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Abiotic factors and distribution patterns of epiphytic lichens inhabiting different altitudes

ASMIDA ISMAIL[1,2,♥], NUR SYAFIQAH ABD HAKIM[2], KHAIRUL ADZFA RADZUN[2], FAEZAH PARDI[1,2], MOHD TALIB LATIF[3], NURUL AIDA KAMAL IKHSAN[4], DEWI HIDAYATI[5], FAEIZA BUYONG[6]

[1]School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA. 40450 Shah Alam, Selangor, Malaysia.
Tel: +6019-307 3168, ♥email: asmida@uitm.edu.my
[2]Institute for Biodiversity and Sustainable Development, Universiti Teknologi MARA. 40450 Shah Alam, Selangor, Malaysia
[3]Department of Earth Sciences and Environment, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. 43600 Bangi, Selangor, Malaysia
[4]Centre of Foundation Studies, Universiti Teknologi MARA. Cawangan Selangor, Kampus Dengkil 43800 Dengkil, Selangor, Malaysia
[5]Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Teknologi Sepuluh Nopember. Jl. Teknik Mesin No.173, Keputih, Sukolilo, Surabaya 60111, East Java, Indonesia
[6]School of Chemistry and Environment, Faculty of Applied Sciences, Universiti Teknologi MARA. 40450 Shah Alam, Selangor, Malaysia

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Abstract. *Ismail A, Hakim NSA, Radzun KA, Pardi F, Latif MT, Ikhsan NAK, Hidayati D, Buyong F. 2024. Abiotic factors and distribution patterns of epiphytic lichens inhabiting different altitudes. Biodiversitas 25: 3084-3094.* Lichens are a symbiotic partnership of two separate organisms, a fungus, and an alga. Although many species of lichens exist in Malaysia, comprehensive data on lichens are still being determined. Thus, this study provides significant knowledge for the researcher to gain more understanding and information on the lichen species in Malaysia and how the abiotic factors affect the community. This study aimed to identify lichen species taxonomically, assess species composition, and compare the lichen species coverage in two sampling stations with contrasting altitudes: Brinchang in Cameron Highland (high altitude, 1,428 m asl.) and Templer Park in Rawang (low altitude, 131 m asl.). This study presented sound evidence on the relationship between lichen species coverage and abiotic factors such as bark pH, relative humidity, temperature, and atmospheric gas (ammonia), which provide knowledge on lichen diversity. Lichen sampling used a 30×50 cm quadrat on the tree trunk, 1 m above the ground. As a result, a species description table was constructed for 18 lichen species from 9 families and 15 genera. Out of these genera, the crustose group dominates at 72%, the foliose group at 22%, and the fruticose group at 6%. The low altitude recorded significantly higher species coverage, 63.47±5.59%, while the high altitude showed lower species coverage, 46.56±3.08%. The mean temperature of low altitudes is significantly higher at 32.01°C compared to the high altitudes (23.87°C). However, the relative humidity and bark pH within these altitudes were not significantly different. The mean concentration of NH_3 at low altitudes was considerably higher at 0.40±0.07 ppm than at high altitudes (0.11±0.04 ppm). It was shown that the fruticose group increased as the altitudes increased. In contrast, crustose lichen is reduced in coverage and number of species compared to high altitudes. This study concluded that inter-related abiotic factors related to the altitudes facilitated the survival of the lichen community in the sampling areas.

Keywords: Abiotic factor, altitude, lichen, species composition, species coverage

INTRODUCTION

Air pollution is one of the world's most significant environmental health threats. According to the Centre for Research on Energy and Clean Air (CREA) and Greenpeace Malaysia (2022), an estimated 32,000 avoidable deaths occur in Malaysia yearly due to air pollution. Biological testing using susceptible species such as lichens can provide an early warning of contaminating effects on biological elements (Tarasova et al. 2017). Lichens are vulnerable to changes in atmospheric chemistry since they directly obtain almost all of their nutrients from the atmosphere by uptake through the thallus surface (Aragón et al. 2019). Hence, lichens are one of the bioindicators for air pollution to determine air quality as they are sensitive to several air pollutants, including sulfur dioxide, fluoride, and especially nitrogen (Varela et al. 2018; Niepsch et al. 2023). Both species composition and community structure modifications have been found in N_2 deposition (Stevens et al. 2012).

Air and humidity are also crucial for developing lichens (Sales et al. 2016; Abas et al. 2018). Lichens do not have root structures as found in high-level plants. Therefore, lichen will absorb all the elements in the air through the cortex (Yildiz et al. 2023). Some lichen species tolerate contaminants like acid rain and heavy metals (Paoli et al. 2014). Moreover, lichen physiology is related to the micro and macroclimate factors as relative air humidity and rainfall are the primary sources of water supply to the lichens (Gauslaa 2014; Di Nuzzo et al. 2022).

According to a study by Asplund and Wardle (2016), lichen requires sufficient nutrients to survive. Nitrogen, carbon, and oxygen are vital nutrients for lichens' development. Nitrogen is essential in the production of protein and organic acids. As with other plants, lichens do not have a specific structure for modifying nitrogen for its use. Therefore, lichen uses cyanobacteria to modify the nitrogen from the air. Moreover, the temperature is vital in determining the distribution of lichen species in an area (Hurtado et al. 2020). According to Abas et al. (2018), lichen was discovered to survive in an area with temperatures as low as -190 and -78°C for several days. Apart from living in low temperatures, some lichens can live in areas of high temperature, such as in deserts where the temperatures can reach up to 60°C and above.

