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USE OF LICHENS AS BIOINDICATORS FOR DETERMINING ATMOSPHERIC HEAVY METAL CONCENTRATION IN MALAYSIA

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Abstract

This study aimed to determine the concentration of heavy metals (Cr, Fe, Co, Ni, Zn, and Pb) that accumulated in the lichen *Dirinaria picta* and in the ambient air of different industrial areas in Peninsular Malaysia. Heavy metal content was analyzed using wet digestion method with concentrated nitric and hydrochloric acids. Samples were analyzed using ICP-MS. Back trajectory analysis was conducted using the Hybrid Single-particle Lagrangian Integrated Trajectory model to track down the transportation routes of contaminants. Principle component analysis (PCA) was also performed to identify possible sources of heavy metals in ambient air where *Dirinaria picta* was sampled. Results showed that Fe accumulated the most, followed by Zn, Pb, Cr, Ni, and Co, in both locations. A significant correlation ($p<0.05$) was found between heavy metal concentration in the air and the lichen. Sumatra, Indonesia could be one of the possible sources of heavy metals in Malaysia. Motor vehicles/industries are the main source of pollutants in ambient air, followed by soil, sea spray, and the earth crust.

Key words: Heavy metals, Lichen, *Dirinaria picta*, Principle component analysis.

Introduction

Anthropogenic activities is the main source of pollution in Malaysia, release toxic gases through industrial waste disposal, fumes from motor vehicles and particulate from forest fire (Anon., 2009). The increasing number of motor vehicles used daily and the booming industrial sector lead to the production of several types of air pollutants in Malaysia. According to the Anon. (2009), the numbers of vehicles registered in Malaysia were 10.6 million in 2000 and increased to 18.5 million in 2009, with an increase of 7.9 million (42%) within 9 years. The levels of heavy metals in ambient air (Norela *et al.*, 2009) must be studied to determine their significance to pollution. Pollution levels can be alternatively determined using bio-indicators for environmental monitoring.

The industrial sector contributes to the increase in pollutant levels in ambient air (Anon., 2011). For instance, the two study areas, namely, Bandar BaruBangi and Bukit Minyak, are the main industrial areas in Selangor and Penang, respectively. Bandar BaruBangi in Selangor, Malaysia, with area of 5118 acres, was originally an oil palm estate (West Country Estate) and developed as a new town by the Selangor state government from 1974 to 1978. The area is divided into residential, business, recreational, and industrial zones. The industrial zone was named as Bandar BaruBangi Industrial Park (Anon., 2011). In Bukit Minyak in Penang, Malaysia, the industrial area was designated for industrial development by the British government in the early part of 1913. The area was subsequently developed by the local municipal council of Malaysia (Anon., 2009).

Lichens, such as *Dirinariapicta*, are derived from mutualistic associations of a fungus and an algae or cyanobacterium and occur as crusty patches or bushy growths on trees, rocks, and bare ground. Lichens are potential bio-indicators because they can absorb water and minerals from rainwater and the atmosphere through their thallus surface. In contrast to the leaves of plants, lichens do not have outer cuticle and stomata that control the intake of pollutants and/or gases from entering the cells. Therefore, lichens can simply absorb and accumulate chemical substances, particularly heavy metals, from the air.

As early as 1866, scholars have studied the use of lichens as biological indicators in France (Nylander, 1866; Nimis *et al.*, 2000) and worldwide thereafter. Other researchers also used lichens to determine the atmospheric levels of heavy metals in central India (Bajpai *et al.*, 2011), Argentina (Gonzales *et al.*, 1996), Singapore (Ng *et al.*, 2005) and Indonesia (Miyawaki *et al.*, 2004). Lichens have also been used to bio-monitor the effects of forest fires in East Kalimantan, Indonesia (Miyawaki *et al.*, 2004) and as a bio-indicator for air quality in Malaysia (Samsudin *et al.*, 2013b). Moreover, *D. picta*, alichen was applied by Universiti Kebangsaan Malaysia (UKM) to assess the content of the atmospheric heavy metals and polycyclic aromatic hydrocarbons (PAH) in the atmosphere (Samsudin *et al.*, 2013a). Shahrizim *et al.* (2011) studied the distribution of lichens at different altitudes of Gunung Machincang, Langkawi, Kedah, Malaysia.

