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# EVALUATION OF TRANSPLANTED LICHENS, *Parmotrema tinctorum* AND *Usnea diffracta* AS BIOINDICATOR ON HEAVY METALS ACCUMULATION IN SOUTHERN PENINSULAR MALAYSIA

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**Abstract:** Two types of epiphytic lichens, *Parmotrema tinctorum* and *Usnea diffracta* were evaluated as a bioindicator for acculumation of heavy metals in Parit Raja and BatuPahat, in southern Peninsular Malaysia. The transplant technique was applied in this study by insisting lichens into a nylon bag and hung 2-3 meters above the ground at the selected study areas for three and five months. The morphological analysis of lichen transplant was discovered by using a Scanning Electron Microscope (SEM). By using theInductively Coupled Plasma Mass Spectrometry (ICPMS), the data collection of qualitative and quantitative analysis for lichen transplants were compared. The morphological analysis of *P. tinctorum* after exposure to air pollution did not show any significant difference with the control, while the *U. diffracta* indicated a significant difference over their controls. Zinc (Zn) and Lead (Pb) have the highest concentrations in Parit Raja and Batu Pahat, respectively, affected by industrial and vehicular activities. The results imply that *P. tinctorum* can be positively compared to *U. diffracta* for identifying the levels of heavy metals, due to the higher capability to accumulate heavy metals without affecting the internal structure. Therefore, *P. tinctorum* was found to be the best bioindicator of air pollution in this study.

Keywords: Transplanted lichens, *Parmotrema tinctorum, Usnea diffracta,* heavy metal.

## Introduction

Over the years, there has been a growing concern in the level of air pollution in Malaysia. The expansion of an industrial area and vehicular activities in the rapid urbanization is the main contributors to the higher Air Pollution Index (API) in Malaysia (DOE, 2010).The level of API is conducted by Continues Air Quality Monitoring (CAQM) and Manual Air Quality Monitoring (MAQM) which needsregular maintenance.

Therefore, biomonitoring is one of the alternative techniques which is simple, inexpensive and does not require complicated equipment for monitoring air quality. Biomonitoring is a technique that uses bio-organisms and/or materials as well known as “biomonitor/bioindicator” to obtain information on a certain characteristic of the biosphere. In biomonitoring surveys, the appropriate biomonitor/bioindicator may be selected based on their accumulative and response in behavior towards the atmospheric compounds. This technique is very useful in assessing the effect of air pollution and the changes in the environment on the biotic component of the ecosystem (Sujetoviene & Sliumpaite,2013). One of the main effects of air pollution on biomonitors is the bioaccumulation of heavy metal elements (Balabanova *et al*., 2012; Goix, 2013; Kularatne & Freitas, 2013). Moreover, sensitive biomonitors may also be used in optical type assessment to reflect the stress caused by contaminants, thus making them good preventive alarm systems (Pignata, 2007; Adamo *et al*., 2003).

Lichens have been extensively utilized as a biomonitor/bioindicatorof air pollution in environmental research.Lichen is a perennial species that has unique characteristics, with no roots or other special organs that will uptake nutrients around them and they also lack a cuticle and are easily distributed worldwide (Sujetoviene & Sliumpaite, 2013). Lichen is slow growing, the symbiosis between fungus and algae or cyanobacterium which enables lichen toadapt to life in many kinds of places. Most lichens have three distinct layers; (1) cortex, the outermost layer, which is composed of lightly packed fungal filaments that protect the algal cells and intense sunlight and other organisms; (2) symbiont layer (next layer) and (3) medulla, a layer of fungal filaments. Due to its simple anatomical structure, lichens have high surface:volume ratio with the absence of a cuticle, making it easy for them to accumulate heavy metals, thus directly concentrating them in tissues (Adamo *et al*., 2003). This fact makes them one of the best bioindicators of air pollution (Garty *et al*., 2001).

Most of the epiphytic lichens belong to one of the three main morphological categories such as fructose, foliose and crustose. *Foliose* (leaf-like) and *fructose* (shrub-like) are commonly chosen for heavy metal deposition studies over *crustose* (crust-forming) because they are effortlessly separated from the substrate (Boamponsem *et al*., 2010; Cansaran-Duman *et al*., 2011; Guttova *et al*., 2011; Szczepaniak & Biziuk, 2003). A type of foliose lichens, *P. tinctorum,* is leafy looking and has roots like a thread that anchor them to treetrunks, rocks and soils. Meanwhile, *U. diffracta* is a genus of greenish fructose lichen that grows in the form of flattened strap-like branches (Adamo *et al*.,2003).

There are several research highlighted on the potential of in-situor transplanted lichens as effective biomonitors of air quality (Kularatne & Freitas., 2013). *P. tinctorum* and *U. diffracta* are epiphytic lichen species that can be used in active and passive biomonitoring of trace elements (Jeran *et al*., 2003). The example of active biomonitoring is the transplantation of unpolluted lichen species in the polluted area at a certain time of exposure. The transplant technique includes the sample collection of lichen species from unpolluted habitats, the cleaning, selection and pre-treatment of the materials and followed by their exposure (Adamo *et al*., 2008).

Therefore, in this study, *P. tinctorum* and *U. diffracta* collected from the Genting Highlands area were transplanted and exposed for five consecutive months in the industrial areas of Parit Raja and Batu Pahat town, in the southern part of Peninsular Malaysia. Genting Highlands is affirmed to be “clean” with respect to air pollution. A nylon bag method was applied in this study which consisted of mesh that contained the water-washed lichens. The advantages of this technique are uniformity of entrapment surface and exposure period, known original concentrations of contaminants in the biomonitors and greater collection efficiency for most elements. In addition, it eliminates the possibility of contamination via root uptake and offers lower cost and higher efficiency (Adamo *et al*., 2008). The aim of this study was to evaluate the impact of air pollution on the morphology of transplanted lichens using *P. tinctorum* and *U. diffracta*. The study was conducted at two different areas, Parit Raja and BatuPahat. To the best of our literature survey, a limited number of studies on lichen as indicators in Malaysia are available (Abad & Awang, 2015; Muhammad *et al*., 2018) specially to evaluate the response of transplanted lichen in polluted areas by investigating the heavy metal accumulation levels.

