Can lichens be indicators for air pollution monitoring in Kandy City, Sri Lanka?

Edirisinghege Sanduni Madushika Edirisinghe[1] & Athukoralage Dona Sarangi Nirosha Priyajeevani Athukorala[1]

[1] Department of Botany, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka

Correspondence: Edirisinghege Sanduni Madushika Edirisinghe, Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka. E-mail: saduniedirisinghe1995@gmail.com

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Abstract

The quality of the surrounding environment significantly impacts lichen diversity and composition, but crustose lichens, particularly those in disturbed environments, can persist. Although the effects of habitat disturbances on the morphology, anatomy, chemical composition, and fertility of lichen species have not been extensively researched, understanding their mechanisms is crucial for understanding their persistence in their environment. The study aimed to identify common lichen species in polluted and semi-polluted environments and compare their morphology, anatomy, chemical composition, and fertility. A two-kilometer stretch along the road that passes through the University of Peradeniya was selected as the semi-disturbed site based on previous records, while the area around Kandy Lake was designated as the polluted site. Using conventional keys, lichen species were identified, and using a hand lens, dissecting microscope, and compound microscope, the morphological traits of the thallus, apothecia, soredia, and isidia, as well as the anatomical features of the thallus, apothecia, and ascospores, were compared between the two sites. Thin-layer chromatography was used to separate and elucidate the secondary metabolites of lichen. Thirty-nine species were discovered on the university site, 15 in the area around Kandy Lake, and species gathered from both locations shared commonalities, including *Physcia* sp., *Parmotrema* sp., *Lecanora* sp., *Graphis* sp., and *Lepraria* sp. The color difference between all species, as measured by mean dE, is visible to the naked eye and changes from a pale to a dark hue from a semi-disturbed site to a polluted one. In comparison to disturbed sites, the majority of species displayed an increased mean area in semi-polluted sites. Layer thickness increases or decreases depending on how well each layer can withstand disruptions. Depsinose has been recognized as a potential class of metabolites generated by lichens present in both locations. Only lake sites exhibit toxic metal compounds, as opposed to semi-disturbed sites. Every pH value was acidic, and most species at the lake site had more acidic pH than those at the university site. There is no specific trend in apothecial number and ascospore numbers in common lichen species in both sites. The current study showed that changes in certain morphological and anatomical biochemical characteristics can be triggered by the environment, suggesting that the quality of the environment significantly affects the growth and reproductive success of lichens, thereby survival or extinction, and proposed that bioindicator and bioaccumulator species concerning the changes occurred.

Keywords: air pollution, *Thallus* morphology and anatomy, secondary metabolites, fecundity.

Os líquenes podem ser indicadores para o monitoramento da poluição do ar em Kandy City, Sri Lanka?

Resumo

A qualidade do ambiente circundante afeta significativamente a diversidade e a composição dos líquenes, mas os líquenes crustosos, particularmente aqueles em ambientes perturbados, podem persistir. Embora os efeitos das perturbações do habitat na morfologia, anatomia, composição química e fertilidade das espécies de líquen não tenham sido extensivamente pesquisados, compreender os seus mecanismos é crucial para compreender a sua persistência no seu ambiente. O estudo teve como objetivo identificar espécies comuns de líquen em ambientes poluídos e semipoluídos e comparar sua morfologia, anatomia, composição química e fertilidade. Um trecho de dois quilômetros ao longo da estrada que passa pela Universidade de Peradeniya foi selecionado como o local semi-perturbado com base em registros anteriores, enquanto a área ao redor do Lago Kandy foi designada como o local poluído. Usando chaves convencionais, as espécies de líquen foram identificadas e, usando lente de mão, microscópio de dissecação e microscópio composto, as características morfológicas do talo, apotécia, sorédia e isídia, bem como as características anatômicas do talo, apotécia e ascósporos, foram comparadas entre os dois locais. A cromatografia em camada fina foi utilizada para separar e elucidar os metabólitos secundários do líquen. Trinta e nove espécies foram descobertas no sítio da universidade, 15 na área ao redor do Lago Kandy, e espécies coletadas de ambos os locais compartilhavam semelhanças, incluindo Physcia sp., Parmotrema sp., Lecanora sp., Graphis sp., e Leprariasp. A diferença de cor entre todas as espécies, medida pela média dE, é visível a olho nu e muda de uma tonalidade pálida para uma tonalidade escura de um local semi-perturbado para um local poluído. Em comparação com os locais perturbados, a maioria das espécies apresentou um aumento da área média em locais semipoluídos. A espessura da camada aumenta ou diminui dependendo de quão bem cada camada pode suportar interrupções. A depsinose tem sido reconhecida como uma classe potencial de metabolitos gerados por líquenes presentes em ambos os locais. Apenas os sítios lacustres apresentam compostos metálicos tóxicos, ao contrário dos locais semi-perturbados. Todos os valores de pH eram ácidos, e a maioria das espécies no local do lago tinha mais pH ácido do que as do local da universidade. Não existe uma tendência específica no número de apotéticos e no número de ascósporos em espécies de líquenes comuns em ambos os locais. O presente estudo mostrou que alterações em certas características bioquímicas morfológicas e anatômicas podem ser desencadeadas pelo ambiente, sugerindo que a qualidade do ambiente afeta significativamente o crescimento e o sucesso reprodutivo dos líquenes, sobrevivendo ou extinguindo, e propôs que as espécies bioindicadoras e bioacumuladoras em relação às mudanças ocorressem.

Palavras-chave: poluição atmosférica, morfologia e anatomia do *Thallus*, metabolitos secundários, fecundidade.

1. Introduction

Globally, air pollution is becoming a greater hazard to public health, particularly for urban residents. Air pollution affects both the environment and people in different ways. The air quality may gradually decline as a result of the rise in dangerous air pollutants such as particles, hydrocarbons, photochemical oxidants, oxides of carbon, sulfur, and nitrogen, as well as other inorganic heavy metals. Additionally, rising greenhouse gas emissions have a direct impact on global warming and, in turn, influence a variety of climate changes. Extended exposure to air pollution can result in more severe health impacts, mostly affecting the respiratory and inflammatory systems, but also increasing the risk of developing more serious disorders, including cancer and heart disease. When it comes to the economy, air pollution affects it since it increases mortality and disease, damages crops and property, and affects tourism because travelers avoid or limit their stays in extremely polluted cities because of climate change.

Kandy is a popular tourist and pilgrimage destination for both foreign and local visitors, and it is also recognized as a World Heritage Site. In the Kandy area, there are roughly 0.12 million residents, 0.1 million of whom commute every day, and over 100,000 vehicles travel through the city (Premasiri et al., 2012). The Kandy region is home to the majority of the region's industry, including public and private administrative organizations. About 120,000 people live in Kandy Town.

