Heavy Metal Concentration Assessment Using Transplanted Lichen *Usnea Misaminensis* at Pasir Gudang, Johor

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Abstract. Heavy metals were produced by motor vehicles and industry as part of rapid urbanization effects. The objective of this study is to assess the concentrations of heavy metals (Cu, Fe, Zn, Pb, Mn, Cr) in Pasir Gudang, Johor using lichen transplanted method. Samples of *Usnea misaminensis* have been transplanted into the metropolitan climate. The lichens were collected from the National Park of Endau Rompin, Johor. It's a remote area. Fifteen sampling sites were chosen and heavy metals were exposed to lichen transplants at these stations for approximately 4-6 weeks. Exposed lichens have been studied using inductively coupled plasma mass spectrometry (ICP-MS) to assess the concentration of heavy metals in each sample. One-way ANOVA has also been used to check that there is major difference in the concentration of heavy metals in Pasir Gudang, Johor. The test revealed that the maximum concentration of iron (Fe) was 84.43 μg / g and the highest concentration of chromium (Cr) is 0.66 μg / g. A mathematical One-way ANOVA study found that there is a substantial difference between the heavy metal concentration with a P-value of 0.0000 < 0.05. The Tukey study has showed that Fe has slightly higher amounts relative to the others. This result suggest that the growing number of passenger cars would often raise the accumulation of heavy metals in the environment. Transplanted lichens may be an effective method to measuring air quality in the metropolitan region of Malaysia.

1. Introduction

Human well-being has been seriously deteriorated due to air contamination exacerbated by urbanization and industrialization in the region. In Malaysia, approaches to resolve environmental concerns have been introduced by the introduction of a range of measures, such as the National Environment Policy (DASN) and the adoption of the Multilateral Environmental Agreement, such as the Paris Agreement 2015 [1]. Such policies have been developed to maintain human well-being in the city and steps have also been initiated to create a sustainable atmosphere. According to figures, 54% of Malaysia's happiness disruption was induced by air pollution, one of the most important factors of human well-being depletion in Malaysia. The Department of the Environment has provided a range of guidelines to be implemented. Yet it is also not enough in terms of estimation. Transport, stationary sources and open burning are the three primary factors of air emissions. The challenge currently confronting the Department of the Environment is that environmental quality in places far from their environmental sampling stations is challenging to assess, and so lichens have been chosen as a possible bio-indicator for this reason.
Heavy metals may be assessed and extracted using two forms: sampling directly from the environment and sampling using a biological proxy. According to Carreras & Pignata [2], high-volume air samplers and glass fiber membranes are used to obtain samples containing heavy metals. Collected samples were washed using a mixture of analytical grade nitric acid and analytical grade hydrochloric acid and analyzed to assess amounts of heavy metals by atomic absorption spectrophotometry. Heavy metals can also be sampled from lichens where selected lichens from a particular sampling location have been taken to the laboratory and examined using atomic absorption spectrophotometry [3]. Heavy metals can also be extracted from the soil within the house, as observed by Abas et al. [4], where heavy metals such as Fe, Pb, Zn, Cu and others within the university building may be detrimental to the safety of the resident.

Lichen is often used as a bio-indicator for air emissions for decades. In Italy, a model named Lichen Biodiversity Index has been created and used to control air quality in the district of Fuenza, Italy [5]. In addition, Loppi & Frati [6] performed work in Central Italy to test nitrogen compounds in lichen foliosis. Foliose-type lichen Hypogymnia physodes was often used and obtained to examine the quality of heavy metals related to traffic pollutants [7, 8]. Transplantation of lichen-type foliosis from remote and healthy air to even more contaminated areas are commonly used to control air quality in some vicinity. For example, Parmotrema tinctorum are often used in Thailand to evaluate airborne impurities near the petrochemical industry complex [9]. Apart from the assessment of outdoor pollution, lichen transplants are often used to measure the level of indoor air quality at which heavy metals such as As, Cd, Cr, Cu, Hg, Ni, and Pb 12 Polycyclic Aromatic Hydrocarbons (PAHs) were also recorded [10, 11]. In Malaysia, lichen research focused more on highland lichens such as Cameron Highland, Gunung Machincang, Bukit Larut, Genting Highland, Fraser Hills, and Pulau Pangkor [1, 12]. In comparison, the work regarded only the biological and chemical portion of the lichen [13, 14]. None of them researched the relationship between lichen and its surroundings, not until 2015, when a report was performed on the distribution of lichen abundance in Kuala Lumpur [4]. Work has shown that lichen abundance patterns are very closely linked to the population level in Kuala Lumpur.

