SPATIAL VARIABILITY OF ARSENIC AND HEAVY METALS IN A HIGHLAND TEA PLANTATION USING LICHENS AND MOSSES AS BIO-MONITORS

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ABSTRACT

A study was carried out to assess the presence and distribution of heavy metal pollutants in Lichen species Platismatia glauca and moss species Meiothecium microcarpum found in a highland tea plantation area in Cameron Highlands, Malaysia. In the study As, Zn, Cu, Pb, Cr, Cd, and Ni were analyzed. The As and heavy metals concentrations were analyzed using multi-elements Energy Dispersive X-rays Fluorescence technique (EDXRF). Results showed that the mean concentration of the metals can be arranged in decreasing order as Zn>Cu>Pb>Cr>Cd>As>Ni for lichen and Zn>Cu>Pb>Ni>Cr>Cd>As for moss. However based on anthropogenic factor, the degree of anthropogenic pollution follows identical decreasing order of Cd>Zn>Pb>As>Cu>Cr>Ni, where the samples were enriched by Cd and Zn. The average effects of As and heavy metals showed the area is not polluted. The correlation and PCA studies on the metals indicated that majorities are of natural origins, except for Cd and Zn. Cd is attributed to phosphate fertilizers, solid waste incineration and fossil fuel burning, while Zn from tires wares and tears and also fossil fuel combustion. Distribution patterns of all As and heavy metals showed very much dependent on types of elements and location factor within the study area.

Keywords: Bio-monitoring, Heavy metals, pollutants distribution

INTRODUCTION

Being one of the most popular highland tourist destinations among locals in Malaysia, Cameron Highlands experiences increment in the number of non-resident visitors and motor vehicles to the area. Coupled with the intensive and uncontrolled clearance of forest to make way for plantations and agricultural lands, the motor vehicles emissions and waste dumping both by residents and non-residents contributed to deterioration of the environment of this area. This issue has raised alarm and concerned among local resident organization and consumer associations due to its negative impacts on environment and human health. The pollutants of natural origin (soil & dust particles) or anthropogenic sources (combustion, vehicular emission and waste) mentioned earlier contained heavy metals and trace elements (Barandovski *et al*., 2008) such as Cr, Cu, Zn, Ni, Cd, As and Pb that could be toxic and cause health risks at high concentration (Zaini Hamzah *et al*., 2011). One alternative way of assessing these long terms deposition levels of atmospheric pollutants is by applying Bio-monitoring technique using certain species of lower plants that are found widespread in the area.

Tea plantations in Cameron Highlands has been established more than sixty years ago. There are abundance growth of mosses and lichens of various species on the tea plants, especially around the stems and branches. Bio-monitoring technique using mosses or lichens to assess the impact of heavy metals pollutants is popular in many countries like Serbia (Dragovic & Mihailovic, 2009), Turkey (Ugur *et al*., 2003), Romania (Lucaciu *et al*., 2004), and even in Antartica (Zvěřina et al., 2014). The method has also been applied in Malaysia around oil refinery area (Mohd Zahari bin Abdullah, *et al*., 2011; Mohd Zahari bin Abdullah, *et al*., 2012) and a coal-fired power station (Nita Salina *et al*., 2013). Recently, with its list of more advantages, lichen-bags technique has been used (Salo, 2014; Petrova, *et al*., 2015). These lower plants are suitable for such monitoring since they are resistant to many substances at higher concentration which are toxic to other plants. Lichen consists of two symbiotic organisms; green alga and fungus (Ugur, *et al*., 2003). Fungus traps water, nutrient and other substances from the ambient environment while the green alga carries out the photosynthesis. Mosses are lack of root system and accumulate water and nutrients largely through the atmosphere (Ugur, *et al*., 2003). Being Bryophyte the leaves of mosses are lack of protective cuticle, hence accommodate mineral nutrition accumulation mainly from wet and dry deposition (Anja *et al*., 2004). The uptake mechanism of lichens and mosses tends to accumulate pollutant as well.

This study assess the heavy metals pollutant levels by applying Biomonitoring method using lichen species *Platismatia glauca* and moss species *Meiothecium microcarpum* that are found to grow extensively and abundantly on the stems and branches of tea plant in the area. The method is coupled to the Energy Dispersive X-rays Fluoresence Technique (EDXRF) for the instrumental multi-elemental analysis of the samples.

METHOD

Meiothecium microcarpum and *Platismatia glauca* samples were collected at fifteen sampling locations within the Shalimar Estate Bharat Tea Plantation, Cameron Highlands, Malaysia. The plantation bordered on the eastern side by the main road to Tanah Rata town, and on the western side by a mountain range, has been established for more than 60 years. At each location samples were collected within an area of about 10 x 10 m. The points were between 100 m to 1100 m from main road on the eastern side, at altitudes ranging from 1198 m to 1302 m from sea level. Within the sampling area there are networks of graveled service roads for the plantation. There is a gully flowing from north-west to south-east direction along the lowest altitude of the valley. The area covered by the study was approximately 600 m x 1200 m. The geographic positions of the sampling locations were determined by GPS. Table 1 and Figure 1 show details of the sampling locations.

