Effects of Cadmium Treatment under Drought Stress on Growth, Cadmium Accumulation, and Photosynthetic Physiology of Buddleja alternifolia Seedlings (Postprint)

Authors: Yan Jiangwei, Li Changxiao, Cui Zhen, Liu Yuan

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Abstract

To investigate the growth and photosynthetic physiological response mechanisms of Buddleja alternifolia Maxim. seedlings to heavy metal cadmium stress under drought conditions, two-year-old B. alternifolia seedlings were used as experimental materials. Two water treatment groups were established: control and drought (soil relative water contents of 65%-60% and 35%-30%, respectively). Under each water treatment condition, three cadmium treatment concentrations were set (0.28, (0.6+0.28), and (1.2+0.28) mg/kg), totaling six treatments. The effects of different water and cadmium treatments on the growth, biomass, photosynthetic parameters, and heavy metal content in B. alternifolia were measured. The results showed that plant survival rate under combined drought and cadmium stress was 100%. Both cadmium stress alone and combined drought and cadmium stress inhibited seedling growth, biomass accumulation, photosynthesis, and chlorophyll content of B. alternifolia to varying degrees, with the reductions in photosynthesis and chlorophyll content being significantly greater than those under cadmium stress alone. Under cadmium stress alone, the maximum cadmium accumulation per B. alternifolia seedling was 69.33 mg/kg, whereas under combined stress it was 50.68 mg/kg. These results indicate that drought stress can exacerbate the effects of cadmium stress on plants, reducing the growth, photosynthetic physiology, and cadmium accumulation capacity of B. alternifolia under combined stress. However, under cadmium stress alone, B. alternifolia exhibited stronger tolerance to cadmium and higher bioaccumulation capacity, and seedlings still accumulated a certain amount of cadmium under combined drought and Cd stress. Therefore, B. alternifolia is a shrub species with great application potential and prospects for landscaping in arid and semi-arid regions and for ecological restoration in Cd-contaminated areas.

Full Text

Preamble

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Effects of Cadmium on Growth, Cadmium Accumulation, and Photosynthetic Physiology of *Buddleja alternifolia* Maxim. Seedlings Under Drought Stress

Yan Jiangwei, Li Changxiao*, Cui Zhen, Liu Yuan
Key Laboratory of Eco-environment in the Three Gorges Reservoir Region of the Ministry of Education, Chongqing Key Laboratory of Plant Ecology and Resources Research in the Three Gorges Reservoir Region, College of Life Science, Southwest University, Chongqing 400715, China

Abstract

This study investigated the growth and photosynthetic physiological response mechanisms of *Buddleja alternifolia* Maxim. seedlings to heavy metal cadmium stress under drought conditions. Using *B. alternifolia* seedlings as experimental material, we established two water treatment groups (control and drought) with soil relative water contents of 60%-65% and 30%-35%, respectively. Under each water condition, three cadmium treatment concentrations were applied (0.28, 0.6+0.28, and 1.2+0.28 mg/kg). A two-factor randomized block design was used with six total treatments and five replicates per treatment. We measured seedling growth, biomass, cadmium content, and photosynthetic parameters.

Results showed that plant survival rate under combined drought and cadmium stress was 100%. Cadmium stress, drought, and combined stress all inhibited growth, biomass accumulation, photosynthesis, and chlorophyll content of *B. alternifolia* seedlings to varying degrees, with inhibitory effects on photosynthesis and chlorophyll content under combined stress being greater than under cadmium stress alone. The highest cadmium accumulation capacity per seedling was 69.33 mg/kg under cadmium stress alone and 50.68 mg/kg under combined stress. These results indicate that drought stress can aggravate the effects of cadmium stress on plants, further reducing growth, photosynthetic capacity, and cadmium enrichment ability under combined stress. However, under cadmium stress alone, *B. alternifolia* seedlings showed stronger tolerance to cadmium and high bioaccumulation capacity. Additionally, under combined stress, seedlings still exhibited cadmium accumulation. Thus, *B. alternifolia* Maxim. seedlings have great potential application in landscaping and phytoremediation of cadmium-polluted areas in arid and semi-arid regions.

