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Morphological and Physiological Responses of Morning Glory (*Ipomoea lacunosa* L.) Grown in a Lead- and Chelate-Amended Soil

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Abstract: Lead (Pb) is one of the most toxic metals in the environment and may cause drastic morphological and physiological deformities in *Ipomoea lacunosa*. The goal of this research was to evaluate some morphological and physiological responses of morning glory grown on a Pb- and chelate-amended soil. Soil samples were analyzed, at Mississippi State University Soil Laboratory, for physico-chemical parameters, such as soil texture (73% sand, 23% silt, 4.4% clay), organic matter (6.24 ± 0.60%), and pH (7.95 ± 0.03), to establish soil conditions at the beginning of the experiments. Five EDTA (ethylenediaminetetraacetic acid) concentrations (0, 0.1, 0.5, 1, 5mM) and four lead (0, 500, 1000, 2000mg/L) treatments were arranged in factorial in a Randomized Complete Block (RCB) design with five replications. Duncan's multiple comparison range test showed that the mean difference values of stomatal density were significant between 500 and 1000mg/L Pb and between 1000 and 2000mg/L Pb. Two way ANOVA (at 1% level) indicated that interaction between Pb and EDTA had a significant effect on the stomatal density and photosynthetic rates, and at 5% level Pb had a significant effect on chlorophyll concentrations. Lowest concentrations of chlorophyll were recorded at 2000mg/L Pb and 5mM EDTA and exhibited a decreasing trend specifically in the ranges of 1000 and 2000mg/L Pb and 1.0 and 5.0mM EDTA. Duncan's multiple comparison range test confirmed that mean differences between the control treatment vs. 2000mg/L Pb, and 500mg/L vs. 2000mg/L Pb were significantly different at $p>0.05$. There was a decrease in leaf net photosynthetic rate with increasing concentrations of Pb from 0 to 2000mg/L. In conclusion, *I. lacunosa* L. plants were grown to maturity in all treatments with no significant and/or apparent morphological disorders, which indicated that this species might be highly tolerant even at 2000mg/L Pb concentrations in the soil.

Keywords: Lead, EDTA, phytoremediation, chlorophyll, stomata, photosynthesis, soil.

Introduction

Lead (Pb) has been used in various forms by ancient civilizations; hence most of its contamination to our environment is anthropogenic. Due to its history as an air emission pollutant, lead has been fairly mobile and is particularly soluble in acid environments [1]. Different plants have different tolerance limits for a variety of elements (including the trace elements). The toxic effect of heavy metals such as Cu, Pb, Cd, Ni on photosynthesis and plant metabolism is well documented [2]. The effects of Pb treatment on thylakoid development in *Populus alba* (poplar) and *Cucumis sativus* (cucumber) plants and growth responses in radish (*Raphanus sativus*) plants are documented in the scientific literature [3, 4]. Decreased levels of chlorophyll were observed in *Brassica oleracea* (cabbage) exposed to <2.5mg/L of copper [5]. For zinc and copper, most vascular plant species exhibited toxicity symptoms at low concentrations ranging from 0.1 to 2.0 mg/L zinc and from 0.02 to 0.06 mg/L copper [6]. Significant reduction in chlorophyll concentrations were reported in most metal (cadmium and lead) treated radish plants [4]. Duckweed plant exhibited some symptoms of toxicity such as reduced growth and chlorosis at higher levels of metal supply, except for Cr [7].

The specific objectives of this study were to evaluate some morphological (necrosis, chlorosis, curling of leaves, stunted growth) and physiological (chlorophyll concentrations and photosynthetic rates) responses of morning glory grown on a Pb- and chelate-amended soil. Data generated will be utilized to further assess the suitability of this species for phytoextraction of Pb-contaminated soils.

Materials and Methods

Experimental Design and Soil Preparation and Analysis

Seeds of morning glory (*Ipomoea lacunosa* L.) were obtained from Azlin Seeds, Leland, MS. Plants were grown in the greenhouse at Jackson State University. Several bags (~18.5kg/bag) of potting mix and delta topsoil were purchased from Hutto's Garden, Jackson, MS and air-dried for 3-4 days under greenhouse conditions. Based from a preliminary experiment, germination and growth of plants were highly responsive to a growth medium consisting of 2/3 potting mix and 1/3 delta topsoil. Plastic pots (1.9L) were filled with approximately two kg of growth medium. A 7-inch plastic saucer was placed beneath each pot to prevent cross contamination among treatments. Any leachate collected in each saucer was poured back into its corresponding pot. Periodically, these saucers were also rinsed with deionized distilled water and were poured back into the respective pots.