Approximately 100,000 people go from outside Kandy to the city for a variety of reasons. The 26 km^2 of Kandy is located 465 m above sea level and 115 km away from Colombo. There is severe traffic congestion as a result. As a result, pollutants from home activities, high traffic, and different industrial operations are contaminating the air quality in Kandy (Premasiri et al., 2012). Its location in a valley between two mountain ranges contributes to the low mixing of the city's atmospheric air. Thus, the first step in putting air pollution management plans into practice is air pollution monitoring. There are three permanent stations in Sri Lanka, which are situated at Kandy City, the Meteorology Department's buildings at Bauddhaloka Mawatha, and Colombo Fort.

Previous studies measured gaseous air pollutant levels in Kandy City using passive sampling techniques. It was found that nitrogen oxide, sulfur dioxide, and ozone concentrations exceeded the Sri Lankan standards in 38%, 53%, and 40% of the 30 samples analyzed, respectively (Elangasinghe and Shanthini, 2008). In Kandy city, the poor air quality level was analyzed in 2006, but the passive sampling device was established in Kandy city in 2018, 12 years after the initial reports by Abeyratne and IIeperuma in 2006. The establishment of the devices and methodologies can be done over a fairly wide area with several sampling points at a reasonable cost. Numerous techniques for monitoring air quality were employed in several industrialized nations, including passive, active, automated point, photochemical sensors, and biomonitoring techniques.

Since Sri Lanka is a developing nation, low-cost monitoring techniques like biomonitoring are required. Lichens have distinct characteristics that set them apart from other biomonitors like fish, bacteria, algae, protozoa, plants, aquatic plants, arthropods, mosses, and bryophytes. Their distribution is global, and they have no roots. Lichens are nourished by the atmosphere, and the components they contain are a reflection of the elements present in the environment in their combined form as gases, dissolved particles, or particulates. Their lack of a cuticle, and a protective covering, caused them to develop defense mechanisms against hostile conditions. Mycobionts and photobionts have a symbiotic connection that results in lichens (Kostryukova et al., 2017). They are very good at storing chemical elements in the body for extended periods and at concentrations higher than what is required for physiologic function. Because lichens appear to be resistant to harsh environments, they can be found in all geographical zones, from deserts to tropical rainforests, and in extreme heat or cold (Giordani; Brunialti, 2015). Lichens are also sensitive to light, air humidity, UV-B radiation, temperature, and airborne chemicals because of their physiology. This sensitivity makes them valuable indicators of air pollution and climate change (Tarhanen et al., 2000; Cornelissen et al., 2001; Kricke; Loppi, 2002; Castello; Skert, 2005).

Lichens have two functions in biological monitoring systems: they are bioaccumulators and sensitive species (bioindicators). Sensitive biomonitors are employed as preventive alert systems and integrators of the stress induced by pollutants. Based on morphological changes, particularly in population abundance, they are based on several physical aspects, such as enzyme-driven processes like photosynthesis and respiration. According to Conti & Cecchetti (2001), cumulative bioindicators are employed to quantify contaminant concentrations in an integrated manner by storing ingested pollutants, heavy metals, and inorganic compounds in their bodies.

The primary objective of this study is to evaluate how Kandy City's air quality affects lichens to potentially create biomonitors. The objectives are to: 1) identify the diversity of lichen species in a particular polluted and semi-polluted site near Kandy Lake; 2) identify the lichen species that are common to both types of sites; and 3) compare the morphological, anatomical, chemical, and fecundity characteristics of common species in polluted and semi-polluted sites.

2. Materials and Methods

2.1 Site selection

Using a random sampling technique, samples of bark and lichen thalli were gathered from the chosen location. The area stretching from Galaha Junction to Senate Hall has been identified as a semi-polluted site. It lies nestled on the gentle slopes near the pristine Hantana mountain range. The University premises are located 8 kilometers away from Kandy city, with minimal vehicular traffic entering and exiting the site, preserving its tranquility. Near Kandy Lake, an area plagued by contamination hosts 60,000 vehicles, accommodates 100,000 visitors, and supports 1.3 million residents. This congestion contributes to elevated levels of NO_2, SO_2, and O_3 exceeding Sri Lankan standard norms (Abeyratne; Ileperuma, 2006).

2.2 Sample collection

To assess the sites, the first two or three field visits took place in January through December of 2019. Using x10 magnifying lenses, lichens were seen and noted on rocks, leaves, and bark — including fallen bark — of randomly chosen trees. Those discovered on downed trees, twigs, and branches were also gathered with a chisel and hammer or a basic cutting knife. Depending on the vegetation, anywhere from 10 to 30 lichen samples were taken at each site. Part of the substrate was collected together with foliose lichens to protect the thallus and rhizines.

Crustose species were eliminated by removing enough bark from the trees, and those that were found on rocks were removed by chipping away portions of the substrate. The lichen specimens were temporarily collected and stored in plain brown paper bags for further identification. Soft tissue that absorbs was used to wrap fragile specimens before they were bagged. Using a random sampling technique, a selection of lichen samples will be gathered from the vicinity of Peradeniya University Premises (~2 km) and Kandy Lake (~2 km). The relevant information, including the locality, sample number, tree number, and collection date, will be labeled on the specimens.

2.3 Identification of lichen species in polluted and semi-polluted sites

Using morphological characteristics of lichens that have been used in Fascinating Lichens of Sri Lanka, Discovering Lichens in Sri Lanka, "Artificial Field Keys to common lichen genera in Thailand" (Wolseley; Aguirre-Hudson, 1995), and a color key to lichens (Wolseley; Aguirre-Hudson, 1997a), the specimens were identified up to the generic level and, where possible, to the species level. To follow the keys, microscopic investigations of freehand sections of thallus and fruiting bodies were also conducted on the majority of lichens. Microscopic observations of freehand sections of thallus and fruiting bodies were also made on most of the lichens to follow the keys.

2.4 Comparison of morphological parameters of common species among polluted and semi-polluted sites

The mean thalli area and color were compared between semi-contaminated and polluted sites under the morphological comparison. First, Nikon Coolpix was used to snap pictures of the thalli to compare their mean color. Next, RGB values were acquired through the Microsoft Office 2007 Paint package. There were three copies made. The most trustworthy RGB value was then chosen for the comparison. Thorali samples from both the semi-contaminated and polluted sites had RGB values determined. Next, DELTA E values were determined by comparing RGB values using Barblecolor software. Ultimately, a standard scale was used to compare the DELTA E values. The first thalli margin was indicated with a sharpened pen and transparent sheath to compare the mean thalli area. And Nikon Coolpix was used to snap pictures. An image software application was used to calculate the mean area.

