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Effects of Some Heavy Metals on Chlorophyll Accumulation in *Barbula lambarenensis*

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ABSTRACT

Samples of moss (*Barbula lambarenensis*) with their substrates collected from Belewu Drive, Oke Odo,Ilorin were taken to the screen house of the University of Ilorin biological garden to monitor the effects and tolerance of this plant to different heavy metals. The moss samples were divided into eleven regimes, widely separated from one another to avoid contaminations. Ten regimes were differently irrigated with 1000ppm and 2000ppm of lead, copper, cadmium, iron and vanadium thrice a week. The eleventh treatment served as the control, and was irrigated with distilled water. It was found that these heavy metals caused some damage to the chloroplasts of this plant as the bright green colours changed light green, yellowish green or brown. The concentrations of the used heavy metals are phytotoxic. In light of this, *Barbula lambarenensis* can serve as a bio-indicator of heavy metals.

INTRODUCTION

Many plants and animals have been used to assess environmental pollution (Kasanen and Venetvaara 1991). Of all, mosses and lichens have been found to be the best bio indicators (Murkherjee and Nuorteva, 1984). The ability of mosses in this regard could be attributed to their small-size, nakedness, habitat, poikilohydric nature and reduced sensitivity to the environment. Generally, bryophytes are well adapted to moist habitats while some are able to survive in areas with little or no rainfall (Carrington, 1997). Bryophytes, especially mosses are ubiquitous as they are equipped with some structural adaptive strategies that enable them to grow successfully where they occur. Their ability to grow on substrates or areas which are inhospitable to higher plants exclude them from intense competition and this gives them an added advantage to serve as good bio indicators. Gradzinski (1990) identified three types of bio indicators: true indicator (pollutants damage the plant proportionally to the concentration of the pollutants); accumulators (accumulate the potentially toxic pollutants); and scales of indicator species (pollutants lead to the disappearance or appearance of certain species).

Environmental pollution has become a matter that requires immediate attention as it affects human health and results in death. Urbanization, civilization and industrialization have worsened this ugly situation of the environment such that there is no more free air again. The pollutants are released from human activities such as agricultural practices (usage of pesticides, fungicides, fertilizers, etc), combustion, sewage and waste disposal, vehicular wear and tear, mining and smelting processes, tetra oxosulphate VI and trioxo nitrate V acid plants. All these produce poisonous pollutants such as SO_2, NO_x, heavy metals, SO_3 that affect both plants and animals. Critical about these pollutants is the ill health associated with the lungs, heart and liver leading to death.

Bates (2000, 2002) reported that mosses are the least sensitive to the major pollutants of the environment. Kakulu (1993) reported that *Polytrichum junipercum* found on the bark of *Azadrachta indica* had been used to assess heavy metal pollution. He confirmed that the concentrations of pollutants in the tree bark correlate with those of the environment. Charabortty *et al*(2006) worked on the use of mosses as bio-monitor of trace element. It has been found that bryophytes growing directly on an alkaline masonry showed enhanced tolerance to SO_2 (Gilbert, 1970). This ability was assumed to be due to $CaCO_3$ concentration of the substrate which was believed to have neutralised the deposition of acid in the soil and pushed the toxic equilibrium from SO_2 dissolving in surface water solution towards sulphite, the least harmful of the three ion forms of SO_2 ; SO_3^{2-}, HSO_3^-, H_2SO_3 in the soil. The toxicity of SO_2 results from the fact that the gas forms highly reactive species on dissolving in water forming SO_3^{2-}, HSO_3^-, H_2SO_3, the most toxic being HSO_3^-, H_2SO_3(Richardson,1981).

Most of the pollutants are silent killers, the need to identify bio -indicators in the environment is required as this would reveal the pollution status of man's environment from time to time. This would equally help in identifying the prevailing danger and thus enable man to take necessary step by checkmating the release of such pollutant and advise the government or people on the necessary steps to take so as to enjoy longetivity and good health. This study was designed to identify the effects of some heavy metals on chlorophyll accumulation in *Barbula* lambarenensi.

