Effects of Thallium and Cadmium Stress on Growth and Photosynthetic Characteristics of Giant Reed (Arundo donax): Postprint

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Abstract

Urbanization and industrial activities have caused widespread contamination of heavy metals such as cadmium (Cd) and thallium (Tl), posing a series of problems to ecosystem functions and human health. Therefore, effective remediation of Cd and Tl pollution represents one of the most pressing environmental issues today. Arundo donax exhibits favorable tolerance to various heavy metals and represents an ideal candidate for phytoremediation applications; however, research on the physiological responses of Arundo donax to Cd and Tl stress remains limited. Consequently, this study employed Arundo donax as experimental material, conducting pot experiments with different concentrations of heavy metal Tl (4, 10, and 20 mg·kg^{1}) and Cd (50, 100, and 200 mg·kg^{1}), and measured plant height, tiller number, chlorophyll content, photosynthetic physiological parameters, as well as Tl and Cd accumulation in Arundo donax to investigate the response mechanisms of Arundo donax to Tl and Cd stress. The results demonstrated that Tl (4-20 mg·kg^{1}) and Cd (50-200 mg·kg^{1}) had no significant effects on plant height, tiller number, or chlorophyll content of Arundo donax ($P > 0.05$). The Tl and Cd contents in Arundo donax exhibited an increasing trend with rising Tl and Cd concentrations; the distribution pattern of Tl content in Arundo donax followed the order root > stem > leaf, while the distribution pattern of Cd content was stem > leaf > root at a Cd concentration of 50 mg·kg^{1}, and root > stem > leaf at Cd concentrations of 100 and 200 mg·kg^{1}, indicating that Tl and Cd were primarily distributed in the roots and that Arundo donax possesses certain enrichment capacity for Tl and Cd. Both Cd and Tl treatments significantly reduced the intercellular CO concentration in Arundo donax leaves; at a Tl concentration of 10 mg·kg^{1}, net photosynthetic rate, stomatal conductance, and transpiration rate were significantly enhanced, and when Cd concentration was 50 mg·kg^{1}, net photosynthetic rate, stomatal conductance, and transpiration rate were significantly enhanced. This study indicates that Arundo donax possesses strong tolerance to heavy metals Cd and Tl and provides a reference for the remediation and restoration of Cd- and Tl-contaminated soils.

Full Text

Effects of Thallium and Cadmium Stress on Growth and Photosynthetic Characteristics of Arundo donax

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Abstract

Urbanization and industrial activities have caused widespread heavy metal contamination, particularly by cadmium (Cd) and thallium (Tl), posing serious threats to ecosystem functioning and human health. Developing effective remediation strategies for Cd and Tl pollution has become one of the most urgent environmental challenges. Arundo donax (giant reed) exhibits strong tolerance to various heavy metals, making it a promising candidate for phytoremediation applications. However, few studies have investigated the physiological responses of A. donax to Cd and Tl stress. This study examined the responses of A. donax to these metals through a pot experiment with varying concentrations of Tl (4, 10, and 20 mg · kg^{1}) and Cd (50, 100, and 200 mg · kg^{1}). We measured plant height, tiller number, chlorophyll content, photosynthetic parameters, and metal accumulation in plant tissues.

The results showed that Tl (4–20 mg · kg^{1}) and Cd (50–200 mg · kg^{1}) had no significant effects on plant height, tiller number, or chlorophyll content ($P > 0.05$). However, Tl and Cd concentrations in A. donax increased with increasing soil metal concentrations. Tl distribution followed the pattern root > stem > leaf, while Cd distribution varied: at 50 mg · kg^{1}, it was stem > leaf > root, but at 100 and 200 mg · kg^{1}, it shifted to root > stem > leaf. These patterns indicate that both metals primarily accumulated in the roots, demonstrating A. donax' s capacity to enrich Tl and Cd. Both metal treatments significantly reduced intercellular CO concentration in leaves. Notably, the 10 mg · kg^{1} Tl treatment significantly increased net photosynthetic rate, stomatal conductance, and transpiration rate, while the 50 mg · kg^{1} Cd treatment produced similar enhancements. These findings demonstrate that A. donax possesses strong tolerance to Cd and Tl stress and can serve as a reference for remediation strategies for soils contaminated with these metals.