(a) (b)

Figure 1: (a) *P. tinctorum* and (b) *U. diffracta* found in Genting Highlands (N3° 24’ 3.924” E 101° 46’ 55.92”)

## Materials and Methods

### Morphological Analysis

The morphological analysis of lichens was conducted by Scanning Electron Microscopy (SEM)**.** The samples were observed at 20 kV through SEM with the lowest magnification of 10-20 X magnifications. This morphological analysis was conducted to examine and compare the effect of air pollution on the internal structure of lichens.

### Lichen Sampling

The lichen sampling method was adopted from Saiki *et al*. (2007) and Geiser *et al*. (2001), *P. tinctorum* and *U. diffracta* lichens were collected from the isolated sites on Genting Highlands, Pahang, Malaysia (N3° 24’ 3.924” E 101° 46’ 55.92”). A suitable knife was utilized during the sample collection. A precautionary step was taken during collection by avoiding dusty, gritty and discolored lichen. Lichens were sampled from homogenous trunks at different heights at range 10-120 cm from the ground. Then, the initial dry weight of lichens was recorded. *P. tinctorum* (Figure 1(a)) is a type of foliose lichen that is leafy looking and has roots like a thread that are anchored to tree trunks, rocks and soils. It has cortex, upper and lower and more or less attached to its substrate. Meanwhile, *U. diffracta* (Figure 1(b)) is a genus of greenish *fruticose* lichen that grows in the form of flattened strap-like branches, with variable shapes (sections from circular to flat) and structure (pendulous strands or hollow upright stalks) (Aprile *et al*., 2011).

### Site Description

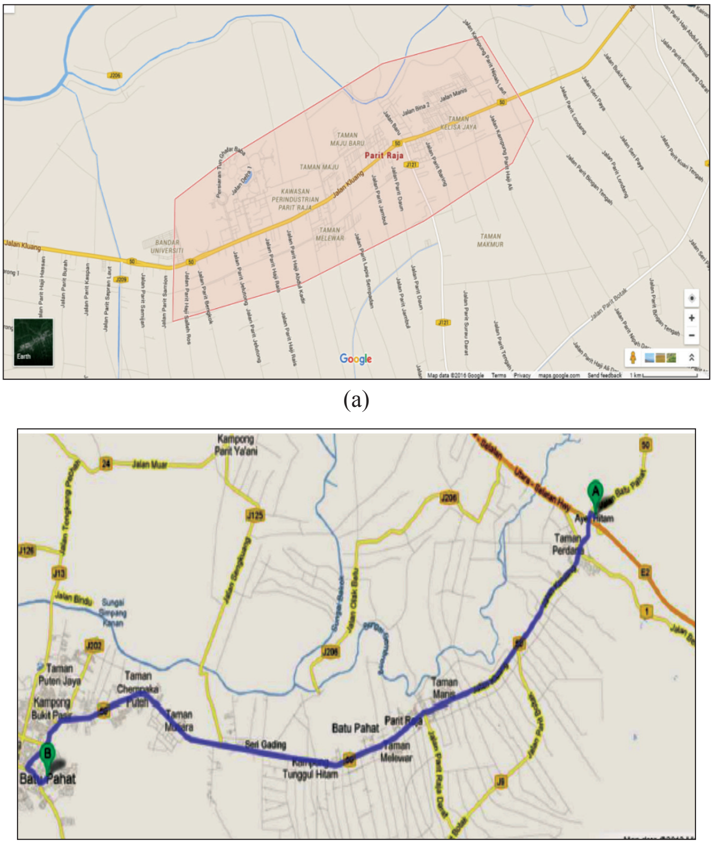
The common sources of heavy metal pollution in ambient air and its surrounding are domestic, transportation and industrial effluents. Several research stated that heavy metal pollution is coming from anthropogenic activities (Mohamed*et al.,* 2014). Therefore, two locations were in this study were on site area of the Material Laboratory, Faculty of Mechanical Engineering, Universiti Tun Hussein Onn Malaysia (UTHM) (N1$^{o}$51’32.3346” E103$^{o}$5’24.306”) and was chosen to represent Parit Raja, Johor, Malaysia and Jalan Teratai (N1$^{o}$51’47.4408” E102$^{o}$57’30.798”) for Batu Pahat site area.

Material Laboratory is exposed to the human-generated air pollutions such as industrial activities and vehicular emission. The distance of this laboratory from the industrial area is approximately 100 m. The types of industry that are in the Parit Raja industrial area are timber, semiconductor and packaging industries. Effluents from industries contain an appreciable amount of metallic cations such as zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), lead (Pb), nickel (Ni) and cadmium (Cd) (Mohamed *et al.,* 2014).

Jalan Teratai represented as anex perimental area site for Batu Pahat is located less than two meters from Federal Route 50 (FT50) in Batu Pahat town. This route has four undivided lanes from Batu Pahat–Air Hitam-Kluang. Report of the Annual Average Daily Traffic (AADT) in 2010 recorded that more than 68,000 vehicles used the Batu Pahat-Parit Raja stretch daily and over 22,000 vehicles went through the Ayer Hitam-Parit Raja stretchdaily. The report also indicated that 80% of air pollutionwascaused by road traffic which contributed to the heavy metals from tyre wear, motor oil, grease, brake emissions and corrosion of galvanized parts and others (Muhammad, 2018). Hence, these locations were selected because they are mainly exposed to anthropogenic activity as emissions of vehicles and factories.

