

Physiological and Biochemical Responses of Evergreen and Deciduous Trees to Combined Drought and Lead Stress: Implications for Urban Green Spaces

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The impact of drought and Pb toxicity were evaluated relative to physiological and biochemical traits of four commonly used urban tree species. The experiment included six treatments: control, 300 ppm Pb, 600 ppm Pb, drought (50% field capacity), drought + 300 ppm Pb, and drought + 600 ppm Pb, applied to *Platanus orientalis*, *Fraxinus excelsior*, *Cupressus sempervirens*, and *Pinus nigra*. Results showed that Pb toxicity – particularly 600 ppm and drought – led to changes in most traits of the plants ($P \leq 0.05$). The co-applied drought and 600 ppm Pb decreased total chlorophyll (Chl, 21%), relative water content (RWC, 23%), while increased malondialdehyde (MDA, 52%), proline accumulation (22%), total soluble sugar (TSS, 54%), superoxide dismutase (SOD, 110%), catalase activity (CAT, 137%), Pb accumulation in roots (1723%), and in shoots (611%) compared to the control. Compared to *C. sempervirens* and *P. nigra*, *P. orientalis* and *F. excelsior* accumulated more Pb and exhibited greater sensitivity to abiotic stress. The heat map results exhibited that traits under drought and Pb stress showed the most significant fluctuation, with TSS explaining the most variation among measured traits.

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INTRODUCTION

Drought and lead (Pb) toxicity represent two major abiotic stresses that adversely affect plant growth, metabolism, and physiological processes, each with distinct mechanistic impacts (Gupta *et al.* 2024; Samanta *et al.* 2024). Drought induces water deficit, leading to reduced cell expansion, stomatal closure, diminished photosynthesis, and increased production of reactive oxygen species (ROS). In response, plants often accumulate osmolytes such as proline and soluble sugars to maintain osmotic balance and cellular stability (Sato *et al.* 2024; Samanta *et al.* 2024). Conversely, Pb stress disrupts nutrient uptake by competing with essential elements like Ca^{2+} , Mg^{2+} , and Fe^{2+} , resulting in metabolic imbalances. It also inhibits chlorophyll synthesis and photosynthetic enzymes, causing chlorosis, stunted growth, and leaf deformation (Collin *et al.* 2022; Gupta *et al.* 2024). Both stresses independently trigger oxidative damage, prompting an upregulation of the antioxidant defense system in plants.

Urban trees are vital for enhancing environmental quality, providing ecosystem services such as air purification, carbon sequestration, microclimate regulation, and phytoremediation (Diener and Mudu 2021; Rahman *et al.* 2022). Deciduous species often contribute to seasonal aesthetics and effective phytoextraction of heavy metals, while evergreen species offer year-round benefits and can stabilize contaminants in roots and woody tissues (Bui *et al.* 2022). Selecting appropriate tree species that can withstand combined environmental stresses is therefore critical for sustainable urban green space development and the remediation of polluted sites.

Previous research has extensively documented the detrimental effects of either drought or Pb stress on various tree species, such as *Platanus orientalis* under water deficit (Akhbarfar *et al.* 2023) and *Theobroma cacao* under Pb contamination (Apraez Muñoz *et al.* 2021). However, in urban environments, trees are frequently exposed to multiple concurrent stresses, yet studies investigating their combined effects—particularly drought and Pb toxicity—remain limited (Zhang *et al.* 2023; Kumar *et al.* 2024). Moreover, comparative analyses of the physiological and biochemical resilience mechanisms between evergreen and deciduous functional groups under such dual-stress conditions are scarce.

This study provides a novel, holistic assessment of the physiological and biochemical responses of two deciduous species (*P. orientalis* and *Fraxinus excelsior*) and two evergreen species (*Cupressus sempervirens* and *Pinus nigra*) to combined drought and Pb stresses. Unlike most prior research focusing on single stressors, this work systematically examines their interaction under controlled open-field conditions, simulating real-world urban scenarios where water scarcity and soil contamination co-occur. The research identifies species-specific tolerance thresholds, phytoremediation potential, and adaptive strategies, offering a refined framework for selecting urban tree species based on resilience to multiple abiotic stresses. These findings deliver actionable insights for urban planners, landscape managers, and environmental policymakers to optimize green infrastructure in regions facing simultaneous challenges of heavy metal pollution and water limitation.

MATERIALS AND METHODS

Experimental Design and Growth Conditions

A factorial experiment was conducted using a completely randomized design (CRD) with three replicates for each treatment in 2023.

The study investigated the interactive effects of abiotic stress and plant species on physiological and biochemical attributes in plants. The first factor was abiotic stress at six levels: Control, 300 ppm Pb, 600 ppm Pb, drought, drought + 300 ppm Pb, and drought + 600 ppm Pb. The second factor was plant species in four levels: *P. orientalis*, *F. excelsior*, *C. sempervirens*, *P. nigra*, resulting in a total of 24 treatments. The three-year-old seedlings of *P. orientalis*, *F. excelsior*, *C. sempervirens*, and *P. nigra* were selected to determine their responses to drought and Pb stresses in an open-field conditions in Karaj, Iran (35°49'57" N and 50°59'29" E). The mean annual temperature and precipitation were 14.2 °C and 207 mm, respectively, in 2023. The experimental soil was obtained from a horticulture field, which consisted of N: 0.30%, P: 15.2 mg kg⁻¹, K: 250 mg kg⁻¹, Pb: 8.5 mg kg⁻¹, pH: 7.20, and EC: 1.5 dS m⁻¹.

Pb was mixed with the soil before transplanting the seedlings into the pots at 300 and 600 ppm. The plants were irrigated at 100% field capacity (FC) for two months and

then drought stress was applied at 50% FC for 60 days. At the end of the experiment, the root and leaf sample were selected for measuring physiological and biochemical attributes.

Chlorophyll Content

The chlorophyll (Chl) content was evaluated using the method described by Arnon (1949). First, 0.1 g of plant leaf sample and 3 mL of 80% acetone were completely crushed in a mortar. The liquid was then diluted until it had a final volume of 15 mL. The mixture was centrifuged at $5000 \times g$ for 10 min to obtain a clear extract. A Shimadzu UV-160 spectrophotometer was employed to measure the absorbance of the samples. The spectrophotometer was calibrated prior to the test using 80% acetone as the blank. Next, the clear extract's absorbance was measured at 645 nm and 663 nm. Using the obtained absorbance values, Eq. 1 was used to calculate the total Chl (mg g^{-1} FW),

$$\text{Total Chl} = [(20.29 \times A_{645}) - (8.02 \times A_{663})] \times V / 1000 \times W \quad (1)$$

where A represents the absorbance at the specified wavelengths, V is the final volume of 80% acetone in mL, and W denotes the weight of the fresh leaf sample in g.

