Comparative Study of Levels of Trace Metals in Moss Species in Some Cities of the Niger Delta Region of Nigeria.

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Abstract

Moss was sampled from moss species at sites at Calabar, Eket, Aba and Port Harcourt. Analysis of samples showed presence of Pb, Hg, Cr, Ni, V, Cu, Cd, Mn, Fe, Zn and As at the time of sampling. Levels of the trace metals were determined. Results showed their levels varied from element to element and from location to location. Level of Fe was the highest in all the locations followed by Zn and Pb. However levels of pollutants were within FEPA(1991) and WHO(2001) threshold limiting values. Levels of trace metals in the moss samples were higher during the wet season than in dry season probably because mosses thrive better in moist, humid and wet environment. A paired t-test analysis at $p \leq 0-05$ (df 4,n =6)confirmed that seasonal variation was significant. Results provide regional baseline information for pollution monitoring .Study recommends use of moss as an effective pollution bio-monitors in the region.

Keywords: *Plantae phylum*, cuticle, bio-monitors, bio-indicators, uptake efficiency, cryptogamic,

1.0 Introduction

The moss is a plant of the plant kingdom Plantae phylum. They grow in forests, on rocks, bare soil, cracks of concrete side walls, un plastered perimeter fences of compounds and uncompleted buildings. Mosses have been used for environmental monitoring as indicator plants of airborne heavy metals (Ruhling and Tyler, 1968, 1973; Nioboer *et al.*, 1972; Little and Martin, 1974; Shelite *et al.*, 1976; Pilegaard 1978; Glydesen *et al.*, 1983; Ruhling *et al.*, 1987; Sardans and Penuela, 2005; Shakya *et al.*, 2004; Anja *et al.*, 2004; Nordic Council of Ministers, 1994; Saxena *et al.*, 2008). Its use for biological monitoring of regional atmosphere depositional and heavy metals contamination of their environment was developed towards the end of the 1960s (Ruhling and Tyler, 1968). The technique is based on the fact that mosses have undeveloped roots and therefore have to obtain minerals from rain and atmospheric particles. They depend entirely on the uptake of nutrients from the atmosphere because of lack of roots, curticle and epidermis. Over the last 20 years mosses have been used as biomonitors and bioindicators (Loppi and Bonini 2000; Fernandez and Carballeira, 2002, Saxena et al, 2008) for producing pollution maps of the area of which heavy metal pollutants are present.

In Niger Delta Region of Nigeria it is suspected that the build up of air pollutants may be anticipated as a result of obvious polluting sources such as heavy petroleum activities, urbanization, rapid industrialization and resulting heavy traffic. There is a need to monitor these pollutants and determine their concentration in the region. Studies in the use of mosses as environmental biomonitors in parts of Nigeria have been reported (Onianwa and Egunyomi, 1983; Onianwa et al, 1986; Kakulu 1993; Fatoba and Oduekun, 2004; Bako et al, 2008). However use of bioindicators in monitoring environmental quality in the West African Sub-region is limited (Bako et al 2008). No study has been carried out in the use of moss as bio monitors in the Niger Delta Region. This study is therefore carried out to identify the trace metals and determine the concentration of trace metals in the Niger Delta Region using moss as bio monitors . The result will provide baseline information for adequate pollution monitoring and control in the region it will also test the effectiveness of the use of moss for pollution monitoring.

2.0 Materials and methods

2.1 Selection of study cities

The cities of the Niger Delta region of Nigeria selected for this study are Calabar, Aba, Port Harcourt and Eket. They are representative of the big cities in the region. The following criteria were considered in the choice of the study cities. Calabar is generally pollution “free” with no serious petroleum activities. It is not heavily industrialized, has moderate traffic density and farming activity. Aba is a heavily industrialized, big commercial city with heavy traffic density with little petroleum activity but highly urbanised. Eket has a lot of petroleum activities by Exxon Mobil and low traffic density. It is not a commercial town. A lot of gas flaring takes place in Eket.Port Harcourt has heavy petroleum exploration and exploitation activities as well as extensive gas flaring. It is a large commercial city, highly urbanised and is faced with a heavy traffic density, bush burning and industrialisation.

Each selected city has its unique characteristics which can be replicated across the region. In addition, all the selected cities are from states which form a contiguous land mass and are located in the Eastern flank of the Niger Delta region of Nigeria as shown in Fig. 1.

2.2 Location of study sites

In each city, the sites selected were located at least 300 metres from main roads and densely populated areas. This was to assist in the collection of samples in areas suspected to be of low deposition of heavy metals. This was also to avoid collection of samples from areas of pollution sources. The direction of the wind was also considered in the selection of study sites.

Wherever possible, the site selection for this study took into consideration the reproducibility of results and other environmental factors such as accessibility, availability of open spaces, and of course areas with minimal local influence from traffic as well as industrial activities. The description of the sites together with geographical coordinates are as shown in Table 1. The map showing the selected study sites is also shown in Fig 1.

