ANALYSIS OF HEAVY METAL CONCENTRATION USING TRANSPLANTED LICHEN *USNEA MISAMINENSIS* AT KOTA KINABALU, SABAH (MALAYSIA)

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Abstract. Heavy metals were emitted from motor vehicles and industries as part of the effects of rapid urbanization. This study aims to measure the heavy metal concentrations (Cu, Fe, Zn, Pb, Mn, Cr) at Kota Kinabalu, Sabah using transplanted lichen. Samples of *Usnea misaminensis* were transplanted to the environment of the urban area. The lichen was collected from Mt. Kinabalu Park which is a remote area. Fifteen sampling stations were selected and transplanted lichens were exposed to heavy metals in those stations for about 4-6 weeks. Exposed lichens were analyzed using the inductively coupled plasma mass spectrometry (ICP-MS) to determine the concentration of heavy metals in each sample. One-way ANOVA also has been used to test whether there is significant variation between heavy metals concentration at Kota Kinabalu, Sabah. The result showed that iron (Fe) has the highest concentration with 84.43 µg/g and chromium (Cr) has the lowest concentration with 0.66 µg/g. A statistical One-way ANOVA test showed that there is a significant variation between heavy metal frequency with P-value is 0.0000 < 0.05. A Tukey test also revealed that Fe has significantly higher concentration compare to the others. These findings prove that the increasing number of motor vehicles will also elevate the concentration of heavy metals in the atmosphere. Transplanted lichen can be the alternative approach in assessing air pollution in Malaysia’s urban area.
Keywords: *lichen, air pollution, urban ecosystem, applied ecology, environmental management, Malaysia*

Introduction

Human well-being has been degrading tremendously due to the air pollution that caused by industrialization and urbanization in the city. In Malaysia, strategies to tackle environmental issues have been enacted by enforcing several policies such as National Policy on Environment (DASN) and the ratification of multi-lateral environmental agreement for example Paris Agreement 2015 (Sulaiman et al., 2018). These strategies were designed in order to sustain the human well-being in the city and also actions have been taken to create a liveable vicinity. One of the most common causes of the degradation of human well-being in Malaysia is air pollution according to statistics 54% of Malaysia’s happiness disturbance was from air pollution. The Department of Environment has outlined a few protocols to be followed. But, in terms of the measurement it still not sufficient. The three major causes of air pollution are transportation, stationary sources and open burning. 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 Carreras and Pignata (2002), High volume air samplers and glass fiber

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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 (Samsudin et al., 2013a). Heavy metals also can be found from the dust inside a building as studied by Abas 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 for 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 research in Central Italy measuring the nitrogen compounds in foliose lichen. Foliose-type lichen *Hypogymnia physodes* also been used and collected to analyse the heavy metals contents due to traffic pollutants (Blicharska et al., 2016; Koroleva and Revunkov, 2017). Transplanting foliose type lichen from remote 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* to 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 heavy metals such as As, Cd, Cr, Cu, Hg, Ni, and Pb also 12 Polycyclic Aromatic Hydrocarbon (PAH) been recorded (Abas, 2015; Protano et al., 2017). In Malaysia, research on lichens more focused on lichens dwelling in the highland such as Gunung Machincang, Cameron Highland, Genting Highland, Fraser Hills, Pulau Pangkor and Bukit Larut (Sulaiman et al., 2018; Abas et al., 2019). 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 the relationship between lichen and its vicinity, not until 2015 where a study on lichen diversity distribution in Kuala Lumpur (Abas and Awang, 2017). The research found that lichen diversity distributions are much related to the population density in Kuala Lumpur.

The study of monitoring air pollution using lichen in Malaysia is still based on lichen diversity and frequency. There is still lacking in using transplanting method to monitor air pollution in Malaysia especially in an urban area. Therefore, the aims of this study are; to analyze the heavy metals contents using transplanted lichen *(Usnea minaminensis)* as the alternative approach in air quality measurement and to determine the relationship between motor vehicles frequency and heavy metals concentration.

Methodology

Area of study

The City of Kota Kinabalu, Sabah (5.9804° N 116.0735° E) is situated at the west coast of Sabah, part of Borneo Island. As one of the largest city in Malaysia, Kota Kinabalu has a total population of 457,326 people with density of 1,463/km² according to census in 2018. Kota Kinabalu has developed into an over-concentrated urban area where recent studies showed the air quality in Kota Kinabalu has depleted.

