Effects of Pb Stress on Chlorophyll Fluorescence Parameters in Leaves of Schima superba and Koelreuteria paniculata Saplings Based on the Lake Model: Postprint

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Abstract

Using one-year-old saplings of the broad-leaved tree species Schima superba and Koelreuteria paniculata as subjects, indoor pot cultivation was employed to prepare three different concentration gradients of PbCl2 solution in the pot soil (L1 < L2 < L3). A comparative study was conducted on the response patterns of chlorophyll fluorescence parameters in leaves of the two saplings under Pb stress, utilizing the Lake model to reveal the operational status of Photosystem II in Schima superba and Koelreuteria paniculata under different Pb concentrations from the perspective of energy balance and allocation, thereby providing data support for rapid diagnosis of Pb tolerance levels in woody plant saplings. The results showed that under three different Pb concentrations, as incident light intensity (PAR) increased, all chlorophyll fluorescence parameters except non-regulated energy dissipation (YNO) varied with PAR in both tested saplings. Relative electron transport rate (rETR) and regulated energy dissipation (YNPQ) exhibited upward trends, whereas Photosystem II (PSII) quantum efficiency (Y) and photochemical quenching (qL) showed downward trends. Simultaneously, the maximum light energy utilization efficiency (Fv/Fm), rETR, Y , and qL of both tested saplings decreased with increasing Pb pollution concentration, while YNPQ and YNO increased with rising Pb pollution concentration. The inhibitory effect of Pb on chlorophyll fluorescence parameters in both tested plants was also reflected in the maximum net photosynthetic rate (Pn). This experiment also revealed that at the L1 concentration, the openness of PSII reaction centers in Schima superba could be maintained at a relatively high level; however, as pollution concentration increased, its light energy conversion capacity became weaker than that of Koelreuteria paniculata. Additionally, Koelreuteria paniculata exhibited higher capacity for regulating energy dissipation and greater sensitivity to Pb stress than Schima superba, further demonstrating that Koelreuteria paniculata possesses higher Pb tolerance than Schima superba. Comprehensive analysis concluded that YNO and YNPQ can serve as evaluation indicators for Pb stress in plants.

Full Text

Preamble

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Effects of Pb Stress on Chlorophyll Fluorescence Parameters in Leaves of *Schima superba* and *Koelreuteria paniculata* Seedlings Based on the Lake Model

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Abstract

This study investigated the responses of chlorophyll fluorescence parameters to lead (Pb) stress in one-year-old seedlings of two broad-leaved tree species, *Schima superba* and *Koelreuteria paniculata*, using a controlled pot experiment. Seedlings were exposed to three PbCl concentration gradients (L1 < L2 < L3) applied to the soil. The Lake model was employed to reveal the functional status of Photosystem II (PSII) under varying Pb stress conditions from the perspective of energy balance and allocation. Our results provide a data foundation for rapid diagnosis of Pb resistance in woody plant seedlings.

The results showed that under all three Pb treatments, the relative electron transport rate (rETR) in both species increased with rising photosynthetically active radiation (PAR), while non-photochemical quenching (NPQ) was down-regulated. However, the actual quantum efficiency of PSII [Y(II)] and photochemical quenching (qL) decreased. Non-light induced energy dissipation

(Y(NO)) remained unchanged. Concurrently, increasing Pb concentration reduced the maximum quantum use efficiency (Fv/Fm), rETR, Y(II), and qL, while NPQ and Y(NO) increased. The inhibitory effects of Pb on chlorophyll fluorescence parameters were also reflected in the maximum net photosynthetic rate (Pn).

At the L1 concentration, *S. superba* maintained relatively high PSII reaction center openness, but suffered severe damage under high Pb stress, indicating weaker light energy conversion capacity compared to *K. paniculata*. The NPQ of *K. paniculata* increased significantly more than that of *S. superba*, demonstrating greater capacity for regulated energy dissipation and higher sensitivity to Pb stress. This further suggests that *K. paniculata* possesses stronger Pb tolerance than *S. superba*. Comprehensive analysis indicated that NPQ and Y(NPQ) could serve as diagnostic indicators for Pb stress and evaluation of plant resistance to Pb.

Keywords: Lake model; Pb stress; *Koelreuteria paniculata*; *Schima superba*; chlorophyll fluorescence; diagnostic indicators

Introduction

Lead (Pb) is a common toxic heavy metal in soils, and its contamination has become a major global environmental issue. In China, the exceedance rate of heavy metals in forest soils reaches 16.1%, with Pb accounting for 1.5% of this contamination. Contaminated forest soils directly harm the plant growth environment, affecting soil microorganisms and enzyme activities. Pb stress inhibits plant photosynthesis and respiration, damages mitochondria, and significantly limits seedling growth with lasting effects throughout the developmental period. Therefore, diagnosing photosynthetic capacity in woody plants at the juvenile stage under Pb stress is crucial for vegetation restoration of contaminated sites.