In addition, Parit Raja is located 14 kilometers from Ayer Hitam and 22 kilometers from Batu Pahat town. According to Miskam & Shafii (2013), Parit Raja was formerly a small town and the main source of economy depended on farming activities due to its topography of having low land surface. However, this area has undergone rapid development in 20 years due to the expansion of the industrial sector in the Batu Pahat district. The transformation involved the changes in land use for housing areas, outlet spaces and social amenities. Figure 2(a) shows the map of Parit Raja and Batu Pahat.

Batu Pahat is located on Federal Route 50 (FT50) as shown in Figure 2(b) and has four undivided lanes from Batu Pahat-Parit Raja-Air Hitam-Kluang. This area consists of a dense vehicle capacity at any one time. Annual Average Daily Traffic (AADT) (San, 2013) reported that the transportation activity indicated that more than 68,000 and over 22,000 vehicles used the main road of Batu Pahat-Parit Raja and Ayer Hitam-Parit Raja main roads, respectively.



(a)

(b)

Figure 2: Location of study areas (a) Parit Raja (b) Federal Route in Batu Pahat

From these activities, it could be said that air pollution was caused by road traffic which contributes to the heavy metals. Also, effluents from the industrial activities were expected to containappreciable amounts of heavy metals such as zinc (Zn), copper (Cu), iron (Fe), manganese (mn), lead (Pb), nickel (Ni) and cadmium (Cd).

### *Preparation and Transplantation of Lichen Sample*

Homogeneous lichens were obtained by mixing carefully a replicate collecting lichens from the same host species and types of substrate locations. The homogeneous lichens were cleaned from soil particles and underwent seven consecutive piles of washing with 50 mL of distilled water within five minutes (Adamo *et al*., 2003; Basile *et al.,* 2008). Then, 2 g of lichens were placed in nylon mesh bags (20 x 20 cm wide, with 1 $mm^2$ mesh) (Figure 3). Then, the packed specimens were brought to a selected site area and were hung at a selected lamp post and tree barks with the height of 2–3 meters above the ground within the period of five months on each selected site area. During the study, specimens were sprayed once a month with 10 mL distilled water to avoid dehydration and to favor bioaccumulation of pollutants. Specimens were retrieved monthly for the morphological changes and the heavy metal concentrations analyses.

Figure 3: Lichen sample in the nylon bag

### *Determination of Heavy Metals*

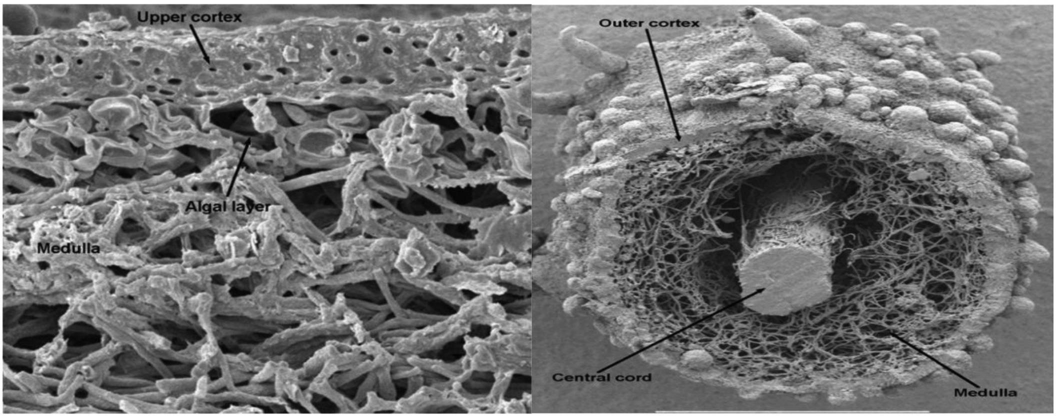
The sample preparation of heavy metal analysis was performed based on a method described by Basile *et al.,* (2008). The amount of 5 mL of concentrated nitric acid ($HNO_3$) and 4 mL of 33 % hydrogen peroxide ($H_2O_2$) was carefully added to 0.5 g of lichen sample. Then, the mixture was heated and slightly stirred until a strong effervescence was produced. When the brown fumes were less dense (7-8 minutes) as observed, the solution was allowed to cool. A pale yellow dissolution and a small white solid quantity in suspension would still remain.

Then, the solution was filtered, washed with 5 mL of diluted hydrochloric acid (HCI). TheInductively Coupled Plasma Mass Spectrometry (ICPMS) (Perkin-Elmer, Elan 9000, USA) was used for the heavy metal analysis. The presented data for heavy metal concentration represents the mean of the triplicate samples.