Keywords: phytoremediation, thallium, cadmium, Arundo donax, photosynthetic characteristics

Introduction

Heavy metal contamination of soils represents a major global challenge, causing soil fertility degradation, crop yield reduction, and water environment deterioration. Thallium is a rare, non-essential metal with extreme toxicity, primarily originating from mining and resource exploitation activities. Cadmium, a toxic silver-white metal widely used in industrial applications, has become one of the most threatening heavy metal pollutants due to its persistence, non-degradability, and strong concealment. Both Tl and Cd exhibit high mobility in the soil-plant system and are readily absorbed and accumulated by plants, subsequently entering the food chain and posing health risks to humans, such as thallium poisoning and "itai-itai" disease. Field investigations in Yangshuo, Guangxi have revealed co-contamination of farmland soils by both metals, prompting this study to examine their individual effects through concentration gradient experiments. While we have initiated field remediation studies for combined pollution in this region, the present work focuses solely on their separate effects to establish a foundational understanding.

Phytoremediation has emerged as a promising approach for heavy metal-contaminated soils due to its cost-effectiveness and ease of implementation. Photosynthesis, as a fundamental metabolic process unique to green plants, provides essential organic compounds, energy, and oxygen, directly influencing plant productivity and crop yield. However, photosynthesis is highly sensitive to environmental factors and vulnerable to heavy metal stress. Therefore, investigating the effects of heavy metal stress on photosynthesis provides a scientific basis for phytoremediation applications.

Arundo donax, a perennial grass species distributed across Jiangsu, Zhejiang, Hunan, Shandong, and Guangxi, is primarily used as a high-quality papermaking raw material. The species exhibits remarkable resilience, tolerating cold, heat, waterlogging, and drought while maintaining vigorous growth in poor or contaminated soils, making it an excellent candidate for revegetating severely polluted sites such as mine tailings and slag heaps. Post-remediation utilization of A. donax for non-food purposes like papermaking or bioenergy production prevents heavy metal transfer through food webs. Additionally, its cultivation provides soil stabilization and ecological restoration benefits, offering significant economic and environmental value. Previous research has demonstrated A. donax' s substantial tolerance to heavy metals, large biomass, extensive root system, and strong adaptability, establishing it as an ideal candidate for phytoremediation.

Most existing studies on A. donax have focused on heavy metal tolerance and accumulation capacity, with some investigations on physiological and ecological characteristics. However, research on photosynthetic responses under heavy metal stress remains limited. While several studies have confirmed A. donax' s tolerance to multiple heavy metals, few have examined its photosynthetic characteristics under Cd and Tl stress specifically. Based on preliminary surveys of contaminated farmland in Sidi Village, Yangshuo County, Guangxi, where soil Cd and Tl concentrations exceed safety standards (5.73-300.3 mg・kg^{1} and 0.5-12.30 mg・kg^{1}, respectively), this study employed pot experiments with varying soil concentrations of exogenous Tl and Cd to investigate their effects on A. donax growth, chlorophyll content, and photosynthetic characteristics. The objective was to explore the toxicity mechanisms of Tl and Cd and provide theoretical support for remediation of contaminated soils.

Materials and Methods

Experimental Design

The experiment utilized A. donax as the test species and red soil collected from the surface layer (0–20 cm) of a garden at the Guangxi Institute of Botany as the growth medium. The basic physicochemical properties of the test soil are presented in , with background Cd and Tl concentrations of 0.014 and 0.003 mg・kg^{1}, respectively. Plastic pots (26 cm × 75 cm × 38.5 cm) were filled with 5 kg of test soil each. At the experiment' s initiation, solutions prepared from TlCl and CdCl were added to the soil and thoroughly mixed. Based on soil environmental quality standards and hyperaccumulation thresholds, three concentration levels were established for each metal with three replicates per treatment: Tl at 4, 10, and 20 mg・kg^{1} (designated Tl1, Tl2, Tl3) and Cd at 50, 100, and 200 mg・kg^{1} (designated Cd1, Cd2, Cd3). A control group without metal addition (CK) was also included, with three replicates per treatment. The experimental plants were tissue-cultured A. donax seedlings of uniform height (15 cm), transplanted into the treated soils. Throughout the experiment, soil moisture was maintained at 90% of field capacity.

Growth Parameters and Chlorophyll SPAD Measurements

Plant height was measured using a tape measure. Relative chlorophyll content (SPAD values) was determined using a SPAD-502 chlorophyll meter (Konica, Japan) at 9:00 AM on mature leaves of consistent position and orientation, avoiding the main leaf vein. Three leaves were measured per treatment, with three measurement points per leaf averaged for the final value.