Many lichens often found on the bark trees are occasionally observed growing on rock or dirt because each type of lichen requires a distinct surface to establish itself (Gaya et al. 2015; Paukov et al. 2022). Aptroot (2012) stated that the classic succession of crustose followed by foliose and then fruticose, which is apparent in rock colonization, was not followed by lichen species on the bark. The first to appear on tree bark is the relatively rapidly growing foliose and fruticose lichens, followed later by crustose lichen. Compared to other types of trees, the physical surfaces of the bark have a more significant impact on lichen growth (Mežaka et al. 2012; Lamit et al. 2015; Kubiak et al. 2016). As trees age, the bark typically produces uneven fractured surfaces, allowing foliose and fruticose lichens to attach to the tree (Stephenson and Steven 2010).

Besides that, the pH of the bark surfaces is one of the significant elements affecting the network shape of corticolous organisms (McDonald et al. 2017; Kovarova et al. 2021). The association between pH and community shape is well known for epiphytic lichens. Epiphytic lichens are the lichen that grows on the branches and trunks of trees. Tree bark is usually recommended as a sensitive and straightforward indicator of air pollution (Alwi et al. 2015). According to Alwi et al. (2015), pollutant concentrations, particularly NH3, are believed to be essential in determining bark pH. Air pollution inside the environment alters the bark pH and offers a higher medium for the algal boom. The high absorption ability of the microalgae makes it easy to build up atmospheric pollution in their cells immediately (Alwi et al. 2015)

MATERIALS AND METHODS

Study area

The study sites are Brinchang, Cameron Highland, and Templer Park, Rawang, Malaysia. Figure 1 shows a map of the area studied.

Brinchang, Cameron Highland, Pahang located at coordinates 4.4910° N, 101.3885° E, lies between 1,070 and 1,830 meters above sea level (m asl.) and experiences mild temperatures, with an average daytime reading of 24°C and an average nighttime reading of 14°C. The average annual rainfall is 2,660 mm, with peaks in May and October. The climate thus was very conducive to a wide range of lichen. About 86% (60,000 hectares) of the area was forested, 8% (5,500 hectares) was under agriculture, settlements occupied 4% (2,750 hectares), and the remainder was used for recreation and other activities (Aminuddin et al. 2005). The climate was favorable for cultivating tea, sub-tropical vegetables, and flowers (under a rain shelter), which was sustained by high fertilizer and manure applications. Consequently, agriculture in this environment was characterized by environmental pollution when the ammonia was dispersed into the air excessively.

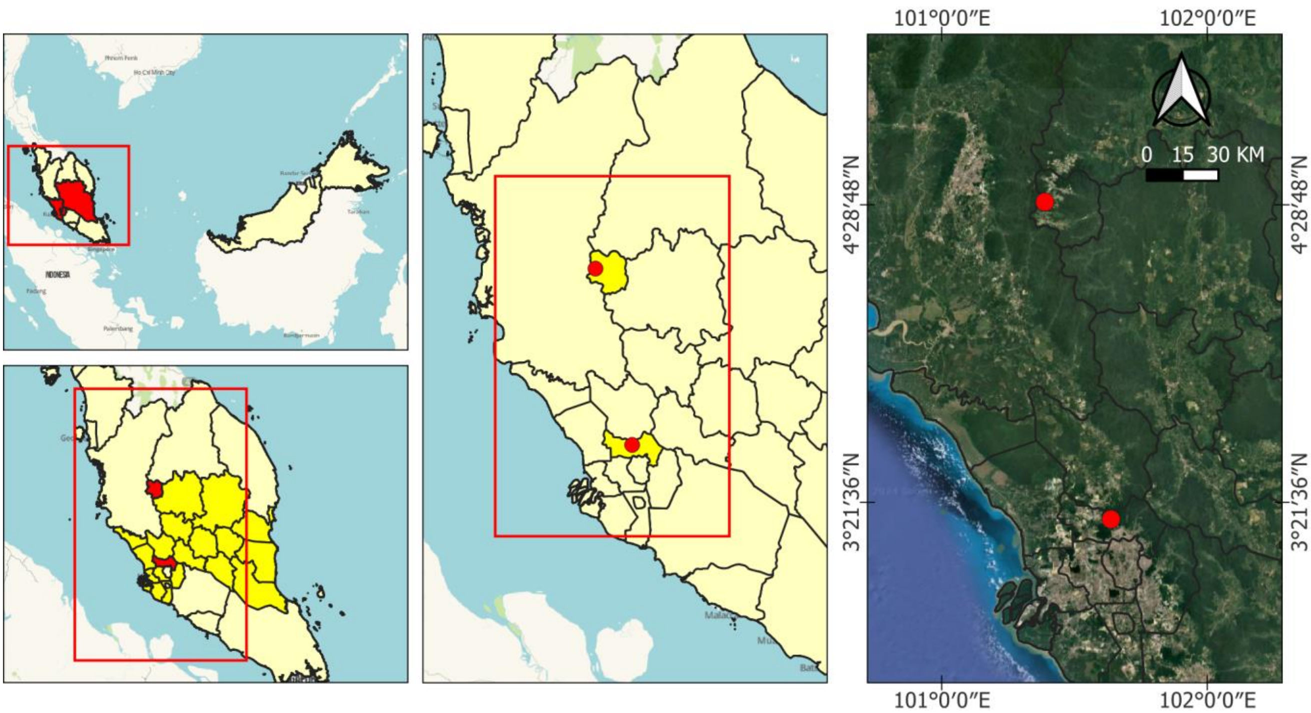


Figure 1. Study sites at Brinchang, Cameron Highlands, Pahang and Templer Park, Gombak, Selangor, Malaysia

Templer Park, Rawang located at coordinates 3.2963° N, 101.6371° E, is a 1,214-hectare forest reserve in Selangor, situated about 100 meters above sea level (m asl.) and 28.9 km from Kuala Lumpur. Throughout the year, daytime temperatures generally reach highs of around 32.4°C, while at night, the average minimum temperature drops to around 23.5°C. The average annual relative humidity was around 78.2%. In recent years, the maximum sustained wind speed has reached 28 km/h (Templer Park Country Club Climate History 2018). Templer Park mainly comprises forest, which constitutes 69.02%, followed by residential at 13.20%, open area at 5.33% and agriculture at 4.51%. The gaseous pollutant levels monitored were low and well within the stipulated limits under the Recommended Malaysian Air Quality Guidelines.