Soil samples were analyzed, at Mississippi State University Soil Laboratory, for physico-chemical parameters, such as soil texture (73% sand, 23% silt, 4.4% clay), organic matter (6.24 ± 0.60%), and pH (7.95± 0.03) to establish the soil conditions at the beginning of the experiments. Five EDTA (ethylenediaminetetraacetic acid) concentrations (0, 0.1, 0.5, 1.0, 5mM) and four lead (0, 500, 1000, 2000mg/L) treatments were arranged in factorial in a Randomized Complete Block (RCB) design with five replications. For each pot, 250mL of the respective concentrations of lead and EDTA were added as aqueous solutions prior to sowing. Five germinated (~5mm radicle) seeds of *I. lacunosa* L. were planted in each pot and were thinned to two plants per pot one week after planting. Plants were periodically watered with 100mL of deionized distilled water for the first two weeks. Beginning at third week of growth, all plants were watered twice a week with 100mL of modified Hoagland's nutrient solution [8]. When the plants were approximately 4-5 weeks old, 250mL of nutrient solution were added 3-4 times a week until the time of harvest. Plants were harvested at 7 weeks after emergence (i.e., at the time of flowering and fruiting).

Chlorophyll Analysis and Photosynthetic Rates

Leaf samples were collected randomly from each treatment and immersed in test tubes filled with water. Experimental peels were obtained from the lower surface of the leaves, prepared as wet mounts and were viewed through an Olympus AH-2 VANOX microscope under 40*10X magnification to obtain stomatal density. Pictures of the stomatal density in each leaf material under different treatments per focal area (frame) were taken. Leaf samples of *L. lacunosa* were collected from different treatments using #4 (7mm diameter) cork borer. A total of ten discs from each treatment and two discs from each leaf were collected for chlorophyll analysis. Leaf discs were obtained from the 4^{th} node, 10^{th} node, and from the uppermost node of the plant. Two leaves from each node and one leaf from the top, uppermost node of the plant were selected. Chlorophyll from leaves was extracted following a standard method [9]. Net photosynthetic rates (μmolCO_2 $m^{-2}s^{-1}$) of selected leaves of *L. lacunosa* were measured using a LiCOR-6200 portable photosynthesis system (LI-COR, Inc., Lincoln, NE). Leaves of similar morphology and position on the stem were used in each measurement. Photosynthetic measurements were made between 10:00 to 13:00 hours (CDT) when the prevailing light intensity was >1600 [μmol photons $m^{-2}s^{-1}$ (PAR, 400-700nm)].

Statistical Analysis

Data were analyzed using ProStat software (version 1.04 for windows 95, 32 bit edition, 1996) to determine means, standard deviations (SD), standard errors (SE), two-way ANOVA with replications, multiple comparison tests (Duncan's Test), and correlations.

Results

Stomatal Density

Stomatal densities on leaf surfaces of *I. lacunosa* L. were significantly affected by different Pb and EDTA treatments. Results on stomatal density are summarized in Table 1 and in Fig. 1A. Minimum number of stomata was observed under 1000mg/L Pb and 5mM EDTA. Duncan's multiple comparison range test showed that the mean difference values of stomatal density were significant between the 500 and 1000mg/L and 1000 and 2000mg/L Pb treatments in 1 and 5mM EDTA amended soils. The two way ANOVA test also confirmed the differences between Pb treatments, and the interaction between Pb and EDTA for stomatal density was significant at the 1% level.

Table 1: Effects of Pb and EDTA concentrations on stomatal density (no./mm^2) of *I. lacunosa* L. leaves; two-way ANOVA with replication.

Source	*DF*	*SS*	*MS*	*F-Value*	*P-Value*
EDTA (mM)	4	5.7	1.425	1.513	0.210
Pb (mg/L)	3	23.7	7.900	8.389*	0.000
EDTA vs. Pb	12	149.3	12.442	13.212*	0.000
Between Groups	19	178.7	9.405		
Error	60	56.5	0.942		
Total	79	235.200	2.977		

* significant at 1% level.