2.5 Comparison of anatomical parameters of common species among the polluted site and semi-polluted site

Lichen's layer thickness was examined between semi-contaminated and polluted sites under anatomical comparison. With a sharpened blade, thin portions were produced. Slender portions were gathered on a watch glass filled with purified water. It was put in distilled water and examined using a compound microscope at either high or medium power.

2.6 Comparison of chemical parameters of common species among polluted and semi-polluted sites

The chemical composition, pH of thalli washing, and secondary metabolites of lichen were studied between polluted and semi-polluted sites. Sterile conditions were used to make acetone extracts. After adding 1 ml of acetone and packing the *Eppendorf* tube loosely until it reached the 0.5 mL mark, pieces of dried thalli (1 mm) were added. The prepared tube was incubated at room temperature for one hour. Using a capillary tube, one millimeter of each acetone extract was spotted on TLC plates. The TLC plate was let to dry for between thirty and sixty minutes. The plate was run through the solvent system of toluene, ethyl acetate, and formic acid (139:83:8 *v/v/v*) in the developing chamber.

The TLC plate was examined using a UV lamp (254 nm and 365 nm). TLC plates were coated with 10% sulfuric acid and baked for 10 min at 110 ºC. Potential chemicals were identified by recording the colors and Rf values of each site. To get rid of inert material like sand, dust, etc., collected samples were repeatedly cleaned in distilled water. The samples were dried in an oven at 80 °C until they reached a consistent dry weight. A motor mill grinder was used to powder and homogenize the ash samples, and a sieve shaker was used to sift the material. An XRF was used to assess the samples' elemental makeup (Gunathilaka et al., 2011). First, 2.5 cm^2 x 2.5 cm^2 of thalli pieces were chosen for each health species, the thalli surface was washed with water, and 10 ml of deionized water was added. After two minutes of stirring, it was given time to settle. With the use of a pH/conductivity meter, the solution's pH was determined.

2.7 Comparison of fecundity parameters of common species among polluted and semi-polluted sites

The number of medium-sized apothecia was counted using low-power microscopy in the field of view, and thin sections of the apothecial structure were obtained. Additionally, samples were carefully crushed on a glass slide to minimize thallus damage, and ten ascus structures were chosen at random for examination at high magnification, with each ascus's ascospore count recorded.

2.8. Lichen transplanting

Every chosen species of lichen (*Physcia* sp.) was gathered. Using a chisel, lichen discs were cut from the bark of hardwood trees that were chosen at random. After being numbered and placed in envelopes, the disks were brought to the lab and photographed. The disks with a consistent diameter of 2.5 cm by 2.5 cm were chosen for transplantation. Next, using exterior wood glue, each disk was set on a 31 cm^2 untreated plywood board. At each site, three lichen samples with a total of eighteen discs were positioned.

2.9 Statistical analysis

Using ANOVA and the two sample *T*-test in the MINITAB-19 software program, statistical analyses were performed to assess the mean thalli area, mean upper cortex, algal layer, medulla layer, and lower cortex, as well as the thalli pH values, mean apothecial number, and mean ascospore number readings.

3. Results

3.1. Lichen identification

Both foliose and crustose species were found in the vicinity of Kandy Lake and the university's grounds during the current investigation. The semi-polluted site had a greater total number of species, whereas the polluted site had the lowest number of species. Every type of habitat was home to lichens, both foliose and crustose. In every environment, fructicose lichens were lacking. Nine foliose species and six crustose species were identified from Kandy Lake. Six of the nine foliose species are members of the *Physcia*genus, two of the *Parmotrema* genus, and one of the *Xanthoria* genus. *Lecanora* sp., *Lepraria* sp., *Graphis* sp., *Trypethelium* sp., *Anthracothecium* sp., and *Megalospora* sp. were crustose species.

Twenty crustose species and nine foliose species were identified from the university location. Out of the 19 foliose species, 6 were found in the *Physica* genus, 7 in the *Parmotrema* genus, 2 in the *Heterdermia* genus, and other species in the *Coccocarpia*, *Leptogium*, *Flavoparmelia*, and *Parmeliopsis* genus. Out of the twenty crustose species, nine were classified as unknown species, and five belonged to the genera *Graphis*, *Lecanora*, *Lepraria*, *Phlyctis*, *Megalospora*, *Pyrenula*, and *Phyllospora*.

Table 1. Summary of identified species from Around Kandy Lake and University premises.

Sites	Total number of species	Growth forms		
		Foliose	Crustose	Fruticose
1. Semi polluted site (U)	39	19	20	AB
2. Polluted site (L)	15	9	6	AB

Note: AB (Absent). Source: Authors, 2024.

3.2 Common lichen species

Five of the identified lichen species from the contaminated and semi-polluted regions were shared by both. These include the foliose growth forms of *Physcia* sp. (family: Physciaceae), *Parmotrema* sp. (family: Parmeliaceae), *Lepraria* sp. (family: Stereocaulaceae), *Lecanora* sp. (family: Lecanoraceae), and *Graphis* sp. (family: Graphidaceae).

3.3. Morphological comparison

ΔE (delta E, dE) represents the difference in how two colors are perceived visually. A measure for figuring out how the human eye interprets color differences is called dE. The mean dE values for each species in both semi-polluted and polluted sites are displayed in (Table 2), along with color changes from semi-polluted to polluted. When *Graphis* sp. (crustose) and *Physcia* sp. (foliose) were found in semi-contaminated and polluted sites, their mean dE values were higher than those of the other species. *Lecanora* sp. exhibits the lowest mean dE (crustose).

The mean dE ranges for each species are represented by the usual scale (Table 3), which shows that all species' mean dE ranges fall between 0 and 100. However, since all species have dE values greater than 1, we can see color changes in all species in both polluted and semi-polluted sites. In all species, the color shifts from a pale to a dark hue at semi-polluted and polluted sites. We can deduce that all colors (ranging from 11 to 49) are more alike than opposites. These findings suggest that air pollution levels have an impact on the thalli color of the chosen species. The mean area of all the chosen lichen species common to semi-contaminated and polluted sites is displayed in (Figure 1).

Table 2. Mean dE values and color changes from semi-polluted to polluted sites of lichen thalli in two selected sites.

Species	Mean Delta E	Color changes from semi-polluted to a polluted site
Lepraria sp.	12.68033	Green – Brown
Graphis sp.	34.5873	Dark brown – Pale brown
Physcia sp.	22.827	Dark green – Grey green
Parmotrema sp.	12.2453	Pale green – Dark green
Lecanora sp.	13.6	Pale brown – Dark brown

Source: Authors, 2024.