Relative Leaf Water Content Measurement

The fully developed leaves from the plants were harvested and weighed in order to obtain fresh weight (FW). The leaves from each treatment were individually immersed in distilled water in closed test tubes at 4°C for 5 h to reach full turgidity. After 5 h the leaves were removed, blotted dry with filter paper, and immediately re-weighed to obtain turgid weight (TW). To determine their dry weight (DW), the leaves were thoroughly oven-dried for 48 h at 70°C . After drying, the leaves were weighed again. The RWC was calculated using Eq. 2 as below (Ritchie *et al.* 1990):

$$\text{RWC} = [(FW - DW) / (TW - DW)] \times 100 \quad (2)$$

Malondialdehyde

The malondialdehyde (MDA) concentration was used to measure membrane lipid peroxidation. Fresh leaf tissues were ground in liquid nitrogen with a 0.1% (w/v) trichloroacetic acid (TCA) on ice. The homogenate was centrifuged at $10000 \times g$ for 10 min at 4°C . An aliquot of the supernatant (0.5 mL) was mixed with 0.5 % (w/v) of 2-thiobarbituric acid in 20 % (w/v) TCA and incubated in a water bath at 95°C for 30 min. The tubes were then placed on crushed ice for 10 min. The absorbance of the solutions was read at wavelengths of 532 and 600 nm. After cooling in an ice bath for 10 min, absorbance was measured at 532 nm and corrected by subtracting the non-specific absorbance at 600 nm. The MDA concentration (nmol g^{-1} FW) was calculated using Eq. 3,

$$\text{MDA} = [(A_{532} - A_{600}) \times V] / (155 \text{ mM}^{-1} \text{ cm}^{-1} \times l \times W) \quad (3)$$

where V is the final reaction volume (mL), l is the path length (1 cm), and W is the fresh weight (g), following Heath and Packer (1968).

Proline Concentration

Proline content was determined following Bates *et al.* (1973). About 0.1 g of fresh leaves was ground and mixed with 10 mL of (3% w/v) sulfosalicylic acid and centrifuged at 10000 g for 5 min. After that, 2 mL of the supernatant liquid obtained was mixed with 2 mL of ninhydrin reagent and 2 mL of pure acetic acid. The mixture was kept in a hot water

bath (100 °C) for 60 min. Then the tubes containing the mixture were cooled using an ice bath. Subsequently, 4 mL of toluene was added to the mixture and stirred with a Vertex for 20 s till two separate layers were formed. The absorbance of the upper layer containing toluene and proline was determined at 520 nm and the standard curve of proline (0, 4, 8, 12, 16 and 20 mg/L) was used to obtain the concentration of proline as $\mu\text{mol g}^{-1}$ FW.

Enzyme Assay

To ascertain the enzymatic activity in plants, the activities of superoxide dismutase (SOD) and catalase (CAT) were examined. Fresh material (0.5 g) was homogenized in 5 mL of ice-cold 50 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA-Na and 1 mM ascorbate at 4 °C. The homogenate was centrifuged at $12\,000 \times g$ for 15 min at 4 °C, and the clear supernatant (crude enzyme extract) was used for assays. SOD activity was determined by monitoring the inhibition of nitro-blue tetrazolium (NBT) reduction at 560 nm in a reaction mixture containing riboflavin (Gupta *et al.* 1993). CAT activity was measured by following the decrease in absorbance at 240 nm due to H_2O_2 decomposition (Aebi 1984). All spectrophotometric readings were taken at 25 °C and corrected against appropriate blanks.

Total Soluble Sugar

To quantify total soluble sugars (TSS), 0.1 g of fresh leaf or root tissue was weighed and immediately placed in 5 mL of 80 % (v/v) ethanol. The suspension was incubated at 80 °C for 30 min with occasional vortexing to extract soluble sugars; this extraction was repeated three times, pooling the supernatants each time to a final volume of ~20 mL. The combined ethanolic extract was evaporated to dryness at 55 °C under a gentle stream of air (or under reduced pressure). The residue was re-dissolved in 2 mL distilled water and stored at –80 °C until analysis. TSS concentration was determined by the anthrone–sulfuric acid method (Van Handel 1968): 0.2 mL aliquot of the aqueous extract was mixed with 0.8 mL of freshly prepared anthrone reagent (0.2 % w/v anthrone in 95 % H_2SO_4), heated at 100 °C for 10 min, cooled on ice, and the absorbance of the blue-green chromophore was read at 620 nm using a Beckman DU-520 spectrophotometer. A glucose standard series (0–100 $\mu\text{g/mL}$) was run in parallel and used to construct the calibration curve; results were expressed as mg glucose equivalents g^{-1} fresh weight (FW).

Pb Accumulation in Plant Tissues

The concentration of Pb in plant tissues was determined using inductively coupled plasma–mass spectrometry (ICP-MS) equipped with a quadrupole mass analyzer, a concentric nebulizer, and a cyclonic spray chamber. The instrument was operated under standard conditions, including an RF power of about 1.4 kW, plasma gas flow of about 15 L min^{-1} , auxiliary gas flow of about 1.0 L min^{-1} , and nebulizer gas flow of about 1.0 L min^{-1} . Pb was quantified by monitoring the ^{208}Pb isotope due to its high sensitivity and minimal spectral interference. Calibration standards were prepared by serial dilution of a certified 1000 mg L^{-1} Pb stock solution in 1% (v/v) HNO_3 , and calibration curves showed excellent linearity ($R^2 > 0.999$).

Quality assurance and quality control (QA/QC) procedures included the use of analytical-grade reagents and ultrapure deionized water ($18.2 \text{ M}\Omega \text{ cm}^{-1}$). Procedural and reagent blanks were analyzed with each batch to assess potential contamination. Internal standards (*e.g.*, ^{103}Rh or ^{115}In) were applied to correct for instrumental drift and matrix effects.

Method accuracy was evaluated using certified reference materials (CRMs) of plant origin, yielding Pb recoveries between 90% and 110%, while precision assessed through replicate analyses showed relative standard deviations (RSDs) below 5%. The limits of detection (LOD) and quantification (LOQ) were calculated as three and ten times the standard deviation of blank measurements, respectively, and all Pb concentrations were reported on a dry weight basis after blank correction (Khosropour *et al.* 2019).

