EFFECTS OF LEAD AND SIMULATED ACID RAIN ON CHLOROPHYLL CONTENTS OF SELECTED TROPICAL MOSSES.

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ABSTRACT

The effects of lead and acid rain on chlorophyll accumulations were investigated in *Archidium ohioense, Bryum coronatum* and *Octoblepharum albidum*. Four regimes of moss colonies were treated with simulated acid rain using dilute H_2S0_4 (pH=5), lead using 1.0 ppm $Pb(N0_3)_2$ solution, interaction of lead and acid and control regime (treated with only distilled water).

Sampling commenced a day after application of each solution at a rate of 1.0 ml per cm^2 of moss coverage. Chlorophylls a and b contents of moss samples were analysed weekly using standard methods. The levels of acid and lead concentration used did not have negative effect on chlorophyll contents in the three mosses. Low chlorophyll a/b ratio was observed in *Octoblepharum albidum*. However, high accumulation of chlorophyll in *O albidum* may be due primarily to the presence of chlorocysts (tissue with abundant chlorophylls). However, lead and lead acid interaction treatments enhanced accumulation of the chlorophylls a,b as well as chlorophyll a/b ratios better than the control in all the three mosses. The study concluded that the selected mosses can be bio accumulators of this heavy metal it is not all levels of heavy metals.

Key Words: Acid Rain, Mosses, Chlorophyll, Lead, Contents

INTRODUCTION

Bryophytes are subjected to environmental stress in some urban areas due to frequent physical changes in the substrata and the chemically aggressive conditions brought about by polluting industries (Ron *et al.*, 1987). The slow growth rate, manageable size and ability to complete life cycle in a short period of time are salient factors responsible for the proliferation of bryophytes (Makinde, 1993; Makinde and Odu, 1994).

The use of various bio-indicators for monitoring air pollution has increased over the years (Anderson *et al.*,1980; Rambaeck and Steinnes, 1980). Bryophytes, especially the mosses with their delicate and uncuticularised plant body, seem to have a great capacity for absorbing and accumulating pollugenic substances from the environment (Shacklette, 1965). Several groups of organisms have been used the boundicators of atmospheric pollution mosses are well known for the monitoring of heavy metal atmospheric deposition in terrestrial ecosystem (Ruhling, 2002).

Determination of chlorophylls a and b is an important parameter in ecophysiological research. Chlorophyll contents in plants is a useful estimation of primary productivity and have been used in bioassays of environmental stresses. As reported by several authors, pollution of the environment by acid and lead (Pb) is a common occurrence (Oluwande, 1977, 1979; Onianwa and Egunyomi 1983 and Ron *et al.*, 1987). (Oluwande, 1977; 1879; Onianwa and Egunyomi; 1983; Ron et al; 1987). This work was carried out to determine the effects of simulated acid and heavy metal (Pb) pollutants on chlorophyll accumulation in selected derived savanna (*A. ohioense* and *B. coronatum* and a forest moss (*O. albidum).* The chlorophyll contents in these mosses will provide information on changes,that may be observed in mosses of the two vegetation zones.

MATERIALS AND METHODS

The study was conducted at the Obafemi Awolowo University, (OAU) Ile-Ife campus (07° 32'N; 04° 31'E) in Southwest of Nigeria. The moss *Octoblepharum albidum* Hedw (a forest moss) was studied on the bark of oil palm tree, *Elaeis guineensis* Jacq at the Parks and Gardens Unit of the Campus while *Bryum coronatum* Schwaegr and *Archidium ohioense* ex C. Muell (two derived savanna mosses) were raised within 13 cm diameter plastic pots in the Screen House of the Department of Botany; O.A.U. Ile-Ife. (at 28.5 ± 1.5°C).

Moss colonies were irrigated with 100 ml water at 48 h intervals for one month after which the three species were divided each into four batches for the following treatment regimes:

(1) 1.0 ppm Pb solution irrigated regime
(2) Dilute H_2S0_4 acid (pH=5) irrigated regime
(3) Pb + Acid regime (Pb solution + Acid solution on above (1:1v/v)
(4) The control (distilled water) regime

The acidity was created with sulphuric acid because the commonest pollutant, sulphur dioxide usually produces sulphurous acid during precipitation in our environment. The lead treatment was added because the heavy metal (Pb) is common additive in gasoline and much of the gases from exhausts of automobiles (Oluwande, 1977; Onianwa and Ajayi, 1987) of the solutions above was used to irrigate one of the four regimes of each moss species at the rate of 1.0 ml per cm^2 cover, for four weeks for the mosses to establish. Thereafter, the effects of various treatments on chlorophyll accumulation were determined even as solution application continued.

