

# Influence of Fe Nutrition on Photosynthesis in Pb Treated *Ailanthus altissima* (Mill.) Swingle Seedlings

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## Abstract

Influence of iron nutrition in Pb-treated *Ailanthus altissima* (Mill.) Swingle seedlings was investigated by measurement of photosynthetic efficiency (Fv/Fm), photosynthetic pigment content, and biomass production. The data of Fe-sufficient and Fe-deficient hydroponically grown plants shows that the presence of Fe in growing media in Pb-treated plants increased Fv/Fm compared to Pb-treated plants without Fe. It was also reduced half time, requiring it to reach maximum fluorescence. Chlorophyll as well as carotenoid content did not decrease in Pb-treated *A. altissima* plants grown with Fe. There was also no significant reduction in root and shoot biomass in all Pb-treated plants when they were constantly supplied with Fe. These results suggest that Fe nutrition increase tolerance to Pb stress in *A. altissima* plants. This finding could be useful for improving recultivation methods on Pb-polluted sites.

**Keywords:** Fe nutrition, Pb treatment, photosynthetic efficiency, *Ailanthus altissima*

## Introduction

Understanding the role of plant nutritional status in toxic metal tolerance could be important for improving the capacity of plants to adapt to polluted soils. In addition to being retained on the leaves, these metals in the soil penetrate and contaminate it, thus affecting plant root function and at higher concentrations can be toxic [1]. Research on the impact of heavy metals on woody species growth in urban and landscape environments have shown that they have various degrees of tolerance [2-6].

Lead (Pb) is one of the most frequent toxic metals. In a very low concentration it can cause changes in the activity of many key enzymes of various metabolic pathways and

inhibit some vital plant processes, such as photosynthesis, mitosis, and water absorption, followed by toxicity symptoms: stunted growth, development on dark leaves, wilting of older leaves, stunted foliage, and short brown roots [7-10].

The reason is occupation of the cell walls binding sites with Pb<sup>2+</sup> in the absence of Fe<sup>3+</sup>, or a direct influence of Pb<sup>2+</sup> on the redox components, affecting enzyme activity [11].

Iron (Fe) is one of the most significant nutrients important for photosynthetic efficiency, chlorophyll synthesis and plant biomass production. Chlorosis induced by Fe deficiency is a common nutritional disturbance of higher plants grown in calcareous and alkaline soils [12].

Chlorophyll fluorescence is a good indicator of efficiency of photosynthetic apparatus in situ, and also a measure of a plant's response to various stresses with negative

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effect to photosynthesis [13-15]. The parameter  $T_{1/2}$  is a measure of the size and accessibility of the plastoquinone pool that accepts electrons from PSII [16-18].

In this study we investigated the effects of Fe nutrition and Pb stress interaction in the fast-growing tree species *A. altissima* (Tree of Heaven). High photosynthetic efficiency and high biomass production of *A. altissima* was observed by Kowarik and Saumel [19]. *A. altissima* is resistant to various types of pollution and adapted to lime-rich soils with a high potential risk of Fe deficiency [20, 21]. It often grows near roads and industrial areas, where it is exposed to the toxic effect of lead that can accumulate in soil and water. It also was determined that Fe-sufficient *A. altissima* plants accumulate significantly more Pb than Fe-deficient plants [12].

Cultured sprouts of *A. altissima* exposed to heavy metals demonstrated a tolerance comparable to species already utilized in phytoremediation [20]. Kovács [22] also recommends *Ailanthus* sp. as well as some other woody species (*Tilia tomentosa* Moench, *Sambucus nigra* L., *Quercus robur* L. etc.) as bioaccumulative indicators. Biomonitoring of heavy metals using plants has been widely applied to detect and to monitor the effects of pollution [10, 23].

Such tree species are appropriate for reclamation purposes. Unlike herbaceous plants, trees repeatedly face stress conditions due to their long lifecycles. After responsive reaction and showing tolerance against environmental stresses, woody plants adapt to these stresses and recover normal growth [24]. Remediation of soils contaminated with Pb using phytoremediation and rhizofiltration technologies appear to have great potential for cleaning Pb-contaminated soils [25].

