Lead Uptake and Growth Responses in *Pistia Stratiotes Linn.* (Quiapo)

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Pistia stratiotes was cultured in hydroponics solutions amended with three different lead (Pb) levels - 2ppm, 4ppm and 8ppm [Pb in the form of Pb $(No_3)_2$]. AAS analysis revealed that Pb accumulation in the plant tissue (BCF) increased with increasing Pb levels in the culture solutions. Pb accumulation had caused the development of chlorotic leaves and decreases in the fresh biomas of the plants. However, there was no interference of the Pb accumulation on the growth responses of Pistia in terms of moisture, chlorophyll, and protein contents. These findings imply that P. stratiotes might be useful in heavy metal decontamination process in industrial and domestic wastewaters. These responses also indicate the potential of Pistia as a lead scavenger and bioremediation tool in the aquatic environment.

INTRODUCTION

Water pollution caused by heavy metals and other toxic and persistent chemicals is a serious problem that exists on a global scale. Heavy metals are a danger to the natural ecosystem because they are not degraded under natural conditions. Industrial and domestic wastewaters that usually contain heavy metals and other toxic chemicals are dumped into bodies of water. Unless these wastes are treated at source or prevented from being discharged into courses of water, freshwater quality can be seriously impaired. It has been reported that toxic chemicals can become readily available to plants when they complex with organic compounds.[1] Plants do not have a mechanism to excrete these substances, so they tend to bio-accumulate and are passed on to organisms in the food chain. These materials often reach concentrations that exceed standard guidelines and their toxicity to aquatic life forms may interfere with their various metabolic and physiological processes.[2]

Some hydrophytes are used as scavengers of lead (Pb).[3] Studies conducted show that *Salvinia* sp., floating aquatic fern, *Elodea nuttallii* (hydrilla), a submerged waterweed, and *Eichornia crassipes*, water hyacinth, has been reported as having the capability of uptaking Pb.[2, 4, 5, 6]

Plants are now being programmed as a bioremediation tool to help remove toxic chemicals from contaminated soil and water. Recent researches indicate uptake of heavy metals into plants may provide cost-effective and environment-friendly approach for decontamination.

This study explored the potential lead-scavenging activity of *Pistia stratiotes*, locally known as *quiapo* or water cabbage. *Pistia* is a hydrophyte that belongs to the taro or *Araceae* family. This plant is widely distributed in the tropics and is used as medicine. Although it contains minute stinging crystal, this plant is a good feed for hogs after boiling.[7] A desirable feature of this plant as Pb scavenger is its good rooting system and ability to multiply profusely.

STATEMENT OF THE PROBLEM

This study aimed to determine the potential of *P. stratiotes* to bioaccumulate Pb and its growth responses to high levels of this metal in culture solution.

Specifically, this study sought to:

1. Petermine and compare the amount of Pb accumulated by *Pistia* grown in culture solutions which are amended with three different concentrations of Pb;
2. Identify the effects of Pb accumulation on the leaf morphology of *Pistia*;
3. Identify the effects of Pb on the fresh biomass and water content of *Pistia*;
4. Determine the extent of Pb interference in the growth responses of *Pistia* in terms of chlorophyll and protein contents.

METHODOLOGY

One hundred fifty pieces of healthy *Pistia*, collected at a pond in Logoy, Talon-Talon, Zamboanga City were cultured in hydroponics solutions prepared by adding 10 grams 20-20-20 grower fertilizer to every 100 liters tap water. Ten viable plants of approximately similar size were cultured in $0.01 m^3$ basins containing hydroponics solutions amended with four different levels of Pb [in the form of lead nitrate, $(Pb(NO_3))_2$]: 0, 2, 4, and 8 ppm Pb. The experiments were conducted in four replicates in a greenhouse under natural photoperiod (daylength) of 12 hours and ambient temperatures of 22 to 35 °C for two weeks. Visual symptoms of Pb injury such as, the presence of chlorotic (yellow) leaves and necrotic (small and curly) leaves were monitored during the culture period. After the culture period, the fresh biomass (weight) of whole individual plant was determined. It should be noted that the initial (before culturing) biomass of each plant was determined.

