DETERMINATION OF TRACE ELEMENTS IN MALAYSIAN LICHENS AS POTENTIAL INDICATORS FOR POLLUTION BY USING INDUCTIVE COUPLE PLASMA EMISSION SPECTROPHOTOMETRY

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Abstract. A study was carried out to determine the concentration of trace elements As, Cd, Cr, Cu, Mn, Ni, Pb, V and Zn in samples of lichens collected from the area in vicinity of Gunung Jerai, Kedah to evaluate the potential of lichens as a pollution indicator. Six species of lichens collected in this study were *Cladia aggregate, Cladonia sp., Heterodarmia flabellata, Parmotrema tinctorum, Parmotrema sp.* and *Usnea flexilis.* The samples were analysed by using the inductively couple plasma - optical emission spectrometer (ICP-OES) Perkin Elmer Optima 4300 DV. Prior to ICP-OES analyses, the samples were cleaned, dried, pulverized and digested in dilute nitric acid solution. The ranges of concentrations (μg/g) in samples of lichens in this study are As (0.16 –0.56), Cd (< 0.005), Cr (0.01– 1.75), Cu (1.07–6.93), Mn (8.17–108.59), Ni (0.09 – 1.08), Pb (0.84 – 9.75), V (0.01 – 2.45) and Zn (17.54– 45.45).

Keywords: Metals, lichens, Jerai, Kedah, ICP-OES

Abstrak. Kajian ini dijalankan untuk mengukur kepekatan unsure surihan As, Cd, Cr, Cu, Mn, Ni, Pb, V dan Zn dalam sample liken yang diperolehi dari kawasan sekitar Gunung Jerai, Kedah untuk menilai potensi sample tersebut sebagai penunjuk pencemaran. Enam spesies liken yang telah disampel adalah pada kalangan *Cladia aggregate, Cladonia sp., Heterodarmia flabellata, Parmotrema tinctorum, Parmotrema sp.* dan *Usnea flexilis.* Sampel tersebut telah diproses dan dihadamkan menjadi larutan sebelum ditentukan kepekatan logamnya dengan menggunakan instrumen plasma gandingan teraruh – spectrometer emisi optik (ICP-OES) berjenama Perkin Elmer Optima 4300 DV. Sebelum analisis berinstrumentasi sample tersebut dibersihkan, dikeringkan, dihancurkan dan dihadamkan dalam larutan cair asid nitric. Julat kepekatan logam tersebut dalam sample kajian ini adalah (μg/g) As (0.16 –0.56), Cd (< 0.005), Cr (0.01– 1.75), Cu (1.07–6.93), Mn (8.17–108.59), Ni (0.09 – 1.08), Pb (0.84 – 9.75), V (0.01 – 2.45) dan Zn (17.54– 45.45).
Kata kunci: Logam, liken, Jerai, Kedah, ICP-OES

Introduction

Plants has been used as bioindicators for many years to detect environmental changes. Lichens are one of the most valuable biomonitors of atmospheric pollution with other growth such as moss and fungi. Most of species of lichen have a wide geographical distribution, which allows for a study of pollution covering wide areas and its high capacity to accumulate metals [1]. The morphology of lichens does not have seasonal variations and accumulation of pollutants can occur all year-round [5].

Lichen are very sensitive to changes in its surrounding environment. Lichens also can absorb pollutants such as trace metals from air borne particles, and then accumulate and saturate the metals. This is due to their structure and anatomy (Hutchinson *et al.* 1996). The weakness of its cuticles enables moist air to be absorbed through its surface. Metals which are absorbed along with the moist air are dissolved in it. The ability of different types of lichens to accumulate metals from the surroundings is different depending upon its species. Lichen can be used as sensitive indicators to estimate the biological effect of pollutants by measuring changes at the community or population level, and as accumulative monitors of persistent pollutants, by assaying their trace element contents [8].

The inductively coupled plasma – optical emission spectrometer (ICP-OES) is an analytical instrument with a sensitivity which is suitable for trace metal analyses. It is widely used in industries such as environment, geology, chemistry, material science, nuclear and clinical research [4].

