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Biomonitoring Potentially Toxic Elements (PTEs) Using Lichen Transplant *Usnea misaminensis*: A Case Study from Malaysia

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Abstract: Urban air pollution has been a major concern due to its impact on global public health. Various techniques for monitoring urban air quality have been developed. However, there is currently a dearth of research on how effective bioaccumulators such as lichen are at monitoring urban air pollution. As a result, the purpose of this research is to investigate the use of *U. misaminensis* as a biological indicator for biomonitoring urban air quality in Malaysia. Three months were spent exposing lichen samples in three Malaysian cities (Kuala Lumpur, Penang, and Johor Bahru). The trace element content and vitality of the lichens were assessed. The results of this study revealed that *U. misaminensis* is an effective biological indicator for measuring 25 elements of air pollutants in metropolitan areas. They also revealed that all 25 elements accumulated in the urban area sample were greater than in the control sample. The vitality rate of lichens dropped in the urban area sample when compared with the control sample, indicating that an increase in elements in the air will impact the vitality rate of any biological component. In this study, two arguments are made: (a) Lichen is an excellent biological indicator, particularly for urban air pollutants such as potentially toxic elements; and (b) traffic is the primary contributor to urban air pollution; hence, the local government requires a better plan and design for urban areas to decrease air pollutants build-up.

Keywords: ecological indicator; lichens; air pollution; traffic; ecosystem management

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1. Introduction

More than 55 percent of the world's population lives in cities, and that figure is anticipated to rise to 68 percent by 2050 [1]. This indicates that the quality of public health will be heavily influenced by the state of the urban environment. Urban air quality has always been one of the most critical concerns to be addressed, and several tactics and methods have been developed to address it [2]. The urban region is also recognised as the primary source of air pollution, accounting for around 78 percent of carbon emissions and significant airborne pollutants produced in this area from diverse sources [3–5]. The growing concentrations of air pollutants such as carbon monoxide (CO), nitrogen dioxide (NO_2), sulphur dioxide (SO_2), and ozone (O_3), as well as suspended particles smaller than 10 µm (PM_{10}), would damage the ecosystem services provided by urban areas [6].

Potentially toxic elements (PTEs) in ambient air particulate matter are mostly caused by automobile emissions and industrialisation [7]. PTEs were shown to have accumulated in the soil, water, and atmosphere in prior investigations, exceeding the criterion for environmental quality [8]. Soltani [9] found Cu, Co, V, Ni, Fe, and Zn to be the most abundant elements in total suspended particle (TSP) matter, $PM_{2.5}$, and PM_{10}. PTEs can be computed using a combination of geogenic and anthropogenic influences [10].

A biological component, such as lichen, can be utilised to assess the level of air pollution in a certain location. Lichens are organisms created by a symbiotic interaction between a fungus (mycobiont) and algae or cyanobacteria (photobiont) [11]. Lichens are an important organism in biomonitoring because of their capacity to absorb foreign particles from the air. This ability is owing to the absence of a cuticle layer in the lichen's thalli, which works to filter out particulate debris in the air in other living creatures [12]. Normally, absorbed particle matter or pollutants hinder the lichen's metabolic activity and, therefore, impair its vitality level [13,14]. Numerous studies have been conducted using lichen to monitor air pollution in the forest [15], mountainous areas [16], and urban areas [17,18] using various biomonitoring techniques such as transplanting technique [19,20], analysis of diversity distribution and richness [21,22]. There have been several studies undertaken in Malaysian urban areas to measure air pollution using lichen [17,19,21,23]. However, a comparative investigation of air pollution in different metropolitan areas utilising transplanted lichen such as *Usnea misaminensis* is currently absent.

The lichen species *U. misaminensis* was used as a bioaccumulator in this study to monitor air quality in three distinct Malaysian urban areas using a transplanting technique. In this study, 25 elements (Al, Ba, Ca, Cd, Co, Cs, Cu, Fe, K, La, Mg, Mn, Na, Ni, Pb, Rb, S, Sb, Sm, Sr, Tb, Th, Ti, V, and Zn) were measured, as well as the vitality rates of the lichen. With this research, we sought to answer the following questions: (1) Is *U. misaminensis* suitable for biomonitoring of atmospheric element deposition? (2) What is the amount of accumulation of those 25 components in the transplanted lichen? (3) How is the vitality rate of the transplanted lichen? (4) Is there a significant association between the amount of accumulation for all components and the vitality rate of the transplanted lichen in the three selected metropolitan areas?

