

Phytoextraction of Lead Using a Hedge Plant [*Alternanthera bettzickiana* (Regel) G. Nicholson]: Physiological and Biochemical Alterations through Bioresource Management

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Abstract: Phytoremediation is a cost-effective and environmentally friendly approach that can be used for the remediation of metals in polluted soil. This study used a hedge plant—calico (*Alternanthera bettzickiana* (Regel) G. Nicholson) to determine the role of citric acid in lead (Pb) phytoremediation by exposing it to different concentrations of Pb (0, 200, 500, and 1000 mg kg^{−1}) as well as in a combination with citric acid concentration (0, 250, 500 μM). The analysis of variance was applied on results for significant effects of the independent variables on the dependent variables using SPSS (ver10). According to the results, maximum Pb concentration was measured in the upper parts of the plant. An increase in dry weight biomass, plant growth parameters, and photosynthetic contents was observed with the increase of Pb application (200 mg kg^{−1}) in soil while a reduced growth was experienced at higher Pb concentration (1000 mg kg^{−1}). The antioxidant enzymatic activities like superoxide dismutase (SOD) and peroxidase (POD) were enhanced under lower Pb concentration (200, 500 mg kg^{−1}), whereas the reduction occurred at greater metal concentration Pb (1000 mg kg^{−1}). There was a usual reduction in electrolyte leakage (EL) at lower Pb concentration (200, 500 mg kg^{−1}), whereas EL increased at maximum Pb concentration (1000 mg kg^{−1}). We concluded that this hedge plant, *A. bettzickiana*, has the greater ability to remediate polluted soils aided with citric acid application.

Keywords: lead; *Alternanthera bettzickiana*; citric acid; phytoremediation; antioxidants

1. Introduction

Industrial and anthropogenic deeds have led to an augmented proclamation of numerous heavy metals, including lead (Pb) persistence in the environment for a long duration, which is why Pb is considered one of the most lethal pollutants [1]. Lead is released in the environment from diverse anthropogenic activities like electric batteries, burning

of coal, gasoline, metallurgical, and paint explosives activities [2]. Whereas the amount of Pb released in the environment is comparatively more than natural release. Nearly 333 times more amount of Pb is released by human activities than natural discharge [3]. Ecological accomplishments such as windblown dust, forest fires, volcanic eruption, and sedimentary rocks are classified as regular causes of Pb contamination in soil. Lead contamination has become a worldwide issue due to its diligence in the environment and the toxicological concerns for wildlife, plants, and human populations [4]. Both types of activities, natural and anthropogenic, such as disposal from municipal sewage sludge, mining practices, fertilizers application, industrial fumes, storage batteries, forest fires, volcanic eruption, smelting of ores, paints, igneous rocks, gasoline, and explosives are the most important contributors of Pb in the environment [5]. Inorganic Pb cannot be degraded even at lower concentration and it is also collected from cultivated soils because different plant organs take it up. However, Pb entered the food chain through marine species while sea animals' uptake Pb from wastewater and accumulate in their body. As a result, consumers are at a high level of health risk [6].

The toxicity of Pb enhances the harmful impacts on plants; affects the defense system and photosynthesis [7]. Antioxidant enzymes like peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) considerably improved cellular redox homeostasis at a better point under Pb stress [8]. This is because the toxicity of lead overproduction of ROS agitates the redox equilibrium [7,9]. The phytoremediation process is the most important for contaminated soil through which many plants can uptake metals and detoxify the pollutants [10,11]. Green plants are used in phytoremediation and their related microorganisms to eradicate environmental impurities that make them inoffensive [12]. These plant-based techniques are used to improve environmental clean-up [13,14]. As associated with outdated physicochemical techniques, like chemical leaching or soil removal, which are costly and cause deleterious ecological effects, the phytoremediation method is possibly effective, cheap, also eco-friendly for renovating contaminated soils. This technology is less expensive rather than most in situ traditional processes [15]. In this technique, metal ions, immersion takes place by plant roots and later move to plant shoots, monitored by shoot yield also discarding [16].

Hence, hyperaccumulator plants that have suitable features for particular soil pollutants are essential to virtually relate phytoremediation techniques. The *Alternanthera bettzickiana* plant was selected for the present study. In tropical America, *A. Bettzickiana*, also known as the calico plant, is a low statured inhabitant plant that is famous for its cultivation as a hedge/border plant [17,18]. It is an annual, ground cover, tender, and tropical perpetual herb 15–30 cm in height. Its selection was based upon its elevated biomass production and capability to grow in different soil textures and pH levels. Furthermore, it is documented that the maximum salt tolerance of *A. Bettzickiana* is reflected by its physiological characteristics. Application of various inorganic and organic chelators, phytoextraction process potential, can be improved which enhances the mobility and availability of Pb [19]. Therefore, chelators also improved the micronutrient taken up by plants as part of their regular physiological actions, which are helpful to plants and also help them to grow with metal toxicity [20,21].

