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Ph.D. dissertation

Physiological Responses and Functional Traits of some Willow, Poplar and Bamboo taxa under Cadmium Stress

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1 INTRODUCTION AND OBJECTIVES

Human activities have caused a multitude of environmental problems, among which heavy metal pollution is particularly concerning. Cd pollution is one of the most widespread and harmful issues, posing significant and often irreversible risks to human health and the environment (Six & Smolders, 2014). Panagos et al., (2013) estimated that over 2.5 million potentially contaminated sites exist within the European Union (EU), based on an analysis of the European Environmental Information and Observation Network for Soils (EIONET-SOIL) data. Urban and industrial waste accounts for approximately 38% of these sites, with mineral oils and heavy metals comprising 60% of the associated pollutants. The primary sources of Cd contamination in soils include vehicle emissions, mining activities, smelting operations, and the excessive application of pesticides and fertilizers. In recent years, environmental legislation aimed at mitigating Cd accumulation in soils has led to a 40% reduction in the use of phosphate fertilizers over the past 15 years. This reduction is projected to decrease soil Cd levels in cereal and potato cropping systems by approximately 15% over the next century (Ballabio et al., 2024). Despite these efforts, an estimated 5.5% of soils in the EU still exhibit Cd concentrations exceeding the critical threshold of 1 mg kg^{-1}.

In China, agricultural practices such as fertilization and sewage irrigation have exacerbated Cd pollution. In the rice-producing regions of southern China, approximately 1.46×10^8 kg of agricultural products are contaminated with Cd, including roughly 5.0×10^7 kg of rice (Mu et al., 2020). Although Cd is not an essential element for plant growth, it is readily absorbed by plants due to its chemical similarity to divalent metal ions such as zinc (Zn), iron (Fe), and calcium (Ca). Once absorbed, Cd can replace the active centers of various enzymes or bind to the hydrophilic groups of proteins, altering the structure of biological macromolecules. These interactions disrupt enzyme systems, impairing plant growth and development. Cd's ability to enter the food chain poses severe health risks to humans, including kidney failure and bone fractures (Bautista et al., 2024). As such, mitigating Cd exposure and limiting its mobility in the natural environment are critical measures for ensuring food and ecological safety. These efforts are essential to protect both food consumers and agricultural practitioners from the hazardous effects of heavy metals. Addressing Cd pollution is therefore imperative for safeguarding public health and maintaining environmental integrity.

The adsorption of heavy metals by plants is primarily influenced by the concentration of free heavy metal ions in the soil. Cd exists in the environment predominantly as an oxide ion (Kubier et al., 2019). Anthropogenic sources of Cd in soil are largely derived from phosphate fertilizers (Wielgusz et al., 2022), as Cd is a common impurity in phosphate minerals and phosphate rocks. It can substitute for Ca in apatite, which constitutes the main component of phosphate (Gnandi & Tobschall, 2002). Cd concentrations in unpolluted soil solutions typically range between 40 and 300 nM (Lux et al., 2011). The concentration and dissolution order of active metals in soil plays a critical role in determining the accumulation of metals in plants (Gupta & Sinha, 2007). Cd exhibits a weak adsorption effect in environments where it competes with other metals and persists in soil solutions with a pH of less than 6.5, unlike other heavy metals which tend to be more readily fixed. Furthermore, Cd often forms stable dissolved coordination complexes with inorganic and organic ligands, inhibiting adsorption and precipitation processes (Kubier et al., 2019). Soil organic matter and pH are key factors controlling the distribution of heavy metals (Qishlaqi & Moore, 2007). The pH of the soil significantly influences the chemical form of heavy metal ions, directly impacting their uptake by plants (Han et al., 2017). Mechanisms such as adsorption, precipitation, and complexation with minerals and soil organic matter mediate these effects (Stein et al., 2021).

Cd concentrations in agricultural and horticultural soils should not exceed 3 $\mu g\ g^{-1}$ to ensure safe plant cultivation (Lawlor, 2004). Plants are more likely to absorb Cd in acidic soils (Tudoreanu & Phillips, 2004). In addition, plants can release root exudates, increasing the bioavailability of Cd (Chen et al., 2016). Mlangeni et al., (2022) observed that rice accumulated high levels of Cd in acidic soils, a phenomenon attributed to the enhanced mobility and bioavailability of Cd at lower pH levels, which facilitates the conversion of Cd from fixed to bioavailable forms. Willow seedlings, which thrive under moderately acidic conditions, also demonstrate significant growth responses to soil pH. Gu et al., (2015) reported that willow seedlings exhibited optimal root length, seedling length, and vigor at a soil pH of 4.8.

Phytoremediation has emerged as a prominent research area in recent years within the broader domains of bioremediation and sustainable remediation. This approach encompasses various mechanisms, including phytoextraction (the absorption of pollutants from the soil by plants, followed by their transport and storage in above-ground organs), phytostabilization (the immobilization of pollutants in the soil to prevent their environmental mobility), phytovolatilization (the absorption of pollutants from the soil and their subsequent release into the atmosphere as volatile compounds), phytodegradation (the metabolism of organic pollutants through plant or rhizosphere microbial activity), and rhizofiltration (the use of plant roots in aquatic systems to filter contaminants from sewage) (Mocek-pł et al., 2023). For non-degradable heavy metals, certain plants can either concentrate them in their accessible above-ground parts or immobilize them in their roots (Liang et al., 2024).

