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STATISTICAL ANALYSIS OF HEAVY METAL CONCENTRATION IN MOSS AND SOIL AS INDICATOR OF INDUSTRIAL POLLUTION

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Abstract: The use of living organism, especially the lower plant as an indicator of the environmental pollution has been recently considered as the complementary to the traditional monitoring techniques. This study was conducted to evaluate the levels of the selected heavy metals deposited in the chosen industrial area and to identify its possible contributors. Mosses and topsoil were collected surrounding the industrial area, only the youngest segments (greenish part) of *Syrrhopodonconfertus* species were selected, which represented the last three years tissue's production. The elements of Fe, Zn, Mn and Ni were analyzed by FAAS. All the data was evaluated by multivariate analysis. The results obtained in this study clearly show that the elements were not evenly distributed throughout the studied area. Some elements such as Fe (moss) and Zn, Mn and Ni (topsoil) showed a somewhat different in their distribution pattern. The highest concentration of elements in mosses and topsoil were recorded for Fe followed with Zn, Mn and Ni. In general, comparison, there were no significant differences of metals content in moss and topsoil in this study. All the mono-elemental pairs between moss and topsoil samples showed a moderate correlation. This strongly suggested that, even so, elements in moss and topsoil were originated from the same origin. In general, based on the PCA results it could be concluded that Fe and Mn were contributed by the local anthropogenic activities, Ni emitted by the oil and fossil combustion and Zn, mostly from traffic vehicle's factor.
Keywords: Heavy metals, Soil, moss, PCA, correlation index.

INTRODUCTION

Air pollution has become a major public concern and are experiencing by most of the developing countries. In general, air pollution is defined as the addition of varied hazardous chemicals, particulate matter, toxic substances and biological organisms into the lower area of the troposphere layer of the atmosphere. There are various factors causing air pollution, but what comes from industries and factories is often considered as the most important factors in air pollution. There are numerous serious ecological implications and health risks associated with industrial air pollution. One of the important groups of air pollutants called as toxic air pollutants and also knew as hazardous air pollutants (HAP). Most of the air toxics originate from anthropogenic sources like mobile sources (e.g., cars, trucks, buses), stationary

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sources (e.g., factories, refineries, power plants), indoor sources (e.g., some building materials and cleaning solvents), and even released from natural sources such as volcanic eruptions and forest fires. Examples of toxic air pollutants include benzene (in gasoline), perchlorethlyene (in dry cleaning chemicals), asbestos, toluene, arsenic, and metals such as zinc, cadmium, mercury, chromium, and lead compounds. All of these pollutants that are inhaled have a serious impact on human health such as affecting the lungs and the respiratory system; they are also taken up by the blood and pumped all around the body. These pollutants are then deposited on soil, plants, and in the water, further contributing to human exposure. It is very difficult for anyone to avoid exposure to the harmful heavy metals that are so prevalent in our environment. Monitoring data and studies related to ambient air quality show that some of the particular air pollutants in several cities in Malaysia are increasing with time and are not always at the acceptable levels (Afroz et al, 2003).

Bio indicators have been considered as a complementary tool in order to monitor the environmental pollution and also could overcome some of the shortcomings to the conventional monitoring techniques which normally done through direct measurement by using electronic devices (Poykio et al, 2000). Bio indicators include biological processes, species, or communities and are used to assess the quality of the environment and how it changes over time. Changes in the environment are often attributed to anthropogenic disturbances (e.g., pollution, land-use changes) or natural stressors (e.g., drought, late spring freeze). Bio indicator species effectively indicate the condition of the environment because of their moderate tolerance to environmental variability. Entire communities, encompassing a broad range of environmental tolerances, can serve as bio indicators and represent multiple sources of data to assess environmental condition in a "biotic index" or "multimetric" approach (Carignan and Villard, 2002).

Based on its special physical and chemical properties (Chakrabortty et al, 2006), moss species has been used as a bio indicator to monitor atmospheric heavy-metal deposition since more than 50 years ago (Palmieri et al, 1997). Mosses have gained the attention of many researchers because mosses, especially the carpet-forming species, obtain most of their nutrients directly from precipitation and dry deposition. In general, the life of moss species is considered as independent of its substrate. Heavy metals deposited from the atmosphere tend to be retained by the mosses for a long period of time. Over the years, a lot of studies were done this alternative technique for the monitoring of the trace metals in air (Tyler, 1990; Conti and Ceccetti, 2001; Onianwa, 2006).