This study aims to determine and compare the concentration of heavy metals (Cr, Fe, Co, Ni, Zn, and Pb) that accumulated in the thallus of *D. picta* and in the ambient air of different industrial areas in Peninsular Malaysia.

Materials and Methods

Study area: The two selected sampling stations in Bandar BaruBangi were a residential area (Section 3) and the Selaman Industrial Park (Section 10) in Selangor, Malaysia. The two other sampling stations selected were the Bukit Minyak Industrial Park and the Botanical Gardens, in Penang, Malaysia (Table 1 and Fig. 1). The Botanical Gardens is adjacent to a montane forest reserve, whereas the three other sampling stations are surrounded by urban areas.

Table 1. Information on the study areas.

Station	Study area	State	Coordinate
S1	Bukit Minyak Industrial Park	Penang	5.307563N, 100.446675E
S2	Botanical Gardens	Penang	5.440988N, 100.287207E
S3	Section 3-Bandar Baru Bangi	Selangor	2.936275N, 101.776093E
S4	Section 10-Selaman Industrial Park	Selangor	2.961789N, 101.753106E

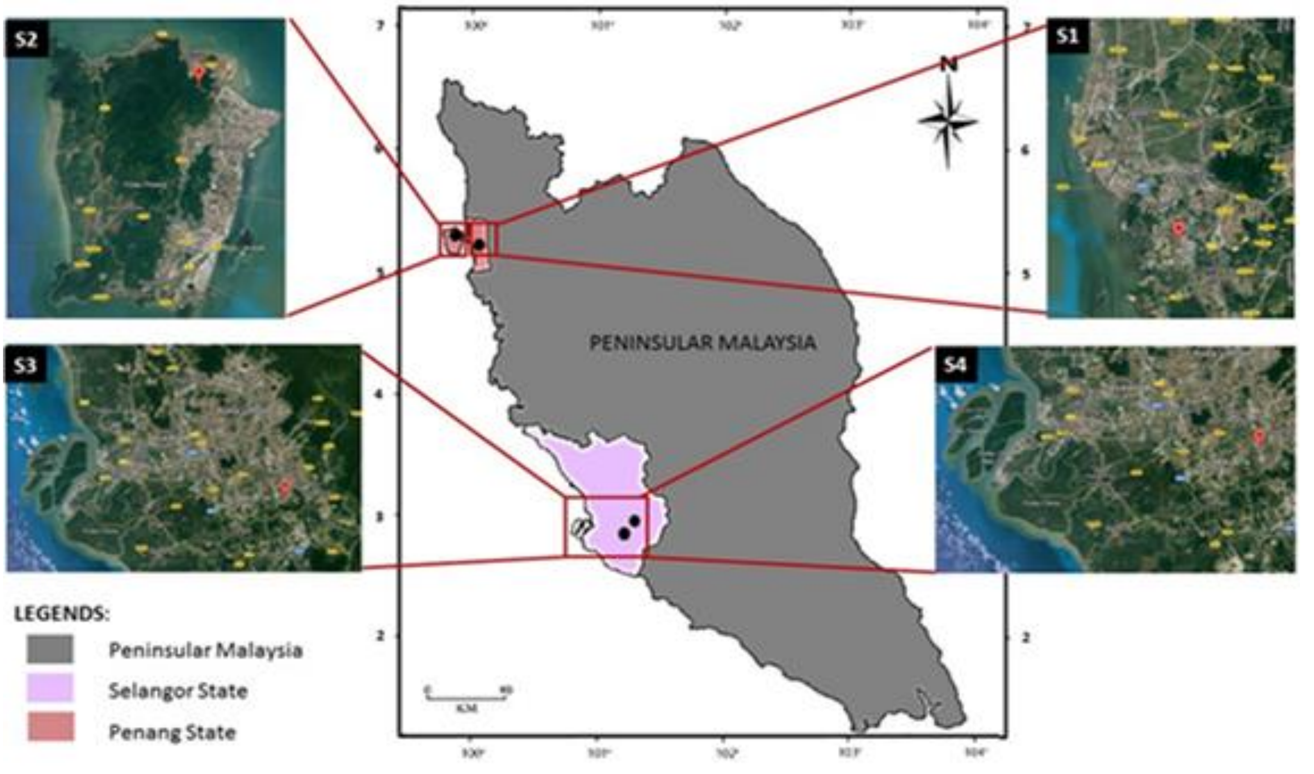

Fig. 1. Map of the study area.

Analysis of airborne trace elements: Cr, Fe, Co, Ni, Zn, and Pb were determined from *D. picta* sampled from the core of the industrial activity areas and were considered potential sources of metal pollution in the study areas.

Sampling and analysis: Lichen sampling and heavy metal analysis: *D. picta* was sampled from the main host plant, the royal palm (*Roystonea regia)*, at 2 m above the ground to avoid contamination by soil. The samples were air dried for 1 day, labeled, and analyzed in the laboratory. Foreign residues attached to the lichen samples were removed to prevent any errors during analysis. The samples were then dried in an oven at 35°C–45°C for 48 h to remove excess moisture, crushed, and powdered.