Compared to traditional remediation methods, phytoremediation offers several advantages, including its in situ applicability, cost-effectiveness, and the absence of secondary pollutants (Ciadamidaro et al., 2022). Current research predominantly focuses on the absorption and enrichment capacities of crops (An et al., 2022), cash crops (Alamer et al., 2022), and aquatic plants (Dalla Vecchia et al., 2020). However, studies on woody plants, particularly forest timber species, ornamental green plants, and economic forest species, remain in their nascent stages. Unlike herbaceous plants, woody plants possess deep root systems, high biomass, and enhanced tolerance to heavy metal stress (Nong et al., 2023). Among woody plants, the genera *Salix* and *Populus* within the *Salicaceae* family are of particular interest. *Salix* encompasses approximately 520 species worldwide, primarily distributed in the temperate and frigid zones of the Northern Hemisphere, while *Populus* comprises around 30–40 species (Dickmann & Kuzovkina, 2014). These genera are prominent energy tree species and are typically cultivated using short-rotation forestry models. They exhibit high nutrient-use efficiency, elevated transpiration rates, rapid growth, substantial biomass production, and the ability to regenerate above-ground parts following harvesting. Furthermore, they hold significant potential for genetic improvement (Christersson, 2010).

The United States initiated research and application of woody plant remediation technology as early as the 1980s, utilizing short-rotation hybrid poplar trees to remediate soils contaminated with petroleum hydrocarbons (Ile et al., 2021). As this technology evolved, tree remediation methods were expanded to encompass water and atmospheric pollution control. The conceptual framework of tree remediation extends beyond the trees themselves to include the soil and associated microbiome as an integrated system. The root systems of most tree species can establish symbiotic associations such as mycorrhizae and nodules with soil microorganisms, thereby enhancing the synergistic effects of plant and microbial remediation. Through the regular harvesting of biomass, pollutants can be effectively removed from the soil. Additionally, fast-growing trees cultivated under a short-rotation model can generate biomass while simultaneously restoring ecosystems. This approach offers the dual benefits of ecological rehabilitation and contributing to the alleviation of the energy crisis (Espada et al., 2022).

As the application of this technology deepens, researchers are exploring the remediation potential of various hybrids while evaluating the capacity of other energy plants to act as sinks for soil pollutants, thereby expanding the species bank. Bamboo, a widely distributed economic tree species in tropical and subtropical regions, has emerged as a potential alternative material for phytoremediation. Beyond its ecological significance, bamboo offers additional benefits in terms of landscape aesthetics, energy production, building materials, and ecological restoration (Liang et al., 2023). Globally, there are over 1,300 species of bamboo, with China hosting more than 500 species, making it the country with the largest area of bamboo forests. According to the results of the ninth national forest resources inventory in China, the area of bamboo forests spans 6.4116 million hectares, accounting for 2.94% of the total forest area. The average biomass of these forests is 65.81 t ha^{-1}, significantly surpassing that of hyperaccumulators (Li & Feng, 2019). Bamboo, as one of the most primitive subfamilies of the *Poaceae*, represents a highly diverse group within this family. It is characterized by a woody stem, well-developed root systems, complex branching, and infrequent flowering. Bamboo exhibits rapid growth, with moso bamboo (*Phyllostachys edulis*) typically reaching heights of 15–20 m within approximately 60 days. The bamboo sheath plays a critical role in supporting and protecting the growth of bamboo shoots (Chen et al., 2022).

Furthermore, the annual biomass output of moso bamboo is noteworthy, reaching 6.0–7.6 Mg C ha^{-1} per year, highlighting its substantial carbon sequestration potential (Zheng et al., 2022). Studies indicate that the absorption capacity of bamboo plants is primarily determined by the underground root system and the whiplash root system. A larger root surface area facilitates the uptake of heavy metals and nutrients (Liu et al., 2014). For instance, the root systems of moso bamboo and Lei bamboo (*Phyllostachys praecox*) concentrate approximately 80% of their length and volume within the 0–40 cm soil layer, optimizing their ability to absorb heavy metals (Zhou et al., 2022; Gao et al., 2024)

Some studies have reported cases of in situ phytoremediation in different regions of Hungary, but these studies are usually based on wastewater irrigation and mixed pollutant treatment (Tőzsér et al., 2018; Tóth et al., 2024). However, there are many hybrids of willow and poplar, and studies on the absorption and accumulation of a heavy metal by different hybrids of native poplar and willow are still lacking, especially on the application potential of different poplars and willows in the remediation of Cd-contaminated soil. On the other hand, due to the long growth cycle of woody plants, not much is known about the heavy metal distribution strategies of woody plants, especially the nutritional element response under Cd stress. Although the genome sequence of poplar and willow has been published, providing valuable references for molecular biology research, there are still many differences in the mechanisms of response to heavy metals (Dos Santos Utmazian et al., 2007).

