CELLULAR COMPARTMENTALIZATION AND HEAVY METAL LOAD IN THE MOSS *Barbula lambarenensis* AROUND A MEGA CEMENT FACTORY IN SOUTHWEST NIGERIA

Ogunkunle, C. O.* **and Fatoba, P. O.**

Department of Plant Biology, University of Ilorin
P. M. B. 1515, Ilorin, Nigeria
*ogunkunle.co@unilorin.edu.ng; +2348062176518
(Received: 21st June, 2012; Accepted: 25th July, 2012)

ABSTRACT

Natural populations of ectohydric moss *Barbula lambarenensis* growing in a cement polluted environment were collected and subjected to Sequential Elution Technique using 0.2M EDTA as the extracting agent, in order to quantify the Pb, Cu, Cr, Cd and Zn contents of the extracellular, intracellular and particulate fractions. The selected heavy metal concentrations in the *B. lambarenensis* extracts were read using Atomic Absorption Spectrophotometry (Buck Scientific 210 VGP). The data obtained were subjected to one-way Analysis of Variance and Duncan Multiple Range Test to separate means that were statistically the same. Results showed that Pb, Cu and Zn had higher concentrations in the intracellular compartment while Cr and Cd were found to be more concentrated in the extracellular compartment of *B. lambarenensis*. In term of their solubility across the cell membrane, Cu (41%), Cr (43%) and Cd (43%) were more soluble; hence high bioavailability and toxicity potential to the moss. Solubility of Pb (49%) and Zn (54%) were relatively low compared to Cu, Cr and Cd and these results into low toxicity to *B. lambarenensis*. It was concluded that moss can be effectively used as biomonitors/bioindicator of environmental pollution due to dust particles from cement factories.

Keywords: Bioconcentration, Toxicity, Bioavailability, Biomonitor, Transmembrane, Solubility

INTRODUCTION

It is a well-established fact that mosses, especially the ectohydric are excellent biomonitors of air quality and some heavy metal depositions (Llamazares, 2010). They do not have the protective cuticle and thick cell walls, as a result of which their tissues are readily permeable to water and minerals, including metal ions (Cencil, 2008). The tissues that make up the cell walls contain numerous active anionic exchange sites which act as efficient cationic exchanges (Brown, 1984). Although, mosses are exposed to different concentrations of pollutants, especially heavy metals but without much pronounced or negative effects (Fatoba *et al.*, 2012). The tolerance of mosses was attributed to the ability of these plants to accumulate toxic elements through their capacities to extracellularly take up metals and also through ion exchange mechanism (Richardson, 1981). Bleuel *et al.* (2005) found that Tillandsia were able to intercept air particles and to accumulate them on their leaf surfaces.

The effectiveness of a particular moss as biomonitors can be assessed by its ability to sorb and retain metals as a measure of total concentrations which is dependent on aerial inputs (Gjengedal and Steinnes, 1990; Ross, 1990) but to understand the environmental relevance of metals to mosses; a detailed knowledge of the compartmentalization of these metals is needed. Pollutants absorbed by bryophytes can be found in the cell (intracellular), cell wall (extracellular) or as particle on plants (particulate matter). Most studies were based on the total metal contents in mosses of which large fractions of the metals may be in the form of insoluble particulates with no environmental relevance (Fernandez *et al.*, 2004). Studies of location and form of heavy metals in mosses may give a meaningful understanding of biomonitoring contaminated environment (Brown and Brumelis, 1996) and also give a clear view of dose-effect relationship in mosses (Brown and Sidhu, 1992; Fernandez *et al.*, 2004). The ratio of the extracellular and insoluble particulate fractions is an apparent way of determining toxicity level of metals in terms of bioavailability and relative solubility and the intracellular fraction is of interest in toxicity studies since it is better related to metabolic effects (Brown and Sidhu, 1992; Brown and Brumelis, 1996). The determination of concentrations of total metal fractions is a blurred way of assessing environmental impact of metals in mosses because bioavailability is not possible to be inferred from it.

The use of Sequential Elution Technique (SET) when EDTA is the displacing agent allows for the determination of the concentrations of metal in the extra and intracellular and insoluble particulate fractions (Fernandez *et al.*, 2004). SET is a useful tool in determining the contents of intracellular and extracellular locations rather than total fraction and is also useful in minimizing the difficulties associated with toxicological risk evaluation (Brumelis *et al.*, 1999). Though, quantification of the metals associated with cellular locations in bryophytes has been established through cation exchange techniques (Brown and Brumelis, 1996; Branquinho *et al.*, 1997) and laboratory studies on uptake of metals from solutions are evident but only few studies have been carried out under field conditions (Brown *et al.*, 1994). This present study was therefore designed to determine the loads and cellular locations of heavy metals in *Barbula lamabarenensis* exposed to heavy metals from cement dusts.

