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LICHENS AS BIOINDICATORS OF ATMOSPHERIC HEAVY METAL POLLUTION IN SINGAPORE

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Abstract. Lichens have been used as bioindicators in various atmospheric pollution assessments in several countries. This study presents the first data on levels of heavy metals (As, Cd, Cu, Ni, Pb, and Zn) in lichens at different locations in Singapore, Southeast Asia. Singapore is a fully industrialised island nation, with a prevailing tropical climate and a population of 4 million people within a confined land area of less than 700 km^2. The ubiquitous lichen species, *Dirinaria picta* was collected from six sample sites across Singapore and analysed for heavy metals using inductively coupled plasma mass spectrometry (ICPMS). No significant relationship existed between metal levels in lichen and soil, indicating that accumulated metals in lichen are primarily derived from the atmosphere. Peak concentrations of zinc (83.55 μg g^{-1}), copper (45.13 μg g^{-1}) and lead (16.59 μg g^{-1}) in lichens were found at Sembawang, Jurong and the National University of Singapore campus which are locations associated with heavy petroleum and shipping industries, and road traffic respectively. The mean heavy metal levels of lichen samples in Singapore were found to be at the upper range of values reported in the literature for temperate countries.

Keywords: heavy metals, lichen, *Dirinaria picta*, Singapore, ICPMS

1. Introduction

Human activities have altered global biogeochemical cycling of heavy metals, resulting in a progressive increase in the flux of bioavailable chemical forms to the atmosphere. There is evidence that increasing exposure to toxic elements in marine and terrestrial organisms is having adverse toxicological consequences (Rana *et al.*, 2004; Katranitsas, *et al.*, 2003) In order to evaluate, minimise and avoid detrimental effects of toxic metals, there has been an emphasis in the use of natural bioindicators to monitor atmospheric quality in both urban and rural environments (Szczepaniak and Biziuk, 2003; Bargagli, 1998).

Recent investigations have shown that lichen species can be used to effectively monitor levels of metal contamination in the atmosphere (González *et al.*, 1996; Loppi *et al.*, 1998; Freitas *et al.*, 1999; Rossbach *et al.*, 1999; Pandey *et al.*, 2002; Jeran *et al.*, 2002; Yun *et al.*, 2003; Nayaka *et al.*, 2003). A key feature of this sentinel group of bioindicators is their ability to reflect prevailing atmospheric pollutant levels in their tissues without significant adverse effects on survival or growth

(Beeby, 2001; Sarret *et al.*, 1998; Nash, 1989). Due to a high surface area:volume ratio, a simple anatomy and the absence of a well-developed root system, lichens rely directly on atmospheric particulate deposition for nourishment and, as a consequence, readily bioaccumulate pollutants from the air (Bargagli *et al.*, 2002). The lack of a waxy, exterior cuticle also facilitates the uptake and retention of aerosols (Sloof, 1995; Nash, 1996). Hence, over the long term, lichens reflect the elemental composition and prevailing concentration of metal ions in the atmosphere (Adamo *et al.*, 2003). Moreover, many lichens are geographically extensive in their distribution which facilitates biomonitoring programmes over extended areas (Hawksworth and Rose, 1976).

To date, almost all studies on the use of lichens as bioindicators of atmospheric pollution have been undertaken in Europe and America, while equivalent studies in Southeast Asia are lacking. This study was therefore undertaken to evaluate heavy metal levels in lichens in Singapore to provide primary data for Southeast Asia. The species used i.e. *Dirinaria picta* is a whitish grey coloured, foliose lichen widely found on the arboreal trunks of Royal Palm trees found throughout the city state of Singapore. Data obtained from this study were compared to similar available data reported for lichens in temperate countries.