Procedures

Quadrat sampling method

Lichen species coverage was calculated using a 30x50 cm quadrat made using clear hard plastic with a 10 cm grid. The quadrat was tied on tree bark 1 meter above the ground, and the lichen species coverage was observed and calculated. For each of the two sites, 15 trees were randomly selected, resulting in a total of 30 trees from which lichen was collected from the bark. Figure 2 shows the quadrat sampling method.

Sample collection

Epiphytic lichens on woody substrates 1 m above the ground were collected using a chisel and hammer from each quadrat and kept in a paper bag. The label of the paper bag contained information including the name and family of the lichen, detail locality, latitude, longitude from where it was collected, date of collection, reference number, and other interesting observations. The relative humidity of the study area was observed during sample collection. Reading for relative humidity was taken using a humidity meter, while the study area's temperature was measured by using an environmental thermometer. After collection, samples were taken to the laboratory and air-dried to prevent humidity-related fungi growth. The lichen samples were preserved in lichen herbarium sheets after the species were identified.

Species identification

The lichen species were identified through a combination of morphological observations and chemical spot tests. Key morphological features such as the structure of the thallus, apothecium, lobes, soredia, medulla, and hymenium, along with the characteristics of the upper and lower surfaces of the lichen, were carefully examined. When species identification was not possible in the field, specimens were brought to the laboratory for further analysis using a dissecting microscope.

Species identification was further assisted by the work of Abas et al. (2018) work. The lichen substances were identified by performing a color spot test. A color spot test is a chemical test used to identify or differentiate lichen species by applying specific reagents to the lichen and observing color changes. The chemical composition of the lichens was determined using color spot tests, a method that distinguishes lichen species by applying specific reagents to the specimen and observing any resulting color changes. Household chemicals were utilized to test the reaction of the lichen's unique compounds. Commonly used reagents for these tests include a 10% solution of potassium hydroxide (K), a 5.25% solution of sodium hypochlorite (C), and Steiner's stable Pd solution of paraphenylenediamine (Pd) (Mulligan 2009). A KC test was also conducted by applying K followed immediately by C to the thallus. All solutions were stored in small dropper bottles. The 10% potassium hydroxide solution was prepared by dissolving 10g of potassium hydroxide (KOH) in 90 mL of distilled water, yielding 100 mL of solution. Spot tests involved applying a few drops of KOH to the upper thallus surface (cortex) or medulla, which had been exposed with a blade, or both. The color change was then observed: if the color turned yellow and then red, it was recorded as K+ yellow to red; if no change occurred, it was recorded as K-.A 5.25% sodium hypochlorite (C) solution was used directly without dilution. A few drops were placed on the lichen's surface, and color changes were observed: if the color turned red or green, it was recorded as C+; no change was recorded as C-. Additionally, a solution of paraphenylenediamine (Pd) was prepared by adding a drop of ethanol (70-95%) to a few crystals of the chemical. When this solution was applied to the lichen sample, a yellow to red color indicated a P+ result.

Positive and negative results were obtained depending on the reactions of the chemical reagents with the lichen's medulla and cortex. The color changes indicated the presence of specific chemical compounds in the lichens. For example, a KC+ red reaction in *Parmotrema tinctorum* was due to the presence of lecanoric acid, while *Dirinaria aegialita* produced a K+ yellow result, indicating the presence of atranorin and chloroatranorin. Similarly, lichens from the genus *Librata* exhibited a Pd+ reaction due to norstictic acid. These secondary metabolites can be extracted from both fresh lichens and those preserved over time (Elix and Worgotter 2008).

