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Phlyctis argena Spreng. Flot. Lichen as Biomonitor of Airborne Heavy Metals Near a Nickel Mining Site in Mindoro Island, Philippines

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ABSTRACT

The aim of this study was to analyze the concentration of cadmium, nickel and lead in Phlyctis argena Spreng Flot. (white paint lichen) which was collected in two sites near a nickel mining area in Barangay Villa Cerveza, Municipality of Victoria, Mindoro Island, and to relate the metal concentration to the local air quality condition. White paint lichen growing on the bark of Artocarpus heterophyllus (jackfruit) trees was collected in two locations (mining site and along roadside) in May, August and November, 2014. Heavy metals in lichens were analyzed by Flame Atomic Absorption Spectrometer. Results showed that Ni was the most abundant metal in two sampling sites. The highest concentration of Cd (mean=4.593 mg/kg, mining site) and Pb (mean=44.02 mg/kg, road side) was observed in May 2014, whereas the lowest concentration of Pb (<0.24 mg/kg, mining site and roadside) and Cd (<0.20 mg/kg, mining site and roadside) was observed in August and November. The possible reason for high metal deposition during the month of May is the continuous mobilization of dust particles containing high concentrations of heavy metals by the wind. Weathering of wastes rocks and ore materials left in mining site which is the most favorable during dry season generates dust particles. Conversely, the highest concentration of Ni (mean=83.597 mg/kg, road side) was detected in November, while the lowest concentration was <0.24 mg/kg in roadside in the month of May. The accumulation of Cd, Ni, and Pb in the lichen varies with season and could be influenced by weather condition dominated by strong wind and little precipitation, and distance from the mining site. This study showed the suitability of white paint lichen as a bioindicator of airborne metals and may help in detecting early signs of air quality deterioration.

Key words: *Airborne heavy metals, Bioindication, Phlyctis argena Spreng Flot.*

1. INTRODUCTION

The presence of airborne heavy metals, particularly in areas with intense industrial activities and fossil fuel consumption such as large-scale mining, power plants, manufacturing plants, and vehicular traffic which are released in the atmosphere as particulate matter, is a public health concern and threat to agricultural production. Studies have shown that exposure to airborne heavy metals is associated to respiratory diseases [1], cardiovascular diseases [2-4], alteration of female reproductive hormone, and mental health stress [5]. In addition, airborne heavy metals can be deposited in soil which in turn can lead to poor plant growth and low crop yield. For this reason, it is imperative to continuously monitor atmospheric contamination due to heavy metals to detect signs of air quality deterioration.

Monitoring of airborne heavy metals and trace elements using conventional air sampling technique is costly most especially air quality monitoring of a large area and remote areas. As an alternative, lichens have been used as a reliable and sensitive biological monitors and indicators of atmospheric pollution because of their ability to accumulate chemical pollutants and metals from the atmosphere [6-9]. But unlike higher plants, lichens do not have roots or stomata and as a result their mineral nutrition depends mainly on wet and dry atmospheric deposition, hence making them as excellent bioindicators of air pollution [6,7,10-13]. Lichen species such as *Cladonia* [14], *Evernia* sp. [15], *Flavocetraria nivalis* [16], *Hypogymnia physodes* (L.) Nyl. [8], *Palmelia sulcata* [17], *Parmotrema reticulatum* [18], *Pyxine cocoes* (Sw.) Nyl. [19], *Ramalina celastri* [11],

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Ramalina lacera (With.) J.R. Laund [20], *Tillandsia capillaries* [21], *Usnea antartica* [6], *Usnea* sp. [15], *Xanthoria parietina* [7,10,22], and *X. parietina* Ochsn. 1928 [13] have been studied to gain understanding of the spatial and temporal distribution pattern of metal pollutants in air, to identify heavy metal sources and as an early warning to detect environmental stress. This study is in line with this research work.