Chlorophyll fluorescence parameters contain vital information about photosynthetic processes, including light energy absorption and conversion, energy transfer and allocation, excess energy dissipation, and photoinhibition status. These parameters are widely used for non-destructive detection of photosystem performance under various stress conditions. The Lake model, developed by Kramer et al. based on the Stern-Volmer relationship, introduces parameters for monitoring PSII stability and deriving measures of non-photochemical quenching processes. The model calculates Y(NPQ) for regulated energy dissipation (reflecting photoprotective capacity) and Y(NO) for non-regulated energy dissipation, enabling evaluation of PSII reaction center openness and photochemical efficiency changes caused by downstream regulation of antenna pigments. Compared to the Puddle model, the Lake model' s chlorophyll fluorescence parameters better describe energy balance and allocation under defined light intensities and plant capacity to cope with excessive excitation energy.

Previous studies have successfully applied the Lake model to research on drought tolerance in crops, photoacclimation, and photoinhibition. However, its application for rapid evaluation of photosystem function in woody plants under heavy metal stress remains unexplored. This study selected *S. superba*, a common evergreen broad-leaved tree in southern China with low Pb tolerance, and *K. paniculata*, a deciduous broad-leaved species known for Pb tolerance. Both are light-demanding species suitable for subtropical regions. Using pot experiments with three Pb concentration gradients, we investigated the dynamic changes in Lake model-based chlorophyll fluorescence parameters under different PAR levels to reveal how energy allocation in the photosynthetic electron transport chain responds to Pb stress and to evaluate Pb resistance from perspectives of photosynthetic activity and photoprotective capacity.

Materials and Methods

Plant Materials

One-year-old container seedlings of *S. superba* and *K. paniculata* were obtained from the campus of Central South University of Forestry and Technology. Seedlings were uniform in growth status, with average heights of 84.1 cm and 15.9 cm, and ground diameters of 7.3 cm and 3.34 cm for *S. superba* and *K. paniculata*, respectively.

Experimental Design

The experiment was conducted in a greenhouse at the West Garden of Central South University of Forestry and Technology. Soil was air-dried, sieved to remove debris, and placed in plastic pots (30 cm height × 26 cm diameter) with 5 kg of dry soil per pot. After transplanting and establishment, PbCl solutions were applied to create three concentration gradients based on pure metal mass: L1: 500 mg/kg, L2: 600 mg/kg, and L3: 900 mg/kg soil. A control (CK) without Pb addition was included. Each treatment had 8 replicates.

The PbCl solution was prepared and sprayed evenly onto the soil surface, ensuring no contact with plant foliage and no leaching. Greenhouse conditions were maintained at 28°C/20°C day/night temperature with 60–70% relative humidity. Plants were watered with pure water as needed, avoiding drainage from pots. Regular weeding and soil loosening were performed.

Chlorophyll Fluorescence and Gas Exchange Measurements

Chlorophyll fluorescence parameters were measured using a portable pulse-modulated fluorometer (Mini-PAM, WALZ) on the 30th day after Pb treatment. Fully expanded, healthy leaves were selected from each pot after 20 minutes of dark adaptation. Rapid light-response curves were generated using actinic light intensities of 0, 105, 237, 349, 553, 796, and 1129 mol m^{2} s^{1} PAR.

Lake model parameters were calculated according to Kramer et al.: - Y(II) = (Fm' - Fs)/Fm' (actual quantum efficiency of PSII) - qL = (Fm' - Fs)/(Fm' - Fo') × Fo' /Fs (photochemical quenching) - Y(NPQ) = 1 - Y(II) - 1/(NPQ + 1 + qL×(Fm/Fo - 1)) (regulated energy dissipation) - Y(NO) = 1/(NPQ + 1 + qL×(Fm/Fo - 1)) (non-regulated energy dissipation) - rETR = PAR × 0.84 × 0.5 × Y(II) (relative electron transport rate)

Maximum net photosynthetic rate (Pn) was measured on the 30th day using a Li-6400 portable photosynthesis system (Li-Cor, USA) between 9:00-11:00. Measurements were conducted at 1000 mol m ² s ¹ PAR, with leaf chamber temperature at 30°C and relative humidity at 40–60%.

Data Analysis

Data were processed using Excel 2013 and Origin 8.0. One-way ANOVA was performed using SPSS 20.0 to analyze differences in chlorophyll fluorescence parameters among Pb concentrations and between species, followed by Duncan' s multiple comparison tests at $P < 0.05$ significance level. Figures were prepared using SigmaPlot 12.5.

Results

Effects of Pb Stress on rETR

The relative electron transport rate (rETR) in leaves of both species increased with PAR in both control and Pb-treated groups, though the magnitude of increase diminished with higher Pb stress. Under L1 and L2 treatments, rETR showed no significant difference from the control at most light intensities ($P > 0.05$). However, at L3, rETR was significantly lower than the first two treatments at light intensities above 349 mol m ² s ¹ ($P < 0.05$). For *K. paniculata*, rETR peaked at 553 mol m ² s ¹ under control and L1 conditions, while for *S. superba*, the peak occurred at 796 mol m ² s ¹. These peaks shifted to lower light intensities with increasing Pb concentration, indicating that Pb stress inhibits rETR and causes photoinhibition to appear earlier at elevated PAR [Figure 1: see original paper].

