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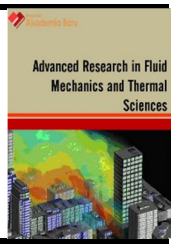

Identification of Most Tolerant Lichen Species to Vehicular Traffic's Pollutants: A Case Study at Batu Pahat

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ABSTRACT

Bio-indicators are organisms that can be used for the identification and qualitative determination of human generated environmental factors. The decreasing population of sensitive lichens in specific regions around the world due to low air quality level has make lichens as a bio-indicator for air pollution. Lichen is a result of symbiotic association of fungus and alga and well known for having wide variety of sensitivity towards environmental stressors such as air quality and climate change. This study aims to identify the most tolerant lichen species to vehicular traffic's pollutant at Batu Pahat urban and suburban areas by using Index of Atmospheric Purity (IAP) method. The color spot test, thin layer chromatography (TLC) profiling and morphological analysis were employed for species identification. The results have shown that Dirinaria picta has been identified as the most tolerant lichen against pollutants from vehicle traffic. The results also indicated that the air quality of Batu Pahat town/urban area could be considered as moderately clean.

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1. Introduction

Air pollution has become major environmental concern in many countries. The increasing of atmospheric pollutants has shown tremendous effect to the environment as compared to the "traditional" pollutants such as sulphur oxides, ozone and nitrogen oxides. Efforts to establish control program to many countries around the world have been reinforced due to the increasing anthropogenic sources of pollutants such as heavy metals, halogenated organic compound and

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polycyclic aromatic compound. One of the important anthropogenic sources of pollutants is from vehicular traffic such as motorcycles, cars, buses, lorries, trucks, etc. A study has indicates that vehicle flow in the road network is an important indicator of atmospheric pollution, as mobile sources are the main producers of emissions of such pollutants especially in urban areas [1]. The similar findings have been reported the correlation between exposure to atmospheric pollutants and its association with respiratory and cardiovascular diseases in human [2,3].

The increasing disappearance of sensitive lichens in specific regions due to the higher level of harmful substances have made lichen group as a bio-indicator for air pollution. Lichen is well known for having wide variety of sensitive towards environmental stressors such as air quality and climate change. It is also very sensitive to wide range of habitat changes mostly from human activities. A previous study has shown that lichens have been used as bio-indicator for air quality since 1866 [4]. Lichen is a result of symbiotic association of fungus and alga. The term “alga” is referred to Cyanobacteriae or Chlorophyceae. Both are classed in Ascomycetes group. Occasionally, the association is either Basidiomycetes or Phycomycetes whereas bio-indicator is the organisms that can be used for identification and qualitative determination of human generated environmental factors [5]. This characteristic has made lichens as useful indicators to monitor the air quality.

The ability of lichens to absorb toxic materials such as sulphur dioxide (SO_2), flourine (F_2), and nitrogen dioxide gas (NO_2) into the thallus system for a longer period has developed the lichens into valuable indicators of air quality [4]. There are several studies performed on lichens as bio-indicators and bio-monitors of atmospheric pollution around the world but there are limited similar studies have been carried out in Malaysia [6,7]. It is beneficial to study about the biodiversity of lichen group as they can show early signs of environmental disruption particularly in atmospheric pollution system [8].

Lichen does not have cuticles to control the exchange of nutrient, gas and water with the external environment. They can only perform absorption from atmosphere and not from the root system. Lichens have slow rate of growth and a slow rate of tissue repair (damaged tissue) [4]. Lichens are used as prevention system to public health especially for air pollution related diseases. In addition; lichen monitoring method is believed to give an alternative to the conventional monitoring system [6]. Thus, this study aimed to identify the most tolerant lichen species to vehicular traffic’s pollutant at Batu Pahat urban and suburban areas by using Index of Atmospheric Purity (IAP) method.

2. Methodology

Methodology consists of two main parts, that are sampling technique and species identification tests, which involves color spot test, thin layer chromatography profile and morphological analysis.

A. Sampling Technique

Sampling techniques was carried out according to the methods described by Freitas and Rodrigues [2], Pinho *et al*., [9] and Loppi *et al*., [10] with modification. Study was done in Batu Pahat area prior to locations which consist of homogenous and healthy host trees and suited the criteria required for the Index of Atmospheric Purity (IAP) method [11]. IAP method is based on number (n), frequency (F) and tolerance of the lichen present in the area under study

$$IAP = \Sigma Fi \quad (1)$$

where F is the frequency of every i^{th} species that is calculated as number of rectangles in the grid used in the study.

Royal palm (Roystonea regia) with circumference greater than 60 cm and situated from three (3) to four (4) meters from the road traffic has been chosen as host tree. Eight (8) host trees were selected at each station and grid is attached from 100 to 130 cm above the ground level. Measuring tape was used to measure the height of the grid. It was attached to the area of bark which facing the same source of air pollutants in different stations. The stations were identified as sample area due to the frequent activity of motorized vehicles Number of vehicles was calculated manually during the observation to determine the level of the exposure. Observation was done during peak hours from 11 am to 3 pm for three days. A survey has been done for a few weeks to identify the most peak hours of vehicular traffic density prior to observation. Four locations were identified based on survey study of average number of vehicular traffic density and observation in order to determine the level of exposure to the pollutant; two in Batu Pahat town, one at the recreational and residential area of Bukit Soga and one at main entrance of Universiti Tun Hussein Onn Malaysia (UTHM) campus.