Chlorophyll contents were extracted with absolute Dimethylsulfoxide (DMSO) according to the methods of Raeymaekers and Glime (1986) modified by Makinde (1991). The extraction was done on 10mg of each moss sample placed in test tube and 5ml Dimethylsulfoxide added before incubation at 67°C in oven for 15hrs. The concentrations of chlorophylls a and b were determined using the formulae of Arnon (1949).

$$\text{Chlorophyll a} = [12.7_{A663}\ 2.7_{A645}) \times sw^{-1}$$

$$\text{Chlorophyll b} = (22.9_{A645}\ 4.7_{A663}) \times sw^{-1}$$

s = amount of solvent used for extraction (ml)

w = dry weight of sample (mg)

RESULTS

Chlorophyll accumulation in *Archidium ohioense* is shown in Figure 1a. A similar pattern of accumulation was observed for the four regimes up to the week after which the acid regime continued to increase while the other regimes declined. On the other hand, chlorophyll b accumulation by *A. ohioense* in lead + acid treated regime was unusually high but decreased sharply in the second week. However, it still maintained the highest value compared to other regimes throughout the experiment. It can be noted that the values decreased like in chlorophyll a after the fourth week except acid regime that continued to increase. (Fig. 1b). Figure 1c shows chlorophyll a/b ratio with similar pattern for all the regimes with values reaching a peak in the third week and thereafter declined till the end of the experiment.

In *Bryum coronatum,* chlorophylls a and b and chlorophyll a/b ratio contents showed similar pattern in that lead and Acid interaction regimes had highest values in all the regimes. (Figure 2a and b). The values for all the regimes declined slightly between the first and second analysis (Fig, 2a), but rose to a peak from second through the fourth week of analysis. It was noted that the interaction (lead + acid regime) continue to increase till the end of