Figure 2. Quadrat sampling method

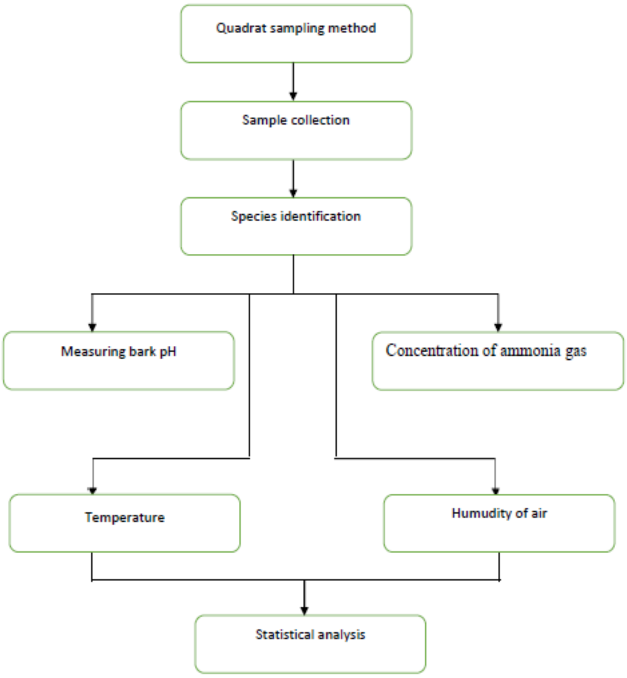


Figure 3. Flow chart of study

Abiotic parameter measurement

Bark pH was measured by removing thin slices from the tree rind surface using a blade. A sample of about 0.5 g was collected in stable dry weather, stored in a plastic bottle, and taken to the laboratory. The thickness of the tested bark pieces is imperative for all estimations. The samples should be as thin as conceivable to gauge the peripheral bark layer with the nearest relationship with the epiphytic vegetation. The bark pieces were grounded with a coffee grinder, and 0.5 g of powder was suspended in 10 mL of deionized water. The blend was inserted into a closed conical flask to prevent the entrance of air CO_2. After 1 hour with incidental shaking, the blend was sifted, and the water's pH was measured with a pH meter. The suspension's pH estimation may likewise be obtained using a unique anode for estimating suspensions recalibrated after each perusing. The convergence of disintegrated protons and, subsequently, the pH esteem relies upon the mass of bark utilized and the volume and nature of dissolvable.

Most ammonia production comes from the agricultural industry, where ammonia is used as a fertilizer. Therefore, the concentration of ammonia gas was observed in this study area. The concentration of NH_3 in the atmosphere was measured using the Aeroqual Ammonia Sensor. Measurements of the concentration of NH_3 in the atmosphere were collected from each tree for both sites near the lichen sample. The mean of data was calculated from the three readings of each tree.

Statistical analysis

ll data collected were analyzed via Statistical Package for Social Sciences (SPSS). The statistical test used for this study was the Independent Samples t-test. This statistical analysis determined the significant difference in abiotic factors between the two study sites. The significant difference in lichen species coverage between the two sites (Brinchang, Cameron Highland, and Templer Park, Rawang) was also conducted using a t-test. Figure 3 illustrates the flowchart of the study.

RESULTS AND DISCUSSION

Taxonomic descriptions of lichens

A total of 18 species of lichens from 9 families and 15 genera were identified. Figure 4 and Table 1 show some of the collected lichen descriptions.

For further species identification process, a chemical spot test was conducted. The results showed positive (changes occurred) or negative (no changes) when the chemical was applied to their medulla or cortex, as shown in Table 2.

Taxonomic composition

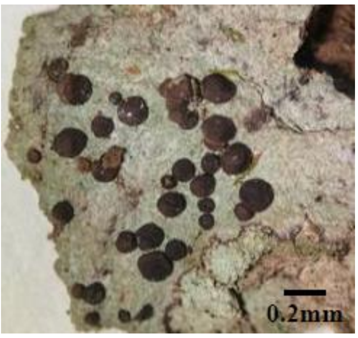
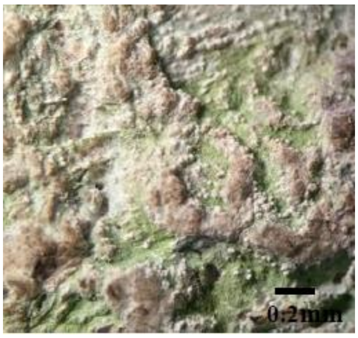
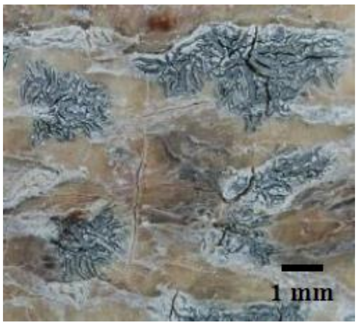
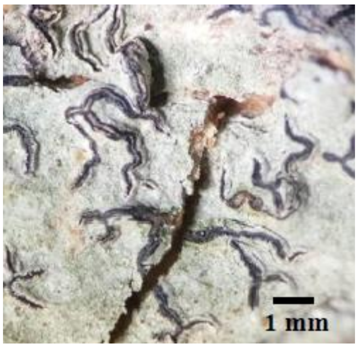
The study identified and recorded 18 species of lichens from 9 families and 15 genera. The family, genus, and species list were listed and appropriately arranged as in Table 3. There are eight families of crustose lichens, three of foliose lichen and one of the fruticose lichens. Based on Table 3, the family Graphidaceae of crustose lichen is dominated by two genera and five species, followed by the family Lecanoraceae, with two genera and two species for crustose lichen. Stereocaulaceae, Caliciaceae, Arthoniaceae, Roccellaceae, Physciaceae, and Stictidaceae each recorded a single genus and species.

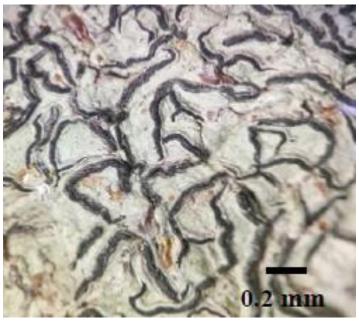
Foliose lichen was dominated by the family Parmeliaceae, which had two genera and two species, while a single genus and species represented the family Physciaceae and Caliceae. On the other hand, fruticose lichens are represented by only one family, genus, and species. The presence of fruticose may be low due to the limited area of sample collection, which focused on tree bark only, in contrast with crustose lichen, in which their habitat was abundant, especially on tree bark. The distribution of crustose and foliose lichen was abundant in Brinchang. The dominant presence of crustose lichen at high altitudes may be due to the condition of sampling sites in an open area that was too humid (Sujetovienė 2017; Mónge-Nájera 2019). In contrast, fruticose lichen was not present at low altitudes, which could be attributed to high temperatures and low relative humidity (Frisch et al. 2015; Sáiz et al. 2020; Belguidoum et al. 2021).