Citric acid (CA) is a naturally occurring, easily biodegradable, and cost-effective low molecular weight organic acid, which can be used as an alternate to synthetic ethylenediaminetetraacetic acid (EDTA) and can greatly enhance metal solubility in the solution medium and subsequent uptake by plant tissues [22–24]. The efficacy of CA for assisting metal ions uptake by plant roots and enhancing the translocation to aboveground plant tissues has already been well documented in the literature [25,26]. Limited information exists concerning the utilization of CA as a chelating agent for phytoextraction of Pb with *L. minor*—common duckweed [27] and some other plants. Further, the type of plant species also affects its applicability through root exudates and inherent metal (e.g., Cd) accumulation traits [28]. However, no such study has been conducted involving *Alternanthera bettzickiana* as a phytoextraction plant. Therefore, the objective of the present study was

to evaluate if CA can enhance the phytoextraction potential of *Alternanthera bettizianna*. The study examined the natural as well as CA-assisted phytoextraction potential of *Alternanthera bettizianna* for remediation of Pb contaminated soils. The study results help in elucidating the phytoextraction potential of CA and onward application of this technology to remediate Pb polluted soils under field conditions. Due to its biodegradable nature, CA application would ultimately serve as an environmentally friendly and sustainable strategy to manage soils contaminated with Pb.

2. Materials and Methods

2.1. Experimental Site

Soil samples were collected from the field of the University of Agriculture, Faisalabad, Pakistan. The random samples of soil were mixed, and a homogenous compost sample was prepared. The experiment was carried out in the Botanical Garden of GC University Faisalabad (GCUF). The soil was grounded with a wooden roller, sieved through 2 mm, and systematically mixed. The physico-chemical characteristics of the soil used in this experiment are presented in Table 1. The parameters employed here were measured using the methods described by [29].

Table 1. Soil physico-chemical properties.

Texture	Clay Soil
Sand (%)	21.0
Silt (%)	16.0
Clay (%)	63.0
EC _e (dS m ⁻¹)	2.79
pH (1: 2.5 soil to water ratio)	7.86
Organic matter (%)	0.54
SAR (mmol _c ⁻¹) ^{1/2}	6.27
HCO ₃ ⁻ (mmol L ⁻¹)	3.33
Available P (mg kg ⁻¹)	2.21
SO ₄ ²⁻ (mmol L ⁻¹)	6.38
Cl ⁻ (mmol L ⁻¹)	2.29
K ⁺ (mmol L ⁻¹)	0.04
Na ²⁺ (mmol L ⁻¹)	3.52
Ca ²⁺ + Mg ²⁺ (mmol L ⁻¹)	3.61
Available Zn ²⁺ (mg kg ⁻¹)	0.76
Available Cu ²⁺ (mg kg ⁻¹)	0.21
Available Pb ²⁺ (mg kg ⁻¹)	0.43

2.2. Experimental Design and Treatments

The experiment was arranged in a complete randomized design where three replicates of lead (Pb) concentrations viz. 0, 200, 500, and 1000 mg kg⁻¹ of soil, the sieved soil were spiked. All plastic pots were filled with 3 kg of clay soil that was saturated from top to bottom with normal irrigation water. The preferred moisture level was maintained at 70%. Citric acid levels (0, 250, 500 µM) were applied four times with an interval of seven days. Four seedlings of *Alternanthera bettizianna* (having a similar size) were grown in each pot. After 15 days of growth of these seedlings, fertilizers were applied in the form of urea, diammonium phosphate (DAP), and potassium bisulfate (K₂SO₄) which was applied as 2.19, 1.36, and 2.40 g per pot, respectively. In the case of controlled plants, the plants were irrigated with tap water as and when required.

2.3. Harvesting of the Plants

After a growing period of 90 days, the plants were harvested. After harvesting, the roots, leaves, and stems were separated. The samples were then first rinsed with normal water, then with 2% HCl solution, and finally with purified water to remove any aerial deposition. The roots and shoots were oven-dried at 70 °C for 48 hours to get constant roots and shoot dry weights and roots dry weight and shoot dry weight were recorded.

2.4. Determination of Pigment Contents

The top-most fully expanded leaves were used to extract the pigments. After eight weeks of the application of Pb toxicity, the plants were harvested for the determination of enzymes such as chlorophyll and carotenoids and were determined by spectrophotometer [30]. The photosynthetic contents were extracted from a known fresh weight of leaves in 85% (v/v) aqueous acetone. The extract was centrifuged at 4000 rpm for 10 min; the supernatant was then taken and diluted with 85% aqueous acetone to get a suitable concentration for spectrophotometric measurements. The extinction was measured against a blank of pure 85% aqueous acetone at a wavelength of 452, 644, and 663 nm.

2.5. Determination of Electrolyte Leakage

The electrical conductivity of leaf samples was determined while *A. bettzickiana* was growing and the electrical conductivity of the leaf samples was measured using the electric conductivity meter. We measured the initial EC₁ and final EC₂. Finally, EL was calculated through the described method by [31]:

$$EL = (EC_1/EC_2) \times 100$$

2.6. Determination of Antioxidant Enzymes and ROS

Antioxidant enzymes such as SOD, POD, APX, CAT, MDA, and H₂O₂ in roots and leaves of plants were determined using a spectrophotometer [4,31]. The leaves and roots of the plants were sampled for enzymatic analysis after 8 weeks of application of treatments. The samples were homogenized in 0.05 M phosphate buffer (pH 7.8) by grinding with a mortar and pestle under a chilled condition with liquid nitrogen. The homogenate was filtered through four layers of muslin cloth and centrifuged at 12,000 rpm for 10 min at 4 °C, and the supernatants were used for measurements of SOD and POD activities.