## Results and Discussion

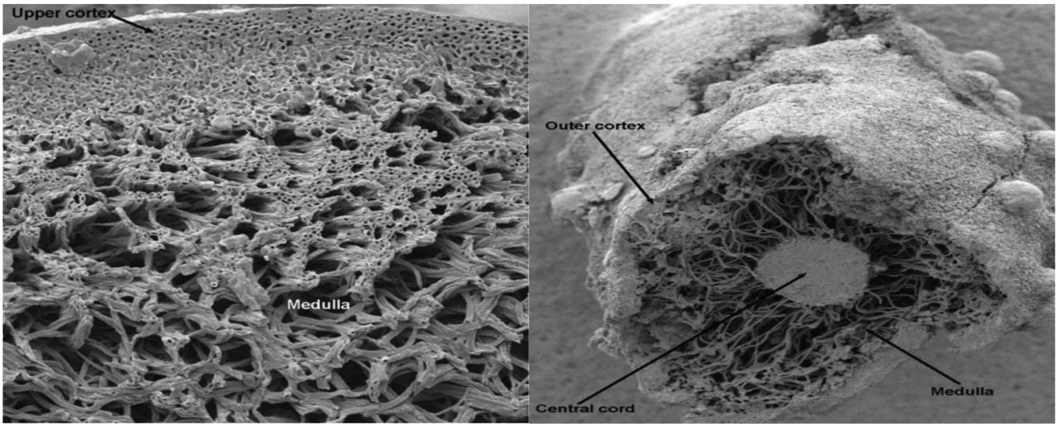
### *Morphological Analysis of P. tinctorumand U. diffractalichens*

Morphological analysis was conductedto examine the effect of air pollution on the morphological internal structure of lichens. Physically, *P. tinctorum* has an ash-green colour on the surface and dark brown below the surface. It has a flat structure and intermittent isidia which were seen around the thallus while *U. diffracta* has a pale greyish-green colour and appears as a shrub-like structure. The morphological internal structures of *P. tinctorum* and *U. diffracta* are shown in Figures 4(a) and 4(b). The normal structure consists of the cortex, alga/symbiont layer and medulla that can be clearly seen as they were collected from the unpolluted area. No solid deposition was observed on the internal structure of both lichens. This result indicates that the collected lichens were not exposed to high concentration of pollutant.



(a) (b)

Figure 4: The morphological structure of internal surfaces of (a) *P. tinctorum* and (b) *U. diffracta* before the transplantation (23, 000 X magnifications)

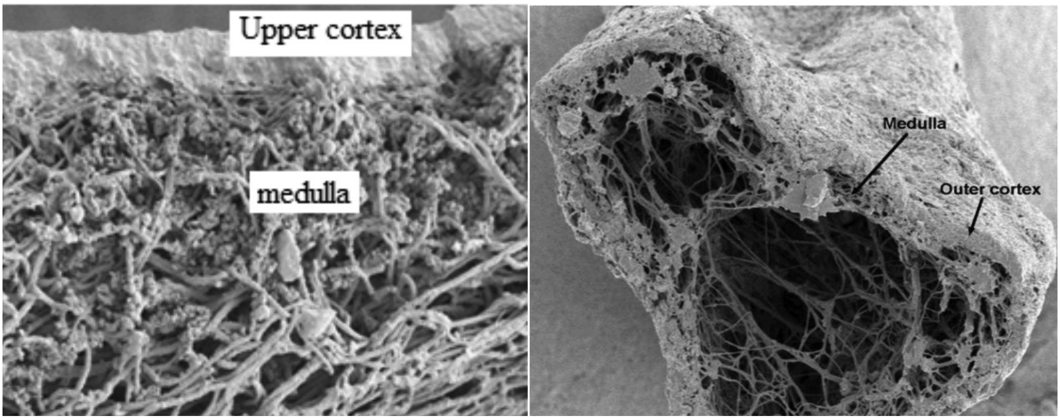


(a) (b)

Figure 5: The morphological structure of (a) *P. tinctorum* and (b) *U. diffracta* after threemonths of exposure (23, 000 X magnifications)

Figures 5(a) and (b) show the morphological internal structure of the transplanted *P. tinctorum* and *U. diffracta* after three months of exposure.*P. tinctorum* has clear structure on the upper cortex, symbiont and medulla layer, with the samecolor that remains as ash green. However, there were some dust and ashes deposited on the surface of the thallus. The presence of dust and ashes observed inside *P. tinctorum* may have originated from the emissions from the factories nearby and smoke from vehicles (Mohamed *et al*., 2014).

The thallus of transplanted *U. diffracta* (Figure 5(b)) was shrunken and reduced to 800 µm from the initial diameter. The outer cortex of the thallus was damaged after three months of exposure. Moreover, the colour changed to brownish-green due to the breakdown of chlorophyll that resulted from exposure to the pollutants (Sujetovienė, 2015). Some parts of the thallus were broken from the main branch and damaged. Dust and ashes were also observed on the thallus, thus promoted chlorophyll degradation of the lichen.



(a) (b)

Figure 6: The morphological structure of (a) *P. tinctorum* and (b) *U. diffracta*after fivemonths of exposure (23, 000 X magnifications)

After a five-month exposure, the colour of *P. tinctorum* thallus remained unchanged. Some deposited dust and ashes were found on the thallus. However, the colour of *U. diffracta* thallus changed to brown. The thallus was fragile and had to be handled with care to avoid major losses of the parts of thallus. Fortunately, the internal structure of *P. tinctorum* could be clearly seen without any structural damage (Figure 6(a)), but the internal structure of *U. diffracta* was damaged due to the absence of the central cord (Figure 6(b)). More solid depositions were observed on the medulla section indicating that too much accumulation of heavy metal and dust particles impurities had taken place.

Overall, it had been shown that *fruticose* lichen (*U. diffracta*) is more sensitive to air pollution or contaminants as compared to fructose lichen (*P. tinctorum*) which can maintain its characteristics after being exposed to pollutants. It also indicated that *P. tinctorum* is the most suitable to be used as a biomonitor as it can accumulate heavy metals and does not show any changes in the physical (thallus color) and the internal structures.