In addition, bamboo has strong adaptability and can not only survive in poor soil but also effectively absorb and fix heavy metals such as Cd, Pb, and Zn through its well-developed root system (Bian et al., 2017). In tropical and subtropical regions, bamboo has been widely used to remediate polluted soil and has performed well in rapidly restoring ecological functions (Tong et al., 2020). For Hungary, bamboo is not only an important species in horticulture but also offers a new option for the remediation of polluted soil. Bamboo's high biomass and multiple harvesting characteristics make it economically and ecologically valuable. However, there are currently few studies on the adaptability of bamboo in the local environment of Hungary, especially in Central European climatic conditions. The cold tolerance and heavy metal absorption efficiency of bamboo have not been fully evaluated. In addition, compared with willows and poplars, there are even fewer studies on the molecular mechanisms involved in the phytoremediation of bamboo. There is a gap in research on nutrient allocation and metabolic regulation of bamboo under heavy metal stress. This provides important scientific value for exploring the gene regulation mechanism of bamboo and its interaction with the environment. At the same time, the introduction of bamboo may also enrich Hungary's phytoremediation technology and provide more diverse options for soil remediation of different pollution types. Therefore, the introduction of bamboo as a phytoremediation material in Hungary not only provides a useful supplement to the current phytoremediation strategy but also provides an innovative direction for solving the problem of heavy metal pollution. This process not only requires collaborative research in ecology, botany, and molecular biology but also the accumulation of practical experience to achieve the sustainable application of bamboo in soil remediation in Hungary.

Objectives to achieve

1) The characteristics of Cd extraction and accumulation by poplar, willow and bamboo, and the distribution of Cd in different organs

2) The contents of N and K in the organs and the physiological changes in the leaves of poplar, willow and bamboo under Cd treatment

3) Changes in soil nitrogen content of poplar, willow and bamboo under Cd treatment
4) Phytoremediation from the perspective of PFT and PES

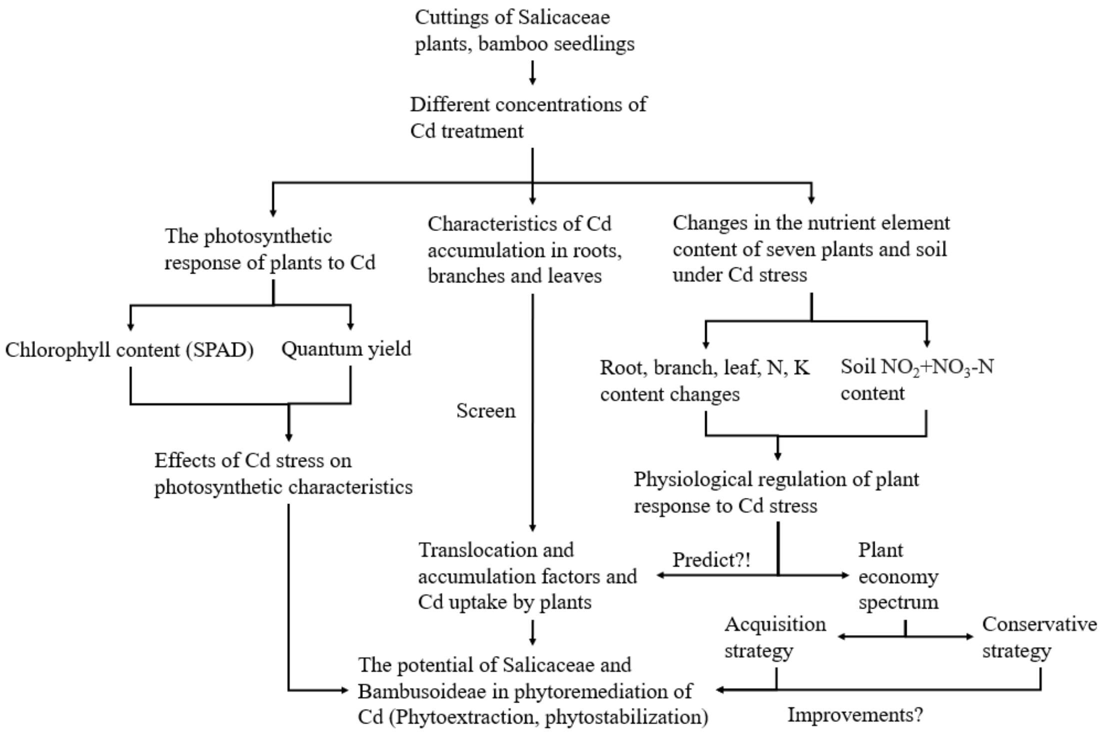

Figure 1. Technical route and research objectives

2 MATERIALS AND METHODS

2.1 Material Cultivation and Processing

The pot experiment was carried out in a ventilated small plastic tunnel with open ends on the farm of the University of Agriculture and Life Sciences in Hungary (47°58′N, 19°37′E) in 2022 and 2023 for 90 days (June to September). Each plastic pot was 15 cm high, with an upper diameter of 19 cm and a lower diameter of 13 cm. The pots were filled with 1 kg of soil for rooting the plants. The soil used in the experiment was purchased from Florimo. Poplar and willow cuttings from a short rotation coppice plantation (latitude 47°58′, longitude 19°37′). Two willow genotypes: 'Csala' (*Salix triandra* × *S. viminalis*), 'Tora' (*Salix schwerinii* × *S. viminalis*), two poplar genotypes: 'Pegaso' (*Populus* × *Generosa* × *P. nigra*), 'AF2' (*Populus deltoides* × *P. nigra*) (diameter: 3±1 cm, length: 20 cm). Three types of bamboo: *Phyllostachys edulis* (Moso), *Dendrocalamus asper*, *Dendrocalamus strictus* were purchased from Yunnan Bamboo Industry Co., Ltd. in August 2021 and 2022, and germinated in pots under greenhouse conditions for 8 months.