The lichen *Phlyctis argena* Spreng. Flot (white paint lichen) is widespread in Barangay Villa Cerveza, Municipality of Victoria, Oriental Mindoro and abundantly grow in tree barks. At the time of this study, nickel mining is active in this study area. We hypothesize that mining activities in this study area could be a significant source of airborne and dust borne heavy metals. Fugitive dust produced by land excavation and mechanical crushing of geological materials and weathering of rocks from the mining site maybe transported by prevailing winds. On the basis of existing literature acknowledging the desirable characteristics of lichen species as sensitive biomonitors and bioindicators of air pollutants, white paint lichen is an appropriate choice for investigating airborne heavy metals. To the best of our knowledge, studies concerning heavy metal analysis in white paint lichen as a reflection of air quality are scarce or have received little attention. There is also no published local studies regarding the use and analysis of heavy metals in white paint lichen in this area, hence this study will serve as a baseline data and will contribute to the growing number of studies employing lichens as biomonitors for airborne heavy metals. This study is limited to analysis of cadmium, nickel and lead in *P. argena* (white paint lichen) collected near a nickel mining area and along a roadside in Barangay Villa Cerveza, Municipality of Victoria, Mindoro Island, and Philippines. An accurate source identification of metals and lichen transplantation to assess the performance of *P. argena* as a biomonitor of heavy metals is beyond the scope of this study. Moreover, the interpretation of data is speculative and limited by unavailability of weather data such as wind speed, wind direction and temperature during lichen sampling, and absence of air quality monitoring devices to systematically measure atmospheric metal concentration in this remote area. Nonetheless, the studies [16,17,23] indicate that lichens are reliable biomonitors of airborne and dust borne heavy metals from mining areas.

2. MATERIALS AND METHODS

2.1. Study Area

The study was conducted in Barangay Villa Cerveza, Municipality of Victoria, Mindoro Island, and Philippines (Figure 1). The Municipality of Victoria is the second class municipality and is bounded by Municipality of Naujan and Municipality of Socorro. It is also adjacent to Naujan Lake National Park, an inland aquatic ecosystem with high endemism and species diversity, and a Ramsar wetland of international importance [24].

2.2. Collection of Lichen Samples

Lichen samples were collected on May 30, 2014, August 28, 2014, and November 30, 2014, employing line transect method. The distance between the sampling point is 100 m. The lichen specimens were identified by Ms. Krystle Angelique A. Santiago of Far Eastern University as *P. argena* (Spreng.) Flot. (Figure 2). Fresh living lichen was collected on the bark of jackfruit trees (*Artocarpus heterophyllus* Lam) at a height of approximately 0.5-0.7 m above the ground in two sites near a mining area within the ancestral land of Mangyan community. Lichens were collected using stainless knife and were collected in jackfruit trees only because at the time of collection the local people prohibited gathering of lichens in other tree species. During May 2014 lichen collection, slash and burn agriculture or *kaingin* was performed by local people. The first sampling site is near a nickel mining area located near Mount Kisluyan. Three sampling stations were chosen in this area. The other sampling sites are a roadside which is 2 km away from nickel mining area and is near a human community. Three sampling stations were chosen in this site. The lichens were kept in paper bags and transported to the laboratory for analysis.

2.3. Analysis of Metals

The lichens were carefully separated from the bark, washed with purified water then distilled water to remove dust and extraneous materials. This was followed by drying lichen samples at room temperature for two days. Cadmium (Cd), lead (Pb), and nickel (Ni) were analyzed following procedures described in USEPA (1996) Method 3050B [25] using Flame Atomic Absorption Spectrometry. Concentrations of metals are expressed in mg kg^{-1}.

2.4. Statistical Analysis

Descriptive statistics such as minimum and maximum values, mean, median, and standard deviation (SD) were determined. The means were compared using two samples: t-test and one-way analysis of variance (ANOVA). Pairwise comparison of means by Tukey simultaneous 95% confidence interval was carried out whenever the ANOVA indicated significant differences between means. The differences were interpreted significant when $p<0.05$. Relationships were assessed using Pearson correlation and linear regression. The statistical analyses were carried using the statistical program MINITAB version 15.