Photosynthetic Physiological Measurements

A LI-6400xt portable photosynthesis system (LI-COR, USA) was used to measure photosynthetic parameters on healthy, pest-free leaves of consistent position and growth status, maintaining natural leaf angles. Three plants were measured per treatment. Parameters included net photosynthetic rate (Pn, µmol・

m ^{2} · s ^{1}), stomatal conductance (Gs, mol · m ^{2} · s ^{1}), intercellular CO concentration (Ci, mol · mol ^{1}), transpiration rate (Tr, mmol · m ^{2} · s ^{1}), and photosynthetically active radiation (PAR, mol · m ^{2} · s ^{1}). Water use efficiency was calculated as WUE = Pn · Tr ^{1} (Nijs et al., 1997).

Tl and Cd Content Analysis

After four months of metal stress treatment, leaves, stems, and roots were harvested from each treatment. Samples were washed with tap water followed by three rinses with deionized water, then oven-dried at 105°C for 30 minutes and subsequently at 80°C to constant weight. Dried samples were ground using a stainless-steel plant grinder and passed through a 20-mesh nylon sieve. Soil samples were air-dried, ground, and sieved for analysis. Both plant and soil samples were digested using a microwave digestion system, and Tl and Cd concentrations were determined using an Agilent 7700e inductively coupled plasma mass spectrometer (7700 series ICP-MS, USA).

Statistical Analysis

Data were organized and calculated using Excel, with results presented as mean ± standard deviation. SPSS 23 was used for one-way ANOVA and least significant difference (LSD) tests for significance assessment at $P = 0.05$. Sigmaplot 12.5 was used for graphical presentation.

Results

Effects of Different Tl and Cd Concentrations on A. donax Growth

The effects of varying Tl and Cd concentrations on A. donax growth are shown in . Plant height was lowest under Cd1 treatment and highest under Tl2 treatment. Tiller number peaked under Cd2 treatment and was lowest under Cd3 treatment. Chlorophyll content was highest under Tl1 treatment and lowest under Tl2 treatment. With increasing Cd concentration, plant height and chlorophyll content followed the pattern Cd3 > Cd2 > Tl1, while tiller number showed Cd2 > Cd1 > Tl3. With increasing Tl concentration, plant height followed Tl2 > Tl3 > Tl1, chlorophyll content showed Tl1 > Tl3 > Tl2, and tiller number exhibited Tl3 > Tl2 = Tl1. Compared with the CK treatment, different Cd and Tl concentrations had no significant effects on plant height or chlorophyll content ($P > 0.05$). However, Cd2 treatment significantly increased tiller number ($P < 0.05$), while other treatments showed no significant differences. Within the tested concentration range, Cd and Tl treatments did not significantly affect growth parameters, indicating strong tolerance of A. donax to Tl and Cd stress.

Accumulation Characteristics of Tl and Cd in A. donax

Changes in Tl and Cd concentrations in A. donax are presented in . Metal accumulation increased significantly with increasing soil concentrations ($P < 0.05$).

Tl distribution consistently followed root > stem > leaf, indicating preferential accumulation in roots and suggesting a retention mechanism that reduces toxicity to aboveground tissues. Under CK and Cd1 treatments, Cd distribution was stem > leaf > root, demonstrating some translocation capacity to aerial parts. However, under Cd2 and Cd3 treatments, the pattern shifted to root > stem > leaf, indicating that at higher concentrations, Cd accumulation was primarily restricted to roots.

Effects of Different Tl Concentrations on A. donax Photosynthesis

Net photosynthetic rate (Pn) serves as a crucial indicator of plant environmental adaptation, with higher values reflecting stronger photosynthetic capacity. As shown in [Figure 1: see original paper], Pn increased with photosynthetically active radiation (PAR) across all treatments, but Tl treatments showed an initial increase followed by a decline. At higher PAR levels, the control treatment exhibited higher Pn than Tl1 and Tl2 treatments. Intercellular CO concentration (Ci) decreased with increasing PAR, with control values exceeding all Tl treatments. Stomatal conductance (Gs) showed Tl2 > Tl1 > Tl3 > CK below 550 mol · m^{2} · s^{1} PAR, shifting to Tl2 > CK > Tl3 > Tl1 between 550–1400 mol · m^{2} · s^{1}. At 1200 mol · m^{2} · s^{1} PAR, Pn followed Tl2 > Tl3 > CK > Tl1, Ci showed CK > Tl3 > Tl2 > Tl1, and Tr (transpiration rate) mirrored Gs patterns as Tl2 > CK > Tl3 > Tl1. All Tl treatments increased water use efficiency (WUE) compared to control, following Tl3 > Tl1 > Tl2 > CK, indicating that Tl stress reduced water consumption while WUE initially decreased then increased with Tl concentration. Although Tl treatments significantly reduced Ci, Tl2 treatment significantly enhanced Pn, Gs, and Tr, demonstrating that Tl stress affects photosynthesis with an initial stimulation followed by inhibition at higher concentrations, confirming strong Tl tolerance in A. donax.