MATERIALS AND METHODS

Site Description

The study was carried out around West African Portland Cement (WAPCo), a mega cement factory in the southwestern part of Nigeria. The study area is situated within latitude 6°50' and 7°00' N and longitude 3°45' and 4°00' E (Gbadebo and Bankole, 2007*)*. The area stands on a low-lying gently undulating terrain with altitude ranging between 30 and 61 m above sea level. The area is characterized by high annual temperature, high rainfall, high evapo-transpiration and high relative humidity which make it to be classified as humid tropical region (Akanni, 1992). The soil type of the study area is ferralitic (Aweto, 1981; Adamson, 1996). The climate is humid tropical climatic zone (Adamson, 1996) and is controlled by the tropical maritime and tropical continental air masses (Aweto, 1981).

Mosses Sampling

Native populations of acrocarpous moss (*Barbula lambarenensis*) within a radius of 1 km around the WAPCo were randomly sampled in ten locations between September and October, 2011 and the co-ordinates of the locations were obtained with the use a GPS (Garmin 72H). The sampling locations were premised on availability of *B. lambarenensis* because the heavy pollution load reduces the rate of availability. The reference samples used to determine the Pollution Load Index were collected at a distance of 12 kilometers away from the cement factory to the north. Samples were harvested from the substrates according to Nordic guidelines (Kubin *et al.*, 2000; Cencil, 2008). Samples were collected from sandcrete materials with stainless knife, substrates were removed and the moss samples were kept in well- labeled envelopes. The moss samples were air-dried for 3 days to ensure total dryness so that all physiological and biochemical processes ceased before the determination of the extent of cellular compartmentalization of heavy metals.

Analysis of Mosses

Soluble extracellularly-bound (apoplastic) metals were obtained by washing 0.5 g of moss samples with 10 ml of 0.2 M Na_2-EDTA (pH 4.5) for 40 min (Branquinho *et al.*, 1997; 1999). After the 40 min-agitation, a second washing with 5 ml of the same 0.2 M was carried out for 30 min; 0.2 M Na_2-EDTA was used to extract several metals without causing changes in membrane permeability in sensitive organisms (Branquinhno *et al.*,1997; Serrano *et al.*, 2011). After the second washing, the samples were filtered through a sintered glass and the residues oven-dried at 80°C.

Soluble intracellularly-bound (symplastic) metals were obtained by washing the residues from 2.3.1 in 10 ml of 0.2 M Na_2-EDTA for 2 hrs. The soluble intracellular metals were easily extracted due to the rupture of the cell membrane during the oven-drying process (Branquinho *et al.*, 1997; 1999).

The particulate fraction of each sample was obtained by digesting the residue from 2.3.2 in 3 ml of 65% HNO_3 on a hot plate at approximately 120°C (Branquinho *et al.*, 1997; 1999). The digestion was completed after all organic materials had disappeared and the released fumes turned colourless. The fraction was then filtered through a sintered glass and labeled appropriately.

The filtrate was then made up to 50 ml in 100 ml beaker. The heavy metal concentrations of Pb, Cu, Cr, Cd and Zn were determined by Atomic Absorption Spectrophotometry (Bulk Scientific 210VGP, USA) at these wavelengths; □: Cd = 228.8 nm; Cu = 324.8 nm; Zn = 213.8 nm; Cr = 357.9 nm and Pb =283.3 nm.

For the validation of elution procedure, certified reference material IAEA-336 was subjected to the same procedures and results obtained were within the specified range.

Data Treatment and Statistical Analysis.

Differences in the metal concentrations in the three compartments were tested with one-way ANOVA and the means were separated in case of any significant difference with Duncan Multiple Range Test. Pollution Load Index (PLI) of metals was calculated according to the formula of Tripathy *et al.* (2009); Kalavrouzioti *et al.* (2012) and Adekola *et al.* (2012).

$$PLI \quad n\sqrt{CF_1 * CF_2 \quad * CFn}$$

The Contamination Factor (CF) is determined by dividing the metal concentration in the moss sample by the metal concentration in the reference moss sample.

$$CFn \quad Cn / Cref;$$

Cn = Metal concentration in moss;

Cref = Metal concentration in reference/background sample

RESULTS AND DISCUSSION

Cellular Location of heavy Metals in *Barbula lambarenensis*

The concentrations of heavy metals recovered in *B. lambarenensis* using sequential elution techniques are presented in Figures 1-5. The intracellularly-bound Pb and Cu (Figures 1&2) rated highest while the particulate fractions had the least recovered concentrations. The bioconcentrations of these two heavy metals in the intracellular and extracellular compartments were high whereas little fractions as insoluble particulates were retained on the moss indicating high affinity of these two metals for transmembrane transport molecules (Vasquez *et al.*, 1999). Environmental risk of these metals can only be evaluated through the assessment of these metals in the cellular locations as asserted by Brown (1995). Fernandez *et al.* (2004) asserted that environmental effects of pollutants in mosses can only be examined through the internal concentrations that best describe the dose-effect relationship. Only the intracellular metal concentrations can best be related to their sensitivities (Brumelis *et al.*, 1999). The high concentration of Pb in the intracellular compartment pose toxicity potential to *B. lambarenensis* but the high concentration of Cu in the same compartment could reflect the requirements of Cu by *B. lambarenensis* since Cu is among the minor nutrients needed for growth. Brown (1987) reported that the pattern of uptake of metals typically reflect the plant's requirements and its characteristic patterns.

