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SHORT COMMUNICATION

Surrogate approach to determine heavy metal loads in a moss species – *Barbula lambaranensis*

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Abstract Biomonitoring using a moss species *Barbula lambaranensis* is an economical method for continuous assessment of atmospheric metal pollution. However, frequent measurement of common heavy metals such as Zn, Cd, Cr, Pb, Cu and Ni in moss can be costly for monitoring large areas. Thus, the aim of the study was to use the surrogate approach to reduce the number of heavy metals required for monitoring. The study found that the Zn load in moss was higher; Pb, Cu and Ni loads were moderate; while Cd and Cr were relatively lower across the study sites. Further, the following surrogates were identified based on PCA: Cu for Cr; Pb for Cd, Cu and Ni; and Cu and Pb for Zn. Quantitative relationships between surrogate loads and the loads of other heavy metals were developed by performing Multiple Linear Regression on a data set constructed using a four level full factorial design. The equations had a relative prediction error and standard error of cross validation below 25% and 1.5%, respectively, indicating that the equations are accurate. However, the cross validated coefficient of determination is relatively low suggesting that the precision of prediction using the equations is low, possibly due to the influence of factors such as climatic conditions on bioaccumulation of heavy metals by moss. Nevertheless, the developed equations can be useful for preliminary investigations.

1. Introduction

Anthropogenic activities release significant amount of harmful pollutants such as heavy metals, the presence of which above the threshold limits in the atmosphere poses adverse ecosystem and human health threats (Wolterbeek, 2002). For example, human exposure to heavy metals can result in a variety of negative health effects such as cancer and kidney disorder (Itoh et al., 2014; Lin et al., 2013). Therefore, it is important to continuously monitor the level of heavy metals in

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atmosphere in order to develop intervention strategies to mitigate the potential negative health impacts.

Most studies on atmospheric heavy metal pollution are based on aerosols collected on filters using air samplers (Chakrabortty and Paratkar, 2006). However, this method requires long-term sampling at a large number of sampling sites, sophisticated expensive technical equipment and has the risk of contamination when determining low concentrations of heavy metals in the atmosphere (Poikolainen, 2004). Biomonitoring is considered as a potentially effective and economical indirect alternative to direct ambient air measurement, especially for monitoring large areas (Celik et al., 2005; Chakrabortty and Paratkar, 2006). Mosses, which are cryptogams that thrive in humid climate, are one such biomonitor that possess suitable properties such as lack of cuticle, due to which water and minerals, including metal ions can readily penetrate into the tissues (Cencil, 2008). Consequently, mosses, especially the ectohydric types such as *Barbula lambaranensis*, *Thidium* spp. and *Funaria hygrometrica* are excellent biomonitors of air quality and heavy metal depositions (Llamazarene, 2010). For example, Mazzoni et al. (2012) reported that metal deposition in an ectohydric moss was significantly correlated with the increased atmospheric metal concentration at Caxias do Sul, Brazil.

In general, Zn, Cd, Pb, Cr, Ni and Cu provide important information about the atmospheric metal pollution (Egodawatta et al., 2013). Thus, continuous assessment of air quality requires frequent measurement of these metal ion loads in moss. However, frequent measurement of these heavy metals can be expensive and time consuming, especially when large areas are assessed. Hence, a feasible approach is the use of representative surrogate heavy metals that can be used to predict the loads of other heavy metals in moss. Therefore, the aims of the study were to: (1) identify a set of surrogate heavy metals to determine the heavy metal loads in an ectohydric moss species *B. lambaranensis*; and (2) develop reliable mathematical equations to relate the loads of surrogate heavy metals to those of the other heavy metals.

2. Materials and methods

2.1. Study site

The study was carried out in three cities, namely Abuja, Kaduna and Ilorin, which encompass varying land use activities in the Guinea savanna zone of Nigeria (Fig. 1). A total of 81 samples (Abuja – 25; Kaduna – 33 and Ilorin – 23) were collected from these study sites.

