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Phytoremediation efficiency of poplar hybrid varieties with diverse genetic backgrounds in soil contaminated by multiple toxic metals (Cd, Hg, Pb, and As)

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A R T I C L E I N F O

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A B S T R A C T

Fifteen poplar varieties were used in a field trial to investigate the phytoremediation efficiency, stress resistance, and wood property of poplar hybrid varieties with diverse genetic backgrounds under the composite pollution of heavy metals. The coefficient of variation and clone repeatability for growth traits and Cd concentration were large. The Cd accumulation of poplar varieties 107 and QHQ reached 1.9 and 1.7 mg, respectively, followed by QHB, Ti, 69, and Pa, in which Cd accumulation reached 1.3 mg. Most of the intra-specific hybrid varieties (69, QH1, SL4, T3, and ZL46) had low Cd concentrations and small biomass, resulting in weak Cd accumulation and low phytoremediation efficiency for Cd-polluted soil. By contrast, the inter-sectional and inter-specific hybrid varieties exhibited better growth performance and accumulated higher concentrations of heavy metals than the intra-specific hybrids. The bioconcentration factor and translocation factor of Hg, As, and Pb were less than 1, indicating that poplars have low phytoremediation efficiency for these heavy metals. The hybrids between section *Aigeiros* and *Tacamahaca* (QHQ and QHB) and the inter-specific hybrid 107 within section *Aigeiros* were more resistant to composite heavy metal stress than the other poplar varieties were partially because of their high levels of free proline that exceeded 93 $\mu g \cdot g^{-1}$ FW. According to the correlation analysis of the concentrations of the different heavy metals, the poplar roots absorbed different heavy metals in a cooperative manner, indicating that elite poplar varieties with superior capacity for accumulating diverse heavy metals can be bred feasibly. Compared with the intra-specific hybrid varieties, the inter-sectional (QHQ and QHB) and inter-specific (107) hybrid varieties had higher pollution remediation efficiency, larger biomass, higher cellulose content, and lower lignin content, which is beneficial for pulpwood. Therefore, breeding and extending inter-sectional (QHQ and QHB) and inter-specific hybrid varieties can improve the phytoremediation of composite pollution.

1. Introduction

With the accelerating pace of economic construction and intensive anthropogenic activities, such as those in industries and agriculture, the problem of soil pollution is becoming increasingly prominent. Heavy metal pollution is one of the major threats to human health, and the human body is exposed to it through the food chain, air, skin, and other means. Farmlands are usually polluted by mining waste residues, pesticides, and sewage discharge, and heavy metal pollution in farmlands needs to be solved urgently (Cheng et al., 2017; Shaghaleh et al., 2024). Compared with water and air pollution, soil pollution has poor capacity for self-cleaning, strong concealment, and long risk accumulation time. Some of the cultivated soil in China is polluted by heavy metals, such as cadmium, copper, mercury, lead, and arsenic (Liu et al., 2012; Qin et al.,

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2021). The methods for the remediation of heavy metal pollution in farmlands include physical, chemical, and biological remediation (Etim, 2012; Sani et al., 2023; van der Ent et al., 2013; Zine et al., 2020; Jalil et al., 2023). Physical and chemical remediation of heavy metal pollution have many drawbacks, including high cost, easy destruction of the soil structure and fertility, and secondary pollution, and certain limitations exist in their actual promotion (Yang et al., 2020). By contrast, phytoremediation has numerous merits, such as large application area, low price, long-lasting repair effect, ease of monitoring, and absence of secondary pollution, so it has high potential for addressing soil pollution (Hu et al., 2016; Tow et al., 2019; Wu et al., 2009; Yong et al., 2010).

Hyperaccumulator plants, such as *Thlaspi caerulescens*, *Sedum alfredi*, *Arabidopsis helleri*, and *Solamum nigrum*, can accumulate heavy metals at high concentrations (Sahito et al., 2024; Yu et al., 2024). However, most of these hyperaccumulator plants are herbaceous and have disadvantages, including small size, slow growth, low biomass, and limited root system, which considerably limit their application in phytoremediation (Bustingorri et al., 2017; van der Ent et al., 2013). Compared with herbaceous plants, woody plants, such as poplar (*Populus*) and willow (*Salix*), have numerous merits, including a vast root system, large biomass, strong capacity for heavy metal enrichment, and high tolerance; therefore, they are important candidate plants for phytoremediation of heavy metal pollution (He et al., 2013; Song et al., 2019; Hao et al., 2020; Sahito et al., 2023). Poplar is one of the major industrial wood species with high economic value and is widely used for pulp and furniture production (Du et al., 2010). Poplar can produce a large wood yield in a short period because of its fast growth, and its stem can accumulate large quantities of heavy metals, thus reducing heavy metals in the soil. The quality and property of wood determine its economic benefits. In the pulp industry, wood property evaluation indices mainly include the contents of lignin, cellulose, and hemicellulose (Plomion et al., 2001; Zhang et al., 2011). From the perspective of wood utilization, clonal variations in the lignin, cellulose, and hemicellulose contents of poplars exposed to heavy metal pollution are scientific issues that must be addressed.

The heavy metal absorption, translocation, and tolerance of poplar varieties exhibit considerable differences. The capacity for Cd accumulation and the stress tolerance of *Populus canescens* are higher than those of *Populus deltoides*, *Populus* × *euramericana*, *Populus alba* × *Populus glandulosa*, *Populus nigra*, *Populus popularis*, and *Populus cathayana* (He et al., 2013). The Pb resistance of *Populus nigra* is stronger than that of *Populus cathayana*, *Populus canescens*, *Populus alba* × *Populus glandulosa*, and *Populus euphratica* (Rahman et al., 2022). Under heavy metal stress, reactive oxygen species (ROS) and malondialdehyde (MDA) can be induced in plant cells, and the contents of osmotic regulatory substances, such as free proline, soluble sugar, and antioxidant enzymes, change to alleviate membrane system damage and oxidative detoxification (Zhang et al., 2016, 2019b). Research has shown that arsenate-tolerant *Populus deltoides* has higher stress tolerance under arsenic stress, exerts less damage on cell morphology and structure, has lower ROS and MDA accumulation, and has stronger photosynthesis and ROS scavenging abilities compared with arsenate-sensitive *Populus* × *euramericana* (He et al., 2015; Shi et al., 2017).

Heterosis is a widespread biological phenomenon that has been widely used for the genetic improvement of crops and trees (Chen et al., 2013; Song et al., 2018). Plant heterosis is manifested not only in the biomass and economic yield of plants but also in their stress tolerance (Chen et al., 2013; Groszmann et al., 2014). Hybrid kenaf has higher tolerance for Pb stress than its parents (Mun et al., 2008). Under Cd exposure, hybrid plants usually show considerable heterosis in terms of Cd bioenrichment and Cd tolerance. A comparative analysis of different rice varieties has found that hybrid rice varieties have a higher Cd accumulation capacity than conventional rice varieties (Sun et al., 2016). The hybrid progeny of nightshade, a typical Cd hyperaccumulation plant, exhibits remarkable heterosis in terms of biomass and Cd enrichment (Lin et al., 2016). Compared with common poplar genotypes, the hybrid poplar has marked advantages in Cd enrichment and tolerance. For instance, *Populus tremula* × *Populus alba*, a hybrid formed by the natural hybridization of *Populus tremula* and *Populus alba*, has a strong capacity of Cd enrichment and tolerance (Ma et al., 2014; He et al., 2015). The Cd enrichment level of *Populus* × *euramericana,* which is produced by the hybridization of *Populus deltoides* and *Populus nigral*, is also higher than that of its parents (Marmiroli et al., 2013).