In April 2022 and April 2023, 30 cuttings of each setting of poplar and willow cuttings were planted in pots for rooting and acclimatization. Because of high temperatures in 2022, only five plants survived in numbers greater than the experimental setup (3 x 6 pots), and AF2 and *D. strictus* only survived in 5 and 3 pots each and were therefore not experimented with. Meanwhile, six well-grown bamboo plants were selected separately, transplanted into pots to ensure proper rooting and acclimatization. After two months, cadmium treatment was carried out. The experiment was carried out from June to September in 2022 and 2023. For the Cd treatment, 1 liter of $CdCl_2 \cdot 2.5H_2O$ solution with concentrations of 50 mg kg^{-1} and 100 mg kg^{-1}, respectively, was divided into 12 equal parts and evenly sprayed on the soil surface. The soil was then allowed to equilibrate for three days. The resulting final total Cd concentrations were therefore 0.29 mg kg^{-1} (CK), 4.17 mg kg^{-1} (T1) and 8.33 mg kg^{-1} (T2). In a completely randomized block design, there were six replicates of each treatment per plant, 90 pots in the first year and 126 pots in the second year. Humidity was maintained at 60–65% during growth, and no fertilizer was applied. At the end of the experiment, the roots, branches and leaves of each plant were harvested. Soil samples were taken from the entire soil profile in each pot using a stainless-steel spatula. The soil samples were thoroughly homogenized, sieved through a 2 mm sieve, and then divided equally into 3 random sub-samples (n=3). The plant root systems were rinsed with deionized water and then dried in an oven at 70°C for 72 hours before being analyzed.

2.2 Measurement methods

2.2.1 Quantitative analysis of elements in plant organs

To determine the total elemental content in plant organs, a stainless steel pulverize was used to grind a dried sample of biomass. Total nitrogen content was determined using the Kjeldahl method (Hungarian standard: MSZ-08-1783-6:1983). The potassium content of the finely ground and dried plant sample was determined after acid digestion (Hungarian standard: MSZ-08-1783-29:1985). The resulting solution was diluted and analyzed using a Shimadzu UV-1800 spectrophotometer. The concentration of potassium was determined by measuring the absorbance and comparing it to a calibration curve. For cadmium analysis, the samples were digested using nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) according to the Hungarian standard MSZ 21470-50:2006:4.1 using a VELP DK20 digestion block. After digestion, the solution was diluted and analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES; JY Ultima 2). The cadmium content was quantified using instrumental reading and calibration curve.

2.2.2 Quantitative analysis of soil NO_2+NO_3-N

The concentration of nitrate (NO_2+NO_3-N) in the soil was determined using a FIAStar 5000 analyzer in accordance with the Hungarian standard MSZ 20135:1999 5.4.3. Soil samples were collected, air-dried and sieved through a 2 mm sieve. An amount of 10 g of prepared soil sample was extracted with 100 ml of 1 M KCl solution. After extraction, the mixture was filtered to obtain a clear filtrate. The filtrate was then analyzed using the FIAStar 5000, which uses flow injection analysis (FIA) and a chemical colorimetric reaction.

2.2.3 Measurement of physiological parameters of leaves

The chlorophyll content (SPAD value) and quantum yield (Q_y) of three mature leaves of each plant were measured every 15 days using the SPAD-502 Konica Minolta portable chlorophyll meter and the PAR-FluorPen FP110 portable fluorometer (due to equipment acquisition, Q_y data will only be available in the 2023 experiment). Chlorophyll content is expressed as a chlorophyll index without units. Measurements were taken from 10:00 a.m. to 3:00 p.m. and followed the operating procedures of the instrument.

2.2.4 Phytoremediation efficiency parameter

The accumulation of cadmium was assessed by calculating the bioconcentration factor values for the root (BCF_r), shoot (BCF_b) and leaf parts (BCF_l) using the following formula:

$$BCF_l = C_{leaf}/C_{Soil}$$

$$BCF_b = C_{branch}/C_{Soil}$$

$$BCF_r = C_{root}/C_{Soil}$$

The ability of cadmium to translocate from the roots to the aboveground part was evaluated by calculating the translocation factor (TF) of the aboveground part. The calculation formula is as follows:

$$TF = C_{leaf+branch}/C_{root}$$

2.3 Data processing

2.3.1 Difference analysis

Analyses and plots were carried out in SPSS (IBM SPSS 27) and Excel (Microsoft 365), respectively. The individual effects of treatment concentration, species, and plant organ and the interactions of the three were assessed using multi-way ANOVA and expressed in the form of graphs and tables. Prior to the ANOVA, the normality of the data and the homogeneity of variances were assessed using the Levene's test to confirm the applicability of the ANOVA. Duncan's post-hoc test was used to detect differences between means. If the data did not meet the requirements of homogeneity, the Games-Howell test was used as a non-parametric alternative, statistical significance was defined as $p \leq 0.05$, and bar charts were used to visualize the results. The data from the experimental results are always presented as mean ± standard deviation.

2.3.2 Correlation analysis

The differences in the physiological functions of leaves under Cd treatments of different concentrations were studied using one-way ANOVA(SPSS 27)and linear fitting and expressed as line graphs (Excel Microsoft 365).

R is used for analysis and plotting; Euclidean distance is used to represent the linear distance between two points in multidimensional space. The similarity or difference between different variables is calculated, and a heat map is generated using the *pheatmap* function. A dendrogram is generated by recursively merging or splitting variables to reveal the potential relationships between variables. The formula is as follows: *x* represents the functional form of each nutrient element, and *y* represents each bioaccumulation and translocation parameter.