The Pb content of whole plants oven-dried at 60 °C for 24 hours was determined by Atomic Absorption Spectrophotometry (AAS), Method 934.07B.[8] The biological concentration factor (BCF) or the accumulation of Pb in the plant tissue was expressed as the ratio of the Pb content in the plant tissue and in the culture solutions initially.[2]

One gram of ground fresh leaf samples from each experiment was oven-dried to constant weight at 100-105 °C. The weight of the dried sample was determined by using an analytical balance with sensitivity of 0.01 g. The moisture content was analyzed and computed as follows:[9]

$$\% \text{ Moisture} = \frac{\text{Weight of wet sample} - \text{Dry weight}}{\text{Weight of wet sample}} \times 100\%$$

Another ground fresh leaf samples from each experiment was analyzed for chlorophyll content and computed as follows:[9]

$$\% \text{ Chlorophyll} = \frac{\text{Weight of chlorophyll residue}}{\text{Weight of fresh sample}} \times 100\%$$

The protein content of the leaf samples was analyzed by Kjeldhal Block Digestion, Method 984.13.[8] One-way Analysis of Variance (ANOVA) was employed to determine whether there was a significant difference in the moisture, chlorophyll, protein, and Pb contents, as well as biomass, among the plants exposed to different Pb levels. The *t*-test for dependent

samples was computed to determine the significance of the difference in the biomass of the plants before and after the culture period. The 0.05 level of significance was used in the statistical computations, which means that the value of the test statistics is significant when its probability level is lesser than or equal to 0.05.

The biological concentration factor (BCF) or the accumulation of Pb in the plant tissue was expressed as the ratio of the Pb content in the plant and in the medium initially. The visual toxicity symptoms were determined based on the frequency and percentage of chlorotic and necrotic leaves in the plants in each of the experiments.

RESULTS AND DISCUSSION

The results of the AAS analysis show that the *Pistia* grown in the different culture solutions varied in Pb content as indicated by the computed F-value 50.8607 at 0.05 level (Table 1). The *Pistia* exhibited high Pb content

Table 1. Pb content in *P. stratiotes* after the culture period.

Pb Level	Mean (ug/g) dry wt	Standard Deviation	Biological Concentration Factor (BCF)
0	67.4496	19.6933	-
2	1025.5650	343.8192	512.7825
4	2479.1513	740.7927	619.7878
8	6282.8769	1842.8537	785.3596

*The F-Value 50.8607 at 0.05 level is significant

Table 2. Visual toxicity symptoms of *P. stratiotes* exposed to different Pb levels in culture solution.

Pb Level	Total No. of Healthy Leaves Before the Culture Period	Visual Toxicity Symptoms In Leaves After the Culture Period	
		Chlorotic Leaves	Necrotic Leaves
0	450	8%	0 %
2	620	14.03%	0 %
4	623	19.10%	0 %
8	627	22.00%	0 %

*Counts were based on all plants in the four replicates.

with increasing levels of the heavy metal in the culture solutions. The plants in the unsupplemented medium (control or 0) had the lowest Pb content. The ability of *Pistia* to accumulate Pb, as indicated by the biological concentration factor (BCF), also increased with increasing levels of the heavy metal.

Chlorosis was evident in the plants after the culture periods (Table 2). Apparently, chlorosis increased with increasing Pb levels. The plants in the unsupplemented medium had lower percentage of chlorotic leaves. Necrosis, however, was not evident in all plants.

The *Pistia* grown in the different culture solutions had different biomass after the culture period as indicated by the computed F-value 5.0802 at 0.05 level (Table 3). The *Pistia* cultured in the 2 ppm and 4 ppm Pb culture solutions had significantly reduced biomass after the culture period. However, the plants in the 8 ppm Pb culture solutions had the same biomass before and after the culture period. The plants in the unsupplemented medium had significantly higher biomass after the culture period.

The plants grown in the different culture solutions had the same moisture content as indicated by the computed F-value of 0.8959 at 0.05 level (Table 4). The plants in the unsupplemented medium also had no significant difference in the amount of moisture when compared with those cultured in the solutions amended with different levels of Pb.

Table 3. The influence of Pb on the fresh biomass of whole plants after the culture period.