Populus species are divided into five sections, namely, *Leuce*, *Aigeiros*, *Tacamahaca*, *Leucoides*, and *Turanga*. *Populus* hybridization can be conducted between or within different species and between or within different sections to produce different types of hybrid varieties, including intra-specific hybrid, inter-specific hybrid, and hybrids of different poplar sections. Positive correlations between parental genetic distances and heterosis have been reported in numerous crops, such as maize, wheat, and pearl millet (Charcosset et al., 1991; Schnable and Springer, 2013; Legarra et al., 2023). According to this hypothesis, high levels of heterosis can be obtained from parents with large genetic distances and expression differences in their stress-responsive genes (Miller et al., 2015; Birchler et al., 2010). In poplars, cross parents from different sections have larger genetic distances than inter-specific and intra-specific parents, and the genetic distance of inter-specific parents is larger than that of intra-specific parents. Disclosing the heavy metal bioaccumulation patterns, stress tolerance, and wood properties of poplar hybrid varieties produced by inter-specific, intra-specific, and inter-sectional hybridization is helpful for breeding poplar varieties with desirable capacity for phytoremediation and ideal growth performance.

In this study, 15 poplar varieties with different genetic backgrounds were used to investigate the phytoremediation efficiency of different poplar varieties in soil contaminated by complex heavy metals (Cd, Hg, As, and Pb) in a mining area in south China. The objective of this study was to investigate the phytoremediation efficiency, stress resistance, and wood properties of different types of poplar hybrid varieties under composite heavy metal pollution. The genetic background of the poplar varieties in this experiment was complex and diverse; and included hybrid varieties between sections and inter-specific and intra-specific hybrid varieties within sections. The results obtained from this study would provide the scientific basis for the selection of poplar varieties with high heavy metal pollution remediation efficiency and excellent wood properties.

2. Materials and methods

2.1. Plant material and experimental design

A field experiment was performed in Longgou Township, Chongyi County, Ganzhou City, Jiangxi Province (114°53′E, 25°69′N) from March to October 2021. The experimental site has a subtropical monsoon humid climate zone. The average altitude is about 300 m, the average annual temperature is ~17 °C, and the annual rainfall is approximately 1600 mm. Due to the mining of non-ferrous metals, the experimental site selected in this study is a typical location contaminated by heavy metals (Cd, Hg, As, and Pb) and covers an area of 0.4 ha. According to the detailed soil investigation data of the experimental site, the soil pH is 4.78; the type of soil is acidic red soil; the concentrations of soil organic matter, total nitrogen, available phosphorus and available potassium were 2.45 %, 0.136 %, 8 mg·kg^{-1} and 55 mg·kg^{-1}, respectively (http://vdb3.soil.csdb.cn/); the Cd concentration in the soil is 1.81 mg·kg^{-1}; and the concentrations of Hg, As, and Pb in the soil reach 1.13, 50.04, and 94.04 mg·kg^{-1}, respectively.

The research materials included 15 *Populus* varieties, and detailed descriptions of the poplars are shown in Table 1. Poplar cuttings with a length of 20 cm were planted in three completely random blocks, with four replicated seedlings in each block for each genotype. A total of 180 experimental plants were used. The planting density was 100,000 plants·ha^{-1}, and the inter-row and intra-row distances were 40 and 25 cm, respectively. Plots were watered to keep the substrate moist;

Table 1
The genetic background and Latin name of the fifteen *Populus* varieties.

Clone	Genetic background	Latin name
QHB	Hybrid between section *Aigeiros* and *Tacamahaca*	*Populus deltoides* × *Populus cathayana*
QHQ	Hybrid between section *Aigeiros* and *Tacamahaca*	*Populus deltoides* × *Populus cathayana*
107	Inter-specific hybrid within section *Aigeiros*	*Populus* × *euramericana*
Ti	Inter-specific hybrid within section *Aigeiros*	*Populus* × *euramericana*
La	Inter-specific hybrid within section *Aigeiros*	*Populus* × *euramericana*
QB1	Inter-specific hybrid within section *Leuce*	*Populus alba* × *(P.alba* × *P. glandulosa)*
QB2	Inter-specific hybrid within section *Leuce*	*Populus alba* × *(P. alba* × *P. glandulosa)*
QB3	Inter-specific hybrid within section *Leuce*	*Populus alba* × *(P. alba* × *P. glandulosa)*
Pa	Intra-specific hybrid within section *Aigeiros*	*Populus deltoides* Marshall
Qg	Intra-specific hybrid within section *Aigeiros*	Populus deltoides Bartr. cv. 'Lux' (I–69/55)
69	Intra-specific hybrid within section *Aigeiros*	*Populus deltoides* Marshall
QH1	Intra-specific hybrid in section *Aigeiros*	*Populus deltoides* Marshall
SL4	Intra-specific hybrid clone in section *Aigeiros*	*Populus deltoides*
T3	Intra-specific hybrid clone in section *Aigeiros*	*Populus deltoides*
ZL46	Intra-specific hybrid clone in section *Aigeiros*	*Populus* × *euramericana*

removal of weeds was carried out weekly to reduce the competition with herbs during the pre-emergence period of cuttings.

2.2. Measurement of growth traits

After 7 months of cultivation, the major growth traits, including tree height and diameter at breast height (DBH), were measured. Then, plant samples were collected in accordance with the Procedural Regulations Regarding the Monitoring of Pollutants in the Produce of Agriculture, Animal Husbandry and Fishery (NY/T 398–2000) of the Chinese government. Soil samples were also collected in accordance with the Technical Rules for Monitoring of the Environmental Quality of Farm and Soil (NY/T 395–2012) of the Chinese government. The soil and plant sample collections were synchronized. Each organ (root, stem, and leaf) of the plant samples were washed and rinsed 3–5 times with ultrapure running water, dried with absorbent paper, and weighed. Subsequently, a 0.3 kg sample of each organ was dried at 70 ℃ to a constant weight and used to measure biomass and heavy metal concentration. For the physiological experiment, fresh leaves from the upper, middle and lower parts of the seedlings were taken mixedly and snap-frozen in liquid nitrogen before stored at −80 ℃. Four individual plants of each genotype in each block were mixed as one replicate for a total of three biological replicates in three blocks. The phenotypic coefficient of variation (CV) and repeatability (R) were used to analyze the genetic variation in poplar. CV and R are calculated as (Silvio et al., 2021)

$$C_{trait} = \frac{\text{Standard deviation}_{trait}}{\text{Mean}_{trait}} \quad (1)$$

$$R = 1 - \frac{1}{F} \quad (2)$$

where F indicates the F value of the analysis of variance.

2.3. Quantification of heavy metals

The heavy metal (Cd, Hg, As, and Pb) contents were determined in accordance with the following national standards of the Chinese government: National Standard for Food Safety–Determination of Cadmium in Foods (GB 5009.15–2014), National Standard for Food Safety–Determination of Lead in Foods (GB 5009.12–2017), National Standard for Food Safety–Determination of Total Arsenic and Inorganic Arsenic in Foods (GB 5009.11–2014), and National Standard for Food Safety–Determination of Mercury in Foods (GB 5009.17–2014). The bioconcentration factor (BCF) and translocation factor (TF) of the heavy metals were calculated based on the heavy metal concentration as follows:

$$BCF_{tissue} = \frac{[Cd / Hg / As / Pb]_{tissue}}{[Cd / Hg / As / Pb]_{soil}} \quad (3)$$

$$TF = \frac{[Cd / Hg / As / Pb]_{above-ground\ parts}}{[Cd / Hg / As / Pb]_{root}} \quad (4)$$

2.4. Measurement of physiological indices in leaves

The leaves of five genotypes (QHB, 107, 69, Pa, and QHQ) that had strong tolerance and bioaccumulation capacities for heavy metals were used to determine MDA, free proline, soluble sugar, and starch contents. The level of free proline was determined based on previously reported methods (Qiu et al., 2022). Fine powder (0.1 g FW) was extracted from the leaves by using 3 % sulphosalicylic acid for 15 min at 100 ℃. After centrifugation (10,000 rpm, 15 min), 1 mL of the supernatant was obtained and added with 1 mL of ninhydrin reagent and 1 mL of glacial acetic acid. The mixed liquid was incubated for 0.5 h at 98 ℃. Subsequently, absorbance was measured by spectrophotometry at 520 nm. A standard curve was obtained with gradient solutions of L-proline (Amresco Inc., OH).