Effects of Different Cd Concentrations on A. donax Photosynthesis

The influence of varying Cd concentrations on A. donax photosynthesis is illustrated in [Figure 2: see original paper]. In control plants, Pn, Gs, and Tr increased with PAR, while Cd treatments showed an initial increase followed by decline. Cd1 and Cd2 treatments produced higher Pn than control, whereas Cd3 treatment resulted in lower Pn and Gs compared to control. At 1200 mol · m^{2} · s^{1} PAR, Pn followed Cd1 > Cd2 > CK > Cd3, while Gs and Ci showed CK > Cd1 > Cd2 > Cd3. Transpiration rate exhibited Cd1 > Cd2 > Cd3 > CK, and WUE followed CK > Cd3 > Cd1 > Cd2, indicating that Cd stress increased water consumption with WUE decreasing then increasing with Cd concentration. Cd treatments significantly reduced Ci and WUE, but Cd1 treatment significantly enhanced Pn, Gs, and Tr, demonstrating that Cd stress affects photosynthesis with concentration-dependent inhibition, yet confirming A. donax' s Cd tolerance.

Discussion and Conclusion

Both Tl and Cd are non-essential elements that can cause irreversible physiological and growth impairments in plants. Due to similar uptake pathways with potassium (K), Tl can disrupt plant nutrient transport when substituting for K, thereby affecting growth. Our results indicate that while Tl and Cd treatments influenced A. donax growth, differences compared to control were not significant, consistent with findings by Pu et al. (2018) and Han et al. (2005). The observed metal distribution patterns, with preferential root accumulation, reflect a tolerance mechanism where roots restrict metal translocation to aboveground parts, maintaining lower aerial tissue concentrations and reducing toxicity.

Heavy metal stress-induced reductions in net photosynthetic rate have been widely documented. Previous research identifies two primary mechanisms: stomatal limitation from partial stomatal closure and non-stomatal limitation from reduced mesophyll cell photosynthetic activity. According to Farquhar and Sharkey (1982), intercellular CO concentration serves as the key criterion for distinguishing these mechanisms. If Pn reduction accompanies decreased Ci and Gs, stomatal factors dominate; otherwise, non-stomatal factors are responsible. Our results show that under Tl and Cd stress, Pn, Tr, and Gs initially increased then decreased with metal concentration, while Ci increased with concentration. This indicates that photosynthetic changes in A. donax under Tl and Cd stress were primarily limited by non-stomatal factors—namely, reduced photosynthetic activity in mesophyll cells—consistent with studies by Li et al. (2016), Sun et al. (2005), and Gu et al. (2008). Under low metal concentrations, A. donax may require additional energy to maintain normal metabolism, but as concentrations increase, this early stimulation transitions to inhibition, reducing photosynthetic rates.

Heavy metals affect photosynthesis through impacts on chlorophyll content and metabolic processes. Appropriate Tl stress can enhance photosynthesis by increasing chlorophyll content, while high concentrations reduce photochemical activity and inhibit photosynthesis (Pu et al., 2018). Low Cd stress may increase chlorophyll content by facilitating absorption of Mg, Fe, K, and P ions, promoting porphyrin ring formation. Conversely, high Cd concentrations reduce chlorophyll content by damaging chloroplast structure and activity, inhibiting photosynthetic pigment formation and impairing photosynthesis (Qin et al., 2000).

Water use efficiency (WUE) reflects the relationship between plant productivity and water consumption, with lower WUE indicating greater water consumption per unit of photosynthate produced. Under Tl or Cd stress, A. donax WUE showed a decreasing then increasing trend with metal concentration. Tl stress reduced WUE compared to control, possibly by inhibiting nutrient transport, while Cd stress increased WUE relative to control, likely due to higher energy demands for metabolic maintenance. Overall, Tl and Cd stress affected photosynthesis and water absorption in A. donax but had minimal impact on growth parameters, demonstrating strong tolerance.

In summary, A. donax exhibits robust tolerance to Tl and Cd stress through two primary mechanisms: first, root systems restrict metal translocation to aerial parts, and second, despite effects on photosynthetic characteristics and WUE, growth remains largely unaffected, suggesting the presence of effective detoxification systems. Future research should investigate antioxidant enzyme system responses under Tl and Cd stress to further elucidate tolerance mechanisms. Additionally, since most contaminated sites involve multiple heavy metals, studies on combined Tl and Cd pollution would better reflect real-world remediation scenarios.

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