R is used for analysis and plotting; Principal component analysis (PCA) is used to project high-dimensional data onto a few orthogonal principal components while retaining as much of the data's primary variation information as possible. The principal components are eigenvectors of the data covariance matrix. The first principal component captures the maximum amount of variation, the second principal component captures the maximum amount of the remaining variation, and so on. The *prcomp* function is used to perform principal component analysis, and the *factoextra* package is used for visualization.

R was used for analysis and plotting; redundancy analysis (RDA) was performed using the *vegan* package. Redundancy analysis is a method that combines multiple regression and principal component analysis to investigate how the response variable (bioaccumulation parameters) is affected by the combined influence of the explanatory variables (nutritional characteristics). The main objective of our study was to explore the potential role of all candidate variables, rather than to construct a simplified predictive model. Therefore, we did not remove collinear variables.

Mantel's test (Pearson's correlation coefficient) from the vegan package was used to explore the relationship between plant nutritional traits and organ bioaccumulation and translocation parameters in plants in the bamboo and willows families. This is a non-parametric statistical method based on a distance matrix that is used to compare the correlation between two matrices. Since the results in the first year varied greatly due to the influence of natural temperature, we used the data from the second year for analysis.

Fisher discriminant analysis (FDA) is used to determine whether nutritional functional traits can be used as the basis for plant classification and their position on the economic spectrum. The method of analysis of variance is used, which not only uses the classification of known samples and the extremum method of multivariate functions to maximise the variance between groups and minimise the variance within groups, but also to find the discriminant function. Data processing and analysis was carried out using SPSS. Eight characteristics of the seven plants from the second year were selected: RN (X1), RK (X2), BN (X3), BK (X4), LN (X5), LK (X6), SN (X7) and SK (X8) for statistical classification and Fisher's discriminant analysis to obtain the discriminant function coefficients and construct the discriminant model.

3 RESULTS AND DISCUSSION

3.1 Difference analysis

3.1.1 Uncertainty in phytoremediation due to differences in field environments

This study revealed significant variations in Cd accumulation and soil nutrient dynamics across different years and plant species, influenced by environmental conditions. In the first year, high temperatures led to increased Cd accumulation in most plants, likely due to enhanced transpiration and root exudation that promoted metal bioavailability. However, some species experienced high mortality during rooting, indicating species-specific heat sensitivity. The *Salicaceae* plants showed a substantial increase in Cd accumulation under specific treatments in the first year, but this trend weakened in the second year, except for 'Csala,' which maintained a high Cd uptake capacity, possibly due to its unique metabolic adaptation. The study also found that high temperatures accelerated potassium mobility in the soil and increased N and K availability in the rhizosphere of *Salicaceae* plants.

In contrast, the second year showed a reversal in soil N and K distribution, with *Bambusoideae* plants exhibiting higher rhizosphere N and K content than *Salicaceae*. This shift suggests that under stable environmental conditions, bamboo plants, with their dense root networks, gradually enhance their nutrient absorption efficiency, while the decline in Cd uptake by *Salicaceae* may have reduced microbial activity, indirectly affecting soil nutrient cycling. The results indicate that phytoremediation efficiency is not only species-dependent but also varies with long-term environmental fluctuations, influencing plant-microbe interactions and soil nutrient availability. Understanding these dynamic changes is crucial for optimizing plant selection and management strategies in practical phytoremediation applications.

3.1.2 Species accumulation specificity of the *Bambusoideae* and *Salicaceae*

This study found significant differences in Cd accumulation capacity and distribution patterns between *Bambusoideae* and *Salicaceae*. *Bambusoideae* primarily accumulated Cd in the roots, with significantly lower aboveground accumulation compared to *Salicaceae*. The total Cd accumulation in plants varied depending on Cd treatment concentration, plant species, and plant organs, with higher Cd concentrations leading to increased accumulation. Willow and poplar species exhibited higher Cd accumulation than *Bambusoideae*, with Cd primarily translocated to leaves, while bamboo retained most of the Cd in its roots. Among the studied species, 'Csala' maintained stable Cd translocation factors across years and treatments, whereas 'Pegaso' and 'Tora' showed higher translocation and bioconcentration factors compared to bamboo.

In both years, *Salicaceae* plants showed higher Cd accumulation in aboveground parts compared to *Bambusoideae*. Bamboo exhibited a relatively stable root fixation strategy throughout the study, limiting aboveground Cd accumulation. In contrast, willow and poplar maintained consistent Cd translocation from roots to shoots under different Cd concentrations. The results highlight that *Bambusoideae* species are more suitable for Cd stabilization in contaminated soil, while *Salicaceae* species are better suited for phytoextraction due to their ability to transport and accumulate Cd in aboveground tissues.

3.1.3 Species-specific response of nutrients between plants and organs

The results showed that there was no significant difference in total N content between the first and second years in Salicaceae, whereas *Bambusoideae* species (Moso and *D. asper*) exhibited significantly lower total N content in the first year compared to the second year. Additionally, the study found no significant effect of Cd treatments on total N content in plants, while significant differences in total K content were only observed in Moso and *D. asper* in the second year. Further analysis of N and K contents in different plant organs indicated that leaves had higher N and K contents than roots and branches across all species. Leaves and roots were also identified as the most sensitive organs to Cd exposure, with leaf nutrient content varying among species. In the first year, N content in Moso and *D. asper* leaves increased with higher Cd concentrations, whereas responses among *Salicaceae* species varied, with 'Tora' showing an increase and 'Csala' and 'Pegaso' showing a decreasing trend.