The levels of soluble sugar and starch were determined using previously reported methods (He et al., 2013). For each sample, 0.1 g of fresh leaves was extracted using 80 % ethanol for 0.5 h at 80 °C, and the mixture was centrifuged (10,000 rpm, 15 min) to obtain the supernatant. The step was repeated three times. The supernatants were combined, the reagent anthrone (2 mL) was added, and the mixture was incubated at 100 ℃ for 7 min. After the mixture was cooled to room temperature, its absorbance was determined by spectrophotometry at 620 nm. The standard curve was obtained by gradient solutions of glucose. The pallet after the extraction of soluble sugar was further extracted by adding perchloric acid to measure the starch level.

For MDA measurement, 0.1 g of fresh leaves was extracted with 10 mL of 5 % trichloroacetic acid (pH 7.8) and 4 mL of 0.6 % thiobarbituric acid at 4 ℃ and incubated for 15 min at 98 ℃. Subsequently, the mixture was cooled immediately and centrifuged (10,000 rpm, 15 min). The absorbance of the obtained supernatant was determined by spectrophotometry at 450, 532, and 600 nm. The formula for calculating the MDA concentration is follows (Zhang et al., 2019b):

$$MDA(mmolg^{-1}FW) = 5 \times \frac{6.452(A_{532} - A_{600}) - 0.559A_{450}}{3.85M} \quad (5)$$

where *M* indicates the sample mass.

2.5. Quantitation of chemical components

The stems of the five genotypes (QHB, 107, 69, Pa, and QHQ) that had strong tolerance and bioaccumulation capacities for heavy metals were selected for the determination of lignin, cellulose, and hemicellulose contents. The contents of lignin, cellulose, and hemicellulose were determined using SinoBest bioassay kits (Shanghai). For the determination of lignin content, fine powder (0.1 g of dry weight or DW) was extracted from the stems by using 1 % acetic acid (10 mL). After centrifugation (10,000 rpm, 15 min), the pallet was soaked in a mixture of 70 % ethanol and 30 % ether for 3 min. The dried pallet was dissolved in 72 % sulfuric acid. Subsequently, the samples were acetylated in accordance with the instructions of the lignin content assay kit, and the absorbance of the mixture was determined by spectrophotometry at 280 nm (Liang et al., 2020). For the determination of cellulose content, fine powder (0.1 g DW) was extracted from the stems by adding a mixture of acetic acid and nitric acid for 25 min at 100 ℃. After centrifugation (10,000 rpm, 15 min), the pallet was dissolved in 10 % sulfuric acid. Subsequently, the samples were processed in accordance with the instructions of the cellulose content assay kit, and the absorbance of the mixture was determined by spectrophotometry at 620 nm. The standard curve was obtained by gradient solutions of glucose. For the determination of hemicellulose content, fine powder (0.2 g DW) was extracted from the stems by using 80 % calcium nitrate (10 mL) for 5 min at 100 ℃. After centrifugation (10,000 rpm, 15 min), the pallet was soaked in hydrochloric acid (2 M) for 45 min at 100 ℃. The mixture was centrifugated again, and the supernatant was obtained. Then, the supernatant was processed in accordance with the instructions of the hemicellulose content assay kit. The absorbance of the mixture was determined by spectrophotometry at 540 nm, and the standard curve was obtained by gradient solutions of D-Xylose.

2.6. Statistical analysis

Statistical analysis was performed with SAS software (SAS Institute, Cary, NC; 1996). The univariate procedure in SAS was used to analyze data normality. A multiple comparison was conducted to determine differences among genotypes by using Tukey's honestly significant difference method as a post hoc test after one-way ANOVA. The Pearson correlation coefficient was employed to analyze the linear correlations between the tissue concentrations of different heavy metals and the biomass of each organ by using Origin 2021. Differences were considered significant when $P \leq 0.05$.

3. Results

3.1. Growth traits of the 15 poplar varieties under the composite contamination of multiple heavy metals (Cd, Hg, As, and Pb)

Under the combined contamination of heavy metals, considerable genotypic differences in tree height, DBH, and biomass were observed in the 15 poplar varieties (Fig. 1, Table 2). In terms of tree height, QHQ (2.95 m) and QHB (2.75 m) were much higher than the other varieties, followed by 107, La, 69, and Ti. In terms of DBH, 107 (10.07 mm), QHQ (11.07 mm), and QHB (10.67 mm) were markedly higher than the other poplar varieties, followed by La, Ti, and Pa. In terms of whole biomass, QHQ (156.54 g), 69 (146.69 g) and Pa (143.15 g) were notable higher than the other varieties, followed by 107, QHB and Ti. In addition, the biomass of all organs (roots, stems and leaves) of poplar in QHQ was significantly higher than that of the other varieties. The CV for tree height and DBH was 0.27 and 0.53, respectively. The repeatability for tree height and DBH was 0.92 and 0.91, respectively (Fig. 1). For the biomass of the different organs, CV ranged from 0.22 to 0.34, and repeatability ranged from 0.93 to 0.97 (Table 2).

Table 2
Biomass of the fifteen poplar varieties growing in fields with multiple heavy metals (Cd, Hg, As, Pb).

Clone	Root (g)	Stem (g)	Leaf (g)	Whole plant (g)
QHB	31.54±0.67 ab	65.08 ±1.35 cd	40.64±1.88 bcde	137.26 ±2.46c
QHQ	33.01±0.31 a	74.83±0.51 a	48.71±1.36 a	156.54±0.87 a
107	29.22±1.34 e	65.02 ±1.88 cd	44.20±1.65 abcd	138.43 ±0.97c
69	30.42±0.60 b	70.04±1.28 b	46.24±0.98 ab	146.69±1.45 b
La	21.93±1.27 d	47.44±1.33 f	41.39±1.15 bcde	110.76 ±0.45 f
Pa	30.00±1.14 b	67.69±1.26 bc	45.46±2.58 abc	143.15±2.06 bc
Qg	21.93±1.09 d	46.38±2.47 f	39.19±3.54 de	107.50 ±3.18 f
Ti	26.22±1.06c	59.21±2.35 e	40.08±1.78 cde	125.51±3.85 d
QB1	14.12±0.26 e	34.03 ±0.78 g	32.07±1.46 g	80.22±0.87 g
QB2	10.27 ±0.18 f	29.64 ±0.86 h	23.48±0.94 h	63.40±0.61 h
QB3	11.52 ±0.44 f	31.93±0.67 gh	25.38±1.79 h	68.82±2.00 h
QH1	20.91±1.11 d	61.56±1.57 de	37.84±1.60 ef	120.32±2.44 de
SL4	20.69±0.95 d	61.74±0.74 de	38.74±1.97 de	121.17±2.00 de
T3	25.81±0.78c	59.40±1.18 e	32.75±2.58 fg	117.95±2.59 e
ZL46	21.20±0.76 d	58.56±1.05 e	28.18±2.51 gh	107.94 ±1.61 f
P-value	**	**	**	**
C.V.	0.30	0.25	0.21	0.23
Repeatability	0.97	0.97	0.93	0.96

Average value of biomass in root, stem, leaf and whole plant in fifteen poplar species under combined conditions of heavy metal (Cd, Hg, As, Pb) for 7 months. The numbers indicate means ± SE (n = 6). Different letters indicate significant differences in one-way ANOVA analysis ($P < 0.05$). The ANOVA results for species are indicated (*, $P < 0.05$; **, $P < 0.01$). C.V., the coefficient of variation among fifteen poplar varieties.