2.7. Determination of Catalase

CAT, EC activity was determined according to methods [32]. The assay mixture (3.0 mL) consisted of 100 µL enzyme extract, 100 µL H₂O₂ (300 mM), and 2.8 mL 50 mM phosphate buffer with 2mM EDTA (pH 7.0). The CAT activity was assayed by monitoring the decrease in the absorbance at 240 nm because of H₂O₂ disappearance ($\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$).

2.8. Determination of Ascorbate Peroxidase

Ascorbate peroxidase (APX) activity was assayed according to the method described [33]. The reaction mixture consisted of 100 µL enzyme extract, 100 µL ascorbate (7.5 mM), 100 µL H₂O₂ (300 mM), and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). The oxidation of ascorbate was determined by the change in the absorbance at 290 nm ($\epsilon = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$).

2.9. Determination of Hydrogen Peroxide and Lead Contents

H₂O₂ was extracted by homogenizing 50 mg leaf or root tissues with 3 mL of phosphate buffer (50 mM, pH 6.5). Then, the homogenate was centrifuged at 6000 rpm for 25 min. To measure H₂O₂ content, 3 mL of extracted solution was mixed with 1 mL of 0.1% titanium sulfate in 20% (v/v) H₂SO₄, and the mixture then centrifuged at 6000 rpm for 15 min. The intensity of the yellow color of the supernatant was measured at 410 nm. H₂O₂ content was computed by using the extinction coefficient of $0.28 \text{ } \mu\text{mol}^{-1} \text{ cm}^{-1}$. Pb contents

in root, stem, and leaves were determined by flame atomic absorption spectrometry, and data were analyzed by using an appropriate statistical package [31]. The digestion of plant samples was done on a hot plate by adding HClO_4 and HNO_3 in a given ratio (1:5) and final Pb contents were recorded by atomic absorption spectrophotometer.

2.10. Statistical Analysis

The three replicate means were used of all values which were used in this study. By the use of (SPSS, IBM, 2009) with version 16.0, the ANOVA was applied. Tukey's post-hoc test was applied to different treatment means to check the standard deviation and significant difference.

3. Results

3.1. Plant Growth and Biomass

The difference in *A. Bettzickiana* growth and biomass parameters, including plants height, roots length, leave the area, the number of seedlings per plant, and dry weights of root and shoot, is given in Figure 1. *A. Bettzickiana* plant presented an important effect of Pb stress during the experiment period dry biomass and growth increased. A decreased plant height, dry biomass, and the leaf area of plants were allied with the maximum application of Pb. At maximum level of CA (500 μM), the plants height increased by 35, 82, 77, 43% and leaf area increased by 32, 97, 71, 64% at Pb levels (0, 200, 500, 1000 mg kg^{-1}), respectively. Furthermore, shoot dry weight increased by 33, 106, 92, 81% and root dry weight increased by 63, 247, 190, 132% at Pb levels (0, 200, 500, 1000 mg kg^{-1}), respectively at CA level (500 μM).

3.2. Photosynthetic Pigment

The effect of CA on *A. bettzickiana* under Pb stress, chlorophyll contents, and carotenoids was measured and presented in Figure 2. All the chlorophyll contents were significantly increased with increasing concentration of CA. At maximum level of CA (500 μM), *Chl a* increased by 44, 141, 118, 77% and *Chl b* increased by 42, 100, 63, 44% at Pb levels (0, 200, 500, 1000 mg kg^{-1}), respectively. Furthermore, carotenoids increased by 32, 117, 62, 50% at Pb levels (0, 200, 500, 1000 mg kg^{-1}), respectively at CA level (500 μM).

3.3. Plant Physiological Parameters (Concentration of H_2O_2 and MDA) and Electrolyte Leakage

The data regarding H_2O_2 in the leaves, roots presented in Figure 3, depict that a significant reduction in root and leaf H_2O_2 was recorded with an increase in CA at all Pb levels. The electrolyte leakage was also controlled with the presence of CA in the rooting media. EL decreased with the addition of CA at all Pb levels (Figure 3). The same is true with MDA in leaves and roots of *A. bettzickiana*. The physiological responses of *A. bettzickiana* under HMs stress have not been evaluated extensively in the literature. However, phytoextraction potential, physiological, and biochemical response of various plant species against HMs stress is not fully understood. Among other HMs, lead (Pb) is an inorganic pollutant with deleterious biotic effects.

3.4. Activities of Antioxidant Enzymes and ROS

Enzymatic activities such as POD and SOD of *A. Bettzickiana* in leaves and roots were without Pb and with CA in the soil. Antioxidant enzyme activities in roots and leaves considerably improved at Pb (500 mg kg^{-1}) and then reduced at Pb concentration (1000 mg kg^{-1}) in Figure 4. At maximum level of CA (500 μM), SOD in root increased by 51, 90, 133, 77% at Pb levels (0, 200, 500, 1000 mg kg^{-1}). Furthermore, POD in root increased by 38, 67, 79, 60% at Pb levels 0, 200, 500, 1000 (mg kg^{-1}), respectively at CA level 500 μM . The electrolyte leakage (EL) was significantly decreased with increasing concentration of CA. At maximum level of CA (500 μM), EL in root decreased by 16, 39, 36, 20% at Pb levels (0, 200, 500, 1000 mg kg^{-1}).

