the background concentration value used as a comparison.

All statistical analyses were performed using SPSS Statistics software (IBM Corp., Armonk, NY, USA), PAST version 4.17, and Microsoft Excel. The use of open analysis platforms is recommended in modern biomonitoring studies to improve analytical repeatability and research transparency (Oksanen et al. 2022).

Spatial analysis and integration of biomonitoring frameworks

To evaluate the spatial distribution patterns of lichen community structure and Pb bioaccumulation in various anthropogenic landscape categories, a spatial analysis based on the geographic coordinates of sampling points was conducted. The coordinates of each observation point were recorded using a Global Positioning System (GPS) and used to generate a distribution map of sampling locations. Integration of spatial data in atmospheric biomonitoring is important because pollutant distribution and indicator organism responses generally show heterogeneous variations at the landscape scale, particularly in urban and peri-urban areas with varying anthropogenic pressures (Niepsch et al. 2024).

The spatial distribution of Pb concentrations in lichen thallus was visualized using the Inverse Distance Weighting (IDW) or Kriging spatial interpolation approach in QGIS software version 3.40.2. The interpolation approach was used to describe the spatial variation patterns of Pb content and identify areas with higher potential for metal deposition. Spatial interpolation methods have been widely used in environmental biomonitoring studies because they can assist in interpreting the relationship between pollutant sources, land use characteristics, and the distribution of atmospheric contaminants (Bahinskyi et al. 2025; Niepsch et al. 2024).

As part of an integrated biomonitoring framework, lichen community structure, Pb content, and environmental variables were analyzed simultaneously to evaluate ecological responses to anthropogenic gradients. The integration of biological and chemical indicators was used to produce more comprehensive interpretations compared to using a single approach. This integrative approach is known to increase the sensitivity of biomonitoring in detecting changes in environmental quality and helps identify factors influencing variations in atmospheric deposition (Yenisoy et al. 2025).

All spatial and multivariate analysis results were then integrated into a biomonitoring framework to assess the potential of lichens as biological indicators of atmospheric pollution in anthropogenic landscapes in Sleman Regency. This approach is expected to yield more representative information on the relationship between changes in community structure, Pb deposition, and local environmental characteristics (Niepsch et al. 2024).

Results

3.1 Lichen Community Structure and Composition

The composition *of lichens* in Sleman Regency consists of nine families, eleven genera, and twelve species. A summary of the taxonomy and characteristics of the macrochemical response (*spot test*) is presented in Table 1.

Table 1
Taxonomic distribution, growth forms, and chemical spot test results of lichens in Sleman

No	Family	Species	Talus Type	Spot Test Results	Spot Test Results	Spot Test Results
No	Family	Species	Talus Type	K	C	P
1	Chrysotrichaceae	*Chrysothrix candelaris*	Leprose	-	-	-
2	Arthoniaceae	*Cryptothecia striata*	Crustose	-	+	+
3	Caliciaceae	*Dirinaria applanata*	Foliose	-	-	-
4	Caliciaceae	*Amandinea punctata*	Crustose	-	+	+
5	Physciaceae	*Phaeophyscia orbicularis*	Foliose	-	-	-
6	Parmaliaceae	*Parmotrema perlatum*	Foliose	+	-	-
7	Parmaliaceae	*Flavoparmelia caperata*	Foliose	-	-	-
8	Stereocaulaceae	*Lepraria lobificans*	Leprose	-	-	+
9	Stereocaulaceae	*Lepraria finkii*	Crustose	+	-	+
10	Graphidaceae	*Graphic script*	Crustose	-	+	-
11	Phlyctidaceae	*Phlyctis argena*	Crustose	-	+	+
12	Physciaceae	*Heterodermia casarettiana*	Foliose	-	+	+

Quantitative analysis of ecological parameters (density, frequency, and importance index) at three research stations showed significant spatial adaptation variations (Fig. 2).