Abiotic parameters and lichen species coverage

Table 4 presents the measurements of abiotic factors (ammonia, bark pH, temperature, and relative humidity) from the sampling sites. Abiotic factors and altitude were identified as key influences on lichen species coverage (Nelson et al. 2015; Nunes et al. 2019). The slight differences in temperature and relative humidity between the sampling sites suggest potential variations in species coverage. These environmental factors play a significant role in promoting lichen diversity.

Table 1. Crustose, foliose, and fruticose lichen with their morphological description form collected from Brinchang, Pahang and Templer Park, Rawang, Selangor, Malaysia (Bates et al. 2001)

Species	Description
 *Lepraria incana*	Thallus is crustose, leprose, irregular, indeterminate, and firmly fastened to the substrate, forming a thin layer of soredia. The upper surface is grey-green and blue-green. *Lepraria incana* have dull soredia.
 *Cryptothecia striata*	Thallus is corticolous, rarely saxicolous, delimited by a distinct byssoid prothallus of white, radiating hyphae; thallus surface cottony, decorticate, greenish grey to greyish white, with storage beige, lacking soredia and have a white medulla.
 *Phaeographis intricans*	Thallus pale fawn, thin, smooth, glossy. Ascomata immersed in raised pale fawn circular to oval stromata 1-3 mm wide—disc black, finely white-pruinose.
 *Graphis casiella*	Thallus pale greyish white, thin, smooth, somewhat glossy. Ascomata inconspicuous, scattered, thin, immersed to semi-immersed, black, with a thin thalline veil or white pruina over the exposed proper exciple, straight or curved, rarely branched, 2-5 (-6) mm long. Thalline margin conspicuous; lips usually closed, rarely becoming slightly open to expose a weakly white-pruinose disc.

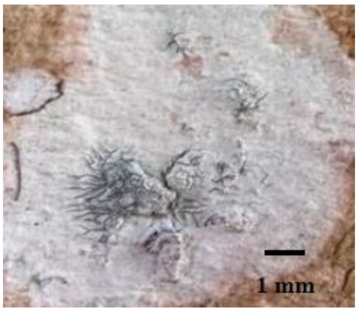


Graphis librata

Thallus pale greyish white, thin, smooth, dull. Ascomata conspicuous, black, scattered, sometimes clustered, straight, curved or sinuous, occasionally branched, semi-immersed to sessile, 0.8-2.0 mm long, 0.15-0.30 mm wide, with a thin thalline margin; lips closed.

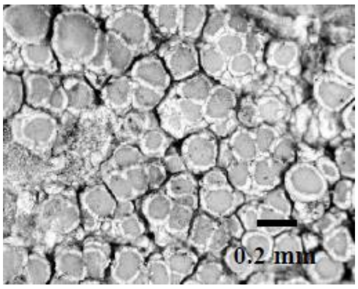
Graphis scripta

The thallus is crustose and continuous to slightly rugose. Its surface is cream, white, pale grey, or greyish green, dull. Its apothecia are raised from the thallus. The disc is narrow to wide and open, dark grey to brown, with a whitish pruina margin.



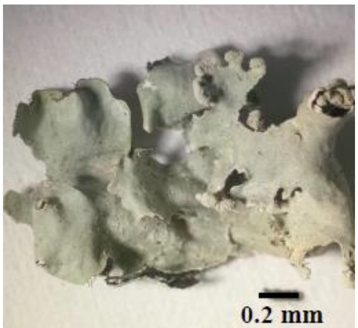
Graphis vestitoides

Thallus off-white, smooth, dull. Ascomata are numerous, conspicuous, scattered, sulcate, sessile, straight, curved, or sinuous, occasionally branched 2-5 (-7) mm long, 0.25-0.50 mm wide, covered by a thin thalline veil.



Lecanora helva (Bates et al. 2001)

Thallus is crustose, continuous rimose-areolate, or verrucose-areolate; prothallus: blackish to bluish brown. Its surface is yellowish white to yellowish-grey or yellowish green, smooth, opaque, pruinose, with an indistinct margin.



Parmotrema tinctorum

Thallus is foliose, loosely adnate. Lobate. Its lobes are sub-irregular, elongate, slightly imbricate, plane, and separate. Have size 10-20 mm wide. The upper surface is grey, smooth, dull centrally, shiny marginally, immaculate, and finely reticulately cracked with age.

Table 2. A chemicals spot test of Potassium hydroxide (K), Calcium hypochlorite, KC, and paraphenylenediamine (PD) test.

Lichen taxa	Chemical reagents			
	K	C	KC	PD
Amandinea efforescens	-	-	-	-
Cryptothecia striata	-	+Red	+Red	-
Dirinaria aegialita	+Yellow	-	-	+Yellow
Usnea ceratina	+Yellow to red	-	+Red	+Red
Graphis caeseilla	-	-	-	+Red
Graphis librata	-	-	-	+Red
Parmotrema tinctorum	+Yellow	-	+Red	-

Table 3. List of families, genera, and species of lichens recorded in Brinchang, Pahang and Templer Park, Rawang, Selangor, Malaysia. Plus and minus results indicate the presence and absence of the lichens at each sampling site.

