

Research Article

Uptake and growth response of water lettuce (*Pistia stratiotes* L.) in lead (Pb)-spiked water

Musa Adamu Ibrahim, Mohammad Sadiu Waziri, Musa Ibrahim, Binta Aliyu Kyari, Bitrus Apagu, Kutarju Jasini Adams

Department of Biological Sciences, University of Maiduguri, P.M.B.1069, Maiduguri, Nigeria

Corresponding author: moosaad8@gmail.com, doi.org/10.55639/607.4252

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ABSTRACT

Discharge of lead (Pb) into water bodies affects water quality, human health and aquatic organisms. Lead is one of the most important toxic heavy metals of environmental concern. This study evaluates the phytoaccumulation rate of water lettuce (*Pistia stratiotes* L.) to [Pb (NO₃)₂] for 40 days. Static water vessels containing various nominal concentrations; 0 mg/L, 25 mg/L, 50 mg/L and 100 mg/L were used in a controlled environment. The results indicated a 21% mean uptake of Pb by *Pistia stratiotes*. There was a strong correlation ($R^2 = 0.996$) between tissue uptake and concentration. Similarly, pH, dissolved oxygen (DO) and electrical conductivity (EC) were concentration-dependent. Chlorophyll contents and biomass reduced significantly ($p < 0.05$) at higher concentrations.

Corresponding author: Musa Adamu Ibrahim, Email: moosaad8@gmail.com
Department of Biological Sciences, University of Maiduguri, P.M.B.1069, Maiduguri, Nigeria

INTRODUCTION

The industrial revolution has increased the generation and discharge of toxic metals into the environment for decades (Obiora *et al.*, 2016; Leblebici *et al.*, 2019). Human and natural processes are the key sources of these toxicants (Obiora *et al.*, 2016; Kumar & Prasad, 2018; Ali *et al.*, 2020). Removal of toxic pollutants is very significant in reducing

the potential effects on human and ecosystem health (Ali *et al.*, 2020). Heavy metals are non-biodegradable and can accumulate at different trophic levels, thereby difficult to remove from living tissue and the environment (Ali *et al.*, 2020). Heavy metals are hazardous toxicants, non-biodegradable and

bioaccumulate in organisms (Victor *et al.*, 2016; Leblebici *et al.*, 2020).

Hyper-accumulators can bioaccumulate metals at about 100 folds concentration compared to non-phyto-accumulator species, and affinity to specific metals varies (Choudhury *et al.*, 2016). Diverse pollutants released usually end up in plants (Pourrut *et al.*, 2011; Shahid *et al.*, 2017; Vareda, *et al.*, 2019). Water lettuce (*Pistia stratiotes* L.) has a short-term vegetative generation in lentic or lotic water (Adomako *et al.*, 2020). The tolerance and bioaccumulation of some heavy metals by *P. stratiotes* to has been reported in different studies (Čadková *et al.*, 2015; Di Luca *et al.*, 2014; Hanks *et al.*, 2015; Lu *et al.*, 2011; Mufarrege *et al.*, 2010; Odjegba and Fasidi, 2004; Vanhoudt *et al.* 2018; Kumar *et al.*, 2018; Lu *et al.*, 2010; 2011). However, some studies reported the uptake and responses of lead by *P. stratiotes* (Vesely *et al.*, 2011; Čadková *et al.*, 2015; Rodrigues *et al.*, 2017; Adomako *et al.*, 2020).

Lead is a highly toxic metal and plants usually absorb it through their roots (Vesely *et al.*, 2012; Vareda *et al.*, 2019; Pires-Lira *et al.*, *et al.*, 2020). There is no known importance of a lead to plants (Vesely *et al.*, 2012). Plants exposed to lead have low biomass (Vesely *et al.*, 2012). *P. stratiotes* has low susceptibility to lead toxicity stress (Vesely *et al.*, 2012). The effect of lead toxicity varies with plant species (Arshad *et al.*, 2008).