Methods

Triplicate samples of lichen were collected from the area in vicinity of Gunung Jerai, Kedah, Malaysia. These samples were cleaned by using distilled water, oven dried for two days at a temperature of 50^0C, grounded into powder using agate mortar and homogenized. About 0.2 g were weighed accurately and digested using 3.0 ml HNO_3 and 1.0 ml H_2O_2. Digestion of samples was carried out in a microwave oven

(Milestone, Easywave). The digested samples were diluted to 50.0 ml using deionised distilled water as preparation for analysis using (ICP-OES).

Trace elements were determined by using inductively coupled plasma-optical emission spectrometry for (As, Cd, Cr, Cu, Mn, Ni, Pb, V and Zn). Operational parameters of the ICP-OES were: gas flow for plasma and penebula at 15 L/min and 0.8 L/min. Penebula pressure was 112 kPa, ICP RF power 1300 Watts, pump flow rate at 2.5 ml/min, the temperature set at 31.5 ^{0}C and for the pump parameter, the rate of flow for sample was 1.5 ml/min.

Results and Discussion

Concentration of trace metals in the various species of lichens of this study and compared to those of earlier studies conducted elsewhere are shown in Table 1. As, Cd, Cr, Cu, Mn, Ni, Pb, V and Zn had been detected in six species of lichens by using ICP-OES.

From this study, the distribution of concentration of trace elements for the six species was found to be uneven. Concentrations of manganese in *Cladonia sp.* were 108.59 $\pm$ 0.69 µg/g, 85.32 $\pm$ 17.67 µg/g in *Heterodarmia flabellate* and 25.16 $\pm$ 0.98 µg/g for *Usnea flexilis*. Zinc was of higher concentrations at 17.54 $\pm$ 1.78 µg/g for *Cladia aggregate*, 36.5 $\pm$ 9.45 µg/g in *Parmotrema tinctorum* and 34.28 $\pm$ 1.57 µg/g in *Parmotrema sp.*

Concentrations of Ni were 0.19 $\pm$ 0.16 µg/g for *Usnea flexilis*, 0.09 $\pm$ 0.13 µg/g for *Cladia aggregate*, 0.57 $\pm$ 0.25 µg/g for *Parmotrema tinctorum*, 0.68 $\pm$ 0.16 µg/g for *Heterodarmia flabellate*, 1.08 $\pm$ 0.1 µg/g for *Cladonia sp*. and 0.38 $\pm$ 0.03 µg/g for *Parmotrema sp*. Concentrations of As and Cd in the lichen were between 0.005 to 0.563 µg/g.

Concentrations in *Cladonia sp*. were 6.93 $\pm$ 0.23 µg/g for Cu, Mn at 108.59 $\pm$ 0.69 µg/g, Ni at 1.08 $\pm$ 0.1 µg/g and Pb at 9.75 $\pm$ 0.30 µg/g. Higher concentrations of V and Zn were measured in *Heterodarmia flabellate* with values of 2.45 $\pm$ 0.61 µg/g and 45.45 $\pm$ 4.77 µg/g. Respectively Cr registered a higher concentration in *Cladia aggregate* with 1.75 $\pm$ 0.29 µg/g. Cd has been detected only in *Usnea flexilis* with 0.005 $\pm$ 0.01 µg/g. As only has been detected in three species of lichen ie 0.16 $\pm$ 0.29 µg/g in *Usnea flexilis*, 0.56 $\pm$ 0.98 µg/g in *Cladia aggregate* and 0.48 $\pm$ 0.82 µg/g in *Cladonia sp.*

Different species have different sensitivities in absorbing and accumulating trace metals. *Cladonia sp.* was sensitive to V and Zn, while *Cladia aggregate* was sensitive to Cr and As. *Usnea flexilis* was sensitive to Cd and Cd could not be detected in the other five species.