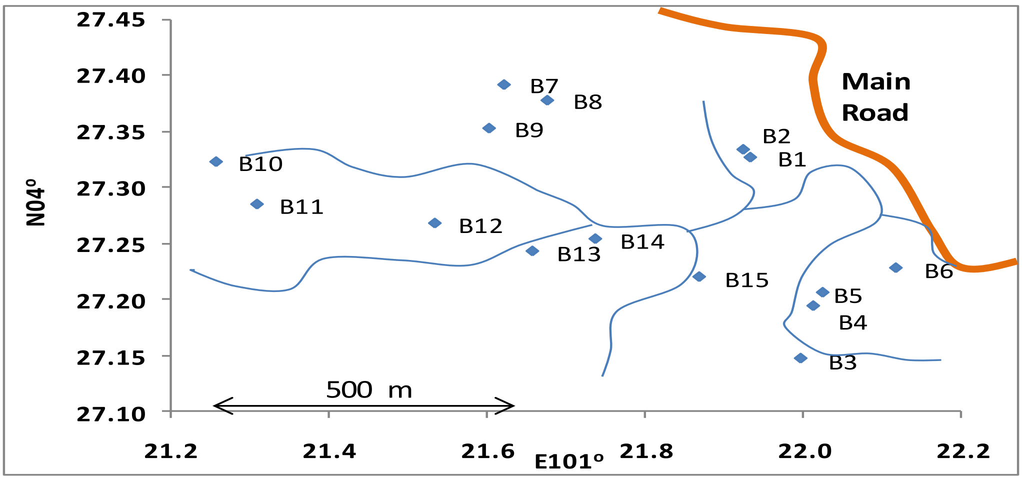

Figure 1. Relative position of sampling locations to main road (thick line) and service roads (thin lines)

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The samples were collected from the stem and branches of tea plants at about 1 m from ground level to ensure only air deposited pollutants on the samples were considered. The physical appearance and morphology of the collected samples were observed to ensure the right species. As for moss only the green to greenish-brown part of moss samples were collected to represent 2 to 3 years period of accumulation (Urgur, *et al.,* 2003). In order to avoid contaminating the samples, disposable polyethylene glove and plastic knife was used during the collecting, and samples were kept in clean sealed plastic bags.

Table 1. Sampling locations showing distance from main road and elevation.

Sample	*N04°*	*E101°*	*Dist. (m)*	*Height (m)*
B1	27.327'	21.934'	150	1200
B2	27.334'	21.925'	150	1230
B3	27.147'	21.998'	300	1200
B4	27.194'	22.014'	250	1198
B5	27.206'	22.026'	240	1206
B6	27.228'	22.119'	100	1224
B7	27.392'	21.621'	530	1237
B8	27.378'	21.676'	500	1246
B9	27.353'	21.602'	550	1256
B10	27.323'	21.255'	1100	1243
B11	27.285'	21.307'	1000	1302
B12	27.268'	21.533'	710	1260
B13	27.243'	21.657'	600	1254
B14	27.254'	21.737'	450	1253
B15	27.220'	21.869'	350	1233

Foreign materials attached to the samples such as dust, dead branches and tree barks were removed. The samples were air dried at room temperature and then oven dried at 60 °C for about 36 hours. The dried samples were ground using agate planetary ball mills in the grinding range of 3-5 minutes at 300 rpm to reduce the particle size to satisfy the condition for homogeneity of the sample (Mohd Zahari bin Abdullah *et al.* 2011). To avoid moisture the ground samples were stored in desiccators.

For EDXRF analysis about 3 g of each dried sample was pressed without binder at 15 tons compression force for 30 s, to turn it into solid thin pellets of diameter about 25.0 mm and 5.0 mm in thickness (Mohd Zahari bin Abdullah *et al*., 2011). At least duplicate pellet were prepared for each sample. All pellets ware stored in the desiccators prior to analyzing using Minipa14 PanAnalytical EDXRF Spectrometer, at 100s measurements. For the elemental concentration analysis samples were measured using Standardless Application of the spectrometer and the respective fluorescence peaks of the elements were compared to the corresponding peaks obtained using Certified Reference Materials (CRM) Tomato Leaves (1573a) and Lichens (IAEA 336), where the pellets were prepared in the same manner as the samples. The spectrometer was optimized earlier, giving the recoveries of between 83.7 % and 102.5 % depending on elements being analyzed (Mohd Zahari bin Abdullah *et al.,* 2011).

RESULTS AND DISCUSSION

Heavy Metals Concentration

Basic statistics of seven heavy metals studied in the fifteen lichen and fifteen moss samples are summarized in box-plots of Figure 2. In the figure the cross mark on the box represents the mean concentration of the respective metals, while the points beyond the whiskers are outliers. The figure shows that only Cu in lichen and Pb and Zn in moss show approaching normal distributions, which is supported by the closeness of the values of median and mean. Others indicated skewed distributions. The distribution among sampling points indicated that Ni in mosses while Pb and Cr in lichens showed greater variability. The mean concentration of the metals can be arranged in decreasing order as Zn>Cu>Pb>Cr>Cd>As>Ni for lichen and Zn>Cu>Pb>Ni>Cr>Cd>As for moss. Except for Ni and Cd, the mean concentrations of heavy metals in lichen always higher than in moss. Statistical t-test at 95% level was also carried out on the concentrations of each elements in lichens and mosses to determine any significant different on their means concentrations in lichen and mosses. The results indicated that all elements studied show significant different of the means concentrations in lichen and mosses except for Cr ($t_{stat} = 1.603 < t_c = 2.119$; $p = 0.128$), Cu ($t_{stat} = 0.522 < t_c = 2.048$; $p = 0.606$) and Zn ($t_{stat} = 1.832 < t_c = 2.080$; $p = 0.081$). These observations might be attributed to the difference in uptake mechanism efficiency between lichen and moss for As, Ni, Pb, and Cd.