Data Analysis

Using Statistical Analysis System (SAS, version 9.2), the means of three data for each treatment were compared using Duncan test, the mean comparison test, at a 5% level of statistical significance ($P < 0.05$). Principal component analysis (PCA) was performed using XLSTAT (Version 2009.6.03, Addin soft, USA). With CIMminer, a heat map analysis was performed online at <https://discover.nci.nih.gov/cimminer/home.do>.

RESULTS

Chlorophyll and Water Content

Total Chl content was significantly different under drought and Pb stresses and varied among the plant species ($P \leq 0.05$). Total Chl content decreased by 4%, 10%, 13%, 17%, and 21%, respectively, for 300 ppm Pb, 600 ppm Pb, drought, 300 ppm Pb + drought, and 600 ppm Pb + drought relative to the control (Fig. 1a). The Chl was also different among the plant species. The highest amount was observed in *P. nigra* with 22% higher than in *P. orientalis*, which had the lowest Chl content (Fig. 1b).

The RWC was significantly affected by the interaction between abiotic stress and plant species ($P \leq 0.05$). It ranged from 68% in (*C. sempervirens* and drought + 600 ppm Pb) to 88.6% (*P. orientalis* without abiotic stress). The drought + 600 ppm Pb treatment resulted in RWC declines of 21%, 23%, 20%, and 27% in *C. sempervirens*, *F. excelsior*, *P. nigra*, and *P. orientalis*, respectively, in comparison to the control (Fig. 1c).

Proline and Total Soluble Sugar

Proline content was affected significantly only by abiotic stress ($P \leq 0.05$), while it didn't show any significant changes among plant species ($P > 0.05$). It increased by 5%, 16%, 11%, 19%, and 22%, respectively for 300 ppm Pb, 600 ppm Pb, drought, 300 ppm Pb + drought, and 600 ppm Pb + drought, compared to the control (Fig. 1a).

The interaction of abiotic stress and plant species was significant ($P \leq 0.05$) on TSS. The treatment of drought + 600 ppm Pb stress led to increased TSS by 56%, 48%, 45%, and 68% in *C. sempervirens*, *F. excelsior*, *P. nigra*, and *P. orientalis*, respectively, relative to the control. The abiotic stress levels showed different effects on TSS. For example, in *P. orientalis*, 12%, 20%, 44%, 54%, and 68% increases were respectively obtained by 300 ppm Pb, 600 ppm Pb, drought, 300 ppm Pb + drought, and 600 Pb +drought relative to the control (Fig. 2b).

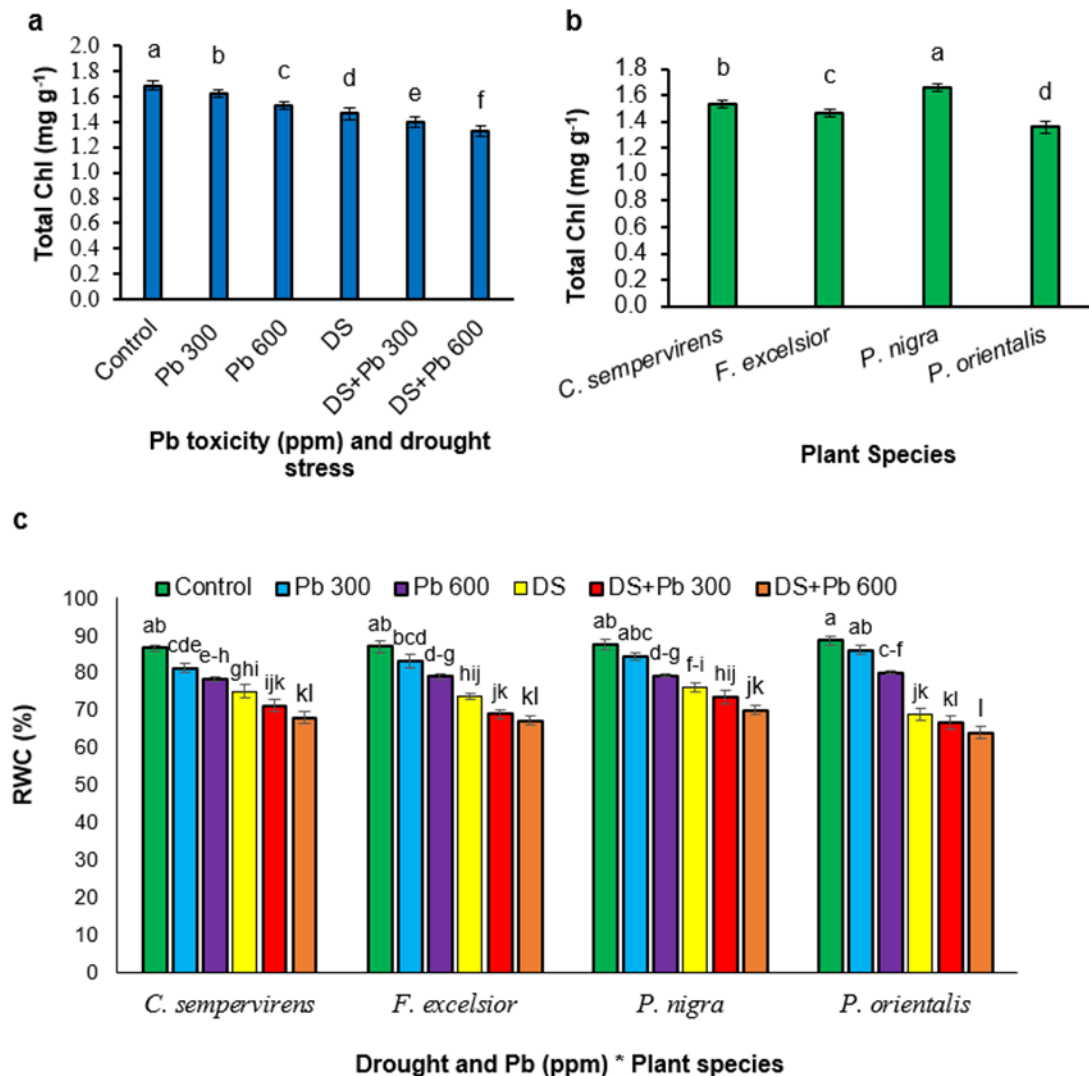


Fig. 1. Chlorophyll (Chl, a & b) and relative water content (RWC, c) of *Platanus orientalis*, *Fraxinus excelsior*, *Cupressus sempervirens*, and *Pinus nigra* under Pb and drought stress (DS). Lowercase letters above the bars indicate significant differences ($P \leq 0.05$) by Duncan test. Error bars represent the standard error.