MATERIALS AND METHODS

Barbula lambarenensis (Pottiaceae) is a bright green, cushion like acrocarpous moss that grows on sandcrete materials under tree canopy or in cool environment supplied with adequate moisture. Although it reproduces sexually but rely mostly on asexual propagules, gemmae for its establishment and expansion (Fatoba 1998). This plant was chosen for this study because of its occurrence in the nooks and corners of the environment *B. lambarenensis* with its sandrcrete substrates were collected along Yinka Belewu Drive, Oke-Odo, Tanke, Ilorin Kwara State, Nigeria These plant collections were taken to the screen house in the University of Ilorin Biological Garden where they were divided into eleven regimes with wide spaces left in between regimes to avoid contaminations and mix-up.

Five different salts: Pb $(NO_3)_2$ $CdSO_4$, $CuSO_4$, $Fe_2(SO_4)_3$ and Va were used for the experiment to furnish five different heavy metals, Required amounts of each salt was weighed and dissolved in a 1litre of distilled water to give 5 and 10 mg/l of each of the metals (Table 1).

Table 1. Preparation of working solutions.

concentration Quantity of salts dissolved in 1 litre of distilled					
	$Pb(NO_3)_2$	$CdSO_4$	$CUSO_4$	$Fe_2(SO_4)_3$	Va
5mg/l	0.00799	0.09270	0.01255	0.01788	0.00500
10mg/l	0.01599	0.01854	0.02510	0.03576	0.01000
Control (water)	-	-	-	-	-

Samples were collected from each regime weekly for chlorophyll determination and fortnightly for microscopic observations. Plant materials collected were extracted separately with 80% acetone, centrifuged and the optical densities read off at 645 and 660 wavelengths of the Spectrophotometer model Campspec M105. The chlorophylls a and b contents of each sample determined with Arnon (1949) formula and total chlorophyll with Strain and Svec (1966) formula. The experiment lasted for three months and the data generated were subjected to Student t – paired test.

OBSERVATIONS AND RESULTS

All the plants irrigated with heavy metal solutions have their bright green colours changed to light green, yellow or brown while the control remained green throughout the period of study. This shows that the heavy metals affected the chloroplast negatively. The change in colouration started at the 4th week of experiment in regimes irrigated with 5mg/l Cd, Fe and Pb, 6th week in regimes irrigated with Va and 8th week in samples irrigated with Cu.. The change was noticed earlier (4th week) in the regimes irrigated with 10mg/l of all the heavy metal solutions except in the regime irrigated with Va that showed in 6th week. The trend of this result showed that Cd, Feiii and Pb are more toxic/ and deleterious to the chloroplast than Va and Cu. The cells and nuclei of the control experiment remained intact throughout the period of study but the cell started collapsing and becoming distorted during the 4th week for Va treatment, 6th week for Cu, Cd, Fe and Pb treatments. The same trend

was observed for the nature of the nuclei except for the Cu treated samples. However, the effects started earlier (4th week) in regimes treated with 10mgll working solutions. In light of this, Va is more toxic than other heavy metals on the foliar cells and nuclei of the plant.

Generally, the trend of toxicity was Va> Cd Pb, Fe iii >Va> Cd> Cu as the foliar colours changed brown, light yellow and pale green respectively.

Table 2 shows the weekly effect of the different heavy metals on chl;orophyll a content of the treated *Barbula lambarenensis*. The treated samples had less chlorophyll a than the control (Table 2). Generally, the chlorophyll a contents of samples irrigated with 5mg/l of heavy metal solution were greater than those of 10mg/l. Both the control and treated followed same trend in respect of the chlorophyll a content. The chlorophyll a increased from the 1st week of experiment to the 5th week then gradually decreased to the end of the experiment. Chlorophyll a in the treated samples (5 and 10mgll) were less than that of the control after the 4th week of the experiment with some being greater than the control during the 1st & 2nd week (Cd, Fe, Pb) while Cu & Va were less for 5mg/l However, the pattern of the relationships of the treated with the control were not clearly defined for 2nd to 4th week (Table 2). Moreover, only the samples treated with 10mgll Fe and Pb had greater chlorophyll a than the control (Table 2). Table 2 further revealed the high chlorophyll a content of samples irrigated with both 5mgll and 10mgll of Cu f rom 2nd week to 4th week.