Figure 3 summarizes the Anthropogenic Factor (AF) for each element based on the mean concentration relative to the background concentration of the element calculated using a method described by Adamu & Nganje (2010). Here, the world's average concentrations of the elements were considered as background. Based on the single factor analysis an AF < 1indicates no pollution, a value of between 1 and 2 indicates potential pollution, while between 2 and 3 represents slightly polluted, and if AF > 3 polluted by the respective element is concluded (Deng, 2012). In the figure, except for Cd and Zn, all AF values are less than 1 either for lichens or mosses. Cd in lichens shows slightly polluted (AF=2.40) and in mosses polluted (AF=3.34) respectively. For Zn in lichens slightly polluted (AF=2.19) is observed while in mosses potential pollution (AF=1.94) is concluded. Enrichment of Cd and Zn could be traced to anthropogenic inputs. The main sources of Cd in air of the area could be attributed to incineration of solid waste, fossil fuel burning and application of phosphate fertilizer (Dragovic and Mihailovic, 2009; Bajpai *et al.*, 2011). Being quite close to the main road and the presence of service roads within the area the enrichment of Zn can be attributed to ware of tires and incomplete fossil fuel burning (Bajpai *et al.*, 2011). Based on AF, the degree of anthropogenic pollution by the elements in lichen and in moss follows identical decreasing order of Cd>Zn>Pb>As>Cu>Cr>Ni. This shows that Cd and Zn are most enriched in both lichen and moss compared to other metals. The average pollution impact based on five elements Cd, Zn, Pb, As and Cu using Pollution Index (PI), a multiple factors analysis as suggested Adamu and Nganje (2010) for lichen and moss are 0.38 and 0.29 respectively. The PI was calculated relative to the maximum tolerable concentration of the elements by plants.

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Thus, although lichen and moss were found to be enriched by Cd and Zn, on average the pollution impact by the five heavy metals is still low.