2.2. Sample collection and testing

Samples of *B. lambaranensis*, which is a common ectohydric moss species found in Nigeria, were collected from different substrates such as walls, floors and rocks with the aid of a spatula into dispensing nylons and properly labeled. Only the green parts of the moss samples were collected without the substrates as per the Nordic guidelines (Kubin et al., 2000). In Abuja, 25 samples were collected randomly at sites adjacent to frequent traffic congestion, major motor parks and markets. Collection of 33 samples in Kaduna was carried out around motor parks, markets, abattoirs and also close to major road intersections; In Ilorin, 13 samples were collected from industrial areas, while another 10 samples were collected close to major road intersections.

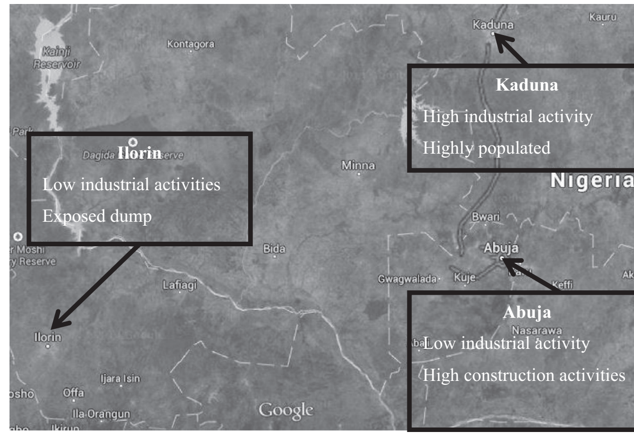

Figure 1 Three study locations.

The unwashed samples were cleaned from extraneous materials in the laboratory, air-dried to constant weight and pulverized into powder using a ceramic mortar and pestle. 5 g of air-dried moss was digested in 50 ml of conc. HNO_3 (Sigma-Aldrich Corporation, Germany) for 30 min on a hot plate till white fumes appeared. Digest was filtered using Whatman No. 42 filter paper into a 50 ml calibrated flask, after which it was diluted with deionized water to make up to the mark. Concentrations of Zn, Cd, Pb, Cr, Ni and Cu in the filtered solution were determined using a Flame Atomic Absorption Spectrophotometer (AAS-Bulk Scientific 210VGP). Calibration of the AAS was performed using a series of working standard solutions and the correlation coefficient (R^2) for the calibration curve of the instrument for each element was above 0.98.

Quality control was ensured by the use of blanks and replicate digestions. Certified reference material (IAEA 336-lichen; International Atomic Energy Agency) was also used to validate the precision of the instrument and the digestion procedure. The percentage recoveries of all the elements were within the range of 85% and 105%.

2.3. Data analysis

The data analyses were performed using Matlab R2009b® (Mathworks Inc., Natick, MA, USA). The outliers in the raw data matrix were identified using the box plot and the outlier free data matrix was checked for the normality using the Quantile–Quantile (*Q–Q*) probability distribution plot. Multi-variate chemometrics techniques, namely Principal Component Analysis (PCA) and Multiple Linear Regression (MLR) analysis, were used to identify the surrogate heavy metals and to develop quantitative relationships between the surrogate heavy metals and other heavy metals, respectively. The relevant Matlab codes are provided in Supplementary Information.

3. Results and discussion

The data matrix consisted of the loads of six heavy metals present in the moss samples collected from 81 study sites.

According to the box plot (Fig. S1 in Supplementary Information), 10 sites contained heavy metal loads with extreme values. Consequently, these sites were excluded from further analysis. Furthermore, the *Q–Q* plots for outlier-free data presented in Fig. S2 in Supplementary Information show that the frequency distribution of the data matrix can be approximated to the normal distribution.

3.1. Heavy metal load variation in moss samples

The heavy metal load variation in the moss samples across the three study sites is presented in Fig. 2. According to Fig. 2, Zn in moss was the highest followed by Cu, Ni and Pb. Zn is generally found in the highest concentration in the atmosphere and is often associated with the vehicular activities suggesting that metal levels in moss samples reflect the actual metal concentration pattern in the atmosphere (Gunawardena et al., 2012; Uno et al., 2013).