Thallus	Families	Genera	Species/ taxa	Templer Park (131 m asl.)	Brinchang (1,503 m asl.)
Crustose	Stereocaulaceae	Lepraria	*Lepraria*	-	+
	Caliciaceae	Amandinea	*Amandinea efforescens*	+	-
	Arthoniaceae	Cryptothecia	*Cryptothecia striata*	+	-
	Roccellaceae	Bactrospora	*Bactrospora metabola*	+	-
	Graphidaceae	Phaeographis	*Phaeographis intricans*	+	-
		Graphis	*Graphis caesiella*	-	+
			Graphis librata	-	+
			Graphis scripta	-	+
			Graphis vestitoides	-	+
	Physciaceae	Rinodina	*Rinodina axydata*	+	-
	Lecanoraceae	Lecanora	*Lecanora helva*	+	-
		Lecidella	*Lectdella elaeochroma*	-	+
	Stictidaceae	Thelopsis	*Thelopsis isiaca*	+	-
			Crustose Total:	7	6
Foliose	Parmeliaceae	Flavoparmelia	*Flavoparmelia caperata*	-	+
		Parmotrema	*Parmotrema tinctorum*	+	-
	Physciaceae	Hyperphyscia	*Hyperphyscia adglutinata*	+	-
	Caliciaceae	Dirinaria	*Dirinaria aegialita*	+	-
			Foliose Total:	3	1
Fruticose	Parmeliaceae	Usnea	*Usnea ceratina*	-	+
			Fruticose Total:	0	1
			Grand Total:	10 (7 Crustose, 3 Foliose & 0 Fruticose)	8 (6 Crustose, 1 Foliose & 1 Fruticose)

Table 4. List of families, genera, and species of lichens recorded in Brinchang, Pahang and Templer Park, Rawang, Selangor, Malaysia

	Altitudes (m asl.)	NH_3 (ppm)	Bark pH	Temperature (°C)	Relative humidity (%)
Temple Park	131	0.40±0.07	05.50±0.20	32.01±0.41	64.51±1.50
Brinchang	1,503	0.11±0.04	5.49±0.07	23.88±0.31	66.62±1.44

There was a significant difference in lichen species coverage between the two sites (p=0.015). Templer Park had higher species coverage compared to Brinchang. Species coverage tends to be higher at lower altitudes and lower at higher altitudes due to the different habitats preferred by lichens. Crustose lichens dominate tree bark at low altitudes, while foliose and fruticose lichens are more prevalent at higher altitudes. Studies by Li et al. (2017) and Khare et al. (2022) both reported an increase in foliose-type lichens with increasing altitude. Further analysis of the same lichen data also highlighted the effects of altitude on both macro-lichen and crustose lichen communities (Saipunkaew et al. 2005; Nascimbene and Marini 2015). The results indicate that in upland areas above 600 m asl., foliose species dominated tree trunks, whereas in lowland areas (250-400 m asl.), trunks were frequently dominated by crustose lichens.

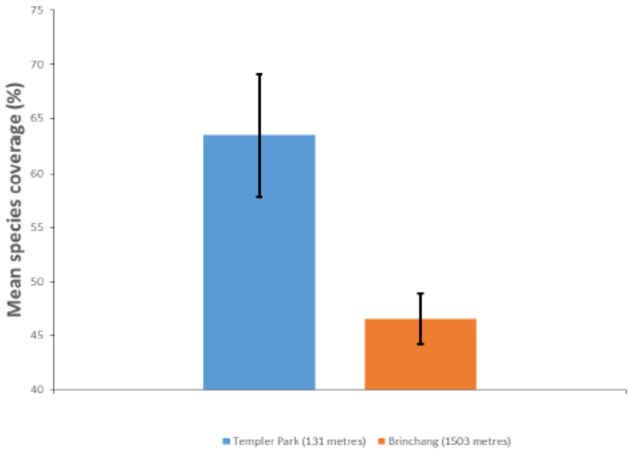


Figure 4. Mean lichen species coverage at low and high altitudes

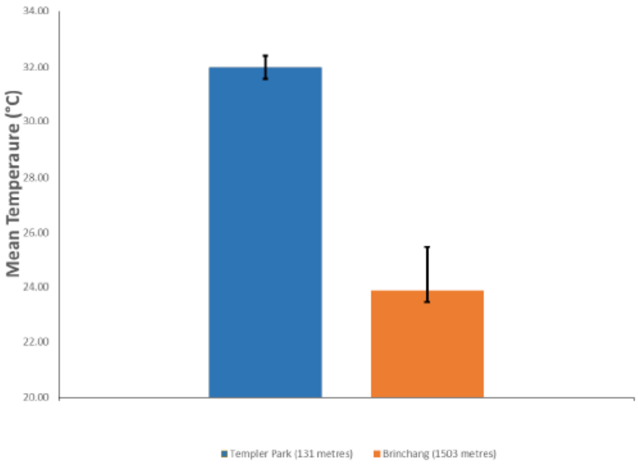


Figure 5. Mean temperature at low and high altitudes

Figure 4 shows that species coverage was higher at low altitude (63.47±5.59%) compared to high altitude (46.56±3.08%). The difference in mean scores between the two sites was statistically significant ($p<0.05$). The variation in species coverage across altitudes underscores the delicate balance lichens maintain with their environment. As elevation increases, species coverage decreases, likely due to the specialized habitats and conditions found at higher altitudes, where foliose lichens become more dominant, adapting to these challenging environments. In contrast, lower altitudes exhibit greater species coverage, with crustose lichens thriving in the less extreme conditions typical of these elevations.

While there is a statistically significant difference in species coverage between the low-altitude and high-altitude sites, as demonstrated in Figure 4, a comprehensive understanding of the ecological factors affecting these communities requires examining various environmental aspects beyond altitude. Temperature and relative humidity play crucial roles in creating microhabitats that support a diverse range of lichen species or favor particular groups. Anthropogenic influences, such as air pollution—particularly ammonia—can significantly impact lichen communities due to their sensitivity to air quality; these pollutants can inhibit lichen growth by damaging the algal partner or altering the bark's chemistry. Additionally, the chemical composition of the bark, particularly its pH, acts as a selective medium, with certain lichen species adapted to thrive on specific substrates, thereby either limiting or promoting their growth. Conducting detailed assessments of local environmental conditions, including measurements of temperature, relative humidity, ammonia, and bark pH, can provide deeper insights into the complex interactions that drive the composition and distribution of epiphytic lichen communities. This holistic approach offers a more nuanced understanding of ecosystem dynamics and the crucial role lichens play within them.