Soils served as recipients and recognized as contaminated if accept any other chemicals or other elements called contaminants (Gowd et al., 2010). Furthermore, soils can act as a natural buffer for the transportation of chemical substances and elements in atmosphere, hydrosphere, and biosphere (Yaylalı-Abanuz, 2011). The existence of heavy metals in soils is continuously increasing due to the rapid man-made activities such as industrialization and urbanization (Li and Feng, 2010). The concentration of heavy metals in soils so much depends on retention capacity of soil, which affecting soil particle surfaces, physical, chemical properties of soils and chemical properties of heavy metals (Soriano et al., 2012). In addition, heavy metals can remain in soils for a very long period of time. At a certain level, they are essential to a human body, but they also can cause toxic effects if exceeded the limit value.

Considering as one of the developing country, Malaysia emphasizes its industry development to progress into a developed country. Manufacturing industries and processing plants are significant contribution to the economic growth, but can cause a major problem to the environment in form of pollution. Industrial estates are dispersed throughout the country, including the state of Pahang, which Gebeng is considered as one of the major industrial areas for the state which covered 8600 hectares of land. Two of the major industries operating in Gebeng are petrochemical processing and manufacturing and rare-earth processing and refining activities. The pollution caused by radionuclide and heavy metals over the Gebeng industrial area can bring adverse effects to living organisms and human. Therefore, it is important to monitor and evaluate the level of pollution in the surrounding area for the benefit of the local residents and related communities. Furthermore, this type of pollution can also affect crops, soil content and the surrounding air. There is a lacked of data obtaining about the level of heavy-metal pollutants related to the Gebeng Industrial Area. Therefore, this study was conducted in order to evaluate the concentrations of heavy metals in mosses and topsoil and to identify the origin of the fall-out of the selected heavy metals.

MATERIALS AND METHOD

Sampling location

All the mosses and soil samples were collected surrounding the Gebeng Industrial Area in Kuantan Pahang, Malaysia as illustrated in Figure 1. The site is located between latitude 3o 57’ 21.84“N and longitude103o 22’15.74”-E with a temperate climate and sometimes influenced by two monsoon seasons, the southwest monsoon (late May to September) and the

Northeast Monsoon (November to March) resulting heavy rainfall to the area. Numerous multinational corporations actively operate their activities in the area, and some of them are BASF chemicals (M) SdnBhd, BP chemicals, Polyplastic Asia Pasific, Petronas MTBE-polypropylene, WR Grace Speciality Chemicals, Kaneka (M), EASTMEN Chemical (M), Cryovac (M), Petronas Chemicals MTBE, Lynas Malaysia.