The research of lichen emission monitoring in Malaysia is still focused on lichen diversity and frequency. There is still a lack of use of transplantation methods to control air quality in Malaysia, especially in the industrial areas. The goal of this research is therefore to analyze the content of heavy metals using lichen transplanted (Usnea minaminensis) as an additional option to air quality assessment and to establish the association between the number of motor vehicles and the accumulation of heavy metals.

2. Methodology

2.1 Study Area
The town of Pasir Gudang, Johor (5.9804 by N 116.0735 by E) is centered on the west coast of Johor, Malaysia. As one of Malaysia's main manufacturing towns, Pasir Gudang has a total population of 46,571 inhabitants with a density of 130 / km2 by census in 2018. Pasir Gudang has developed into a rather-concentrated industrial area where previous findings have shown that the air quality in Pasir Gudang has been reduced.

2.2 Sampling Procedures
Lichen Usnea minaminensis samples were obtained from rural areas (unexposed from harmful air pollution) of Endau Rompin National Park, Johor, Malaysia. All experiments were separated into 45 tests at 15 sampling sites and only one sample was omitted from the separation to be the control sample. Table 1 shows the location of 15 sampling stations randomly selected all over Pasir Gudang City Centre, Johor. This work was carried out using the lichen transplantation process, where lichen Usnea minaminensis was extracted from the remote region and then deposited at sampling locations for approximately 1 month (from 20 August 2019 to 20 September 2019). Approximately 50 g of lichen samples were placed in paper bags and placed on trees 1.5 m above the ground facing the source of
pollution. After 2-4 weeks, all samples were gathered and sent to the laboratory for the study of the accumulation of heavy metals [15, 16].

Table 1. Coordinates for lichen *Usnea misaminensis* transplantation at study sites

| Site | GPS Coordinates                  |
|------|----------------------------------|
| 1    | N 1° 27.93’ E 103° 53.13’       |
| 2    | N 1° 27.09’ E 103° 54.34’       |
| 3    | N 1° 26.09’ E 103° 54.91’       |
| 4    | N 1° 27.89’ E 103° 58.32’       |
| 5    | N 1° 28.44’ E 103° 58.28’       |
| 6    | N 1° 29.42’ E 103° 55.69’       |
| 7    | N 1° 27.76’ E 103° 56.09’       |
| 8    | N 1° 27.04’ E 103° 53.94’       |
| 9    | N 1° 27.81’ E 103° 54.53’       |
| 10   | N 1° 28.58’ E 103° 53.75’       |
| 11   | N 1° 29.99’ E 103° 54.08’       |
| 12   | N 1° 29.97’ E 103° 54.94’       |
| 13   | N 1° 30.32’ E 103° 54.96’       |
| 14   | N 1° 31.26’ E 103° 54.98’       |
| 15   | N 1° 31.36’ E 103° 55.72’       |