The study was conducted at the identified locations using grid of 30 x 50 cm subdivided into 10 rectangles (Figure 1). Every rectangle consisting lichen species were recorded and identified. The frequency of the species was recorded in a form/table. The form was provided for each tree unit and total of the frequencies of species were calculated using the data recorded in the form.

Fig. 1. Grid of 30 x 50 cm subdivided into 10 rectangles attached to tree bark

B. Color Spot Test

Color spot test was conducted by chemical reagents that are potassium hydroxide (K) and sodium hypochlorite (C). For the K and C tests, a few drops of 10% solution of potassium hydroxide and sodium hypochlorite were used respectively. For the KC and CK tests, a few drops of solution of K followed by C or C followed by K were used alternately on the same fragments of the spots [12].

C. Thin Layer Chromatography Profile

The sample preparation prior to thin layer chromatography (TLC) profiling was adapted from Rahman *et al.*, [13] with modification. The samples were kept in air dried condition at room temperature and foreign debris on thallus was removed. Samples were grounded into powder

using a ceramic mortar and pestle. Grounded powders were extracted using acetone solution at room temperature for approximately three days. The samples were then filtered through Whatman no. 93 (125 mm) filter paper and extracts were placed in the fumes chamber to dry. The excess samples powder was soaked again for another three days. The previous extracts were filled in the same beakers and placed in the fumes chamber to be dried for collecting the crude extract materials.

Thin layer chromatography (TLC) analysis was done according to the method of Culberson and Kristinsson [14] with modification. Chromatograms were developed in glass tank on aluminium plate coated with 0.20 mm Kieselgel 60 F_{254} (Merck, Germany) to a height of 5 cm. The TLC has been performed by employing two types of solvent system (A and C). Solvent system A consists of toluene, dioxane and acetic acid with ratio of 180:45:5, while solvent system C consists of toluene and acetic acid with ratio of 170:30. The developed plates were air-dried to evaporate the solvents followed by visualizing under UV light (254 nm) and sprayed with 10% H_2SO_4. Subsequently, plates were heated until the colors developed. The retention factor (R_F) of each spot was recorded and calculated. The calculated R_Fs were compared with standard value to identify the presence of lichen substances.

D. Morphological Analysis

A light microscope (Olympus, UK) was used for morphological analysis of the thallus [12].

3. Results and Discussions

Table 1 shows the observation of the average number of vehicles on selected stations

Table 1
Sampling locations and average number of vehicles.

Number of Station	Location of Sampling	GPS Location	Average Number of Vehicles / hour
1	Dataran Penggaram	N 1° 50' 51.81" E 102° 56' 4.3548"	739 ± 1.00[a]
2	BP Mall Entrance	N1° 51' 48.6216" E102° 57' 42.8976"	621 ± 2.08[b]
3	UTHM Entrance	N1° 51' 9.954" E103° 5' 11.6118"	585 ± 2.89[c]
4	Bukit Soga	N1° 50' 53.8584" E102° 57' 37.9872"	27 ± 3.79[d]

[a – d] Mean within each row with different letters differ significantly ($p < 0.05$). Each values presented as mean ± standard deviation (n = 3).

Data distribution of lichens species at four different stations are summarized in Table 2. The data has indicated that lichen A has the highest number of colonies identified in every station. In Station 4, species A dominates the distribution as it has the highest number of colonies identified among other stations. However it reduces in number of colonies identified from 79 (Station 4) to 32

(Station 1) as the average number of motor vehicles per hour increase significantly ($p < 0.05$) from 27 (Station 4) to 739 (Station 1).

Table 2
Number of identified lichen species at sampling locations.

Number of Station	Number of Lichen Colonies						
	A	B	C	D	E	F	G
1	32	-	-	-	-	-	-
2	39	-	-	-	-	-	-
3	56	4	2	2	5	-	-
4	79	52	18	13	6	30	12

Lichen B which marked as second highest number of colonies found at sampling locations, also shows reducing number of species collected as it reduces from 52 (Station 4) to 4 (Station 3). The result also indicates that no lichen B is observed at Station 1 and Station 2 as the number of vehicles increased. The other species (lichen C, D and E) have also shown similar pattern of reducing. However, the lichen F and G (present only at station 4) may show higher sensitivity compared to other species in this study.

Seven (7) species of lichens were observed at Station 4 indicating that the air quality of the station is acceptably good compared to other stations. It can be deduced based on the lowest number of vehicles observed at the station, as Bukit Soga is a recreational and low density residential area. In contrast, Dataran Penggaram (Station 1) and BP Mall (Station 2) demonstrated that only one species was identified at both of the station. This might be due to the higher concentration of air pollutants from mobile vehicle traffic as Dataran Penggaram is located at the central of Batu Pahat town. This area is considered as busiest place due to several public facilities such as banks, post-office, retail shops, petrol station, and etc. are located nearby the sampling station.

