EnvironmentAsia
The international journal by the Thai Society of Higher Education Institutes on Environment

EnvironmentAsia 12(3) (2019) 85-90
DOI 10.14456/ea.2019.48
ISSN 1906-1714; ONLINE ISSN: 2586-8861

Using Lichen (*Dirinaria* sp.) as Bio-Indicator for Airborne Heavy Metal at Selected Industrial Areas in Malaysia

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Received: August 17, 2019; Revised: January 8, 2019; Accepted: June 9, 2019

Abstract

In Malaysia, air pollution still measured using instrumental approach. Hence, this study used lichen as biological indicator to assess air pollution. Study has been conducted in four different locations which are Rumah Tumbuhan, UKM (Control station), Section 51 Industrial Area, Petaling Jaya, Spring Crest Industrial Area, Batu Caves and Nilai Industrial Area, Negeri Sembilan. This study used lichen *Dirinaria* sp.; an endemic species of tropical country; as the biological indicator. Heavy metal concentrations in lichen samples been analyzed using ICP-OES and number of motor vehicles were recorded for every sampling location. One way ANOVA and Pearson's correlation used to test the relationship between heavy metals and sampling locations also relationship between heavy metals and motor vehicles. Result shows that heavy metals such as Cr, Fe, Cu, Ni, Zn and Pb have been recorded. One way ANOVA test shows there is significant relationship between heavy metals and sampling locations where F is 2.7728 and P value is 0.0001 (99% significant level). Pearson's correlation also shows the relationships between all recorded heavy metals with number of motor vehicles where all the P value is < 0.05. This study found that lichen can be used as the alternative approach in determining the heavy metals content in the environment and it also cheaper and time saving rather than using instrumental approach.

Keywords: Heavy metals; Lichen; Air pollution; Environment; Urban ecosystem

1. Introduction

Human well-being has been degrading tremendously due to the air pollution that caused by industrialization and urbanization in the city. In Malaysia, The Sustainable Development Goals has been tackled in order to sustain the human well-being in the city and also action has been taken to create livable vicinity (Abas *et al*., 2018b). To tackle the issue of the degradation of human well-being in Malaysia due to air pollution, The Department of Environment has outlined few protocols to be followed (DOE, 2018). But, in term of the measurement it still not sufficient. The three major causes of air pollution are transportation, stationary sources and open burning (Jassim *et al*., 2018). The problem they face now is that the quality of air in areas far away from their air monitoring stations is difficult to determine, hence lichen has been selected as a potential bio-indicator for this purpose.

Heavy metals can be measured and analyzed using two approaches which are sampling directly from the atmosphere and sampling using biological indicator. According to Gharaibeh *et al*. (2010), High volume air samplers and glass fiber filters were used to collect the samples containing heavy metals. Collected samples were digested using a mixture of analytical grade nitric acid and analytical grade hydrochloric acid, and analyzed to evaluate the levels of heavy metals by atomic absorption spectrophotometry. Heavy metals also can be sampled from lichen where selected lichen from specific sampling location brought to the lab and analyzed using atomic absorption spectrophotometry (Wahid *et al*., 2013). Heavy metals also can be found from the dust inside building as studied by Ali *et al*. (2017) where they stated heavy metals such as Fe, Pb, Zn, Cu and As are existed inside the university building which can bring harm to the occupant health.

Lichen has been used as bio-indicator for air pollution since decades ago. In Italy, a standard called Lichen Biodiversity Index been developed and used to monitor air pollution in the district of Faenza, Italy (Cioffi, 2009). Also, Loppi and Frati (2006) conducted a research in Central Italy measuring the nitrogen compounds in foliose lichen. Foliose-type lichen *Hypogymnia physodes* also been used and collected to analyze the heavy metals contents due to traffic pollutants (Koroleva and Revunkov, 2017). Transplanting foliose type lichen from remoted and clean air area to much more polluted area is widely used to monitor the air pollution in certain vicinity. As examples, in Thailand they used *Parmotrema tinctorum* tp monitor airborne trace elements near a petrochemical industry complex (Boonpeng *et al*., 2017). Apart of assessing outdoor pollution, transplanted lichen also used to indicate the level of indoor air quality where heave metals such as As, Cd, Cr, Cu, Hg, Ni, and Pb also 12 Polycyclic Aromatic Hydrocarbon (PAH) been recorded (Kara, 2018).