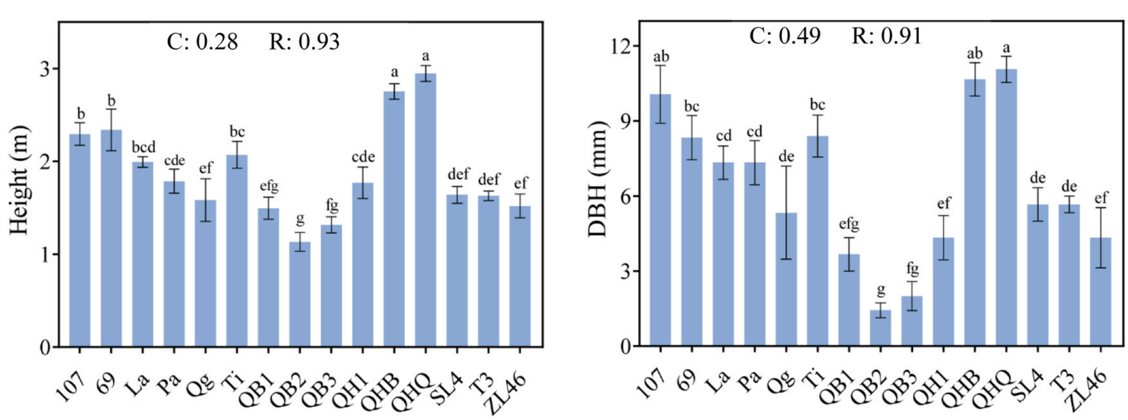

Fig. 1. Growth traits of the fifteen poplar varieties growing under composed pollution of multiple heavy metals (Cd, Hg, As, Pb). The bar indicates mean ± SE (n = 3). Different letters on the bars indicate significant differences in one-way ANOVA analysis ($P < 0.05$). C, coefficient of variation (C.V.) of the fifteen poplar varieties; R, clone repeatability of the fifteen poplar varieties. DBH, diameter at breast height.

3.2. Bioaccumulation of multiple heavy metals (Cd, Hg, As, and Pb) in the 15 poplar varieties

The bioaccumulation of Cd was more vigorous than that of Hg, As, and Pb (Figs. 2 and 3, Table 2). The Cd concentrations of the different organs exceeded the soil Cd concentration (1.81 mg·kg^{-1}), resulting in BCFs and TFs larger than 1, which met the criteria of hyper-accumulators. The BCF of Cd was the highest in variety 107 and reached about 10 for the aerial part. TF was higher in varieties 69, La, and ZL46 than in the other varieties (Fig. 4). The Cd concentration in the leaves was markedly higher than that in the roots and stems (Fig. 2). The most efficient varieties for Cd accumulation per plant were 107 and QHQ whose efficiency reached 1.9 and 1.7 mg, respectively, followed by QHB, Ti, 69, and Pa whose efficiency reached 1.3 mg (Fig. 3). The amount of Cd accumulation per hectare was 204 and 171 g·ha^{-1} in the poplar varieties 107 and QHQ, respectively, and reached 130 g·ha^{-1} in the varieties QHB, Ti, 69, and Pa (Fig. 5).

The concentrations of Hg, As, and Pb in the leaves of all the poplar species were lower than those in the soil (Fig. 2), resulting in a BCF lower than 1. The root Hg concentrations were higher in the varieties Pa, QB1, and QB2 than in the others. Hg and As accumulation in the aboveground parts was the highest in Pa, and Pb accumulation was the highest (exceeded 220 μg) in Pa and QHQ (Fig. 3). With regard to the total amount of heavy metal bioaccumulation per unit area, Cd accumulation was the highest in 107 (204 g·ha^{-1}), followed by QHQ (171 g·ha^{-1}). Hg accumulation was the highest in Pa, QHQ, and QHB, and all values exceeded 0.3 g·ha^{-1}. As accumulation was the highest in Ti and reached 13 g·ha^{-1}. Pb accumulation in Pa, QHQ, and QHB exceeded 30 g·ha^{-1} (Fig. 5). For Hg, the aerial-part BCFs were high in Pa, QB1, and QB3, and the Hg TF was high in Ti. The BCF of As for the aerial part was the highest in QB2 (Fig. 4).

3.3. Correlation analysis of heavy metal concentration and biomass in each organ

The Cd concentration in the roots was positively correlated with the As, Pb, and Hg concentrations. The Pb concentration in the roots was positively correlated with the root concentration of As. The tissue concentrations of Cd, As, and Pb in the roots were negatively correlated with root biomass (Fig. 6). In the stems, no significant correlations were found among the different heavy metals. The As concentration in the leaves was positively correlated with the Pb and Hg concentrations. The tissue concentrations of Cd, As, and Hg in the leaves were negatively correlated with leaf biomass.

3.4. Physiological indicators in the leaves of the five representative poplar varieties

Under the combined contamination of the four heavy metals, QHB and 107 had higher levels of free proline, soluble sugar, and MDA compared with the other varieties (Fig. 7). Variety 69 had a higher level of soluble sugar and starch and lower MDA and free proline contents compared with the other species. Pa had a higher level of free proline (112.8 μg·g^{-1} FW) than varieties 69 and QHQ but had the lowest starch content among all the varieties. Starch content was higher in QHQ than that in QHB and Pa (Fig. 7). With regard to the physiological indices of the five poplar varieties, CV ranged from 0.1 to 0.2, and the repeatability values exceeded 0.9.

3.5. Contents of lignin, cellulose, and hemicellulose in the stems of the five representative poplar varieties

Substantial differences were found in the contents of lignin, cellulose, and hemicellulose among the poplar varieties (Fig. 8). Under the combined contamination of the multiple heavy metals (Cd, Hg, As, and Pb), the cellulose contents in QHB and 107 (276.2 and 264.87 mg·g^{-1}, respectively) were higher than those in the other poplar varieties. Among all the varieties, Pa had the lowest cellulose content (196.13 mg·g^{-1}). Variety 107 had the highest hemicellulose content (223.03 mg·g^{-1}), followed by QHB and Pa. The lignin contents of 69 and Pa were higher than those of the other poplar varieties and reached 228.33 and 215.30 mg·g^{-1}, respectively. For the contents of lignin, cellulose, and hemicellulose, CV ranged from 0.1 to 0.13, and the clone repeatability value was 0.95. These results indicate that the selection of

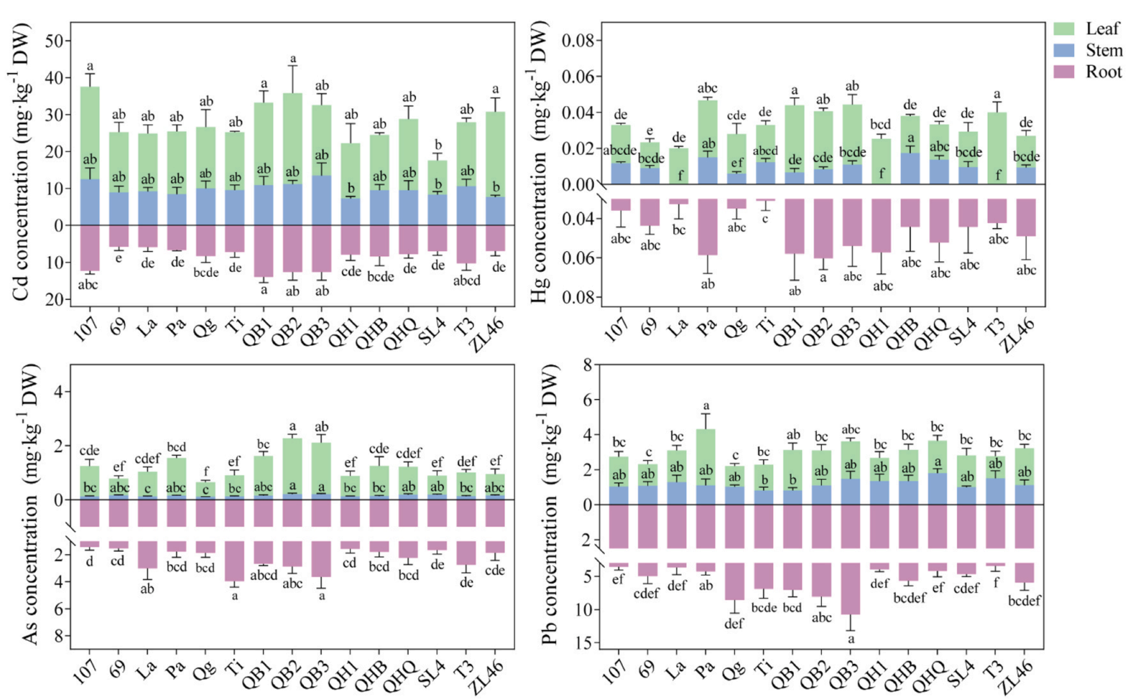

Fig. 2. The concentration of Cd, Hg, As, Pb in different organs (root, stem, leaf) of the fifteen poplar varieties growing under composed pollution of multiple heavy metals (Cd, Hg, As, Pb). The bar indicates mean ± SE (n = 3). Different letters on the bars indicate significant differences in one-way ANOVA analysis ($P < 0.05$).