Fig. 4. Four studied location (a) Station 1 (b) Station 2 (c) Station 3 (d) Station 4

A previous study has indicated that the density of traffic showed a statistically significant positive correlation with vehicle pollutants, that are carbon monoxide (CO), benzene, 1,3-butadiene and nitrogen dioxide (NO_2) [1]. In addition, the station was surrounded by three to four storey buildings and therefore the particulate materials and black carbon from the motorized vehicles can be trapped by the lichens thallus, In addition, the location can be considered as leeward side of the

area [15]. Nonetheless, the observation of lichen community at station 2 showed not much different in number when compared to Station 1. This could be due to the location of host tree that located at about three (3) to four (4) meters from the federal road, traffic light, bus stop and parking area. Meanwhile, Station 2 is located at windward area since the residential area and retail shops location opposite the station consist of single storey buildings. Station 3 indicated as better area for lichen survival compared to Station 1 and 2, since there is no building surrounded the area, and the number of vehicles passing the area was significantly lower ($p < 0.05$) from Station 2. Figure 4 shows the selected locations observed in this study.

Table 2 has shown that lichen A community were present at all stations. This could be indicated that lichen A is highly resistant against vehicular traffic's pollutant in comparison to other species identified in this study. Therefore, further study was conducted to identify the species of lichen A. Table 3 indicates the results of chemical test for lichen identification.

Table 3
Chemical test for identification of lichen A.

Station	Type of test			
	K	C	KC	CK
1	+	-	-	-
2	+	-	-	-
3	+	-	-	-
4	+	-	-	-

Lichen A of all station showed positive reaction with K reagent where yellow color has been seen immediately after dropping the reagent into the cortex of the specimens. However other tests gave negative reaction. It can be deduced that lichen A has shown similar characteristic of *Dirinaria* sp. [12]. Further analysis for routine identification of lichen's product by TLC method was performed to confirm the genus of lichen A [10] (Figure 3).

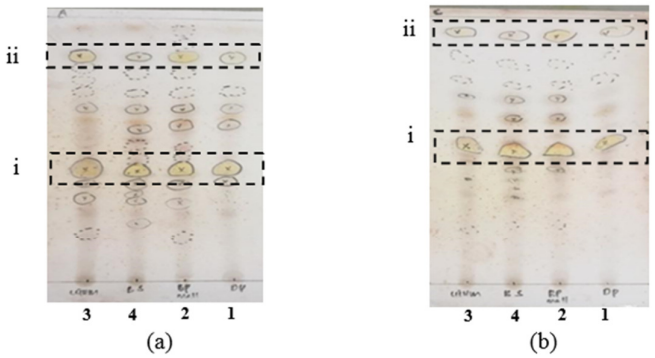

Fig. 3. TLC profile of lichen A from all stations and with compound i and ii. (a) Solvent system A; (b) Solvent system C

Table 4 summarizes data obtained from the TLC test. TLC analysis was done to determine the presence of divaricatic acid and atranorin. This is due to literature report which indicated that these two lichen substances were among the marked compounds in *Dirinaria sp.* [12].

R_F values and color of compound i and ii were found in an accordance with divaricatic acid and atranorin respectively (Solvent system A: 39 and 75; Solvent system C: 51 and 79; yellow) [16] and (Solvent system A: 41 and 70; Solvent system C: 48 and 67; yellow) [14]. It is unlikely that these R_F

values could be reproduced exactly in another laboratory, so the slightly different in R_F value is still could be accepted [14].

Morphological study was conducted to ensure the type of species A as *D. applanata* and *D. picta* is morphologically related to each other [17]. Result showed that lichen A has thallus from two (2) to seven (7) cm wide but not longitudinally plicate or rugrose, tightly adnate, lobes were slightly flat and overlapping, while apices are not flabellate (Figure 4). These characteristics are identical to *D. picta* as described by Jayalal *et al*., [12].

Fig. 4. Habit (a) and close-up of the lower surface (b) of Dirinaria picta

Table 4
The TLC analysis of lichen A

Compounds	Solvent System R_F x 100 values		Spot Coloration With H_2SO_4 and Heat
	A	C	
i	38	50	Yellow
ii	74	73	Yellow

Thus, this study has shown that the most tolerant lichen species to vehicular traffic's pollutant at all study area of Batu Pahat was *D. picta*. This result was in accordance with Elix [18] which reported that this species distributed from pantropical to subtropical regions. Study by Abas [6] also indicated that *D. picta* was the most dominant lichen species at Kuala Lumpur area.

4. Conclusion

This study deduced that lichens can be used as bio-indicator of air pollution. It is due to the ability of specific lichens species to survive and exhibit response to atmospheric pollutants from different emission sources including gaseous from motorized vehicle traffic. *D. picta* has been identified as the most tolerant lichen species at the studied area due to their presence at all locations albeit higher number of motorized vehicles passing nearby the location. Since *D. picta* was categorized as lichen with moderately resistant to air pollution [7,19,20], so it can be concluded that the air quality of town/urban area of Batu Pahat could be considered as moderately clean.

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