Further, Cd and Cr were found in low quantity. Pajak and Jasik (2011) also reported that Zn in moss was found in a higher concentration followed by Pb, while Cd concentration was relatively lower. A similar trend was also reported by Koz et al. (2012). It is worthy to note that there was no significant difference in the load of each heavy metal between Abuja, Kaduna and Ilorin. This suggests that the difference in the land use characteristics between study sites did not have any significant influence on the heavy metal loads.

3.2. Identification of potential surrogate parameters

The outlier-free data matrix was subjected to PCA in order to identify the potential surrogate parameters. The data matrix consisted of six heavy metals (variables) for 71 study sites (objects). The biplot for the first two PCs is presented in Fig. 3. In the PCA biplot, the angles between the vectors corresponding to Cu and Cr loads are acute suggesting a strong correlation between Cu and Cr. Thus, Cu can be used as a surrogate for Cr. This can be attributed to similar solubility characteristics of these metals across the cell wall as Ogunkunle and Fatoba (2012) have reported that Cr and Cu have a solubility of 41% and 43% in *B. lambaranensis*, respectively. Similar finding has also been reported by Fernandez et al. (2004).

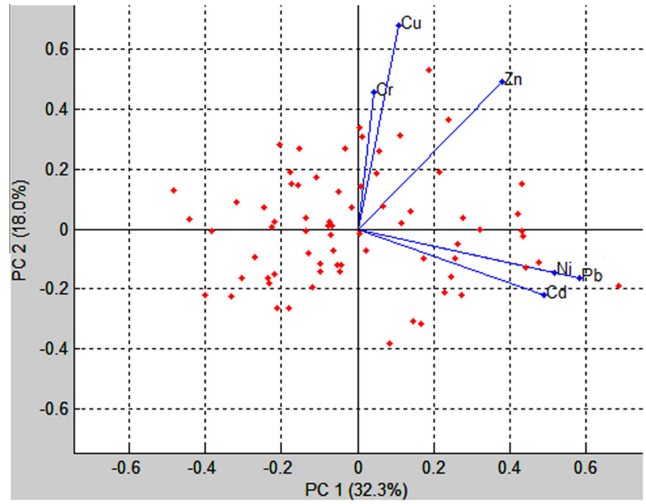

Figure 3 PCA biplot for the identification of surrogate parameters.

Similarly, there is a strong correlation between Ni, Pb and Cd since the angles between their corresponding vectors are acute. Consequently, Pb was selected as the surrogate heavy metal for Ni and Cd. Furthermore, the angles between Zn and other heavy metals are acute indicating that Zn is strongly associated with other heavy metals. Therefore, Cu and Pb can