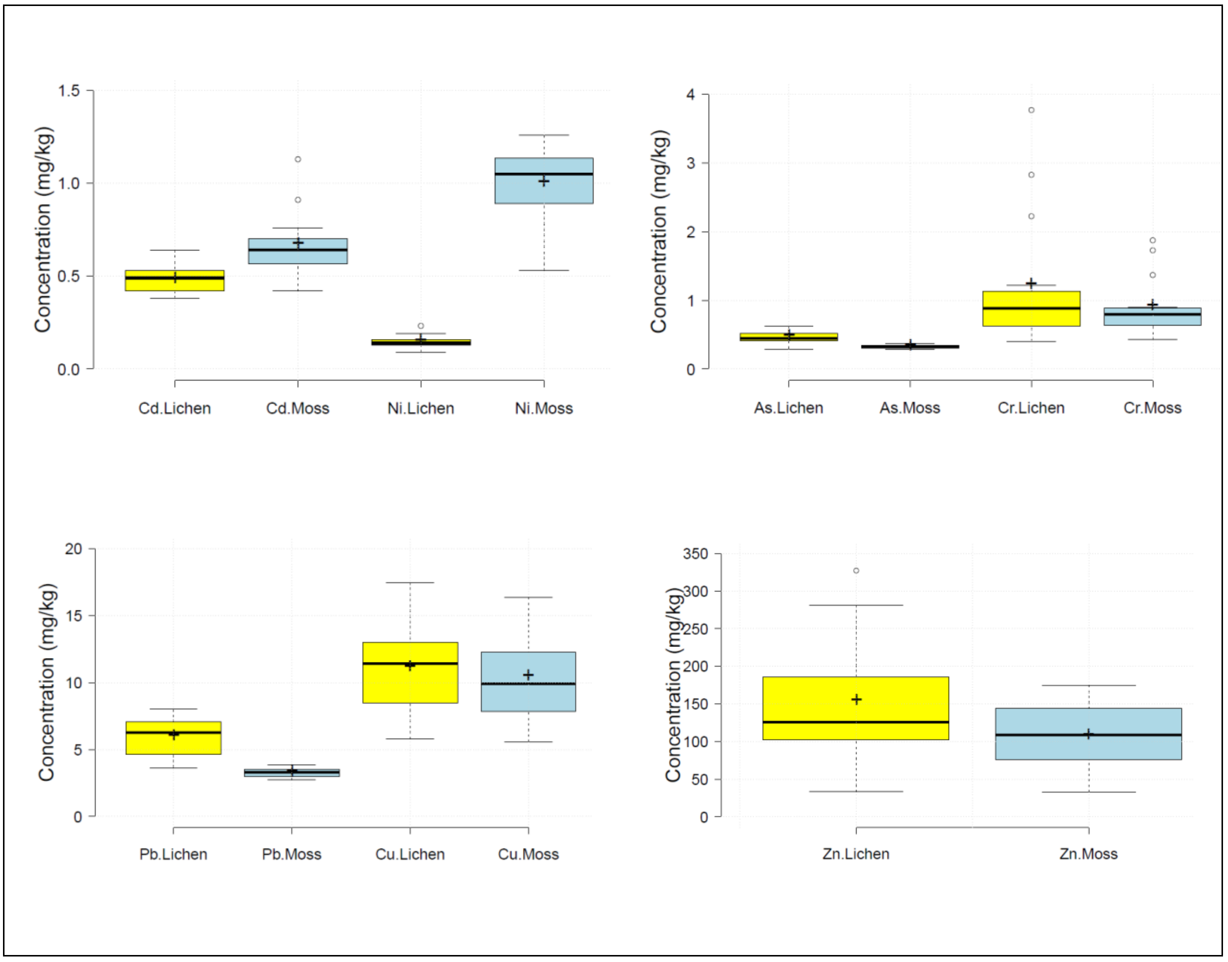

Figure 2. Box-plots of heavy metals concentrations in lichens and mosses

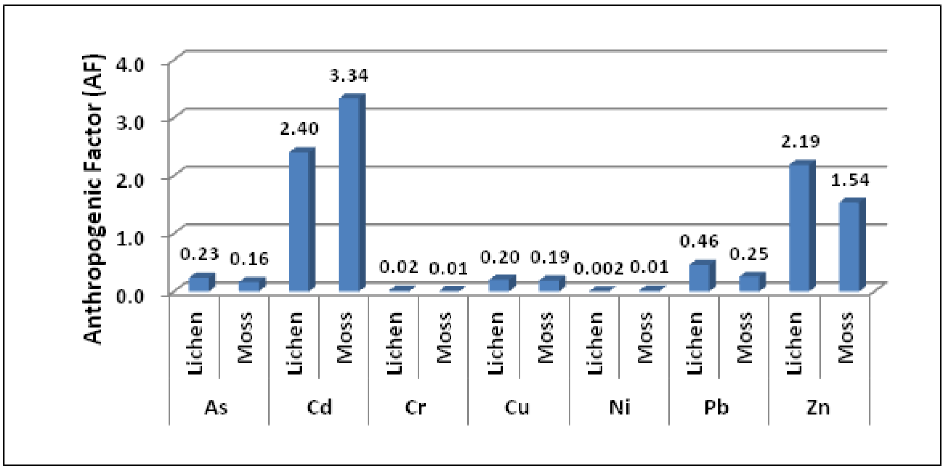

Figure 3. Summary Anthropogenic Factor for heavy metals in lichen and moss samples.

The relationship among the metals studied was investigated using Pearson Correlation Coefficient as well as Principal Component Analysis (PCA). As can be seen in Table 2 (above diagonal), Cd and Ni show strong positive correlation ($r = 0.615$) to one another, while As and Pb ($r = 0.385$) and Ni and Pb ($r = 0.360$) show moderate correlation in lichen.

With r = 0.505 Cd also shows strong correlation with Ni, in moss (Table 3, below diagonal). The same strong correlation is also observable between Zn and Cr (r = 0.431). However Ni shows relatively moderate correlation with Pb (r = 0.363) and Zn (r = 0.397).

Table 2. Pearson Correlation Coefficients between heavy metals in Lichen (above diagonal) and moss (below diagonal)

	As	*Cd*	*Cr*	*Cu*	*Ni*	*Pb*	*Zn*
As	1	-0.045	-0.138	-0.174	0.277	**0.385**	-0.089
Cd	0.267	1	-0.243	-0.259	**0.615**	0.162	-0.355
Cr	-0.187	-0.214	1	0.192	-0.357	-0.729	-0.054
Cu	0.154	0.076	-0.283	1	-0.408	-0.017	0.221
Ni	-0.048	**0.505**	0.049	-0.408	1	**0.360**	-0.535
Pb	-0.146	**0.384**	0.032	0.307	**0.363**	1	0.143
Zn	0.199	-0.088	**0.431**	-0.459	**0.397**	-0.081	1

Results for PCA are shown separately in Table 3 and Table 4 for lichen and moss. The top three highest eigenvalues of the principle components (PC) were retained. The total variance explained by the three PC was 89.5% for lichen and 86.9% for moss. For lichen, the first factor is loaded with As, Cd, Cu, Ni and Pb, while Cr in the second factor. No obvious association of heavy metals was observed in the third factor. Although the first factor elements could be associated to human activities, however the less enrichment (low AF) showed by As, Cu, Ni and Pb indicated that they are mainly represent the wind-blown dust contribution from soil of the study area (Dragovic and Mihailovic, 2009). The accumulation of Cr in lichen exhibit airborne transfer from sources such as diesel combustion from diesel-fueled vehicles (Bajpai *et al.*, 2011), that are the most common form of transportation for agricultural products in the area.

Table 3. PCA variance and factor loadings of Heavy Metals in Lichen

Component	*Eigen-values*	*% of Variance*	*Element*	*Loading*		
				1	*2*	*3*
1	1.359	53.7	As	**0.435**	-0.031	0.060
2	0.673	26.6	Cd	**0.434**	-0.072	0.084
3	0.234	9.2	Cr	0.171	**0.972**	0.127
4	0.127	5.0	Cu	**0.426**	0.029	-0.876
5	0.065	2.6	Ni	**0.463**	-0.105	0.364
6	0.042	1.6	Pb	**0.445**	-0.191	0.270
7	0.004	0.1	Zn	-0.013	0.001	0.013

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The first two PCs for moss do not indicate any association between metals however the third factor is loaded with Cd and Ni (Table 4). This observation is consistent with the corelational study described earlier. Based on the anthropogenic factor (AF), Cd was enriched, not Ni. As mentioned earlier Cd originated from solid waste incineration and phosphate fertilizers known for having strong affinity to organic matter, especially at the moss exchange sites (Dragovic and Mihailovic, 2009). Ni in moss originated from the same source as those found on lichen.