Keywords: drought; cadmium stress; *Buddleja alternifolia* Maxim. seedlings; photosynthetic characteristics; cadmium accumulation characteristics

Introduction

Cadmium is one of the heavy metal elements with the strongest biological toxicity, and its accumulation in plants inhibits photosynthesis, destroys various protective enzyme activities, and ultimately leads to physiological metabolic disorders. Through the food chain, cadmium accumulation threatens human health. Compared with traditional remediation methods, phytoremediation technology utilizes plants to immobilize and extract heavy metals from soil, offering advantages including low cost, environmental protection, and public acceptance, making it the preferred method for remediating heavy metal-contaminated soil. To date, numerous studies have focused on screening heavy metal hyperaccumulator plant resources and investigating plant physiological and biochemical responses under single or combined heavy metal stress.

With the development of mining and industrial activities in western China, soils in arid and semi-arid regions have suffered varying degrees of cadmium pollution. Plants in these regions often face the dual stress of soil drought and cadmium pollution, yet research on plant response mechanisms to this combined stress remains relatively limited. *Buddleja alternifolia* Maxim. is a typical native tree species in the arid and semi-arid regions of northwest China, with strong ornamental value and excellent adaptability, making it a promising candidate shrub for ecological restoration in these areas. This study used *B. alternifolia* as experimental material to investigate its growth, photosynthetic pigment content changes, and cadmium accumulation under single and interactive drought and cadmium stress, aiming to clarify the tolerance and adaptability of this species to dual stress and provide a scientific basis for its potential application in phytoremediation of cadmium-contaminated soils in arid and semi-arid regions.

Materials and Methods

Experimental Materials

The experiment used two-year-old *B. alternifolia* seedlings collected from the Yinchuan Botanical Garden in Ningxia. Seedlings had an average height of (28.79±1.01) cm and base diameter of (1.42±0.10) mm. The experimental soil was sandy loam taken from the cultivated soil layer (0-20 cm) of the Yinchuan Botanical Garden. Initial nutrient contents of the soil are shown in .

Experimental Design

Each pot contained 17.5 kg of air-dried soil. Based on the National Soil Environmental Quality Standard (GB15618-1995) and pollution levels in Ningxia region, cadmium was added in solution form as $CdCl_2 \cdot 2.5H_2O$ at concentrations of 0.28 (soil background value), 0.6+0.28, and 1.2+0.28 mg/kg, designated as T0, T1, and T2, respectively. Following the rainfall pattern classification for arid and semi-arid regions and recent precipitation data from Yinchuan, two water gradients were established: a conventional water supply group (control) with soil water content maintained at 60%-65% of field capacity, and a drought treatment group with soil water content maintained at 30%-35% of field capacity. Soil relative water content was controlled using the weighing method. After one month, three seedlings were transplanted into each pot. There were six experimental treatments: CT0, CT1, CT2, DT0, DT1, and DT2. After three months, complete plants were harvested for measurement of various indices while maintaining relatively stable soil water conditions.

Growth Index Measurement

Plant height and base diameter were measured with a steel ruler before and after stress. Plant height relative growth increment was calculated. Plants were harvested and separated into roots, stems, and leaves. Fresh weight was measured, then samples were dried at 105°C for 15 minutes, followed by 85°C until constant weight to determine dry weight and biomass.

Cadmium Content Determination

Dried root, stem, and leaf samples were digested using a microwave digestion system (Spewed Wave MSE-4, Berghof). Cadmium content was determined using inductively coupled plasma optical emission spectrometry (ICAP6300, Thermo Fisher, USA).

Photosynthetic Parameter Measurement

Photosynthetic parameters were measured using a GFS-3000 portable photosynthesis system (WALZ, Germany). Based on preliminary experiments, measurements were taken on sunny days between 9:00-12:00 on the third fully expanded leaf from the top of each seedling. Using red-blue light as the source, measured indices included net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Sc), and intercellular CO concentration (Ci).

Chlorophyll Content Determination

Leaves used for photosynthetic measurement were extracted to determine chlorophyll content. A TU-1901 spectrophotometer was used to measure absorbance at 663 nm and 645 nm, and total chlorophyll content was calculated.

Data Processing

The bioconcentration factor (BCF) was calculated as: BCF = (cadmium content in root or aerial part) / (cadmium content in soil). The translocation factor (TF) was calculated as: TF = (average cadmium content in aerial part) / (average cadmium content in root). Data were analyzed using two-way ANOVA with SPSS 22.0 software. Differences among treatments were tested using Tukey' s method ($P = 0.05$). Microsoft Excel 2010 and Origin 8.5 were used for data processing and graphing.