2.3 Sampling of moss

Sampling of moss was carried out during the wet and dry seasons in 2006. Specifically, moss samples were collected in the months of July, August and September for the wet season and in the months of January, February and March during the dry season. For each month, moss samples were collected randomly from six locations in the site selected for sampling. To avoid contamination from the soil, only epiphytic moss species were collected for the study. Moreover, epiphytic mosses appeared to survive even at extremely dry sites (Halleraker *et al.*, 1998) and were readily available for sampling. Moss species were collected randomly from 2 to 2.5 m high from the ground of uncompleted buildings, unplastered perimeter fences, storey buildings and such structures within the sample area. The samples were collected in plastic bags and taken to the laboratory for further treatment.

Table 1:Location of sampling sites

S/N	State	City	Code	Coordinates	Location	Description
1	Cross River	Calabar	CAQI	5^0 01.479N 8^0 19.878 E	Located about 1km east of CFTZ, cal.	Open filed in an undeveloped plot. Little vehicular emission
2	Akwa Ibom	Eket	EAQ2	4^0 34.956 N 7^0 57.798 E	Esit Urua Ekit Eket LGA	Open field. No bush burning activities. Little vehicular traffic.
3	Abia	Aba	AAQ3	15^0 03.29 N 7^0 19.18 E	Around ICS power generation plant. Alaoji Road, Aba	Open area. No bush burning. Little vehicular emission.
4	Rivers	Port Harcourt	PAQ4	4^0 50.535N 7^0 15.245 E	Near Afam Power station, PH	Open field. No bush burning activities. Littles vehicular emission.