At site 1, the community structure was dominated exclusively by the genus *Lepraria*, with the highest densities recorded by *Lepraria finkii* (0.309 ind/m²) and *Lepraria lobificans* (0.240 ind/m²). Sensitive species such as *Phaeophyscia orbicularis* and *Phlyctis argena* were completely absent from this site. In contrast, at locations 2 and 3, there was a shift in absolute dominance to the species *Phaeophyscia orbicularis*, which reached a density value exceeding 0.3 ind/m² at location 2. The species *Phlyctis argena* (0.223 ind/m²) was observed only colonizing the area of location 2. Meanwhile, species such as *Cryptothecia striata* and *Parmotrema perlatum* consistently showed the lowest density, frequency, and importance index values at all three study locations (< 0.05 and importance index < 10%), indicating their high sensitivity to landscape anthropogenization.

3.2 Host Tree Specificity and Diversity Indices

Diversity analysis based on host tree species showed the significance of bark microhabitats (Table 2).

Table 2
Lichen diversity indices across different host tree species.

Host Tree Species	Shannon-Wiener (H')	Simpson Dominance (D)	Species Richness (S)	Evenness (E)
Polyalthia longifolia (Glodokan tiang)	2.211	0.128	1.547	0.890
Mangifera indica (Mango)	2.054	0.145	1.339	0.849
Roystonea regia (Palm)	1.848	0.197	1.296	0.803

The Glodokan tiang tree (*P. longifolia*) supported the highest diversity (H' = 2.211) and the highest evenness index (E = 0.890), indicating its bark structure provides optimal microhabitat stability for multispecies colonization. In contrast, the palm tree (*R. regia*) showed the lowest diversity (H' = 1.848), but with the highest Simpson's dominance value (D = 0.197), proving that the palm substrate surface tends to be selected and can only be dominated by a handful of tolerant species.

3.3. Multivariate Ordination (CCA)

The results of the CCA ordination analysis (Fig. 3) confirmed that the spatial distribution and structure of the lichen community were significantly controlled by opposing environmental gradients ($p < 0.01$).

The ordination map separates communities into three main ecological groups:

1. **High Physico-Chemical Parameter Group**: Located in the upper right quadrant, includes *Flavoparmelia caperata, Graphis scriptaa, Lepraria lobificans, Parmotrema perlatum, Cryptothecia striata*, and *Lepraria finkii*. The distribution of this group is strongly influenced by temperature vectors, light intensity, and atmospheric Pb concentration.
2. **Topographic Generalist Group**: Located at the center of coordinates, represented by *Heterodermia casarettiana* and *Dirinaria applanata*, whose distribution is controlled by the height factor (Elevation).
3. **Urban Micro-Climate Group**: Located in the lower left quadrant, where *Phaeophyscia orbicularis* and *Chrysothrix candelaris* are strongly correlated with humidity levels, while *Phlyctis argena* and *Amandinea punctata* cluster at the extremes in the direction of the PM10 and PM2.5 particulate air pollutant vectors.

3.4. Lead (Pb) Bioaccumulation and Contamination Factor (CF)

Measurement of Pb accumulation in dry thallus detected a very strong positive linear correlation (r = 0.89; $p < 0.001$) between the internal Pb concentration of the tissue and its relative Contamination Factor (CF) value (Table 3).

Table 3
Lead (Pb) concentration and Contamination Factor (CF) among lichen species.

Species	Pb Concentration (mg/kg)	Contaminant Factor (CF)
Flavoparmelia caperata	7.27	0.3635
Graphis scriptaa	6.96	0.348
Lepraria lobificans	6.5	0.325
Phaeophyscia orbicularis	4.99	0.2495
Phlyctis argena	3.21	0.1605
Parmotrema perlatum	3.08	0.154
Chrysothrix candelaris	3.06	0.153
Cryptothecia striata	2.99	0.1495
Lepraria finkii	2.52	0.126
Dirinaria applanata	2.22	0.111
Heterodermia casarettiana	2.14	0.107
Amandinea punctata	1.73	0.0865
	Average	**0.19446**

Flavoparmelia caperata (7.27 mg/kg), *Graphis scriptaa* (6.96 mg/kg), and *Lepraria lobificans* (6.50 mg/kg) functioned as the strongest Pb accumulators with CF values that soared far above the population average (Mean CF = 0.194). In contrast, the lowest accumulation was found in *Amandinea punctata* (1.73 mg/kg) with a minimum CF value of 0.0865, indicating a low metal retention capacity or a minimum level of local exposure in its microecological niche.