Pb Level	Mean Biomass		Mean Difference
	Before Culture	After Culture	
0	29.4350	30.3417	0.9067
2	30.7313	26.6270	-4.1042
4	28.1993	25.9890	-2.2103
8	29.0885	28.2312	-0.8572

*The F-value 5.0802 and F-Probability .0022 at 0.05 level is significant

Table 4. The influence of Pb on the moisture content of *P. stratiotes* after the culture period.

Pb Level	Mean	Standard Deviation
0	93.3597	0.5268
2	93.3747	0.5421
4	92.7396	1.7079
8	93.4384	0.3106

*The F-value 0.8959 at 0.05 level is not significant

Table 5. The influence of Pb on the chlorophyll content (%) of *P. stratiotes* after the culture period.

Pb Level	Mean %	Standard Deviation
0	0.3611	0.1222
2	0.4133	0.1700
4	0.4685	0.1000
8	0.4367	0.1227

*The F-value 0.6311at 0.05 level is significant

Table 6. The influence of Pb on the protein content (%) of *P. stratiotes* after the culture period.

Pb Level	Mean %	Standard Deviation
0	2.2233	0.3138
2	2.2475	0.1612
4	2.3288	0.3040
8	2.3200	0.2451

*The F-value 0.2942 at 0.05 level is not significant

The *Pistia* grown in the different culture solutions had the same amount of chlorophyll as indicated by the computed F-value of .6311 at 0.05 level (Table 5). The plants in the unsupplemented medium also showed no significant difference in the amount of chlorophyll when compared with those cultured in the Pb-amended solutions.

The plants cultured in the solutions amended with different Pb levels had the same protein content as indicated by the computed F-value of 0.2942 at 0.05 level (Table 6). The plants in the unsupplemented medium also had the same amount of protein when compared with those cultured in the solutions amended with Pb.

DISCUSSION

The *Pistia*, apparently, can accumulate Pb from the culture solutions as indicated by the presence of heavy metals in the plant tissues. The degree of accumulation increased with increasing Pb levels in the solution. The BCF also increased with increasing Pb levels in the culture solutions. Studies show that the capacity of some hydrophytes to accumulate Pb is related to the apoplastic binding of the heavy metal in their tissues.[10, 11] It is believed that metal tolerance phenomenon is due to an increased capacity of the plants to synthesize metal chelating peptides, such as phytochelatins, which would completely prevent oxidative stress in metal tolerance.[12]

Within the culture period of two weeks, necrosis was not evident; however, chlorosis was noted in all the plants. Chlorosis was lower in plants cultured in the unsupplemented medium but increased with increasing Pb levels in the culture medium. Many chlorotic leaves were observed to have prematurely fallen out of the plant body. Such observations can be due to leaf senescence enhancement resulting from Pb intoxication.[13] It is presumed that chlorosis and necrosis are the results of the impairment of metabolic and nutritional processes of the plants.[6]

Statistical analysis show that the presence of Pb in the culture solutions had interfered with the biomass of *Pistia*. There was a significant decrease in the biomass of the plants cultured in the 2 ppm and 4 ppm. culture solutions. This might be explained by the premature fallout of chlorotic leaves during the culture period. However, the plants in the 8 ppm culture solution had the same biomass before and after the culture period. This observations might indicate the capability of *Pistia* to uptake, bioaccumulate, and yet maintain a normal physiological and metabolic functions. Both the experimental and control plants had the same moisture contents after the culture periods. This implies that the presence of Pb did not impair the ability of the plant to uptake and store water.

The cultured *Pistia* had the same chlorophyll contents, in contrast with the investigation on *Pisum sativum* (pea plants), grown in Pb-amended solution, which revealed a reduction of chlorophyll in the plant.[14] It was also reported that the presence of Pb in leaf tissues could interfere with the initial steps of chlorophyll synthesis.[13]

The plants in the different experiments had the same protein content, indicating that Pb accumulation in the plant tissue had no effects on the plant's ability to synthesize protein. Similar observation was noted in the plants grown in the unsupplemented medium. The results of this experiment is in contrast with the observations that one of the adverse effects of Pb presence in the plant tissue is its capability of binding with delta-aminolevulinic acid dehydrase, an enzyme that catalyzes the initial chemical reaction to produce chlorophyll.[13] The amount of chlorophyll is correlated with the plant's ability to synthesize protein.