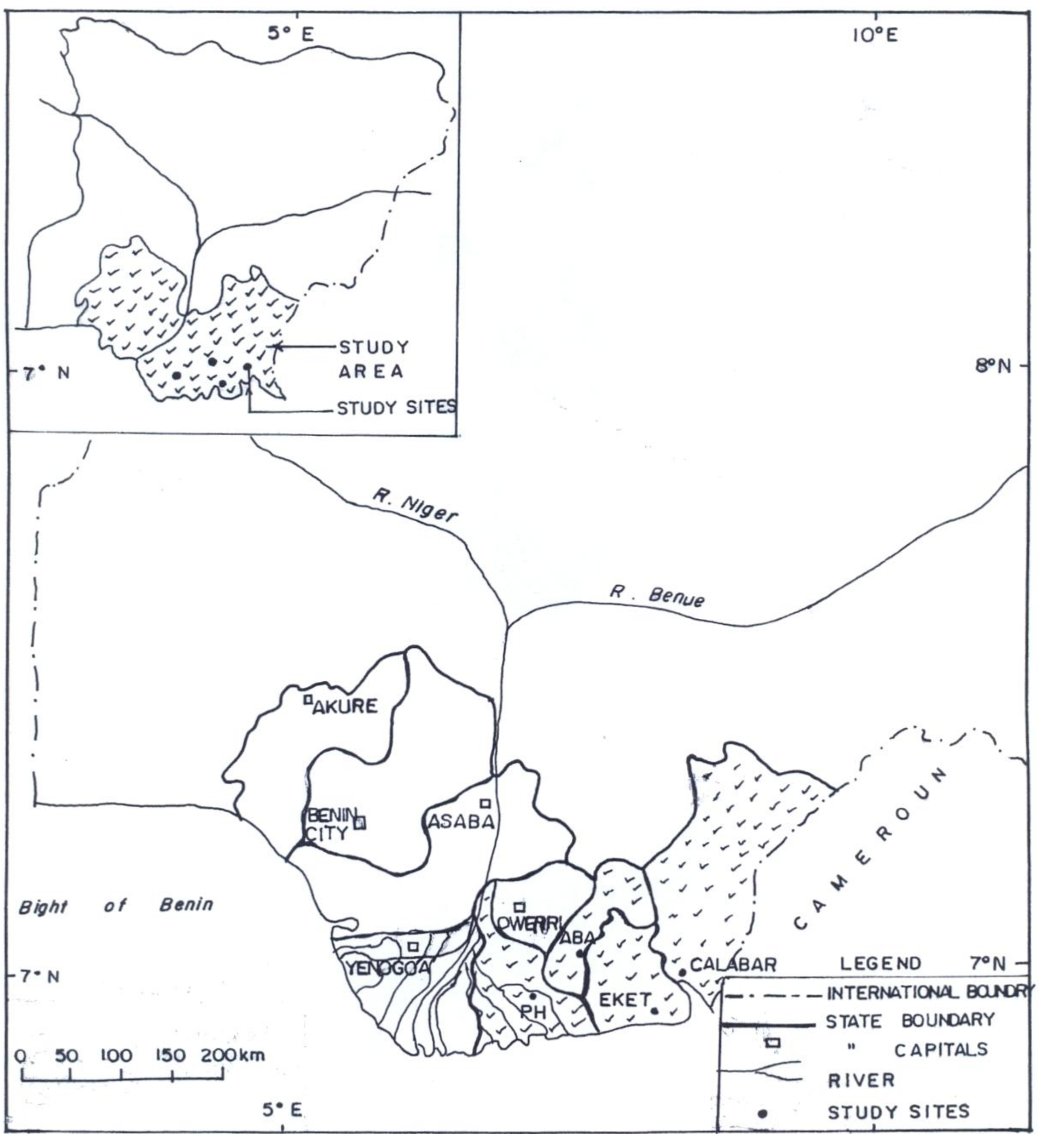


Fig. 1: Location of study area on the map of Niger Delta region

2.4 Preparation and analysis of moss samples

The method of Shakya *et al.* (2001) was used. Impurities adhering to the surface of the moss sample were removed very carefully under dry conditions. For analysis, only the green and greenish brown parts of the moss plants were used since they are generally intended to represent a period of about 3-5 years. Their metal content is generally considered to reflect the atmospheric deposition during that period (Ruhling and Steinnes, 1998). The samples were dried at 50^{o}C in an oven for 24 hours and the representative samples of each moss species were used for analyses in triplicates. One gramme of each representative sample was transferred into an open quartz tube for digestion. Concentrated HNO_3 (5 cm^3) was added to each tube and the mixture was left at room temperature overnight. The temperature of the mixture was then raised to 50^{o}C for 2 hours after which it was further raised to 160^{o}C for another 4 hours. The solution was filtered through Whatman type 589/2 filter paper with blue ribbon and the volume of the filtrate was diluted to 25 cm^3 with deionized water (Sawidis *et al.*, 1993; Chettri, 1997). The metal contents in the filtrate were determined using an atomic absorption spectrophotometer (Perkin Elmer 2380).

3.0 Results

3.1 Mean levels of trace metals in moss species.

The mean levels of the trace metals in moss at the period of sampling are presented in Table 2. At all the locations, the level of Fe was the highest followed by Pb and Zn. The mean levels are presented in Fig. 2.

3.2 Seasonal variability of mean levels of trace metals in moss species

The mean levels of trace metals in moss for the wet and dry season are presented in Table 3. Levels are higher in the wet season than the dry season. The levels of Fe were highest at all locations. This was followed by those of Zn. The comparative trends are presented in Fig. 3. A paired t - test analysis at $p \leq 0.05$ (df = 4, n=6) reveals that the variation in the mean levels of trace metals for the dry and wet season was all significant except that of Hg at Calabar. This is presented in Table 3.

4.0 Discussion

4.1 Mean levels of trace metals in moss species

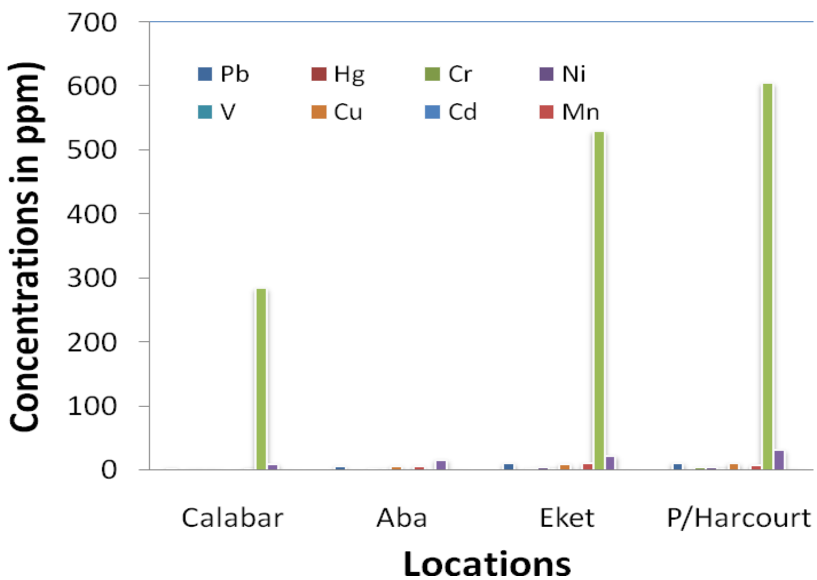
The moss samples exhibited significant variation in the mean levels of the trace metals with locations. This result is in agreement with that obtained by Olajire (1998) who carried out a survey of heavy metals deposition in Nigeria using the moss monitoring method. He reported that the results from the moss samples exhibited significant variations in the levels of metal ions with type of sampling site. The significant variation suggests that ambient levels of atmospheric pollutants depend upon strength of the sources, efficiency of their dispersion and other meteorological conditions (WHO, 2001). Strong variation in the levels of trace metals in mosses was also reported in a study in Kathmandu Valley by Shakya *et al.* (2001). It may be due to differing degree of tolerance to heavy metals (Brown and Sidhu, 1992) and the element specific uptake capacity (Ross, 1990). Other factors include morphological and physiological properties of the immediate environment, age of the moss and the metal supply in the environment (Chakraborty *et al*., 2006).