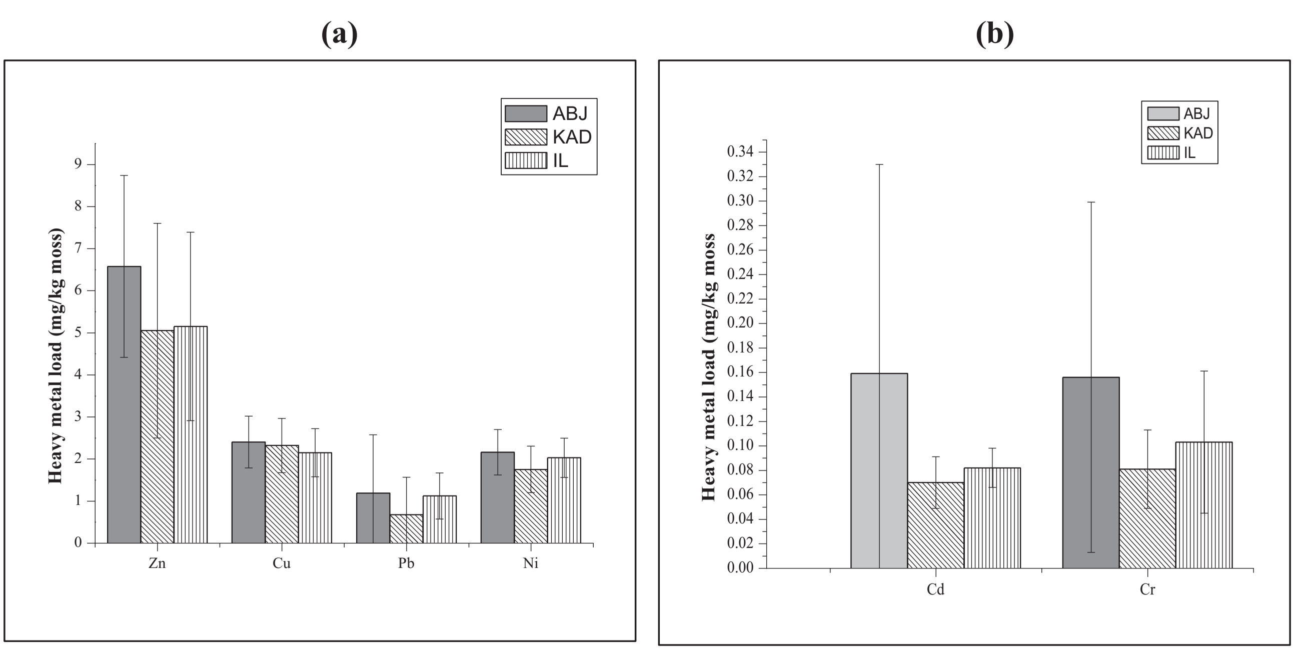

Figure 2 Heavy metal load variation across the study sites (ABJ – Abuja; KAD – Kaduna; IL – Ilorin): (a) for Zn, Cu, Ni and Pb and (b) Cd and Cr.

also be used as the surrogate for Zn loads in moss. The strong association of Zn with other metals can be due to the fact that Zn can be readily displaced by other elements from the active sites of moss since Zn forms only a weak and temporary bond with the extracellular exchange sites (Samecka-Cymerman et al., 1997; Ogunkunle and Fatoba, 2012). Consequently, Zn can relate well with other available metals.

3.3. Design of experiments

Design of experiment or experimental design is used to study the effects of certain independent variables on the dependent variables by systematically varying the independent variables (Deming and Morgan, 1993). A description of experimental design is given in the Supplementary Information. In this study, a four level full factorial experimental design was used to systematically vary the surrogate heavy metal loads (independent variables) to develop reliable mathematical relationships to predict the loads of the rest of heavy metals in moss (dependent variables). However, unlike well-controlled laboratory experiments, it is difficult to precisely control the variation of surrogate heavy metal loads determined through a field experiment because of the spatial and temporal variability in the field. To overcome this problem, the corresponding averages of their four quartiles were taken as the four levels of independent variables (Pb and Cu loads), i.e. average of the first quartile as Level 1, average of the second quartile as Level 2 and so on. The resulting design of experiment is given in Table 1.

3.4. Mathematical relationships for heavy metal loads based on the surrogate heavy metal loads

The mathematical relationships were developed using the Multiple Linear Regression (MLR) technique. The mathematical equations were validated using the Leave-One-Out Cross Validation (LOOCV) method and the reliability of the equations was assessed using the relative prediction error (RPE), standard error of cross-validation (SECV) and cross-validated coefficient of determination (Q^2). The related formulae are shown below (Gunawardena et al., 2014):

$$\mathrm{RPE} = \sqrt{\frac{\sum(Y_{\mathrm{predicted}} - Y_{\mathrm{measured}})^2}{\sum Y^2_{\mathrm{measured}}}} \times 100\% \tag{1}$$

$$\mathrm{SECV} = \sqrt{\frac{\sum(Y_{\mathrm{predicted}} - Y_{\mathrm{measured}})^2}{N}} \tag{2}$$

$$Q^2 = \left[1 - \frac{\sum(Y_{\mathrm{predicted}} - Y_{\mathrm{measured}})^2}{\sum(\bar{Y} - Y_{\mathrm{measured}})^2}\right] \times 100\% \tag{3}$$

where $Y_{\mathrm{predicted}}$ is the load of a dependent variable (mg/kg) predicted using the developed mathematical relationship, Y_{measured} is the load of the dependent variable present in the moss samples determined using the field sample collection (mg/kg), N is the number of samples and $\bar{Y}$ is the mean of Y_{measured}.