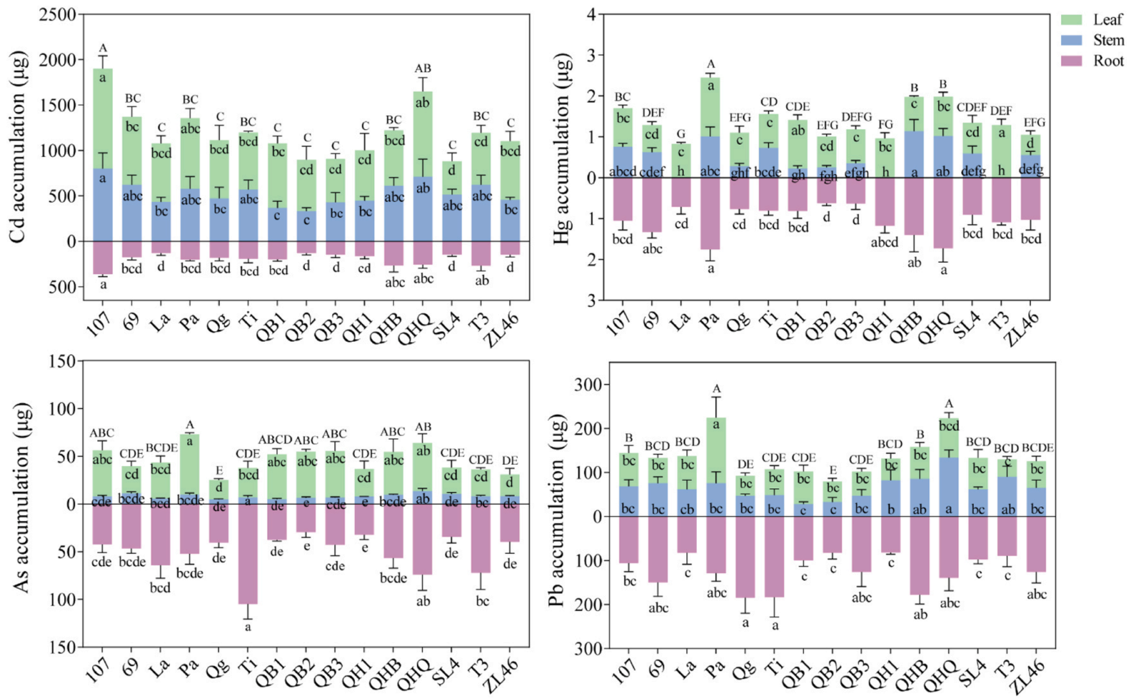

Fig. 3. The accumulation of Cd, Hg, As and Pb in different organs (root, stem, leaf) of the fifteen poplar varieties growing under composed pollution of multiple heavy metals (Cd, Hg, As, Pb). The bar indicates mean ± SE (n = 3). Different letters on the bars indicate significant differences in one-way ANOVA analysis ($P < 0.05$). Lowercase letters represent the differences among the organs, and uppercase letters represent the differences of the aerial parts.

desirable poplar varieties with high cellulose contents and low lignin contents under composite heavy metal conditions is feasible and has high genetic potential.

4. Discussion

4.1. Cd bioconcentration and phytoremediation efficiency of the 15 hybrid poplar varieties

The typical Cd hyperaccumulators have the following criteria: (1) the concentration of Cd in the aboveground part of the plant reaches or exceeds 100 mg·kg^{-1}, (2) BCF (ratio of plant Cd bioconcentration to soil Cd concentration) is greater than 1.0, (3) Cd TF (ratio of Cd concentration in the aboveground part to that in the root) is greater than 1, and (4) strong Cd tolerance so that plants can withstand high concentrations of Cd stress and maintain normal growth. However, these criteria can vary depending on the types and extent of soil contamination (He et al., 2015; Ma et al., 2016; Dou et al., 2019; Shen et al., 2022). In this study, the Cd concentration in the roots and aboveground parts of the different clones of poplar ranged from 5.8 mg·kg^{-1} DW to 20.5 mg·kg^{-1} DW, which was much higher than the Cd concentration in the contaminated soil (1.81 mg·kg^{-1}). Moreover, BCF was 5–11, and TF exceeded 1. These results show that poplar has a capacity for Cd bioconcentration and can thus be used as a candidate Cd hyperaccumulator under heavy metal pollution. However, the concentration of Cd in this experiment (40 mg·kg^{-1}) did not reach the threshold (100 mg·kg^{-1}) for typical hyperaccumulators possibly because the Cd concentration in the soil of this study was low (1.81 mg·kg^{-1}).

Previous studies have compared the phytoremediation efficiencies of different tree species and found that poplar has a more prominent Cd accumulation capacity than other woody species, such as elm, pine, artemisia, and willow (Evangelou et al., 2012; Van Nevel et al., 2013; Pilipović et al., 2019). Some poplar species have stronger Cd accumulation ability and Cd tolerance than other species (He et al., 2013; Jakovljević et al., 2014). However, most previous studies focused on a single type of heavy metal pollution, and only a few studies have been conducted on genotype variations in phytoremediation efficiency in soil contaminated by compound heavy metals. Under the composite pollution (Cd, Hg, As, and Pb) in the present study, the CV and clone repeatability of poplar were large for various traits, including Cd concentration in different organs and Cd accumulation per plant. Further analysis showed that Cd accumulation per plant was related not only to the concentration of Cd enrichment but also to biomass; the two factors jointly determined the capacity for Cd bioaccumulation. The Cd accumulation advantage of variety 107 was mainly due to its high Cd concentration, that is, the Cd concentrations in the leaves and stems exceeded 35 and 10 mg kg^{-1} DW, respectively. By contrast, the Cd accumulation advantage of variety QHQ was mainly due to its superior biomass; QHQ had the highest biomass accumulation among all the tested varieties. Although the Cd concentration of QB1, QB3, and QB2 was high, the biomass accumulation per plant of these varieties was low, leading to low Cd accumulation in the whole plant. The accumulation of Cd in SL4 was also low mainly because of the low Cd concentration of this variety.