At all locations, the trace metal with the highest mean level was Fe at Calabar (284.6 + 31.29 ppm), Aba (420.5+73.6ppm), Eket (529.85 + 95.08 ppm) and Port Harcourt (604.96 + 78.4ppm.)Study on baseline heavy metal levels in river sediments within Okitipupa, Ondo, in the Niger Delta region of Nigeria reveals that the range in the level of Fe (70.62 - 799.97 mg/kg) was the highest among 12 trace metals in the area of study (Aiyesanmi, 2008). From the study, Fe was consistently found to be the most abundant trace metal in the sediment consistent with reports for the Niger Delta Area and Ondo State Coastal waters (Asaolu, 1998: Kakulu and Osibanjo, 1988) and in top soils of Abeokuta (Odukoya *et al*., 2000). This is in agreement with the dominant level of Fe in the region in the study.

Table 2: Mean levels of trace metals in moss. Values are given as mean ± SD and values in bracket represent ranges (ppm)

	Calabar	Aba	Eket	P/Harcourt
Pb	3.50± 1.27	6.68 ± 2.04	10.57 ± 1.77	11.12± 3.15
	(2.23 – 5.15)	(4.15 – 9.25)	(8.49 – 12.63)	(8.16 – 14.44)
Hg	0.03 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
	(0.02 – 0.06)	(0.05 – 0.06)	(0.03 – 0.05)	(0.02 – 0.04)
Cr	1.69 ± 0.44	0.03 ± 2.20	2.91 ± 1.25	4.23± 2.07
	(1.22 – 2.17)	(3.41 – 8.45)	(1.56 – 4.25)	(2.24 – 6.6)
Ni	0.97 ± 0.29	1.93 ± 0.04	4.34 ± 0.94	5.27 ± 2.01
	(0.65 – 1.29)	(1.54 – 2.50)	(3.37 – 5.55)	(3.26 – 7.51)
V	1.12 ± 0.49	2.35 ± 0.68	3.64 ± 1.0	3.46 ± 1.12
	(0.54 – 9.32)	(1.67 – 3.32)	(2.55 – 4.97)	(2.23 – 4.6)
Cu	5. 07 ± 0.44	6.63 ± 1.22	10.10 ± 2.99	10.43 ± 1.93
	(4.64 – 5.56)	(5.27 – 7.88)	(7.37 – 14.24)	(8.45 – 12.82)
Cd	0.21 ± 0.08	0.38 ± 0.15	0.56 ± 0.18	0.52 ± 0.17
	(0.12 – 0.30)	0.24 – 0.58	(0.38 – 0.79)	(0.34 – 1.69)
Mn	3.51 ± 0.99	6.58 ± 1.87	10.91 ± 1.55	8.51 ± 2.01
	(2.54 – 4.54)	(4.65 – 8.62)	(9.47 – 12.65)	(6.34 – 10.62)
Fe	284.6 ± 31.29	420. 5 ± 73.61	529.85 ± 95.08	604.96 ± 78.4
	(257.5 – 322.65)	(345.60 – 488.56)	(445.24 – 645.5)	(534.5 – 699.8)
Zn	9.89 ± 1.07	16.55 ± 4.25	22.78 ± 5.80	31.81 ± 10.67
	(8.67 – 11.54)	(12.45 – 21.43)	(17.34 – 28.95)	(22.6 – 48.2)
Co	0.6 ± 0.17	0.97 ± 0.26	0.05 ± 0.21	1.64 ± 0.28
	(0.41 – 0.82)	(0.67- 1.25)	(0.84 – 1.25)	(1.34 ± 1.95)
As	0.32 ± 0.09	0.42 ± 0.18	0.52 ± 0.14	0.87 – 0.29
	(0.21 – 0.42)	(0.24-0.66)	(0.38 – 0.72)	0.56 – 1.25