Results

Effects of Cadmium Treatment on Growth and Biomass of *B. alternifolia* Seedlings Under Drought Stress

Under single cadmium stress, aboveground biomass of *B. alternifolia* seedlings in the conventional water supply group decreased by 23.15% and 25.21% compared to the control group at T1 and T2 concentrations, respectively. Main stem increment showed significant decreases only at T2 concentration. However, root biomass and root-shoot ratio showed significant decreasing trends with increasing cadmium concentration. Under combined drought and cadmium stress, aboveground biomass and main stem increment both decreased significantly with increasing cadmium concentration, while root-shoot ratio showed no significant differences among different cadmium concentration treatments.

Cadmium Accumulation and Translocation in *B. alternifolia* Seedlings

Under single cadmium stress, cadmium contents in roots, stems, and leaves of *B. alternifolia* seedlings all increased significantly with increasing soil cadmium concentration. Under combined drought and cadmium stress, cadmium contents in roots and stems increased significantly with soil cadmium concentration, while leaf cadmium content showed no significant difference between DT1 and DT2 treatments. At T0, T1, and T2 cadmium concentrations, root cadmium content under single stress was 12.01, 34.15, and 19.53 mg/kg, respectively, which was significantly lower than the 29.43, 15.87, and 9.23 mg/kg observed under combined stress. Stem cadmium content under single stress was higher than under combined stress but not significantly so. Leaf cadmium content under single stress was significantly higher than under drought treatment alone.

The bioconcentration factor in aboveground parts of *B. alternifolia* seedlings under single cadmium stress was significantly greater than under combined stress at T1 and T2 concentrations. Root bioconcentration factor increased significantly with soil cadmium concentration, with values under single stress being greater than under combined stress. The translocation factor decreased significantly with increasing cadmium concentration under both stress conditions, with values under single stress being significantly greater than under combined stress.

Effects of Combined Drought and Cadmium Stress on Photosynthetic Physiology of *B. alternifolia* Seedlings

Both drought and combined drought-cadmium stress significantly affected leaf net photosynthetic rate (Pn) of *B. alternifolia* seedlings. Under single cadmium stress, Pn decreased significantly with increasing cadmium concentration by 28% and 48% at T1 and T2, respectively. Under combined stress, Pn of DT1 and DT2 groups decreased by 57%, 59%, and 80% compared to the control group.

Transpiration rate and stomatal conductance of *B. alternifolia* seedlings also decreased significantly with increasing cadmium concentration. Drought stress significantly reduced transpiration rate, and when different cadmium concentrations were applied, the decline in both transpiration rate and stomatal conductance became more pronounced. Under cadmium stress alone, transpiration rates at T1 and T2 were only 46% and 24% of the control, respectively, while stomatal conductance was maintained at 45% and 32% of the control.

Intercellular CO concentration (Ci) was significantly affected by both stress treatments. Under single cadmium stress, Ci decreased significantly with increasing cadmium concentration by only 5% and 13% at T1 and T2. However, under combined stress, Ci increased significantly with cadmium concentration.

Both cadmium treatment alone and combined drought-cadmium treatment significantly affected photosynthetic pigment content in *B. alternifolia* seedlings. Under single cadmium stress, chlorophyll a content decreased significantly by 13.60% at T2, while chlorophyll b content decreased but not significantly. Total chlorophyll content decreased significantly with increasing cadmium concentration. Under combined stress, both chlorophyll a and total chlorophyll content decreased with increasing stress levels, with total chlorophyll content decreasing by 21% and 31% at DT1 and DT2, respectively.

The chlorophyll a/b ratio under all treatment groups was significantly higher than the control. Under single cadmium stress, the chlorophyll a/b ratio at T1 and T2 increased by 15% and 7%, respectively, compared to the control. Under combined stress, the chlorophyll a/b ratio increased significantly with soil cadmium concentration.

Discussion

Biomass production is of great importance in the phytoremediation of heavy metal-contaminated soils, as it not only affects remediation efficiency but also represents a comprehensive indicator of the plant' s environment. This study found that both cadmium stress and combined drought-cadmium stress inhibited growth and biomass accumulation in *B. alternifolia* seedlings, with combined stress showing greater inhibitory effects. The stress conditions also altered dry matter allocation patterns. The significant decrease in root-shoot ratio under cadmium stress occurred primarily because roots are the first organs to contact cadmium, showing growth inhibition through shorter roots, fewer lateral roots, and decreased root biomass. Unlike the significant decrease in root-shoot ratio with increasing cadmium concentration under cadmium stress alone, combined stress showed no significant changes in root-shoot ratio across different cadmium concentrations. This may be because drought stress mainly affects aboveground growth while cadmium stress primarily affects belowground growth, resulting in relatively balanced inhibition of both aboveground and belowground biomass under combined stress and thus no significant change in root-shoot ratio. Despite significant effects on plant growth under both single and combined stress, *B. alternifolia* seedlings maintained high survival rates, indicating strong resistance to both drought and cadmium stress.