Electrolyte Leakage and Malondialdehyde

Electrolyte leakage (EL) was significantly affected only by abiotic stress ($P \leq 0.05$) and did not show significant changes between plant species ($P > 0.05$). The EL increased in response to stress, indicating greater membrane damage under these conditions. It increased by 5%, 17%, 18%, 22%, and 29%, respectively for 300 ppm Pb, 600 ppm Pb, drought, 300 ppm Pb + drought, and 600 Pb +drought relative to the control (Fig. 3a).

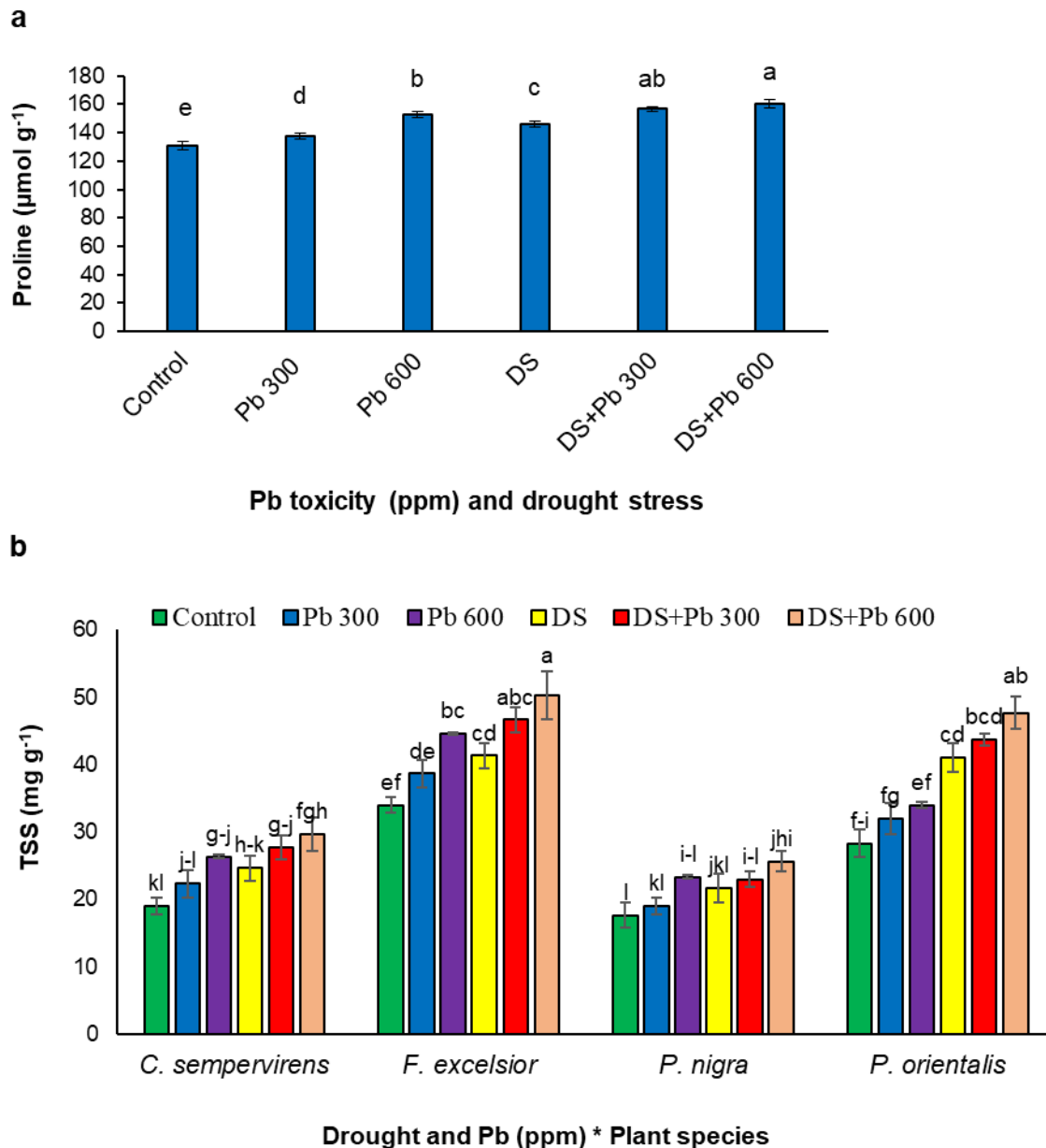


Fig. 2. Proline (a); and total sugar content (TSS, b) of *Platanus orientalis*, *Fraxinus excelsior*, *Cupressus sempervirens*, and *Pinus nigra* under Pb and drought stress (DS). Lowercase letters above the column show the significant differences ($P \leq 0.05$) by Duncan test. Error bars represent the standard error.

MDA was significantly affected by abiotic stress and plant species ($P \leq 0.05$). Exposure to Pb and drought stress led to a notable increase in MDA levels, indicating higher lipid peroxidation under these conditions. It increased by 7%, 29%, 37%, 46%, and 52%, respectively, to 300 ppm Pb, 600 ppm Pb, drought, 300 ppm Pb + drought, and 600 ppm Pb +drought relative to the control (Fig. 3b). Among the plant species, *P. nigra* exhibited the minimum MDA accumulation (Fig. 3c).