### *Determination of Heavy Metals*

### *Accumulation of Heavy Metals Before Transplanting*

The types and concentrations of heavy metals inside *P. tinctorum* and *U.diffracta*lichen species were analyzed for five months. Table 1 shows the initial presence of heavy metal types and their concentrations in lichens before the transplantation process. The initial concentrations (µg/g) of Cu, Zn and Pb in both lichens were found to be higher compared to other metal elements for both lichen species with ascending order as Cu > Zn >Pb> Cr > Ni > Cd. This ascending orser may possibly may possibly be affected by human activities such as transportation and agricultural activities in the Genting Highlands.

The highest concentration of Cu was related to the agricultural activities which were utilizing a fungicide or herbicide and manure materials which contained excess copper (Garty

Table 1: Heavy metal concentrations (µg/g) in the *P. tinctorum* and *U. diffracta* before the transplantation process

| | Cd | Cr | Cu | Ni | Pb | Zn |
|---|---|---|---|---|---|---|
| ***P. tinctorum*** | 0.05 ±0.01 | 0.32±0.06 | 1.90±0.21 | 0.15±0.03 | 0.65±0.12 | 1.14±0.01 |
| ***U. diffracta*** | 0.02±0.01 | 0.20±0.01 | 0.58±0.14 | 0.11±0.02 | 0.52±0.04 | 0.42±0.02 |

*et al*., 2001). Concentrations of Zn and Pb were correlated to the transportation activities such as combustion exhaust, galvanized parts and railings, fuel and oil, brake linings and rubber tyres. The source of Zn was from tyre-wear particles (Terry *et al*., 2004). The other contaminants such as Cd, Cr and Ni had less of concentration in both lichen species, indicating the lowest anthropogenic activity in the Genting Highlands area.

The wind factor is a driving force that resuspends natural soil material into a fine mixture of light particulates (dust) transportable over a long distance and precipitable on lichens and other plants. Genting Highlands has a spring-like climate. Thus it can be related to this pollutant as it had arisen naturally from the earth due to weathering and erosion of parent rocks and then transported by wind and concentrating the air, it might have also come from earth crust, topsoil and rocks (Garty *et al*., 2001).

### *Accumulation of Heavy Metals by Lichens in the Parit Raja Area*

Table 2 indicates the level of heavy metal concentration in both transplanted lichen species that was monitored in the Parit Raja site for the five-month of exposure and compared with the control (pre-existing concentration). Zn has the highest concentration followed by Pb, Cr, Cu, Cd and Ni in both transplanted lichen samples.

The concentrations of heavy metal elements in lichens increased during the five months of monitoring. The initial value of Zn concentration in *P. tinctorum* and *U. diffracta* was 1.14±0.01 μg g$^{-1}$and 0.42±0.02 μg g$^{-1}$, respectively (Table 1). However, after five months, the highest concentration of heavy metals recorded was Zn and with concentrations in *P. tinctorum* and *U. diffracta* at 537.5 ± 3.16 μg g$^{-1}$ and 185.89 ± 3.24 μg g$^{-1}$, respectively. The lowest heavy metal concentration recorded after five months was Ni with 3.77 ± 0.03 μg g$^{-1}$ (*P. tinctorum*) and 1.91 ± 0.07 μg g$^{-1}$ (*U.diffracta*).

These findings may be the result of the main industrial activities of timber and textile production in the Parit Raja site. Zn was used as the main preservative to prevent wood from decaying and used in the dyeing process. Besides that, the highest Zn concentration in the environment might have been associated with automobile tyre and incomplete combustion of fossil fuel, while the Pb was predicted to be from motor vehicle activity. Cr could possibly

Table 2: Concentration of heavy metals (mean ± standard deviation) (μg/g) in determined *P. tinctorum* and *U. diffracta* after five months of exposure in Parit Raja

| Months | Cd | Cr | Cu | Ni | Pb | Zn |
|---|---|---|---|---|---|---|
| | | | *P. tinctorum* | | | |
| **1** | 0.85±0.04 | 2.53±0.25 | 2.50±0.36 | 0.88±0.17 | 5.54±0.42 | 114.6±5.12 |
| **2** | 1.46±0.02 | 4.10±0.03 | 3.09±0.14 | 1.60±0.05 | 11.31±0.13 | 238.9±5.23 |
| **3** | 2.46±0.12 | 5.99±0.11 | 3.72±0.06 | 2.37±0.04 | 16.16±0.22 | 354.7±2.19 |
| **4** | 3.03±0.05 | 8.74±0.15 | 4.31±0.09 | 3.01±0.03 | 23.73±0.09 | 474.4±6.52 |
| **5** | 4.02±0.09 | 10.82±0.20 | 4.88±0.11 | 3.77±0.03 | 28.93±0.97 | 537.5±3.16 |
| | | | *U. diffracta* | | | |
| **1** | 0.11±0.01 | 2.29±0.12 | 0.62±0.03 | 1.20±0.02 | 2.95±0.06 | 90.49±0.46 |
| **2** | 1.07±0.05 | 3.38±0.09 | 1.23±0.02 | 1.41±0.03 | 7.39±0.06 | 129.96±0.58 |
| **3** | 1.24±0.15 | 3.45±0.06 | 2.05±0.06 | 1.52±0.17 | 17.89±0.04 | 146.12±0.14 |
| **4** | 1.81±0.12 | 4.56±0.07 | 2.78±0.09 | 1.64±0.06 | 20.23±0.11 | 174.71±1.38 |
| **5** | 1.84±0.02 | 4.79±0.17 | 3.01±0.19 | 1.91±0.07 | 20.67±0.38 | 185.89±3.24 |

be due to the use of preservative from the timber and textile factories and semi-conductor factory. The presence of Cu was also due to its use in the timber industries. The contaminations of Cd might be related to the production of the semiconductor in the industrial area. Cd is used in the production of the wafer, which is one of the semiconductor components. Cadmium tellurides (CdTe) are the most common photovoltaic (PV) materials used in the mass production of thin film modules at present (Mohamed *et al*., 2014). Heavy metal pollutants probably originated from wind-transported particulate emissions from local farming (applied pesticides and fertilizers), traffic, landfills and industries in and near Parit Raja and from the town itself. It might have been caused by the traffic passing through because Cd may come from lubricating motor oil, tyres and galvanized parts of vehicles (Carreras & Pignata, 2002; Miskam & Shafii, 2013;). This result is similar to the previous study done by Miskan & Shafii (2013) and Radin *et al*. (2014).