Cost-effective methodologies and green technology are required for the remediation of heavy metal-polluted water (Ali *et al.*, 2020). Thus, the objectives of this study were to evaluate the potential phytoaccumulation of lead and eco-physiological stress in water lettuce (*Pistia stratiotes*) in water bodies of arid areas that experience high seasonal temperatures and to provide cost-effective that can be adopted easily by the locals to reduce lead pollution in their waters.

MATERIALS AND METHODS

Collection of *Pistia stratiotes* and exposure site

P. stratiotes of similar sizes were obtained from a pond at the University of Maiduguri Teaching Hospital (UMTH) Staff Quarters. The plants were acclimatized for 12 days before the exposure. The experiment was conducted at the Botanical Garden of the Department of Biological Sciences, University of Maiduguri, Nigeria. A Completely Randomized Design (CRD) with 5 treatments, and 3 replicates containing 3 plants/vessels.

Preparation of Lead (Pb) concentrations and exposure to *P. stratiotes*

The stock was prepared by dissolving 15.98 g of lead (ii) nitrate [Pb (NO₃)₂] in a 1000 mL flask that contained 1% v/v HNO₃ (AnalaR® grade). Other treatments (Control (0), 25, 50 and 100 mg/L) were prepared with distilled water using the dilution principle ($C_1V_1 = C_2V_2$) Where; C_1 = concentration of the stock, V_1 = volume of the stock, C_2 = working concentration to prepare and V_2 = volume of working concentration to prepare (volume of the volumetric flask) (Adesanmi *et al.*, 2020). The initial weight of day zero (day 0) was measured before the exposure. At 40 days post-exposure (dpe), the plants were removed, washed with distilled water, and weighed. Homogenized parts (root and leaves) of the plants were oven-dried for 6 hours at 60°C. The dried biomass was ground to a fine powder using mortar and pestle, sieved (2.2 mm mesh size), and stored in polythene bags before being used for acid digestion.

Digestion of plant tissue for heavy metal analysis

Half a gram (0.5 g) of the dry biomass of different treatments was pulverized and put in porcelain crucibles in a muffle furnace at 450°C for 6 hours until the samples turned to greyish-white ashes. Five millilitres (5ml) of 2N 70% HNO₃ (AnalaR® grade) was added to each sample in a test tube. The solution was filtered

with a filter paper (Whatman No. 450), and transferred into a 100ml graduated flask. Distilled water was added to the mark. The filtrate was transferred to a glass vial. The total lead (Pb) concentration in the samples was analysed using Atomic Absorption Spectrophotometer (AAS) Buck Sci. 210® Model.

Determination of fresh weight and chlorophyll content

At forty days post-exposure (40 dpe), samples from different treatments were collected, blotted with a paper towel and weighed (Mettler electronic balance). Chlorophyll content (chlorophyll-a, chlorophyll-b and total chlorophyll) were determined at 40 dpe using the method of Arnon (1949). Hundred (100) mg of fresh leaves were macerated in 5ml of 80% acetone. The absorbance was measured

with a spectrophotometer at 645nm, 663nm, and 663nm for chlorophyll-a, chlorophyll-b and total chlorophyll, respectively. The concentration was calculated according to the following equations:

$$\text{Chl a } (\mu\text{g/ml}) = 12.7 (A_{663}) - 2.69 (A_{645})$$

$$\text{Chl b } (\mu\text{g/ml}) = 22.9 (A_{645}) - 4.68 (A_{663})$$

$$\text{Total chl } (\mu\text{g/ml}) = 20.2 (A_{645}) + 8.02 (A_{663})$$

Where; chl = chlorophyll, A = absorbance, numbers that followed letters = wavelength (nm).

Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) using statistical software (DSASTAAT® ver.1.101). Duncan’s multiple range test (DMRT) was used to separate the means at $p < 0.05$. The Results are presented as mean ± standard deviation (SD).