Table 4. PCA variance and factor loadings of Heavy Metals in Moss

Component	*Eigen-value*	*% of Variance*	*Element*	*Loading 1*	*Loading 2*	*Loading 3*
1	1.190	59.1	As	-0.364	0.146	-0.213
2	0.292	14.5	Cd	-0.453	0.224	**0.573**
3	0.267	13.3	Cr	-0.275	-0.952	0.097
4	0.129	6.4	Cu	-0.522	0.064	-0.642
5	0.090	4.5	Ni	-0.367	0.099	**0.443**
6	0.042	2.1	Pb	-0.423	0.089	-0.084
7	0.004	0.2	Zn	0.023	0.004	0.015

Heavy Metals Distribution

To have some ideas on the distributions pattern of the heavy metals in lichen and moss of the study area, distribution plots for all the studied metals are presented in Figure 4 to Figure 10. In the plots only the minute scales of the longitude (horizontal axis) and latitude (vertical axis) are shown, while the concentration is in mg/kg dry-weight . Areas located on the upper-right corner of the plots are close to the main road while on the western side is bordered by mountain range (Compare to Figure 1).

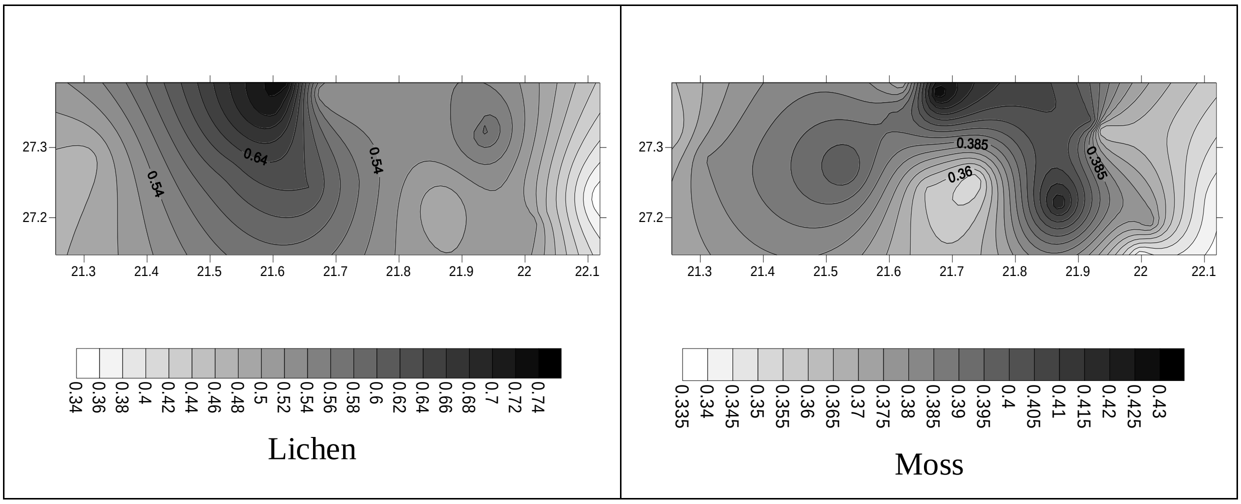

Figure 4. Geographical distribution of As (mg/kg) in lichen and moss.

Obviously seen from Figure 4, As is consistently higher in lichen than in moss although statistical test shows no significant difference between the means. Near the central-north of the study area the concentration in lichen is almost twice than in moss. However, generally these results are lower than a similar study, using lichen by Bajpai *et al*. (2012) in India but order of magnitude similar to those by Lucaciu *et al*. (2004) using moss in Romania. As for distribution patterns both lichen and moss agrees to one another, with low concentration near the main road and highest concentration recorded near the central-north area. These high concentration areas are relatively on low lying altitudes. Being ubiquitous element this would facilitate leached As-contained soil dust from the eroded bare slopes near the main road to accumulate within the area.

Contrary to As, concentrations of Cd in lichen samples (mean value of 0.48±0.07 mg/kg) are generally lower than those in moss samples (mean value of 0.67±0.17 mg/kg), despite no significant difference between them. Values in lichens and mosses of the present study are comparable to those by Lucaciu *et al*. (2004) however lower than a study in India (Bajpai *et al*., 2012) and Serbia (Dragovic and Mihailovic, 2009). The highest distribution in both type of samples are recorded around B15, within the valley of the study area. Being originated from fertilizer application as well as vehicular emission the windblown transfer mechanism tends to accumulate them in air at lower altitudes of the study area. This consistent observation of spatial distribution in both lichen and moss is shown in Figure 5.