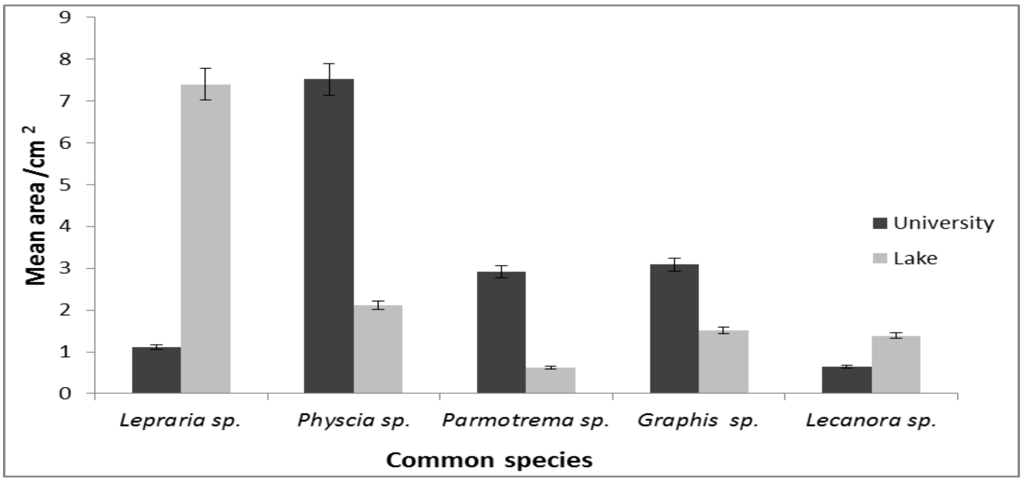


Figure 1. The mean area of the common lichen species found in semi-polluted and polluted sites. Source: Authors, 2024.

Compared to university sites, the majority of species at the Lake Site had lower mean areas. Yet, the mean value of *Lepraria* sp. is larger in the lake site than in the semi-polluted location. *Parmotrema* sp. at the lake location has the lowest mean area, while *Physcia*sp. on university property has the largest mean area. The lake site has a higher *Lepraria* sp. area than the university site. The area of the university site is larger than that of the lake site for *Physcia* sp., *Parmotrema* sp., and *Graphis* sp. The *Lecanora*sp. and *Lepraria* sp. area in the Lake Site is larger than that of the University Site. Additionally, there are notable differences in *Lepraria* sp., *Physcia* sp., *Parmotrema* sp., and *Graphis* sp. between the two sites. (p = 0.041, p = 0.001, p = 0.017, p = 0.124 and p = 0.001).

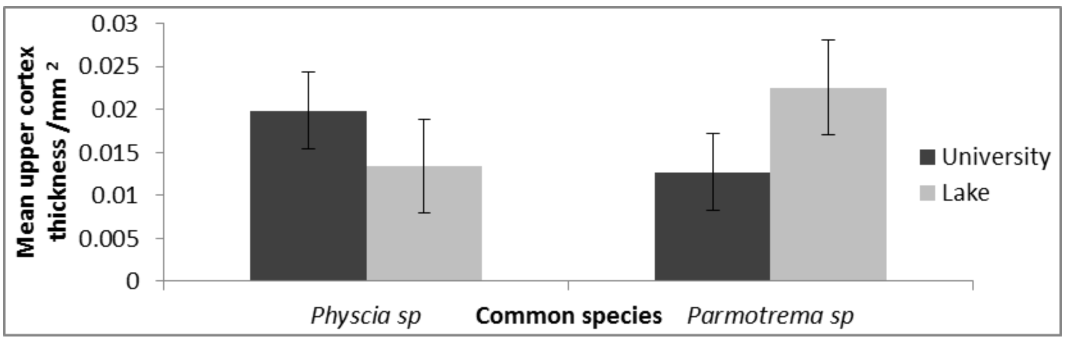
3.4 Anatomical comparison

The mean upper cortical thickness of common lichen species in semi-contaminated and polluted sites is displayed in Figure 2. *Physcia* sp. and *Parmotrema* sp. had different mean upper cortical thicknesses at the Lake Site. In comparison to the university site, the mean upper cortical thickness of *Physcia* sp. in the lake was smaller.

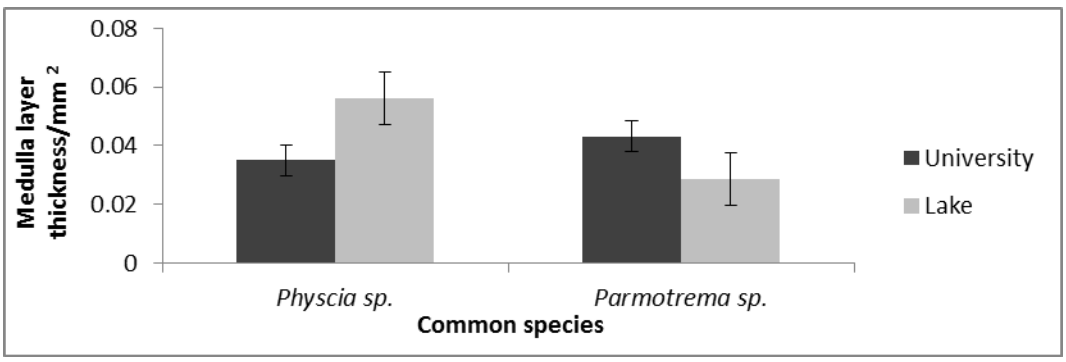
Parmotrema sp. at the university location had a mean upper cortical thickness that was less than that of the lake site (p = 0.014). Between the two places, *Parmotrema* sp. differs significantly. *Parmotrema* sp. has significantly increased compared to *Physcia* sp. The mean algal layer thickness of common lichen species in semi-contaminated and polluted sites is displayed in (Figure 2). *Parmotrema*sp. had a mean algal layer thickness that was higher at the university site than *Physcia* sp. The mean algal layer thickness in *Physcia* sp. was greater at the university site compared to the lake site. There is a noticeable rise in *Parmotrema* sp. between the two sites. *P* is equal to 0.023.

The university site had a mean algal layer thickness greater than the lake site for *Parmotrema* sp. The average thickness of the medulla layer for common lichen species in semi-contaminated and polluted sites is shown in (Figure 2). In comparison to the university site, the mean medulla layer thickness of *Physcia* sp. was greater at the Lake Site. The University Site has a larger mean medulla layer thickness than the Lake Site in *Parmotrema* sp. For both species, there were notable differences between the two locations. (*Physcia* sp. = 0.003 and p = 8.297×10^{-5} for *Parmotrema* sp.)