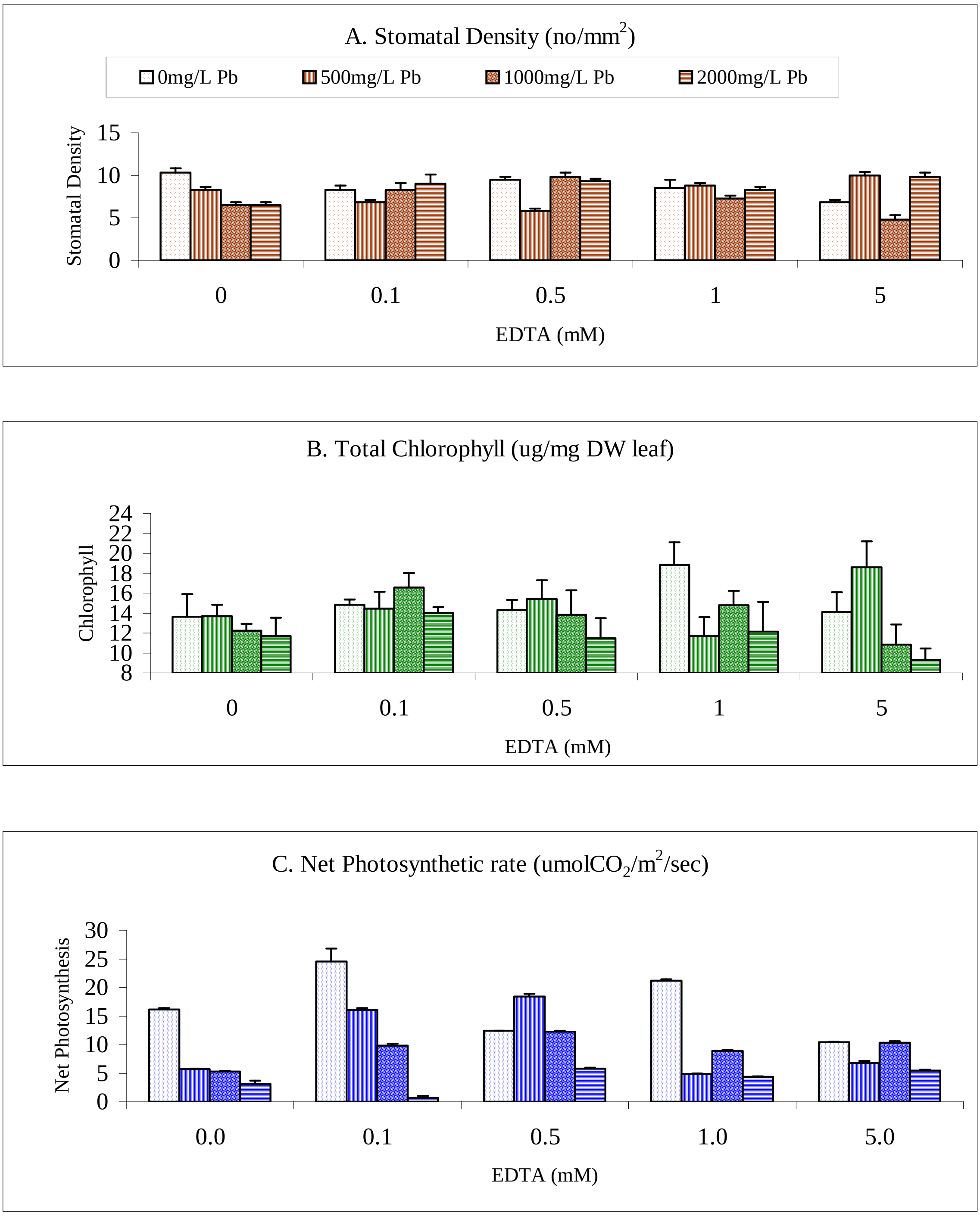

Fig. 1: Effects of various concentrations of Pb and EDTA on (A) stomatal density, (B) total chlorophyll, and (C) the net photosynthetic rate of *Ipomoea lacunosa* (L.). Vertical bars indicate standard error of the mean of 4 replications.

Chlorophyll

The effects of Pb and EDTA concentrations on total chlorophyll contents of *L. lacunosa* leaves are presented in Fig. 1B and Table 2. The two way ANOVA test indicated that Pb had a significant effect on chlorophyll concentrations at the 5% level of significance. Lowest concentrations of chlorophyll were recorded at 2000mg/L Pb and 5mM EDTA and exhibited a decreasing trend specifically between 1000 and 2000mg/L Pb and 1.0 and 5.0mM EDTA. The Duncan's multiple comparison range test confirmed that mean differences between the control treatment vs. 2000mg/L Pb, and 500mg/L vs. 2000mg/L Pb were significantly different at $p<0.05$.

Table 2: Effects of Pb and EDTA concentrations on total chlorophyll (µg/mg dry weight of leaf) of *I. lacunosa* L.; two-way ANOVA with replication.

Source	*DF*	*SS*	*MS*	*F-Value*	*P-value*
EDTA (mM)	4	47.58	11.89	0.893	0.474
Pb (mg/L)	3	140.57	46.85	3.518*	0.020
EDTA vs. Pb	12	251.500	20.95	1.574	0.124
Between Groups	19	439.665	23.14		
Error	60	799.110	13.31		
Total	79	1238.775	15.681		

*significant at 5% level.