The mean chlorophyll a contents of the samples irrigated with 5mgll & 10mg/l of Cu and Pb were statistically the same with that of the control. This shows that these two heavy metals did not impair the chloroplast of *B. lambarenensis*. Samples irrigated with 5mg/l & 10mg/l of Cd , Fe and Va had lower chlorophyll a con tents that were statistically different at 0.05% and 0.01% levels of significance than those of the control. This shows that the chloroplasts were affected by these treatments. The worst effect was manifested in regime treated with Va.the effects of these heavy metals on chlorophyll a of *B. lambarenensis* was Va> Cd >Fe >Cu > Pb (Table 2)

Table 3 shows the effects of different heavy metals on the chlorophyll b contents of *B. lambarenensis*. Generally, chlorophyll b was generally less than chlorophyll a contents of this plant. Like chlorophyll a,, chlorophyll b contents of the treated and the control followed same trend of an increase from 1st week to the 5th week which subsequently reduced to the end of the experiment (Table 3). Samples irrigated with 5mg/l & 10mg/l of Cu &Cd and 10mgll of Fe had greater chlorophyll b than the control during the 1st week of experiment. This is also true for 5mg/l Cu, Fe & Pb and 10mgll of Fe

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for the 2 week of experiment. Statistical analysis revealed that the chlorophyll b contents of samples irrigated with 5mg/l of Cu, Pb and Va and 10mgl l of Pb were statistically the same with the control though less numerically. Moreover, , the 5mgll of Cd , Fe and 10mgll of Fe were found to be statistically less than that of the control at 5% level of significance while those of 10mgll of Cd & Va were statistically less than the control at both 5% and 1% levels ofsignificance.The trend of toxicity on chlorophyll b contents was Cd>Fe>Va>Pb_3Cu> control (Table 3).

The effects of different heavy metals on the total chlorophyll contents of *B. lambarenensis* are shown in Table 4. The total chlorophyll contents of samples irrigated with 5mgll of Cd, Fe, Pb and 10mgll of Fe were greater than the control during the 1st week while

those of 5mgll & 10mgll of Cu were greater from 2nd to the 4th week (Table 4) .Samples of all the regimes showed same trend, an increase from 1st week to 5th week which subsequently

reduced to the end of experiment. The total chlorophyll content of samples irrigated with 5mgll & 10mgll of Cu and Pb were found to be statistically the same with the control. Those irrigated with 5mgll & 10mgll of Cd Va & Fe were statistically lower than the control (Table 4). The trend of action was Cd>Va>Fe>Pb>Cu> control. Generally, all the treatments had negative effects on the chloroplasts leading to lower contents.

DISCUSSION

The changes in the green colouration and the distortion of the foliar cells and nuclei of *Barbula lambarenensis* as observed in this study is a manifestation of the detrimental effects of the heavy metals used. This fact is hinged on the maintenance of the bright green colouration, and intact cells of the control experiment. Sergio (1987) indicted pollutants such as $S0_2$ and heavy metals in the disappearance and changes in the flora around urban and industrial areas. Neolith Chemical Ltd (1988) directed that aqueous solutions of heavy metals such as lead , bitsmuth, copper or mercury is necessary to prevent moss growth on masonry substrates This further shows the effects of the heavy metals. Martin & Coughtrey (1982) reported that metals vary in their toxicity and degree of toxicity is proportional to their concentration. This is also observed in this study as the 10mg/l was found to be more toxic than 5mgll of heavy metal solution used. The results observed in this study compared favourably with the findings of Gulvag *et al* (1974). Brown *et al* (1986) observed that low

concentration of mercury inhabits photosynthesis temporarily, increase respiration, reduces chlorophylls a and b concentrations and also causes substantial loss of intracellular potassium from *Rhytidiadephous squarrosus*. The reduction of chlorophylls a and b and total chlorophylls observed in this study agreed with the findings of Brown & Whitehead (1986). These reductions would certainly reduce the photosynthetic process of this plant.