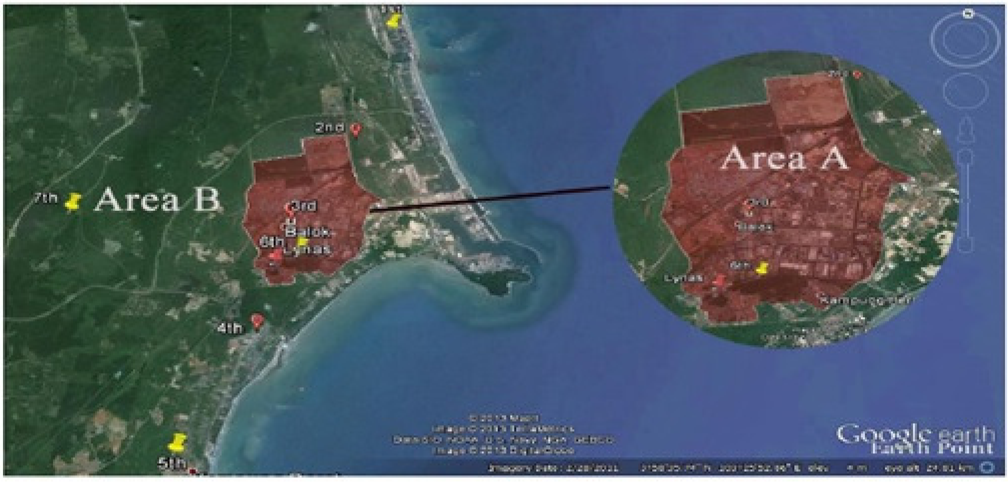

Figure 1: The sampling stations surrounding Gebeng industrial area

Moss SamplingAnd Chemical Treatment

Sampling was done according to the work done by Ruhling and Tyler (2004) with some amendments to adapt with the local conditions, especially in relation to the topography of the area and the number of samples needed as a whole. Moss samples were collected in an open area and at least 100 meter away from the main road. In a whole, 14 moss samples, *Syrrhopodonconfertus* species, were collected at seven selected sampling stations that scattered around the study area. Only epiphytic moss species were considered for this study in order to avoid some contamination from the soil. Moreover, the epiphytic mosses could survive even at extremely dry sites (Halleraker, 1998) and were available for sampling in the selected study area. Each sampling point covered 50 x 50 m2 area. All the sample materials were collected in plastic bags and transported to the laboratory (UniversitiTeknologi MARA, Pahang) for treatment and analysis. In the laboratory, mosses were cleaned and dried at 40 ^{o}C overnight without washed and homogenized. For the metal analysis, only the green and greenish brown parts of the moss were used, as they generally are intended to represent a period of about three to four years of accumulation. All the moss samples were mineralized by acid digestion. The representative samples (about 1 gram dry weight) were placed in an open quartz tube and was mixed with five ml concentrate HNO3 (Merck), and the mixture was left to room temperature for overnight. The samples were heated at 40 o C for two hours and reheated at 160 o C for another two hours. The nearly dried moss sample (slurry) was cooled to room temperature and was diluted with 0.1M HNO3 to the final volume. The solution was then filtered through Whatman type filter papers, and the filtrate was maintained to 25 ml with double deionized water (Abdullah, 2011). The samples were saved in a polyethylene bottle for further analysis.

Soil Sampling and Chemical Treatment

A total of 14 topsoil samples (0–20 cm depth) were collected from seven different locations surrounding the Gebeng industrial area at the exact locations where the mosses were taken. Each soil sample consisted of four subsamples obtained within 1 m2 in each sampling site, and next stored in polyethylene bags for transport and storage. The soil samples were air-dried and then sieved through a 1.0-mm polyethylene sieve. Portions of the soil samples (approximately 20 g) were further ground to pass through a 100-mesh polyethylene sieve. Approximately, 0.50 g (100 mesh) of each soil sample was digested with a mixture (1:5, v/v) of two mL concentrated HClO4 and 10 mL HF. The mixtures were heated under hot plate until the mixture became near dryness. Finally, one ml of HClO4 was added and the mixtures were heated until the appearance of white fumes. The final residue was dissolved with 0.1 M HNO3, transferred into polyethylene volumetric flask (25 mL) through 0.45-µm filters, and afterwards diluted to the mark. The rests of the soil samples were later stored in polyethylene bags in a desiccator for further analysis.

Metal Analysis and Quality Control

The concentrations of studied metals (Fe, Mn, Ni and Zn) in all samples were analyzed by using flame atomic absorption spectrometry (FAAS). The quality control of the analytical procedure was carried out by analyzing the standard reference material Pine Needle SRM 1575a and SRM-CC141. The standard reference material (SRM) was analyzed exactly the same as what was applied to the actual samples. Three replicate measurements were made for each SRM in order to get the average value. The results obtained shows that all the measurements were not differed by more than 10 %.

RESULTS AND DISCUSSION

Heavy Metal Concentrations In Moss And Topsoil Sample

Table 1: Element concentrations (mean value) in moss and topsoil samples at all stations

	Moss Samples, mg/kg dry weight				Soil Samples, mg/kg dry weight			
Sampling station	Fe	Zn	Mn	Ni	Fe	Zn	Mn	Ni
St1	8871	63	34	18	2467	34	38	4
St2	1893	52	59	29	4500	63	97	17
St3	1469	46	60	11	9268	88	57	5
St4	2679	80	73	14	7865	19	36	7
St5	8338	94	65	17	5221	55	42	4
S6	4318	67	84	20	7950	43	110	27
St7	3551	63	63	15	7145	27	37	17
Average	4445.6	66.4	62.6	17.7	6345.1	47.0	59.6	11.6
% RSD	67.51	24.53	24.55	32.55	37.39	50.34	52.15	76.95