The samples of lichen in this study has been taken from bark of trees and as epiphytic lichens. Lichens absorbed metal elements not only from the air but also from its substrate. This lichens grow on the outer bark of trees, which were made up of dead and dried materials. The place that they were growing on did not influence the absorption and accumulation of trace metals from the air.

Different species show different concentrations. Rate of accumulation of specific metals depends on amount of metal present in the air. The concentration of metals would be lower if the lichen were located further from the pollution sources. However the location of Gunung Jerai is quite far ie about 100 km away from potential sources of air pollution which normally originated from human settlements and industrial estates.

The source of pollution can attributed to industrial factories, motorized vehicles, power generation, heaters and waste incineration. Pollutants that are released to the air are usually inorganic gases like sulphur dioxide, hydrogen sulfide, nitrogen dioxide, ozone and others. Besides that, organic gases can also be released such as hydrocarbons, inorganic particles like metal oxides and organic particles such as ash.
In this study it was not possible to compare levels of metals studied in similar lichen species obtained from polluted places with those detected in this study location which is an area quite pristine in characteristics. Nevertheless, the data obtained are vary much of value to be used for future comparisons when researchers could sample the same species of lichens found in ‘polluted’ areas. We believe, the values obtained in this study are representative of ‘relatively clean’ areas in Malaysia; and will also be of use as important inputs into databases on metal content in lichens of Malaysia.

These were compared with values of similar metals in other species of lichens that had been sampled and analysed by other researchers in other parts of the world as a general comparison to give some idea on the order of magnitude of samples that had been obtained from ‘clean’ and ‘polluted’ areas, wherever possible.

Main sources that contribute to air borne trace metal pollutants originated from motorized vehicles. This is due to the location of the samples that has been taken near access route for vehicles. *Cladonia sp.* is the most sensitive lichen and could be used as pollution indicator.

Table 1 Concentration of trace metals in lichens of this study and compared with those of earlier studies elsewhere

Metal	Lichen species	Place, Country	Concentration (µg/g)	Reference or Research Group
As	*Parmotrema tinctorum*	UKM, Bangi, Malaysia	1.72 ± 0.41	[7]
	Cladia aggregate *Cladonia sp.* *Heterodarmia flabellate* *Parmotrema sp.* *Usnea flexilis*	Gunung Jerai, Malaysia	0.56 ± 0.98 0.48 ± 0.82 n.d., < 2.00 ng/g n.d., < 2.00 ng/g 0.16 ± 0.29	This study, 2006
Cd	*Cladia aggregate* *Cladonia sp.* *Heterodarmia flabellate* *Parmotrema tinctorum* *Parmotrema sp.* *Usnea flexilis*	Gunung Jerai, Malaysia	n.d, < 0.1 ng/g n.d, < 0.1 ng/g n.d, < 0.1 ng/g n.d, < 0.1 ng/g n.d, < 0.1 ng/g 0.005 ± 0.01	This study, 2006
Cr	*Lepraria sp.*	UKM, Bangi, Malaysia	2.37 ± 0.47	[7]
	Cladia aggregate *Cladonia sp.* *Heterodarmia flabellate* *Parmotrema tinctorum* *Parmotrema sp.* *Usnea flexilis*	Gunung Jerai, Malaysia	1.75 ± 0.29 1.39 ± 0.25 0.25 ± 0.15 0.47 ± 0.23 0.01 ± 0.01 0.53 ± 0.73	This study, 2006
Cu	*Parmotrema praesorediosum*	UKM, Malaysia	5.85 ± 1.06	[7]
	Cladia aggregate *Cladonia sp.* *Heterodarmia flabellate* *Parmotrema tinctorum* *Parmotrema sp.* *Usnea flexilis*	Gunung Jerai, Malaysia	1.07 ± 0.52 6.93 ± 0.23 4.94 ± 0.76 3.34 ± 0.37 3.05 ± 0.43 1.93 ± 0.06	This study, 2006
Mn	*Lepraria sp.*	UKM, Bangi, Malaysia	12.81 ± 0.71	[7]
	Cladia aggregate *Cladonia sp.* *Heterodarmia flabellate* *Parmotrema tinctorum* *Parmotrema sp.* *Usnea flexilis*	Gunung Jerai, Malaysia	8.17 ± 0.45 108.59 ± 0.69 85.32 ± 17.67 32.69 ± 0.34 28.59 ± 0.36 25.16 ± 0.98	This study, 2006
Ni	*Parmotrema tinctorum*	UKM, Bangi, Malaysia	1.63 ± 0.17	[7]
	Cladia aggregate *Cladonia sp.* *Heterodarmia flabellate* *Parmotrema sp.* *Usnea flexilis*	Gunung Jerai, Malaysia	0.09 ± 0.13 1.08 ± 0.10 0.68 ± 0.16 0.38 ± 0.03 0.19 ± 0.16	This study, 2006
Pb	*P. baltimoresis* *Xanthoparmelia conspera* *Cladina subtanuis*	Plummers Island, USA	1893.5 1647.5 272.0	[6]
	Lepraria sp.	UKM, Bangi, Malaysia	18.92 ± 1.55	[7]
	Cladia aggregate *Cladonia sp.* *Heterodarmia flabellate* *Parmotrema tinctorum* *Parmotrema sp.* *Usnea flexilis*	Gunung Jerai, Malaysia	0.84 ± 0.59 9.75 ± 0.30 6.97 ± 0.27 7.44 ± 0.73 6.67 ± 1.08 3.20 ± 0.46	This study, 2006