Chromium and Cd were found to be largely present in the extracellular compartment (Figures 3&4). The large quantities in the extracellular compartments seems to be an adaptation to facilitate survival/growth in the presence of high metal loads (Satake *et al.*, 1988). Since metals that bound extracellularly exhibit high affinity for extracellular binding sites but have less serious effects on metabolism than metals taken up into the cell (Vasquez *et al.*, 1999).

The cellular locations of different Zn fractions across the locations are presented in Figure 5. The highest concentration was found in the intracellular compartment while insoluble particulate rated next to it. This seems to explain the free transport of Zn across the cell wall as it has a weak binding force towards the extracellular (anionic) exchange sites or is always temporarily bound to the sites and is displaced by other elements that have a higher affinity for the sites or that present at higher concentrations in the environment (Samecka-Cymerman *et al.*, 1997).

Considering the ratio of particulate fractions to the sum of particulate fractions and extracellular concentrations of the heavy metals (Table 1), Cu, Cr and Cd seemed to have relatively high solubility (ratio value 41%, 43% and 43% respectively) in *B. lambarenensis* as postulated by Fernandez *et al.* (2004). The lower the ratio values, the higher the solubility across the cell membrane (Fernandez *et al.*, 2004). This also applies to Pb but less soluble in *B. lambarenensis* compared to other metals studied (ratio value of 49%); Zn has the least solubility with the ratio value that is more than 50% (Table 1), hence exhibiting low solubility. Since the ratio for Cr and Cd were the same, it could be inferred that the pathway of Cr and Cd to *B. lambarenensis* may probably be through the same cement particles and have the same bioavailability according to Fernandez *et al.* (2004). The ratio values of the metals below 50% explained their great bioavailability and relatively high bioconcentrations in *B. Lambarenensis*.

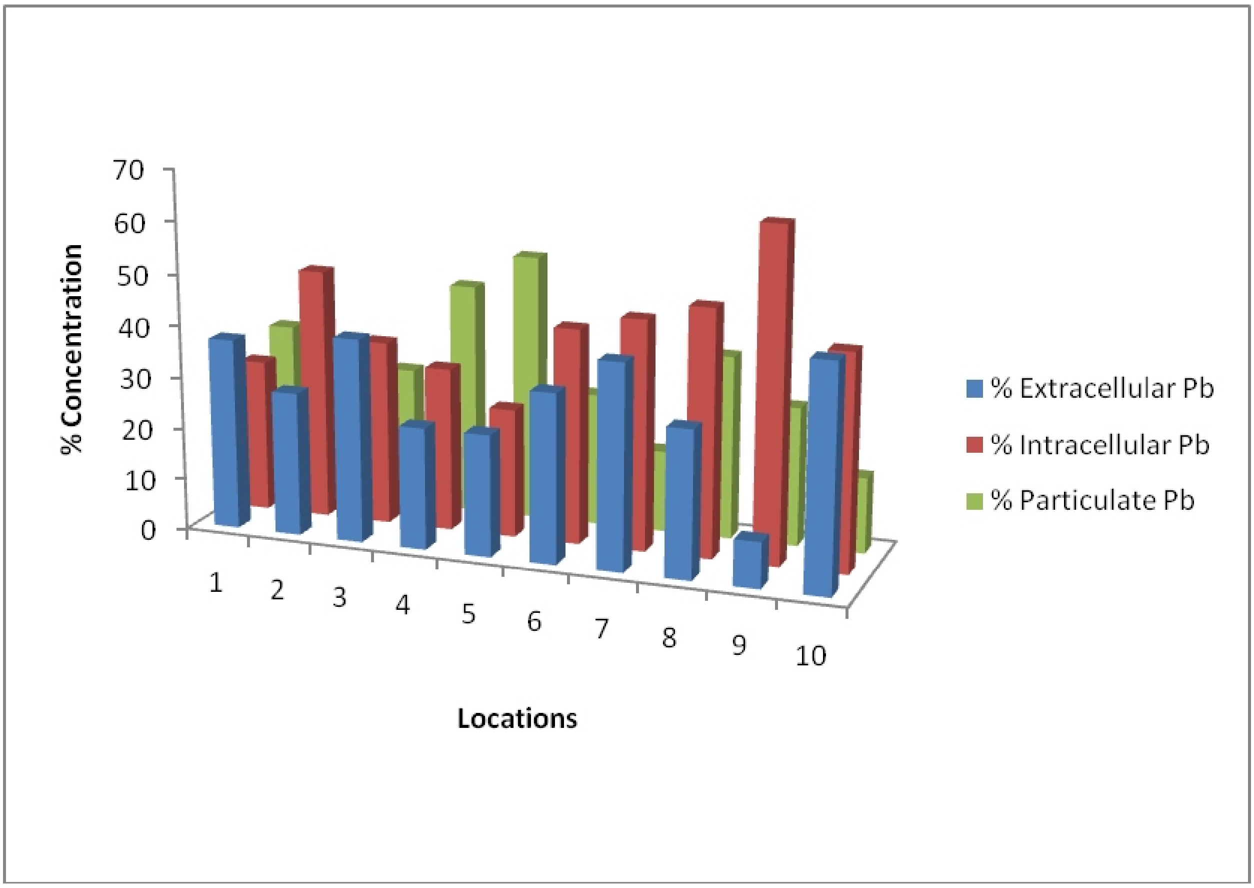

Fig. 1. Percentage Distribution of Pb in the Different Compartments of *B. lambarenesis* Growing Around WAPCo.