The objectives of this work was to determinate how Fe nutritional status in Pb-treated *A. altissima* plants influence photosynthetic efficiency, photosynthetic pigment content, and growth. This study may contribute to understanding interactions of Fe nutrition and Pb toxicity in tree plants.

## Material and Methods

### Plant Material and Experimental Design

Samaras of *A. altissima* were collected from a tree in the city of Belgrade in the wasteland area. Seeds were germinated in the dark at 25°C in Petri dishes on filter paper with distilled water. The experimental design was carried out according to Đunisijević Bojović et al. [12], presented in Fig. 1. At the stage of primary leaves, development seedlings were transferred to a preculture in half-strength modified Hoagland nutrient solution (5 plants per 3 L pot) containing (in mmol/L): 0.35  $K_2SO_4$ , 0.05 KCl, 1.0  $Ca(NO_3)_2$ , 0.25  $MgSO_4$ , 0.05  $KH_2PO_4$ , and (in  $\mu mol/L$ ): 5  $H_3BO_3$ , 0.25  $MnSO_4$ , 0.25  $ZnSO_4$ , 0.1  $CuSO_4$ , 0.005  $(NH_4)_6Mo_7O_{24}$ , and 20  $Fe^{III}EDTA$ . Plants were grown in a growing chamber with a photosynthetic photon flux density of 250  $\mu mol \cdot m^{-2} \cdot s^{-1}$  at plant height, photoperiod 16/8h, temperature 25/18±2°C, relative humidity 60±3%. The nutrient solutions were renewed completely every 5 days and continuously aerated, and pH of the solutions was adjusted to 6.5.

### Evaluation of Photosynthetic Efficiency

To determine the effects of Pb treatment on photosynthetic efficiency (Fv/Fm) the method of induced chlorophyll fluorescence kinetics of photosystem II was used (F<sub>o</sub>, non-variable fluorescence; F<sub>m</sub>, maximum fluorescence; F<sub>v</sub>=F<sub>m</sub>-F<sub>o</sub>, variable fluorescence;  $T_{1/2}$ , half the time required to reach maximum fluorescence from F<sub>o</sub> to F<sub>m</sub>). Measurements were taken with the aid of a portable plant stress meter (BioMonitor S.C.I. AB, Sweden), as described by Krause and Weis [17]. Chlorophyll was excited for 2 to 5 s by actinic light with a photon flux density of 200 and 400  $\mu mol \cdot m^{-2} \cdot s^{-1}$ . Prior to measuring, samples were adapted to the dark for approximately 30 minutes in order to maxi-

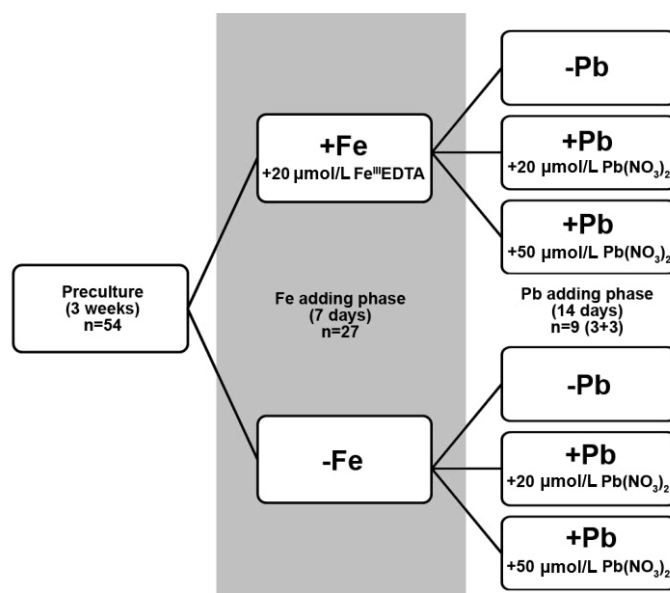


Fig. 1. Schema of the experiment design presents chemical compound, concentration, duration of the treatments, and number of plants (n) in each group. (+) indicates the presence of the noted chemical compound in the treatment; (-) indicates its exclusion.