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Sampling procedures

Lichen *Usnea minaminensis* samples were collected from remote area (unexposed from excessive air pollution) of Mt. Kinabalu Park, Sabah, Malaysia (6.0192 N 116.5369 E). All samples were segregated into 45 samples for 15 sampling locations and only one sample was excluded from the segregation to be the control sample. *Table 1* and *Figure 1* show the location of 15 sampling stations that have been selected randomly around the City Centre of Kota Kinabalu, Sabah. This research was conducted using lichen transplanting method where lichen *Usnea minaminensis* was taken from remote area then placed on selected stations for approximately 1 month (from 20th Aug 2018 to 20th Sept 2018). About 50g of lichen samples were put in a punctured paper bags and placed on trees 1.5 m above ground facing the source of air pollution. After that, all samples were collected and brought to the lab for heavy metals concentration analysis (Nimis et al., 2002; Abas et al., 2016).

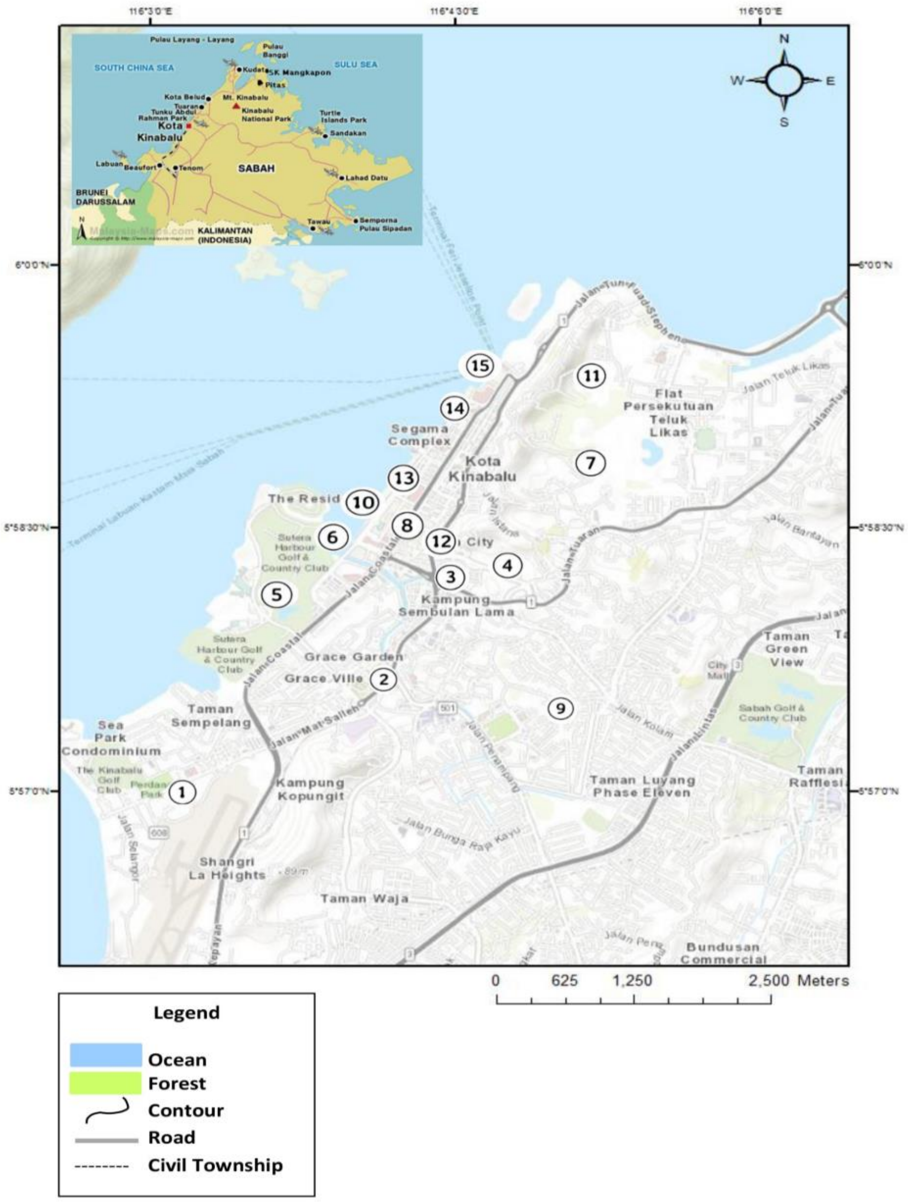

***Figure 1.** Location of the sampling stations*

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Table 1. *Coordinate for sampling locations*