3.5 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was conducted to determine the relationship between environmental variables and pollution at the research site. The variables analyzed included Pb concentration, temperature, humidity, light intensity, PM 10, PM 2.5, and altitude.

The PCA results show that there are three principal components with eigenvalues > 1, which collectively explain 78.22% of the total variation in the data (Table 4). The first principal component (PC1) explains 44.78% of the variation in the data, while PC2 and PC3 explain 17.79% and 15.66%, respectively.

Table 4
Total variance explained by PCA results

Principal Component	Eigenvalue	Variance Explained (%)	Cumulative Variance (%)
PC1	3.134	44,778	44,778
PC2	1,245	17,785	62,563
PC3	1,096	15,660	78,224

Based on the rotated component matrix, PC1 shows high loadings on the variables PM 10, PM 2.5, and Pb. These three components indicate a strong influence of environmental pollution factors on variations in research data (lichen species). PC2 correlates with temperature and humidity, while PC3 is related to light intensity and location altitude. These results indicate that variations in environmental parameters at the research location are influenced by a combination of anthropogenic and microclimatic factors.

Discussion

4.1. Anthropogenic Gradients as Controllers of Lichen Community Damage

Variations in lichen community structure at three research sites in Sleman Regency indicate that anthropogenic pressures can affect lichen ecological conditions. The dominance of the genus *Lepraria* at site 1 (urban traffic), accompanied by the absence of sensitive species such as *Phaeophyscia orbicularis* and *Phlyctis argena*, confirms that motor vehicle emissions significantly impact epiphytic lichen communities. Lichens with a leprose growth form characterized by a disorganized, powdery thallus surface without a clear cortical structure give the genus *Lepraria* a very high surface area to volume ratio. These morphological characteristics enable it to efficiently trap particulate matter such as PM10 and PM2.5 and tolerate extreme drought stress and high temperature fluctuations typically found along major highway corridors such as the North Ring Road of Yogyakarta.

In contrast, the restricted distribution *of Phlyctis argena* to Station 2 (High Elevation) suggests that this species may serve as a reliable indicator for areas with low anthropogenic disturbance. Habitats at higher elevations generally maintain lower ambient temperatures, higher relative humidity, and reduced exposure to pollutants from vehicle emissions. These microclimatic conditions meet the physiological requirements of sensitive *crustose lichens*, which lack the adaptive mechanisms to survive under intense chemical stress and the urban *heat island effect* found in high-traffic zones. The sharp decline in species richness from the rural to urban gradient observed in this study is in line with the global trend documented by Taurozzi et al. (2024), where increasing urbanization systematically eliminates sensitive epiphytic taxa, leaving behind very simple communities dominated by pollution-tolerant generalists.

4.2. Host Tree Architecture as a Microhabitat Modulator

Variations in lichen species diversity among three host trees provide evidence that substrate characteristics play a significant role in modulating lichen colonization. The Glodokan Tiang tree

(*Polyalthia longifolia*) supported the highest Shannon-Wiener diversity (H' = 2.211) and evenness (E = 0.890). The raux architecture type of *P. longifolia* allows for sunlight filtering, so that the light intensity reaching the trunk is not too high. The Glodokan Tiang bark has a rough and hard structure, making it easier for lichen to attach. These substrate conditions can optimize microhabitat conditions by increasing moisture retention and providing physical attachment points for lichen ascospores.