RESULTS

Total Pb accumulation in the tissue increased significantly with an increase in the level of

exposure ($R^2 = 0.996$). An average transfer factor (TF) of 21% at termination (40 days post-exposure) was observed (Figure 1).

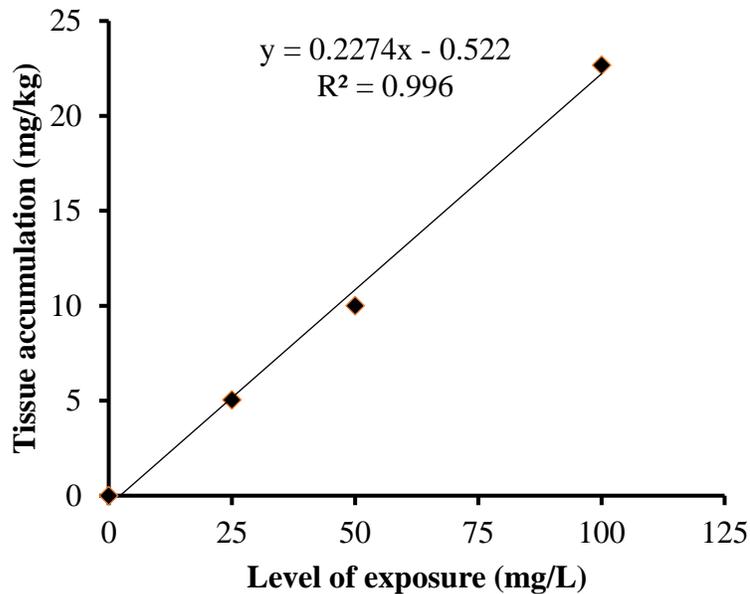


Figure 1: Tissue accumulation of *Pistia stratiotes* to lead (Pb) 40 days post-exposure

There was a significant ($p < 0.05$) decrease in pH at 50 mg/L and 100 mg/L. The treatment

group (25 mg/L) showed a significantly ($p < 0.05$) lower dissolved oxygen (DO) compared

to other treatments. However, the electrical conductivity increased significantly with the increase in concentration (Table 1).

Table 1: Physicochemical parameters of lead-spiked water-containing *Pistia stratiotes* at forty days post-exposure (40 dpe)

Treatment(mg/L)	Parameter ($\bar{X} \pm SD$)		
	pH	DO (mg/L)	EC ($\mu\text{S/cm}$)
0	8.6 \pm 0.00 ^a	3.61 \pm 0.00 ^{bc}	0.59 \pm 0.00 ^c
25	8.5 \pm 0.06 ^a	3.51 \pm 0.08 ^c	0.64 \pm 0.01 ^c
50	8.3 \pm 0.06 ^b	3.87 \pm 0.17 ^a	0.65 \pm 0.02 ^b
100	8.2 \pm 0.17 ^b	3.74 \pm 0.02 ^{ab}	0.68 \pm 0.01 ^a

Means followed by the same superscript in the same column are not significantly different using Duncan's multiple range test (DMRT), $p < 0.05$

The concentration of lead in tissues of the exposed *Pistia stratiotes* at forty days post-exposure (40 dpe) reduced significantly depending on the concentration of lead in the

water. However, the average transfer factor and uptake rate was closely the same across all the treatments (Table 2).

Table 2: Tissue retention of lead by *Pistia stratiotes* (n = 3) at forty days post-exposure (40 dpe)

Treatment (mg/L)	Tissue concentration (mg/kg)	TF	Uptake (%)
0	0.0 \pm 0.00 ^d	–	–
25	5.0 \pm 0.06 ^c	0.20	20.12
50	10.0 \pm 1.15 ^b	0.20	20.00
100	22.7 \pm 1.53 ^a	0.23	22.67

TF = Transfer factor. Means followed by the same superscript within the same column are not significantly different using DMRT, $p < 0.05$.