Heavy metal accumulation and translocation capacity are important indicators for evaluating plant suitability for phytoremediation, second only to biomass production. Different plant parts and different growth media affect heavy metal accumulation capacity. This study found that maximum cadmium accumulation per seedling was 69.33 mg/kg under single stress but only 50.68 mg/kg under combined stress. This difference occurs because under drought conditions, the bioavailability of cadmium in soil decreases as cadmium storage forms shift from loosely bound to tightly bound states. Plant cadmium uptake is closely related to soil cadmium bioavailability, which is the main reason for this difference. We also found that under single cadmium stress, cadmium accumulation in different plant parts followed the order root > leaf > stem, while under combined stress it was root > stem > leaf. For most non-tolerant or non-hyperaccumulator plants, most cadmium absorbed by roots is confined to root tissues, a general strategy to prevent cadmium translocation to aboveground parts and avoid damage. However, under cadmium stress, *B. alternifolia* seedlings can utilize detoxification mechanisms to reduce cadmium toxicity, including leaf compartmentalization and formation of heavy metal phytochelatins. These processes mostly occur in leaves, making them a storage reservoir for cadmium in aboveground parts. Under combined stress, more cadmium was stored in stems compared to leaves. This allocation strategy is important for reducing cadmium toxicity to aboveground parts, as stems have weaker physiological functions than leaves. Both strategies demonstrate plant tolerance to cadmium, enabling survival and biomass production.

Changes in photosynthetic physiology are among the most sensitive adaptive characteristics of plants to environmental changes, with photosynthesis being a direct indicator. Under drought or cadmium stress, plant Pn decreases significantly with increasing stress degree and duration. In this study, Pn of *B. alternifolia* seedlings decreased significantly under both stress conditions, with greater reduction under combined stress than under cadmium stress alone, indicating strong photosynthetic adaptability even under single cadmium treatment. The decline in Pn under stress occurs through two main mechanisms: stomatal limitation and non-stomatal limitation. According to Farquhar and Sharkey' s criteria, if Pn decreases while Ci also decreases, the limitation is stomatal; if Pn decreases while Ci remains unchanged or increases, the limitation is due to decreased photosynthetic capacity of leaf mesophyll cells. This study showed that under single cadmium stress, Pn decreased while Ci also decreased significantly, indicating stomatal limitation as the main cause. Cadmium accumulation affects stomatal cell wall elasticity and disrupts synthesis of plant hormones like abscisic acid in guard cells, causing stomata to remain closed and limiting photosynthesis. Under combined stress, however, Ci increased significantly as cadmium concentration increased while Pn decreased, indicating that non-stomatal factors gradually became limiting factors for photosynthesis, causing damage to photosynthetic organ structure and function and decreasing mesophyll cell assimilation capacity.

Photosynthetic pigment content is an important physiological parameter, with chlorophyll being crucial for light energy absorption, transfer, and conversion in photosynthesis. This study found that different stress conditions significantly reduced chlorophyll content in *B. alternifolia* seedlings, with greater reduction under combined stress than under single cadmium stress. Different stress types likely affect chlorophyll through different mechanisms. Drought can destroy chloroplast ultrastructure and affect enzymes involved in chlorophyll synthesis, while cadmium, as a positive cation, competes with essential elements like Fe, Zn, and Mg that have the same valence state, blocking their transport to leaves and interfering with chlorophyll synthesis. The two stresses have synergistic effects on chlorophyll damage. Interestingly, the chlorophyll a/b ratio under stress conditions was significantly higher than the control. A higher chlorophyll a/b ratio indicates more thylakoid stacking and higher light energy utilization efficiency, suggesting that *B. alternifolia* seedlings can improve light energy utilization efficiency to maintain growth under stress by increasing the chlorophyll a/b ratio.

Conclusion

Combined drought and cadmium stress significantly affected two-year-old *B. alternifolia* seedlings, but all seedlings survived well, demonstrating multiple positive adaptive characteristics. *B. alternifolia* is a shrub species with great application potential and promise for landscaping in arid and semi-arid regions and for phytoremediation of contaminated soils, as it still accumulates certain amounts of cadmium even under combined stress conditions.

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