SD = Standard deviation



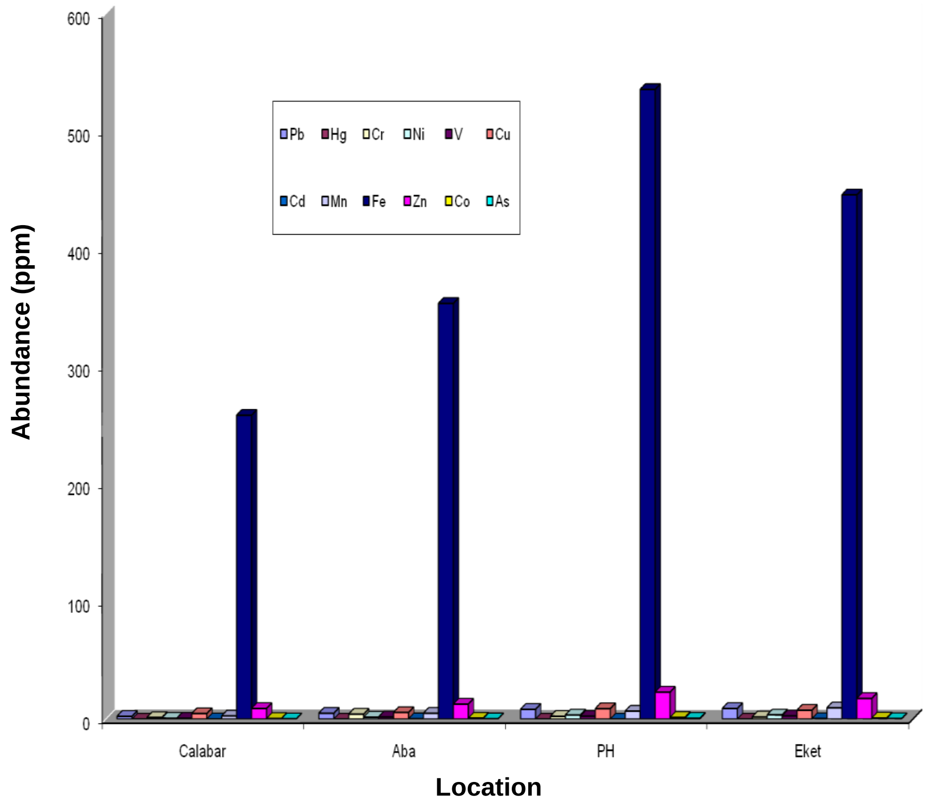
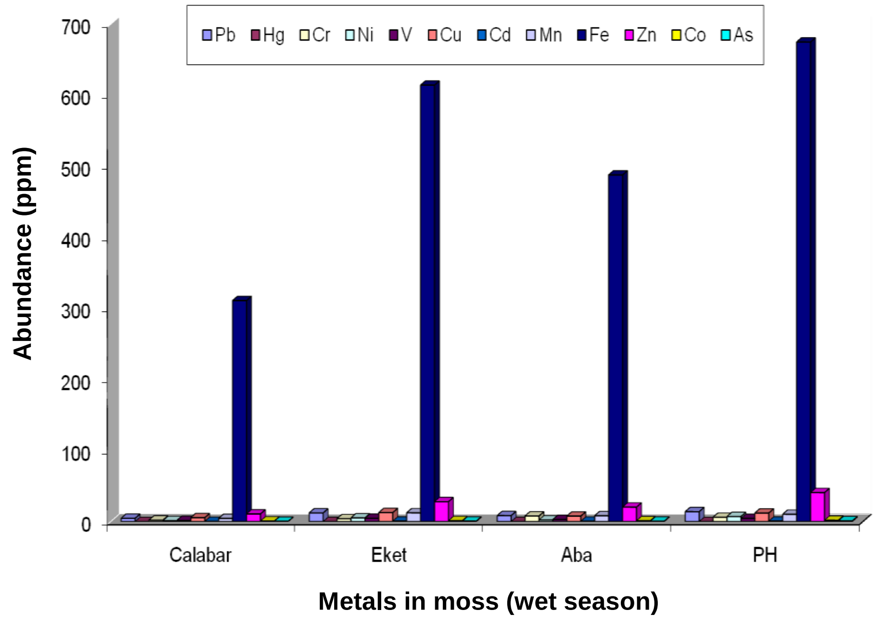


Fig. 2: Mean level of trace metals in moss

Table 3;Seasonal variation of mean level of trace metals in moss (ppm)

	Wet	Dry	P≤0.05	Rmk	Wet	Dry	P≤0.05	Rmk
	Calabar				Aba			
Pb	4.61	2.37	0.002	Sig	8.42	4.94	0.007	Sig
Hg	0.03	0.02	2.112	NS	0.06	0.05	0.000	Sig
Cr	2.07	1.30	0.002	Sig	7.61	4.17	0.033	Sig
Ni	1.23	0.71	0.004	Sig	2.28	1.58	0.004	Sig
V	1.56	0.69	0.002	Sig	2.92	1.78	0.010	Sig
Cu	5.47	4.68	0.001	Sig	7.70	5.57	0.003	Sig
Cd	0.28	0.14	0.003	Sig	0.50	0.25	0.004	Sig
Mn	4.41	2.61	0.000	Sig	8.24	4.92	0.001	Sig
Fe	310.87	258.33	0.009	Sig	487.58	353.42	0.000	Sig
Co	0.74	0.46	0.008	Sig	1.20	0.74	0.001	Sig
Zn	10.77	9.00	0.014	Sig	20.37	12.72	0.000	Sig
As	0.39	0.24	0.003	Sig	0.58	0.26	0.002	Sig
	Eket				Port Harcourt			
Pb	12.15	8.99	0.001	Sig	13.96	8.27	0.000	Sig
Hg	0.05	0.03	0.047	Sig	0.03	0.02	0.374	Sig
Cr	4.03	1.79	0.001	Sig	6.08	2.38	0.001	Sig
Ni	5.16	3.51	0.002	Sig	7.09	3.45	0.000	Sig
V	4.59	2.69	0.004	Sig	4.45	2.45	0.001	Sig
Cu	12.65	7.55	0.007	Sig	12.01	8.85	0.015	Sig
Cd	0.71	0.41	0.004	Sig	0.67	0.37	0.001	Sig
Mn	12.31	9.52	0.000	Sig	10.29	6.72	0.001	Sig
Fe	613.98	445.71	0.001	Sig	674.60	535.32	0.001	Sig
Co	1.24	0.86	0.000	Sig	1.88	1.39	0.001	Sig
Zn	28.01	17.55	0.000	Sig	40.77	22.85	0.009	Sig
As	0.63	0.40	0.000	Sig	1.10	0.64	0.024	Sig