The measured concentration of heavy metals deposited in the moss and topsoil samples from the seven different sampling stations are shown in Table 1. There are notable differences in the distribution of the studied elements in both moss and soil samples. The results obtained in this study clearly shown that all the measured elements were unevenly distributed throughout the sampling stations. For moss samples, the element of Fe was measured with the highest variation which recorded almost to 68 % (RSD). Meanwhile, Zn and Mn were measured with the smallest variation (both are identical) that was closed to 25 % RSD. The highest variation of the measurement of heavy metals in the topsoil sample was recorded also for Ni with 76.95 % RSD while the lowest was recorded for Fe with 37.39% of RSD. The highest concentration of Fe, Zn, Mn and Ni in moss samples were recorded as 8871, 94, 84 and 29 mg/kg dry weight respectively. For topsoil samples, the highest concentration was recorded as 9268, 88, 110 and 27 mg/kg dry weight for Fe, Zn, Mn and Ni respectively.

Correlation of Heavy Metals In Moss And Topsoil Samples

In general, mosses and soil were highly considered as the most preferable media in term to evaluate the fall-out of atmospheric heavy metals in the surrounding area. Mosses have the intrinsic characteristic that allows the absorption of elements directly from the atmosphere (Markert et al. 1999). Mosses are able to absorb and retain substantial quantities of heavy metals through dry deposition and precipitation because of large surface area of leave. Meanwhile, the direct collection and analysis of the deposition by evaluating soil sample will provide some advantages and considered as the conventional approach. In this study, the moss and soil samples were utilized in order to evaluate the fall-out of the selected elements in an area surrounding the hi-tech industrial area of Gebeng.

Table 2: Relationships among heavy metal concentrations in moss and topsoil samples.

Moss			Topsoil			Moss-topsoil		
Elemental pair	Pearson (r)	*t*-test	Elemental pair	Pearson (r)	*t*-test	Elemental pair	Pearson (r)	*t*-test
Fe - Zn	0.575	.008	Fe - Zn	0.188	.000	Fe - Fe	-0.682	.348
Fe - Mn	-0.441	.008	Fe - Mn	0.122	.000	Zn - Zn	-0.482	.188
Fe - Ni	-0.016	.008	Fe - Ni	0.243	.000	Mn - Mn	0.445	.785
Zn - Mn	0.281	.610	Zn - Mn	0.349	.336	Ni - Ni	0.474	.088
Zn - Ni	-0.152	.001	Zn - Ni	-0.148	.012			
Mn - Ni	-0.042	.001	Mn - Ni	0.771	.002			

Table 2 shows, the correlation coefficient and the t-test values for all the elemental pairs in moss and topsoil samples measured in this study. The measured correlation coefficient clearly indicates that almost all the studied elements that were deposited in moss samples are not strongly correlated among them except for the pairs of Fe-Zn and Fe-Mn, which were recorded 0.575 and -0.441 respectively. The fact that both Fe and Zn are considerably moderately correlated was supported by the t-test value which was evaluated as 0.61, which means that, in general, the two samples are not significantly different. This strongly suggested that most of the elemental in moss was possibly contributed by the various types of emitters except for Fe, Zn and Mn. Fe and Mn was found as negatively correlated in the group. The low correlation for the elemental pairs of Ni-Fe, Ni-Zn, Ni and Mn-Ni clearly reveals that the metal of Ni was contributed by the other sources compared to the Fe, Mnand Zn. For the topsoil samples, almost all the elemental pairs show very low correlation among all the elements except for the pair of Mn-Ni, which was strongly correlated (r = 0.771), but the distributions of these two elements are significantly differences. The t-test values for all elements in soil samples were recorded the values below than 0.05 except for the pair of Zn and Mn. Although Mn is often is considered associated with terrigenous sources and Zn to the anthropogenic sources (Chapman et al, 2003), in this study the result obtained clearly shows that Zn-Mn having a low correlation and there are not significant differences for their distribution in soil ($p > 0.05$).This reveals that, except for ZN-Mn, the distribution's patterns of all elements in topsoil samples are significantly differences ($p < 0.05$) and almost perfectly uncorrelated between them ($r < 0.3$). This can be related to the fact that the metal content in the topsoil sample was largely depended on the soil parent materials and the meteorological factors.