2. Materials and Methods

2.1. Sample Collection and Preparation

Usnea misaminensis (Vain.) Motyka (Voucher No. BL143/2009) was the lichen species used in this research. *U. misaminensis*, a fruticose lichen species, are abundant in tropical highland ecosystems. The lichen sample was obtained in the Bukit Larut highlands area of Perak (4.8623° N, 100.7930° E) (Figures 1 and 2a), which is free of any potential air pollution. This area's *U. misaminensis* has been employed for biological monitoring, and it has been discovered that the chemical composition of the area offers an overview of an air-pollution-free environment [17]. Bukit Larut is situated in the Bintang Range in Peninsular Malaysia's northwestern region. The region is hilly, with three peaks, the tallest of which is Gunung Hijau at 1448 m (4751 ft), followed by Gunung Biong at 1218 m (3996 ft) and Wray's Hill at 1020 m (3350 ft). The average annual temperature in Bukit Larut ranges between 15 and 25 degrees Celsius during the day and can reach 10 degrees Celsius at night. This area also receives the most rainfall in Malaysia, with annual precipitation reaching up to 5800 mm (230 in).

The lichen was then cleaned to ensure that no extraneous material interfered with the study's findings. The sample was then rinsed three times with distilled water. It was then placed in a mesh bag made specifically for this investigation [24]. The weight of the lichen sample to be placed in the net bag was predicted to be between 20 and 50 g. A total of ten net bags per urban area (for a total of thirty net bags of transplanted lichen) and three controls were chosen. The transplants were delivered immediately to the exposed locations. The controls were placed in a laboratory and maintained at room temperature in sealed paper bags until they were analysed.

2.2. Sample Exposure

The exposure period for the lichen *U. misaminensis* sample began on 1 March 2021 and terminated three months later. Ten sample sites were chosen for each location, with all of the selected sites having the highest concentrations of air contaminants. This study used Bukit Bintang, Bangsar, and Cheras as the key sample locations in Kuala Lumpur City (Figures 1 and 2b). Meanwhile, in Penang City (Figures 1 and 2c), sample locations were chosen in the areas of Georgetown, Bayan Lepas, and Gelugor. Skudai, Stulang, and Setia Tropika were chosen as sample locations for Johor Bharu (Figures 1 and 2d). These locations were chosen because the region is one of the most polluted in Malaysia, and the highways in those cities are among the busiest in the country. Therefore, the selected sample sites are appropriate locations for evaluating lichen element composition reactions to vehicle emissions in the context of severe air pollution.