In the second year, the nutrient content in plant organs stabilized as the influence of high temperature stress diminished. Only Moso bamboo exhibited a significant increase in leaf N content under T1 treatment, while *D. strictus* leaves showed no significant response to Cd treatments. In contrast, *Salicaceae* species displayed more complex patterns, with N content in 'Csala' and 'Tora' leaves decreasing significantly under Cd treatment, but with inconsistent trends in the magnitude of decrease. Furthermore, K content in 'Csala' and 'Tora' showed different responses, with one increasing and the other decreasing. These findings highlight the differences in Cd accumulation and nutrient metabolism responses between *Bambusoideae* and *Salicaceae*, reflecting variations in Cd uptake, transport, and nutrient distribution strategies between the two plant groups.

3.2 Correlation analysis

3.2.1 The stimulating effect of Cd on plant photosynthesis

This study found that Cd had a positive stimulatory effect on chlorophyll content in some plant species, particularly in the first year. 'Csala', Moso, and *D. asper* all exhibited increased chlorophyll content under Cd stress, with this effect persisting in Moso and 'Csala' under T2 treatments in the second year. Additionally, Moso showed an increase in quantum yield, suggesting its ability to maintain higher photosynthetic efficiency even under high Cd concentrations. However, the extent of Cd-induced photosynthetic stimulation varied significantly among species, with differences not only in chlorophyll content but also in photochemical efficiency.

In the second year, 'Csala' continued to show increased chlorophyll content but did not exhibit significant changes in quantum yield, and under T2 treatment, a decreasing trend was observed. This suggests that prolonged Cd exposure may gradually suppress photosynthetic efficiency in some species. The results indicate that Cd can have a stimulatory effect on plant photosynthesis under specific conditions, but the extent of this effect depends on species differences, Cd concentration, and environmental factors. Moso demonstrated a strong adaptability to Cd contamination, maintaining higher photosynthetic efficiency across different treatments.

3.2.2 The connection between the functional traits of nutrients and phytoremediation

This study analyzed the differences in Cd accumulation and translocation strategies between plant species based on their organ-level responses. The results showed that *Bambusoideae* primarily accumulated Cd in the roots, while *Salicaceae* species translocated more Cd to aboveground organs. Heatmap clustering results indicated that Moso bamboo had the highest Cd accumulation in the roots, whereas branches restricted further translocation. exhibited more accumulation in the stem, whereas *D. strictus* showed significant Cd accumulation in both roots and stems, with limited translocation to leaves. Among *Salicaceae*, 'Csala' and 'Tora' exhibited strong Cd translocation to aboveground parts, while 'Pegaso' displayed a balanced accumulation across all organs. 'AF2' had high Cd accumulation in roots and stems but lower translocation to leaves. These differences highlight distinct Cd allocation strategies between species, with some plants emphasizing root fixation and others favoring aboveground translocation.

Nutrient content analysis further revealed significant differences in N and K allocation strategies. Moso bamboo exhibited a strong correlation between leaf N and K content and Cd accumulation, indicating a preference for retaining nutrients in leaves. *D. asper* had a more conservative strategy, with roots accumulating higher N and K while limiting Cd translocation to aboveground organs. *D. strictus* prioritized root Cd accumulation, while its leaves maintained stable N levels, reducing Cd effects on photosynthesis. Among *Salicaceae*, 'Csala' and 'Tora' exhibited a strong positive correlation between leaf N content and Cd translocation, supporting efficient Cd movement to aboveground parts. 'Pegaso' displayed a balanced strategy, with roots and stems contributing equally to Cd accumulation. 'AF2' followed a similar pattern, with roots and stems jointly promoting Cd accumulation while limiting translocation to leaves. These findings indicate that different species adopt distinct resource allocation strategies in response to Cd stress, influencing their phytoremediation potential.

3.2.3 Nutrient functional traits serve as a predictor of phytoremediation

This study explored the potential of functional traits as predictors for phytoremediation efficiency in poplar and bamboo species. Mantel analysis showed that stem potassium content in Bambusoideae was significantly correlated with the translocation factor of the three bamboo species, suggesting that BK could serve as a valid predictor for Cd transport efficiency in bamboo. Similarly, in Salicaceae, branch nitrogen content was significantly correlated with Cd TF, indicating its potential as a key predictor for Cd translocation in poplars and willows. These findings suggest that specific nutrient traits may provide more precise indicators for selecting plant species suitable for phytoremediation.

The study further highlighted the role of BK in bamboo as a critical factor influencing

Cd transport, while BN in Salicaceae reflected the importance of branches as an intermediate site for Cd accumulation and redistribution. The high BN content in Salicaceae species may be associated with greater Cd sequestration and translocation capacity, contributing to their effectiveness in phytoextraction. These results emphasize that functional trait screening in phytoremediation should be species-specific and adapted to different environmental conditions. While this study provides initial insights, further research is needed to test a broader range of plant species and refine predictive trait models for optimizing phytoremediation strategies.

4 CONCLUSION AND RECOMMENDATIONS

4.1 Effect of high temperature in pot plant experiments

The results of this study demonstrate that temperature exerts a significant impact on the phytoremediation capacity of plants, with different species exhibiting distinct responses under varying thermal conditions. Overall, high temperatures exerted both promoting and inhibitory effects on the remediation efficiency, which contributed to the interannual variations observed during the experiment.