3. RESULTS AND DISCUSSION

3.1. Cadmium

The highest concentration of Cd was found in one sampling station in a mining site in the month of May while it fell to <0.20 mg kg^{-1} in November for

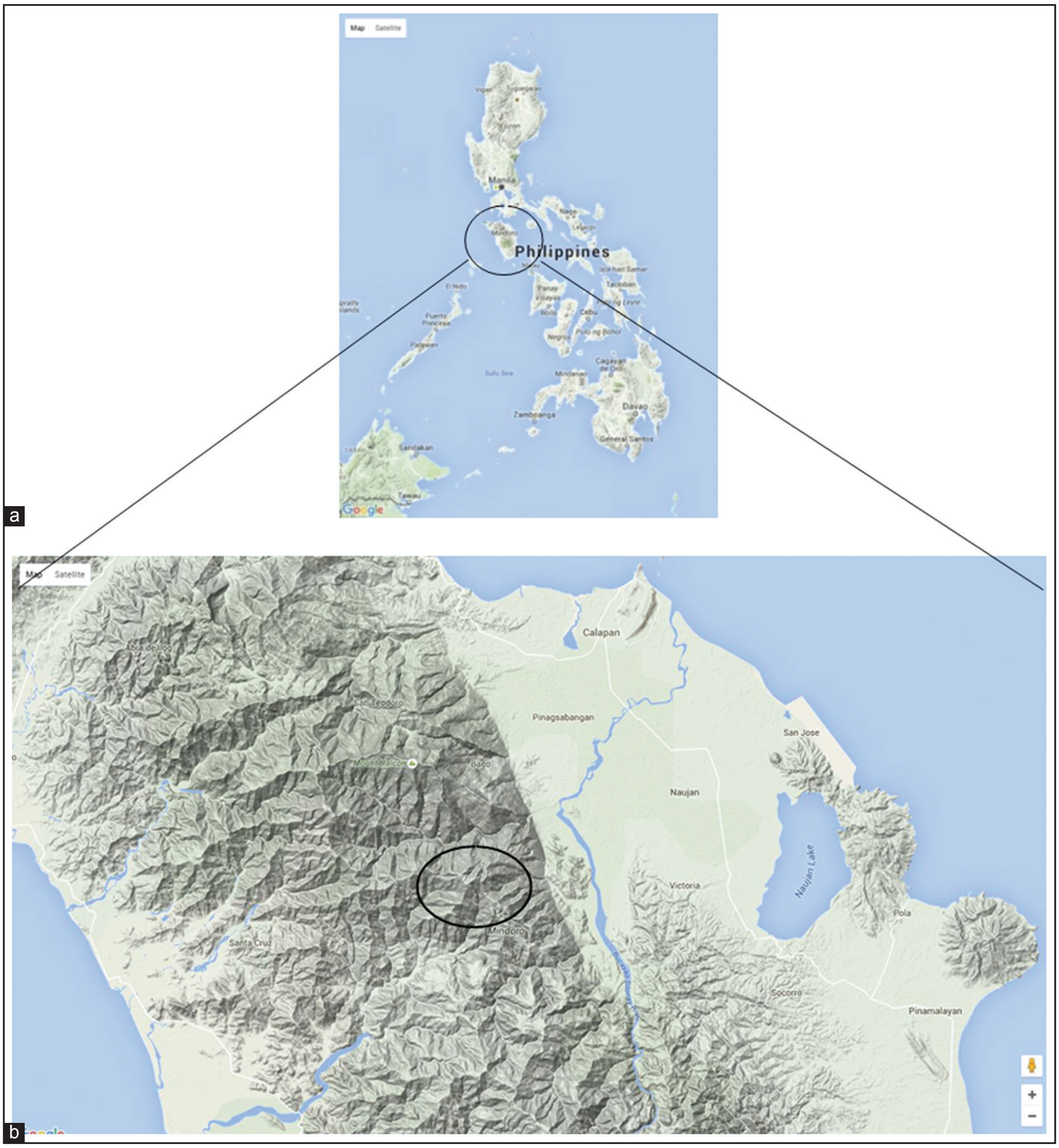

Figure 1: (a) Location of Mindoro Island in the Philippine map. (b) Location of Municipality of Victoria in Mindoro map.

both mining site and road side. Cd concentration in mining site for the months of May, August and November ranged from 1.56-4.88 mg kg^{-1} (mean=2.89 mg kg^{-1}, median=2.37 mg kg^{-1}, SD=1.32), 0.61-3.02 mg kg^{-1} (mean=1.82 mg kg^{-1}, median=1.86 mg kg^{-1}, SD=0.65 mg kg^{-1}), and <0.20 mg kg^{-1}, respectively. For road side, the range of cadmium concentration for the months of May, August and November are 1.09-2.82 mg kg^{-1} (mean=1.80 mg kg^{-1}, median=1.81 mg kg^{-1}, SD=0.55 mg kg^{-1}), 0.88-2.59 (n=9, mean=1.908 mg kg^{-1}, median=2.32 mg kg^{-1}, SD=0.66 mg kg^{-1}), and <0.20 mg kg^{-1}, respectively (Table 1).