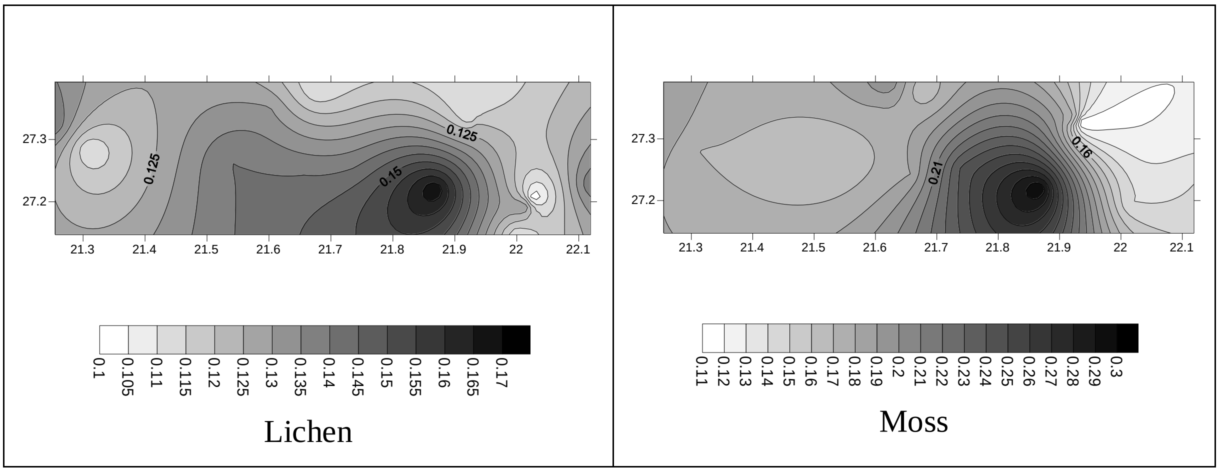

Figure 5. Geographical distribution of Cd (mg/kg) in lichen and in moss.

Chromium was found to be higher in lichens and mosses collected at locations near the main road compared to locations near the mountain range on the western side. Although generally concentrations in mosses (average 0.90±0.42 mg/kg) are lower than in lichens (1.61±0.64 mg/kg), identical distribution trend is observed for both monitors (Figure 6). This exhibits the airborne origin of Cr from heavy vehicular activities on the main road, a result similar to the observation by Bajpai *et al*., (2011). The nearly two times higher concentrations in lichens compared to mosses could be attributed to different absorption efficiency of the bio-monitors for the element.

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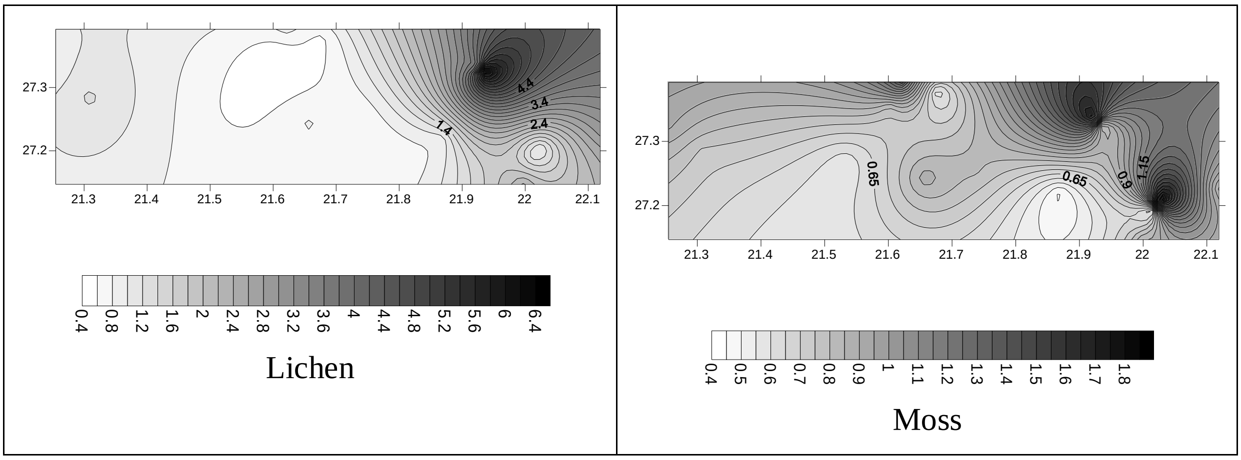

Figure 6. Geographical distribution of Cr (mg/kg) in lichen and in moss.

In Figure 7 the trend of higher concentration in lichen (mean 5.96±1.46 mg/kg) than in mosses (mean 3.30±0.37 mg/kg) still prevails for Pb. On average these values are two to three folds lower than other studies in India (Bajpai *et al*., 2012) and Romania (Lucaciu *et al*., 2004). Distribution wise higher concentration of Pb was found in areas near the middle and close to the mountain range, hence away from the main road. This could be attributed to the fact that unleaded petrol introduced in Malaysia more a decade earlier being used for motor-vehicles using the road. In the plantation area diesel (leaded) powered vehicles are main mode of transportation for workers and agricultural products.