2. Materials and Methods

2.1. STUDY SITES AND SAMPLE COLLECTION

Singapore is located at the southern tip of the Malaysian Peninsula, between latitudes 1°09′N and 1°29′N and longitudes 103°36′E and 104°25′E. The city state comprises one major island and about 60 smaller ones and is separated from mainland Malaysia by the Straits of Johor, and from Indonesia by the Straits of Singapore (see Figure 1). As a result of its equatorial location and maritime exposure, Singapore's climate is characterized by a uniform temperature (25–34°C) and abundant rainfall (average 2549.7 mm) (NEA, 2004). The country has a population of approximately 4 million persons within a land area of less than 700 km^2. Singapore is a fully industrialised nation, comprising major industries such as electronics, petrochemicals and biotechnology.

Six sites, including the campus of the National University of Singapore, Sembawang Park, Jurong, Fort Canning, Lower Pierce Reservoir, and Changi, were chosen to represent a spatial cross section of the main island and varying levels of human activity and environmental disturbance. Further details on sample locations are given in Table I and Figure 1. At each site, the lichen, *D. picta,* was collected from six separate trees at a height of between 0.1 and 2 m above ground level. At each site, the top five cm of top-soil was also collected for analysis of heavy metals and correlation with lichen metal concentrations. Sampling was conducted between February and March 2004, and lichen specimens were collected using

TABLE I
Sample locations and general characteristics

Site no.	Site name	Description of activities
1	National University of Singapore (NUS)	Approximately 800–1000 m away from open sea. Chemical industries are located to the southwest of the site and heavy industries are found at the north-western direction. Low traffic intensity site, but 500 m away from main road used by industrial vehicles.
2	Jurong (J)	Main industrial area in Singapore – computer & electronics, petroleum refining, metal processing food, construction, chemical & biomedical (JTC, 2003)
3	Fort Canning Park (FC)	19-hectare urban park in the middle of Singapore's Central Business District
4	Sembawang Park (Sem)	15-hectare park adjacent to northern coast, facing Johor's maritime port at a distance of 700 m away.
5	Lower Pierce Reservoir Park (LPRP)	Part of the central water catchment area of Singapore adjacent tropical secondary forest.
6	Changi (C)	200 m away from one of the world's busiest airports located on the Eastern most part of Singapore with moderately-heavy vehicle traffic

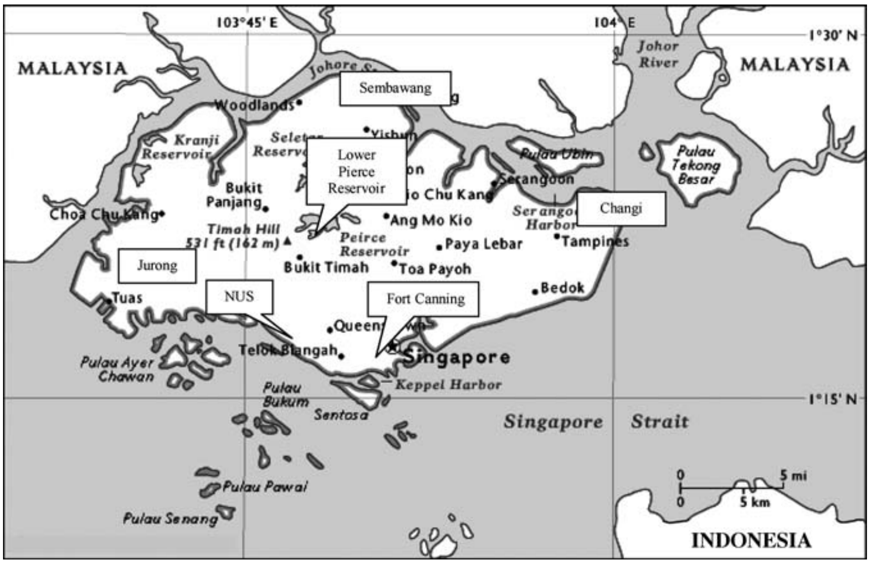

Figure 1. Singapore and lichen sample locations (6).

plastic forceps with subsequent storage in Petri dishes that were sealed prior to laboratory analysis.