Temperature

This study highlights a pattern in species distribution that favors greater species richness at lower elevations. The increase in Physciaceae in temperate regions has been associated with rising temperatures and greater nutrient availability (Aptroot 2004; Coyle and Hurlbert 2016). In this study, Physciaceae were predominantly found at low altitudes, with their absence noted at higher altitudes. Templer Park recorded a slightly higher number of species (10) compared to Brinchang (8).

Figure 5 shows that Templer Park, at lower altitudes, recorded higher temperatures than Brinchang, located at higher altitudes. The mean temperature at lower altitudes is significantly higher at 32.01±0.41°C compared to 23.88±0.31°C at higher altitudes. This significant temperature disparity ($p<0.05$) suggests that lower elevations may provide a more conducive environment for a wider range of lichen species by facilitating optimal metabolic rates and photosynthesis processes, which are essential for lichen growth and reproduction.

Relative humidity

Brinchang recorded slightly higher relative humidity compared to Templer Park, with relative humidity being lower at low altitudes (64.51±1.5%) than at high altitudes (66.62±1.44%). Based on Figure 6, the difference in mean relative humidity between the two sites was not statistically significant ($p>0.05$). Despite the higher humidity at higher altitudes, species coverage was actually higher at lower altitudes, where humidity was lower. This contrasts with findings from past studies, such as Saipunkaew et al. (2005), which associated Parmeliaceae with upland sites in the Chiang Mai region where both rainfall and relative humidity were higher. The current study suggests that other environmental factors at lower altitudes may contribute more significantly to the observed higher species coverage, indicating a complex interaction between humidity and lichen distribution.

Ammonia

According to Greaver et al. (2022), NH_3 provides nutrients to lichens, but high exposure levels can cause physiological stress and mortality, impacting the local community's diversity and composition. Lichens lack stomates to regulate gas exposure, contributing to stress and damage as NH_3 levels rise (Ming et al. 2019).

Different lichen types have varying sensitivities to pollution (Manninen et al. 2023). Notably, Figure 7 shows the NH3 concentration in Templer Park is significantly higher than in Brinchang, with a higher concentration at lower altitudes (0.40±0.07 ppm) compared to higher altitudes (0.11±0.04 ppm), indicating a statistically significant difference ($p<0.05$). This suggests a potential stressor affecting species diversity. Despite this, the higher species coverage at lower altitudes indicates some lichen species may have adaptive mechanisms to cope with or benefit from increased NH3 levels, mitigating the negative impacts of ammonia exposure.

Templer Park, situated closer to urban areas and approximately 28.9 km from Kuala Lumpur, the capital city of Malaysia, exhibits higher ammonia concentrations than Brinchang. The study identifies 10 lichen species in Templer Park, including 7 crustose, 3 foliose, and no fruticose types, while in Brinchang, 8 species were noted - 6 crustose, 1 foliose, and 1 fruticose. Sujetovienė (2017) explains that fruticose lichens thrive only in pristine air, indicating their high sensitivity to pollutants, whereas foliose lichens withstand a minor level of air pollution, and crustose lichens are the most resilient, capable of surviving in more polluted environments. This distribution pattern aligns with the observations from the study, demonstrating the dominance of crustose lichens in both locations, likely due to their ability to prosper in areas with higher pollution levels. The absence of fruticose lichens in Templer Park can be attributed to the elevated ammonia concentration, corroborating the hypothesis that such environments are inhospitable to the most pollution-sensitive lichen species.

Bark pH

The study shows tree bark's pH does not significantly affect lichen distribution. pH is generally considered the primary property of the substratum that lichens respond to, and nitrogen can change the pH of the lichen's environment when it reacts with water. Another study by Greaver et al. (2022) stated that if dry NO_2 reacts with water in the cytosol, it will result in nitric acid (HNO_3). This strong acid may further dissociate in water from NO_3 and release H+, causing acidification. The pH changes caused by the reaction between water and NH3 or NO_2 occur where protonation occurs, therefore, within the lichen or the external habitat. Additionally, if the above reactions occur with water on the tree bark, pollution exposure will change the tree's pH bark.

Consequently, the pH of tree bark has been identified as an important factor related to epiphytic lichen community composition in areas with high NH_3 concentrations (Van Herk et al. 2003; Paoli et al. 2015), even if nitrogen is the principal pollutant. Nevertheless, bark pH alone cannot explain certain features (Aptroot 2004). Based on Figure 8, the bark pH of the two sites was almost similar. The analysis of bark pH shows minor variation between altitudes, suggesting its limited role in influencing lichen diversity directly. The similarity in pH values (mean pH 5.5033±0.20 at low altitudes and 5.4887±0.07 at high altitudes) underscores the potential of other factors, like temperature and ammonia concentration, as being more critical in shaping species coverage across different altitudes.