Copper contaminated soils (10mug/g) was found to inhibit the protonemal growth of *Funaria hygrometrica* (Richardson, 1981). He further stated that SO_2 progressively changed green colouration of lichens and mosses to whitish brown as one moved from mildly to the highly polluted zone. A resultant colour change was observed in *Fontinalis squamosa* which absorbed metal ions from contaminated water (Mclean and Jones, 1975). Farooqui *et al* (1993) reported that lead, cadmium, chromium, copper, zinc and aluminum are phytotoxic. This also confirmed the toxicity of Pb, Cd and Cu which were used in this study. The initial higher chlorophyll contents observed in some samples could be due to some damage done to the chloroplasts.

Mosses usually have efficient mechanism for absorbing metals and other pollutants from their environments with or without any destructive impact. This puts them at an advantage over other plants. The greater part of the metal contents of mosses is however not taken up and is not located within the cells but accumulates extracellularly (Richardson, 1981). The use of mosses as bio indicator could be attributed to their tolerance to heavy metals (Kakulu,1993) and the ability to accumulate toxic elements (Brown, 1984; Rao, 1982;

Kovacs *et al.,* 1993). Brown (1984) reported that metals accumulate in form of particulate matters on the surfaces of the gametophores which are rough, providing many crevices for these tiny particles. Moreover, extracellular cation exchange capacity of bryophytes helps in explaining their metal uptake ((Brown and Bates, 1998).

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Table 2. Effect of different heavy metals on chlorophyll a content of *Barbula lambarenensis*.

Concentration of Metal (mg/l)		Weekly Concentration of Chlorophyll (mg/g)												
		Week												
		1	2	3	4	5	6	7	8	9	10	11	12	× ± SD
5	Control	3.272	5.459	4.297	10.391	10.413	9.707	7.399	7.029	6.126	3.395	3.231	3.512	6.185± 1.293
	Cu	2.894	10.461	5.973	15.764	9.354	8.527	4.374	3.219	3.113	2.396	2.304	2.862	5.936± 2.059[a]
	Cd	5.355	3.944	4.343	6.484	4.309	2.514	2.597	2.397	1.235	1.043	0.977	1.049	3.020± 1.361
	Fe	5.330	4.443	6.541	6.667	7.834	6.088	4.356	2.822	1.534	0.690	1.379	1.489	4.097± 1.566
	Pb	4.594	5.466	10.132	9.021	7.063	6.090	6.433	5.234	3.364	2.947	2.533	3.308	5.515± 1.548[a]
	Va	2.584	3.974	3.943	7.548	3.799	2.690	2.764	1.559	1.134	0.898	0.714	0.830	2.703± 1.401
10	Cu	2.811	11.127	6.314	11.069	6.823	4.995	5.447	3.974	3.645	3.357	3.025	3.229	5.484± 1.711[a]
	Cd	2.357	1.054	2.205	2.550	3.633	1.334	2.268	1.192	0.900	0.767	0.752	0.934	1.066± 0.958
	Fe	6.766	7.243	2.890	4.086	7.147	3.571	2.810	2.214	1.725	1.722	1.396	1.577	3.595± 1.496
	Pb	3.397	4.254	2.854	6.400	3.887	5.690	9.453	8.311	5.802	4.533	3.825	4.331	5.228± 1.146[a]
	Va	2.582	1.356	2.076	6.022	2.123	0.806	1.214	1.050	1.123	0.808	0.740	0.896	1.729± 1.224

Values with same superscript are statistically the same at 0.05 level of probability.

Table 3. Effect of different heavy metals on chlorophyll b content of *Barbula lambarenensis*.