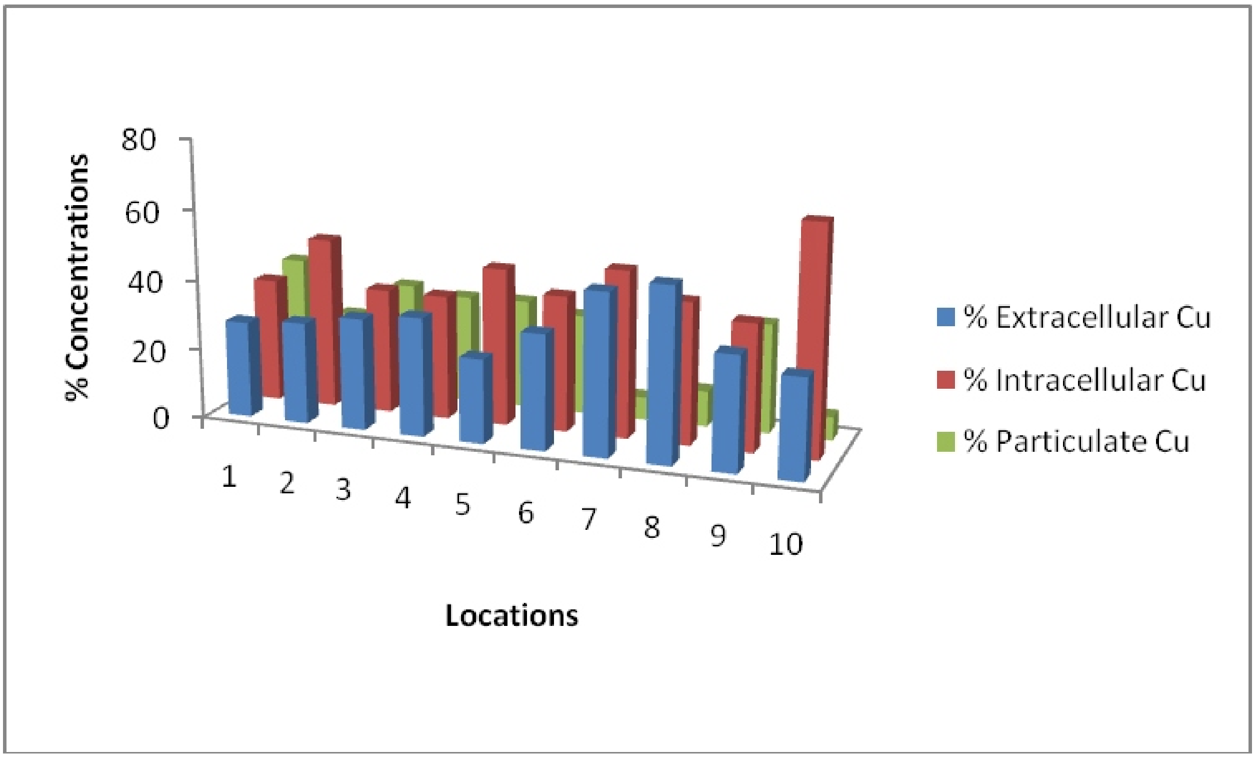

Fig. 2. Percentage Distribution of Cu in the Different Compartments of *B. lambarenesis* Growing Around WAPCo.

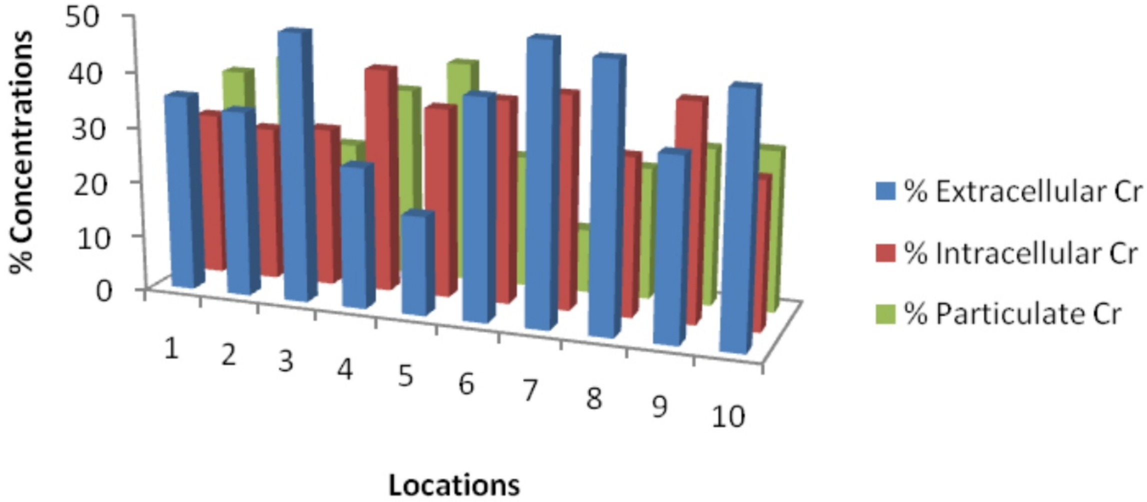

Fig. 3. Percentage Distribution of Cr in the Different Compartments of *B. lambarenesis* Growing Around WAPCo.