4.2. Influence of the genetic background of poplar hybrids on their phytoremediation efficiency

The genus *Populus* is usually classified into five sections, namely, *Aigeiros*, *Leucoides*, *Leuce*, *Tacamahaca*, and *Turanga* (Cervera et al., 2005; Zhang et al., 2019a). In this study, the 15 poplar hybrid varieties had wide genetic backgrounds, which included inter-sectional hybrids between sections *Aigeiros* and *Tacamahaca* (QHQ and QHB), inter-specific hybrids within section *Aigeiros* (107, La, Ti), inter-specific hybrids within section *Leuce* (QB1, QB2, and QB3), and intra-specific hybrids within section *Aigeiros* (69, QH1, SL4, T3, ZL46, Qg, and Pa; Table 1). The results showed that the hybrid vigor for juvenile stem volume was stronger for the inter-specific crosses between *P. tremuloides* and *P. tremula* than that for the intra-specific crosses (Li et al., 1998). Most of the inter-specific and inter-sectional hybrid varieties, such as 107, QHQ, QHB, and Ti, showed large biomasses and high capacities for Cd accumulation. Variety 107 was an inter-specific hybrid variety

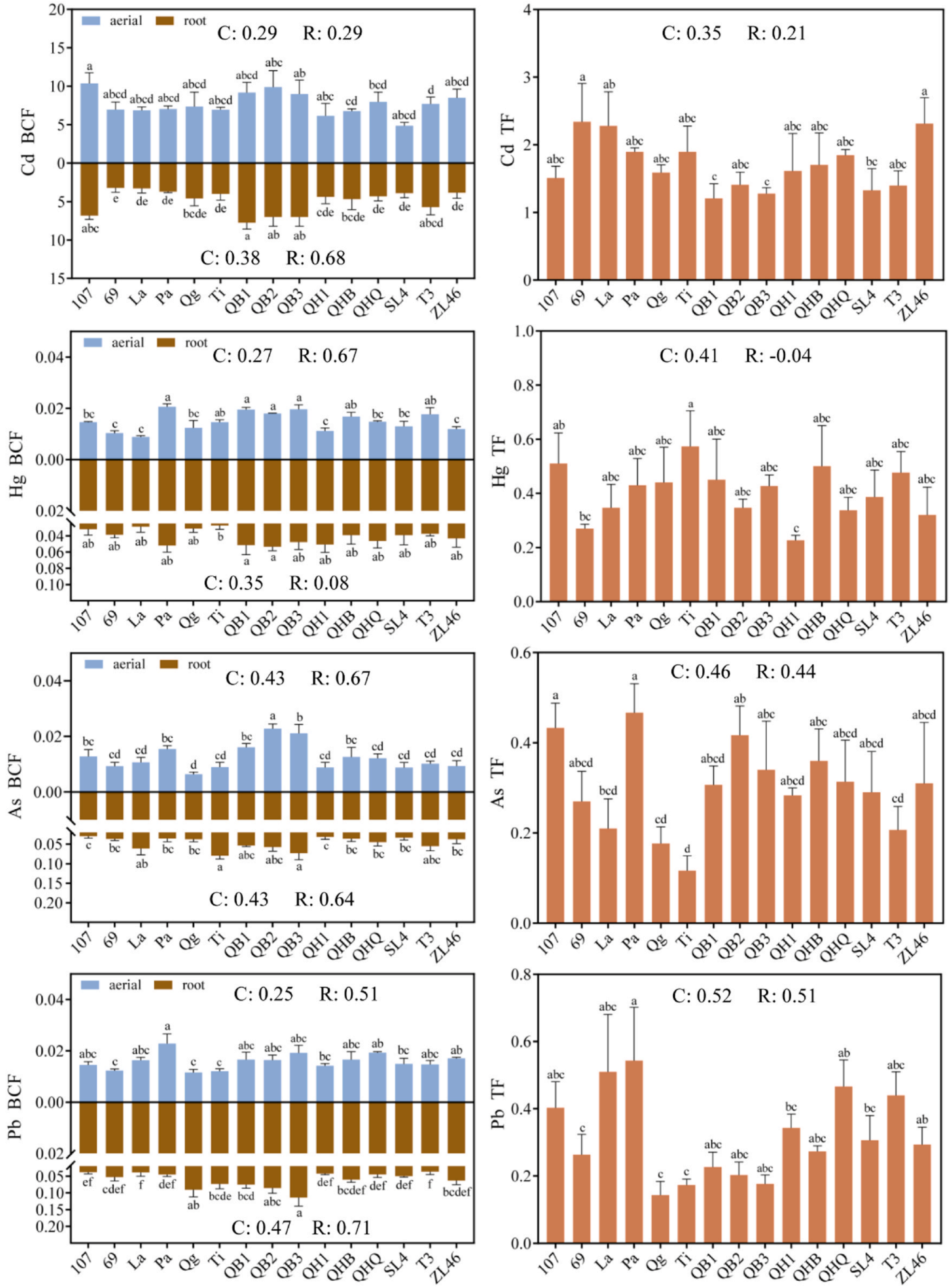

Fig. 4. The bioconcentration factor (BCF) of aerial part and root and the translocation factor (TF) of the fifteen poplar varieties. The bar indicates mean ± SE (n = 3). Different letters on the bars indicate significant differences in one-way ANOVA analysis ($P < 0.05$). C, coefficient of variation (C.V.) of the fifteen poplar varieties; R, clone repeatability of the fifteen poplar varieties.

between *Populus deltoides* M. and *Populus nigra* L., both of which belong to section *Aigeiros*. QHQ and QHB were hybrid varieties between sections *Aigeiros* and *Tacamahaca*. By comparison, most of the intra-specific hybrid varieties (69, QH1, SL4, T3, ZL46, Qg, and Pa) had low Cd concentrations and small biomasses, resulting in minimal Cd accumulation per plant and inefficient remediation of Cd-polluted soil.

Positive correlations between heterosis and genetic distance in inbreeding parental lines have been widely observed in many plant species (Boeven et al., 2020; Legarra et al., 2023; Würschum et al., 2023). By using a novel quantitative genetic model based on an intra- and inter-specific factorial mating design between *P. tremuloides* and *P. tremula*, a study found that heterosis is the result of the

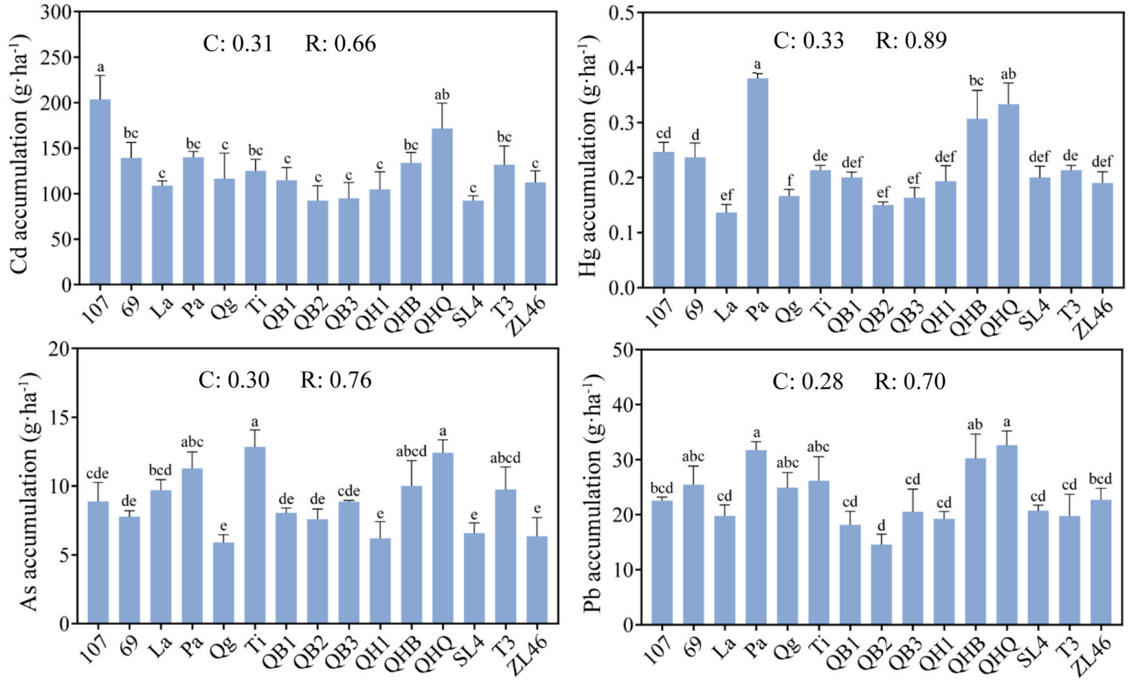

Fig. 5. The total bio-accumulation of Cd, Hg, As and Pb per unit area in fifteen varieties. The bar indicates mean ± SE (n = 3). Different letters on the bars indicate significant differences in one-way ANOVA analysis ($P < 0.05$). C, coefficient of variation (C.V.) among fifteen poplar varieties; R, clone repeatability of the fifteen poplar varieties.