The result of two-sample t-test indicates that mean Cd concentration in mining site and roadside for the month of May are significantly different (t=2.29, df=10, p=0.045) while the mean Cd concentration in mining site and roadside for the month of August are not significantly different (t=−0.28, df=15, p=0.780). The results suggest that white paint lichen can accumulate Cd and can be used as Cd biomonitor.

3.2. Nickel

The highest Ni concentration was detected in the month of November in both mining site and roadside. Ni concentration in mining site for the months of May, August and November 2014 ranged from 8.44-20.54 mg kg^{-1} (mean=1.56mgkg^{-1},median=15.80mgkg^{-1},SD=4.68), 6.90-14.98 mg kg^{-1} (mean=11.19 mg kg^{-1}, median=11.18 mg kg^{-1}, SD=2.502 mg kg^{-1}), and 39.76-62.32 mg kg^{-1} (mean=52.96 mg kg^{-1}, median=56.51mgkg^{-1},SD=7.57mgkg^{-1}),respectively. For roadside, the range of cadmium concentrations for the months of May, August and November are 0.23-7.88 mg kg^{-1} (mean=3.17 mg kg^{-1}, median=3.39 mg kg^{-1}, SD=2.68 mg kg^{-1}), 11.17-20.83 (mean=15.13 mg kg^{-1}, median=15.23 mg kg^{-1}, SD=3.42mgkg^{-1}),and57.79-86.31mgkg^{-1}(mean=72.91mg kg^{-1}, median=74.30 mg kg^{-1}, SD=10.22 mg kg^{-1}), respectively (Figure 3). In comparison to Cd, the highest accumulation of Ni was observed in the month of November (rainy season) and it seems reasonable to assume that frequent rainfall event during wet season favored accumulation of Ni in lichen.

Two-sample t-test showed that Ni concentration in mining site and roadside are significantly different in the month of May (t=6.22, df=12, p=0.000), August (t=-2.79, df=14, p=0.015) and November (t=-4.71, df=14, p=0.000). One-way ANOVA using Tukey method showed that Ni concentration in the month of May, August and November in mining site and roadside are significantly different (f=203.08, p=0.000) (Figure 3). Pearson correlation indicates that Ni and Cd have a negative correlation (−0.712, p=0.000) (Figure 4). The results indicate that white paint lichen can accumulate Ni and may be used as airborne biomonitor for Ni.

Table 1: Concentration of cadmium in *P. argena* (white paint lichen) collected in mining site and roadside on the month of May, August and November 2014.

Site/sampling station	Month		
	May 2014	August 2014	November 2014
Mining site			
Station 1	4.59 (0.253)	1.64 (1.24)	<0.20
Station 2	1.96 (0.405)	1.91 (0.31)	<0.20
Station 3	2.12 (0.402)	1.91 (0.05)	<0.20
Road side			
Station 1	1.70 (0.974)	2.18 (0.44)	<0.20
Station 2	1.79 (0.415)	1.57 (0.90)	<0.20
Station 3	1.92 (0.216)	1.97 (0.66)	<0.20

Values are mean concentration (n=3) expressed in mg kg^{-1}. Values in parenthesis are standard deviation

AQ4 **Table 2:** Concentration of nickel in *P. argena* (white paint lichen) collected in mining site and roadside on the month of May, August and November 2014.