In contrast, the King Palm (*Roystonea regia*) showed the lowest diversity (H' = 1.848) but with the highest Simpson's dominance (D = 0.197). The King Palm has a corner architecture type, namely a monopodium stem with lateral inflorescences and no branches. This results in a fairly high light intensity reaching the stem. The King Palm's bark tends to be dense, smooth, and perpendicular, making lichen attachment less strong. Furthermore, the periodic micro-peeling process causes severe physical disturbances for slow-growing lichens (Nurchayati & Ardiyansyah, 2021; Rahmawati et al., 2022). This evolutionary pressure acts as a secondary substrate filter, selecting only *foliose forms* with aggressive adhesive properties such as *Dirinaria applanata* and *Phaeophyscia orbicularis*. These dominant species have rhizines *or* tightly attached thalli that are able to maintain attachment despite unstable substrate conditions. This substrate specificity emphasizes that biomonitoring frameworks must carefully control host tree species, as the physical properties of the bark can exacerbate or mitigate physiological stresses caused by regional air pollution.

4.3. Lead (Pb) Bioaccumulation Mechanism and Contamination Levels

A strong linear correlation ($r = 0.89$; $p < 0.001$) between the internal Pb concentration of thallus tissue and the calculated *Contamination Factor (CF) highlights the efficiency of lichens as passive biomonitors. Flavoparmelia caperata* (7.27 ± 0.45 mg/kg) and *Graphis scriptaa* (6.96 ± 0.38 mg/kg) emerged as the most potent bioaccumulators, exhibiting a “High Contamination” status (CF > 0.3). Pb accumulation in lichen involves a two-stage process:

1. **Extracellular Cation Exchange**: Negatively charged functional groups (such as carboxyl, hydroxyl, and phosphoryl groups) present in the fungal cell walls of lichen symbionts bind free divalent lead cations (Pb^{2+}) deposited from the atmosphere electrostatically.
2. **Intracellular Particulate Entrapment**: Microparticles from vehicle exhaust gases and industrial dust are physically trapped within the intercellular spaces of the medulla, where they are slowly dissolved by lichen acids, mobilizing metal ions into the thallus matrix.

The very low accumulation observed in *Amandinea punctata* (CF = 0.09, indicating very low Pb accumulation) suggests a morphological exclusion mechanism *or* spatial avoidance strategy, where the species strictly occupies protected microcracks in the bark that are physically safe from wind-borne pollutant deposition. Compared with international benchmarks, the maximum Pb level in Sleman (7.27 mg/kg) is lower than those reported in highly industrialized European megacities such as Manchester (> 20 mg/kg as reported by Niepsch et al., 2024). Nevertheless, the elevated baseline value relative to the control site signals a significant and persistent anthropogenic Pb flux into Sleman's tropical atmosphere, driven primarily by fossil fuel combustion and urban vehicle traffic.

4.4. Principal Component Analysis (PCA)

Based on Principal Component Analysis (PCA), the results of the reduction of seven environmental variables into three main components were obtained, which could explain 78.22% of the total variation in the data. This total percentage indicates that the PCA model has good capabilities in presenting the relationship patterns between environmental variables at the research location.

The first principal component (PC1) is dominated by PM10, PM2.5, and Pb variables, indicating that air pollution is the main factor influencing environmental conditions. High concentrations of airborne particulates and Pb in the air indicate anthropogenic pressures stemming from motor vehicle activity, industrial emissions, and other urban activities. Areas with high levels of anthropogenic activity create interactions between airborne particulates and heavy metals, as atmospheric particulates can act as a transport medium for heavy metals in the environment.

The second component (PC2) relates to temperature and humidity, which describes microclimate factors affecting habitat conditions. Variations in temperature and humidity play a crucial role in determining the stability and influence of lichen communities. Stable microclimate conditions generally support lichen growth, while changes in temperature and humidity can cause physiological stress on the lichen thallus.

The third component (PC3) relates to light intensity and altitude, reflecting the influence of topography and habitat openness. Light intensity can influence photosynthesis in lichens, while altitude is associated with changes in temperature and humidity. Variations in these two factors will lead to differences in the distribution and structure of lichen communities between research locations.

Overall PCA results indicate that environmental conditions at the study site were influenced by a combination of air pollution, microclimate, and habitat characteristics. Air pollution was the dominant factor potentially influencing lichen community structure and the organisms' ability to bioaccumulate heavy metals.