Each 1 mL sample of powdered lichen (collected from every sampling station) was placed in a conical flask and added with 3 mL of distilled water. The mixture was digested with 5 mL of concentrated nitric acid and 15 mL of concentrated hydrochloric acid. The samples were heated slowly until the volume decreased by approximately 50%. The samples were filtered through a filter paper and rinsed with 5 mL of concentrated nitric acid. Each filtrate residue was added with 5% nitric acid to obtain a final volume of 100 mL. The samples were analyzed using ICP-MS, and the experiment was conducted three times.

Air sampling and heavy metal analysis: Air sampling was carried out using the Graseby high-volume air sampler fitted with fiber glass filter paper (204 mm × 254 mm). Air was continuously drawn at the rate of 1.13 m^3 per minute for 8 h over a period of 3 days. The filter paper was divided into two parts. The second part was cut into small pieces, placed into a 150 mL beaker, and digested by adding 15 mL of acid (from the mixture of 30 mL of concentrated HNO_3 and 20 mL of $HClO_4$ mixed at the ratio of 3:2). The sample was then added with 50 mL of 2% HNO_3 and digested to ensure the oxidation of all carbon compounds. The sample was cooled, filtered under a fume hood, and placed into a 250 mL volumetric flask. The filtrate was diluted to 250 mL by adding distilled water and analyzed through ICP-MS.

Data analysis: Data were statistically analyzed using Pearson correlation test (SPSS version 2.0) to determine the relationship between heavy metal concentration in the lichen and that in the air. Moreover, heavy metal concentration was compared between that in the lichen and in the air in relation to the number of moving vehicles recorded. Back trajectory analysis was conducted using the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model to track down the transportation routes of contaminants in the monitoring locales. Principle component analysis (PCA) was performed to identify possible sources of heavy metals in the ambient air collected at the four stations where *D. picta* was sampled.

Table 2. Related studies on the use of lichens as bio-indicators.