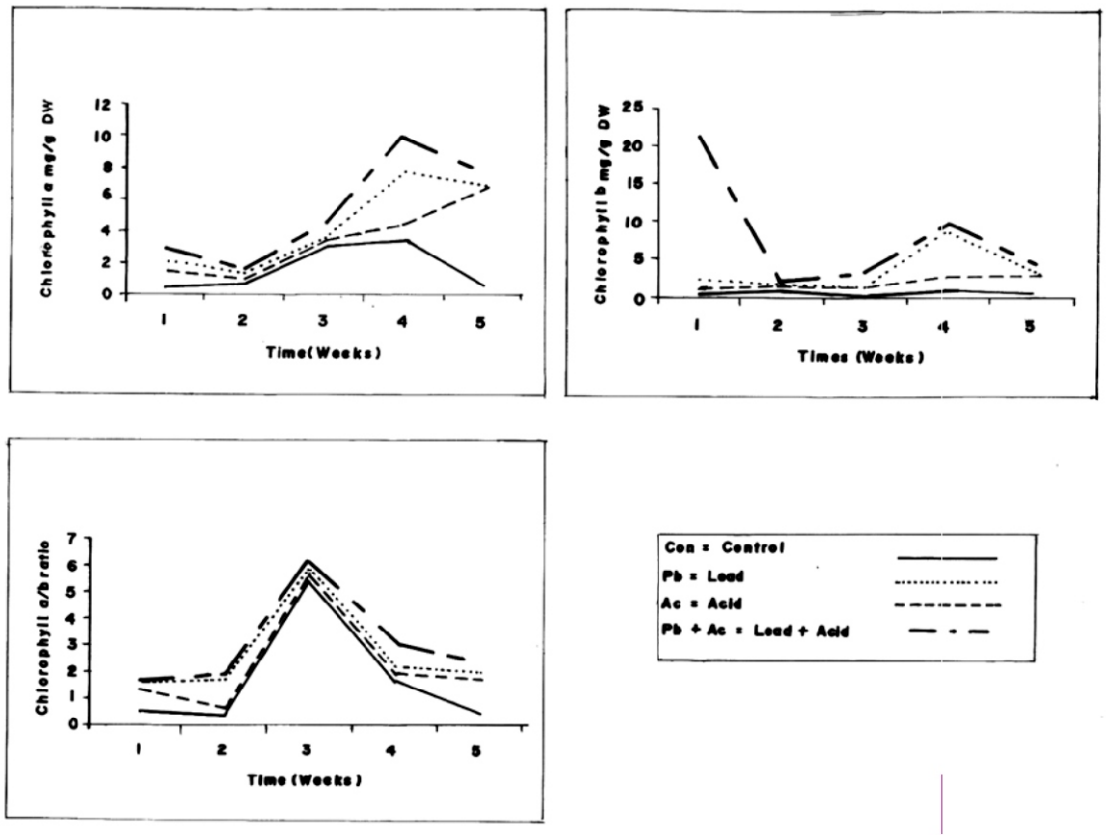

Figure 1: The effects of acid, lead and acid-lead interactions on chlorophylls a and b, and a/b ratio in *Archidium ohioense*.

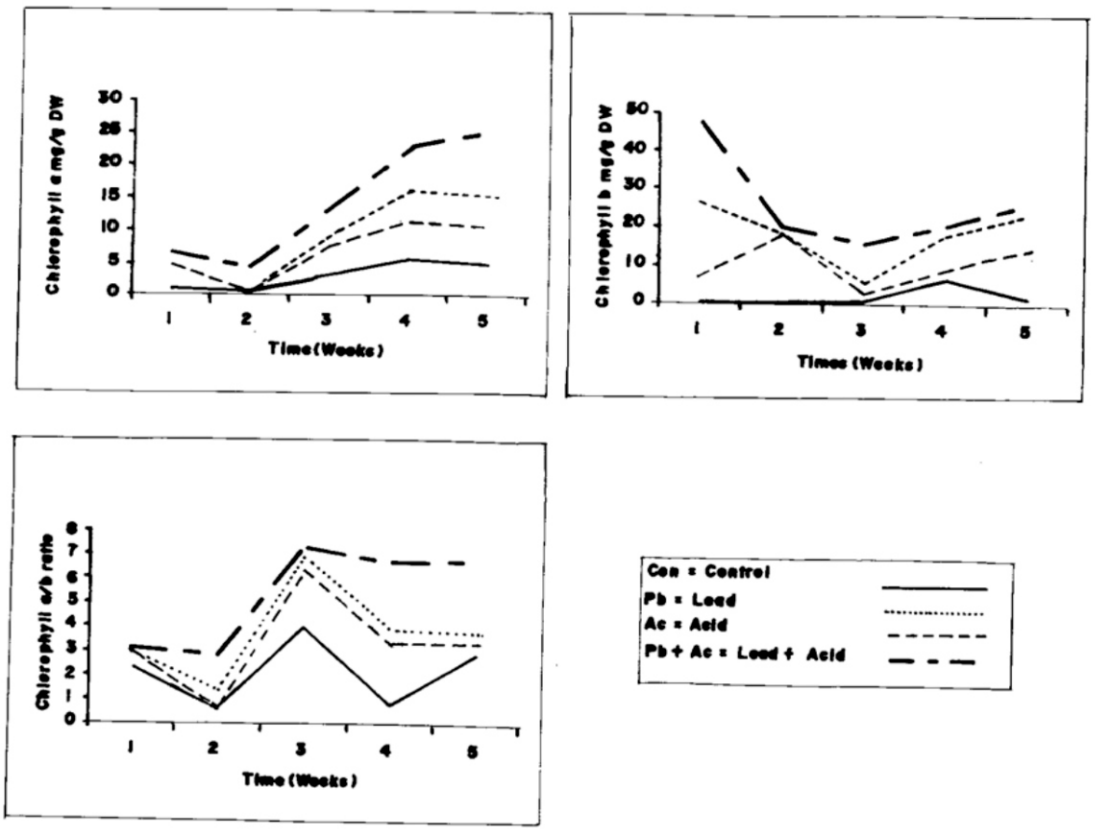

Figure 2: The effects of acid, lead and acid-lead interactions on chlorophylls a and b, and a/b ratio in *Bryum coronatum*.

analysis while the other regimes slightly decreased. Chlorophyll b accumulation by *B. Coronatum* in lead^{+} acid regime was highest but curiously declined between first and the third analysis. Lead treated regime also recorded high accumulation of chlorophyll b even higher than acid regimes. Figures 3a and 3b show chlorophylls a and b in *Octoblepharum albidum*. The lead + acid interaction regime recorded the highest values while the pattern of accumulation was similar to what obtained in *A. ohioense* and *B. coronatum*. In the three species studied, the differences observed were significant after the third week of investigation (P=0.05).