V	*Parmotrema praesorediosum*	UKM, Bangi, Malaysia	3.02 ± 0.66	[7]
	Cladia aggregate *Cladonia sp.* *Heterodarmia flabellate* *Parmotrema tinctorum* *Parmotrema sp.* *Usnea flexilis*	Gunung Jerai, Malaysia	n.d,< 0.5 ng/g 1.57 ± 0.07 2.45 ± 0.61 2.45 ± 0.61 0.53 ± 0.04 0.01 ± 0.01	This study, 2006
Zn	*Umbilicarin pustulata*	Finland	3000 –10000	[2]
	Cladonia rangifera	Sweden	51-204	[3]
	Lepraria sp.	UKM, Bangi, Malaysia	61.31 ± 4.48	[7]
	Cladia aggregate *Cladonia sp.* *Heterodarmia flabellate* *Parmotrema tinctorum* *Parmotrema sp.* *Usnea flexilis*	Gunung Jerai, Malaysia	17.54 ± 1.78 33.26 ± 3.06 45.45 ± 4.78 36.5 ± 9.45 34.26 ± 1.57 21.01 ± 1.67	This study, 2006

Lichens had been established as good indicators of air pollution in many references and again in this study where they are quite easily available in various parts of the country, at various altitudes and settings, but ht echallenge that remains is to sample similar species that could be found in different kinds of habitats with different levels of pollution.

Conclusion

From the analysis of this study, it can be concluded that the trace metals (As, Cd, Cr, Cu, Mn, Ni, Pb, V and Zn) in six species of lichens could be detected by the ICP-OES instrument, a technique with good sensitivity.

Results indicate that trace elements have an uneven distribution in the six species of the lichens studied. Mn is the element that was present at the highest concentration among all six species with a concentration of 108.59 ± 0.69 µg/g for *Cladonia sp.* Ni was the trace element that has the lowest concentration of 0.09 ± 0.13 µg/g in *Cladia aggregate*. From the data, *Cladonia sp*. was found to be the most sensitive in its ability to accumulate metals, while *Cladia aggregate* is the least sensitive as it only absorbed metals at lower concentrations. Cd was detected in *Usnea flexilis* compared to five other species. Gunung Jerai seems to have a good air quality because the concentrations that had been detected in lichens were low.

For future studies it is recommended that sampling should be done in different locations. Sample of lichens of the same species should be collected in an area that is polluted and other areas where the level of pollution is variable. This is to enable the observation of absorption of metals and compare the concentration of pollutants at different locations. Also to evaluate the potential of lichens as a pollution indicator.

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