Literature	Lichen species	Description
Molina & Vicente (1998)	- *Xanthoriaparietina* - *Everniaprunastri* - *Ramalinafarinacea*	The results are discussed on the possibility of using specific lichens as bio-indicators of air pollution in the Madrid area. Analysis of the three different lichen species used as bio-indicators of acid rain (*R. farinacea*, *E. prunastri*, *X. parietina*)
Varrica *et al.* (2000)	- *Parmeliaconspersa* - *Xanthoriacalcicola*	Estimation of the contribution of natural, volcanic and anthropogenic sources to metal content in lichens from two of the most active volcanic areas in Sicily
Carreras & Pignata (2002)	*Usneaamblyoclada*	Investigation of the content of some heavy metals in the transplanted thalli of the targeted lichen to evaluate the relative air quality of the area
Loppi & Pirintsos (2003)	*Parmeliacaperata*	The survey was undertaken with the aim of using epiphytic lichens as sentinels for heavy metal deposition at selected forest ecosystems of central Italy
Loppi *et al.* (2004)	*Flavoparmeliacaperata*	The study was done to test whether the changes in the air pollution status, as expressed by the biodiversity of epiphytic lichens and the accumulation of heavy metals in the thalli of *Flavoparmelia caperata*, were detected at intervals over a period of a few years
Backor & Fahselt (2004)	*Cladoniapleurota*	Discovering the proportion of heavy metals associated with photobiont and mycobiont cells for determination of the concentration of metals in whole lichens
Mendil *et al.* (2009)	14 different lichens and moss	Trace metal analyses in environmental samples of lichen and moss i.e. Cu, Mn, Fe, Zn, Pb, Cd, Cr and Ni
Sujetovienė (2010)	- *Physciatenella* - *Parmeliasulcata*	An investigation on whether NO_2 emitted by traffic can influence the content of nitrogen in lichen thalli, and monitoring of the content of sulphur and carbon. For the above purpose, the distance from a highway was regarded as the main parameter for providing the effect
Stamenković *et al.* (2010)	22 lichen species	Different lichen species were used as bio-indicators to establish different air pollution levels
Freitas *et al.* (2011)	- *Flavoparmeliacaperata* - *Parmotremachinense* - *Puncteliasubrudecta*	This study aimed at finding out and understanding how lichens (abundance and diversity) could be affected by atmospheric pollution in Porto with emphasis on the importance of their reliable utilization towards environmental conservation and public health protection

Table 3. The concentrations of heavy metals in *Dirinaria picta* at the studied stations.

Sampling station		Element, µg/g dry wt (ppm)					
		Cr	**Fe**	**Co**	**Ni**	**Zn**	**Pb**
S1	St. dev.	1.62	1156	0.118	0.45	21.45	1.19
	Max	8.19	5550	0.441	2.88	129.00	6.66
	Average	3.98	3580	0.143	1.83	92.70	4.23
S2	St. dev.	21.19	157.70	0.02	1.48	26.59	1.38
	Max	44.80	498.00	0.08	4.37	76.60	4.31
	Average	21.1	329	0.0616	2.68	49.2	2.96
S3	St. dev.	4.39	234.75	0.48	2.41	35.93	1.43
	Max	16.42	1840	1.169	7.506	169.3	17.8
	Average	11.09	1565	0.7331	5.362	127.2	16.49
S4	St. dev.	7.56	641.66	0.27	2.09	55.12	12.59
	Max	33.02	2632	1.009	11.14	187.6	45.08
	Average	21.1	1721	0.6501	7.818	141.3	27.28

Results and Discussion

Assessment of the concentration of trace heavy metals in *D. picta*: Table 2 presents previous studies that investigated the use of lichens as bioindicators. Table 3 shows of the concentrations of heavy metals accumulated by the lichen in the study areas. *Dirinaria picta* effectively accumulated six major elements, namely, Cr, Fe, Co, Ni, Zn, and Pb.

Fe showed the highest average concentration in the lichen samples collected at all test stations, whereas Co was the least abundant. The Fe content was the highest in *D. picta* sample collected from the Bukit Minyak Industrial Park (3580 µg/g dry weight) and the lowest in the sample derived from the Botanical Gardens, Penang (329 µg/g dry weight). The Co concentration varied from 0.0616 µg/g dry weight (Botanical Gardens, Penang) to 0.7331 µg/g dry weight (Section 3, Bandar BaruBangi). Overall, the trace element concentration among all stations was distributed in the following order: Fe> Zn>Pb> Cr> Ni> Co.