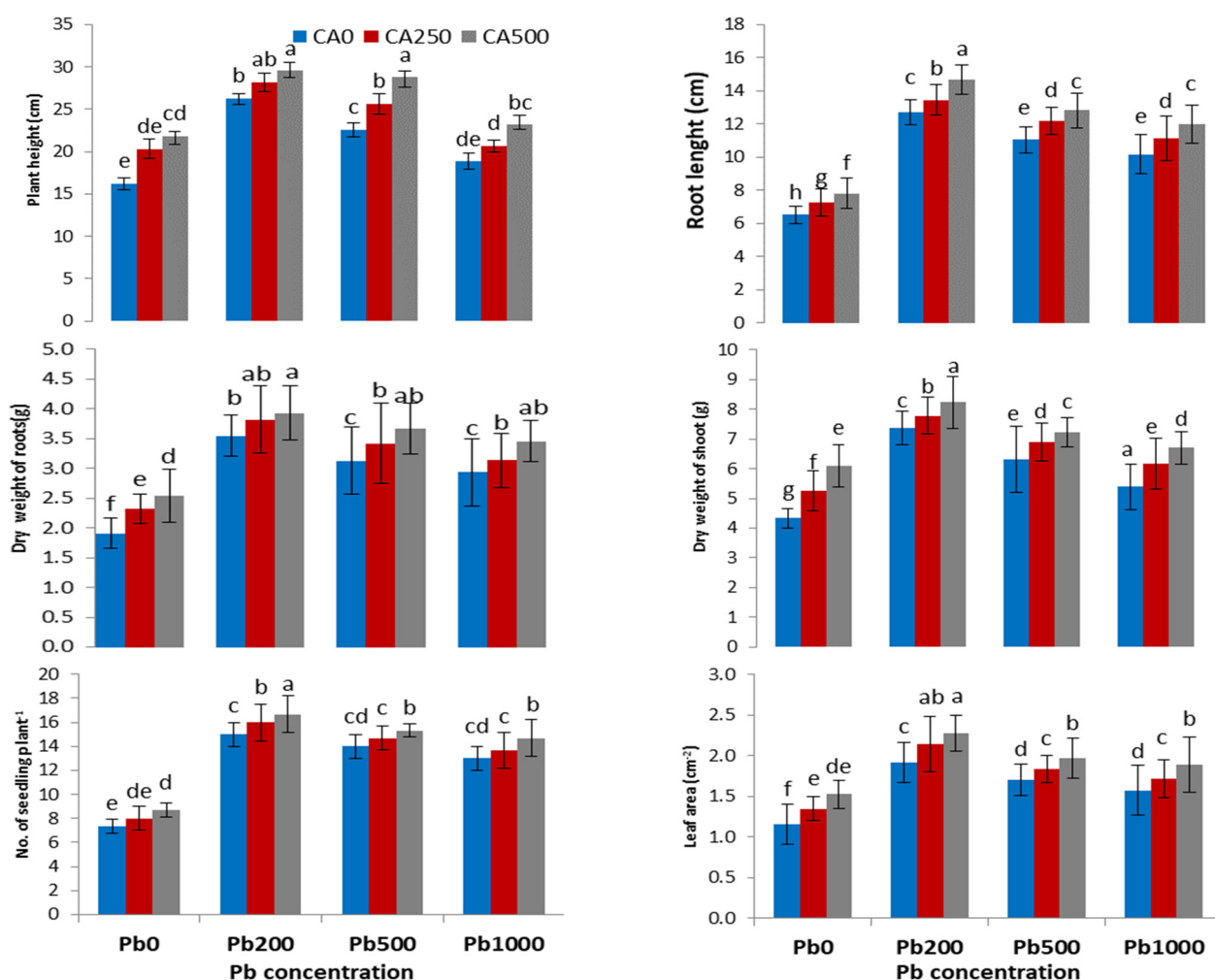


Figure 1. Effect of citric acid and Pb treatments on the plant-on-plant growth parameters of *A. bettzickiana* (Regel) G. Nicholson. Citric acid was applied at 0, 250, and 500 (μ M) and Pb application rates were 0, 200, 500, 1000 (mg kg^{-1}). Bars sharing the same letters do not differ significantly $p < 0.05$.