The chlorophyll contents (a, b and total) were significantly ($p < 0.05$) higher with an increase in treatment concentration. The chlorophyll-a content at 0 and 25 mg/L was significantly higher than other concentrations. However,

chlorophyll-b content decreases with an increase in the concentration of lead. The total chlorophyll contents at 50-100 mg/L were significantly low at $p < 0.05$ (Figure 2).

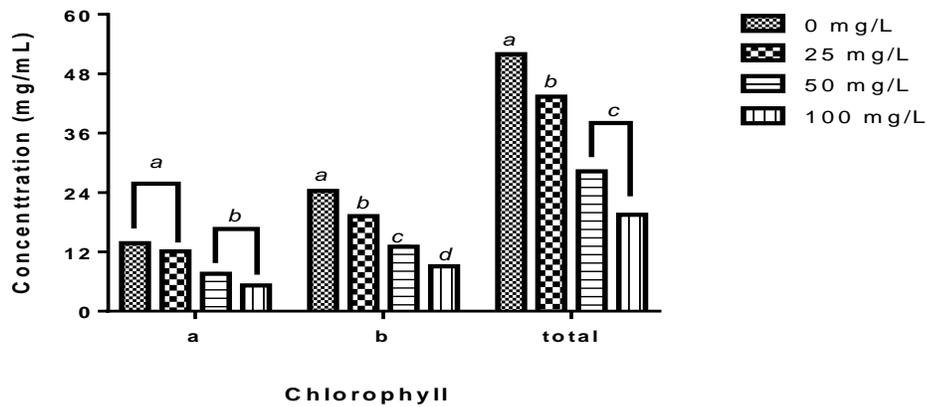


Figure 2: Chlorophyll contents of *P. stratiotes* forty days after exposure (40dpe) to lead-spiked water. **Key:** The same bars with the same letter were not significantly different using DMRT ($p < 0.05$).

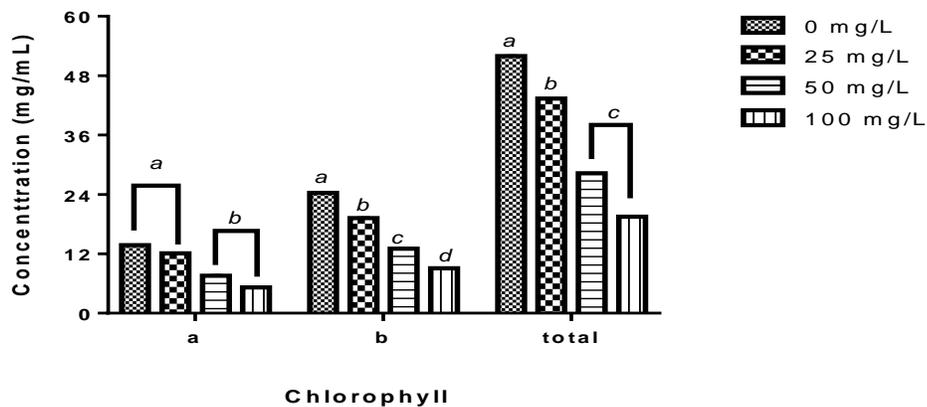


Figure 2: Chlorophyll contents of *P. stratiotes* forty days after exposure (40dpe) to lead-spiked water. **Key:**The same bars with the same letter were not significantly different using DMRT ($p < 0.05$)

The average biomass of the *P. stratiotes* at day one was not significantly ($p < 0.05$) different. However, at 40 days post-exposure, there was a significant increase

in biomass at lower concentrations (0 mg/L and 25 mg/L), while no significant difference was observed at 50 mg/L and 100mg/L (Figure 3).