Site/sampling station	Month		
	May 2014	August 2014	November 2014
Mining site			
Station 1	8.79 (0.60)	9.80 (3.74)	48.7 (8.44)
Station 2	15.2 (1.95)	12.9 (1.81)	50.6 (7.22)
Station 3	19.0 (1.75)	10.9 (0.47)	59.5 (2.52)
Road side			
Station 1	< 0.24	14.4 (2.84)	62.1 (5.38)
Station 2	3.36 (0.48)	12.5 (1.65)	83.6 (3.58)
Station 3	5.91 (2.04)	18.5 (2.90)	73.0 (5.50)

Values are mean concentration (n=3) expressed in mg kg^{-1}. Values in parenthesis are standard deviation

3.3. Lead

The highest concentration of Pb was detected in one sampling station in mining site in the month of May, while it fell to <0.24 mg kg^{-1} in both mining site and roadside in the month August and November. Pb concentration in mining site ranged from 21.62 to 51.18 mg kg^{-1} (mean=33.36 mg kg^{-1},

Figure 2: *Phlyctis argena* Spreng Flot. in the bark of *Artocarpus heterophyllus* Lam (jackfruit tree).

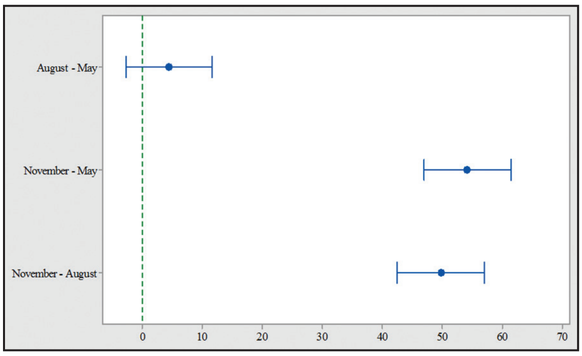

Figure 3: Tukey simultaneous 95% confidence interval for Ni concentration in *Phlyctis argena* lichen in the month of May, August and November 2014. If an interval does not contain zero, the corresponding means are significantly different.

median=33.80 mg kg^{-1}, SD=10.11) in the month of May. For roadside, lead concentration in the month of May is 2.38-12.92 mg kg^{-1} (mean=8.18 mg kg^{-1}, median=6.42 mg kg^{-1}, SD=3.71 mg kg^{-1} (Table 3). In comparison to Cd and Ni concentration, the highest accumulation of Pb was observed in the month of May and this may be explained by greater dust dispersion and low rainfall frequency during the dry season. On the other hand, the low Pb concentration in the month of August and November could be attributed to low vehicle emissions or low fuel combustion due to less frequency of truck activities in the mining site during the rainy season. Pb (as tetraethyl) is a component of diesel motor fuel and could be the probable source of airborne Pb [10,26]. Other sources of Pb are metal industries, battery factories, chemical industries, cement plants [21], smelter emissions [27], and municipal solid wastes combustion [15].

Two-sample t-test showed that lead concentration is significantly higher in mining site than in roadside (t=7.02, df=10, p=0.000). Pearson correlation indicates that Pb and Cd have significantly weak positive correlation (r=0.484, p=0.000) (Figure 5). This may suggest co-deposition or similar sources for both elements [8]. It is worth mentioning that Cd is used as diethyl-cadmium in the production of tetraethyl lead, hence atmospheric Cd could originate from vehicle emissions [22]. Furthermore, Cd has a low crustal abundance [17] thus supporting our data that Cd is not soil-borne but anthropogenic origin. Other sources of Cd are metal industries, battery factories, chemical industries and cement plants [21]. Significant positive correlation was obtained in *Cladonia* lichens (r=0.79, p<0.001) [14] and in *X. parietina* (r=0.56, p<0.05) [10]. On the other hand, in this study, Pearson correlation indicates a negative correlation between Pb and Ni ($^{-}$0.310, p=0.0023) (Figure 6). On the contrary, obtained positive correlation of Pb and Ni (r=0.632)

Table 3: Concentration of lead in *P. argena* (white paint lichen) collected in mining site and roadside on the month of May, August and November 2014.

Site/sampling station	Month		
	May 2014	August 2014	November 2014
Mining site			
Station 1	23.4 (2.62)	<0.24	<0.24
Station 2	32.7 (2.84)	<0.24	<0.24
Station 3	44.0 (8.55)	<0.24	<0.24
Road side			
Station 1	8.63 (5.54)	<0.24	<0.24
Station 2	5.96 (0.66)	<0.24	<0.24
Station 3	9.94 (3.40)	<0.24	<0.24

Values are mean concentration (n=3) expressed in mg kg^{-1}. Values in parenthesis are standard deviation

in *X. parietina* lichen [22]. The results indicate that white paint lichen can be used for detecting presence of airborne lead.