4.5. Environmental Implications and Applications of Biomonitoring

The integration of ecological community metrics and chemical bioaccumulation data offers a comprehensive diagnostic tool for urban environmental management. Traditional instrumental air quality monitoring networks provide real-time data with high precision, but are economically limited and have low spatial density. The local mapping of lichen community degradation and the CF index developed in this study provides a low-cost alternative with high spatial resolution.

The high pollution zone identified at Station 1 can be utilized by the Sleman Regency Environmental Agency (DLH) as an "early warning system" *to* optimize urban spatial planning. For example, the implementation of green infrastructure, specifically the planting of host trees such as *Polyalthia longifolia* along high-traffic corridors, can serve a dual purpose: expanding the surface area for biological interception of particulates while providing a living matrix for long-term ecological monitoring. These nature-based *solutions* bridge the gap between basic ecological research and applied urban environmental policy in the tropical regions of developing countries.

Conclusion

This study successfully demonstrated that the epiphytic lichen community structure and Pb bioaccumulation capacity in Sleman Regency are highly responsive to anthropogenic landscape gradients. Clear striking differences in community structure between highly polluted urban commuter areas, which are strongly dominated by the genus *Lepraria*, and high-altitude areas with more even diversity. *Flavoparmelia caperata* and *Graphis scripta species* were identified as potential bioindicators of Pb heavy metal accumulation. This spatial-multivariate integrative approach is recommended for use as a complementary system for long-term ambient air quality monitoring in tropical regions.

Declarations

Funding

Not applicable

Author Contribution

Wahida Aulia Rahma wrote the result and discussion, Andhika Puspito Nugroho wrote the introduction and material methods

References

1. Asta J, Erhardt W, Ferretti M, Fornasier F, Kirschbaum U, Nimis PL, Purvis OW, Pirintsos S, Scheidegger C, van Haluwyn C, Wirth V (2002) Mapping lichen diversity as an indicator of environmental quality. In: Nimis PL, Scheidegger C, Wolseley P (eds) Monitoring with lichens—monitoring lichens. Springer, Dordrecht, pp 273–279. https://doi.org/10.1007/978-94-010-0423-7_20
2. Borcard D, Gillet F, Legendre P (2018) Numerical ecology with R. 2nd edn. Springer, New York. https://doi.org/10.1007/978-3-319-71404-2
3. Brodo IM, Sharnoff SD, Sharnoff S (2016) Lichens of North America. Yale University Press, New Haven
4. Çobanoğlu G, Kaan T (2024) Biomonitoring of atmospheric heavy metals in native lichen Xanthoria parietina around Salda Lake (Burdur–Turkey), a special environmental protection area. Air Qual Atmos Health 17:2789–2800. https://doi.org/10.1007/s11869-024-01602-6
5. Correggia M, Moretti S, De Ceglie C, Zambon S (2024) Standard operating procedure for the determination of trace elements by inductively coupled plasma mass spectrometry. Open Res Eur