### *Accumulation of Heavy Metals by Lichens in the Batu Pahat Area*

Table 3 presents the concentrations of heavy metals in *P. tinctorum* and *U. diffracta* in Batu Pahat town area. The highest concentration of heavy metals is noted to be Pb followed by Zn > Cu > Ni> Cr >Cd for both species after five months of exposure.

The concentration of Pb is found to be higher in *P. tinctorum* (245.64 ± 0.81 µg $g^{-1}$) than in *diffracta* (14.33 ± 0.10 µg $g^{-1}$). Smoke released by passing vehicles could have possibly contributed to the high amount of Pb in *P. tinctorum*. The study area is located along Federal Route 50 (FT50) which has four undivided lanes from Batu Pahat–Air Hitam-Kluang. This area has the capacity volume of dense vehicles in a day, thus causing lead to be a major pollutant in this area due to vehicular emissions. Different biomonitoring studies carried out using lichens have shown that Pb is a widespread pollutant and one of the most important emission sources. Pb is a well-known pollutant derived mostly from emissions from motor vehicles using leaded petrol and from the manufacture of batteries, metal products, paints and ceramic products (Ng *et al*., 2005).

The concentration of Zn in *P. tinctorum* and *U.diffracta* is 57.24 ± 0.99 µg/gand 6.65 ± 0.22 µg/g,respectively. The presence of Zn pollutant comes from the tyre-wear particles (Basile *et al*., 2008) as well as from combustion exhaust, galvanized parts and railings, fuel and oil, brake

Table 3: Concentration of heavy metals (mean ± standard deviation) (µg/g) in determined *P. tinctorum* and *U. diffracta* after five months of exposure in Batu Pahat

| Months | Cd | Cr | Cu | Ni | Pb | Zn |
|---|---|---|---|---|---|---|
| | | | *P. tinctorum* | | | |
| 1 | 0.38±0.02 | 0.36±0.03 | 12.69±0.14 | 1.17±0.04 | 48.99±0.19 | 20.11±0.11 |
| 2 | 0.53±0.04 | 0.59±0.02 | 23.73±0.03 | 1.65±0.02 | 97.72±0.66 | 29.96±0.68 |
| 3 | 0.71±0.01 | 0.82±0.07 | 29.62±0.11 | 1.88±0.08 | 141.27±0.04 | 36.04±0.05 |
| 4 | 0.97±0.03 | 1.16±0.23 | 35.62±0.17 | 2.13±0.05 | 195.87±5.31 | 48.42±0.45 |
| 5 | 1.13±0.02 | 1.42±0.04 | 40.51±0.13 | 3.61±0.02 | 245.64±0.81 | 57.24±0.99 |
| | | | *U. diffracta* | | | |
| 1 | 0.13±0.03 | 0.24±0.05 | 1.84±0.07 | 0.61±0.02 | 5.31±0.01 | 1.73±0.03 |
| 2 | 0.23±0.02 | 0.40±0.07 | 2.24±0.08 | 0.88±0.03 | 10.00±0.19 | 2.37±0.03 |
| 3 | 0.29±0.09 | 0.44±0.14 | 2.50±0.11 | 1.02±0.10 | 13.41±0.04 | 3.85±0.08 |
| 4 | 0.38±0.07 | 0.62±0.01 | 3.09±0.13 | 1.32±0.02 | 13.91±0.03 | 4.91±0.17 |
| 5 | 0.39±0.04 | 0.65±0.12 | 3.71±0.02 | 1.43±0.03 | 14.33±0.10 | 6.65±0.22 |

linings and rubber tyres (Miskam & Shafii, 2013). The concentration of Cu in *P. tinctorum* is 40.51 ± 0.13 µg/g and *U. diffracta* is 3.71 ± 0.02 µg/g, respectively. Cu can be distributed by wind and commonly originated from the semiconductor production. There are several semiconductor-based industries in the study area. Thus, the absorption of Cu by the lichens in our studies could potentially occur.

The concentration of Ni in *P. tinctorum* and *U. diffracta* is 3.61 ± 0.02 µg/gand 1.43 ± 0.03 µg/grespectively. Ni is also produced by smoke emissions from factories and vehicles. The data recorded for Cr in *P. tinctorum* was 1.42 ± 0.04 µg/g and *U. diffracta* was 0.65 ± 0.12 µg/g after the five-month period. The Cr contamination could be related to the production of electronic components in the industrial area in Batu Pahat. Cr was also produced through burning activities of the residents living nearby and smoke emitted from vehicles (Basile *et al*., 2008; Garty *et al*., 2001).

The concentration of Cd is 1.13 ± 0.02 µg g$^{-1}$ (*P. tinctorum*) and 0.39 ± 0.04 µg g-$^{1}$ (*U.diffracta*). Cr may be also originated from burning activities by the nearby residents and smoke from the vehicles. The highest level of heavy metal concentration was detected in *P. tinctorum*in both transplantation areas. From the analysis, it was found that the concentrations of Zn and Pb are higher in Parit Raja, while the concentrations of Pb, Zn and Cu are higher in Batu Pahat.