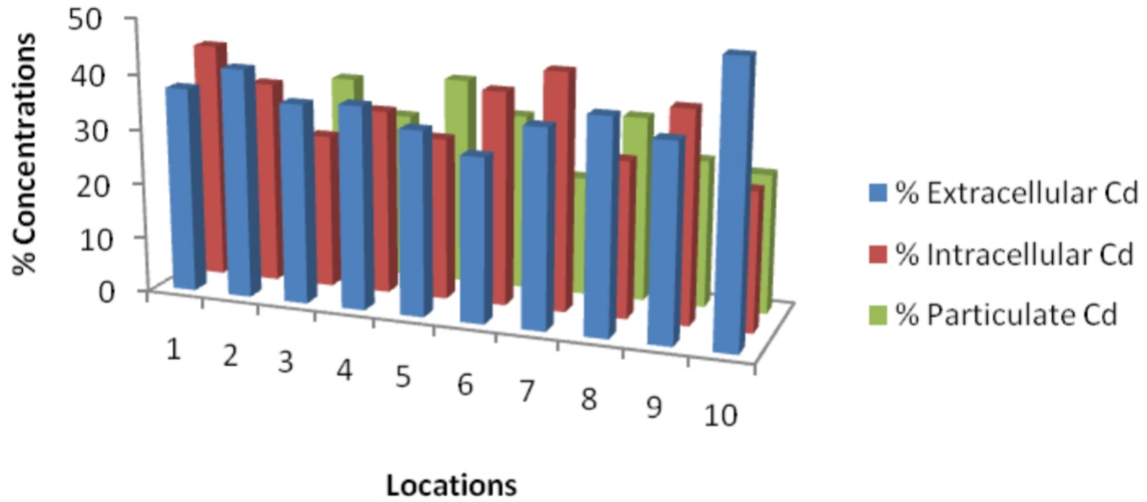

Fig. 4. Percentage Distribution of Cd in the Different Compartments of *B. lambarenesis* Growing Around WAPCo.

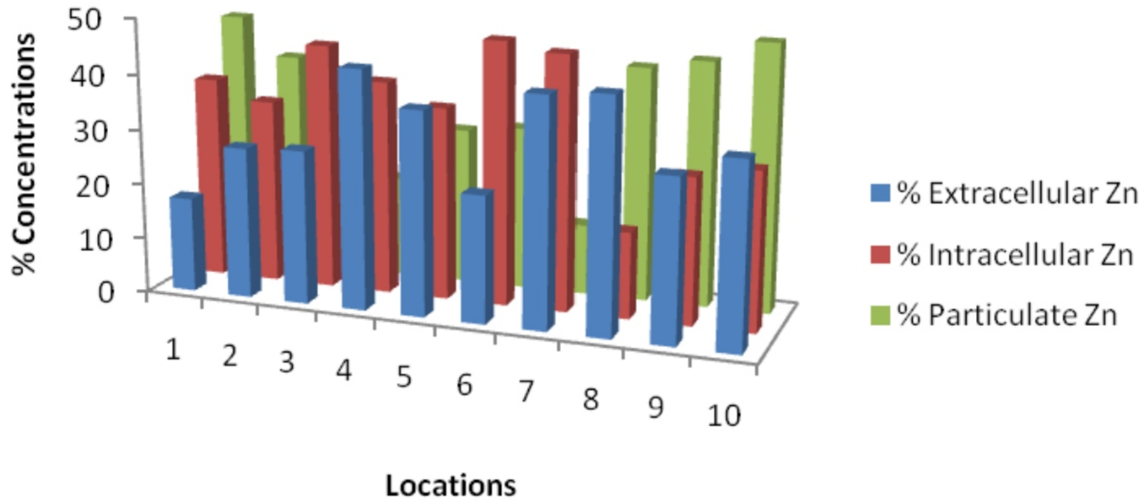

Fig. 5. Percentage Distribution of Zn in the Different Compartments of *B. lambarenesis* Growing Around WAPCo.

Table 1. Concentrations of Some Selected Heavy Metals (in %) in Different Cellular Compartments of *B. Lambarenensis*

Heavy metal	% Extra Contents	% Intra Contents	% Particulates	*Ratio value
Pb	30.49±10.3	41.03±11.4	29.88±11.7	49% -High solubility
Cu	33.50±8.1	42.76±9.3	23.73±11.6	41% -High solubility
Cr	37.43±10.6	32.98±5.3	28.79±8.7	43% -High solubility
Cd	37.39±5.4	34.26±6.5	28.35±6.3	43% - High solubility
Zn	28.15±8.8	35.19±9.8	33.66±12.2	54% -Low solubility

*Ratio value = Ratio of particulate fraction to the sum of extracellular and particulate fractions

Metal Load and Pollution Load Index of Mosses.