Sampling location	Location name	Coordinates
1	Perdana Park, Tanjung Aru	5.9518N 116.0531E
2	Sabah State Mosque	5.9588N 116.0673E
3	Karamunsing Flyover	5.9698N 116.0740E
4	Nenas Hill Road	5.9693N 116.0808E
5	Sutera Harbour	5.9635N 116.0611E
6	Imago Mall & The Loft	5.9708N 116.0647E
7	Anjung Senja	5.9745N 116.0674E
8	Sinsuran	5.9793N 116.0732E
9	Gaya Street	5.9842N 116.0773E
10	KK Sky City	5.9706N 116.0806E
11	Signal Hill	5.9823N 116.0822E
12	Bandaran Berjaya	5.9768N 116.0753E
13	KK Central Market	5.9826N 116.0729E
14	Suria Sabah	5.9870N 116.0778E
15	Jesselton Point	5.9905N 116.0813E

Laboratory analysis

After about 2-4 weeks, those samples were collected and placed in sealed containers and were taken to the laboratory to be analysed. In the lab, the samples were air dried for about two days in the open air. Then, those samples were heated in the oven for another two days at about 50 °C in in order to remove any excessive moisture. Any debris or dust on the samples was then picked out using clean tweezers before it then has been crushed into powder form. 1.0 g of each samples from the control area and all sampling stations later were weighed for the digestion process. After that, the digestion process was carried out by mixing 5 mL concentrated nitric acid and 15 mL perchloric acid into one conical flask. The samples were then heated on a hot plate until the initial volume was reduced into half. Then, the samples were then cooled off and later rinsed using 5 mL concentrated nitric acid before filtered into the 150 mL conical flask using Whatman No. 42 filter papers. During the filtration process, the samples were rinsed with 5% nitric acid and the filtrates were diluted to 50 mL using 5% nitric acid. The dilution later placed in Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to detect heavy metals such as copper (Cu), manganese (Mn), lead (Pb), zinc (Zn), iron (Fe) and chromium (Cr).

Statistical analysis

One-way ANOVA been used in this research to determine whether there are any statistically significant differences between heavy metals concentration among all sampling stations. Tukey Test was also performed to check significant comparisons between each of the heavy metals.

Results and discussion

Table 2 shows the concentration of heavy metals determined in the lichen samples transplanted and collected from all 15 sampling stations including a control sample.

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Table 2. *Concentration of heavy metals*

Sample	**Concentration of heavy metals in lichen *Usnea minaminensis* (µg/g)**					
	Fe	Zn	Mn	Cu	Pb	Cr
Control	14.96 ± 0.11	1.77 ± 0.23	0.93 ± 0.14	0.51 ± 0.12	0.40 ± 0.03	0.38 ± 0.09
1	68.54 ± 0.08	17.02 ± 0.12	16.73 ± 0.07	1.54 ± 0.21	1.41 ± 0.13	1.48 ± 0.14
2	64.30 ± 0.12	18.55 ± 0.11	15.33 ± 0.18	1.47 ± 0.03	1.29 ± 0.21	1.39 ± 0.34
3	64.57 ± 0.33	15.81 ± 0.23	15.69 ± 0.02	1.61 ± 0.28	1.37 ± 0.14	1.51 ± 0.11
4	57.66 ± 0.34	15.63 ± 0.34	16.43 ± 0.33	1.44 ± 0.16	1.22 ± 0.06	1.43 ± 0.17
5	60.12 ± 0.15	16.57 ± 0.19	12.85 ± 0.24	1.51 ± 0.16	1.40 ± 0.15	1.33 ± 0.18
6	71.47 ± 0.27	19.29 ± 0.02	22.61 ± 0.18	2.42 ± 0.14	3.02 ± 0.27	1.87 ± 0.03
7	82.09 ± 0.19	20.38 ± 0.16	24.75 ± 0.21	2.39 ± 0.08	4.11 ± 0.24	1.90 ± 0.16
8	76.32 ± 0.26	18.44 ± 0.08	19.68 ± 0.04	2.50 ± 0.12	3.67 ± 0.34	2.22 ± 0.25
9	84.43 ± 0.04	18.07 ± 0.21	20.28 ± 0.25	2.22 ± 0.32	3.73 ± 0.03	2.38 ± 0.09
10	44.55 ± 0.06	9.54 ± 0.34	6.49 ± 0.27	0.98 ± 0.21	1.08 ± 0.17	0.66 ± 0.09
11	32.78 ± 0.13	13.66 ± 0.23	10.70 ± 0.14	1.33 ± 0.20	1.19 ± 0.16	0.91 ± 0.33
12	63.17 ± 0.21	14.91 ± 0.09	17.19 ± 0.11	1.49 ± 0.10	2.62 ± 0.24	1.67 ± 0.22
13	79.89 ± 0.14	15.31 ± 0.10	18.87 ± 0.31	1.64 ± 0.31	1.31 ± 0.24	1.93 ± 0.17
14	69.51 ± 0.11	16.83 ± 0.11	14.61 ± 0.01	1.71 ± 0.04	2.77 ± 0.30	1.47 ± 0.13
15	63.73 ± 0.26	15.72 ± 0.12	15.79 ± 0.07	1.39 ± 0.03	1.84 ± 0.09	1.55 ± 0.06