### *The Comparison of Accumulation of Heavy Metals Between P. tinctorum* **and** *U. diffracta*

In order to compare the accumulation rate by the two biomonitors, the total accumulated heavy metal concentration of lichen. *P. tinctorum* and *U. diffracta* in five months were compared with the initial heavy metal content before the transplantation (control) as shown in Table 4. These values defined as the average accumulation rate.

Similarly, the heavy metal concentrations in *P. tinctorum* and *U. diffracta* increased after being exposed for five months, thus confirming that these organisms are capable ~~to~~of accumulating trace elements. Conversely, the heavy metal concentrations in *P. tinctorum* were found to be higher compared to *U. diffracta* for both locations. The speculation of interspecific differences in heavy metal accumulation between *P. tinctorum* and *U. diffracta* are related to their morphological, anatomical and physiological features, such as metal-absorbing abilities, surface area, size of intercellular space, the permeability of cell membrane and pH level. Therefore, it is clear that fructose lichen of *P. tinctorum* has the potential to accumulate large amounts of heavy metals compared with foliose lichen (*U.diffracta*) for the above-mentioned reasons.

Furthermore, it can point out that the heavy metal concentrations for both locations showed constant increment in *P. tinctorum* for the duration of five months. Nevertheless, for *U. diffracta* the increment showed a discontinuous trend starting in the fourth and fifth month of exposure. The pattern of heavy metal concentrations in the Parit Raja site differs from that of Batu Pahat site due to the different industrial activities carried out at these two locations. This trend could be that *P. tinctorum* (foliose type; leaf-like) had a large surface area-to-volume compared to *U. diffracta* (fructose type; shrub-like). Therefore, the transfer of heavy metal accumulation is efficiently and simply embedded into the *P. tinctorum* when compared to *U. diffracta* thallus due to the surface characteristics. Several mechanisms such as ion exchange, particulate trapping, hydrolysis, extracellular electrolyte and intracellular uptake are involved during the transfer process of heavy metal accumulation (Ismail *et al*., 2014).

In addition, *P. tinctorum* showed no major internal structural changes (cortex, medulla and symbiont layer) after being exposed to the pollutants during the five months of exposure, though *U. diffracta* showed internal structural changes in the third and fifth months. This changeable structure occurred due to the different cell wall of lichens. The cell wall is mostly involved in the process of mass and

Table 4: Average accumulation of heavy metals in *P. tinctorum* and *U. diffracta* after five months of exposure in Parit Raja and Batu Pahat (µg $g^{-1}$)

| Location Site | Heavy Metal | *P. tinctorum* | *U. diffracta* |
|---|---|---|---|
| Parit Raja | Cd | 3.97 | 0.35 |
| | Cr | 10.50 | 4.59 |
| | Cu | 2.98 | 2.43 |
| | Ni | 3.62 | 1.80 |
| | Pb | 28.28 | 17.91 |
| | Zn | 536.36 | 185.47 |
| BatuPahat | Cd | 1.08 | 0.35 |
| | Cr | 1.10 | 0.45 |
| | Cu | 38.60 | 3.15 |
| | Ni | 3.45 | 1.30 |
| | Pb | 245.00 | 13.80 |
| | Zn | 56.10 | 6.20 |

charge balance (Nieber *et al*., 1978), hence indicating that *fruticose* lichen has low ability to survive in a polluted area and would be unsuitable to be used as a bioindicator of air. *P. tinctorum* has been shown to be the best bioindicator of air pollution as it can accumulate high amount of heavy metals with no effect on its internal structure as compared to *U. diffracta.*

## Conclusion

The accumulation of heavy metals by transplanted lichens represented by *P. tinctorum* and *U. diffracta* at Parit Raja and Batu Pahat areas was successfully investigated. These lichen species positively responded to the heavy metal accumulation levels. The result indicated that the concentration levels of Zn and Pb were the highest in Parit Raja and Batu Pahat areas, respectively. Based on the morphology structure, *P. tinctorum* performed well with higher accumulation of heavy metals without altering the internal structure when compared to *U. diffracta*.

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## References

Abad, A., & Awang, A. (2015). Penentuan tahap pencemaran udara di Malaysia menggunakan pendekatan penunjuk bio (Liken): Kajian kes Bandar Baru Bangi. *Malaysian Journal of Society and Space*, *11*, 67-74.

Adamo P.., Giordano, S., Vingiani, S., Cobianchi, R. C., & Violante, P. (2003). Trace element accumulation by moss and lichen exposed in bags in the city of Naples (Italy). *Environmental Pollution*, *122*, 91-103.

Adamo, P., Bargagli, R., Giordano, S., Modenesi, P., Monaci, F., Pittao, E., & Tretiach, M. (2008). Natural and pre-treatments induced variability in the chemical composition and morphology of lichens and mosses selected for active monitoring of airborne elements. *Environmental Pollution*, *152,* 11-19.

Aprile G. G., Catalano I., Migliozzi A. & Mingo A. (2011). Monitoring epiphytic lichen biodiversity to detect environmental quality and air pollution: The case study of

Roccamonfina Park (Campania Region - Italy). *Air Pollution - New Developments*, Anca Maria Moldoveanu, IntechOpen, *DOI: 10.5772/17907*.

Balabanova, B., Stafilov, T., Sajn, R. & Baceva, K. (2012). Characterisation of heavy metals in lichen species *Hypogymniaphysodes* and *Everniaprunastri* due to biomonitoring of air pollution in the vicinity of copper mine. *International Journal of Environmental Research, 6,* 779-794.

Basile, A., Sorbo, S., Aprile, G., Conte, B., & Castaldo Cobianchi, R. (2008). Comparison of the heavy metal bioaccumulation capacity of an epiphytic moss and an epiphytic lichen. *Environmental Pollution, 151*, 401-407.