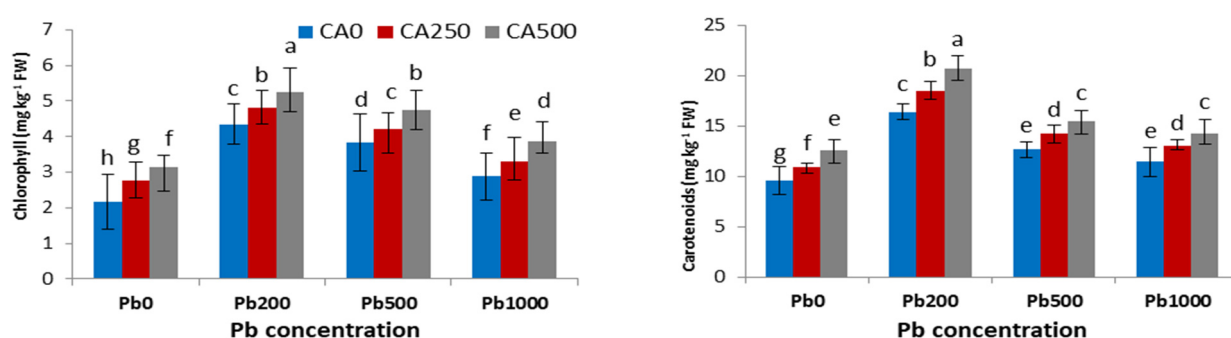


Figure 2. Effect of citric acid and Pb treatments on the plant on chlorophyll and carotenoids of *A. bettzickiana* (Regel) G. Nicholson. Citric acid was applied at 0, 250, and 500 (μ M) and Pb application rates were 0, 200, 500, 1000 (mg kg^{-1}). Bars sharing the same letters do not differ significantly $p < 0.05$.

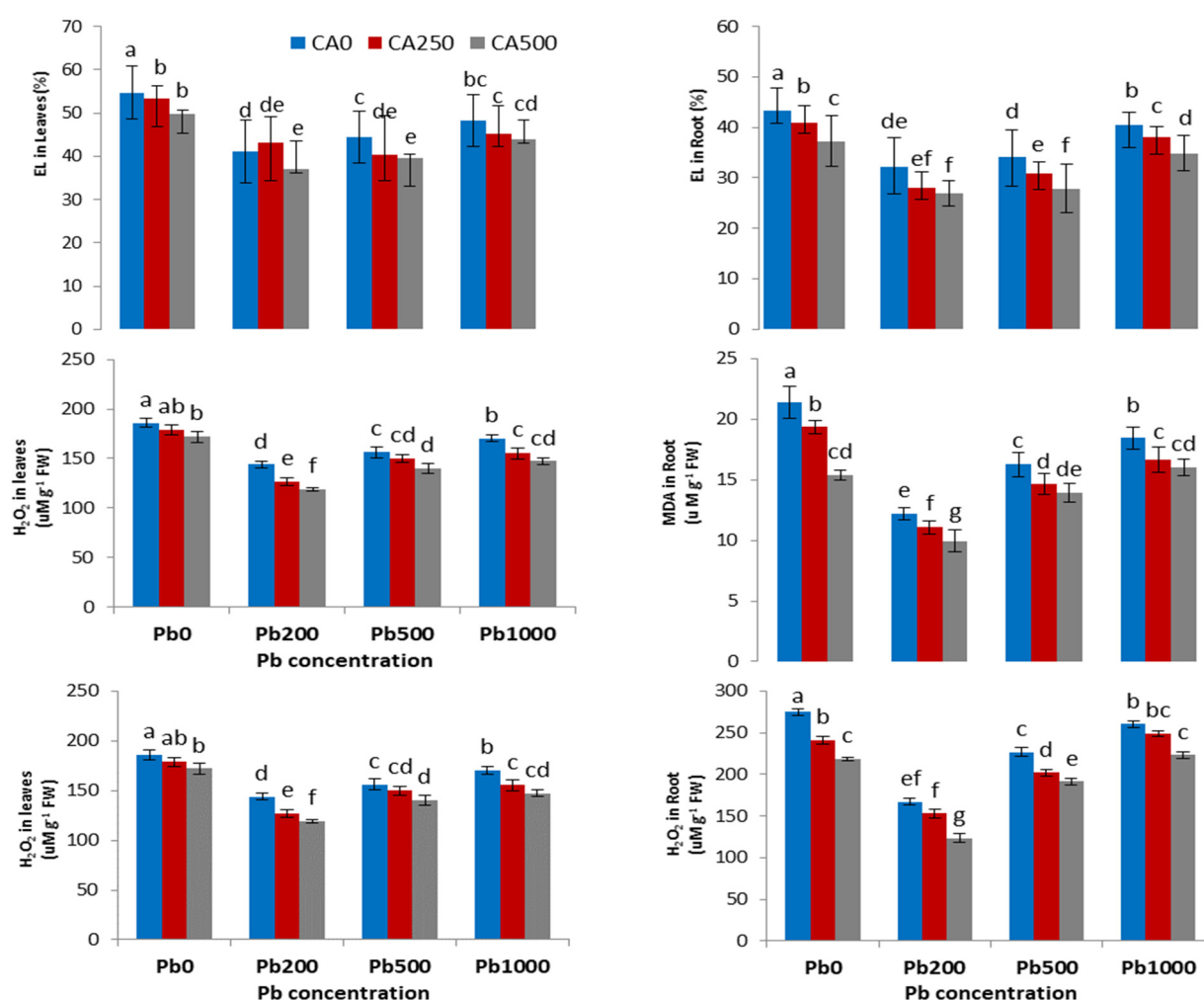


Figure 3. Effect of citric acid and Pb treatments on the plant on physiological parameters of *A. bettziackiana* (Regel) G. Nicholson. Citric acid was applied at 0, 250, and 500 (μ M) and Pb application rates were 0, 200, 500, 1000 (mg kg^{-1}). Bars sharing the same letters do not differ significantly $p < 0.05$.