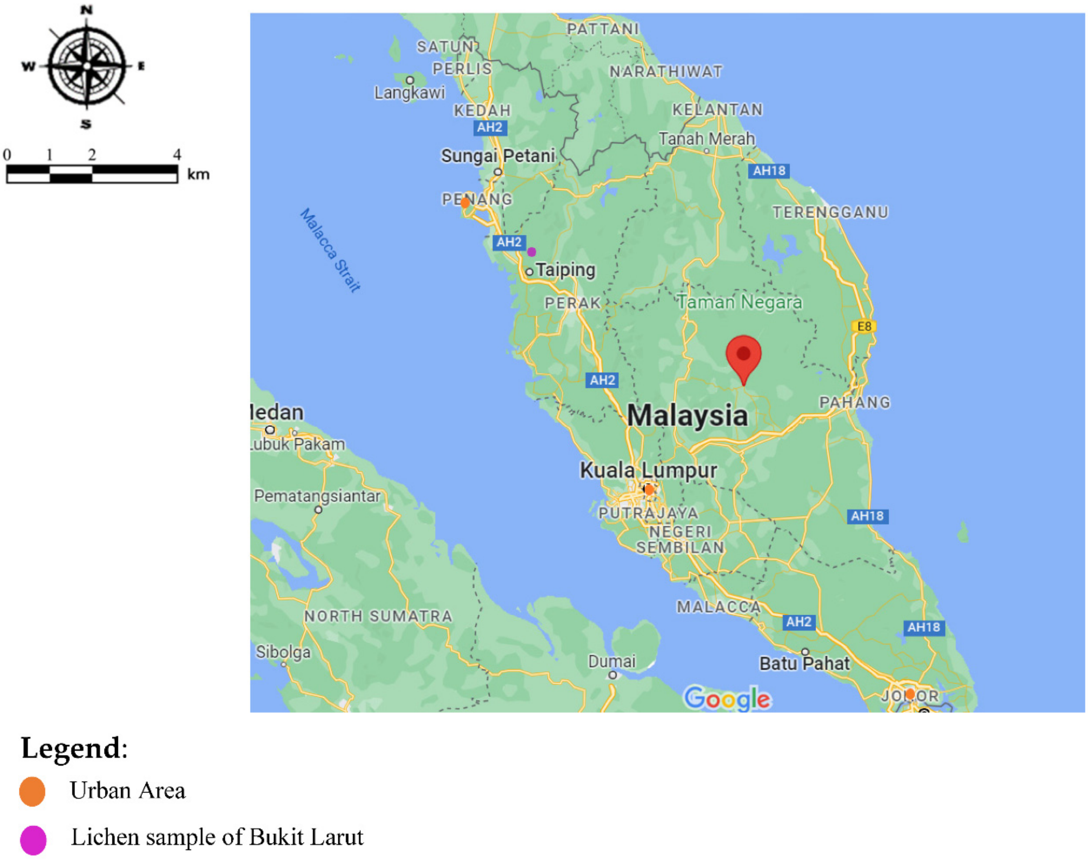


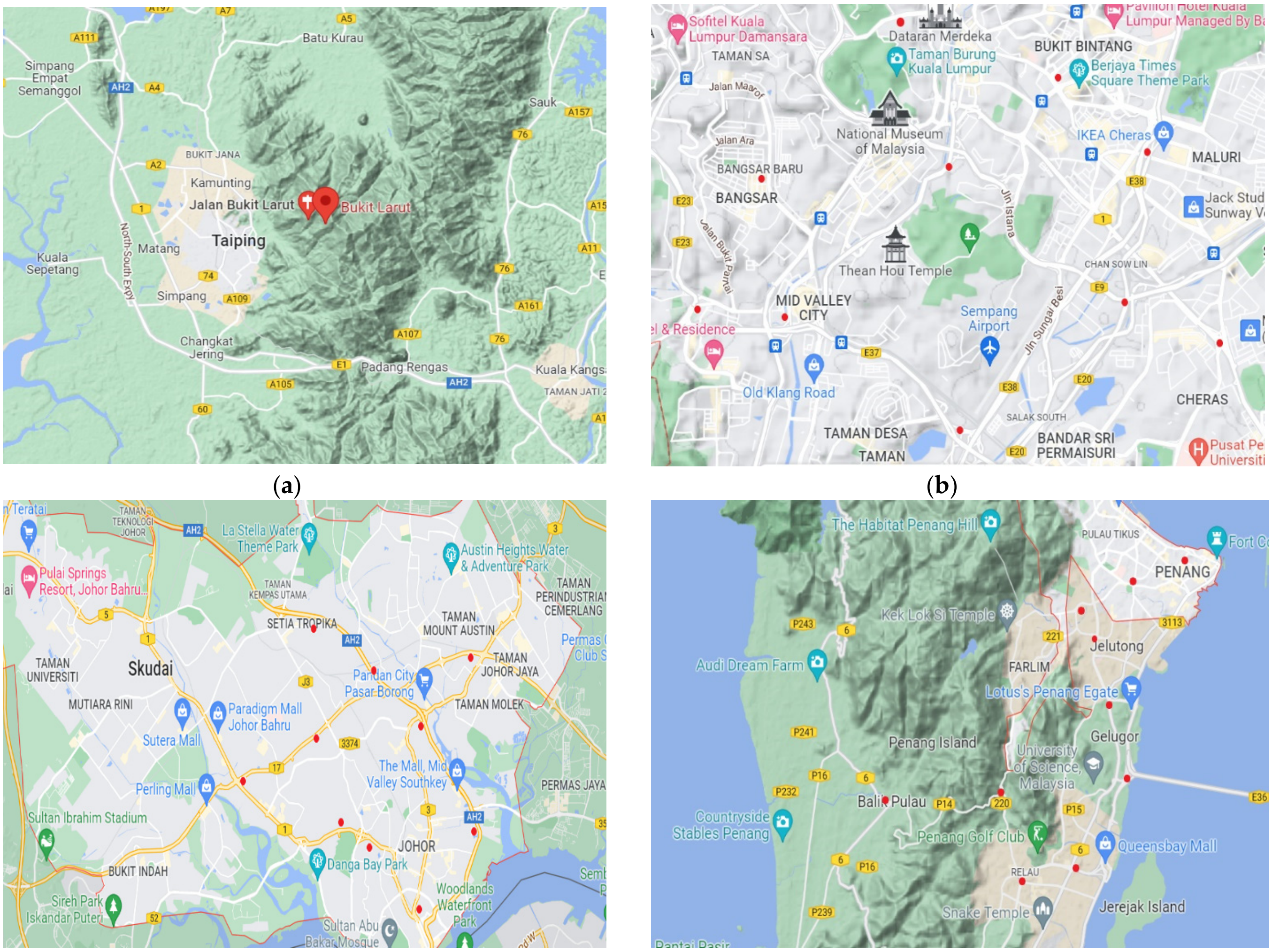
Figure 1. Map of sampling location (Peninsular Malaysia) in larger scale.