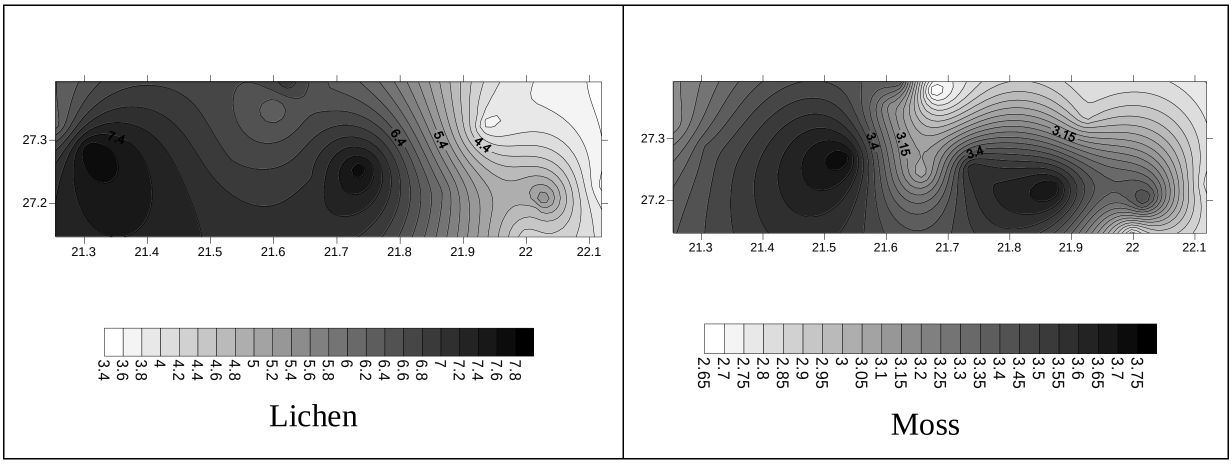

Figure 7. Geographical distribution of Pb (mg/kg) in lichen, and in moss.

Mean concentrations of Cu in lichens and mosses are 11.1±3.3 mg/kg and 10.4±3.4 mg/kg respectively, comparable to the value of 11 mg/kg in a study by Lucaciu *et al*. (2004). However, besides the significant difference between the means of Cu in lichens and mosses, the distribution pattern of Cu also differs significantly as shown in Figure 8. Lichens showed highest concentration in areas close to the mountain range and lower concentration in the middle sections of the study area. While, Cu in mosses showed higher concentrations in middle areas closer to the main road and relatively lower near the mountain range. This could be explained by the fact that the AF for Cu showed no enrichment due to anthropogenic factor, therefore its presence in lichens and mosses represents soil contribution associated with the soil mineral particles (Dragovic and Mihailovic, 2009) of the sampling locations.

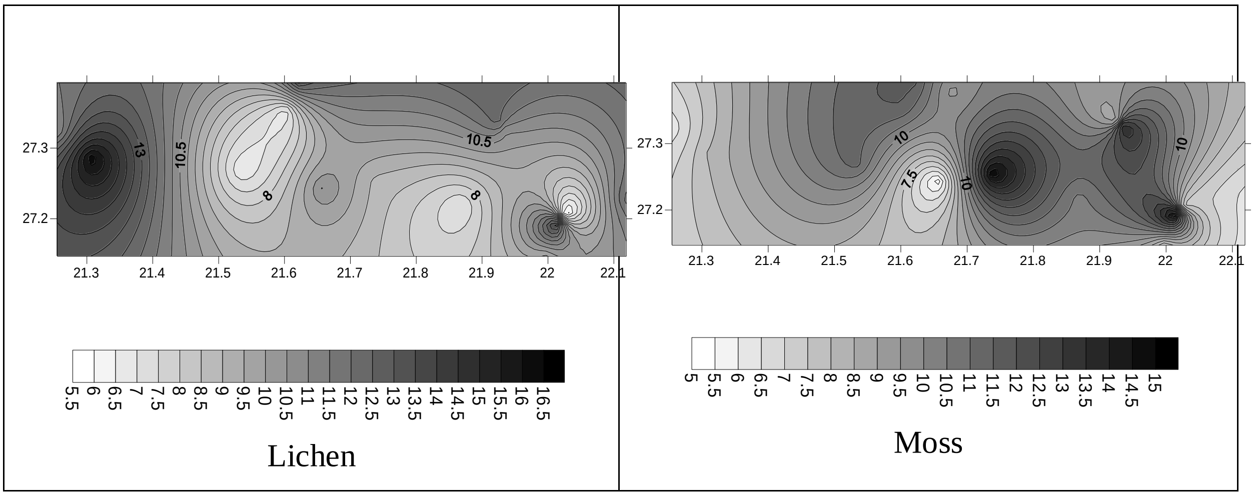

Figure 8. Geographical distribution of Cu (mg/kg) in lichen, and in moss.

The mean concentration of Zn in lichens is 153±86 mg/kg as compared to 108±44 mg/kg in mosses (Figure 9), highest being around B11 for lichens and around B4 for mosses. These means are higher than similar study on mosses in Serbia (Dragovic and Mihailovic, 2009) but comparable to those in India (Bajpai *et al.*, 2012). In terms of distributions higher concentrations were observed in lichens and mosses collected at sections near the middle and towards the mountain range. Zn can also be attributed to soil contributions (Dragovic and Mihailovic, 2009), however being enriched (AF > 1) in lichens and mosses of the area, the possible sources could be wears and tares of tires and also incomplete burning of fossil fuels (Bajpai *et al*., 2012) that frequent the service roads within the plantation area.