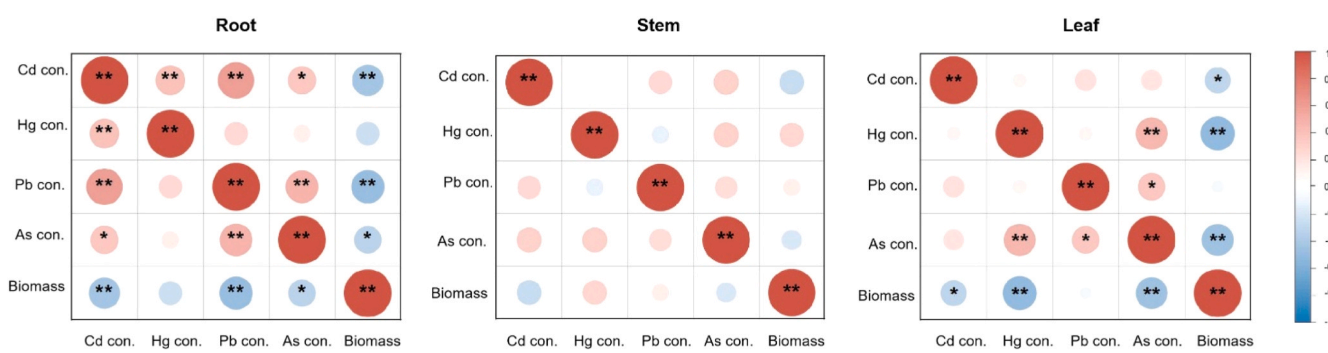

Fig. 6. Correlation co-efficient between the concentration of Cd, Hg, As, Pb and the biomass of different organs (root, stem, leaf). Cd con., Cd concentration; Hg con., Hg concentration; Pb con., Pb concentration; As con., As concentration. *, $P < 0.05$; ** $P < 0.01$.

overdominance of the interaction of two alleles, each from a parent at a homological locus (Li et al., 1998). In the present study, the genetic background of the poplar hybrid varieties considerably affected their capacity for Cd bioaccumulation and phytoremediation efficiency. The large capacities for Cd bioaccumulation and the phytoremediation potential of inter-specific and inter-sectional hybrid varieties are presumably due to the active overdominance of the interaction of two parental alleles from different species or sections (Li et al., 1998; Zhang et al., 2019a). On the basis of these results, inter-specific and inter-sectional hybridizations are recommended for the breeding of elite hybrid varieties with a favorable capacity for Cd bioaccumulation and phytoremediation.

Under the composite contamination of heavy metals in the present study, the BCF and TF of Hg, As, and Pb were less than 1, indicating that the capacity for the bioaccumulation of these heavy metals was low. Previous research demonstrated that certain species of *Brassica juncea*, *Zea mays*, and *Ambrosia artemisiifilia* are good accumulators of Pb (Muthusaravanan et al., 2018). The accumulation of As in the stems of *Pteris cretica* can exceed 400 mg As kg^{-1} DW, which is much higher than the values in our study (Shen et al., 2022). Large CVs and clonal repeatability values were obtained for the concentration and whole-plant accumulation of Hg, As, and Pb, suggesting that bioaccumulation of Hg, As, and Pb in poplar can be increased via genetic improvement (Table 3). The inter-specific and inter-sectional hybrid varieties were dominant not only in Cd accumulation but also in Hg, As, and Pb bioaccumulation, which may benefit the phytoremediation of soil contaminated by multiple heavy metals (Cd, Hg, As, and Pb). Moreover, the root Cd concentration was positively correlated with the root As, Pb, and Hg concentrations, indicating that poplar absorbs different heavy metals in a synergetic manner. Therefore, elite poplar varieties with an improved capacity for accumulating heavy metals should be bred, and this can be achieved through the hybridization of different poplar sections, such as *Aigeiros* and *Tacamahaca*, and various poplar species.

4.3. Stress tolerance and wood composition of poplar varieties under composite heavy metal stresses

The bioaccumulation capacity and remediation efficiency of plants for heavy metals are determined by two factors: tissue concentration of

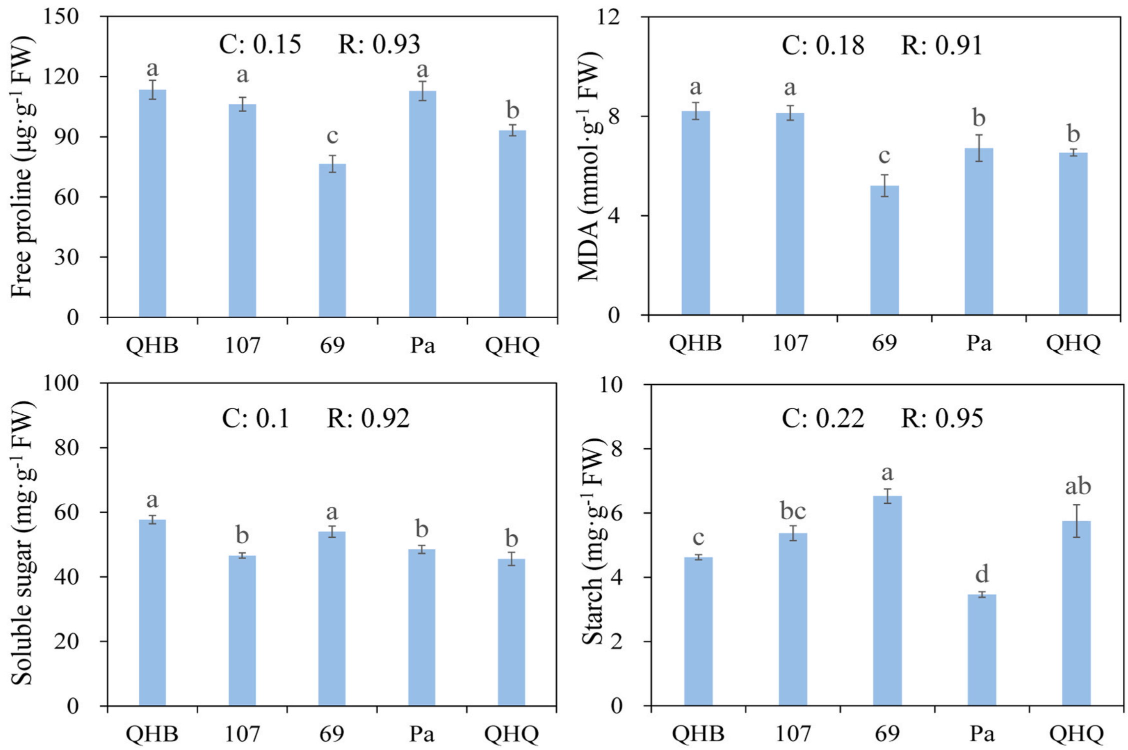

Fig. 7. Physiological parameters (free proline, soluble sugar, starch and MDA) in leaves of the representative five poplar varieties growing under composed pollution of multiple heavy metals (Cd, Hg, As, Pb). The bar indicates mean ± SE (n = 3). Different letters on the bars indicate significant differences in one-way ANOVA analysis ($P < 0.05$). C, coefficient of variation (C.V.) among five poplar varieties; R, clone repeatability of the five poplar varieties. MDA, malonaldehyde.