To guarantee that exposure to pollutants would be at an optimal level, all bags were put at a height of 1.5 to 3 m. The transplants were exposed and recovered using slightly modified procedures to those employed by Bari et al. [25] and Nannoni et al. [26]. For each sampling station, one bag of lichen thalli was placed at the nearby tree branches, 2–3 m above the earth. At three-month intervals, one composite sample of each lichen species was collected from each exposure location. To reduce bag variability, the composite sample (15–20 thalli, 4.0–6.0 g) was a combination of thalli drawn at random from four bags (3–5 thalli each bag). The collected samples were packed in plastic bags and numbered according to the exposure site and time.

2.3. Analysis Procedure for Elements

The lichen samples were analysed after the exposure period in the laboratory using a light microscope to detect the presence of foreign materials, and they were then cleaned before proceeding with the processes. As lichen thalli have a high propensity to absorb and retain potentially toxic elements from the environment, *U. misaminensis* thalli were chosen for trace element analysis.

Lichen samples were prepared by taking them out of their net sacks. Then, 20 g samples were extracted in plastic flasks with 3 mL 70% nitric acid (HNO_3), 0.2 mL 60% hydrofluoric acid (HF), and 0.5 mL 30% hydrogen peroxide (H_2O_2) in a microwave decomposition system (Milestone Ethos 900) at 280 °C and 797.7psi. ICP-MS (PerkinElmer Sciex, Elan 6100) was used to estimate and quantify the concentration level of chosen elements (Al, Ba, Ca, Cd, Co, Cs, Cu, Fe, K, La, Mg, Mn, Na, Ni, Pb, Rb, S, Sb, Sm, Sr, Tb, Th, Ti, V, and Zn). The analysis's quality was assessed using the IAEA-336 (Lichen) Standard Reference Material manual [27].



(**a**) (**b**)

(**c**) (**d**)

Figure 2. (**a**) Location of Bukit Larut (location for lichen *U. misaminensis* is sampled); (**b**) sampling sites at Kuala Lumpur; (**c**) sampling sites at Johor Bahru; (**d**) sampling sites at Penang.

2.4. Lichen's Vitality Rate Measurement

Chlorophyll (Chl.)-α fluorescence emission analysis was utilised to assess the vitality rate of lichen samples after the exposure period. The performance of the lichen samples' photosynthetic components was assessed and quantified using the primary concept of photochemical quantum, that is, F_V/F_M, where $F_V = F_M - F_0$ is the fluorescent variable, and F_M and F_0 are the maximum and minimum values of the Chl.-α fluorescence. Furthermore, the performance index formula was used to determine the total index of photosynthetic performance (PI_{ABS}). After reactivating the samples for 24 h, they were sprayed with mineral water and kept at 16 °C under ambient light (70 μmol m^{-2} s^{-1}). Afterward, the samples were doused with water again and left in the dark for 10 min without light. The samples were then exposed to a saturating light (3000 μmol m^{-2} s^{-1}) for a few seconds, and the fluorescence emission was measured. A plant efficiency analyser (Handy PEA, Hansatech Ltd., King's Lynn, Norfolk, UK) was used to make the measurements [24].