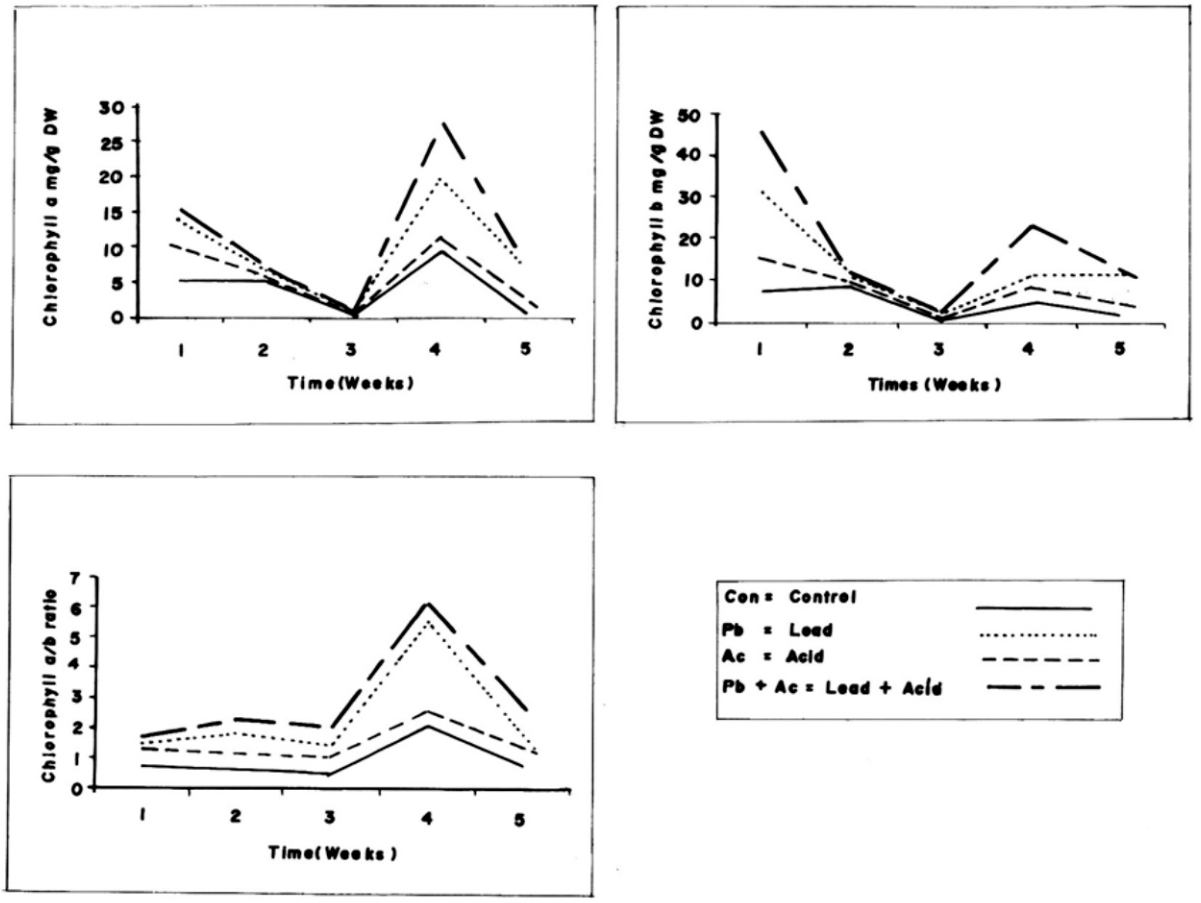

Figure 3: The effects of acid, lead and acid-lead interactions on chlorophylls a and b, and a/b ratio in *Octoblepharum albidum*.

DISCUSSION

The patterns of chlorophyll accumulation reported in this work clearly show that the mosses were not negatively affected by the treatments. Further studies with increased concentrations of Pb and Acid may probably show contrary effects.

The level of lead used (1.0 ppm) appeared not to be toxic enough to do physiological damage to the mosses. Germination of *Funaria hygrometrica* spores have been delayed for 2 days by 0.7 ppm lead tetraethyl, while a 12-days delay was caused by 66 ppm concentration of it. Anatomical feature that may be of advantage to bryophytes is their relatively thick cells which are thicker than that of any higher plant leaves (relative to the size of protoplast). Previous work on extraction of chlorophyll in bryophytes by Makinde (1991) has revealed the need for longer time of extraction (12 hr) even at 67°C with DMSO compared with much shorter periods required with leaves of higher plants.