Table 1. Photosynthetic efficiency (Fv/Fm) and half the time required to reach maximum fluorescence ( $T_{1/2}$ ) in the treatments with  $Pb(NO_3)_2$  (50  $\mu$ mol/L and 20  $\mu$ mol/L) in the absence (-) or presence (+) of Fe (20 mM  $Fe^{III}EDTA$ ) in *A. altissima* hydroponically grown seedlings.

Treatments	Fv/Fm $\pm$ SE	$T_{1/2}\pm$ SE
-Fe+Pb 50	0.702 $\pm$ 0.036 <sup>c</sup>	604.667 $\pm$ 40.7073 <sup>a</sup>
-Fe+Pb 20	0.752 $\pm$ 0.095 <sup>b</sup>	247.000 $\pm$ 8.9730 <sup>b</sup>
-Fe-Pb control	0.785 $\pm$ 0.010 <sup>a</sup>	246.330 $\pm$ 13.4164 <sup>b</sup>
+Fe+Pb 50	0.784 $\pm$ 0.069 <sup>a</sup>	252.875 $\pm$ 11.5935 <sup>b</sup>
+Fe+Pb 20	0.818 $\pm$ 0.015 <sup>a</sup>	261.800 $\pm$ 9.3259 <sup>b</sup>
+Fe-Pb control	0.829 $\pm$ 0.005 <sup>a</sup>	255.220 $\pm$ 10.2005 <sup>b</sup>

ANOVA, values are means  $\pm$ SE (standard error) of 10 replicates. Means followed by a different letter are significantly different at  $P<0.05$ .

mize the oxidation of the primary quinone electron acceptor pool of PSII and to enable the full relaxation of any rapidly recovering fluorescence quenching. Fifty-four plants were analyzed by measuring parameters in 9 plants per treatment on the completely developed 3<sup>rd</sup> leaf from the top.

#### Photosynthetic Pigments Determination

Leaf tissue fresh weight (FW) of 1g was homogenized with 5-10 ml of acetone and a little of quartz sand for 3 min. To prevent acidification of the solution, a small amount of  $MgCO_3$  was added. After homogenization the content was filtered through a glass filter. Pigment extracts were quantitatively transferred to flasks and diluted up to 25 ml with acetone. Absorptions of the prepared extracts were read on the spectrophotometer (Thermo, Type evaluation 300 UV-Vis Spectrophotometer) at wavelengths of 662, 644, and 440 nm and then calculation was performed according to Wetstein [26] formula. Pigment concentrations for chlorophylls (Chl *a* and Chl *b*) and carotenoids were expressed as  $mg\cdot g^{-1}$  (FW).

Table 2. Chlorophylls and carotenoids content ( $mg/g$  FW) in leaves of *A. altissima* hydroponically grown seedlings influenced by Fe presence (20 mM  $Fe^{III}EDTA$ ) and  $Pb(NO_3)_2$  (50  $\mu$ mol/L and 20  $\mu$ mol/L) treatment.

Treatments	Chlorophyll <i>a</i> $mg/g$ FW	Chlorophyll <i>b</i> $mg/g$ FW	Chlorophyll <i>a+b</i> $mg/g$ FW	Chlorophyll <i>a/b</i> ratio	Total carotenoids $mg/g$ FW
-Fe+Pb 50	0.455 $\pm$ 0.1031 <sup>bc</sup>	0.127 $\pm$ 0.0319 <sup>c</sup>	0.582 $\pm$ 0.1350 <sup>c</sup>	3.621 $\pm$ 0.1000 <sup>a</sup>	0.160 $\pm$ 0.0419 <sup>ab</sup>
-Fe+Pb 20	0.304 $\pm$ 0.0018 <sup>c</sup>	0.135 $\pm$ 0.0077 <sup>c</sup>	0.439 $\pm$ 0.0059 <sup>c</sup>	2.263 $\pm$ 0.1417 <sup>ab</sup>	0.120 $\pm$ 0.0010 <sup>b</sup>
-Fe-Pb control	0.542 $\pm$ 0.0036 <sup>b</sup>	0.158 $\pm$ 0.0038 <sup>bc</sup>	0.700 $\pm$ 0.0074 <sup>bc</sup>	3.429 $\pm$ 0.0585 <sup>ab</sup>	0.163 $\pm$ 0.0019 <sup>ab</sup>
+Fe+Pb 50	0.792 $\pm$ 0.0120 <sup>a</sup>	0.420 $\pm$ 0.0097 <sup>a</sup>	1.213 $\pm$ 0.0218 <sup>a</sup>	1.881 $\pm$ 0.0145 <sup>b</sup>	0.253 $\pm$ 0.0482 <sup>a</sup>
+Fe+Pb 20	0.813 $\pm$ 0.0038 <sup>a</sup>	0.446 $\pm$ 0.1810 <sup>a</sup>	1.260 $\pm$ 0.1774 <sup>a</sup>	2.189 $\pm$ 0.8980 <sup>ab</sup>	0.195 $\pm$ 0.0030 <sup>ab</sup>
+Fe-Pb control	0.757 $\pm$ 0.0380 <sup>a</sup>	0.267 $\pm$ 0.0700 <sup>b</sup>	1.024 $\pm$ 0.1087 <sup>ab</sup>	3.009 $\pm$ 0.6489 <sup>ab</sup>	0.210 $\pm$ 0.0107 <sup>ab</sup>