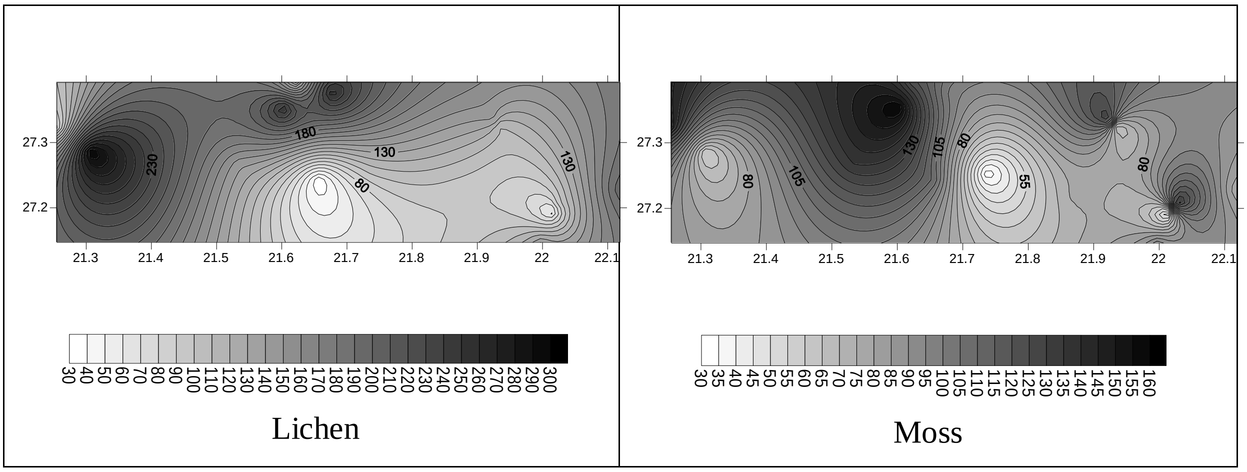

Figure 9. Geographical distribution of Zn (mg/kg) in lichen, and in moss.

In Figure 10 the mean concentrations of Ni in lichens and mosses together with the distribution patterns are shown. The low concentrations in lichens and mosses compared to the results of Dragovic and Mihailovic (2009) and Lucaciu *et al*. (2004) indicated that the presence of Ni is associated to inputs from soil mineral particles, just like in the case of Cu. This is enhanced by the fact that Zn was not enriched (AF<1) in the lichens and mosses samples. As a consequence, the distribution of Ni in lichens and mosses of the study area would be influenced by the distribution of Ni contents in soil. The distribution patterns in lichens and mosses are quite similar with higher concentration on the southern sections of the study area. However, the observation that on average Ni in mosses is about seven times higher than in lichens can be explained by the different in absorption capacity or efficiency of the two bio-monitors for Ni.

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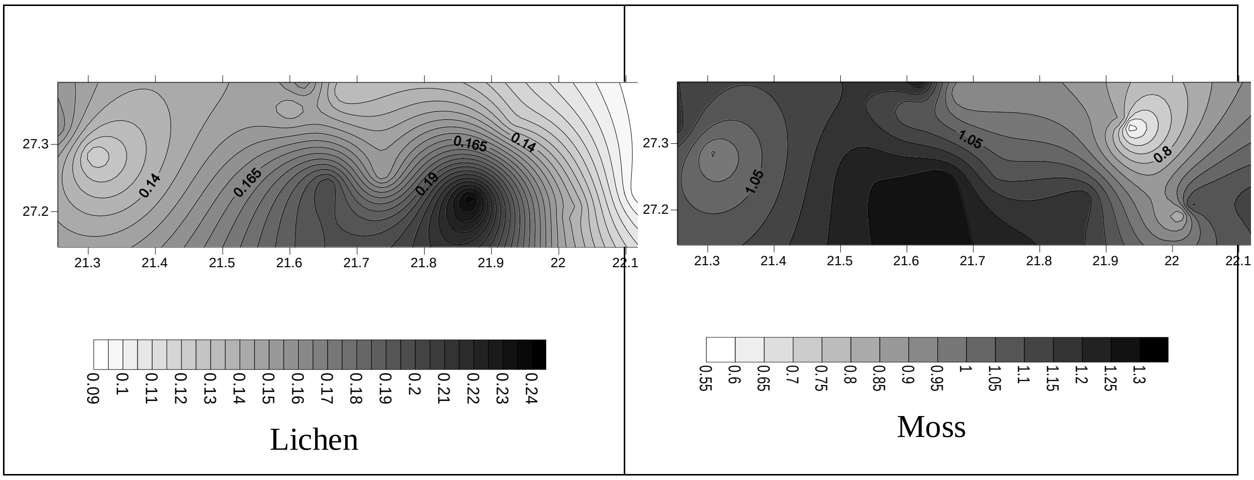

Figure 10. Geographical distribution of Ni (mg/kg) in lichen, and in moss.

CONCLUSIONS

Results of the study indicated the presence of heavy metals pollutants suspended in the air and are adsorbed on to, and absorbed by lichens (*Platismatia glauca*) and mosses (*Meiothecium microcarpum*) found in the study area. Based on Anthropogenic Factor (AF) Cd and Zn are enriched in both lichens and mosses. The correlation and PCA studies indicated that these enrichments are due to different sources; Cd mainly from application of phosphate fertilizers, solid waste incineration and fossil fuel combustion, while Zn from wears and tears of vehicles tires as well as fossil fuel combustion. However based on pollution index (PI) of the studied heavy metals the area can be categorized as not polluted. Other heavy metals are mainly of natural origins being suspended in air particulates by development activities like lands opening and terracing, as well as road construction and widening works that render the ground bare. The particulates were wind-blown to the study area, giving the distribution patterns of the metals in lichens and mosses. Another aspect of the study also found that lichens and mosses show different absorption efficiency for Cr and Ni.

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