In Malaysia, research on lichens more focused on lichens dwelling in the highland such as Gunung Machincang, Cameron Highland, Genting Highland, Fraser Hills and Bukit Larut. In addition, these researches only touched about the ecological and chemical part of the lichen (Din *et al*., 2010; Zulkifly *et al*., 2011). None of them studied about the relationship between lichen and its vicinity, not until 2015 where a study on lichen diversity distribution in Kuala Lumpur (Abas, 2015). The research found that lichen diversity distributions are much related with the population density in Kuala Lumpur. Also, there are few researches that focused on air pollution in urban area using lichen as the bio-indicators such as study in Bandar Baru Bangi (Abas and Awang, 2015), Klang (Abas *et al*., 2018a) and Batu Pahat (Khairuddin *et al*., 2018).

The aim of this study is to determine the heavy metal concentration using lichen as the alternative approach in air quality assessment and to analyze the relationship between motor vehicles frequency and heavy metals concentration.

2. Materials and Methods

2.1 Sampling

Lichens were collected from one control station and three selected urban stations. Rumah Tumbuhan, UKM was selected as the control station because of its location that remoted from the urban area, still sustaining the natural vicinity and less anthropogenic activity (Wahid *et al*., 2013). For sampling in the urban area, three industrial areas has been selected as the urban station which are a) Section 51 Industrial Area, Petaling Jaya, b) Spring Crest Industrial Area, Batu Caves and c) Nilai Industrial Area, Negeri Sembilan. These three selected urban areas are known as congested traffic and industrial area. Each location comprises of 4 sampling sites to have variety set of data. Figure 1-4 shows the location of each station and sampling sites. Lichens that collected were from genus of *Dirinaria*. *Dirinaria* sp. had been recorded in many urban areas such as Kuala Lumpur (Abas, 2015),

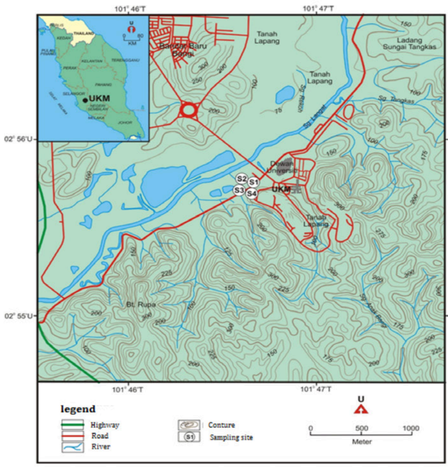

Figure 1. Rumah Tumbuhan, UKM (Control Station)

Figure 2. Section 51 Industrial Area, Petaling Jaya

Figure 3. Spring Crest Industrial Area, Batu Caves

Figure 4. Nilai Industrial Area, Negeri Sembilan

Bandar Baru Bangi (Abas and Awang, 2015) also at Penang and Selangor (Norela *et al.*, 2018). The procedures in collecting lichens for further analysis are: i) lichens collected on tree bark near to the road < 10 m, ii) selected tree bark must be facing to the road, iii) lichens must be in the range of 1-2 m from the ground and 4) motor vehicles also counted for comparative test.

2.2 Heavy Metals Analysis

The collected lichens were cleaned, oven dried at 60°C for 48 h and ground for further analysis. The ground samples then were homogenized. About 1 g of samples was weighted accurately for 3 replicates and digested using wet acid digestion procedure. The acid mixture used for digestion is 5.0 mL nitric acid 65% and 2.0 mL hydrogen peroxide 30%. After that, the mixtures were heated on the hot plate at 90°C for 4 h. The residue of digestion then was rinsed with 1 mL hydrochloric acid 2% and diluted to 100 by deionized water before carrying out the ICP-OES (Optima 4300 DV, Perkin Elmer) analysis.

Table 1. Average of heavy metals concentration

Sampling location	Heavy metals µg/g (ppm)					
	Cr	Fe	Cu	Ni	Zn	Pb
Rumah Tumbuhan, UKM (Control Station)	1.17±0.03	908.00±0.47	0.16±0.02	3.29±0.09	88.58±0.17	30.34±0.16
Section 51 Industrial Area, Petaling Jaya	24.67±0.15	5836.59±0.52	1.08±0.08	9.91±0.17	183.89±0.23	67.66±0.15
Spring Crest Industrial Area, Batu Caves	13.55±0.09	3493.49±0.31	0.83±0.06	7.59±0.20	152.67±0.19	48.93±0.20
Nilai Industrial Area, Negeri Sembilan	11.75±0.10	2673.38±0.28	0.61±0.08	4.62±0.22	117.27±0.15	39.31±0.18

Table 2. Number of motor vehicles at sampling location

Sampling location	Number of Motor vehicles (per hour)
Rumah Tumbuhan, UKM	33
Section 51 Industrial Area, Petaling Jaya	1942
Spring Crest Industrial Area, Batu Caves	1547
Nilai Industrial Area, Negeri Sembilan	1022

2.3 Statistical Analysis

One-way Analysis of Variance (ANOVA) was used in order to analyze the relationship of heavy metals content between control stations and sampling stations. Pearson's correlation also used to analyze the relationship between heavy metals content and motor vehicles frequency.