In the first year, elevated temperatures enhanced the Cd accumulation capacity of most plants, leading to a significant improvement in overall remediation efficiency. Under high-temperature conditions, plant metabolism accelerated, facilitating the transport of water and solutes, which in turn promoted the translocation of Cd from soil to aboveground tissues. Consequently, the total Cd accumulation across treatment groups was generally higher in the first year. However, high temperatures also negatively affected the survival and rooting rates of certain species, such as *D. strictus* and 'AF2,' resulting in increased mortality during the early growth stages and constraining further improvements in remediation performance.

In the second year, when temperatures were milder, significant interspecies differences in Cd accumulation capacity became apparent. Some taxa, such as 'Csala' and Moso, maintained high levels of Cd accumulation, whereas others experienced a decline in their remediation performance. These variations likely stem from the differential adaptability of species to changing thermal conditions. For instance, 'Csala' continued to exhibit high Cd accumulation in the second year, reflecting its robust adaptive mechanisms to environmental variability. Conversely, other species may have lacked sufficient adaptive traits or experienced reduced stimulation under milder conditions, leading to diminished remediation efficiency. Additionally, moderate temperatures appeared to enhance root development in certain species. For example, *Bambusoideae* plants exhibited higher soil nitrogen in the second year, likely due to their enhanced root absorption capacity and more efficient resource utilization under stable environmental conditions.

The differential responses of species to temperature variations significantly influenced the outcomes of this study. Certain plants exhibited stimulated photosynthesis under high-temperature conditions, characterized by increased chlorophyll content and improved photochemical efficiency. For example, Moso demonstrated exceptional photosynthetic efficiency and remediation performance under combined heat and Cd stress. However, as the experiment progressed, prolonged exposure to high temperatures and Cd stress began to exert inhibitory effects on some species. For instance, while 'Csala' maintained increased chlorophyll content in the second year, its photochemical efficiency began to decline, indicating that prolonged stress might have compromised the stability of its photosynthetic system.

4.2 Species-specificity of Cd accumulation

The results of this study indicate significant differences in Cd accumulation capacity and distribution patterns between *Bambusoideae* plants and *Salicaceae* plants, reflecting their distinct strategies for adapting to polluted environments. *Bambusoideae* plants primarily accumulate Cd in their roots, with significantly lower accumulation observed in aboveground organs compared to *Salicaceae*. This "low translocation-high fixation" strategy enables *Bambusoideae* plants to effectively reduce the toxic effects of Cd on photosynthesis and other physiological processes, thereby maintaining high tolerance levels in contaminated environments. However, this approach also limits the translocation of Cd to aboveground tissues, resulting in relatively lower overall Cd accumulation capacity.

In contrast, *Salicaceae* plants exhibit a strong capacity for Cd translocation and accumulation in aboveground organs, adopting a "high translocation-high accumulation" strategy. In polluted environments, *Salicaceae* plants rapidly translocate absorbed Cd from roots to aboveground tissues, maintaining high accumulation efficiency. Experimental results demonstrated that some *Salicaceae* species maintained stable translocation factors and accumulation coefficients under varying Cd treatment conditions, highlighting their strong adaptability in Cd uptake and translocation.

The differences in Cd accumulation characteristics between bamboo subfamily and *Salicaceae* plants determine their suitability for different applications in phytoremediation. *Bambusoideae* plants are well-suited for phytostabilization, effectively immobilizing Cd and preventing its migration and dispersion in the environment. In contrast, *Salicaceae* plants, with their superior Cd absorption and translocation capabilities, are better suited for phytoextraction applications. These species-specific characteristics provide diverse options for addressing varying scenarios in pollution management.

4.3Effect of Cd on the content of N and K in plants

The results of the study indicate that the impact of Cd on N and K content in plants varies significantly and exhibits distinct patterns across different plant species and organs. Overall, Cd treatment had no significant effect on the total N content in plants but caused notable changes in the distribution of N and K among plant organs. Leaves, as the primary organs for photosynthesis and transpiration, consistently exhibited higher N and K content compared to roots and branches. Furthermore, leaves and roots were the most sensitive organs to Cd stress.

In the first year of the experiment, Cd significantly influenced leaf N content in Moso and , with LN increasing significantly as Cd concentration rose. In contrast, *Salicaceae* plants displayed diverse response patterns. For instance, the leaf N content of 'Tora' increased with increasing Cd concentration, whereas 'Csala' and 'Pegaso' showed a decreasing trend. These differences reflect the distinct adaptive strategies employed by *Salicaceae* plants under Cd stress. Additionally, Cd had a pronounced effect on K content in roots, underscoring the role of roots as the primary target for Cd-induced disruption of potassium metabolism.

In the second year, as high-temperature stress diminished, nutrient content changes in plant organs became more stabilized. While Moso exhibited an increase in leaf N content under certain treatments, the leaf N content of and *D. strictus* showed low sensitivity to Cd treatment. Compared to bamboo subfamily plants, *Salicaceae* plants exhibited more complex changes in N and K content. For example, the leaf N content of 'Csala' and 'Tora' decreased significantly under Cd treatment, but the extent and pattern of this decrease were inconsistent. Similarly, leaf K content displayed contrasting trends, with 'Csala' showing an increase and 'Tora' a decrease.