The mean lower cortical thickness of common species in semi-contaminated and polluted sites is displayed in (Figure 2, d). The higher Lake site had a mean lower cortical thickness across all species compared to the university site. *Physcia* sp. mean thickness was not significantly different across the semi-polluted and lake sites, although it was higher in the former. P is equal to 0.735. The difference between the mean thickness of *Parmotrema* sp. at the lake site and the university site is statistically significant (p = 0.043).



a)



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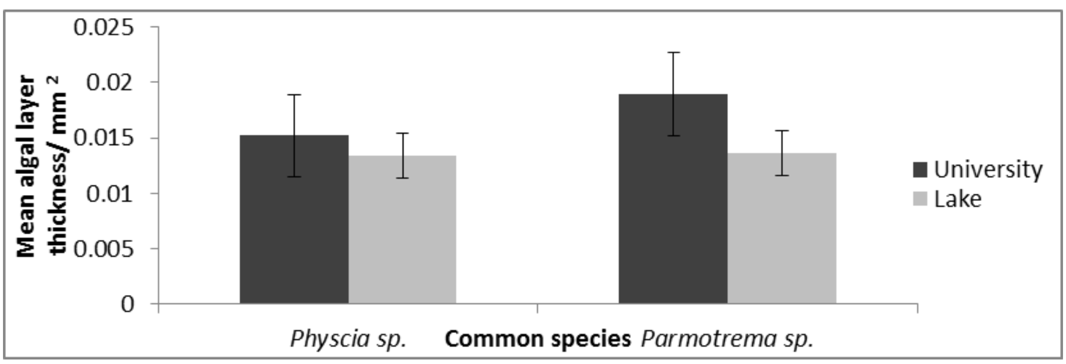


c)

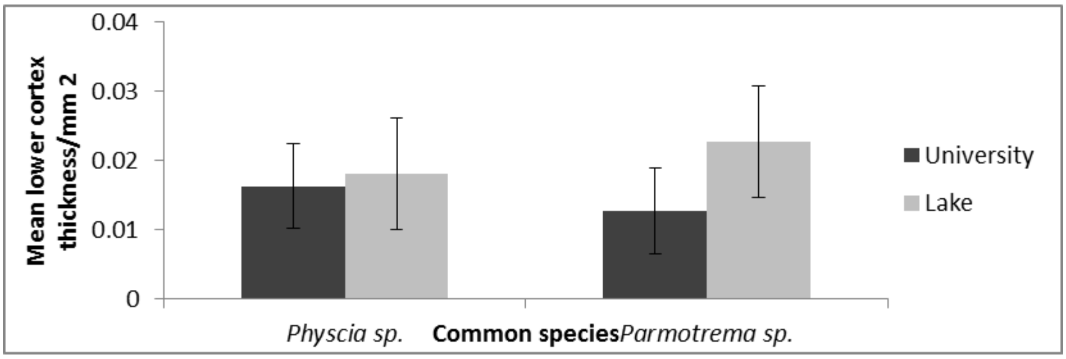
d) 

Figure 2. a) Mean upper cortex thickness of the common lichen species found in semi polluted and polluted site. b) Mean algal layer thickness of the common lichen species found in semi-polluted and polluted sites. c) Mean medulla layer thickness of the common lichen species found in semi-polluted and polluted sites. d) Mean lower cortex thickness of the common lichen species found in semi-polluted and polluted sites. Source: Authors, 2024.

3.5 Chemical comparison

When *Graphis* sp. secondary metabolites are compared between the two sites, they are found in the same groups in both species (Pigments, Anthraquinone, Depside, Depsidone, Dibenzofuran, and Pulvinic acid). Only the lake site in sp. has depsidone, and only the lake site contains xanthone and usnic acid. Usnic acid is present in both locations of *Parmotrema* sp., whereas depsidones are exclusively present at the university site. Depside, Depsidone, and Pulvinic acid are present in both locations of *Lepraria* sp. Every species in both sites has metallic compounds of Fe, Ca, and K. Cu, Cr, and Mn are only observed at the Lake Site in *Parmotrema* sp. Sr is only found in the university site of *Lecanora* sp., whereas Cu, Cr, and Mn are only found in the lake site.

Cu and Mn are exclusively visible in the Lake Site of *Physcia* sp. Except Fe, Ca, and K, Ti and Ba are visible at both sites in *Graphis* sp. Ba, Ti, and Zr can only be seen in the university site of *Lepraria* sp., whereas Mn can only be seen in the lake site. The thalli samples' pH levels were all less than 7 mean acidic. The pH of the Lake Site was higher (more acidic) than the University Site for *Graphis* sp. University pH was higher (more acidic) than Lake Site pH in *Lecanora* sp. University samples of *Physcia* sp. were more basic (more acidic) than lake samples. Lake samples had higher acidity levels than university site samples in *Parmotrema* sp. Most of the time, lake sites have more acidic pH values than university sites. Additionally, there was a significant difference (p = 0.001, 0.006) between the two sites of *Physcia* sp. and *Parmotrema* sp.

Table 3. Possible secondary metabolites present in lichen samples from semi-polluted to polluted sites of lichen thalli in two selected sites.

Graphis sp.		*Physcia* sp.		*Parmotrema* sp.		*Lepraria* sp.	
University	Lake	University	Lake	University	Lake	University	Lake
Pigment	Pigment	Depside	Anthraquinone	Depside	Xanthone	Depside	Depside
Anthraquinone	Anthraquinone	Depsidone	Xanthone	Depsidone	Usnic acid	Depsidone	Depsidone
Depside	Depside	Pigment	Usnic	Pigment		Anthraquinone	Pigment
Depsidone	Depsidone	Pulvinic	Steroid	Pulvinic acid		Triterpenoid	Usnic acid
Dibenzofuran	Dibenzofuran			Anthraquinone		Pulvinic acid	Pulvinic
Pulvinic acid	Pulvinic acid			Xanthone			Xanthone
				Usnic acid			

Source: Authors, 2024.

Table 4. Possible chemical compounds present in lichen samples from semi-polluted to polluted sites of lichen thalli in two selected sites.

Parmotrema sp.		*Lecanora* sp.		*Physcia* sp.		*Graphis* sp.		*Lepraria* sp	
University	Lake	University	Lake	University	Lake	University	Lake	University	Lake
Fe	Fe	Fe	Fe	Ca	Fe	Fe	Fe	Fe	Fe
Ca	Ca	Ca	Ca	Fe	Ca	Ca	K	Ca	Ca
Bi	Br	K	K	K	K	K	Ca	Ba	K
Zr	Cu	Sr	Cu		Cu	Ti	Ti	K	Mn
K	Cr		Mn		Mn	Ba	Ba	Ti	
	Mn		Cr					Zr	

Note: Fe - Iron, Ca - Calcium, Bi - Bismuth, Zr - Zirconium, K - Potassium, Cu - Cooper, Mn - Manganese, Ba - Barium, Cr - Chrome, Ti - Titanium. Source: Authors, 2024.