2.2. SAMPLE PREPARATION

In the laboratory, the unwashed lichen samples were dried in an oven at 40 ± 2 °C for 48 h and subsequently sorted to remove dead or senescent tissue and extraneous material (adhering bark, other lichen species, soil particles). All six lichen samples from each site were crushed and homogenized in a plastic mortar and three replicate sub samples were analysed. Soil samples were oven dried at 50 ± 2 °C for 48 h and passed through a 2 mm sieve. All samples were stored at 3 ± 2 °C prior to analysis.

A 0.2 g processed lichen sample (dry weight equivalent) was digested (Tuncel *et al.*, 2004) in a microwave assisted extraction unit (MarsX, CEM, Matthews, N.C., USA) with 10 ml of HNO_3 using a closed XP-1500 Teflon vessel for 45 min via a two-step microwave enhanced digestion (MED) procedure: pre-digestion at 95 °C, 0 PSI for 15 min; digestion ramp to 200 °C, 800 PSI for 15 min, held at 200 °C, 800 PSI for 15 min. Similarly, 0.5 g of a homogenized soil sample was digested using another microwave oven (Milestone, *Ethos* D, Monroe, C.T., USA) with a mixture of 9 ml HNO_3 and 3 ml of HF in a closed vent medium pressure vessel with a ramp to 180 °C at 600 W for 10 min and held at 180 °C, 600 W for 10 min.

2.3. SAMPLE ANALYSIS

After cooling, digestion solutions were diluted to 50 ml with ultrapure water. The solutions were then analysed for total concentrations of As, Cd, Cu, Ni, Pb, and Zn by inductively coupled plasma mass spectrometry (ICPMS). A Perkin-Elmer (*ELAN 6100*) ICPMS (West Coast Analytical Service, C.A., USA), equipped with a standard pneumatic nebulizer and an automatic sampler (AS 90) was used for analysis. The instrument was operated according to the manufacturer's recommended specifications for instrumental parameters and sample matrix modifications (detailed ICPMS operating parameters can be found in Diegor and Longerich, 2000). Prior to analysis of lichen and soil samples, the ICPMS was calibrated with a multi-element standard (J.T. Baker Trace Metal Standard 1 Plasma Std-Matrix 5% HNO_3) that was diluted with 2% nitric acid to 5 ng/ml, 50 ng/ml, 100 ng/ml and 200 ng/ml. All metal concentrations are reported as $\mu g\ g^{-1}$ based on dry weight.

2.4. QUALITY CONTROL

For quality assurance purposes, two blank solutions of 10 ml HNO_3 were prepared to determine the method detection limits (MDLs) of each analyte, and were used for every eight samples analysed. MDLs were calculated as follows:

$$MDL = C_{\text{Blank\_ave}} + 3^{*} SD_{\text{Blank}}$$

Values of all sample analytes were above detection limits indicating the overall success of laboratory protocols.

The accuracy of absolute metal concentrations in the samples was ascertained by analysing samples of lichens spiked with concentrations of 2 and 10 ppm of each heavy metal analysed prior to digestion with HNO_3. All spiked samples were dried in a laboratory oven at 50 °C before digestion. The percentage recovery of analytes was calculated as follows:

$$R = [(C_s - C)/S] \times 100\%;$$

where R: percent (%) recovery; C_s: fortified sample concentration; C: sample background concentration; and S=concentration equivalent of analyte added to fortify the sample.

To evaluate the accuracy of the soil digestion and analytical procedure, the certified reference material MESS-3 (National Research Council, NRC, Canada) was digested and analyzed with the same matrix and procedure as that of soil samples.

2.5. STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) was used to determine significant differences of analysed elements (at $p < 0.05$) between sample sites. To establish possible correlations between metal concentrations in the lichens and soils, the Spearman's rank correlation coefficient (r) was calculated.

3. Results and Discussion

3.1. QUALITY CONTROL

The MDL values were satisfactory for As, Cu, Ni, and Zn (Table II). Summary data for spiked lichen samples and soil certified reference material are given in Table III. Percentage recovery of analytes spiked at 2 and 10 ppm are within the EPA certified range of 70–130% (EPA 1991). In general, concentrations of heavy metals in lichen samples were all above detection limits (Table II). High precision percentages (average of all six sites) were also recorded for each of the different heavy metals (Table II). Summary data for the certified soil reference material are given in Table III. Data indicates that the laboratory procedure for the digestion of samples to analysis using ICP-MS was satisfactory.