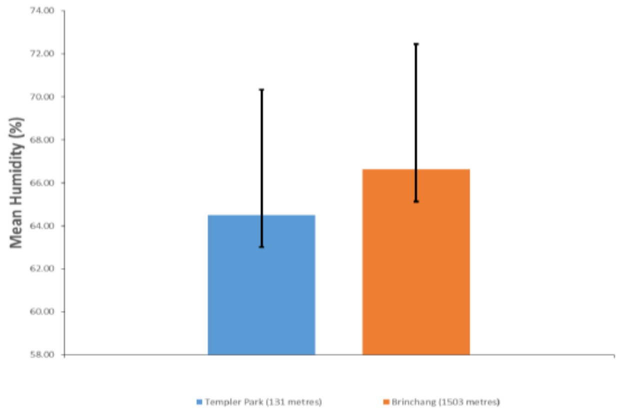


Figure 6. Mean relative humidity at low and high altitudes

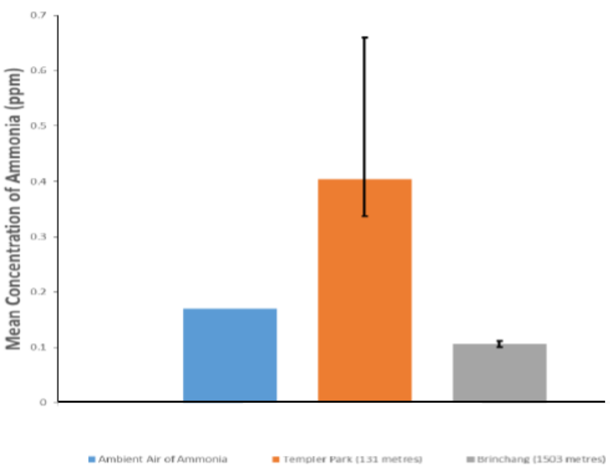


Figure 7. The mean concentration of ammonia at low and high altitudes

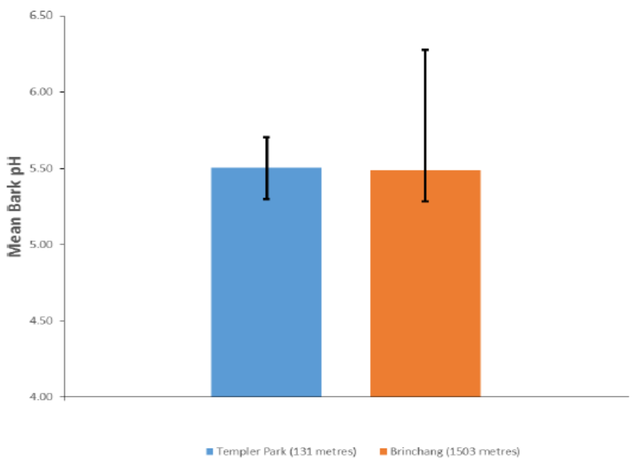


Figure 8. Mean bark pH at low and high altitudes

The crustose lichens, including *Amandinea efforescens, Cryptothecia striata, Bactrospora metabola, Phaeographis intricans, Rinodina axydata, Lecanora helva*, and *Thelopsis isiaca*, are dominant at lower altitudes due to their resilience to milder conditions and higher tolerance to ammonia concentrations, which many crustose species can endure due to their lower sensitivity to pollution. Foliose lichens, such as *Flavoparmelia caperata, Parmotrema tinctorum, Hyperphyscia adglutinata*, and *Dirinaria aegialita*, have larger surface areas for photosynthesis and water retention, allowing them to adapt to varying light conditions and relative humidity levels. These species are more common at mid-altitudes, where these environmental factors are moderate. *Usnea ceratina*, the fruticose lichen identified, thrives at higher altitudes due to its high surface area to volume ratio, facilitating efficient moisture absorption and light capture, which are critical in the more extreme conditions of higher elevations. This pattern of distribution highlights the specialized adaptations of each growth form to specific environmental conditions.

While this study primarily examines the effects of temperature, relative humidity, ammonia, and bark pH across different altitudes on lichen species composition, it is crucial also to consider the roles of microclimate variations and specific substrate conditions. These factors can significantly influence lichen communities, providing a more nuanced understanding of their distribution and abundance. Additionally, acknowledging the broader range of air pollutants beyond sulfur dioxide, fluoride, and nitrogen will offer a more thorough assessment of environmental impacts on lichen communities. Furthermore, variations in land use and specific human activities in the sampling areas may also affect lichen species composition and community structure. Including these considerations in future analyses will contribute to a more holistic and comprehensive understanding of lichen ecology across different altitudes.

In conclusion, this study recorded 18 species of lichens from 9 families and 15 genera. The crustose group dominates at 72%, the foliose group at 22%, and the fruticose group at 6%. Species coverage is high at low altitudes due to different habitats inhabited by lichens. It was shown that the fruticose increased as the altitudes increased while, in contrast, crustose lichen was less in high altitudes. However, the lichen species coverage was not just affected by the altitudes; it was also facilitated by abiotic factors such as relative humidity and temperature, bark pH, and ammonia concentration. Significant differences exist between abiotic factors (temperature and ammonia concentration) and altitudes. The factors influencing lichen species coverage are complex and interconnected. Temperature facilitates a broader range of species at lower altitudes and affects the species's coverage due to the need for sunlight for lichen to undergo photosynthesis. Therefore, the number of species at lower altitudes (10) was slightly higher than at higher altitudes (8). While ammonia's role is dual-faceted, presenting both a challenge and an opportunity for lichen species, they can be sensitive to various pollution levels, depending on their type. The abundance of crustose lichen in the area with a higher concentration of ammonia is associated with its least sensitive characteristic towards pollution. Relative humidity and bark pH, while essential for lichen life, do not display a significant direct impact on species diversity, according to the study's findings. This comprehensive analysis underscores lichens' adaptability and resilience in various environmental conditions and highlights their importance in maintaining ecosystem balance. These findings are crucial for environmental and conservation strategies, particularly in Malaysia and similar regions, where preserving diverse lichen communities can bolster ecosystem resilience, serve as bioindicators, and help mitigate climate change and pollution effects, ultimately ensuring long-term ecological health.

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