Fe was found to be the most abundant heavy metal at all study areas. This finding could be due to the fact that

Fe, which is required by all organisms, occurs naturally at higher levels in the environment compared with other heavy metals. A previous study reported the dominance of Fe over other metals evaluated (Uluozlu *et al.*, 2007; Bajpai *et al.*, 2011). The concentrations of almost all heavy metals were higher in the lichen samples collected from the industrial areas than those from the residential areas (Section 3, Bandar BaruBangi) and the Botanical Gardens (Penang). This finding could be due to significant gas emissions from motor vehicles, as evident on the higher number and frequency of vehicular movements in the industrial areas).

The concentrations of all the studied trace elements in the lichen samples obtained from the Botanical Gardens, Penang were higher than those in the samples collected from the other stations. Moreover, the concentrations of Cr (21.1 µg/g dry weight) and Ni (2.68 µg/g dry weight) in the sample collected from the Botanical Garden were higher or comparable with those in samples derived from the other three stations because the site was formerly a granite quarry.

Concentrations of heavy metals in ambient air: Table 4 shows the concentrations of trace elements (Cr, Fe, Co, Ni, Zn, and Pb) measured in the ambient. Overall, the concentrations of all elements in the ambient air were higher in the samples from the industrial areas than those in the samples from the non-industrial areas. The average concentration of Fe was the highest among all ambient air samples collected from all stations, whereas Cr, Co, and Ni were the least abundant. The average concentration of trace elements was distributed in the following order: Fe> Zn>Pb> Cr> Ni> Co. The distribution of all element studied was the same between the samples of lichen (*D. picta*) and ambient air. Hence, a relationship possibly exists between the concentrations of heavy metals in the ambient air with that in *D. picta*.

Table 5 lists the standard concentrations of heavy metals based on the Ambient Air Quality Criteria Act (AAQA) 1994, Texas Commission on Environmental Quality (TCEQ), the Environmental Quality Act (EQA) 1974, and the California Environment Protection Agency (CEPA). The concentrations of all heavy metals determined in the ambient air sample were lower than the standard permissible level. However, the concentrations of Cr, Co, Ni, and Pb at S1 and the concentration of Cr at S3 exceeded the standard permissible concentration level. This finding could be due to the influence of industrial activities.

Table 5. The standard permissible concentration level of heavy metal in ambient air.

Heavy metal	Standard concentration ($\mu m/m^3$)	Reference
Fe	0.005	AAQC
Pb	0.500	AAQC
Cr	0.010	TCEQ
Ni	0.015	TCEQ
Cu	1.000	TCEQ
Zn	1000	EQA
Co	0.005	CEPA

Table 4. Concentration levels of heavy metals in the ambient air of the sampling stations.

Sampling station		Element, $\mu g/m^3$ (ppm)					
		Cr	Fe	Co	Ni	Zn	Pb
S1	St. dev.	0.015	8.88	0.0016	0.052	0.027	0.225
	Max	0.096	20.00	0.0033	0.110	0.108	0.862
	Average	0.080	9.75	0.0015	0.050	0.082	0.602
S2	St. dev.	0.051	0.29	0.0001	0.013	0.050	0.048
	Max	0.231	3.12	0.0003	0.046	0.230	0.324
	Average	0.141	2.72	0.00017	0.0238	0.130	0.356
S3	St. dev.	0.005	0.04	0.0000	0.001	0.660	0.003
	Max	0.024	0.32	0.0005	0.009	1.530	0.012
	Average	0.020	0.28	0.0004	0.007	0.860	0.008
S4	St. dev.	0.006	0.00	0.0023	0.001	0.041	0.014
	Max	0.014	0.32	0.0219	0.008	0.165	0.030
	Average	0.007	0.32	0.0193	0.008	0.120	0.132
Standard conc.		**0.010**	**Minimal expose limit**	**0.0050**	**0.015**	**1000**	**0.500**