2.5. Data Analysis

In this study, R Software was utilised for non-parametric analysis. The Mann–Whitney U test ($p < 0.05$) was used for each experiment to examine the significance of the degree of collectivity (or variation in photosynthetic parameters) relative to the control sample. This was carried out to demonstrate the difference between samples that were placed in the same position and measured dependent on their surroundings (Kuala Lumpur, Johor Bahru, and Penang).

For a better understanding of trace element assemblage data in the lichen, in this study, the *exposed-to-control* (EC) ratio, developed by Frati et al. [28]), was used. The EC ratio is a ratio between the concentration of each element after the exposure and in control samples prior to exposure. The proposed scale is <0.25 for severe loss; 0.25–0.75 loss; 0.75–1.25 normal; 1.25–1.75 poor assemblage; and >1.75 assemblage. The EC ratios were also used to investigate trace elements with values >1.25 to detect any possible contamination from the indoor environment.

3. Results

Table 1 illustrates the trace element concentrations accumulated in the lichen *U. misaminensis*, as well as the photosynthetic characteristics (an indication of vitality) after three months of exposure in three different metropolitan regions (Kuala Lumpur, Penang, and Johor Bahru). In most components, Kuala Lumpur City has higher concentrations than the other two urban areas.

Table 1. The coordinate for all sampling stations in the three selected urban areas.

Sampling Stations	Kuala Lumpur (Coordinate)	Johor Bahru (Coordinate)	Penang (Coordinate)
1	3°08′47.4″ N 101°42′43.5″ E	1°32′59.5″ N 103°42′46.0″ E	5°25′52.4″ N 100°18′42.7″ E
2	3°08′44.6″ N 101°41′58.3″ E	1°31′50.4″ N 103°44′52.4″ E	5°27′15.3″ N 100°17′40.1″ E
3	3°09′02.0″ N 101°41′34.2″ E	1°32′19.9″ N 103°47′08.2″ E	5°23′55.7″ N 100°18′30.2″ E
4	3°07′54.9″ N 101°40′19.8″ E	1°31′22.1″ N 103°45′48.2″ E	5°22′49.1″ N 100°18′28.1″ E
5	3°06′56.3″ N 101°40′40.3″ E	1°30′13.0″ N 103°41′36.2″ E	5°21′25.9″ N 100°18′37.8″ E
6	3°06′18.7″ N 101°41′38.1″ E	1°30′57.8″ N 103°43′21.0″ E	5°19′53.7″ N 100°17′47.5″ E
7	3°06′52.7″ N 101°43′39.7″ E	1°29′24.5″ N 103°43′58.4″ E	5°17′39.7″ N 100°15′23.6″ E
8	3°07′08.0″ N 101°42′59.3″ E	1°29′19.8″ N 103°44′26.6″ E	5°17′11.7″ N 100°14′11.1″ E
9	3°08′11.3″ N 101°43′10.3″ E	1°27′58.1″ N 103°45′39.3″ E	5°21′18.7″ N 100°16′17.1″ E
10	3°07′59.9″ N 101°41′41.0″ E	1°29′44.9″ N 103°46′58.4″ E	5°21′02.5″ N 100°14′05.3″ E

The data on *exposed-to-control* (EC) ratios (the difference in concentration level between the elements before and after exposure to their respective environments) demonstrated that there was a substantial rise for all 25 elements (Figure 3) (Al, Ba, Ca, Cd, Co, Cs, Cu, Fe, K, La, Mg, Mn, Na, Ni, Pb, Rb, S, Sb, Sm, Sr, Tb, Th, Ti, V, and Zn).