The null hypothesis test was performed on the data set to study if there were significant differences in the element concentrations between moss and topsoil samples. The results of this study clearly show that all the single elements pair (moss-topsoil) are moderately correlated and their concentration distributions in the both media are not significantly differences ($P > 0.05$). This strongly suggested that the presence of these particular elements in the studied area was possibly influenced by the same meteorological factors and contributed by the identical sources. Therefore, the present of Fe and Mn in moss and topsoil samples in this study may be associated primarily to re-suspension of soil, road dust, forest fire and so forth. On the other hand, Ni and Zn can be associated mainly to anthropogenic sources because even when present naturally in soils they are considered as trace elements (Monaci et al, 2004).

Multivariate Analysis

In order to identify the possible origin that contributed to the observed studied metals such as earth crustal, marine, vegetation, industry, etc., principal component's analysis (factor analysis) was applied to the data (metals in moss and topsoil) obtained in this study. The statistical approached also has provided a multivariate graphical representation of the overall metal distribution in the study area. From the analysis of the PCA data, it is possible to get a correlation between the studied elements and gain insight into the potential origin. The results of principal component's analysis for the whole concentration data in moss and topsoil are presented in Table 3. Based on the eigenvalue gained in this study, two principal components (PC1 and PC2) were observed to be significant in each case. The cumulative explained variance for the first two PCs ranging from 73.3% and 76.0% in topsoil and moss respectively. The data of the loadings for the individual elements confirms that all the metals are well represented by these two PCs.

Principal Components Analysis of Moss Sample

The PCA data as shown in Table 3 clearly reveals that the distributions of the four elements in the moss samples were defined by the first two PCs, PC1 and PC2, which explain almost 74 % of the total variance in the data.PC1 is mainly dominated by Fe and Mn (negatively correlated) while PC2 by Zn and Ni. The dominant factor loading of Fe in the first PC strongly suggest that the origin of Fe and Mn could be associated to the local emission sources such as ore mining and metallurgical plant (Mmolawa, 2011). When referred to the Gebeng industrial area, the abundance of these two elements in atmosphere was possibly contributed by the rare-earth industry and iron and steels related industries. The higher of factor loading of Zn in the second PC would suggest that the existences of these metals in the moss samples are highly associated to the human activities such as metal industry and fossil-fuel combustion and identified as air pollution markers in several investigations (Belivermis et al, 2008).The inversely correlation of Ni in the second PC may be related to the Gebeng Oil and Petroleum processing plant whereby the main emission source of Ni is oil and coal burning as well as steel industry and smelters.

Table3: Eigenvalues, metal loadings and explained variance of Principal Components (PC) of moss samples

PCs	Eigenvalues			Component matrix		
	Total	% of Variance	Cumulative %	Element	PC1 Factor loading	PC2 Factor loading
1	1.622	40.550	40.550	Fe	0.915	0.328
2	1.311	32.774	73.324	Zn	0.337	0.891
3	.947	23.664	96.988	Mn	-0.755	0.571
4	.120	3.012	100.000	Ni	0.058	-0.427

Principal Components Analysis of topsoil sample

The relationships among all of the studied metals were evaluated based on the data that was treated by principal component analysis, PCA. The total of variance and component matrices is given in Table 4. The first two PCs were chosen according to Kaiser’s criterion, which accepts only component with eigenvalue higher than one as significant data. The total variance given by the first two PCs was almost 76 %. The first PC that contributed 47.4 % of the variance was characterized by the metal of Ni and Mn. The second PC was mainly dominated by Zn and Fe. All elements in PC1 and PC2 were considered highly correlated in their particular component due to the high positive loadings in their group. The highest loading of Ni in the first PC clearly reveals that the elements in the first PC were contributed by the anthropogenic factors. Ni was considered as the fingerprint to the oil and coal burning as applied to V. The high correlation of Mn in the first PC can be related to the leaching from the higher plants (Berg et al, 1995b). The data of the component matrices for the second PC possibly represent the soil contribution. A good correlation for Zn and Fe in the PC2 would suggest the contribution of metal industry. The high loading of Zn metal in the group reveals the contribution of lead and zinc smelters. Emission from non-ferrous metal plant in Gebeng industrial area also contributed to the zinc deposition pattern.