TABLE II
Method detection limit (μg g^{-1}) and precision, expressed as the relative standard deviation (%) of ICP-MS analytical data

Element	MDL (μg/g)	Precision (%)
As	0.215	93.75
Cd	0.009	95.74
Cu	0.253	96.44
Ni	0.963	93.75
Pb	0.159	96.62
Zn	2.330	97.25

TABLE III
Heavy metal recoveries (%) for lichen and soils

Specimen	Spiking concentration	(ppm)	As	Cd	Cu	Ni	Pb	Zn
Lichen	Recovery (%)	2.0	99.15	97.58	120.5	119.7	121.0	88.63
		10	102.2	96.15	113.4	116.2	113.5	86.11
Soil ($n = 4$)	Recovery (%)		91.50	124.6	93.81	96.80	99.05	74.20

[a]Spiked values are all within the USEPA certified range of 70–130%.

3.2. LICHEN AND SOIL ANALYSIS

Heavy metal concentrations in lichen samples are compared with respective soil data for each collection site in Table IV. Regression correlations between lichen and soil metal levels for the six sites are not significant ($p > 0.05$) demonstrating that accumulated heavy metals in lichens predominantly derived from the atmosphere and are not influenced by splash or suspension of soil particulates. For all sites, the mean concentrations of metals in lichens were present in the following order: Zn > Cu > Pb > Ni > As > Cd. Jørgensen (2000) lists gasoline combustion and incineration as key environmental sources of heavy metals in the urban atmosphere. Vehicular traffic as well industrial emissions in Singapore, are the most likely anthropogenic sources contributing to the presence of these elements in the local and regional atmosphere.

Zinc was present in lichen samples at a mean concentration of 65.6 μg/g. Zn is a relatively abundant and naturally occurring metal, but may also be released into the atmosphere during manufacturing and processing activities, such as metal extraction in the petroleum refining industry, the production of corrosion-resistant alloys and brass, and in galvanising steel and iron products (EPA, 2003a). Other anthropogenic emission sources include incinerators, traffic, pesticides (zinc carbamates), fertilisers, coal burning power plants (Scerbo *et al.*, 2002). Relatively high

TABLE IV

Heavy metal concentrations ($\propto$g g^{-1} d.w.) in lichens ($n = 6$) and soil ($n = 3$) sampled in Singapore

Site		As	Cd	Cu	Ni	Pb	Zn
NUS	Lichen	1.83 ± 0.02	0.28 ± 0.03	37.6 ± 0.61	10.8 ± 0.19	15.7 ± 0.10	83.6 ± 3.60
	Soil	5.43 ± 0.32	0.06 ± 0.08	10.8 ± 0.44	3.26 ± 0.24	21.2 ± 1.57	24.2 ± 2.21
J	Lichen	2.0 ± 0.09	0.23 ± 0.01	40.0 ± 1.27	11.5 ± 0.29	16.6 ± 0.36	79.0 ± 0.80
	Soil	21.9 ± 1.64	0.07 ± 0.01	15.6 ± 2.07	2.34 ± 0.10	19.5 ± 1.19	31.3 ± 5.19
FC	Lichen	0.45 ± 0.04	0.14 ± 0.01	11.8 ± 1.14	2.52 ± 0.29	2.83 ± 0.10	63.2 ± 4.50
	Soil	15.1 ± 2.28	0.07 ± 0.01	5.22 ± 0.28	2.08 ± 0.31	20.0 ± 1.01	21.3 ± 1.10
Sem	Lichen	2.06 ± 0.14	0.23 ± 0.01	45.1 ± 0.98	3.06 ± 0.19	10.3 ± 0.29	74.2 ± 1.70
	Soil	6.03 ± 3.82	0.05 ± 0.01	7.17 ± 0.55	1.52 ± 0.32	13.6 ± 0.56	10.7 ± 0.43
LPRP	Lichen	0.75 ± 0.04	0.15 ± 0.00	21.4 ± 0.32	3.87 ± 0.14	11.7 ± 0.14	49.4 ± 0.21
	Soil	4.26 ± 0.03	0.08 ± 0.00	6.13 ± 0.51	2.08 ± 0.22	16.6 ± 0.49	13.2 ± 0.87
C	Lichen	0.71 ± 0.07	0.16 ± 0.01	41.4 ± 1.33	3.18 ± 0.38	14.0 ± 1.42	44.2 ± 0.59
	Soil	2.57 ± 0.32	0.07 ± 0.01	7.18 ± 0.54	1.10 ± 0.15	25.6 ± 2.49	13.4 ± 1.30