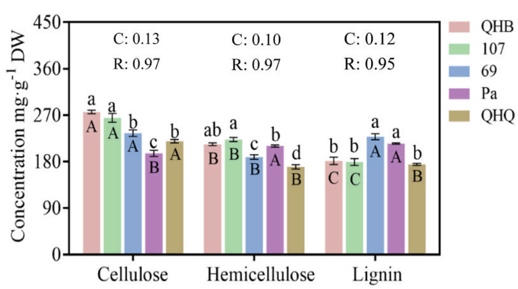

Fig. 8. The content of lignin, cellulose and hemicellulose in stem of the representative five poplar varieties growing under composed pollution of multiple heavy metals (Cd, Hg, As, Pb). The bar indicates mean ± SE (n = 3). Different letters on the bars indicate significant differences in one-way ANOVA analysis ($P < 0.05$). Lowercase letters indicate differences between varieties of the same substance, and uppercase letters indicate differences between the contents of different substances in the same variety. C, coefficient of variation (C.V.) among five poplar varieties; R, clone repeatability of the five poplar varieties.

heavy metals and plant biomass (Chen, 2013; Du and Groover, 2010; Etim, 2012; Liang et al., 2020; Mun et al., 2008; Namdjoyan et al., 2011; Rostami and Azhdarpoor, 2019; Schnable and Springer, 2013; Silvio et al., 2021; Tow et al., 2019; Zhang et al., 2020). Plants with high tolerance are minimally influenced by heavy metals and thus show high growth rates and large biomasses under heavy metal stress (Gao and Zhu 2004; Zine et al., 2020; Bakshe and Jugade 2023). In the present study, some varieties had a large biomass and a high tissue concentration of heavy metals. Specifically, the hybrid varieties between sections *Aigeiros* and *Tacamahaca* (QHQ and QHB) and the inter-specific hybrids within section *Aigeiros* (107, La, and Ti) exhibited superior growth performance and higher biomass compared with the intra-specific hybrid varieties, indicating that these poplar varieties are highly tolerant to composite heavy metal pollution. The Cd concentration of these varieties ranked high or medium–high. By comparison, the intra-specific hybrids, such as Qg, T3, and ZL46, had small biomasses presumably because they had low tolerance for composite heavy metal pollution. Notably, the inter-specific hybrids (QB1, QB3, and QB2) within the section *Leuce* had high bioconcentrations of heavy metals (Cd, Hg, As, and Pb), but their biomasses were generally low, which resulted in weak abilities to accumulate heavy metals at the whole-plant level. This result can be explained as follows: poplars from the section *Leuce* are mainly distributed in high-latitude areas with a cool climate (Zhang et al., 2019a). Hence, the growth performance of these varieties is unfavorable for the current field experiment, which involved a low-latitude area with a hot and humid climate.

Heavy metal exposure can induce the synthesis of various ROS, which trigger membrane lipid peroxidation and protein inactivation, leading to abnormal plant growth (Namdjoyan et al., 2011; Zhang et al., 2020). MDA is the final product of membrane lipid peroxidation, and it can reflect the degree of membrane lipid peroxidation (Qureshi et al., 2024; Tauqeer et al., 2016; Zulfiqar et al., 2024). Osmoregulatory substances, such as free proline, soluble sugars, and starch, can maintain the balance of water potential in plant cells, protect the plasma membrane and the activity of metabolic enzymes, and participate in detoxification (Xu et al., 2013; Guo et al., 2019). In the present study, QHB and 107 produced higher levels of free proline and soluble sugar, and QHQ accumulated a higher starch content than the intra-specific varieties did, thus mitigating the oxidative damage of cells and enhancing the tolerance of poplar to composite heavy metal stress. This result partially explains why the hybrids between sections *Aigeiros* and *Tacamahaca* (QHQ and QHB) and the inter-specific hybrids (e.g., 107) were more resistant to composite heavy metal stress than the intra-specific varieties were. Meanwhile, it was concluded that 107 was the most resistant one to heavy metals among the experimental poplar varieties.

Wood cellulose and lignin contents are the main breeding target characteristics for poplar pulp wood (Gómez-Monedero et al., 2017).

Table 3
Coefficient of variation and clone repeatability of Cd, Hg, As and Pb concentration.

		Concentration		Accumulation per-plant	
		C.V.	Repeatability	C.V.	Repeatability
	root	0.38	0.68	0.41	
Cd					0.70
	stem	0.32	−0.28	0.37	0.32
	leaf	0.36	−0.31	0.36	0.59
	root	0.35	0.12	0.44	
Hg					0.66
	stem	0.69	0.85	0.82	0.88
	leaf	0.36	0.81	0.31	0.80
	root	0.44	0.64	0.48	
As					0.72
	stem	0.30	0.53	0.37	0.72
	leaf	0.48	0.82	0.42	0.66
	root	0.47	0.69	0.41	
Pb					0.52
	stem	0.42	−0.28	0.52	0.58
	leaf	0.40	0.51	0.21	0.60

Data indicates the coefficient of variation and clone repeatability of Cd, Hg, As and Pb concentration and accumulation in poplar ($n = 3$). C.V., the coefficient of variation among fifteen poplar varieties.

Cellulose content plays a decisive role in the pulping rate, and a high lignin content leads to a low pulping rate and a high production cost (Zhu et al., 2010; Maisterra et al., 2024). The causal relationship between enhanced lignin content and decreased Cd accumulation has been detected in plants (Dong et al., 2023). In the present study, the hybrids between sections *Aigeiros* and *Tacamahaca* (QHQ and QHB) and the inter-specific hybrid within section *Aigeiros* (107) had favorable phytoremediation efficiency and biomass, high cellulose contents, and low lignin contents, which are good pulpwood traits. The application of these poplar varieties in fields with composite heavy metal pollution can benefit not only phytoremediation efficiency but also pulpwood production efficiency. High CV and clone repeatability values for biomass and cellulose content were detected in these poplar varieties, indicating the feasibility of breeding new elite poplar varieties with high yield, good wood properties, and high remediation efficiency via the comprehensive selection of multiple traits.

5. Conclusion

Cd accumulation per plant was related not only to the concentration of Cd in the different poplar varieties, but also to biomass; the two factors jointly determined the capacity for Cd bioaccumulation. The Cd accumulation advantage of variety 107 was mainly due to its high Cd concentration, and the Cd accumulation advantage of the variety QHQ was mainly due to its biomass. In general, the hybrids between different poplar sections and the inter-species hybrids showed better growth performance and higher bioconcentrations of heavy metals compared with the intra-specific hybrids. Specifically, the hybrids between sections *Aigeiros* and *Tacamahaca* (QHQ and QHB) and the inter-specific hybrids within the section *Aigeiros* (107, La, and Ti) exhibited ideal growth performance; indicating that these poplars are highly tolerant to composite heavy metal pollution. Poplars absorb different heavy metals in a synergetic manner, thus desirable poplar varieties with a large capacity for the phytoremediation of composite pollution by multiple heavy metals can be bred feasibly. The hybrids between poplar sections (QHQ and QHB) and the inter-specific hybrid (107) had superior remediation efficiency for composite heavy metal pollution and favorable wood properties with high cellulose contents and low lignin contents, which are favorable for the pulpwood industry. Interestingly, soil pollution can be controlled without compromising economic value by breeding inter-specific and inter-sectional poplar varieties with good wood properties and high phytoremediation efficiency.

CRediT authorship contribution statement

Chaobo Hu: Writing – review & editing, Data curation. **Zhiyong Wang:** Investigation. **Yi Zhang:** Writing – review & editing. **Qimeng Heng:** Investigation, Data curation. **Xuelian He:** Investigation. **Jean Wan Hong Yong:** Writing – review & editing. **Yawei Jiang:** Investigation. **Xintong Wang:** Investigation. **Junfeng Fan:** Writing – review & editing. **Mengge Li:** Writing – original draft, Investigation, Data curation. **Turki M. Dawoud:** Investigation. **Siddiq Ur Rahman:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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