3. Results and Discussion

In this study, the heavy metals founds in lichen samples are Fe, Cr, Ni, Zn, Pb and Cu. Table 1 shows the average concentration of heavy metals content that found in *Dirinaria* sp. from all sampling sites for each location. The highest concentration is Fe followed by Zn, Pb, Cr, Ni and the lowest is Cu. All of the sampling stations have higher average concentration of heavy metals significantly compare with the control station ($P<0.05$). All of the heavy metals been recorded the highest in Section 51 Industrial Area then followed by Spring Crest Industrial Area, Batu Caves, Nilai Industrial Area, Negeri Sembilan and the lowest is the control station. Table 2 shows the number of motor vehicle recorded in each sampling station. One way ANOVA analysis for heavy metals shows there are significant differences between heavy metals concentration to the sampling locations ($P<0.05$). Pearson's correlation test also shows there are significant relationships between the frequency of motor vehicles with the heavy metals concentration in all sampling stations where r value for Cr is 0.9568, Fe is 0.9528, Cu is 0.9976, Ni is 0.9624, Zn is 0.9707 and Pb is 0.9562.

Three of the listed heavy metals (Fe, Zn and Cu) are known as the essential metal content where defined as very important in the growth of biological component. Even called as common metals, Fe also produced in large concentration by motor vehicles. Study of Monaci *et al.* (2000) stated that Fe is very rich in the leaded petrol and diesel oil, even unleaded petrol emission contain Fe in some lesser extent. That's why the sampling location with the highest number of motor vehicles passing by has the highest number of Fe. Fe has the greater number compare to all of the other heavy metals due to it excessive contents in the soils and the ability of its which can be easily oxidized to the air (Olivia and Espinosa, 2007). Zn in the other way cannot be found as greater as Fe in the vicinity. Though it also one of the essential metals, but its concentration is less higher compare to Zn. According to Zhao (2018), Zn can be emitted to the atmosphere from the corrosion process of the tire of the vehicles and also can be hazardous if found at largest number in the atmosphere. Cu is a trace element in most soils. It is an essential element for plants, animals, and people, but it also has a toxic element, which has been a major concern, to all organisms. There is strong evidence that shows Cu is one of the pollutants emitted to the atmosphere (Monaci *et al*., 2000).

The other three heavy metals (Cr, Ni and Pb) are called as non-essential metal in our vicinity which means just a little of these heavy metals in the growth of biological component. Cr is well known as the main component in car coating and painting. The deterioration of the painting or coating might be the main source of Cr in the atmosphere (Hjortenkrans, 2008). Ni emitted to the atmosphere from two main components of motor vehicles which are tire and lining brake (Monaci *et al*., 2000). The corrosion between tire and asphalt from the road makes the Ni emitted to the atmosphere and later enter the food cycle of the biology (Hjortenkrans, 2008). Pb is the major compound and very excessive in the leaded petrol and diesel. The emission of fuel cell contains high concentration of Pb and it will be hazardous to the biology when occur at high concentration (Olivia and Espinosa 2007).

4. Conclusion

Based on this study, heavy metals occurrences are very much related with the density of traffic in the particular location. More congested the location with motor vehicles, the higher it will be accumulated with heavy metals. Heavy metals in all lichen samples from study area showed significant relationship with motor vehicles frequency where increment in motor vehicles frequency will also increase heavy metals in lichen. Motor vehicles have been the main contributor in emitting heavy metals to the atmosphere which is later ending up in the ecosystem such soils and water. In Malaysia, we only have the regulations for freshwater and marine towards heavy metals content but no regulations for heavy metals in the atmosphere which is something that can be ponder and should be discussed in the future.

The utility of lichen as the biological indicator should be enhance in Malaysia. Practically, lichens are much more relevant where we can find it on almost tree bark in Malaysia. So there is no need for observation station for air quality or portable machine in conducting air quality assessment generally, heavy metals analysis, specifically.

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