NUS, National University of Singapore; J, Jurong; FC, Fort Canning Park; Sem, Sembawang Park; LPRP, Lower Pierce Reservoir Park; C, Changi.

values of zinc found in Sembawang, and especially in Jurong, which coincides with the presence of several local chemical and metallurgical based industries. The highest mean concentration of copper (45.1 μg g^{-1} dw; see Table IV) was found in lichen collected in Sembawang site, followed by Jurong 40.0 μg g^{-1} dw. Shipment of copper concentrates at the shipyard adjacent to the Sembawang sampling site is a possible local contamination source in addition to the local use of copper for electrical purposes, as well as in alloys, pyrotechnics and construction materials.

Elevated levels of lead (11.86 μg/g) were also found in lichens collected from Jurong. Lead is listed by Goyer and Clarkson (2001) as a major toxic metal that causes multiple effects in human beings. Constant and excessive exposure to lead ($>$5 ppm) pollution may result in chronic and/or peripheral neuropathy especially in children (WHO, 2001). Although Singapore's National Environmental Agency (NEA) reported in the year 2000 that atmospheric lead levels are within the WHO range, these measurements do not reflect accumulated levels over time. In addition to Jurong, lichens collected from the campus of the National University of Singapore, and Changi showed elevated concentrations of lead. The largest source of lead in the atmosphere in the past in Singapore has been from leaded gasoline combustion as well as the manufacture of batteries, metal products, paints, and ceramic products. Metal accumulation in lichens at these locations reflects the influence of metallurgical and petrochemical industries operating nearby, as well as emissions from aeroplanes at the Changi International Airport. Higher concentrations of Ni were also found in lichen samples from Jurong (11.5 μg/g) relative to the other six sites (2.52–10.6 μg/g). Although, nickel is ubiquitous in nature and occurs mainly in the form of sulphide, oxide and silicate minerals, it is enriched in crude oil. The proximity of major petrochemical refineries to the Jurong sample site is the likely cause for the elevated levels of Ni in the atmosphere. It should be noted that coal-fired emissions cannot be expected to contribute to Ni levels in the local atmosphere since energy production in Singapore is predominantly based on clean-burning natural gas combustion (NEA, 2002). Electroplating, steel and alloy production, which includes nickel as a constituent or by-product, are also present in the Jurong industrial area.

Elevated concentrations of arsenic were recorded in lichen samples collected at Jurong and Sembawang sites (Table IV), although concentrations are comparatively lower than other metals (mean 1.30 μg/g; median 1.29 μg/g). Arsenic may be released to the atmosphere via both natural and anthropogenic sources, where human sources include the smelting of metals and the combustion of fuels, especially low-grade brown coal (Merian, 1984). Higher levels of As in lichens from Sembawang and Jurong to other are indicative of localised industrial sources of this metal in Singapore. Maximum Cd levels are incidental in samples collected from both Sembawang and the NUS campus. Cadmium is used mainly as an anticorrosive agent, and electroplated onto steel, and related compounds are commonly used as pigments in plastics, electric batteries and components (EPA, 2003b). However, the distribution of this metal is not restricted to urban or industrialised areas in Singapore, as elevated concentrations were also found in lichens from non-industrialised areas, including Fort Canning Park and the Lower Pierce Reservoir Park where adjacent construction of new roads and buildings may be responsible for the release of cadmium to the local atmosphere.