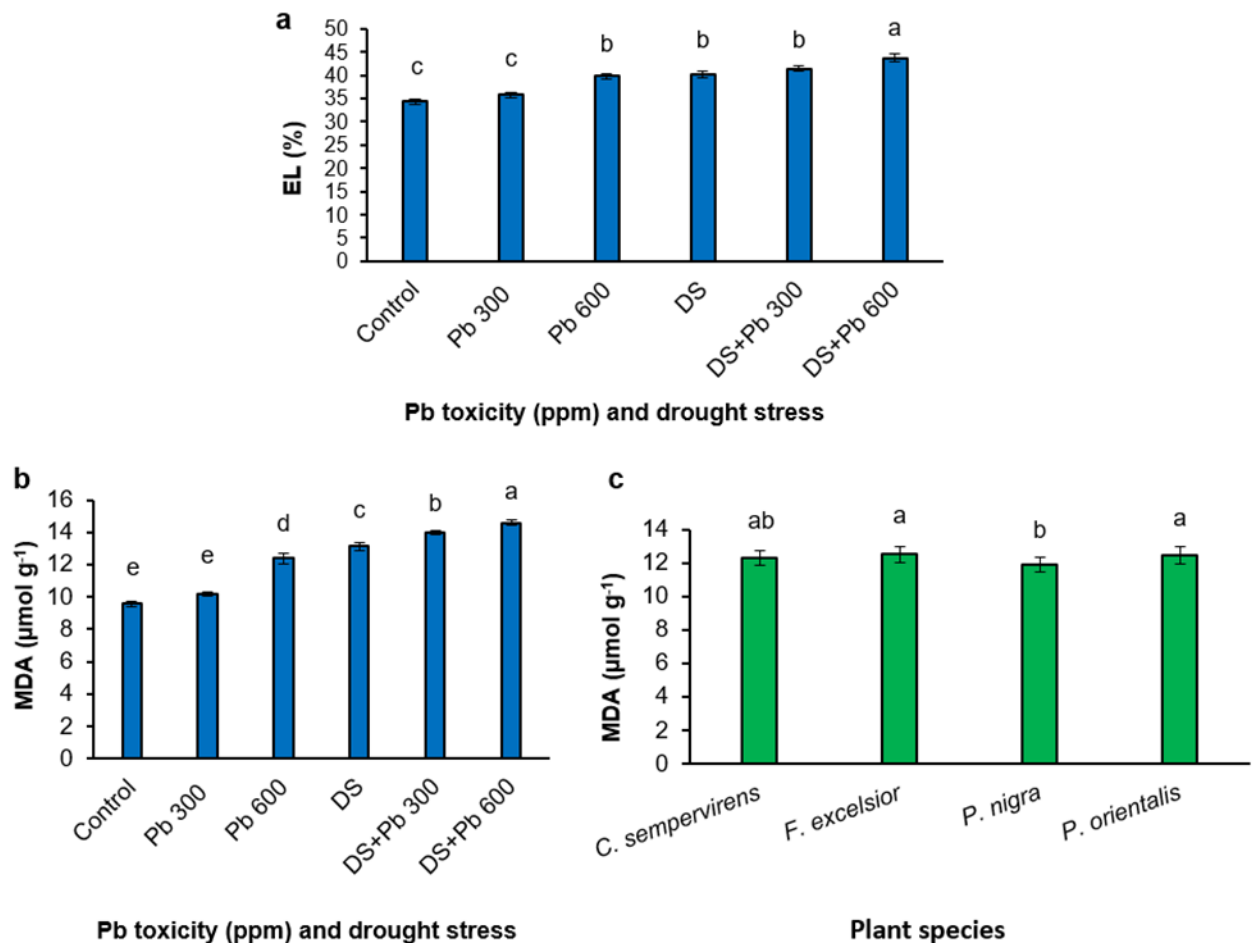


Fig. 3. Electrolyte leakage (EL, a); and malondialdehyde (MDA, b & c) of *Platanus orientalis*, *Fraxinus excelsior*, *Cupressus sempervirens*, and *Pinus nigra* under Pb and drought stress (DS). Lowercase letters above the bars shows the significant differences ($P \leq 0.05$) by Duncan test. Error bars represent the standard error.

Antioxidant Enzymes

The SOD activity responded significantly to both abiotic stress and plant species ($P \leq 0.05$). The Pb and drought elevated SOD levels, with drought exerting the stronger effect. Compared with the control, SOD rose by 10%, 57%, 73%, 81%, and 100% under 300 ppm Pb, 600 ppm Pb, drought, 300 ppm Pb + drought and 600 ppm Pb + drought, respectively. Across species, *P. orientalis* and *F. excelsior* exhibited the highest activities; *P. orientalis* surpassed *P. nigra* by 12 % (Fig. 4a).

The interaction of abiotic stress and plant species was significant ($P \leq 0.05$) on catalase (CAT) activity. All plant species exposed to drought and 600 ppm Pb represented the maximum CAT activity. The of drought + 600 ppm Pb stress enhanced CAT activity by 120%, 145%, 108%, and 183% in *C. sempervirens*, *F. excelsior*, *P. nigra*, and *P. orientalis*, respectively, relative to the control. The abiotic stress levels showed different effects on CAT activity (Fig. 4b).