Photosynthesis

Results indicated that there was a decrease in leaf net photosynthetic rate with increasing concentrations of Pb from 0 to 2000mg/L (Fig. 1C, Table 3). Two-way ANOVA results indicated that the effect of EDTA, Pb concentrations, and the interaction between Pb and EDTA on net photosynthesis were highly significant at the 1% level. Results also showed that within each Pb treatment, there was a decreasing photosynthetic rate as EDTA concentration increased. Levitt (1980) described metals in the environment as stress factors in that they cause physiological reaction changes which may result in the total inhibition of plant growth. He also indicated that resistance to heavy metals can be achieved by tolerance mechanisms which allow a plant to survive the effects of internal stresses associated with such heavy metals.

Table 3: Effects of Pb and EDTA concentrations on leaf net photosynthetic rate (µmol $CO_2 m^{-2} sec^{-1}$) of *I. lacunosa* L. leaves (two-way ANOVA with replication).

Source	*DF*	*SS*	*MS*	*F-Value*	*P-value*
EDTA (mM)	4	257.122	64.28	64.163*	0.000
Pb (mg/L)	3	1297.766	432.58	431.797*	0.000
EDTA vs. Pb	12	776.295	64.69	64.573*	0.000
Between Groups	19	2331.183	122.69		
Error	40	40.073	1.00		
Total	59	2371.256	40.191		

* significant at 1% level.

Discussion

Tolerance is conferred by the possession of special physiological mechanisms, which collectively enable a plant to function normally even in the presence of high concentrations of potentially toxic elements [10]. *Ipomoea lacunosa* L. may be one of these metal-tolerant plant species, which in our experiments survived and continued normal growth and development even at 2000mg/L Pb and 5mM EDTA amendments to the soil. Pb-chelates have been shown to accumulate in interveinal areas of dicot leaves specifically in the anticlinal walls of the leaf epidermis [11]. It is possible that this Pb-chelate deposition may have direct toxic effects on stomatal ontogeny, thereby reducing stomatal density. A decrease in stomatal density can lead to reduced stomatal conductance. Reduced stomatal conductance has been shown in most cases to reduce photosynthetic rate because of a reduction in the supply of CO_2 for the rubisco enzyme [12, 13]. Duckweed has been shown to exhibit some deleterious consequences of toxicity such as chlorosis at higher levels of Pb, Cu, Cd, and Ni [7]. There were no visible signs of metal-induced toxicity (chlorosis) in the present study even though there was a decrease in leaf total chlorophyll contents at 1000 and 2000mg/L Pb treatments in combination with 1 and 5mM EDTA (Figure 1B). In a similar study, it was found that there was no significant reduction in total chlorophyll content in Pb-treated (500-1000ppm Pb) radish plants [4].

The effects of toxic metals on chlorophyllase (an enzyme which decomposes chlorophyll) activity is significant. Some metals such as Cd, Pb, Mn, Co, Ni and their mixture enhanced the activity of chlorophyllase on chlorophyll-a decomposition more than that on chlorophyll-b [14]. Other studies have also reported Pb poisoning of leaves leading to chlorosis in plants growing on metalliferous mine sites [15, 16]. The direct effects of Pb or oxidative stress due to the increased iron concentration may be responsible for changes in thylakoids of cucumber, while the Pb (at higher concentrations)-induced Mn deficiency may be connected with stress-induced changes in poplar plants. If stomatal density and chlorophyll contents are decreased at high Pb levels, there is reduced stomatal CO_2 conductance and PAR absorbance by leaves [3]. Reduced stomatal conductance has been shown in most cases to reduce photosynthetic rate because of less CO_2 for the rubisco enzyme [12, 13, 17]. Carbon dioxide is one of the major factors that dictates photosynthetic rate in plants, and directly depends upon the stomatal aperture and density. In the present study, stomatal density was reduced at higher concentrations of Pb and EDTA, which might have had an impact on the availability of CO_2 and the associated depression in leaf net photosynthetic rates at these levels of Pb and EDTA. Results of the present study show the link between stomatal density, total chlorophyll, and leaf net photosynthetic rate (Fig. 1A-C), all of were low at the higher concentrations of Pb (1000, 2000mg/L) and EDTA (1.0, 5.0mM). This indicates that Pb had either a direct or indirect toxic effect resulting in the reduction of number of stomata, leaf chlorophyll content, and leaf net photosynthesis, by itself or in association with other elements (synergistic effect) in the nutrient medium, which could not be determined based on current research results. Metals such as Cu, Pb, Cd, and Ni have an effect on metal-sensitive processes such as photosynthesis [2]. A previous study also demonstrated that Pb depressed photosynthesis in corn and soybean but did not indicate clearly the mechanism of Pb toxicity on photosynthesis [18].

Conclusion

Ipomoea lacunosa L. plants were able to grow to maturity in all treatments with no significant and/or apparent morphological disorders. This indicated that this species is highly tolerant to lead even at 2000mg/L Pb concentrations in soil. With its demonstrated tolerance to moderate levels of Pb, this species can be further evaluated for its suitability as a potential species for phytoextraction of Pb-contaminated soils.

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