The heavy metal loads found in *B. lambarenensis* around the surrounding of the mega cement factory are given in Table 2. Extremely high mean values of metal contents were recorded; particularly for Pb and Cu (11.9mg/kg and 5.6mg/kg respectively) and these values are comparable to the results reported by Fatoba *et al.* (2012) in Southwest of Nigeria. According to the Analysis of Variance and Duncan's Test, Pb concentration was statistically greater than other heavy metals while Cr, Cd and Zn were statistically similar at p= 0.05. This could be hinged on the possibility of Cr, Cd and Zn being attached on the same particle and deposited on *B. lambarenensis* as Jozwiak and Jozwiak (2009) reported that heavy metals are usually attached to dust particle when they are released especially those of PM 10.

The data in Table 2 cursorily gave the general picture of the air quality around the cement factory. Most mosses especially ectohydric mosses derive their necessary nourishment from the atmosphere, since they have not developed a real root system and lack transport vessels (leptoid and hydroid) as opposed to endohydric mosses. Contaminants are therefore absorbed through the surface of their leaves. In mosses, there can be a close correlation between the concentration of pollutants and atmospheric deposition, since absorption from the substratum can be ruled out (Cencil, 2008). Gbadebo and Bankole (2007) reported very high loads of these potentially toxic metals in their study in the air and dusts around this cement factory. The report of Gbadebo and Bankole (2007) corroborated the findings of this study that the atmosphere around the cement factory was laden with heavy metals.

Moreover, the Contamination Factor (CF) was estimated with reference to the background concentrations from 12 km away to the cement factory. In general, *B. lambarenensis* was moderately polluted in all the studied locations with a CF range of 1.14-1.84. The values of Contamination Factors and the Pollution Load Index (PLI) (Table 3) showed that the atmospheric quality around the cement factory was polluted. PLI values between 1-3 shows slight to moderate pollution while value greater than 3 depicts severe pollution (Daud *et al.*, 2006; Ahiamadjie *et al.*, 2011). Statistical analysis of the heavy metal contents showed that Pb was statistically greater than Cu which in turn was greater significantly than Cr, Cd and Zn which were statistically the same at 5% level of significance (Table 2). All the heavy metals studied moderately polluted the mosses around the cement factory and this suggests that the atmosphere of WAPCo is moderately polluted with these heavy metals.

Table 2. Mean (± standard deviation) Concentration (mg/kg) of Selected Metals in *B. lambarenensis* Samples Across the Locations

Sample	Co-ordinates	Pb	Cu	Cr	Cd	Zn
M1	$6^{O}48^{I}76^{II}$N, $3^{O}36^{I}57^{II}$E	10.00±0.86	7.65±0.32	2.55±0.32	1.75±0.13	4.10±0.14
M2	$6^{O}49^{I}14^{II}$N, $3^{O}36^{I}56^{II}$E	13.30±0.27	7.60±0.16	2.70±0.28	2.05±0.44	1.65±0.23
M3	$6^{O}48^{I}03^{II}$N, $3^{O}36^{I}51^{II}$E	6.85±0.01	6.45±0.06	2.80±0.14	1.80±0.01	1.80±0.10
M4	$6^{O}48^{I}22^{II}$N, $3^{O}37^{I}27^{II}$E	8.70±0.10	6.50±0.13	3.35±0.07	1.50±0.16	2.20±0.07
M5	$6^{O}49^{I}05^{II}$N, $3^{O}36^{I}97^{II}$E	19.10±1.29	6.25±0.06	3.65±0.21	1.20±0.07	2.30±0.17
M6	$6^{O}49^{I}62^{II}$N, $3^{O}37^{I}89^{II}$E	13.10±0.01	5.95±0.03	5.05±0.01	2.20±0.01	3.05±0.04
M7	$6^{O}49^{I}14^{II}$N, $3^{O}37^{I}32^{II}$E	11.20±0.01	3.90±0.06	2.60±0.13	1.40±0.03	3.15v0.03
M8	$6^{O}48^{I}42^{II}$N, $3^{O}38^{I}07^{II}$E	7.00±0.01	4.45±0.07	3.15±0.06	1.95±0.06	2.25v0.01
M9	$6^{O}48^{I}46^{II}$N, $3^{O}36^{I}48^{II}$E	20.1±0.01	4.00±0.01	2.30±0.01	1.70±0.07	1.70±0.08
M10	$6^{O}48^{I}28^{II}$N, $3^{O}36^{I}10^{II}$E	10.25±0.04	3.00±0.01	2.25±0.01	2.00±0.08	2.10±0.07
	Mean	11.9^{a}±4.58	5.6^{b}±1.62	3.0^{c}±0.83	1.8^{c}±0.31	2.4^{c}±0.78
Ref.	$6^{O}51^{I}28^{II}$N, $3^{O}39^{I}41^{II}$E	6.15±0.07	3.65±0.01	2.15±0.03	1.55±0.04	2.00±0.03

Note: Mean values in the same row with different superscripts are significantly different at 5% probability level

Table 3. Contamination Factors (CF) and Pollution Load Index (PLI) of Metals in ***Barbula lambarenensis*** Across the Locations.