The table shows that the heavy metals' concentration in all of the lichen samples from sampling stations are higher compared to the control sample. Iron (Fe) has the highest concentration in all lichen samples with sampling station number 9 has the highest concentration and number 11 has the lowest concentration, followed by manganese (Mn) (highest is number 7 and the lowest is number 10), zinc (Zn) (the highest is number 7 and the lowest is number 10), lead (Pb) (the highest is number 7 and the lowest is number 10), copper (Cu) (the highest is number 8 and the lowest is number 10) and the lowest is chromium (Cr) (the highest is number 9 and the lowest is number 10). Among all sampling stations, sampling station number 7 have the highest accumulation of heavy metals in the lichen samples, followed by number 8, number 9, number 6, number 13, number 14, number 1, number 2, number 15, number 3, number 4, number 5, number 11 and the lowest is sampling station number 10.

Table 3 shows the one-way ANOVA analysis result. Based on the table, it has been shown that p-value is < 0.05 meaning that there is significant variation between heavy metal groups. A Tukey post hoc test has revealed that the concentration of Fe in the transplanted lichen is significantly higher compare to the other heavy metals, while Zn and Mn has lower significant differences in concentration but higher compare to the other 4 and Cu, Pb and Cr also has lower significant difference in concentration but higher towards the other heavy metals as shown in *Table 4*.

Table 3. *One-way ANOVA result*

Source of variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between groups	44196.1640	5	8839.2328	135.5519	0.0000	2.3157
Within groups	5868.8303	90	65.209225			
Total	50064.9943	95				

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Table 4. *Post hoc test (Tukey HSD) result*

Heavy metal		Sig. value
Fe	Zn	.000
	Mn	.000
	Cu	.000
	Pb	.000
	Cr	.000
Zn	Fe	.000
	Mn	1.000
	Cu	.000
	Pb	.000
	Cr	.000
Mn	Fe	.000
	Zn	1.000
	Cu	.000
	Pb	.000
	Cr	.000
Cu	Fe	.000
	Zn	.000
	Mn	.000
	Pb	1.000
	Cr	1.000
Pb	Fe	.000
	Zn	.000
	Mn	.000
	Cu	1.000
	Cr	1.000
Cr	Fe	.000
	Zn	.000
	Mn	.000
	Cu	1.000
	Pb	1.000

Our results demonstrate that heavy metals concentration in lichen *Usnea minaminensis* closely reflected the heavy metals deposition in the City Centre of Kota Kinabalu, Sabah. We presumed that heavy metals accumulation in lichen samples were due to automobile traffic pollution. Based on previous studies in the urban area of Malaysia, there was a high correlation between heavy metals accumulation in lichen and automobile traffic (Abas and Awang, 2015; Abas and Awang, 2017; Abas et al., 2018). A similar pattern has been found in many other areas of the world, that is, that air pollution caused by heavy metal deposition generally tends to be higher in urban zones with more traffic, than in rural areas with less traffic (Benitez et al., 2019). All those heavy metals usually come from anthropogenic activities such as industrial activity and motor vehicles emission. In addition, in densely populated urban area heavy metals are

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trapped by buildings and prevent it from flew away from the urban vicinity (Samsudin et al., 2013b; Amil et al., 2016).

Iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) are categorized as an essential nutrient for organism as those metals are needed for growth and body development. Whereas, lead (Pb) and chromium (Cr) are known as non-essential heavy metal to living things and only exist in the surroundings due to industrial activity and automobile traffic emission. All of the heavy metals showed a similar pattern, with higher concentration was found in lichen samples of highly dense urban area than in nearby forest or highland. We also found that the heavy metals concentration varied depending on the location of the sampling station. Sampling stations that nearby the city centre such as Anjung Senja, Sinsuran and Gaya Street showed higher heavy metals concentration than sampling stations that located in rural areas such as Signal Hill and KK Sky City.

All the heavy metals are so much related to the density of automobile traffic where Zn deposition in lichens typically related to increases of traffic along traffic routes serving inner city urban areas. Air pollution from Zn can typically be ascribed to tyre wear, and this metal is also a common component of antioxidants used as dispersants to improve lubricating oils. Lead was originated from the fuel combustion of automobile traffic and its concentration is much higher in diesel oil.

Conclusion

This research shows that lichen *Usnea minaminensis* can be used to determine the heavy metal deposition in urban area. Besides that, there is significant variation between heavy metals in Kota Kinabalu. The concentration of heavy metals in Kota Kinabalu is varied, where location nearby to the city centre has higher concentration than nearby the rural or forest area.

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