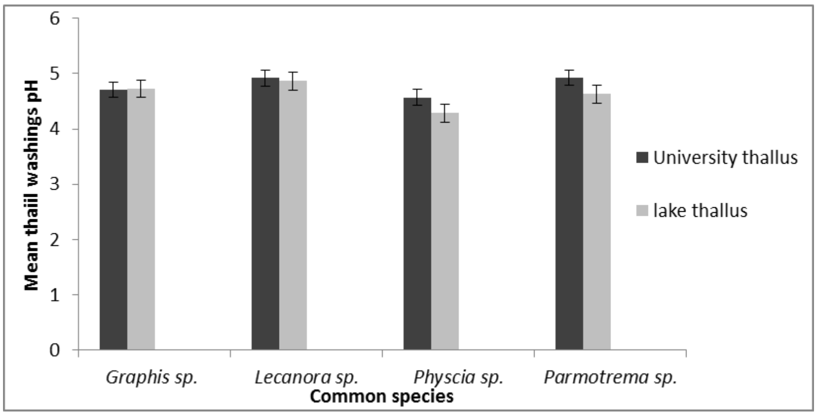


Figure 3. Mean thalli washings pH of the common lichen species found in semi-polluted and polluted sites.

3.6 Fecundity comparison

Lecanora sp. had a greater mean apothecial number than *Physcia* sp. For *Lecanora* sp., the meaning apothecial number was higher in the lake site compared to the university location, whereas the mean apothecial number was higher in the lake site for *Physcia* sp. Between the two *Lecanora* sp. sites, there is a significant difference (p = 0.077). For every species, there was no variation in the number of ascospores between the two sites. All species' ascus structures contain eight ascospores.

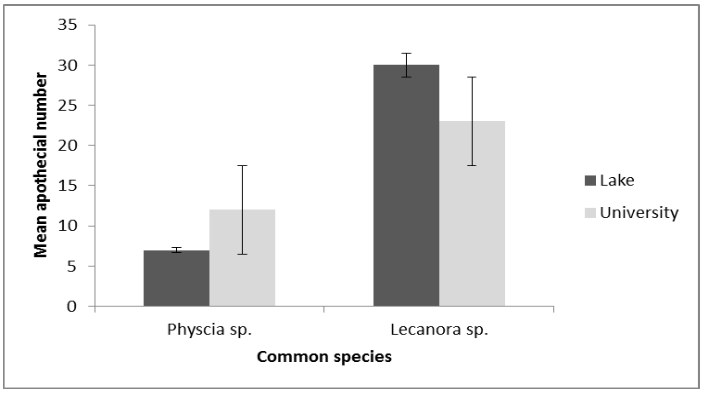


Figure 4. Mean apothecial number of the common lichen species found in semi-polluted and polluted sites.

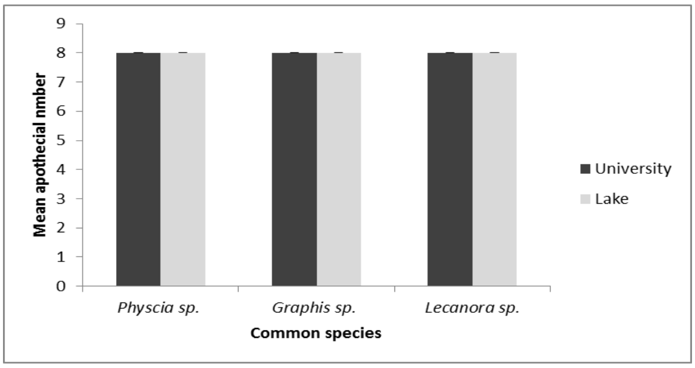


Figure 5. Mean ascospore number of the common lichen species found in semi-polluted and polluted sites.

4. Discussion

The current study compared the morphological, anatomical, chemical, and fecundity parameters of common lichen species collected from the polluted site and semi-polluted sites by considering the previous records of air quality levels in Kandy City (Abeyratne; Ileperuma, 2006; Elangasinghe; Shanthini, 2008). The PM 10 level within the Peradeniya Botanical Garden premises, 300 meters away from Kandy Peradeniya road, was higher than that of the previous reports on PM 10 levels around Kandy Lake (more specifically, the Sagaraja Mawatha main road in the city by Kandy Lake, which is situated within the boundaries of Kandy city).

Additionally, there was a concentration of PM 10 at the University of Peradeniya along the Peradeniya – Doluwa – Gampola road, which is situated 6 km away from Kandy town along the Kandy-Colombo highway, which is closer to the selected semi-polluted site (Elangasinghe; Shanthini, 2008). This could also be the case for other air pollutants of traffic origin. Additionally, the Kandy Lake sampling site was situated on the hillside that borders the basin and views Kandy town. As a result, it can be said that the University location has less air pollution than the Lake site.

The majority of the plants in the study's semi-polluted sites are members of the Physciaceae, Parmeliaceae, Graphidaceae, and Lecanoraceae families. According to earlier research, the majority of the species in those groups are susceptible to pollution (Backor; Loppi, 2009). They are frequently employed as bioindicator organisms to evaluate the quality of the air at specific locations. Because of the existence of species that are susceptible to pollutants, it might be inferred that both locations are disturbed and contaminated. Additionally, there were a lot of species overall in the semi-polluted area, with the majority being crustose species. Additionally, there is little difference in the amount of foliose and crustose in polluted sites. Compared to foliose and fruticose lichens, crustose lichens are known to withstand hard environments better. But the foliose that grows in the contaminated area needs to be a tolerant species.

The absence of fruticose species at both locations raises the possibility that both habitats are contaminated and disturbed. Out of the three lichen growth forms, fruticose lichens are generally considered to be the most susceptible to air pollution, followed by foliose lichens, which are immediately sensitive, and crustose lichens, which are more resilient to pollution. Only foliose and crustose species were found in both sites, indicating a modest level of air quality.

Yavuz & Çobanoğlu (2019) conducted the first bio-monitoring study in Isparta using lichens as a bioindicator. *Physcia* sp., *Lepraria* sp., *Graphis* sp., and *Parmotrema* sp. were previously employed in the majority of biomonitoring projects (Benítez et al., 2019; Pitakpong; Maungsan, 2018; Stamenković et al., 2016). According to Benítez et al. (2019), *Parmotrema arnoldii* is a recommended organism for cost-effective air pollution monitoring in tropical nations. Here, it may be demonstrated that *Parotrema* sp. is a useful organism for biomonitoring. Additionally, the five species that were chosen for this study have already been employed as bioaccumulators and bioindicators.