4.4 Understand plant strategies of Cd stress from functional traits

This research indicated that functional traits provide a deeper understanding of plant adaptive strategies under Cd stress and reveal differences in Cd accumulation and distribution among species and plant organs. The experimental results demonstrate that different plants exhibit distinct trait differentiation and resource allocation strategies under Cd stress, which directly influence their remediation capacity in polluted environments.

Bambusoideae, such as Moso, adopted a "high acquisition-low translocation" strategy by increasing the N and K content in leaves, thereby limiting the upward translocation of Cd from roots to aboveground parts. This reduces the toxic effects of Cd on critical physiological functions in leaves. In contrast, employed a "conservative strategy" by enhancing N and K accumulation in roots, fixing more Cd in the root zone, and slowing its upward translocation. Additionally, different organs exhibit distinct adaptive differentiation, with roots primarily focused on maintaining stability, while stems display more diverse resource allocation strategies.

Salicaceae plants exhibited more complex adaptations. For instance, functional traits of 'Csala' and 'Tora' emphasized the rapid translocation of Cd to aboveground parts, with leaf N content showing a significant positive correlation with Cd translocation and accumulation. This "high translocation-high accumulation" strategy enhances remediation capacity in aboveground organs while alleviating Cd toxicity in roots. 'Pegaso' adopted a "balanced strategy," utilizing coordinated action between roots and stems to achieve efficient Cd accumulation and distribution.

Furthermore, the study revealed that functional traits can serve as important predictors of plant remediation capacity. For example, stem K content in *Bambusoideae* plants showed a significant correlation with Cd translocation factors, while branch N content in *Salicaceae* plants correlates with Cd accumulation capacity. These findings highlight the predictive potential of functional traits in assessing plant responses to Cd stress, providing a scientific basis for optimizing phytoremediation strategies and applications.

5 NEW SCIENTIFIC RESULTS

5.1 Differences in Cd accumulation of bamboo, poplar and willow

Differences in Cd accumulation of bamboo, poplar and willow

This study presents the first scientific investigation into the significant differences in Cd accumulation capacity and distribution patterns between *Bambusoideae* and *Salicaceae* taxa under Cd stress. Based on adaptive strategies and functional trait performance, *Bambusoideae* species primarily adopted a "low translocation-high fixation" strategy, with Cd predominantly accumulated in the roots. In contrast, *Salicaceae* taxa exhibited a "high translocation-high accumulation" strategy, rapidly translocating Cd from roots to leaves and branches, thus achieving higher Cd extraction efficiency.

5.2 The first time using new bamboo plant material in Cd phytoremediation

According to our findings two *Bambusoideae* species, *Dendrocalamus asper*, and *D. strictus* were used for the first time for phytoremediation purposes and proved that under high temperature conditions *D. asper* was more suitable for phytoremediation experiments especially for phytostabilization by the roots.

5.3 The role of functional traits in heavy metal phytoremediation

In my dissertation, the concept of functional traits is introduced for the first time in heavy metal phytoremediation, and the understanding and application of plant economic spectrum in heavy metal phytoremediation are discussed at three levels: plant, organ and nutrient element. These traits influenced the uptake, translocation and accumulation capacity of heavy metals by modulating the resource acquisition, allocation and utilization strategies of plants.

5.4 Nutrient functional traits as predictors of heavy metal phytoremediation

This dissertation concluded that branch potassium (BK)and branch nitrogen (BN) have the potential to serve as effective predictors of heavy metal transport efficiency in *Bambusoideae* and *Salicaceae* taxa, respectively. The results demonstrate that functional traits such as BK and BN are closely linked to the translocation and accumulation of heavy metals, providing a practical tool for assessing phytoremediation potential. Additionally, leaf nitrogen has been identified as a viable predictor for translocation factor in willow, highlighting its significance in evaluating the phytoextraction capacity of specific taxa.

6 SCIENTFIC PUBLICATIONS

Impact factor journals:

Liang, Z., Neményi, A., Kovács, G. P., & Gyuricza, C. (2023) Potential use of bamboo resources in energy value-added conversion technology and energy systems. *GCB Bioenergy*, 2023, 15(8): 936-953. https://doi.org/10.1111/gcbb.13072

Scopus Rank: D1 IF:5.9 Cited: 12

Liang, Z., Neményi, A., Kovács, G. P., & Gyuricza, C. (2024). Incorporating functional traits into heavy metals phytoremediation: the future of field-based phytoremediation. *Ecological Indicators*, *166*, 112262. https://doi.org/10.1016/j.ecolind.2024.112262

Scopus Rank: D1 IF:7.0 Cited: 6

Chan KN, Veres A, Liang Z, Kisvarga S, Neményi A. A Shoot Phenological Study of Certain *Phyllostachys* Bamboo Taxa Under Central European Climatic Conditions. *Plants* (Basel). 2024 Dec 23;13(24):3592. https://doi: 10.3390/plants13243592.

Scopus Rank: Q1 IF:4.0

Non-Impact factor journal:

Liang, Z., Kovács, G. P., Gyuricza, C., & Neményi, A. (2022). Potential use of bamboo in the phytoremediation of heavy metals: A review. *Acta Agraria Debreceniensis*, *1*, 91-97. https://doi.org/10.34101/actaagrar/1/10311 Cited: 7

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Posters:

Using bamboo for the phytoremediation of heavy metals : a review (Shanghai China)

Conferences:

Global Cleaner Production Conference 2023. 12. 9- 2023. 12. 12 (Shanghai China)

31st Biomass Conference & Exhibition 2023. 6.5 - 2023. 6. 8 (Bologna Italy)

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