		Contamination Factors				
Sample	**Co-ordinates**	**Pb**	**Cu**	**Cr**	**Cd**	**Zn**
M1	$6^{O}48^{I}76^{II}$N, $3^{O}36^{I}57^{II}$E	1.6	2	1.2	1.1	2.0
M2	$6^{O}49^{I}14^{II}$N, $3^{O}36^{I}56^{II}$E	2.2	2.1	1.3	1.3	0.8
M3	$6^{O}48^{I}03^{II}$N, $3^{O}36^{I}51^{II}$E	1.1	1.8	1.3	1.2	0.9
M4	$6^{O}48^{I}22^{II}$N, $3^{O}37^{I}27^{II}$E	1.4	1.8	1.6	0.9	1.1
M5	$6^{O}49^{I}05^{II}$N, $3^{O}36^{I}97^{II}$E	3.1	1.7	1.7	0.8	1.1
M6	$6^{O}49^{I}62^{II}$N, $3^{O}37^{I}89^{II}$E	2.1	1.6	2.3	1.5	1.3
M7	$6^{O}49^{I}14^{II}$N, $3^{O}37^{I}32^{II}$E	1.8	1.1	1.2	0.9	1.6
M8	$6^{O}48^{I}42^{II}$N, $3^{O}38^{I}07^{II}$E	1.1	1.2	1.5	1.3	1.1
M9	$6^{O}48^{I}46^{II}$N, $3^{O}36^{I}48^{II}$E	3.3	1.1	1.1	1.1	0.8
M10	$6^{O}48^{I}28^{II}$N, $3^{O}36^{I}10^{II}$E	1.7	0.8	1.0	1.3	1.0
	CF (mean)	1.84±*0.76*	1.53±0.45	1.42±0.38	1.14±0.22	1.19±*1.58*
Pollution Load Index		1.81	1.46	1.38	1.11	1.14

CONCLUSION

There was a clear trend of decreasing concentration of the extracellular, intracellular and particulate fractions of Pb, Cr and Cd in *B. lambarenensis*. Also the intracellular fraction reflected the relationship between dose and effect best. It is evident that the raised level of heavy metal concentration in *B. lambarenensis* was due to the activities of the cement factory as the background levels of studied heavy metals were relatively lower than the recovered concentrations in the moss. From the present study, it may be deduced that mosses are good biomonitors/indicators of atmospheric quality.

REFERENCES

Adamson, K. Y. 1996) Towards an Environmental Action Plan for Ogun State. World Bank Assisted Project Report, pp. 35-72

Adekola, F.A., Inyinbor, A.A. and Abdul-Raheem, A.M.O. 2012. Heavy metals distribution and speciation in soils around a mega cement factory in north-central, Nigeria. *Ethiopian Journal of Environmental Studies and Management*. 5(1): 11-19

Ahiamadjie, H., Adukpo, O.K., Tandoh, J.B., Gyampo, O., Nyarku, M., Mumuni, I. I., Agyemang, O., Ackah, M., Otoo, F. and Dampare, S. B. 2011. Determination of the elemental contents in soils around diamond cement factory, Aflao. *Research Journal of Environmental and Earth Sciences, 3(1)*: 46-50

Akanni, C. O. 1992. *Climate. In: Ogun State in Maps*. Onakomaya, S. O., Oyesiku, K. and Jegede, J. (Eds). Rex Charles Publisher, Ibadan. pp. 187

Aweto, A. O. 1981. Fallowing and Soil Fertility Restoration in South-west Nigeria. *Malaysian Journal of Tropical Geography*, 3: 1-6

Bleuel, C., Wesenberg, D., Sutter, K., Miersch, J., Braha,B., Barlocher, F. and Krauss, G. J. 2005. The use of aquatic moss *Fontinalis antipyrectica* L.ex Hedw as a bioindicator for heavy metals, Cd^{2+} accumulation capacities and biochemical stress response of two *Fontinalis* species. *Science of the Total Environment*, 345:13-21.

Branquinhno, C., Brown, D. H., Maguas, C. and Catarino, F. 1997. Uptake and its effects on membrane integrity and chlorophyll fluorescence in different lichen species. *Environ. Exp. Bot*, 37:95-105

Branquinho, C., Catarino, F., Brown, D. H., Pereira, M. J. and Soares, A. 1999. Improving the use of lichens as biomonitors of atmospheric metal pollution. *The Science of the Total Environment*, 232: 67-77

Brown, D. H. 1995. Sequential elution procedure for establishing the cellular distribution patterns of metal in crytogamic plants. In: Munawar, M., Hanninen, O., Munawar, N., Kavenlampi, l., Brown, D. (Eds). *Bioindicators of Environmental Health, Ecovision World Monographs Series*, SBP Academic Publishing, Amsterdam, pp. 203-209.