3:90. https://doi.org/10.12688/openreseurope.16049.2

6. Ellis CJ (2024) Host tree characteristics and lichen community assembly across environmental gradients: recent advances and future directions. Lichenologist 56:1–14
7. Harikrishna S, Mukherjee S (2024) Biomonitoring with lichens: evaluating heavy metal accumulation and environmental quality assessment. Environ Ecol 42(1):1–15
8. Işik V, Yildiz A (2022) The heavy metal biomonitoring study using lichen Xanthoria parietina in Ankara Province (Turkey). EQA—International Journal of Environmental Quality 52:1–14. https://doi.org/10.6092/issn.2281-4485/15828
9. Legendre P, Legendre L (2012). Numerical ecology. 3rd edn. Elsevier, Amsterdam
10. Lücking R, Hodkinson BP, Leavitt SD (2021) The 2020 classification of lichenized fungi in the Ascomycota and Basidiomycota: approaches, progress, and challenges. Bryologist 124(3):307–339. https://doi.org/10.1639/0007-2745-124.3.307
11. Mitra S., Arka Jyoti Chakraborty, Abu Montakim Tareq, Talha Bin Emran, Firzan Nainu, Ameer Khusro, Abubakr M. Idris, Mayeen Uddin Khandaker, Hamid Osman, Fahad A. Alhumaydhi, Jesus Simal-Gandara. 2022. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity, Journal of King Saud University - Science, 34(3): 1–21.
12. Muvida AH (2020) Diversity and spatial distribution of epiphytic lichens as bioindicators of air quality in urban and suburban areas. Master's Thesis, Universitas Gadjah Mada, Yogyakarta.Niepsch D, Clarke LJ, Jones RG, Tzoulas K, Cavan G (2024) Lichen biomonitoring to assess spatial variability, potential sources and human health risks of polycyclic aromatic hydrocarbons and airborne metal concentrations in Manchester, UK. Environ Monit Assess 196(11):727. https://doi.org/10.1007/s10661-024-12522-4
13. Oksanen J, et al. (2022) vegan: Community Ecology Package. R package version 2.6. https://CRAN.R-project.org/package=vegan
14. Orange A, James PW, White FJ (2010) Microchemical methods for the identification of lichens. British Lichen Society, London
15. Parzych, A., Zdunczyk, A., & Astel, A. (2016). Epiphytic lichens as bioindicators of air pollution by heavy metals in an urban area (northern Poland). Journal of Elementology, 21(3).
16. Singh KP, Gupta RK, Singh P (2019) Lichen taxonomy and identification methods: a practical approach. Springer, Singapore
17. Taurozzi D, et al. (2024) Passive biomonitoring of airborne microplastics using lichens along an anthropogenic pollution gradient. Environ Int 187:108677. https://doi.org/10.1016/j.envint.2024.108677
18. Thomas JV Editor(s): Simon Asher Levin. 2001. Encyclopedia of Biodiversity: Fungi. Elsevier, pages 141–163.
19. Thomson JW (2019) American Arctic lichens: keys and notes for the lichen flora of the Arctic tundra. University of Wisconsin Press, Madison

20. Turekian, KK, KH Wedepohl. 1961. Distribution of elements in some major units of the Earth's crust. Geological Society of America, Bulletin 72, 175–192.
21. US EPA (2014) Method 6020B: Inductively coupled plasma–mass spectrometry. United States Environmental Protection Agency, Washington DC
22. Yang, J., Oh, S. O., & Hur, J. S. (2023). Lichens as bioindicators: assessing their response to heavy metal pollution in their native ecosystem. Mycobiology, 51(5), 343–353.
23. Yenisoy S, Dörter M (2025) Review of the role of elemental and isotopic composition of lichens in air quality assessment. Atmos Pollut Res 18:102830.
24. Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1(1):3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.