Lichens are useful markers of heavy metal deposition, especially those belonging to the family Parmeliaceae (e.g., species of the genus *Parmotrema*). concur well with other research that measured heavy metal deposition indirectly using different *Parmotrema*species, such as *Parmotrema chinense*, *Parmotrema crinitum*, *Parmotrema reticulatum*, and *Parmotrema tinctorum* (Benítez et al., 2019). According to Giordani et al. (2012), lichens are thought to be sensitive bioindicators of biodiversity dynamics, forest architecture, climate change, and air pollution. They are organisms that grow slowly, so they can stay in one spot for a very long time. They absorb contaminants and moisture through the thallus surface because they lack roots and a protective layer (Garty, 2001; Wolterbeek et al., 2003). The levels of air quality for each element are indicated by the quantities of atmospheric contaminants in the thalli body (Bari et al., 2001).

There were other factors influencing the morphological, anatomical, chemical, and fertility changes in lichens besides the sites' air quality levels. The characteristics of a specific element, height, topographic features of the places, wind direction, humidity, temperature, and other environmental elements influence the alterations as well as the distance to the source of pollution (Çobanoğlu, 2015; Parzych et al., 2016). Compared to other plants, lichen species are more susceptible to environmental changes because of their high sensitivity to contaminants (Nimis et al., 2002; Dymytrova, 2009).

A site's transition from a semi-polluted to a polluted state is directly impacted by high particulate matter depositions on the thallus. Lichen development and fertility are inhibited by changes in ambient air quality brought on by air pollution (Valina et al., 2019). Thus, it could be the cause of the contaminated site's smaller thalli area than the semi-polluted site. The thallus size of the semi-polluted location was larger than that of the polluted site, suggesting that the lichens' development rate and thallus size can be influenced by harsh environmental conditions. The majority of lichen diversity surveys revealed that rural areas have higher lichen richness and variety indices than urban areas (Pitakpong; Maungsan, 2018).

The majority of research on bioindicators is mostly focused on abundance and diversity indices. There is currently a dearth of research on lichen bioindicators of air pollution, particularly regarding the impact on the tallus's morphological and anatomical characteristics (Valina et al., 2019). Because these cortical structures have negatively charged anionic sites, heavy metals tend to collect on the surface layers of lichen cell walls, with only trace amounts of supplied metals often found intracellularly in algal and medulla layers. It has been discovered that lichens have an upper cortex layer that contains water-repellent proteins (Wessels, 2000; Dyer, 2002; Scherrer et al., 2002). These proteins are exclusively produced by the mycobiont in the cortex and medulla layers, and they may help further shield the thalli from the indiscriminate intracellular penetration of solutions containing heavy metals (SkowroNska, 2002). Worldwide, air quality evaluations and monitoring programs have made extensive use of changes in lichen diversity as an indication of environmental conditions (Djekic et al., 2017).

It has previously been noted that populations of rock-growing *Ramalina capitata* that face south exhibit thicker thalli, mostly as a result of thicker medullas, as well as a greater capacity to retain water (Pintado et al., 1997). The contaminated site's increased medulla thickness suggested that it had more thallus porosity than the semi-polluted site, as well as a higher water storage capacity. Because of the severe drought and dry circumstances, polluted sites require a larger capacity for storing water than semi-polluted sites (Valladares, 1994; Souza-Egipsy et al., 2000).

The morphological changes caused by the high acidity conditions in the polluted site include a progressive reduction of the surface amorphous layer, which is primarily made up of conglutinated and thickened hyphal walls on the surface. These walls are joined together to form an outside surface that is roughly smooth. It showed that the upper cortex, medulla layer, and lower cortex were thicker in polluted sites than in semi-polluted sites, owing to the high acidity conditions there (Anglesea et al., 1982). Additionally, there is a connection between drying and acidic conditions and the density of the fruiting body (Ahmadjian, 1973). The apothecial density of *Lecanora*sp. was lower in polluted sites than semi-polluted sites because of the high acidity conditions in the former.

According to earlier reports, the upper and lower thalli surfaces may have different metal absorption capacities (Goyal; Seaward, 1981a, 1982b). In a contaminated site with a high metal supply, the bottom surface's capacity increased while the top surface's capacity decreased. It indicated that lower layers have a larger capability for accumulation than upper surfaces. The findings show that the polluted site has a higher medulla layer and lower cortical thickness than the semi-polluted site. It suggests that metals are abundant in the contaminated area and that these metals build up on the thalli's bottom surfaces. Furthermore, it suggested that the mycobiont had a higher ability for accumulation and translocation than the phycobiont (Goyal; Seaward, 1982a). Under conditions of high metal supply, rhizins were shown to acquire the highest amounts of metals (Goyal; Seaward, 1981).

The polluted site exhibits reduced thalli size and original length, as well as increased thickness in the lower, medulla, and upper cortex, likely due to metal tolerance and detoxification of the thalli. A thicker medulla would shield the phycobiont from direct exposure to harmful compounds at a polluted site, whereas the smaller thallium size in the Lake Site would minimize the interception and absorption area to the metal compounds while preventing the accumulation of the metals. Conversely, foliose has an equal surface area, which encourages an equal buildup of metals (Gailey; Lloyd, 1986).

A sizable, comparatively elevated surface area in a contaminated *Lepraria* sp. and *Lecanora* sp. is linked to acidic precipitation in reaction to both improving air quality and rising pollution levels (Leblanc et al., 1974;

Henderson-Sellers; Seaward, 1979; Seaward, 1980; Nash, 1988; Oksanen et al., 1990; Cislaghi; Nimis, 1997). Metal accumulation was thought to have an impact on and/or determine thalli size and thickness as well as the relative thicknesses of algal and fungal tissue layers (Goyal; Seaward, 1982; Brown, 1991).

Four lichen species were used to study the effects of air pollution on morphology and reproduction. The results showed that in polluted sites, thallus size decreased and darkened, the upper cortex thickened, and the number of apothecia per unit area rose. (Canas et al., 1997). Comparing the *Physcia endochrysea* to the less contaminated site revealed a few modest differences. The results showed a considerable difference between the semi-polluted and polluted sites. Additionally, they discovered that there was little difference in the upper and lower cortex thickness between the sites among the four species they had chosen.

In *Physcia* undulate, there was no decrease in thallus size, but there was a decrease in upper cortex thickness in the polluted site. The lower cortex thickness was identical across all sites. Ultimately, nevertheless, they concluded that spore counts were lower, apothecia counts were higher, and lichen diversity was lower in metropolitan locations (polluted sites). The majority of the species, overall, displayed higher thallus areas in semi-polluted sites compared to polluted sites, increased apothecial numbers in polluted sites, and no change in spore numbers, according to the data.