Table 4: Eigenvalues, metal loadings and explained variance of Principal Components (PC) of moss samples

PCs	Eigenvalues			Component matrix		
	Total	**% of Variance**	**Cumulative %**	**Element**	**PC1 Factor loading**	**PC2 Factor loading**
1	1.894	47.357	47.357	Fe	0.237	0.524
2	1.148	28.689	76.046	Zn	-0.076	0.935
3	.891	22.266	98.312	Mn	0.862	0.344
4	.068	1.688	100.000	Ni	0.981	-0.072

Comparison of Heavy Metals Origin In Moss And Topsoil Samples

The data of PCA that was treated to the moss and topsoil metals concentration is summarized in Table 5. This analysis was done in order to overview the potential sources that were contributed some levels of heavy metals into the study area. As shown in Table V, the significant numbers of components were chosen based on the Kaiser's criterion which the component with the eigenvalue higher than 1 is considered. Therefore, three PCs were selected to present the distribution of metals in moss and topsoil samples, which explain almost 86 % of the total variance in the data set. The loadings of every metal are also presented in Table 5.

The first PC, PC1 includes Fe (moss), Fe (topsoil), Mn (moss) and Mn (topsoil) as dominant elements, which contributed 39.01 % of the total variance. These two elements, Fe and Mn are usually associated to the natural sources such as soil dust, wind-blown and mineral processing activities. The second PC that explained for 25.6 % of the total variance were dominated by Ni (moss and topsoil). Both Ni in soil and Ni in moss samples show a very similar factor loading, which indirectly indicates that they were possibly contributed by the same sources. Ni is strongly believed as one of the products of power plants and a specific tracer for oil combustion. The local that their operation mainly depends on the oil and fossil based combustion were suspected to reflect the spatial distribution of Ni to the surrounding area. PC3 is characterized by only the element Zn which contributed almost 21 % of the total variance in the data set. Both Zn in moss and Zn in topsoil show significant loadings in this third PC, and they are inversely correlated. With no significant factor loadings of this element in the other PC, there is a possibility that the existence of Zn in the third PC did not correlate to the possible sources presented by PC1 and PC2. The deposition of Zn is also attributed to the mining and processing of zinc ores and zinc smelting (Lucaciu et al, 2004). In this study, the metal of Zn in moss and topsoil samples was suspected originated from the traffic exhaust, tires and brake wear of the transportation vehicles that moving around the area (Fujiwara et al, 2011).

Table 5: Eigenvalues, metal loadings and explained variance of Principal Components (PC) for the mixture of samples

PCs	Eigenvalues			Component matrix			
	Total	**% of Variance**	**Cumulative %**	**Element**	**PC1 (Factor loading)**	**PC2 (Factor loading)**	**PC3 (Factor loading)**
1	3.121	39.01	39.01	FeM	**-0.662**	-0.192	0.555
2	2.049	25.60	64.61	ZnM	-0.004	-0.184	**0.889**
3	1.703	21.32	85.93	MnM	**0.852**	0.368	0.285
4	0.725	9.064	94.99	NiM	-0.431	**0.849**	-0.074
5	0.343	4.289	99.28	FeS	**0.971**	-0.147	-0.182
6	0.057	0.718	100.00	ZnS	0.031	-0.012	**-0.797**
				MnS	**0.887**	0.188	-0.294
				NiS	0.382	**0.850**	0.056

****XM : metal X in moss and XS: metal X in soil samples.*

Conclusions

The results that were discussed in this study clearly reveal that the mosses and topsoil were useful materials in order to evaluate the heavy metal's level in our surrounding environment. The analysis of mosses enabled to provide a complementary data related metal deposition from various sources. By using mosses and topsoil, it was possible to characterize the spatial distribution of heavy-metal deposition surround the studied area. The area was found not being heavily polluted with the studied elements. In general, the introduction of the studied elements in moss and topsoil, as presented by the spatial distribution and strengthened by the Pearson correlation and t-test analysis, indicates that the metals were contributed by a mixture of sources and activities. Most of the metals were suspected emitted by the local industrial activities that operate surrounding the Gebeng Industrial Area. The results obtained in this study could be considered as a first attempt in this country in terms to evaluate metal's pollution by applying bio monitoring technique. However, in order to get more accurate results, a fold number of samples should be increased and a comparative study with bulk deposition is still important. Besides that, simultaneous study of different lower plants species could be useful.

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