2.3 Laboratory Analysis

After around 2-4 weeks, the samples were gathered and stored in sealed containers and sent to the UKM laboratory for examination. In the test, the samples were air-dried in the open air for around two days. These samples were then heated in the oven for another two days at about 50°C to remove any excess moisture. Some residue or dust on the surfaces was then collected using sterile tweezers before being ground into powder shape. Afterwards, 1.0 g of each sample from both the control area and all sampling stations were weighed for the digestion process. Subsequently, the digestion procedure was executed by mixing 5 mL of concentrated nitric acid and 15 mL of perchloric acid into one conical flask. The samples also were heated in an oven until the initial volume was halved. The samples were then cooled off and then rinsed with 5 mL of concentrated nitric acid before being filtered into the 150 mL conical flask using the Whatman No. 42 filter paper. Throughout the filtration cycle, the samples were rinsed with 5 per cent nitric acid and the filters filtered with 5 per cent nitric acid to 50 mL. The dilution was later used in Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to detect heavy metals such as chromium (Cr), copper (Cu), manganese (Mn), lead (Pb), iron (Fe) and zinc (Zn).

2.4 Statistical Analysis

One-way ANOVA was used in this study to assess if there are statistically meaningful variations in the concentration of heavy metals in both sampling stations. Tukey Test was also conducted to look for major differences between each of the heavy metals.

3. Result and Discussion

Table 2 displays the amount of heavy metals in lichen samples transplanted and obtained from all 15 sampling sites, plus the test study.

Table 2. Concentration of heavy metals

| Sample | Concentration of heavy metals in lichen *Usnea misaminensis* (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|
The table shows that the concentration of heavy metals in all lichen samples from sampling stations is 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.

| Sample | Concentration of heavy metals in lichen Usnea minaminensis (µg/g) |
|--------|---------------------------------------------------------------|
|        | Fe   | Zn    | Mn   | Cu   | Pb   | Cr   |
| 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 | 3.67±0.34 |
| 8      | 76.32±0.26 | 18.44±0.08 | 19.68±0.04 | 2.50±0.12 | 3.73±0.03 | 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 | 3.13±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 findings of this analysis indicate that the accumulation of heavy metals in lichen Usnea minaminensis strongly matched the concentration of heavy metals in Pasir Gudang City Centre, Johor. This research concluded that the concentration of heavy metals in lichen samples was related to agricultural practices and traffic emissions. Based on the previous studies in the Malaysian urban region, there was a strong association between the deposition of heavy metals in lichens and industrial and traffic activities [4, 17, 18]. A common trend has been observed in many other parts of the world, that is, air contamination induced by heavy metal accumulation appears to be higher in metropolitan areas with much more traffic than in rural areas with fewer traffic [19]. Both of these heavy metals typically originate from anthropogenic processes, such as manufacturing production and pollution from passenger cars. In addition, heavy metals are trapped in densely populated urban areas by buildings that prevent them from flying away from urban areas [20, 21].

Iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) are known as critical nutrients for the organism, as these metals are required for growth and body production. Whereas lead (Pb) and chromium (Cr) are...
considered as non-essential heavy metals for living beings that occur mainly in the environment as a consequence of human activity. All heavy metals showed similar patterns, with higher concentrations found in lichen samples of highly dense urban areas than in the nearby forest or highlands. The research also showed that the concentration of heavy metals differed based on the location of the sampling station. Sampling stations situated in the metropolitan city core have recorded a higher concentration of heavy metals than sampling stations found in rural areas.

All heavy metals have too much to do with the amount of automobile travel, that the accumulation of Zn in lichens is usually correlated with the rise of travel along road lines servicing inner-city metropolitan centers. Dust emissions from Zn may usually be due to tire wear, and this metal is also a popular component in antioxidants used as emulsions to boost lubricating oils. Lead derived from the gasoline burning of the vehicle traffic and its content in diesel oil is far greater.

4. Conclusion
This work indicates the lichen Usnea minaminensis may be used for the assessment of heavy metal accumulation in urban areas. In addition, there is a significant variation between heavy metals in Pasir Gudang. The concentration of heavy metals in Pasir Gudang differs, with a higher concentration in the region of the city center than in the region of the agricultural or woodland regions.

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