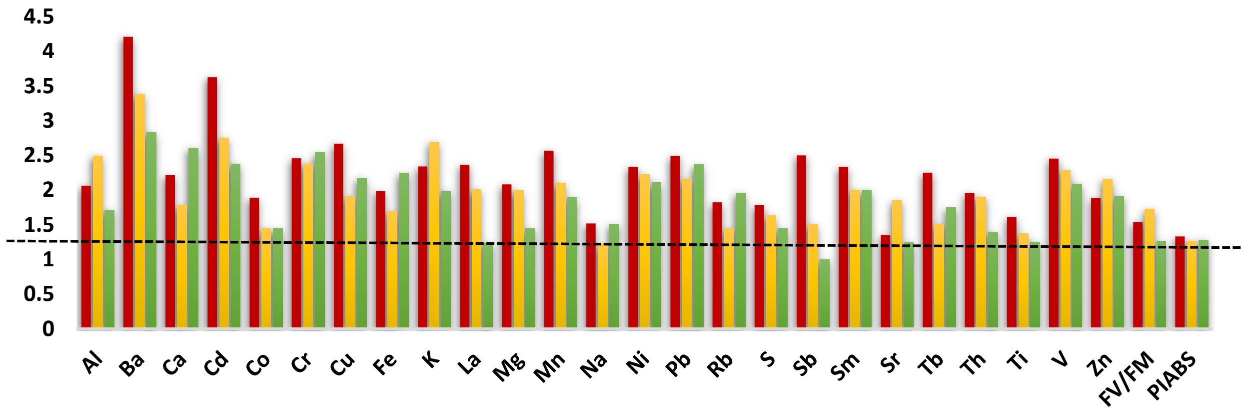


Figure 3. Exposed-to-control (EC) ratios of samples transplanted in Kuala Lumpur, Johor Bahru, and Penang. The dashed line indicates a significant accumulation (threshold EC > 1.25).

The vitality level of the lichen was tested by comparing photosynthetic performance between each sample after exposure to their distinct surroundings, and the results (Table 2) revealed that there was no significant difference in the vitality of lichens between the three selected metropolitan locations. It should also be highlighted that the photosynthetic performance index (PI_{ABS}) increased in each sample from the area owing to the high humidity during the exposure time ($p < 0.05$).

Table 2. Element concentrations (μg g^{-1}) and photosynthetic parameters (potential quantum yield of primary photochemistry—F_V/F_M and performance index—PI_{ABS}) in the lichen *Usnea misaminensis* before and after exposure (average ± standard deviation) (Mann–Whitney U test, $p < 0.05$, significant value is shown in Bold).

		Control (n = 3)	Kuala Lumpur (n = 10)	Johor Bahru (n = 10)	Penang (n = 10)
	Al	15.87 ± 0.18	**32.73 ± 0.24**	**39.55 ± 0.71**	**27.19 ± 0.19**
	Ba	0.29 ± 0.001	**1.22 ± 0.0012**	**0.98 ± 0.0009**	**0.82 ± 0.002**
	Ca	5.12 ± 0.02	**11.34 ± 0.17**	**9.13 ± 0.21**	**13.32 ± 0.11**
	Cd	0.008 ± 0.001	**0.029 ± 0.0001**	**0.022 ± 0.0002**	**0.019 ± 0.0001**
	Co	0.09 ± 0.001	**0.17 ± 0.003**	**0.13 ± 0.002**	**0.11± 0.002**
	Cr	0.24 ± 0.02	**0.59 ± 0.06**	**0.57 ± 0.05**	**0.61 ± 0.02**
	Cu	0.54 ± 0.01	**1.44 ± 0.08**	**1.03 ± 0.02**	**1.17 ± 0.02**
	Fe	11.09 ± 0.31	**22.01 ± 0.22**	**18.77 ± 0.11**	**24.89 ± 0.19**
	K	10.18 ± 0.22	**23.81 ± 0.58**	**27.34 ± 0.43**	**20.19 ± 0.1**
	La	1.88 ± 0.023	**4.44 ± 0.09**	**3.78 ± 0.08**	**2.33 ± 0.02**
	Mg	3.47 ± 0.015	**7.22 ± 0.001**	**6.91 ± 0.008**	**5.02 ± 0.001**
	Mn	0.46 ± 0.001	**1.18 ± 0.0002**	**0.97 ± 0.0001**	**0.87 ± 0.0001**
Elements (μg/g)	Na	2.71 ± 0.018	**4.11 ± 0.006**	**3.31 ± 0.003**	**4.09 ± 0.004**
	Ni	0.09 ± 0.001	**0.21 ± 0.008**	**0.20 ± 0.006**	**0.19 ± 0.001**
	Pb	0.98 ± 0.001	**2.44 ± 0.0051**	**2.12 ± 0.004**	**2.32 ± 0.007**
	Rb	0.045 ± 0.001	**0.082 ± 0.0003**	0.065 ± 0.0004	**0.088 ± 0.0002**
	S	6.67 ± 0.023	**11.88 ± 0.051**	**10.92 ± 0.048**	**9.63 ± 0.022**
	Sb	0.002 ± 0.0001	**0.005 ± 0.0001**	0.003 ± 0.0001	0.002 ± 0.0001
	Sm	0.003 ± 0.0001	**0.007 ± 0.0001**	**0.006 ± 0.0002**	**0.006 ± 0.0001**
	Sr	0.082 ± 0.0001	**0.111 ± 0.0023**	**0.152 ± 0.0011**	**0.1019 ± 0.0018**
	Tb	0.0004 ± 0.0001	**0.0009 ± 0.0001**	**0.0006 ± 0.0001**	**0.0007 ± 0.0001**
	Th	0.068 ± 0.0001	**0.133 ± 0.072**	**0.129 ± 0.052**	0.0944 ± 0.022
	Ti	6.81 ± 0.007	**10.98 ± 0.025**	**9.33 ± 0.017**	**8.54 ± 0.022**
	V	0.042 ± 0.0001	**0.103 ± 0.0001**	**0.096 ± 0.0001**	0.0877 ± 0.0001
	Zn	2.11 ± 0.019	**3.98 ± 0.021**	**4.56 ± 0.019**	**4.03 ± 0.020**