Concentration of Metal (mg/l)		Weekly Concentration of chlorophyll (mg/g)												
		Week												
		1	2	3	4	5	6	7	8	9	10	11	12	× ± SD
5	Control	1.620	2.961	2.096	6.0731	5.4733	3.214	3.517	2.426	2.868	3.678	3.519	3.919	3.447± 1.133
	Cu	1.737	5.587	3.144	8.124	5.185	3.013	2.068	2.275	1.832	1.833	1.692	1.596	3.173± 1.436[a]
	Cd	2.298	2.621	2.430	3.241	7.065	3.836	1.781	1.723	0.552	0.119	0.056	0.270	2.166± 1.409
	Fe	1.585	3.029	3.066	3.436	4.671	2.882	2.732	3.275	0.486	0.148	0.148	0.349	2.150± 1.214
	Pb	1.636	3.418	4.043	4.913	4.517	3.079	2.427	2.606	2.177	0.556	0.358	0.489	2.648± 1.249[a]
	Va	0.756	2.676	2.008	4.733	6.859	5.537	2.223	2.448	0.786	0.523	0.523	0.482	2.446± 1.476
10	Cu	1.921	5.911	3.654	5.889	4.803	1.949	4.105	3.815	1.964	1.773	1,570	1.570	3.233± 1.296[a]
	Cd	1.650	1.473	0.874	1.557	3.722	2.706	1.793	1.948	0.916	0.812	0.730	0.838	1.584± 0.947
	Fe	1.559	4.845	0.731	3.686	4.420	3.525	2.447	2.251	1.029	0.573	0.624	1.565	2.271± 1.232
	Pb	1.070	2.879	2.694	4.367	3.628	2.963	5.060	3.509	3.251	1.747	1.460	2.018	2.887± 1.088[a]
	Va	1.790	2.107	0.943	4.068	6.951	3.274	1.348	1.190	0.265	0.249	0.208	0.358	1.895± 1.418

Values with same superscript are statistically the same at 0.05 level of probability.

Table 4. Effect of different heavy metals on total chlorophyll content of *Barbula lambarenensis*.

Concentration of Metal (mg/l)		Weekly Concentration of Total chlorophyll (mg/g)												
		Week												
		1	2	3	4	5	6	7	8	9	10	11	12	× ± SD
5	Control	4.124	7.105	5.389	13.904	13.400	10.846	9.197	7.940	7.577	6.018	5.744	6.326	8.130± 1.770
	Cu	3.912	13.539	7.690	20.145	12.270	9.694	5.427	4.650	4.177	3.584	3.384	3.763	7.686± 2.297[a]
	Cd	6.441	5.553	5.717	8.198	9.727	4.962	3.704	3.488	1.505	0.968	0.859	1.105	4.352± 1.715
	Fe	5.798	6.322	8.093	8.520	10.563	7.558	5.991	5.193	1.695	1.323	1.273	1.539	5.322± 1.786
	Pb	5.234	7.509	11.621	11.758	9.790	7.730	7.447	6.609	4.686	2.928	2.412	1.560	6.632± 1.854[a]
	Va	2.801	5.625	5.018	10.380	9.124	7.054	4.228	3.425	1.626	1.200	0.878	1.108	4.372± 1.785
	Cu	4.004	14.373	8.417	14.306	9.840	5.800	8.091	6.618	4.733	4.014	3.769	4.045	7.334± 1.957[a]
	Cd	3.392	2.157	2.589	3.471	6.254	3.464	3.442	2.686	1.545	1.343	1.260	1.505	2.759±

														1.186
10	Fe	6.967	1.025	3.033	6.599	9.775	6.031	4.462	3.796	2.327	2.075	1.701	2.671	4.971± 1.715
	Pb	3.748	6.035	4.713	9.110	6.383	7.298	12.244	9.947	7.642	5.279	4.443	5.349	6.849± 1.589[a]
	Va	3.692	2.960	2.543	8.536	7.816	3.520	2.181	1.967	1.162	0.888	0.796	1.033	3.091± 1.605

Values with same superscript are statistically the same at 0.05 level of probability.