Figures

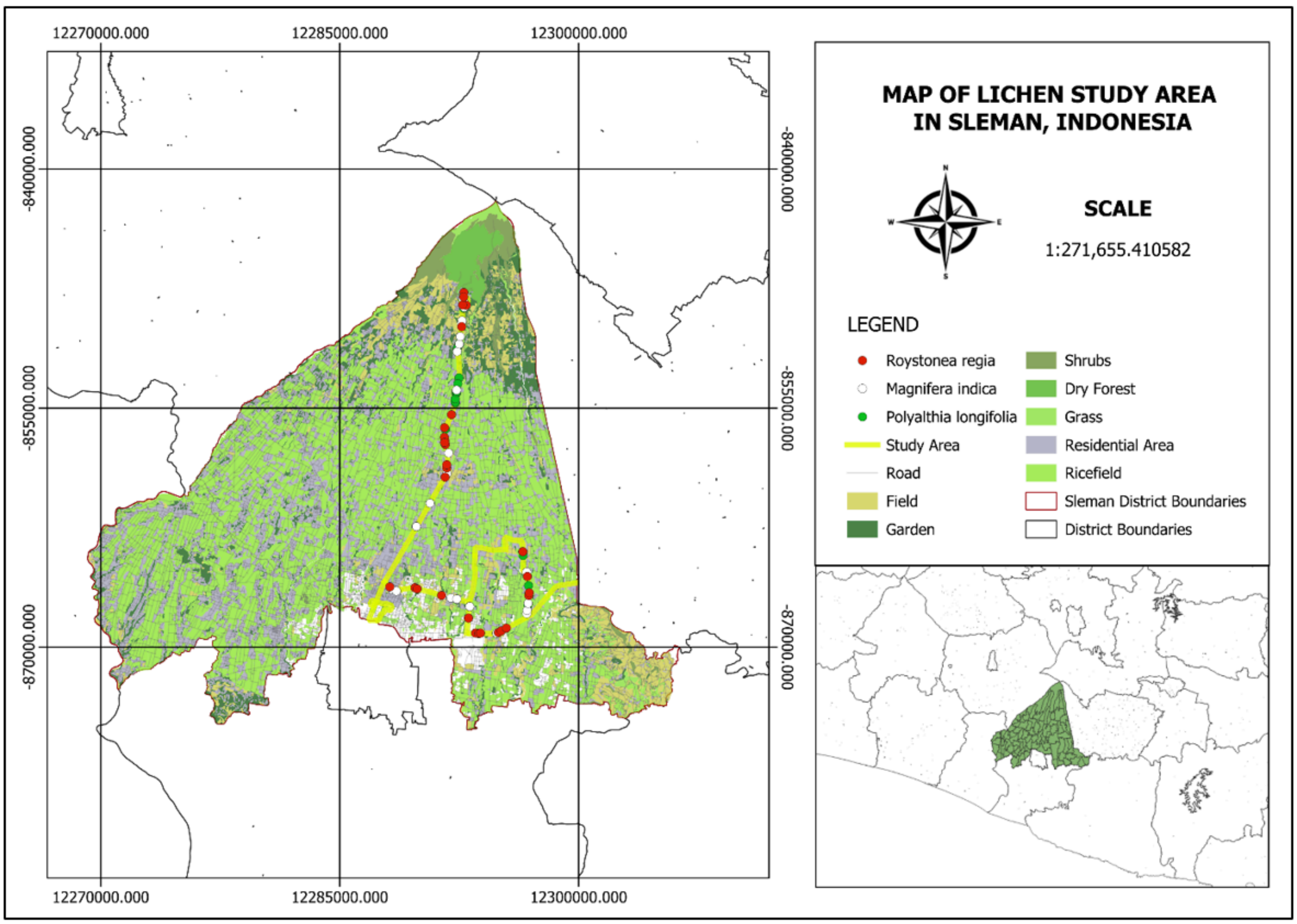

Figure 1

Map of research locations and distribution of sampling points

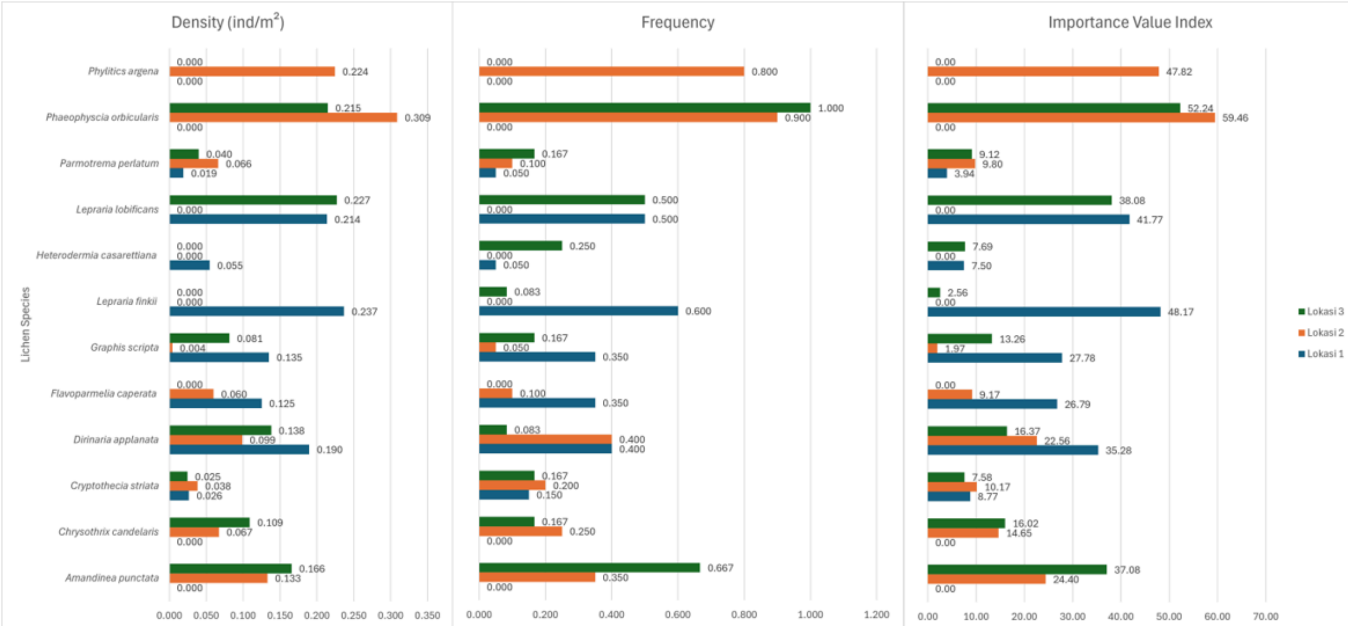