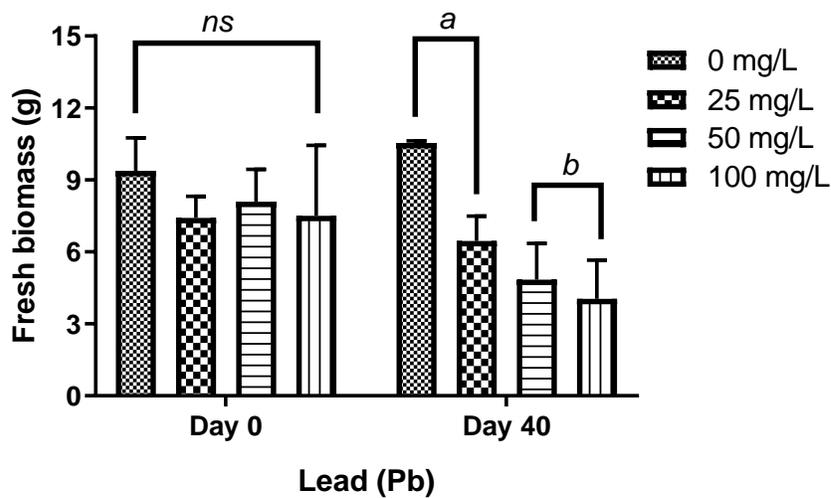


Figure 3: Change in biomass of *P. stratiotes* after exposure to lead-spiked water.

Key: The same bars with the same letter were not significantly different using DMRT ($p < 0.05$). *ns* = not significant.

DISCUSSION

Removal of pollutants from the environment depends on some factors such as exposure time, concentration, temperature and the species of plant (Pourrut *et al.*, 2011). The high tissue uptake and moderate transfer factor indicate tolerance of *P. stratiotes* to lead (Pb) stress at the concentrations used and its phytoremediation potentials in the present study. In a similar study using some floating aquatic plants (*Lemna minor* and *Azolla pinnata*),

Mohamed *et al.* (2021) observed less efficient removal of lead by *Lemna minor* compared to *Azolla pinnata*.

A reduction in plant biomass indicates a disruption of the physiological mechanisms of the plant. Biomass can also be constrained by high doses of lead exposure (Gichner *et al.*, 2008; Islam *et al.*, 2008; Piotrowska *et al.*, 2009; Singh *et al.*, 2010; Vesely *et al.*, 2012). Low biomass indicates disruption of metabolic and photosynthetic activities exerted by heavy metal stress (Das *et al.*, 2014). Similarly, Mohamed *et al.* (2021) reported that *L. minor* has low biomass for some heavy metals

including lead during 20 days of incubation time.

The tissue retention and concentrations tested in this study were contrary to the findings of Mohamed *et al.* (2021) that observed *L. minor* exposed to 25 mg/L lead for 20 days of incubation, which recorded more accumulation rate compared to other higher concentrations; 50 mg/L and 100 mg/L.

In other studies, lead was reported to reduce plant biomass and chlorophyll-a and b in exposed rapeseed and clover showed (Shah *et al.*, 2020). Chlorophyll production, photosynthetic activity, and absorption of other essential nutrients are all affected by a decline in total chlorophyll concentration. (Vesely *et al.*, 2012). It was earlier reported that the reduction of chlorophyll-a and b implies toxicity stress in exposed plants (Das *et al.*, 2014).

CONCLUSION

Though the exposure period was not too long, the uptake rate of lead by *Pistia stratiotes* observed in this study has demonstrated that *Pistia stratiotes* play a significant role in the clean-up of lead-polluted water. The condition and other confounding factors evaluated that are cost-effective can be studied further for optimum phytoremediation. Phyto-extraction and transformation of lead compounds and other inorganic pollutants are environmentally friendly as compared with chemical methods that use chemicals which might be harmful to other components of the environment. These are; high cost, high energy demand/wastage, and requires skilled personnel during operation and maintaining the treatment systems.

CONFLICT OF INTEREST

We declare that there is no conflict of interest.

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