4. Data Comparison to Other Countries

Extensive lichen biomonitoring of atmospheric heavy metal contamination has been performed in other countries, but this study represents the first report for Singapore and Southeast Asia. Metal concentrations in lichens from Singapore are compared to available data from temperate countries in Table V. Local levels of metals in lichens are comparable to ranges reported from other temperate countries (Nimis *et al.*, 2000; Riget *et al.*, 2000; Chiarenzelli *et al.*, 2001; Bargagli *et al.*, 2002; Allen-Gil *et al.*, 2003; Di Lella *et al.*, 2004). Based on available data, Singapore ranks second highest among the six countries listed for all metals in lichen samples except Cd, where it ranks fourth. These observations are most likely to be the result of differences in the industrial status of the various countries, although other factors including prevailing climatic and environmental conditions can be expected to influence the atmospheric transport and accumulation of heavy metals in lichens.

5. Conclusion

It is noteworthy to point out that heavy metal accumulation in lichens is a consequence of accumulated levels in the cells or on the surface of the lichen thallus over

TABLE V
Metal Concentrations in lichens reported for various countries

Country		As	Cd	Cu	Ni	Pb	Zn
		Heavy metal (μg g^{-1} d.w.)					
Kosovo[a]	Mean	3.05	1.08	37.73	13.51	48.67	149.53
	Range	0.91–11.61	0.41–3.48	11.4–188.6	3.2–48.7	9.1–282.1	8–272
Russia – Norilsk[b]	Mean	0.22	0.12	1.52	1.52	1.26	20.5
	Range	0.11–0.44	0.0–0.38	1.12–12.79	0.83–10.20	0.78–5.79	9.73–29.6
Central Italy – Colline Metallifere[c]	Mean	0.36	0.26	5.77	2.65	3.88	34.7
	Range	0.12–1.27	0.06–0.69	3.94–9.17	1.03–8.00	0.68–11.20	25.90–57.70
Canada – Central Barrenlands, Nunavut[d]	Mean	0.42	0.15	3.61	3.25	6.44	22
	Range	0.15–1.00	0.06–0.24	0.73–15.80	1.84–6.68	0.76–19.70	15–44
Greenland[e]	Mean	<0.14	0.09	0.79	<1	1.72	17.65
	Range	<0.1–0.16	0.05–0.16	0.45–1.32	<1–1.8	0.32–4.29	6.48–36.9
North-Eastern Italy – Veneto[f]	Mean	0.99	0.27	9.91	2.24	7.66	33.56
	Range	0.10–3.73	0.11–1.15	2.9–47	0.41–8.7	0.8–34.1	20–99
Singapore (This study)	Mean	1.3	0.2	32.88	5.83	11.86	65.58
	Range	0.45–2.06	0.14–0.28	11.75–45.13	2.52–11.54	2.83–16.59	44.17–83.15

[a] Di Lella *et al*. (2004).
[b] Allen-Gil *et al*. (2003).
[c] Bargagli *et al*. (2002).
[d] Chiarenzelli *et al*. (2001).
[e] Riget *et al*. (2000).
[f] Nimis *et al*. (2000).

several years. As lichens can be regarded as perennial and sentinel organisms, this also explains why elevated concentrations of heavy metals are found in lichens. For the first time in Singapore, lichens have been used to detect spatial variations in heavy metal deposition from the local atmosphere. Analysis of soil showed a lack of positive correlation with lichen metal content implying that the accumulated metals are primarily derived from the atmosphere. The spatial differences in the concentrations of metals analysed reflect prevailing levels of atmospheric pollution in the various parts of Singapore. Samples of *D. picta* indicate relatively elevated concentrations of Zn, Cu, Pb, Ni, As and Cd in industrialised areas of Singapore, principally Jurong in the west and Sembawang to the north of the main island. Comparison of metal levels in lichen samples from Singapore with data reported for temperate countries shows that concentrations are mostly in the upper range, despite differences in prevailing climatic conditions. This study supports the more extensive use of *D. picta* as a bioindicator species of atmospheric heavy metal contamination in the wider tropical region of Southeast Asia.

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