Boamponsem, L. K., Adam, J. I., Dampare, S. B., Nyarko, B. J. B., & Essumang, D. K. (2010). Assessment of atmospheric heavy metal deposition in the Tarkwa gold mining area of Ghana using epiphytic lichens. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms,* 268, 1492-1501.

Cansaran-Duman, D., Aras, S., Atakol, O., & Atasoy, I. (2012). Accumulation of Trace Elements and the Assessment of the Genotoxicity in the *Lichen Pseudevernia* furfuracea Transplanted to a Polluted Site in Ankara. *Ekologi*, *21*, 1-14.

Carreras, H. A., & Pignata, M. L. (2002). Biomonitoring of heavy metals and air quality in Cordoba City, Argentina, using transplanted lichens. *Environmental Pollution*, *117,* 77-87.

San, H. P. (2013). *Influence of Speed and Flow Rate on Road Accidents: A Case Study Along Federal Route 50 (FT 050).* Universiti Tun Hussein Onn Malaysia, Malaysia. Thesis. *http://eprints.uthm.edu.my/4387/1/HOR_PEAY_SAN.pdf*

Garty, J., Weissman, L., Cohen, Y., Karnieli, A., & Orlovsky, L. (2001). Transplanted Lichens in and around the Mount Carmel National Park and the Haifa Bay Industrial Region in Israel: Physiological and Chemical Responses. *Environmental Research, 85,* 159-176.

Geiser, L. H., Jovan, S. E., Glavich, D. A., & Porter, M. K. (2010). Lichen-based critical loads for atmospheric nitrogen deposition in Western Oregon and Washington Forests, USA. *Environmental Pollution, 158,* 2412-2421.

Goix, S., Eléonore Resongles, E., Point, D., Oliva, P., Duprey, J. L., Galvez, E. D. L., Ugarte, L., Huayta, C., Prunier, J., Zouiten, C., & Gardon, J. (2013). Transplantation of epiphytic bioaccumulators (*Tillandsia capillaris*) for high spatial resolution biomonitoring of trace elements and point sources deconvolution in a complex mining/smelting urban context. *Atmospheric Environment, 80,* 330-341.

Ismail, I., M. Mostafa, T., Sulaymon, A., & Abbas, S. (2014). Bisorption of heavy metals: A review. *Water, Air, Soil and Pollution,47,* 189.

Jeran, Z., Jaimovi, R., Batič, F., & Mavsar, R. (2002). Lichens as integrating air pollution monitors. *Environmental Pollution*, *120*, 107-113.

Kularatne, K. I. A., & de Freitas, C. R. (2013). Epiphytic lichens as biomonitors of airborne heavy metal pollution. *Environmental and Experimental Botany, 88*, 24-32.

Miskam, N., & Shafii, H. (2013). Indicators and Index for Rural Area: Case Study at PekanParit. *IOSR Journal of Humanities and Social Science (IOSR-JHSS)*, *12*, 75-82.

Mohamed, R., Saphira, R. M., Aziz, C., Hanim, F., Kassim, M., & Hashim, A. (2014). An assessment of selected heavy metal concentrations (Pb, Cu ,Cr ,Cd ,Ni , Zn ) in university campus located in industrial area, *ARPN Journal of Engineering and Applied Sciences*, *9*, 2734-2730.

Muhammad, N., Hashim, N.H., Kahiruddin, N.A., Yusof, H., Jusoh, S., Abad, A., Talip, B.A., Abdullah, N., & Din, L. (2018). Identification of most tolerant lichen species to vehicular traffic's pollutants: a case study at Batu Pahat, *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences,42*, 57-64.

Ng, O. -H., Tan, B. C., & Obbard, J. P. (2007). Lichens as bioindicators of atmospheric heavy metal pollution in Singapore. *Environmental and Monitoring Assessment, 123*, 63-74.

Nieboer, E., Richardson, D. H. S., & Tomassini, F. D. (1978). Mineral uptake and release by lichens: An overview. *The Bryologist, 81,*226-246.

Saiki, M., Alves, E. R., & Marcelli, M. P. (2007). Analysis of lichen species for atmospheric pollution biomonitoring in the Santo Andre municipality, Sao Paulo, Brazil. *Journal of Radio analytical and Nuclear Chemistry*, *273*, 543-547.

Sujetovienė, G. (2015). Monitoring lichen as indicators of atmospheric quality. In D. K. Upreti, P. K. Divakar, V. Shukla, & R. Bajpai (Eds.), *Recent Advances in Lichenology: Modern Methods and Approaches in Biomonitoring and Bioprospection, Volume 1* (pp. 87-118). New Delhi: Springer India.

Sujetoviene, G., & Sliumpaite, I. (2013). Response of *Evernia prunastri* transplanted to an urban area in central Lithuania. *Atmospheric Pollution Research, 4,* 222-228.

Szczepaniak, K., & Biziuk, M. (2003). Aspects of the biomonitoring studies using mosses and lichens as indicators of metal pollution. *Environmental Research, 93*, 221-230.

Terry, R. S., Smith, J. E., Sharpe, R. G., Rigaud, T., Littlewood, D. T. J., Ironside, J. E., Rollinson, D., Bouchon, D., MacNeil, C., Dick, J.T.A., & Dun, A. M. (2004). Widespread vertical transmission and associated host sex-ratio distortion within the eukaryotic phylum Microspora. *Proceedings Biological sciences, 271,* 1783-1789.

Wolterbeek, B. (2002). Biomonitoring of trace element air pollution: Principles, possibilities and perspectives. *Environmental Pollution, 120*, 11-21.