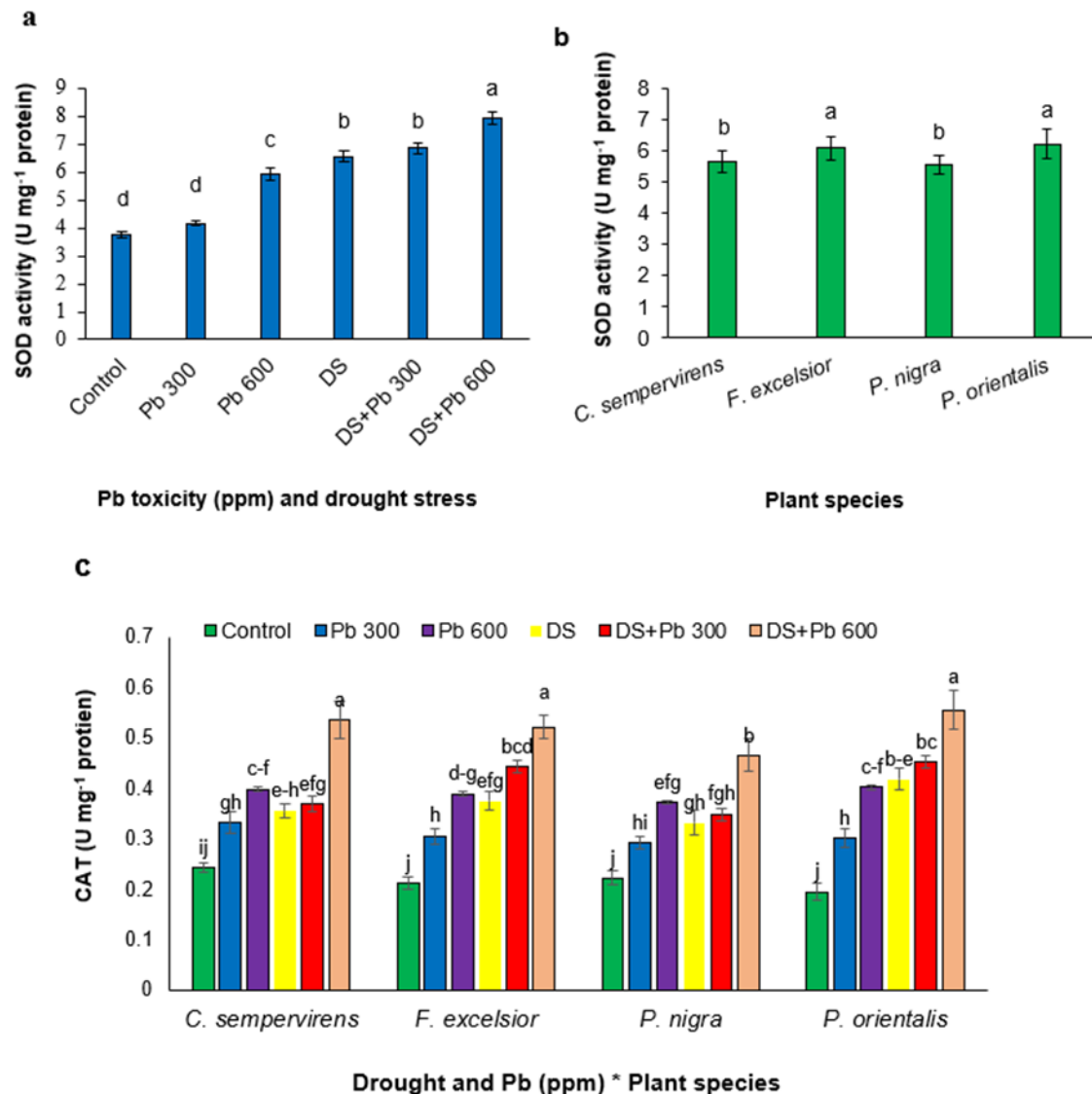


Fig. 4 Superoxide dismutase (SOD, a & b) and catalase (CAT, c) of *Platanus orientalis*, *Fraxinus excelsior*, *Cupressus sempervirens* and *Pinus nigra* under Pb and drought stress (DS). Lowercase letters above the bars show the significant differences ($P \leq 0.05$) by Duncan test. Error bars represent the standard error.

Pb Accumulation in Plant Tissues

Pb accumulation on roots and leaves was significantly influenced by interaction of abiotic stress and plant species ($P \leq 0.05$). A significant increase in Pb uptake and retention in both roots and leaves was shown by the maximum Pb accumulation, which was seen under the combined drought + 600 ppm Pb treatment. When compared to the control, Pb accumulation in roots during drought + 600 ppm Pb stress rose by 1595%, 2159%, 1564%, and 1755% in *C. sempervirens*, *F. excelsior*, *P. nigra*, and *P. orientalis*, respectively (Fig. 5a).

Compared to the control, drought + 600 ppm Pb led to elevated Pb accumulation in leaves by 651%, 603%, 476%, and 721% in *C. sempervirens*, *F. excelsior*, *P. nigra*, and *P. orientalis*, respectively. According to these findings, Pb accumulation differed between roots and leaves and was species-dependent. A significant increase in Pb uptake and

retention in both roots and leaves was shown by the maximum Pb accumulation, which was seen under the combined drought + 600 ppm Pb treatment (Fig. 5b).

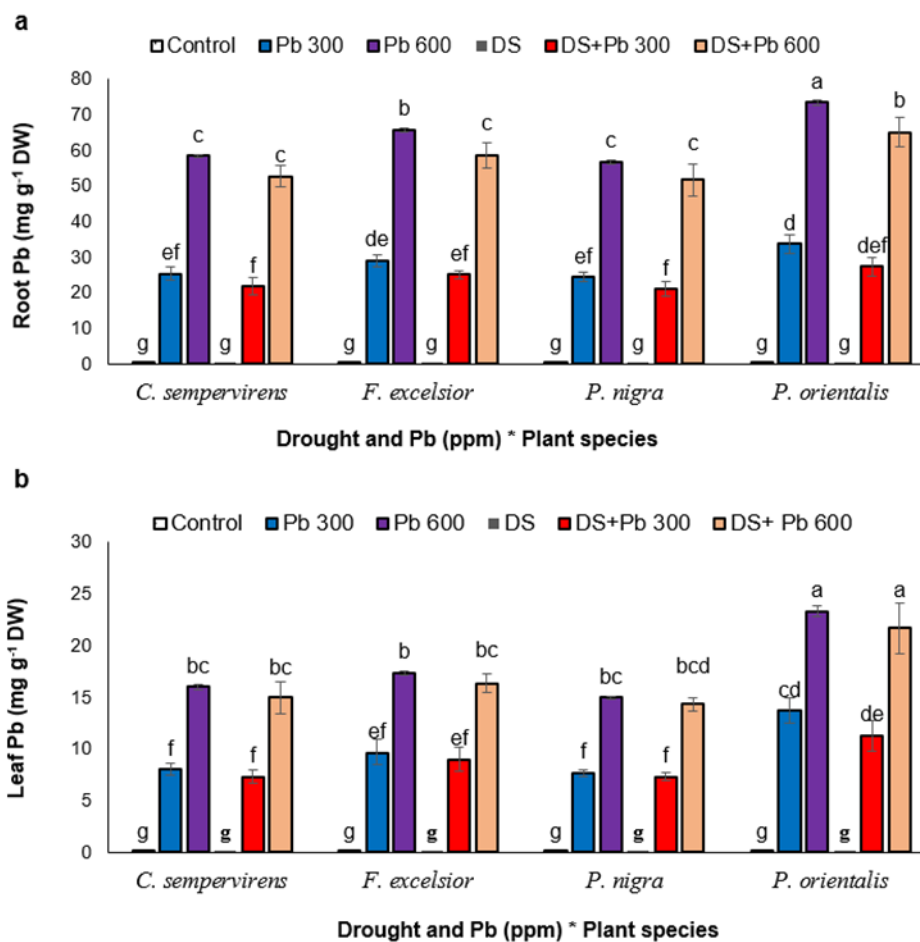


Fig. 5. Pb concentration in of roots (a) and leaves (b) of *Platanus orientalis*, *Fraxinus excelsior*, *Cupressus sempervirens* and *Pinus nigra* under Pb and drought stress (DS). Lowercase letters above the bars show the significant differences ($P \leq 0.05$) by Duncan test. Error bars represent the standard error.

Principal Component Analysis

The first axis (F1) accounted for 97% of the changes in the PCA results related to abiotic stress, but the second axis (F2) only explained 1.8%. F1 supported all the traits and treatments, while F2 supported only 600 ppm Pb among the treatments (Fig. 6a).

The PCA results showed that F1 accounted for 82.8% of the variation in plant species, while F2 explained only 13%. F1 was associated with most evaluated traits except proline, which was supported by F2. F1 strongly supported *F. excelsior*, *P. nigra*, and *P. orientalis*, while F2 was mainly associated with *C. sempervirens*. These results suggest that *C. sempervirens* exhibit unique trait variations that differ from other species, as captured by the F2 (Fig. 6b).