ANOVA, values are means  $\pm$ SE of 3 replicates. Means followed by a different letter are significantly different at  $P<0.05$ .

#### Shoot and Root DW Determination

After two weeks treatment plants were dried at 70°C to constant weight. Shoot and root dry weight (DW) per plant was measured (g) and root/shoot ratio calculated.

#### Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using the statistical software Statistica 6 (StatSoft, Inc., Tulsa, OK, USA). Mean and standard errors (SE) for estimated parameters were determined, and analysis of variance was applied for determination of differences between groups. The canonical discriminant analysis (CDA) was applied to maximize differences among six treatment groups.

### Results

#### Photosynthetic Efficiency

In seedlings that were subjected to Fe deficiency there was a significant decrease of Fv/Fm in Pb treated plants. At the treatment without Fe and with Pb 50  $\mu$ mol/L (-Fe+Pb50) there was the lowest photosynthetic efficiency compared to the control plants (-Fe-Pb). The highest photosynthetic efficiency was in control, Pb untreated group with Fe presence (+Fe-Pb) (Table 1). For the parameter  $T_{1/2}$  it was noticed significantly ( $P<0.05$ ) higher value in Fe deficient plants treated with Pb 50  $\mu$ mol/L. This parameter was more than two times higher than in all other treatments and in control plants (Table 1).

#### Photosynthetic Pigments Content

In Fe-deficient Pb treated seedlings there was a decrease of Chl *a*, Chl *b*, Chl *a+b* content compared to the control plants (-Fe-Pb). The same tendency was noticed in individual carotenoids content but it was not significant at the total carotenoid level. In Fe-sufficient plants, treatments of Pb 50  $\mu$ mol/L and Pb 20  $\mu$ mol/L increased Chl *b* content significantly, compared to control plants (Table 2).

Table 3. Influence of Fe presence (20 mM Fe<sup>III</sup>EDTA) and Pb(NO<sub>3</sub>)<sub>2</sub> (50 μmol/L and 20 μmol/L) treatment on root/shoot dry weight per plant and ratio of *A. altissima* seedlings.

Treatments Fe <sup>III</sup> EDTA Pb(NO <sub>3</sub> ) <sub>2</sub>	Shoot DW (g)	Root DW (g)	Root/Shoot ratio
- Fe+Pb 50	0.48±0.071 <sup>b</sup>	0.29±0.049 <sup>ab</sup>	0.60±0.023 <sup>a</sup>
- Fe+Pb 20	0.68±0.155 <sup>b</sup>	0.34±0.057 <sup>ab</sup>	0.50±0.624 <sup>a</sup>
- Fe-Pb control	0.52±0.122 <sup>b</sup>	0.23±0.055 <sup>b</sup>	0.44±0.072 <sup>a</sup>
+Fe+Pb 50	0.88±0.136 <sup>ab</sup>	0.44±0.055 <sup>ab</sup>	0.51±0.032 <sup>a</sup>
+Fe+Pb 20	0.86±0.026 <sup>ab</sup>	0.39±0.100 <sup>ab</sup>	0.45±0.112 <sup>a</sup>
+Fe-Pb control	1.04±0.262 <sup>a</sup>	0.53±0.154 <sup>a</sup>	0.52±0.037 <sup>a</sup>

Values are means±SE of 5 replicates. Means followed by different letters are significantly different at P<0.05.