The chlorophyll a/b ratio was lower in *O. albidum* compared to the other two savanna mosses *(A. ohioense* and *B. coronatum*). This result agrees with those of Alberte *et al.* (1976) and Makinde (1991) that lower chlorophyll a/b ratio occurs in shade plants than in sun plants. The study may have confirmed mosses as good tools for biogeochemical prospecting Shacklette (1965) and in studying regional deposition of heavy metals (Ruhlings and Tyler, 1968, 1969, 1971 and

2002). The lack of an effect of lead in this study may be due partly to low lead levels (1.0 ppm Pb) used in wetting the mosses. This agrees with report of Raeymaekers and Glime (1986). Rao (1982) reported that initial binding of heavy metals to the cation exchange sites of the cell wall immobilizes the heavy metals and might reduce their damaging effect as long as saturation has not been reached.

In conclusion, the levels of lead and acid applied to the three investigated mosses in this study were not high enough to cause stress or physiological damage as shown in chlorophylls accumulated.

REFERENCES

Alberte, R.S., McClure, P.R and Thornber, J.P. 1976. Photosynthesis in trees organization of chlorophyll and photosynthetic unit size in isolated gymnosperm chloroplast *Plant Physiology* 58:341-344

Anderson,L.E. Hoymand A.M.F; and Johnson, I 1978. Atmospheric heavy metal depositon in the Copennagen area *Environ. Pollut.* 17: 133-151

Arnon, D 1949. Copper enzymes in isolated chloroplasts, polyphenoxidases in *Beta vulgaris Plant Physiology* 24:1-15

Makinde, A.M. 1991 Studies of the seasonal changes in Bryophyte population of derived savanna in South-Western Nigeria *Unpub. Ph.D. Botany Thesis* O.A.U. Ile-Ife.

Makinde, A.M. 1993 Thermotolerance of selected mosses of South Western Nigeria Savanna. *Nigerian Journal of Botany* 6:21-25

Makinde, A.M and Odu E.A. 1994 Phenological Studies of Selected Savanna Mosses of Southwestern Nigeria *Experientia* 50:616-619.

Oluwande, P.A. 1977 Automobile traffic and airpollution in a developing country (An example of affluence caused environmental problems) *Int. J. Environ. Stud.* 11:197 203

Oluwande, P.A. 1979. Automobile exhaust problems in Nigeria *Ambio* 8: 26-29.

Onianwa P.C. and Ajayi, S.O. 1987. Heavy metal contents of epiphytic acrocarpous mosses within inhabited sites in Southwest Nigeria. *Environment International* 13:191-196

Onianwa, P.C. and Egunyomi A. 1983. Trace metal levels in some Nigerian Mosses used as indicators of atmospheric pollution.

Ron E, Masimpaka, V.Vincenter J. and Granzov de la Cerda, I. 1987 Urban bryophytes in Spanish towns *Symposia Biologica Hungarica* 35:727-755

Raeymaekers, G (1986) Chlorophyll extraction in mosses through Dimethylsulfoxide (DMSO) *Bryological Times* 38: 4-5

Raeymaakers, G and Glime J.M. 1986 Effects of simulated acid rain and lead interaction on the phenology and chlorophyll contents of *Pleurozium schreberi* (Brid) Mitt. *Journal Hattori Bot.* Lab 61:525-541.

Rambaeck, J.P and Steinnes, E. 1980 Karterin av tungmetallnedfall in Norge ved analyses av mose. Institut for Atom-energi *Work Report* 40:1-5 + 11 APP.

Rao, D.N. 1982 Responses of bryophytes to air pollution in: *Bryophyte Ecology (*Ed. A.J.E. Smith) Chapman & Hall London pp 445-471

Richardson, D.H.S 1981. *The Biology of mosses.* Blackwell Scientific Pub. London 220pp.

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

Ruhling, A and Tyler, G 1969 Ecology of heavy metals: a regional and historical study *Bot Notiser* 122:248-259

Ruhling, A and Tyler, G 1971 Regional differences in the deposition of heavy metals over Scandinavia *J. Appl. Ecol.* 8: 497-507

Ruhling, A. 2002. A European survey of atmospheric heavy metal deposition in 2000-2001. *Environ. Pollut.* 120:23-25.

Shacklette, H. 1965. Bryophytes associated with mineral deposits and solutions in Alaska *U.S. Geol. Surv. Bull.* 1198.