3.5. Metal Uptake

Enhancing the concentration of Pb in soil considerably improved the uptake of Pb in shoots and roots. The effects of CA on *A. bettziackiana* under Pb stress, Pb concentration in the root, and shoot were measured as given in Figure 5. The Pb concentrations were significantly decreased with the maximum application of CA. At the extreme level of CA (500 mL), Pb concentration in root and shoot decreased.

4. Discussion

4.1. Growth Parameters

In plants, ecotoxicity induced by heavy metals depends upon numerous factors like plant genotype, plant species, the concentration of heavy metals, and exposure duration [34]. Metabolic processes of plants were interfered by Pb, which ultimately leads to the deterioration of plant's growth and development [2], and mostly to a reduction in protein synthesis and photosynthesis and devastation at the cellular as well as subcellular levels [35]. Similar toxic effects of Pb were reported in *Brassica napus* [10] and *A. bettziackiana* [8,36]. Alike to other organic acids, the AA growth-promoting effect is established in the literature where organic acids like citric acid (2.5, 5, and 10 mM), ascorbic acid (5 mM), and glutamic acid (2.5 and 5 mM) were used for *Brassica napus*, sunflower, *Solanum nigrum* L., and *Lemna minor* L. [37,38], respectively in the existence of heavy metals stress. Lead is the most toxic HMs which stimulated various ultra-structural and physiological damages

in plants [2]. The reduction in the plant biomass is exposed in Figure 1. HMS toxicity appeared because of the intrusion of Pb with the metabolism of the plant and also physiology [39,40]. The citric acid accumulation of Pb stressed plants considerably improved the plant biomass and development described in Figure 1. This might have occurred because of the significant role of citric acid in assisting many metabolic processes of the plant. Plant biomasses were enhanced in the *Solanum nigrum* L. plant by using CA concentration [37]. This study was furthermore verified by [41] and [42] who described the same promotion role of CA for the development of plants with Cd and Mn stress in *Brassica napus* L and *J. effusus*, respectively.