Based on the findings at the Lake Site, where the top cortex thickness was higher than at the University Site, it can be inferred that the thick layer may have developed as a defense against the infiltration of air contaminants. Furthermore, the algal layer dies back at polluted sites due to chlorophyll degradation, resulting in a higher algal layer thickness than in semi-polluted sites. Chlorophyll deterioration was previously seen in the vicinity of industrial areas, with particular attention paid to the deposition of Al, Ca, Cu, and Fe on the surface of lichens. These deposits have an impact on the algal layer's dieback and cause obvious necrotic alterations (blackening) in the thallus structure. Thus, the thickening of the algal layer and the dark thallus in the Lake Site demonstrated the significant deposition accumulation on the thallus surface.

Consequently, thylakoid degeneration at a polluted site may be influenced by the deposition (Paoli et al., 2010; Pirintsos et al., 2011). Chemical components can settle on the lichen's surface as dry dust particles, dissolved material, or suspended material in precipitation. Additionally, following deposition, they might stay imprisoned in the medulla layer's intracellular spaces, which would impact the physicochemical processes (on exchange) (Garty et al., 1979). Rainfall during a dry spell helps remove accumulation. Since the majority of the samples in this case were taken during the rainy season, it's possible that the majority of the hazardous heavy metals were removed from the thallus. The majority of the Ca, Fe, and Ti gathered as a result of dust deposition from quarrying operations and cement works at the cement mill have a major impact on the lichen thallus' ability to photosynthesize. In those days, a lot of construction was seen in the Kandy area. Consequently, it could be the cause of the detection of Ca and Fe in both semi-polluted and polluted areas.

Compared to methods based on lichen abundance and diversity, the current study's methodology is comparatively quick and easy to implement (Stamenković et al., 2016). According to numerous studies, heavy metals like zinc (Zn), copper (Cu), lead (Pb), and cadmium (Cd) are some of the most harmful air pollutants. However, only Cu, Mn, and Cr were discovered in the Lake Site in the current investigation. This could be because Kandy is free of industrial and power plant pollution.

These pollutants, such as cadmium, copper, lead, and manganese, have been linked in several studies to both direct and indirect exhaust emissions from motor vehicles caused by the combustion of fuel and lubricant. Indirect sources of these emissions include catalytic converters, particulate filters, suspension, lubricating oils, engine corrosion, and tire wear and tear. According to Pungin & Dedkov (2017), *Xanthoria parietina* and *Physcia adscendens* are regarded as eutrophication indicator species. It has been demonstrated that the lichen bioindicator communities and local climate variables are correlated (Duman et al., 2015; Bolshunova et al., 2017; Bajpai et al., 2018). Depending on the climate, different lichen species have demonstrated varying degrees of susceptibility to air pollution; nonetheless, nitrogen oxide and sulfur dioxide have typically been found to be harmful to them (Danesh et al., 2011; Duman et al., 2015; Stamenković et al., 2016).

Lepraria sp. is a species of crustose lichen that is thought to be worldwide and resistant to air pollution (Taufikurahman et al., 2010). The findings showed that all species in both sites have metallic compounds in the form of Fe, Ca, and K. Cu, Cr, and Mn are only observed at the Lake Site in *Parmotrema* sp. Sr is only found in the university site of *Lecanora* sp., whereas Cu, Cr, and Mn are only found in the lake site. Cu and Mn are exclusively visible in the Lake Site of *Physcia* sp. Except Fe, Ca, and K, Ti and Ba are visible at both sites in *Graphis* sp. Ba, Ti, and Zr can only be seen in university settings in *Lepraria* sp., whereas Mn can only be seen at the Lake Site. Only a small portion of Ca and dust deposition occurs intracellularly; the majority occurs extracellularly (Garty;

Spitz, 2011). Metals linked to dust can exacerbate intracellular oxidizing conditions and the production of free radicals, which can damage thylakoids, cause lipid peroxidation in plant cell membranes, and hasten cell senescence (Mehta et al., 1992; Foyer et al., 1994).

The results of the metal compounds show that Fe, Ca, and K are present in every site and every species. It can be because of the location's closeness to the ocean or the elements' accessibility in the crust of the earth (Gunathilaka, 2011). K was discovered at both locations, which would indicate a high level of air pollution. Furthermore, Ca serves as a reliable marker of airborne dust from highways (Laaksovirta; Olkkonen, 1979). Land preparations, exposed land masses, and unpaved roadways may be excellent sources of calcium from the thallus (Hissler et al., 2008). There are some more development projects in Kandy City.

Due to the abrasion of metals from vehicle exhaust, the development of roadside environments, iron mine smelting, and flying ash (burning coal) all increase the availability of Fe in thallus (Rühling et al., 1994). Ti was discovered in both locations, which could be related to industrial uses (white paint pigment). It also demonstrated how contaminated and disturbed those two locations are. Due to metal abrasion from car exhaust, the roadside environment may be resentful of all harmful components. Cu was only found in the contaminated area, mostly as a result of the Kandy Lake area's vehicle exhaust. Pb, Cu, Ti, Fe, and Zn are examined as heavy metals in certain projects (Gunathilaka et al., 2011). The results showed that although heavy metals were present in both sites, the majority of the hazardous heavy metals were only present in the polluted site.

The build-up of heavy metals and other contaminants on the thallus area is caused by a rise in the concentration of biosorbent, which raises the concentration of absorbed ions due to an increase in the adsorption area. Here, the majority of the species are abundant on the surface of the university site, although the contaminants may be low in the semi-polluted area. Because of the high concentration of pollutants, heavy metals in all species are only visible in the Lake Site. It is recommended that future research focus on measuring SO_2, NO_2, and O_3 to verify the degree of pollution.

5. Conclusions

The *Thallus* shape, anatomy, chemistry, and fecundity of the lichens may be impacted by the amounts of air pollution in two locations. Furthermore, the rise or fall depends on how each parameter helps the system endure shocks. The diversity of lichens is larger at university sites than at contaminated ones. The study demonstrated that the chosen *Physcia* sp., *Parmotrema* sp., *Graphis*sp., and *Lecanora* sp. can be used to measure atmospheric air quality. Consequently, the study's significance lies in the possibility of developing lichens as bioindicators or bioaccumulators to measure air quality. The biomonitoring method is economical and environmentally benign. It is recommended that future research focus on measuring SO_2, NO_2, and O_3 to verify the degree of pollution. Subsequent research endeavors ought to focus on quantifying SO_2, NO_2, and O_3 to validate the degree of pollution, gathering data every month, and utilizing the spore rain method for in vitro spore germination.

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7. Authors' Contributions

Edirisinghege Sanduni Madushika Edirisinghe: write the article, conduct the experiment, data collection, and analysis, development and design the methodology. *Athukoralage Dona Sarangi Nirosha Priyajeevani Athukorala*: conceptualization came up with the research idea, supervision throughout the research project, provision of study materials, development and designs of the methodology, and acquisition of financial support, review, and editing.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Not applicable.

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