Relationship between the concentration of heavy metals in *D. picta* and that in the ambient air: A correlation test was conducted to confirm the hypothesis that heavy metals accumulated by lichens are derived from the concentration of those metals in the air. Table 3 shows the correlation results. Despite the low level of significance, a relationship was found between contaminants that accumulated in *D. picta* with that in the ambient air (Fig. 2). However, the differences in the concentrations of metals that accumulated in *D. picta* compared with that in the ambient air exhibited the following pattern: Fe> Zn>Pb> Cr> Ni> Co. Further studies must be conducted to quantitatively distinguish the actual capacity of *D. picta* as bio-adsorbent for heavy metal contamination in the air.

Trajectory analysis: The route of pollutants that contribute to the concentration of heavy metals in the ambient air in the vicinity of the four stations was determined through back trajectory analysis using the HYSPLIT model. As shown in Fig. 3(a) and (b), contaminants were derived from the same direction around the west coast area (the ocean). A similar pattern was observed between S3 and S4, but the paths of the contamination sources were from Sumatra, Indonesia (Fig. 3c and 3d).

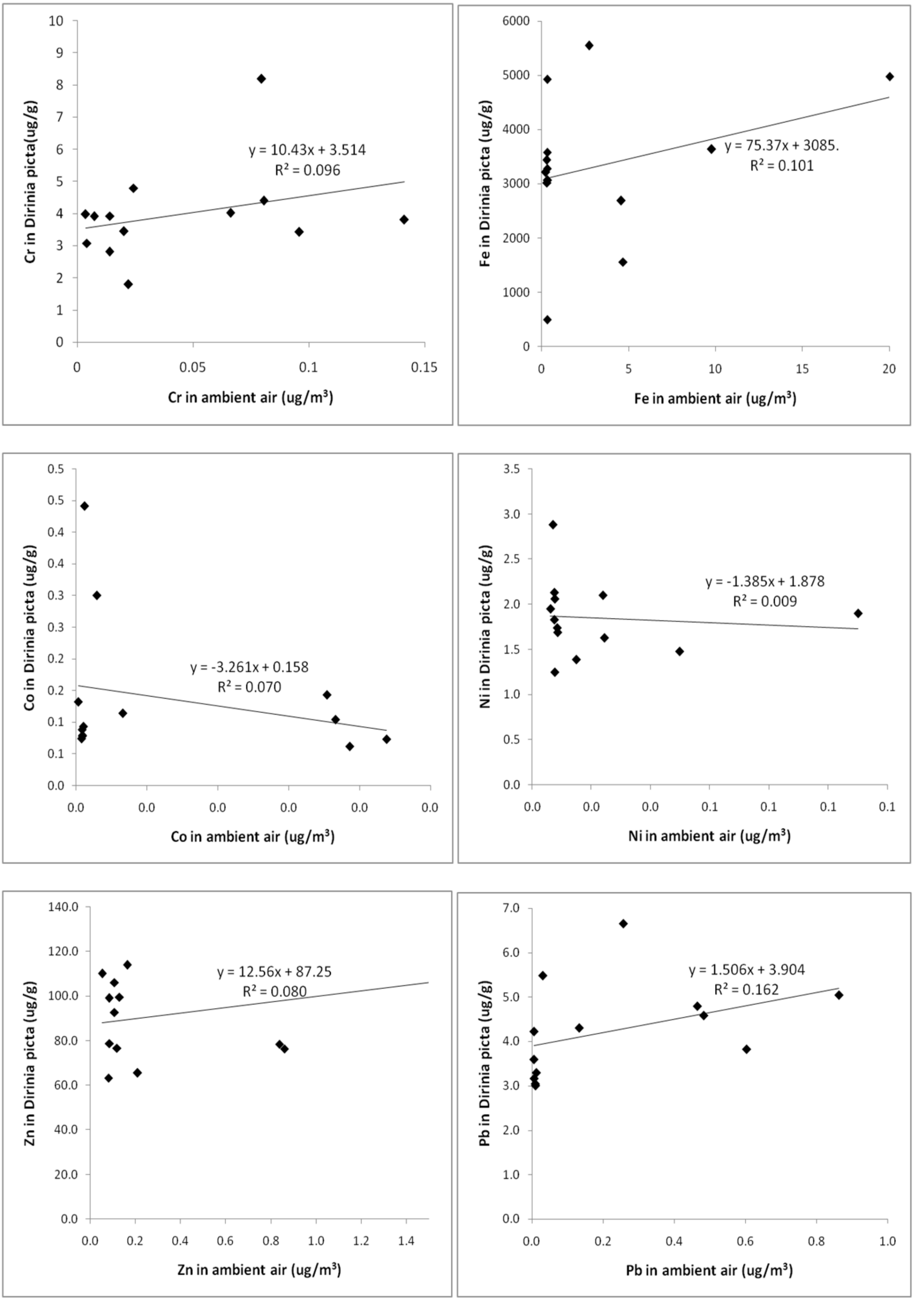

Fig. 2. Heavy metal concentration in *Dirinaria picta* in relation to the heavy metal concentration in ambient air.