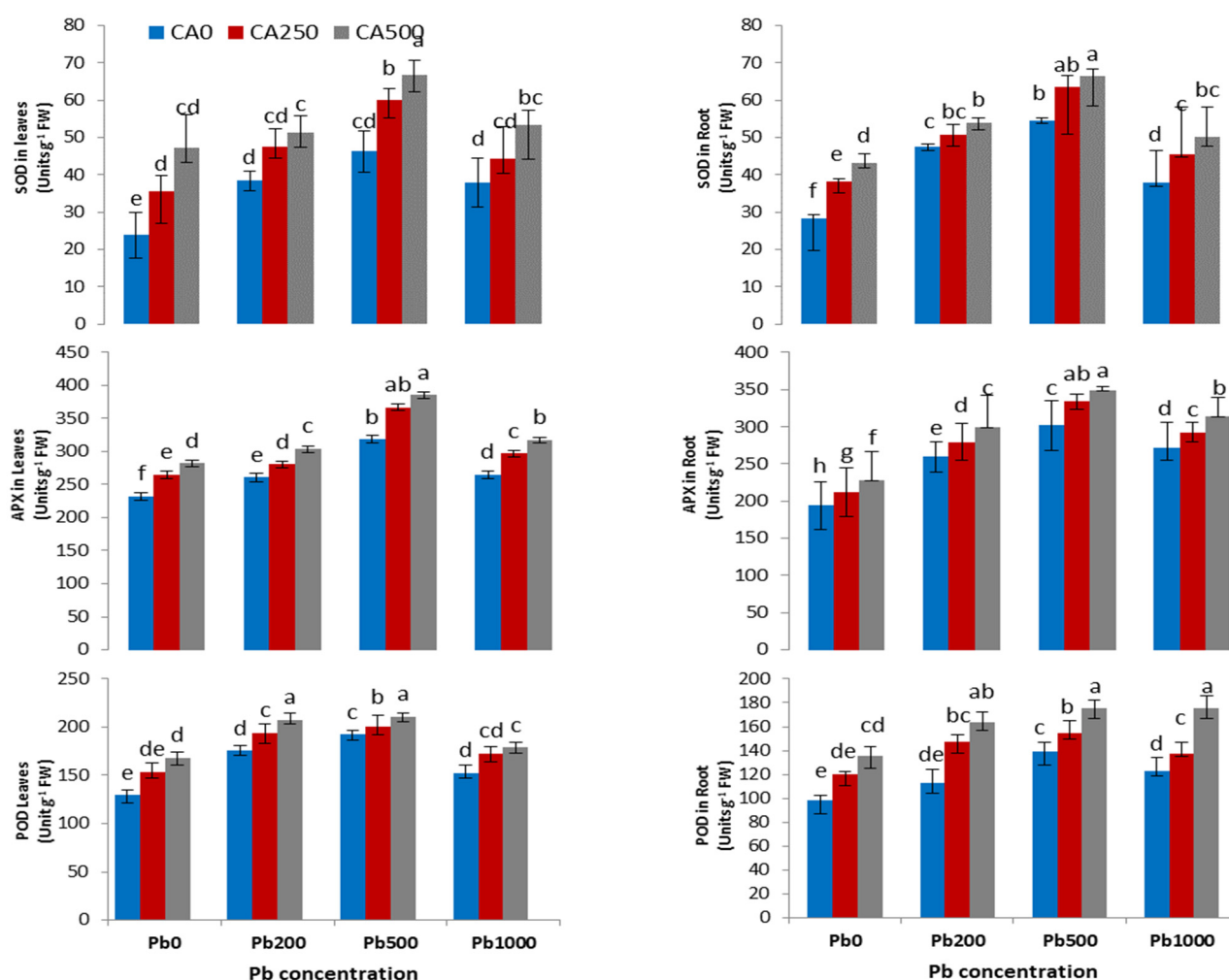


Figure 4. Effect of citric acid and Pb treatments on the plant on enzymatic parameters in leaves and roots of *A. bettziackiana* (Regel) G. Nicholson. Citric acid was applied at 0, 250, and 500 (μ M) and Pb application rates were 0, 200, 500, 1000 (mg kg^{-1}). Bars sharing the same letters do not differ significantly $p < 0.05$.

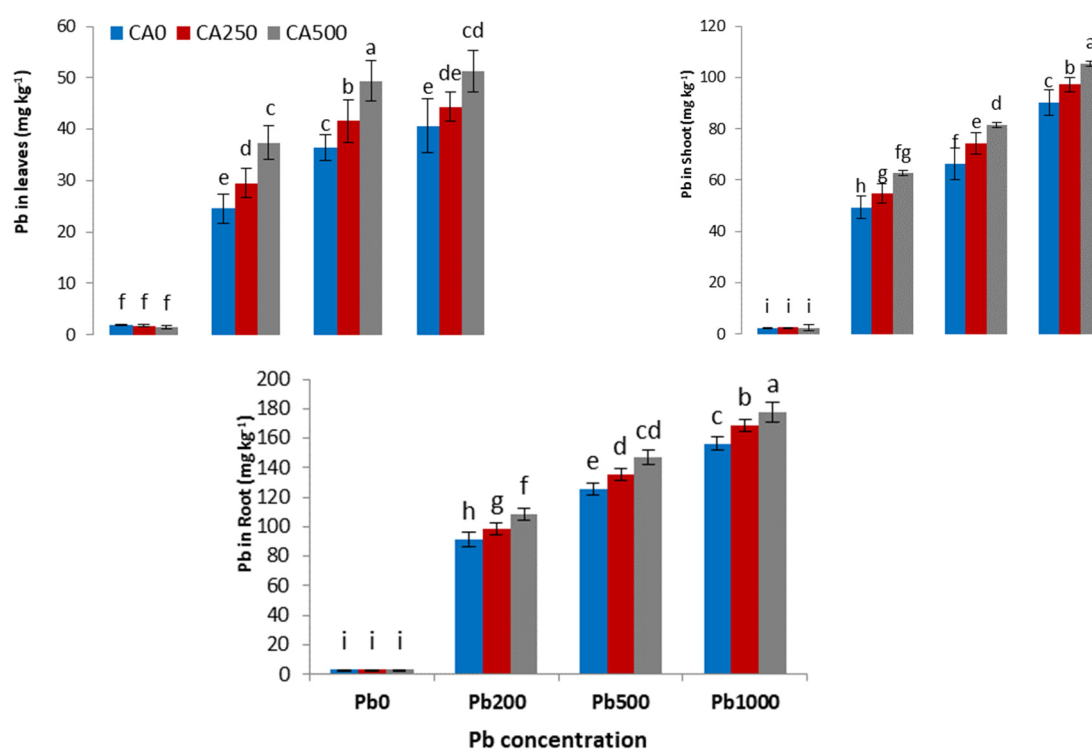


Figure 5. Effect of citric acid and Pb treatments on Pb concentrations in the root, shoot, and leaves of *A. bettzickiana* (Regel) G. Nicholson. Citric acid was applied at 0, 250, and 500 (μ M) and Pb application rates were 0, 200, 500, 1000 (mg kg^{-1}). Bars sharing the same letters do not differ significantly $p < 0.05$.

4.2. Chlorophyll Contents

Reduced chlorophyll contents were reported due to Pb stress by imposing negative effects on the photosynthetic efficiency and transpiration rate of plants. Disturbance in protein complexes, photosynthetic pigments, and chloroplast was reported by various researchers because of the increase in chlorophyllase activity under stress imposed by heavy metals [6,43]. Moreover, lead toxicity stimulated the negative effects on photosynthetic pigments. This could be the result of damage to the photosynthetic chloroplast, apparatus, and protein complex whenever plants indicated metal toxicity [17]. Therefore, chlorophyll breakdown occurs because of an increase in the activity of chlorophyllase due to metal toxicity [44] (Figure 2). However, application of CA reverses this effect and significantly enhances the chlorophyll contents of *A. Bettzickiana*.