RMK = REMARK; SIG = SIGNIFICANT; NS = NOT SIGNIFICANT; DF = 4, N =



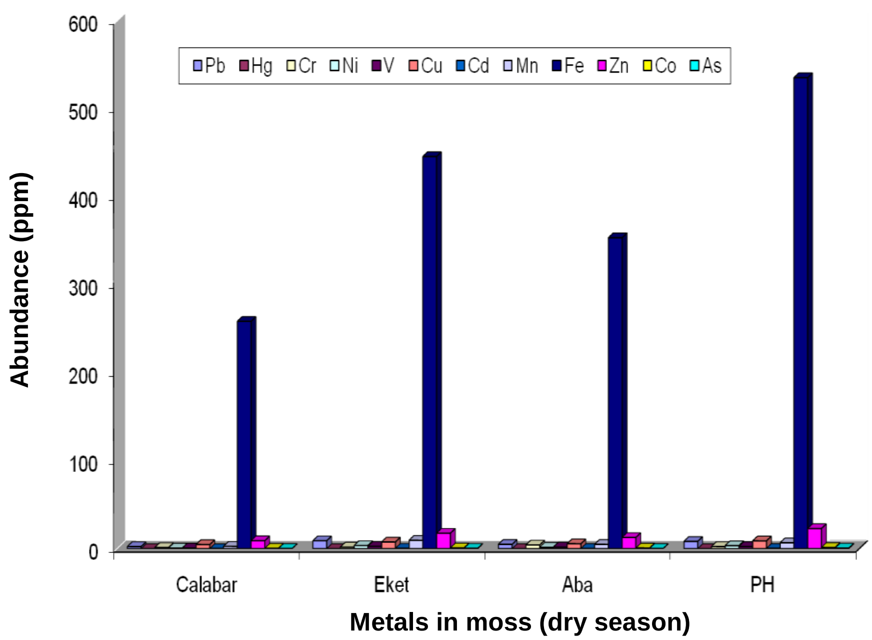


Fig. 3: Seasonal variation in the level of trace metals in moss

4.2 Explanation

The result of analysis of trace metals has revealed that for almost all the metals investigated, leaching is important and has a significant influence on the uptake of metals. Fe does not seem to be significantly affected by leaching process (Cerburnis and Valiulis, 1999). According to the authors, absence of leaching results in rather stable high uptake efficiency and this may be responsible for the high mean level of Fe in moss at the locations.It is necessary to note that mosses do not necessarily reflect monthly or short-term deposition. Mosses do not also reveal information about a particular period and can hardly be used for that purpose. As a living organism, it is strongly influenced by microclimatic and microenvironmental conditions which have an influence on the quantitative uptake of metals from precipitation. Mosses are long term integrator of atmospheric trace metal deposition (Cerburnis and Valiulis, 1999). They accumulate metal loads over a period of their life time.

4.3 Seasonal variability of mean levels of trace metals in moss analysis

The mean levels of trace metals in moss are higher in the wet season than in the dry season. Mosses are small cryptogamic plants that thrive well in damp areas marked with high humidity and frequent rainfall (Chakraborty *et al.,* 2006). Moisture is required because the sperm of mosses must swim to be transported to the egg (Wallace, 1981) for its continual growth. Mosses have high cation-anion exchange property during which process cations and anions become attached to the functional organic groups in the cell wall primarily through chelation (Rao, 1984). Laboratory tests have revealed that cations exchange is a very fast process in carpet forming mosses suggesting that the level measured in this species reflects the effects of the deposition of rain water prior to sampling rather than the results of long term accumulation (Brown and Brumelis, 1996). Reimann *et al.* (1999) in a study carried out in the Kola Peninsula in North West Russia reported that the concentrations of many of the elements in mosses are more closely related to the chemical composition of rain water than in the annual deposition levels (Bargagli, 1998). Mosses obtain nutrients from wet and dry deposition (Steines *et al.,* 1992; Chakraborty *et al.,* 2006) but a high proportion of the pollutant load accumulates in mosses through wet deposition (Berg *et al.,* 1995; Chakraborty *et al.,* 2006). This observation is in agreement with the findings in this study. In a study on assessment of metal deposition in Ilorin metropolis, Nigeria using mosses as bio-indicators, Fatoba and Oduekun (2004) reported that they collected their moss samples toward the end of November, 2002 when, according to them, the rain was over in Ilorin. This does not give the benefit for comparison of seasonal variation of the levels of the trace metals in the study. Bako *et al.* (2008) reported that the use of mosses as bioindcators in monitoring environmental quality in the West African sub region is limited. More experimental data is therefore required to support the finding particularly in these study particularly in West African sub region with similar seasons.