### Effect on Plant Biomass

Results obtained in this study show that in all Pb-treated *A. altissima* plants there was no significant reduction in shoot and root biomass compared to control plants. However, there was significant difference in control, Pb-untreated plants grown with Fe (+Fe-Pb) compared to control plants without Fe (-Fe-Pb). Also, root/shoot ratio didn't show statistical differences (Table 3).

### Canonical Discriminant Analysis

Standardized coefficients of analyzed parameters obtained by canonical discriminant analysis (CDA) among six treatment groups (-Fe+Pb50, -Fe+Pb20, -Fe-Pb-control, +Fe+Pb50, +Fe+Pb20, and +Fe-Pb-control) were examined in order to obtain an estimate of their respective contributions to the general variability and separation of the groups.

Five significant functions were obtained but the first two canonical axes were taken into account because they described 97.8% of total discrimination. Ordination along the first canonical axis showed that the samples with Fe and samples without Fe were clearly separated. Along the second axis the sample -Fe+Pb20 was separated from -Fe+Pb50 and -Fe-Pb control samples while +Fe samples did not show any segregation (Fig. 2).

### Discussion

#### Photosynthetic Efficiency

The process of photosynthesis is adversely affected by Pb toxicity. Plants exposed to Pb ions show a decline in photosynthetic rate, which results from distorted chloroplast ultrastructure, restrained synthesis of chlorophyll,

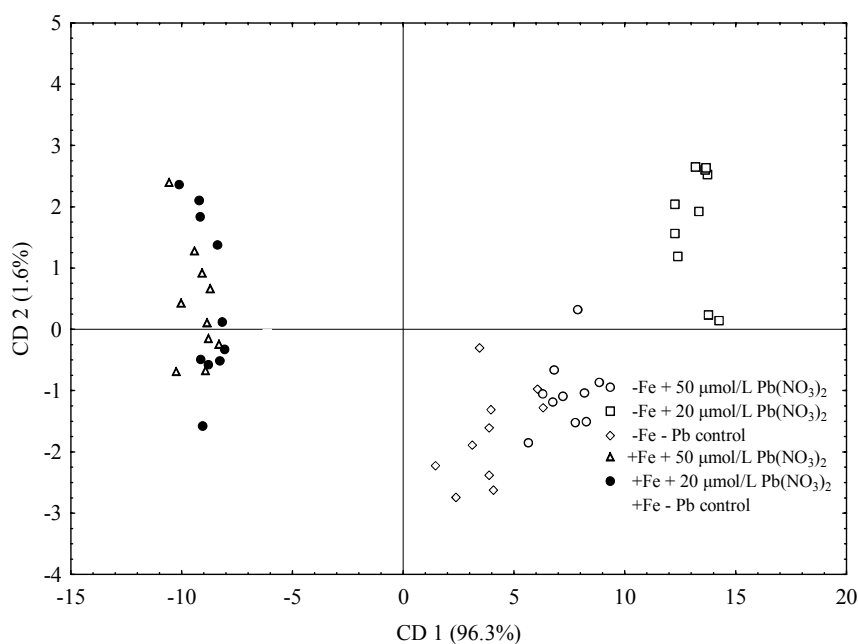


Fig. 2. Canonical discriminant analysis (CDA) of the basic components for the two treatments (-Fe and +Fe) of Pb-treated plants (Pb 20 μmol/L and Pb50 μmol/L) of *A. altissima* seedlings based on the variations of Fv/Fm, Chl *a*, Chl *b*, carotenoids, shoot and root dry weight. Samples with Fe and samples without Fe were clearly separated; sample -Fe+Pb20 was separated from -Fe+Pb50 and -Fe-Pb control samples while +Fe samples did not show any segregation.

plastoquinone and carotenoids, obstructed electron transport, inhibited activities of Calvin cycle enzymes, as well as deficiency of CO<sub>2</sub> as a result of stomatal closure [27, 28].