Brown, D. H. 1987. The location of mineral elements in lichens: implications for metabolism: In: Cramer, J. (Ed.) *Progress and Problems in Lichenology in the Eighties*. Berlin-Stuttgard, pp. 361-375.

Brown, D. H. 1984. Uptake of mineral elements and their use in pollution monitoring. *The Experimental Biology of Bryophytes*, 229-255.

Brown, D. H., Avalos, A., Miller, J. E. and Bargagli, R. 1994. Interactions of lichens with their mineral environment. *Crypt. Bot.*, 4:135-142

Brown, D. H. and Brumelis, G. 1996. A biomonitoring method that determines the cellular distribution of metals in moss. *Science of the Total Earth*, 187:153-161

Brown, D. H. and Sidhu, M. 1992. Heavy metal uptakes, cellular location and inhibition of moss growth. *Cryptogam Bot.*, 3:82-85

Brown, D. H. and Wells, J. M. 1990. Physiological effects of heavy metals on the moss *Rhytiadelphus squarrosus*. *Ann. Bot.* 66: 641-647.

Brumelis, G., Brown, D. H., Nikodemus, O. and Tjarve, D. 1999. The monitoring and risk assessment of Zn deposition around a metal smelter in Latvia. *Environmental Monitoring and Assessment,* 58: 210-212

Cencil, R. M. 2008. Guidelines for the use of native mosses, transplanted mosses and soils in assessing organic and inorganic contaminant fallout. *JRC Scientific and Technical Reports* 44103, Official Publications of the European Communities, 1-135.

Daud, M., Khalid, N., Iqbal, J. and Ahmad, S. 2006. Assessment of atmospheric pollution level using *Asclepias procera* leaves as biomonitor. *Radiochim. Acta,* 95:423-431.

Fatoba, P. O., Ogunkunle, C. O. and Okewole, G. A. 2012. Mosses as biomonitors of heavy metal deposition in the atmosphere. *International Journal of Environmental Sciences*, 1(2):56-62

Fernandex, J. A., Aboal, J. R., Couto, J. A. and Carballeira, A. 2004. Moss bioconcentrations of trace elements around a FESI smelter:modeling and cellular distribution. *Atmospheric Environment,* 38:4319-4329

Gbadebo, A. M. and Bankole, O. D. 2007. Analysis of potentially toxic metals in airborne cement dust around Sagamu, Southwestern Nigeria. *Applied Science,* 7: 35-40

Gjengedal, E. and Steinnes, E. 1990. Uptake of ions from artificial precipitation. *Environ. Monit. Assess.*, 14:77-87.

Jozwiak, M. A. and Jozwiak, M. 2009. Influence of cement industry on accumulation of heavy metals in bioindicators. *Ecological Chemistry and Engineering*, 16(3): 323-344

Kalavrouziotisa, I, K., Koukoulakis, P. and Kostakioti, E. 2012: Assessment of metal transfer factor under irrigation with treated municipal wastewater. *Agricultural Water Management,* 103:114-119

Kubin, E., Lippo, H. and Poikolainen, J. 2000. Heavy metal loading. In: Mälkönen E (ed) Forest condition in a changing environment The Finnish Case. *Forestry Sciences 65;* Kluwer Academic Publishers, Dordrecht, pp. 60-71.

Llamazarene, A. P. 2010. Improving the use of sequential elution technique in terrestrial mosses. Unpublished Ph. D Thesis, Universidade De Santiago De Compostela, Pg. 9. ISBN 978-84-9887-630-7

Richardson, D.H.S. 1981. The biology of mosses. *Blackwell Scientific Publishers*, Oxford, pp.171-181.

Ross, H. B. 1990. On the use of mosses *Hylocomium splendens* and *Plewozium schreberi* for estimating atmospheric trace metal deposition. *Water Air Soil Pollut.*, 50:63-76.

Samecka-Cymerman, A., Marczonek, A. and Kempers, A. J. 1997. Bioindication of heavy metals in soil by liverworths. Archives of environmental Contamination and Toxicology, 32:162-171.

Satake, K., Shibata, K., Nishikawa, M. and Fuwa, K. (1988): Copper accumulation and location in the moss *Scopelophila cataracte*. *J. Briol.* 15:353-376

Serrano, H. C., Pinto, M. J., Martins-Loucao, M. A. and Branquinho, C. 2011. How does an Al-hyperaccumulator plant respond to a natural field gradient of soil phytoavailable Al?. *Sci. Total Environ.* Doi:10.1016/j.scitotenv.2011.06.036

Tripathy, D. P., Panigrahi, D. C. and Singh, G. 2009. Determination of soil pollution index and soil infiltration rates in some non-fire and fires areas of Jharia

Coalfield. *Ninth International Ventilation Congress*, New Delhi, India. 10th-13th November, 2009. htpp://dspace.nitrkl.ac.in/dspace

Vasquez, M. D., Lopez, J. and Carballeira, A. 1999. Uptake of heavy metals to the extracellular and intracellular compartments in three species of aquatic bryophytes. *Ecotoxicology and Environmental Safety*, 44:12-24