Figure 2

Density, frequency, and importance value index chart

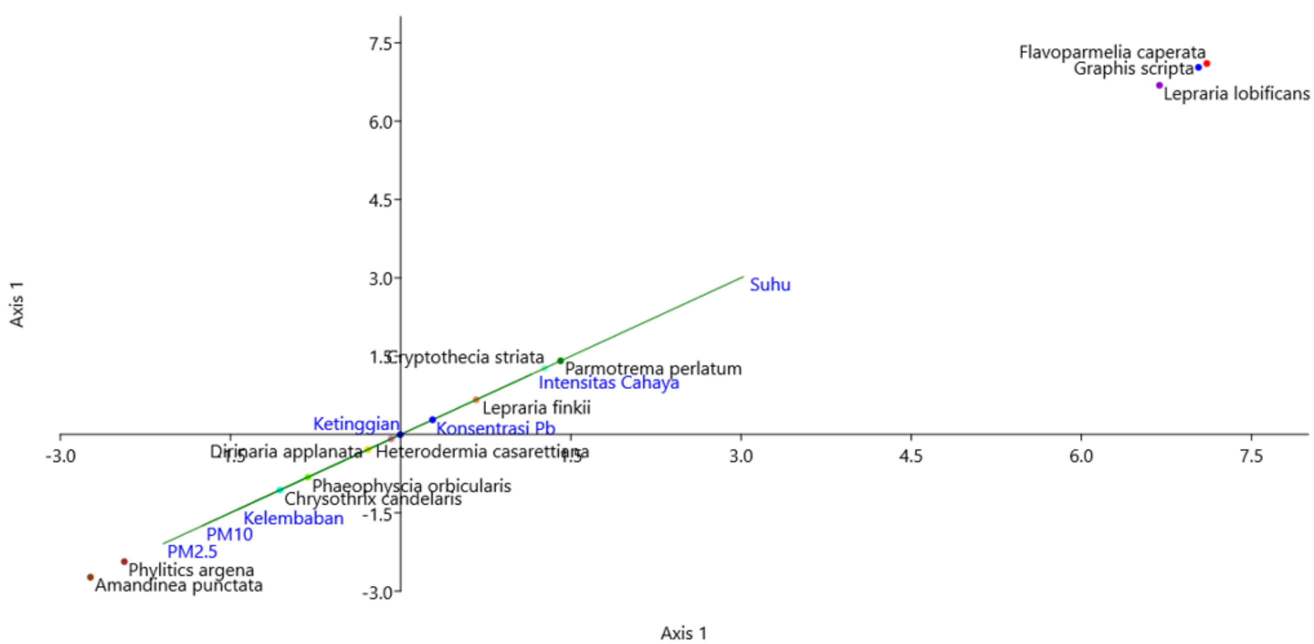

Figure 3

Canonical Correspondence Analysis Ordination Diagram