CONCLUSION

Based on the results of the study, the following conclusions were drawn:

1. *P. stratiotes* has the capacity to uptake Pb, and the level of accumulation depends upon the concentration of the heavy metal in the culture solution. The biological concentration factor of the heavy metal increased along with the Pb level in the culture solution.

2. In terms of growth responses to Pb accumulation:
   a. There was no interference of Pb accumulation on the biomass of *P. stratiotes*.
   b. There was no interference of Pb accumulation on the moisture content of *P. stratiotes*.
   c. There was no interference of Pb accumulation on the chlorophyll and protein contents of *P. stratiotes*.

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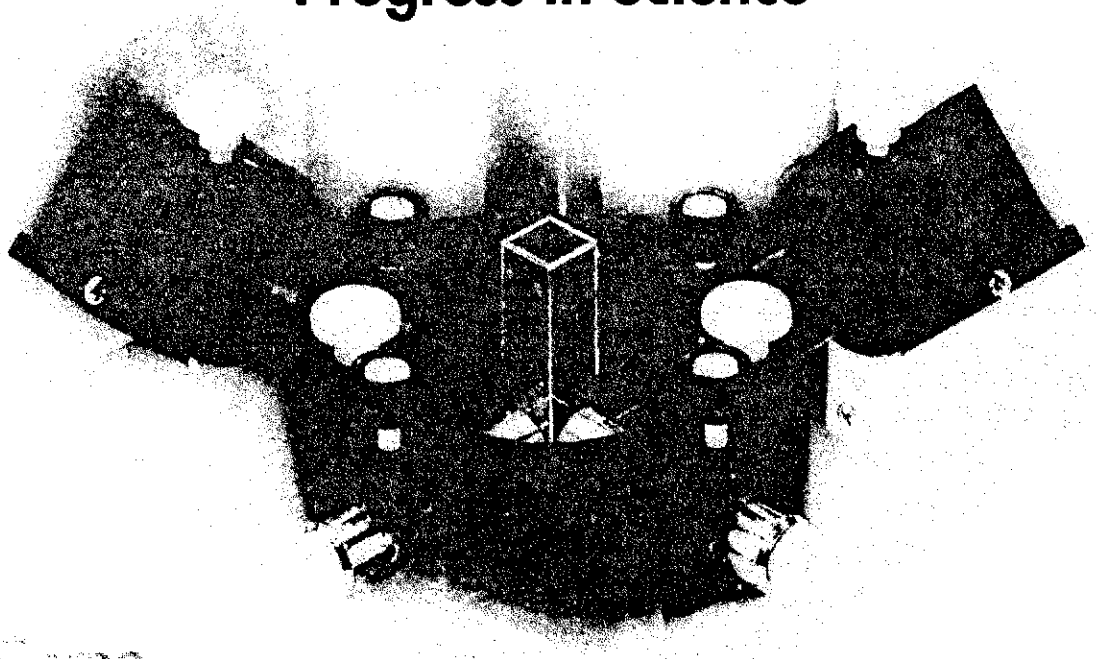

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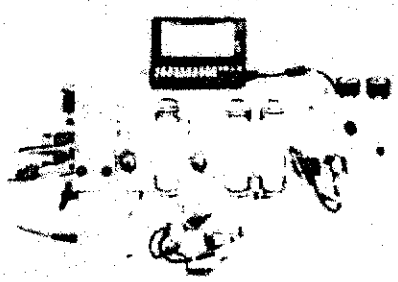

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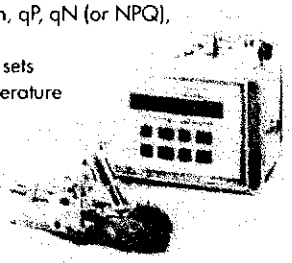

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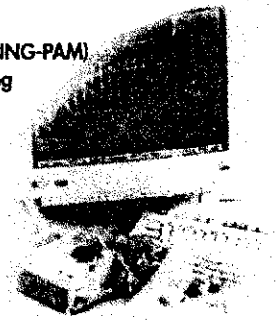

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