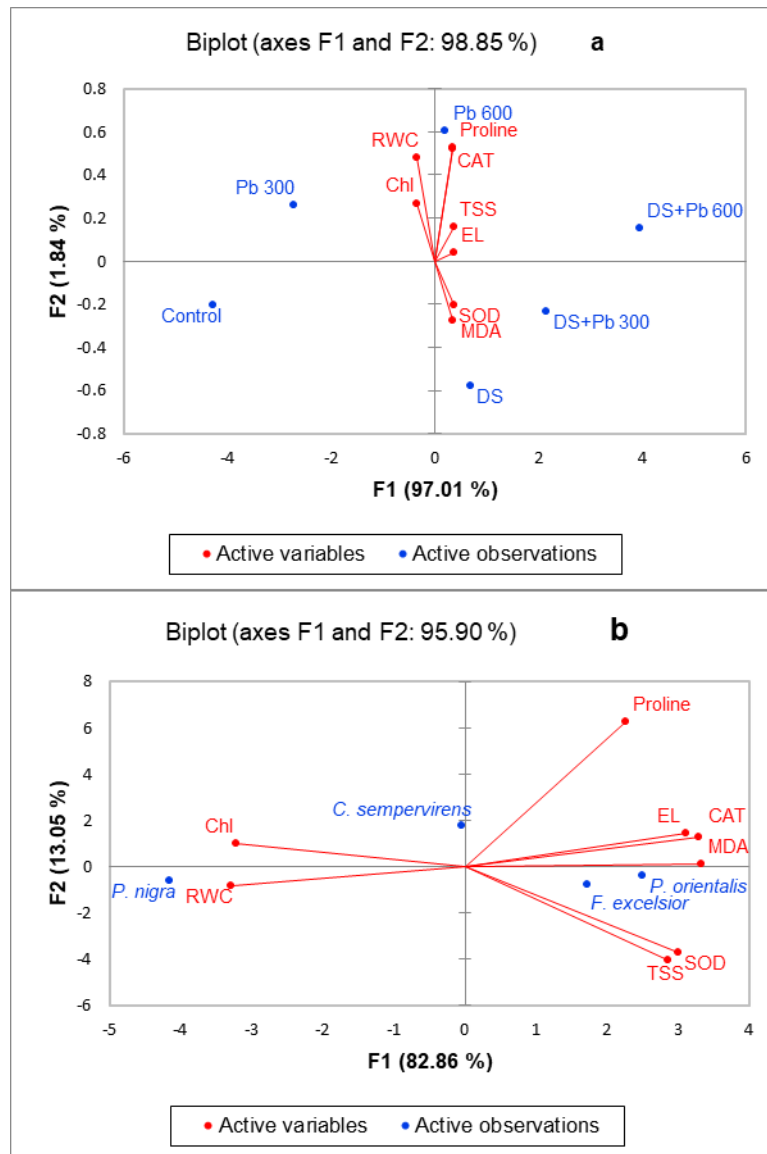


Fig. 6. Principal component analysis (PCA) for Pb and drought stress (DS, a) and plant species including *Platanus orientalis*, *Fraxinus excelsior*, *Cupressus sempervirens*, and *Pinus nigra* (b). Chl: chlorophyll, RWC: relative water content, TSS: total soluble sugar, EL: electrolyte leakage, MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase

Heat Map

The heat map results of abiotic stress showed that CAT and SOD represented the highest variability among measured traits under drought and Pb stress, followed by TSS and MDA. Proline showed the least variability. Two main clusters were identified; cluster 1 comprised the control and 300 ppm Pb treatments, while cluster 2 included 600 ppm Pb, drought, drought + 300 ppm Pb, and drought + 600 ppm Pb treatments (Fig. 7a).

According to the plant species heat map data, TSS accounted for the greatest degree of variability among the parameters that were measured. RWC, EL, and proline had the least amount of variation. There were two major clusters found: cluster 1 included *C. sempervirens*, while cluster 2 included *F. excelsior*, *P. nigra*, and *P. orientalis* (Fig. 7b).

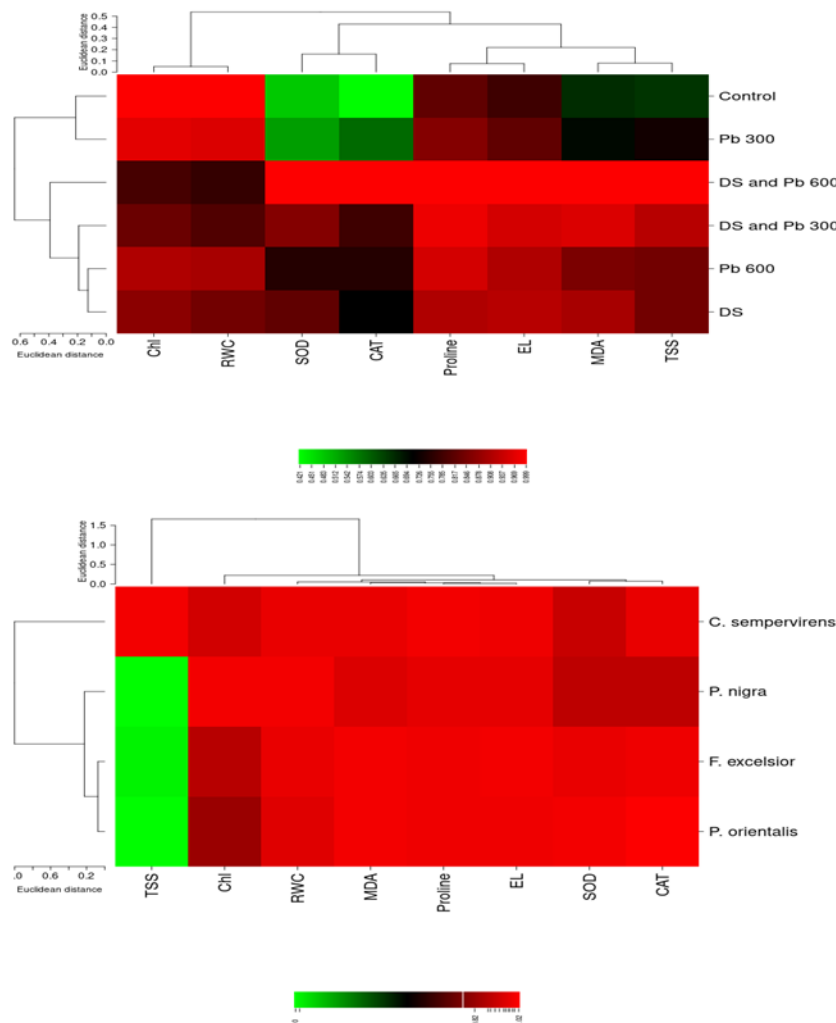


Fig. 7. Heat map for Pb and drought stress (DS, a) and plant species including *Platanus orientalis*, *Fraxinus excelsior*, *Cupressus sempervirens*, and *Pinus nigra* (b). Chl: chlorophyll, RWC: relative water content, TSS: total soluble sugar, EL: electrolyte leakage, MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase

Discussion

Drought stress and Pb toxicity both reduced Chl content in all plant's species. Drought leads to decreased Chl synthesis, increased lipid peroxidation, enhances chlorophyllase activity, and downregulates the genes involved in Chl biosynthesis. It also leads to increased ROS production, damage to chloroplast membranes, and downregulation of genes associated with photosynthesis (Anwar *et al.* 2023). Pb stress replaces essential ions in Chl molecules, increases ROS production, inhibits enzymes involved in Chl biosynthesis, and suppresses photosynthesis gene expression. Both stresses directly and indirectly reduce Chl content, impairing photosynthesis and limiting plant growth (Hussain *et al.* 2021). Akhbarfar *et al.* (2023) showed a significant reduction in Chl content of *P. orientalis* plants under drought at 50% FC. The current work, however, demonstrated a novel method of using both Pb and drought in the four primary tree species, demonstrating the various responses of these plants to abiotic stress. *P. nigra* and *C. sempervirens* had higher Chl content compared to *P. orientalis* and *F. excelsior* due to their evergreen leaves, higher chloroplast density, adaptation to environmental stress, and stable photosynthetic

system (Sim *et al.* 2021). Evergreen trees retain their leaves for several years and are adapted for long-term photosynthesis, which allows them to maintain higher Chl levels even under stress conditions. In contrast, deciduous trees shed their leaves annually and prioritize rapid growth and turnover, resulting in lower Chl retention. They also tend to have a higher carotenoid-to-chlorophyll ratio, which contributes to their comparatively lower Chl content (Qin *et al.*, 2024).

Drought and Pb stress significantly reduced RWC in the plants. Drought leads to reduced water availability and uptake, increased transpiration rate, impaired root function and water transport, and increased production of ROS (Akhbarfar *et al.* 2023). According to Shahmohammadi *et al.* (2024), a significant drop in RWC in *Taxus baccata* was caused by drought at 40% FC. In addition, Hojjati *et al.* (2024) found a significant decline in RWC (22% under drought at 30% FC), similar to the 21 to 27% decline observed under drought and 600 ppm Pb in the present work. Pb stress disrupts water uptake and transport, alters stomatal function, and causes membrane damage and increased water loss. It also interferes with ion homeostasis, reducing K^+ and Ca^{2+} levels, leading to water efflux from cells and further decreasing RWC. Both stress conditions can lead to reduced CO_2 intake and limited photosynthesis, affecting plant health and overall water status (Alavian *et al.* 2024). Deciduous seedlings have lower RWC due to physiological and structural differences, making them more vulnerable to soil drying and prioritizing rapid growth during favorable seasons, affecting their water economy under specific environmental conditions (Chondrogiannis and Grammatikopoulos 2025).

Proline accumulation is crucial for plant stress tolerance, acting as an osmolyte, antioxidant, membrane and protein protector, metal chelation, stress signaling, and energy source during recovery. It helps maintain cellular water balance, neutralize ROS, stabilize cell membranes, and protect against heavy metal damage (Kijowska-Oberc *et al.* 2023; Crespo-Barreiro *et al.* 2025). Proline also plays a role in stress signaling, triggering defense responses and regulating stress-responsive genes like P5CS. After stress conditions subside, accumulated proline can be broken down to provide energy and carbon skeletons for metabolic recovery (Patel *et al.* 2022).

With variations in plant species, TSS rose during abiotic stress. Drought caused greater effects than Pb on TSS accumulation. Drought results in severe osmotic stress and metabolic shifts in plants, impacting sugar accumulation. Dehydration and reduced cell turgor trigger the synthesis of osmolytes like TSS to maintain water balance (Hernandez *et al.* 2021). Drought stress reduces photosynthesis, forcing plants to rely on stored carbohydrates and sugar metabolism for energy (Zahedi *et al.* 2022). Pb stress affects enzyme activity, nutrient uptake, and oxidative stress, but does not induce direct water loss. Because deciduous trees have different stress response mechanisms, they have more TSS than evergreen trees (Afzaal *et al.* 2023). Evergreen species reduce water loss and metabolic changes because of their higher photosynthetic rates and structural adaptations. Because of their greater water conservation, structural robustness, and long-term energy stability, *P. nigra* and *C. sempervirens* are more resilient to Pb stress and drought (Shao *et al.* 2022).

Drought and Pb stresses increased SOD and CAT activities. This is due to oxidative stress to overproduction of ROS. These ROS can damage membranes, proteins, and DNA, causing cell death. Plants activate their antioxidant defense system, including SOD and CAT, to detoxify ROS and protect cellular structures (Yu *et al.* 2023; Torun *et al.* 2024). This protective mechanism enhances stress tolerance and maintains cellular homeostasis (de Souza-Vieira *et al.* 2024). Evergreen plants had lower SOD and CAT activities

compared to deciduous plants. Evergreen plants are more tolerant to environmental stresses due to their water conservation, stronger structural defenses, and stable metabolism. They produce less ROS and require less antioxidant enzymes. Conversely, deciduous plants are more sensitive to stress, leading to higher ROS production (Khosropour *et al.* 2019; Ghadirnezhad Shiade *et al.* 2023).

Under soil Pb stress, deciduous plants accumulated more Pb. It is due to higher transpiration rates, nutrient transport efficiency, and root uptake capacity in deciduous plants accumulated compared to evergreen plants (Rahman *et al.* 2022). These factors increase water uptake and Pb absorption, allowing more Pb to be transported to aboveground tissues. Evergreen plants, on the other hand, have lower transpiration rates, slower nutrient cycling, and protective mechanisms, reducing Pb accumulation and making them more resistant to Pb stress (Khosropour *et al.* 2019; Rahman *et al.* 2022).

CONCLUSIONS

1. The findings highlighted clear functional differences between evergreen and deciduous urban tree species under combined drought and lead stress. Evergreen species demonstrated stronger physiological stability and greater tolerance to dual stress conditions, whereas deciduous species exhibited a higher capacity for lead accumulation, indicating stronger phytoremediation potential.
2. The interaction of drought and lead intensified stress effects, compromising photosynthetic performance and plant water status while enhancing oxidative stress responses.
3. Species-specific responses further confirmed that *Platanus orientalis* and *Fraxinus excelsior* are more effective for lead uptake, while *Cupressus sempervirens* and *Pinus nigra* are better suited for maintaining resilience and canopy sustainability in contaminated, water-limited environments.
4. Therefore, an integrated planting strategy that combines tolerant evergreens for structural stability with selected deciduous species for targeted Pb removal can enhance the effectiveness and sustainability of urban green spaces exposed to multiple abiotic stresses.

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