5.0 Conclusions

Study has revealed presence of twelve trace metals from moss samples at the time of sampling. The metals are Pb,Hg,Cr,Ni,V,Cu,Cd,Mn,Fe,Zn,Co and As.

Their levels have been determined and show significant variation from metal and from location to location. This probably shows differences in the kind of anthropogenic activities in the region.

Fe is the most dominant metal in all the study location followed by Zn and Pb.

Seasonal variation reveals higher level of trace metal in the wet season probably because mosses thrive better in moist, humid and wet environment. Results provide baseline information for a realistic regional atmospheric pollution monitoring. The study also recommends use of moss as an effective bio-monitors in the region.

References

Aiyesanmi, A. F. (2008). Baseline heavy metals concentration in River sediments within Okitipupa, South East belt of the Nigerian Bitumenous sand field. *Journal of Chemical Society of Nigeria*, 33(2): 29 – 41.

Anja, H.T., Agneta, M., Burton, S. & Higgs, D. E. (2004). Field and laboratory exposures of two moss species to low level metal pollution. *Journal of Atmospheric Chemistry,* 49(1-3): 111-120

Asaolu, S. S (1998). *Chemical Pollution of Coastal Waters of Ondo State*. Ph.D Thesis, Federal University of Technology, Akure., Nigeria.

Bako, S. P., Afolabi, S. and Funtua, I. I. (2008). Spatial distribution and heavy metal content of some bryophytes and lichens in relation to air pollution in Nigeria's Guinea Savanna. *International Journal of Environment and Pollution,* 33(2-3): 195 – 206

Bargagli, R. (1998). *Trace Elements in Terrestrial Plants. An Ecophysiological Approach to Biomonitoring and Biorecovery*. New York: Spiniger verlag, Berlin.

Berg, T., Royset, O. and Steinnes, E. (1995). Moss (*Hylocomium splendens)* used as biomonitors of atmospheric trace element deposition: stimation of uptake efficiencies. *Atmospheric Environment*, 29: 353 – 360

Brown, D. H. and Brumelis, G. (1996). A biomonitoring method using the cellular distribution of metals in moss. *The Science of the Total Environment,* 187: 153 – 161.

Brown, D.H. and Sidhu, M. (1992) Heavy metal uptake, cellular localization and inhibition of moss growth. *Cryptogram Botaniser*, 3: 82 – 85

Cerburnis, D. and Valiulis D (1999). Investigation of absolute metal uptake efficiency from precipitation in moss. *The Science of the Total Environment,* 226: 241 – 253.

Chakraborty, S., Jha, S. K., Paramik, V. D & Paratker, G. T. (2006). Evaluation of air quality by means of biomonitors. *Aerosol and Air Quality Research,* 6(3): 247 – 258

Chettri, M. K. (1997). Responses of lichen *Cladonia Convoluta (Lam.)* and *Cladonia rangiformis (L.) Hoffm* to the effect of toxic heavy metals. *Scientific Annals of the School of Biology. Appendix No. 59*. Thessaloniki: Aristotle University of Thessaloniki Greece

Fatoba, P. O. and Oduekun, T. I. (2004). Assessment of metal deposition in Ilorin metropolis using mosses as bioindicators. *Nigerian Journal of Pure and Applied Science,* 19: 1549 – 1552

Fernandez, J. A. & Carballeira, A. (2002). Biomonitoring metal deposition in Galicia (North West Spain) with mosses; Factors affecting bioconcentration. *Chemosphere,* 46: 535-542.

Glydesen, H., Pilegaard, K., Rasmussen, L. and Ruhling, A. (1983). Moss analysis used as a means of surveying the atmospheric heavy metal deposition in Sweden Denmark and Greenland in 1980. *Bulletin SNV PM,* 1670-1675.

Halleraker, J. H., Reimann, C., de Caritat, P., Finne, T. E., Kashulina, G., Niskaavaara, H. and Bogatryrev, I. (1998). Reliability of moss (*Hylocomiumsplendens* and *Pleuzorium schreberi*) as a bioindicator of atmospheric chemistry in the Barents region; Interspecies and field duplicate variability. *The Science of Total Environment,* 218: 123-139

Kakulu, S. E, Osibanjo, O. and Ajayi, S. O. (1988). Trace metals content of fish and shell fishes of the Niger Delta Area of Nigeria. *Environmental International,* 13: 247 – 251

Kakulu, S. E. (1993). Biological monitoring of atmospheric trace metal deposition in North Eastern Nigeria. *Environmental Monitoring and Assessment,* 28(2): 137 - 140

Little, P. and Martin, M. H. (1974). Biological monitoring of heavy metal pollution. *Environmental Pollution,* 6: 1-19

Loppi, S. & Bonini, I. (2000). Lichens and mosses as biomonitors of trace elements in areas with thermal strings and funmarole activity (Mount Amiata, Central Italy), *Chemosphere,* 41: 1333-1336.