Effects of Pb Stress on Y(II)

The actual quantum efficiency of PSII [Y(II)] decreased with increasing light intensity in all treatments. Compared to the control, Y(II) declined significantly under all Pb concentrations ($P < 0.05$), with the reduction magnitude following L3 > L2 > L1. At L1, Y(II) values were comparable between species, but at L2 and L3, *S. superba* showed significantly lower Y(II) than *K. paniculata* ($P < 0.05$), suggesting higher cyclic electron transfer efficiency in *S. superba* but greater sensitivity of its reaction centers to Pb stress [Figure 2: see original paper].

Effects of Pb Stress on qL

Photochemical quenching (qL) decreased with increasing PAR in all treatments. Under Pb stress, qL was significantly lower than the control at all light intensities ($P < 0.05$). The reduction magnitude was greater in *S. superba* than in *K. paniculata*, particularly at L2 and L3 ($P < 0.05$). At L1, *S. superba* maintained relatively high reaction center openness, but its light energy conversion capacity was weaker than *K. paniculata* under higher Pb concentrations [Figure 3: see original paper].

Effects of Pb Stress on Y(NPQ)

Regulated energy dissipation [Y(NPQ)] increased with both PAR and Pb concentration in both species. *K. paniculata* showed significantly higher Y(NPQ) than *S. superba* at all Pb concentrations ($P < 0.05$), indicating stronger photoprotective capacity. Although *S. superba* exhibited substantial Y(NPQ) increases at L2 and L3, its values remained lower than *K. paniculata* at equivalent light intensities, demonstrating *K. paniculata*' s superior ability to regulate energy dissipation [Figure 4: see original paper].

Effects of Pb Stress on Y(NO)

Non-regulated energy dissipation [Y(NO)] showed minimal change with PAR but increased with Pb concentration. *S. superba* exhibited significantly higher Y(NO) than *K. paniculata* across all treatments ($P < 0.05$), with elevation magnitudes following L3 > L2 > L1. This suggests *S. superba* relies more on non-regulated energy dissipation, indicating lower photoprotective capacity compared to *K. paniculata* [Figure 5: see original paper].

Effects of Pb Stress on Fv/Fm and Pn

Maximum photochemical efficiency (Fv/Fm) decreased with increasing Pb concentration in both species, with reductions in *S. superba* (16-27% at L1, 27-31% at L2, 35-65% at L3) exceeding those in *K. paniculata* (19-20% at L1, 28-41% at L2, 62-82% at L3). At all concentrations, *K. paniculata* maintained significantly higher Fv/Fm than *S. superba* ($P < 0.05$). Similarly, Pn decreased with Pb concentration, with *K. paniculata* showing significantly higher values than *S. superba* at L2 and L3 ($P < 0.05$) .

Discussion

Biotic and abiotic stresses affect various photosynthetic processes, with changes reflected in chlorophyll fluorescence parameters. Under stress, photosynthetic reaction centers undergo reversible inactivation or irreversible damage, reducing primary light energy conversion efficiency and causing severe photoinhibition that diminishes electron transport activity and ultimately decreases carbon fixation efficiency.

Our Lake model-based analysis revealed that Pb stress reduced qL and Y(II) in both species, indicating decreased openness of PSII reaction centers and reduced effective quantum yield, which led to significantly lower photosynthetic electron transport efficiency. This inhibition of electron transport reduced carbon assimilation and Pn. The decline in Fv/Fm and Y(II) with increasing Pb concentration was accompanied by rising Y(NPQ), suggesting activation of photoprotective mechanisms. However, the magnitude of these responses differed between species.

At low Pb concentration (L1), *S. superba* maintained relatively high reaction center openness, but suffered severe damage at higher concentrations, reflecting weaker light energy conversion capacity. In contrast, *K. paniculata* exhibited significantly greater Y(NPQ) increases, demonstrating stronger regulated energy dissipation capacity and higher Pb tolerance. The earlier appearance of photoinhibition peaks at lower PAR under elevated Pb stress indicates that Pb toxicity accelerates photoinhibition, likely due to excessive reactive oxygen species (ROS) production damaging PSII reaction centers.

Comprehensive analysis suggests that Y(NPQ) and NPQ can serve as effective indicators for diagnosing Pb stress and evaluating resistance in woody plants. While Y(NO) also responded to Pb stress, its suitability as a diagnostic indicator is limited due to the existence of multiple electron transport pathways in photosynthesis. The consistent patterns observed in rETR, Y(II), and qL across Pb concentrations confirm that *K. paniculata* possesses superior Pb resistance attributable to robust photoprotective mechanisms, whereas *S. superba* is more vulnerable to Pb-induced photosynthetic damage.

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Note: Figure translations are in progress. See original paper for figures.