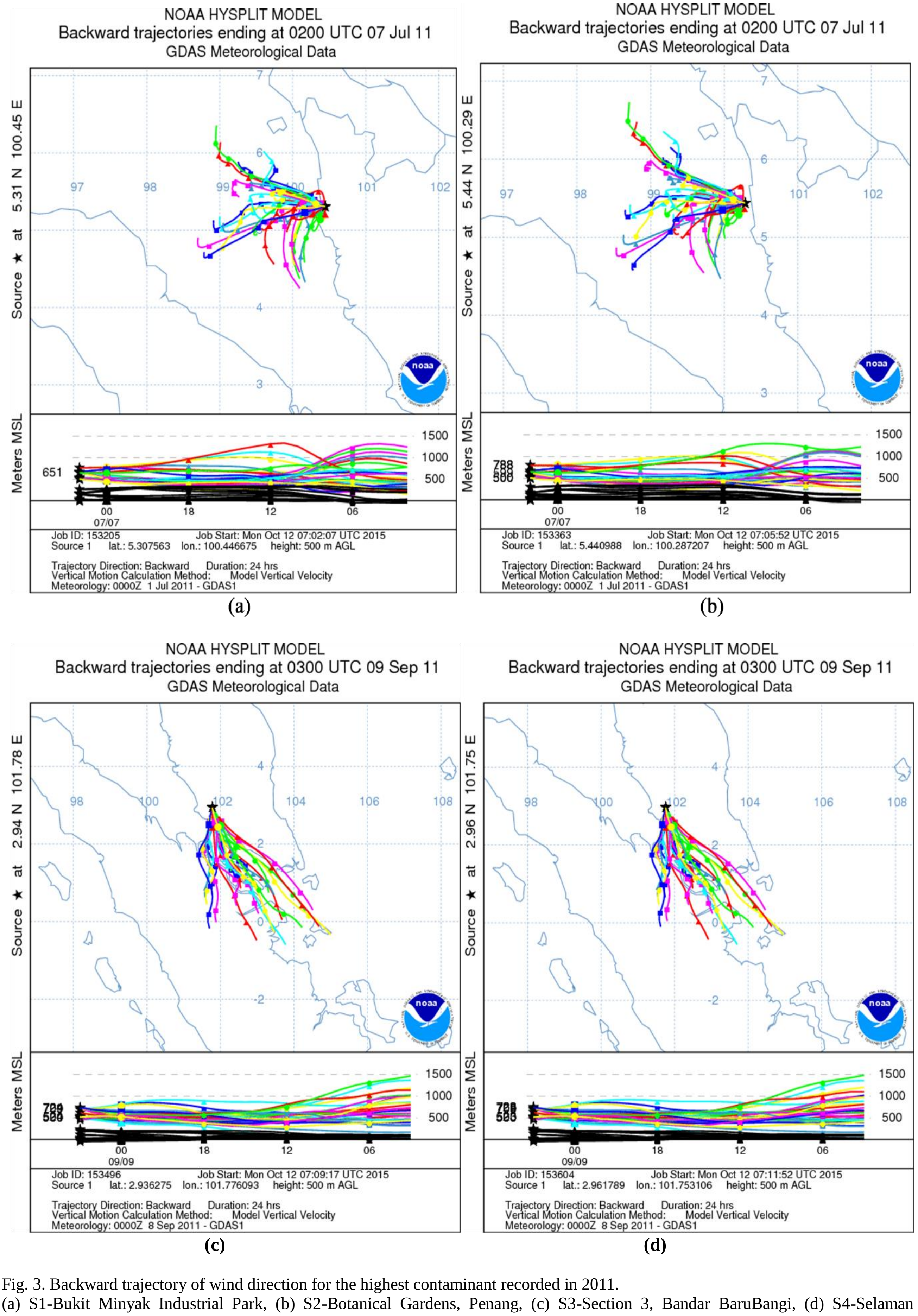

Fig. 3. Backward trajectory of wind direction for the highest contaminant recorded in 2011.
(a) S1-Bukit Minyak Industrial Park, (b) S2-Botanical Gardens, Penang, (c) S3-Section 3, Bandar BaruBangi, (d) S4-Selaman Industrial Park Section 10.

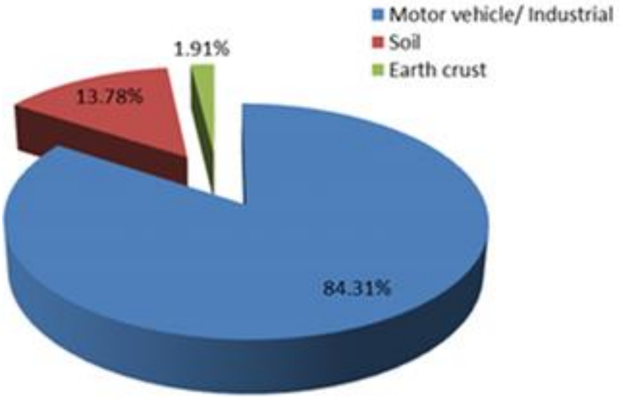

Fig. 4. Source apportionment of heavy metals in the ambient air.

Source apportionment of heavy metals: PCA was conducted to identify the possible sources of heavy metals in the ambient air collected among the four stations where *D. picta* was sampled. Analysis was performed at the four major sources with high loading factor through varimax rotated factor method (Kothai *et al.*, 2008). Fig. 4 shows that motor vehicles/industries were the main source of pollutants in the ambient air and contributed 84.31% of the pollutants. Meanwhile, soil contributed 13.78%, followed by sea spray (1.16%) and earth crust (0.75%). Motor vehicles and industries were considered the major source of pollution due to the high value of the loading factor of heavy metals (high loading factors of Zn, Ni and Cr). Hence, these metals can be considered as the highest contributor considering that the study sites were surrounded by industrial areas and congested traffic. Soil was the second highest contaminant source and had high loading factor for Fe and Co. These crustal elements were considered the pollution source, whereas Pb and Fe were the elements of the earth's crust.

Conclusion

This study determined the concentrations of heavy metals that accumulated in the lichen (*D. picta*) and in the ambient air. The concentrations differed between industrial and non-industrial areas. Almost all elements tested were found at high concentrations in stations located close to industrial areas. The concentrations of Cr and Ni in the samples collected at Botanical Garden, Penang were higher or comparable with those in the samples derived from the three other stations because the site was formerly a granite quarry. Sumatra, Indonesia could be one of the possible sources of heavy metals. Motor vehicles and/or industries were considered the main sources of pollutants in the ambient air, followed by soil, sea spray, and the earth's crust.

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