In our research Pb treatment significantly ( $P < 0.05$ ) decreased photosynthetic efficiency only in Fe absence, while Fe presence did not affect a significant decrease of this parameter in all Pb-treated *A. altissima* seedlings. This indicates that Fe nutrition has a more important influence on photosynthetic efficiency than Pb stress. Significantly decreased Fv/Fm fluorescence ratio was also reported by Morales et al. in Fe deficient dark adapted leaves of *Pyrus communis* L. [29]. Maximum photosynthetic quantum efficiency of PSII (0.829) was measured in *A. altissima* control samples. This is in accordance with common Fv/Fm value for non treated plants of 0.830, obtained by Powels [30] and similar to optimum mean values for non-stressed deciduous trees of 0.843, obtained by Bjorkman and Demmig [31].

$T_{1/2}$ , a predictor of the rate of photochemical reactions, should be changed in an opposite course from Fv/Fm [30] tendency or pattern proved by our results.

#### Photosynthetic Pigment Content

In our study, both types of chlorophyll as well as carotenoid content did not decreased in Pb-treated *A. altissima* plants grown with Fe. It was also found that the presence of Fe and Pb together significantly ( $P < 0.05$ ) increased chlorophyll b content in comparison to treatments without Fe. A lesser decrease of carotenoids than chlorophyll concentration was also observed in hydroponically grown Fe-deficient *Quercus suber* L. seedlings [32]. It was previously reported that environmental stresses, such as heavy metals contamination of soils, can also induce Fe deficiency in some plant species by influence on reduction of Fe (III) to Fe (II) [33] and needle chlorosis in spruce seedlings [34].

In our research high chlorophyll content of *A. altissima* seedlings is probably related to higher photosynthetic efficiency. Similarly, lower chlorophyll content did not affect photosynthetic efficiency. This is in accordance with the findings of Abadia et al. [35] that the low chlorophyll content in iron-deficient leaves showed no sustained decreases in PSII efficiency, measured after dark adaptation.

This could be related to the fact that the substitution of the central atom of chlorophyll, magnesium, by heavy metals (Pb, Cu, Hg, Cd, Ni, or Zn) is possible and seem to be an important damage mechanism in stressed plants. This substitution prevents photosynthetic light-harvesting in the affected chlorophyll molecules, resulting in a breakdown of photosynthesis [36]. Results obtained in our study show high chlorophyll *a/b* ratio in -Fe+Pb50 treatment and low ratio in +Fe-Pb50 treatment, which could be a good indicator of the important role of Fe in adaptation mechanisms of *A. altissima* to the stressful conditions. It was also reported that the higher Chl *a/Chl b* ratio in *Geranium macrorrhizum* L. and *Doronicum columnae* Ten. in two years post-fire period, indicating that these plants have higher photosynthetic activity and seems to have very analogical adaptation patterns in stress conditions [37].

#### Effect on Plant Biomass

There was no significant reduction in root and shoot biomass in Pb-treated plants with adequate Fe supply. This probably means that Pb treatment did not affect biomass production significantly. However, the presence of Fe improved aboveground biomass in untreated plants about twice compared to plants grown without Fe.

It is hypothesized that two-week exposure to Pb treatment blocks functioning of a specific iron transport system, activated under Fe deficiency. It was previously reported that Pb and Cd pollution did not affect diameter growth reduction in *Picea abies* (L.) Karst. [38]. But in less tolerant species as *Thespesia populnea* L., seed germination, seedling growth, and dry weight were reduced after treatments with lead and cadmium [39]. Plants tolerant of toxic metals have a variety of mechanisms involved in their detoxification [40]. It was proved that phosphorus in some phosphate compounds could enhance resistance to Pb stress in Pb-contaminated soils [41]. Also, it was reported that mycorrhizas could protect woody plant (silver birch) seedlings from elevated environmental levels of Cu and Pb [42].

#### Conclusion

According to photosynthetic efficiency, chloroplast pigments content and biomass production *A. altissima* can be considered as Pb-stress-tolerant species and could be successfully grown in sites contaminated with lead for reclamation purposes. Fe presence in growing media enhances Pb stress tolerance in *A. altissima* seedlings.

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