4.3. Antioxidant Enzyme Parameters and Reactive Oxygen Species

In plants, Pb toxicity was previously observed in terms of higher MDA production and higher activities of antioxidant enzymes in *A. bettzickiana* [8], in *Brassica napus* [10], and in *S. nigrum* by [45]. Similar results were reported by [46] for *A. bettzickiana* when exposed to Cd stress. The addition of AA along with Pb enhanced the defensive mechanisms of a plant which assisted in overcoming the lipid peroxidation produced by Pb stress. An alike mechanism was documented by [46] in *A. bettzickiana* under citric acid and Cd. Some antioxidant enzymes (APX, SOD, and CAT), along with other metabolites, achieve a precise role in the adaptation and tolerance of plants to Pb ecotoxicity [47]. At 10 mM Pb, the activities of antioxidant enzymatic tended to decline while MDA production continued to rise, which resulted in reduced nutrient uptake and disruption of plant metabolic pathways [22,46]. Enhanced MDA production is generally observed as a sign of severe oxidative stress beneath metal stress which ultimately destroys the plant cells [48,49]. Some important metabolites and also antioxidant enzymes take part in an essential

role in the development of plants and adaptation due to metal stress. Oxidative stress changes enzymatic activities, which are important to alleviate metal toxicity [50].

Reduction in the antioxidant enzyme activities and oxidative parameters are shown in Figure 3. Reduction in the antioxidant enzymatic activity and also oxidative parameters due to lead stressed plants were observed in the recent research and this reduction was measured due to the Pb uptake in the plant cells. The enhancement of the antioxidant enzyme activities with citric acid and Pb described that CA could enhance the enzyme activities in *A. Bettizikianna*. Ref. [42] described the remarkable role of citric acid by increasing the antioxidant enzyme activities, hence, intervening metal-induced oxidative toxicity in *J. effusus* plant. The improvement in H₂O₂, MDA, and electrolyte leakage due to toxicity of metal for the damage of lipid peroxidation and plasma membrane, can inhibit plant development [46]. In the recent study, the decrease in hydrogen peroxide, EL, and MDA by using citric acid documented the positive role of CA besides the Pb stress and it shows that CA plays an important role alleviating oxidative stress caused under Pb contamination. Some related results of the positive role of CA were reported in *J. effusus* [42].

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4.5. Lead Uptake

In-plant tissues, heavy metals accumulation is associated with their concentration level in the environment. In the current study, the concentration level of Pb in all parts of *A. bettzickiana* increased with the cumulative concentration of applied Pb in soil. The greater uptake of lead in roots compared to stems and leaves was because of the direct contact of roots to Pb in soil [16]. Our findings agreed with those of [8,46], who established the phytoextraction potential of *A. bettzickiana* for Pb and Cd, correspondingly (Figure 5).

Though some studies recommended that Pb generally accumulated in the roots, and only a slight fraction can be translocated to the aerial parts of plants [19,51]. *A. bettzickiana* plant accumulated Pb from media [8] like other plants such as *B. napus*, *L. minor*, and *Typha latifolia* [10,38]. On the other hand, some plant species like *T. orientalis* constrained the Pb accumulation in roots [16]. AA addition under Pb stress meaningfully increased the Pb uptake and its accumulation in roots, leaves, and stems, comparable to the findings stated by [45]. Organic acids and AA offer the protons and electrons and built complexions to be readily uptaken by the roots of the plant [45,52]. Ref. [45] established that indole-3-acetic acid suggestively improved the uptake of Pb, Zn, and Cd in *Solanum nigrum*.

Metal uptake in the plant is directly correlated to the Pb levels in plant development. In a recent study, Pb concentration in a part of the plants, such as the root and shoot of *A. Bettzickiana*, was improved and shown in Figure 5 [53]. As we enhanced the Pb contents in the soil, our finding of more metal accumulation of the Pb level in roots rather than the shoots and leaves showed the potential that can help the metal-induced damages [42]. In *A. Bettzickiana*, CA application considerably improved the metal uptake and its concentration in stem, root, and leaves in comparison with the lead alone treated plant. The results related to P dynamics in different parts of the *A. Bettzickiana* show that CA application enhanced the phytoextraction potential of Pb from the Pb spiked soil. Despite the fact that application of CA significantly enhanced the phytoextraction potential of *A. Bettzickiana*, this study was conducted under controlled conditions. Its onward confirmation under natural field conditions involving Pb contaminated soil would be helpful in authentication of these results and its further recommendation at a large scale. Moreover, rigorous studies involving different climatic and soil conditions would also be helpful in its confirmation and authentication under natural field conditions.

5. Conclusions

Citric acid played an important part in enhancing the development of *A. bettzickiana* in tolerating Pb toxicity. The Pb stress decreased the plant biomass, plant development, chlorophyll content, antioxidant enzymes, and increased the oxidative stress parameters of plants. While the application of citric acid considerably enhanced the photosynthetic contents and morphological parameters by inhibiting the oxidative damage of cells caused under Pb stress. Our results also showed that *A. Bettzickiana* plant can discharge a significant quantity of pollutants such as Pb and is rigorous in working like hyper-accumulator plants confirming the study hypothesis. The application of CA remarkably improved the amount of Pb uptake by this hedge plant via enhancing plant growth and antioxidant defense system of plants. However, we suggest further field investigations to explore the detailed mechanism regarding the phytoremediation of Pb through *A. bettzickiana* assisted by CA under natural field conditions.

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