The MLR parameters and error values are presented in Table 2. According to Table 2, the RPE values for the dependent variables are less than 25%. Previous studies suggest that a RPE over 35% can be considered as satisfactory for a complex natural system, where controlling various influential factors is difficult, in contrast to a laboratory environment (Egodawatta et al., 2013; Gunawardena et al., 2014). For example, Gunawardena et al. (2014) reported a RPE of 39% for the prediction of heavy metal loads present on urban road surfaces. Similarly, SECVs for the investigated heavy metals are less than 1.5, which is relatively lower than the values reported in previous studies (Miguntanna et al., 2010; Gunawardena et al., 2014). The low error values indicate that the heavy metal loads in moss can be accurately predicted based on the surrogate heavy metal loads. However, the Q^2 are relatively low (Table 2), especially for Zn and Cr, indicating that the precision of the values predicted by the equations is low (Ruiz et al., 2008). This can be expected since bioaccumulation of heavy metals by moss can exhibit significant variability depending on the physical, chemical and climatic conditions. However, low Q^2 reduces the reliability of the prediction and thereby limits the application of the equations to preliminary investigations.

Table 1 Full factorial four level design for the development of mathematical relationships.

Independent variables		Dependent variables			
Cu	Pb	Zn	Cd	Ni	Cr
1.52 (1)[a]	0.27 (1)	3.30	0.09	1.69	0.09
1.91 (2)	0.42 (1)	6.16	0.07	1.83	0.09
2.41 (3)	0.23 (1)	4.78	0.07	2.13	0.07
2.97 (4)	0.22 (1)	3.62	0.08	1.60	0.08
1.53 (1)	0.63 (2)	4.57	0.09	1.66	0.06
2.04 (2)	0.54 (2)	5.42	0.08	1.98	0.07
2.48 (3)	0.53 (2)	4.21	0.08	1.86	0.08
3.24 (4)	0.51 (2)	3.46	0.07	1.75	0.07
1.13 (1)	0.83 (3)	6.04	0.10	2.40	0.05
2.06 (2)	0.86 (3)	5.04	0.10	2.21	0.08
2.53 (3)	0.87 (3)	4.54	0.08	1.85	0.09
3.04 (4)	0.86 (3)	3.42	0.09	1.74	0.08
1.68 (1)	1.45 (4)	4.42	0.10	2.24	0.06
2.05 (2)	1.35 (4)	2.32	0.10	2.03	0.07
2.47 (3)	1.55 (4)	6.04	0.14	2.25	0.07
3.04 (4)	1.66 (4)	5.79	0.11	2.46	0.07

Note: [a]Corresponding level is given in the brackets.

Table 2 Multiple Linear Regression parameters.

Dependent Variable	Regression parameters			[a]RPE	[b]SECV	[c]Q^2
	Constant	Pb	Cu			
Cd	0.073	0.031	−0.003	11	0.01	66
Zn	5.2	0.48	−0.44	22	1.1	9.2
Ni	1.9	0.40	−0.10	9.3	0.19	51
Cr	0.067	−0.01	0.01	13	0.01	16

Note: a – relative prediction error; b – standard error of cross-validation; c – cross-validated coefficient of determination.

4. Conclusions

The primary conclusions derived from this study are:

- Zn load in moss samples collected from the study sites is higher than the loads of other heavy metals. Pb, Ni and Cu are present in moderate amount, while Cd and Cr are relatively lower.

- The site specific characteristics do not have a significant influence on the heavy metal loads in the moss.
- Cu load in moss can be used as a surrogate for Cr load, while Pb load is a suitable surrogate for Ni and Cd. Zn load can be represented by both Cu and Pb loads.
- Mathematical equations developed in this study to relate the surrogate heavy metal loads in moss to the loads of other heavy metals are accurate since the error terms such as relative prediction error and standard error of prediction are low. However, the application of these equations is restricted to preliminary investigations due to the low precision characterized by low cross validated coefficient of determination.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jksus.2015.11.002.

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