Table 2. *Cont.*

		Control (n = 3)	Kuala Lumpur (n = 10)	Johor Bahru (n = 10)	Penang (n = 10)
Vitality Rate	F_V/F_M	0.81 ± 0.0003	**0.244 ± 0.0001**	**0.399 ± 0.0002**	0.222 ± 0.0012
	PI_{ABS}	0.172 ± 0.0003	**0.2289 ± 0.0002**	**0.2176 ± 0.0003**	**0.2199 ± 0.0001**

4. Discussion

The level of air pollution in urban areas is usually correlated with the total population and traffic in the area. According to Abas et al. [19], the concentration and variety of air pollutants in urban areas are due to traffic, combustion and agricultural, fuel burning, dust, and industrial activity. As a result, the accumulation of potentially toxic elements concentration in the transplanted lichen was greater than that in the control sample, which was exposed to ambient conditions. The same held true for the vitality rate of transplanted lichen—namely, samples from the urban environment demonstrated a decline when compared with the control sample. This demonstrates that the lichen *U. misaminensis* is as dependable as other air pollution instruments (machine or biological indicator) and can be used to measure urban air pollution. The ability of lichen to survive for long periods of time under any condition while absorbing any elements that pass through them makes lichen a good biological indicator to determine the level of air pollution in that particular area [20]. According to Abas [12], lichen species are useful biological indicators of air pollution, although they are only found in a few species. As a result, *U. misaminensis* is one of the few lichen species that may be utilised as a biological indicator of air pollution.

This study was focused on the accumulation of potentially toxic elements, with the idea that the concentrations of these elements in lichen exposed to urban pollution represent the circumstances and current status of the chosen location. The EC ratio indicated that all of the elements were present in high concentrations (EC ratio between 2.20 and 2.75) in all three urban areas, particularly Al, Ba, Ca, Cd, Cr, Cu, Fe, K, La, Mn, Mg, Ni, Pb, S, Sb, Sm, Tb, V, and Zn. The concentrations of components in the transplanted lichen were greatly influenced by vehicle traffic. Numerous studies have revealed an increase in element accumulation in lichens with increasing proximity to highways [29–31]. Vehicles may release a variety of potentially toxic elements into the atmosphere, including Cd, Cu, Fe, Pb, Ni, Sb, V, and Zn, through fossil fuel burning, tyre and brake pad abrasion, corrosion, lubricating oils, gasoline additives, etc. [32,33]. Ratier et al. [34] discovered that road traffic and agriculture were responsible for the bioaccumulation of Cu, S, and Sb in *Xanthoria parietina*, despite the fact that the sample was conducted away from the main roadsides. Furthermore, vehicle traffic's suspension and resuspension of a mixture of soil and road dust might result in the formation of terrigenous components. Boamponsem et al. [35] discovered, for example, that emissions from road traffic and roadside dust constitute a substantial source of 18 elements collected in transplanted lichen *Parmotrema reticulatum.*