Nioboer, E., Ahmed, H. M., Puckett, K. J. and Richardson, D. H. S. (1972): Heavy metal content of lichens in relation to distance from a nickel smelter in Sudbury, Ontario: *Lichenologist,* 5: 292-304.

Nordic Council of Ministers (1994). *Atmospheric Heavy Metal Deposition in Europe Estimation Based on Moss Analysis*. Copenhagen: Nordic Council of Ministers.

Odukoya, O. O., Bamgbose, O. and Arowolo T. A. (2000). Heavy metals in top soils of Abeokuta dump sites. *Global Journal of Pure and Applied Sciences,* 6(4): 651 – 653.

Oniawa, P. C. and Egunyomi A. (1983). Trace metal levels in some Nigerian mosses as indicators of atmospheric pollution. *Environmental Pollution Series B, Chemical and Physical,* 5: 71 – 8

Oniawa, P. C., Ajayi, S. O. and Osibanjo, O. (1986). Accumulation patterns of heavy metals in forest mosses from the South West Region of Nigeria. *Environmental Pollution series B, Chemical and Physical,* 11(1): 67 – 68

Pilegaard, K. (1978) Airborne metals and SO_2 monitored by epiphytic lichens in an industrial area. *Environmental Pollution,* 17: 81 –92

Reimann, C., Halleraker, J. H., Kashulina, G. & Bogatyrev, I. (1999). Comparison of plants and precipitation chemistry in catchments with different levels of pollution in Kola Peninsula, Russia. *Science Total Environment,* 342/244: 169 – 191.

Rao, D. N. (1984). *Response of Bryophytes to air Pollution*. London: Chapman and Hall.

Ross, H. B. (1990). On the use of mosses *(Hylocomium splendens and Pleurozium schreberi*) for estimating atmospheric trace metal pollution. *Water, Air and Soil Pollution,* 50: 63 – 76

Ruhling, A. and Tyler, G. (1968). An ecological approach to the lead problem. *Botaniska Notiser,* 121: 321-342.

Ruhling, A. and Tyler, G, (1973). Heavy metal deposition in Scandinavia. *Water, Air and Soil Pollution,* 2: 445 – 455.

Ruhling, A., Rasmussen, L., Pilegaard, K., Makinen, A. and Steinnes, E. (1987). Survey of atmospheric heavy metal deposition in the Nordic Countries in 1985- monitored by moss analysis. *Nordic Council of Ministers,* 15: 21 – 44

Ruhling, A. & Steinnes, E. (1998). Atmospheric heavy metal deposition in Europe 1995-1996. *Nordic Council of Ministers,* 15: 10-17

Sardans, J. & Penuela, J. (2005). Trace element accumulation of the moss *Hypnum cuppressiforme Hedw*. and the trees *Quercus Ilex L*. and *Pinus halepensis Mill*. in Catalonia. *Chemosphere,* 60: 1293 – 1307.

Sawidis, T., Zachariadis, G., Stratis, J. and Ladoukakis, E. (1993). Mosses as biological indicators for monitoring of heavy metal pollution. *Fresenius Environmental Bulletin,* 2: 193 – 199

Saxena, D. K., Srivastava, K. and Singh, S. (2008). Retrospective metal data of the last 100 years deduced by moss, *Barbula sp* from Mussoorie City, Garhwal Hills, India. *Current Science,* 94(7): 901 – 904.

Shakya, K., Chettri, M. K. and Sawidis, T. (2001). Appraisal of some mosses for biomonitoring airborne heavy metals in Kathmandu valley. *Eco print,* 8(1): 69-75.

Shakya, K., Chettri, M.K. and Sawidis, T. (2004). Ambient air quality study team 2004 final report in Assessment of ambient air quality in selected urban areas of Nepal with estimated burden of diseases. Submitted to *Nepal Health Research Council (NHRC*), Kathmandu.

Shelite, J., Akselsson, R. and Winchester, J. W. (1976). Trace element similarity groups in North Florida Spanish moss: Evidence for direct uptake of aerosol particles. *Journal of Geophysics Research,* 81: 1047 – 1050.

Steinnes, E., Rambaek, J. P. and Hanssen, J. E. (1992). Large scale multi-element survey of atmospheric deposition using naturally growing moss as biomonitor. *Chemosphere,* 25: 735– 752

Wallace, R. A. (1981). *Biology, the World of Life*. California: Scott, Foreman and Co.

World Health Organization (WHO) (2001). *Guidelines for Air Quality*. Geneva: World Health Organization.