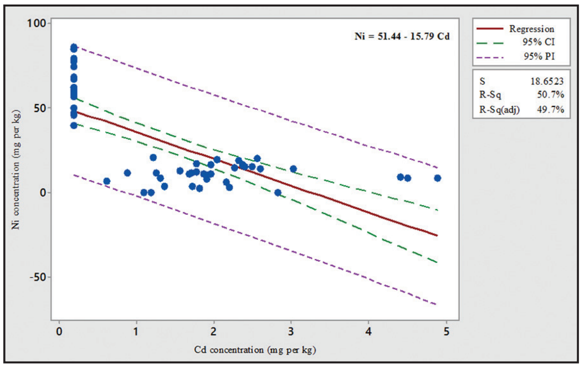

Figure 4: Relationship between Ni and Cd concentration. The regression equation is shown on the upper right side (bold). The solid line is the regression line. The inner and outer broken lines refers 95% confidence interval and 95% predicted interval, respectively.

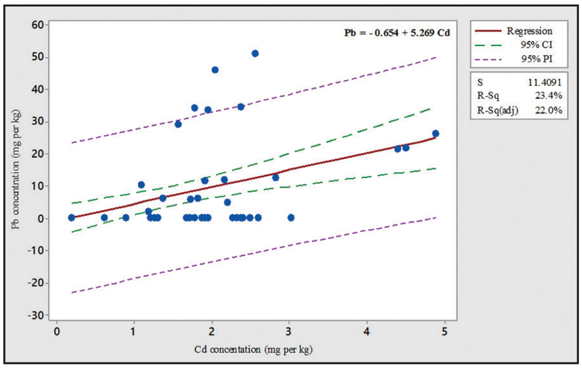

Figure 5: Relationship between Pb and Cd concentration. The regression equation is shown on the upper right side (bold). The solid line is the regression line. The inner and outer broken lines refer 95% confidence interval and 95% predicted interval, respectively.

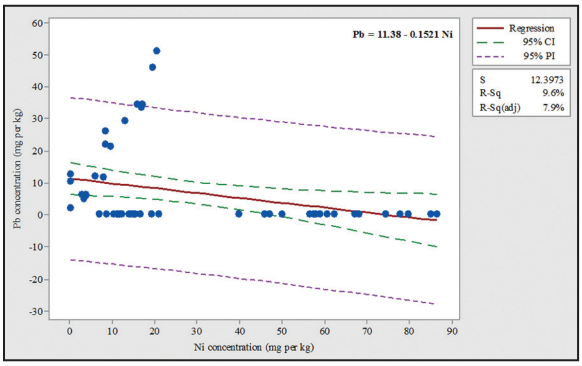

Figure 6: Relationship between concentration of Pb and Ni. The regression equation is shown on the upper right side (bold). The inner and outer broken lines refer 95% confidence interval and 95% predicted interval, respectively.

4. CONCLUSION

This study concludes that *P. argena* lichen is capable of accumulating cadmium, nickel and lead, and thus may be used as biomonitor of airborne metals in remote areas near a mining site. The levels of cadmium, nickel and lead in white paint lichen could depend on season and climatological conditions such as frequency of rainfall event, temperature, wind speed, and wind direction. Previous studies suggest that the accumulation of metals in lichens are significantly influenced by distance from source [16,21,28], local climate and microclimate conditions [6], and altitude [15]. The degradation of geologic materials in mining site such as ore materials and waste rocks through chemical and the physical process produces dust borne metals. Mobilization of dust particles is favored by dry climate, scarce vegetation cover and strong wind [16]. Since white paint lichen was collected in the summer season, the dry climate and high wind velocity could be the possible reason for the high concentration of Cd and Pb in the month of May. In a related study, Kularatne and de Freitas [18] found out that the accumulation of heavy metals in thallus of *P. reticulatum* lichen was highest during the summer season and lowest during winter. Furthermore, the results of their study suggest that the rate of on-thallus heavy metal accumulation in *P. reticulatum* decreases both with increasing rainfall and a greater number of wet days within each season. On the contrary, the uptake of atmospheric nutrients and accumulation of metals in lichens could be also favored by low temperature and high rainfall [21] and possibly temperature inversion [29]. This reason could be used to explain the observed highest accumulation of Ni in the month of November.

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