On the other hand, the concentrations of the potentially toxic elements in the three urban areas showed that a few of the elements such as Al, Ca, Fe, K, Mg, Pb, Ti, and Zn had higher levels than the other potentially toxic elements. According to Aguilera et al. [36], elements such as Al, Fe, Pb, and Ti are emitted from traffic due to fuel combustion meanwhile, and Ca and Mg normally originate from industrial emissions and also agricultural activity. As previously stated, these three places are severely congested and are also among Malaysia's major industrial cities. Among the three selected urban regions, only Penang has significant agricultural activity near the city. According to Alias et al. [37], industrial activities in the city such as the generation of power and cement, waste management and incineration, and extensive livestock production are responsible for dangerous substance emissions into the air, water, and soil. Large-scale industrial activities deplete scarce resources, employ harmful chemicals, and emit pollution that harms human health and the environment. Urban areas such as Johor Bahru and Penang are also known as coastal cities where the geographical location is nearby the marine area. According to Briffa et al. [38], marine potentially toxic elements (PTEs) likely contribute to the increase in PTEs in coastal areas. However, in the case of Johor Bahru and Penang, the effects of the marine PTEs were insignificant due to the location of the selected sampling stations, which are far from the beach and closer to the road [39].

In this study, the parameter Chl.-α fluorescence revealed that the lichen samples were considerably influenced by the urban air pollutants from the three selected urban areas, referring to the vitality level of the lichen sample. Lichen vitality is sensitive to the rate of humidity in their surroundings, according to Viera et al. [40] The collection of pollutants in the air will significantly reduce humidity in that location, causing the temperature to rise [6]. This will essentially alter the vitality rate of the lichen species in that location. Several studies have shown that the vitality rate of lichens decreases with the accumulation of air pollutants. For example, Nannoni et al. [26] used *Evernia prunastri* to gain insight into the lichen vitality as possibly affected by both element deposition or lichen element content and further ambient atmospheric conditions (temperature, precipitation, SO_2 levels). It was discovered that the conductivity of the lichen sample's membrane cell decreases due to increased SO_2 concentrations in the surrounding environment.

The rate of element accumulation and lichen viability varied amongst the three metropolitan areas studied (Kuala Lumpur, Penang, and Johor Bahru). Kuala Lumpur, Malaysia's largest metropolis, had the highest concentration of potentially toxic elements in practically every element and also had the lowest lichen vitality rate. According to Halim et al. [2], Kuala Lumpur has 5.3 million inhabitants, accounting for 18% of Malaysia's total population. Then, it is reasonable to predict that the frequency of vehicle motors and industrial operations in Kuala Lumpur will be the greatest in Malaysia when compared with other cities. This also implies that lichen can serve as biological indicators of urban air pollution in the same way that current technologies are used to measure air pollution.

5. Conclusions

This study was conducted to biomonitor air pollution using *U. misaminensis* as transplanted lichen in three selected urban areas in Malaysia—namely, Kuala Lumpur, Penang, and Johor Bahru. The results of this study confirmed that *U. misaminensis* is an efficient bioaccumulator for determining the air pollution level in urban areas. We also found that all of the 25 elements accumulated in samples from urban areas in higher concentrations compared with the control sample, due to the traffic, industrial activities, and fuel burning. Lichen's vitality rate also decreased in samples from these urban areas, compared with the control sample, which showed that the increase in air pollutants affects the vitality rate of any biological component. However, the use of technology does not reveal how biological components react and respond in terms of their physiological changes. Therefore, the use of bioaccumulators such as lichen could benefit urban planners, local authorities, and also the urban community to anticipate, predict, and analyse the condition of their urban area and eventually plan and execute a better urban and residential planning.

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