

## Comparison of Cadmium Accumulation in Upright and Leaning Poplar Trees

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Poplar 69 (*Populus deltoides* Bartr. cv. 'Lux' (I-69/55)) was chosen for a pot experiment to study the growth and the extraction of Cd from soil to various parts of the one-year-old trees growing in upright and artificial leaning (45°) patterns under different Cd stress. The results indicated considerable tolerance of both upright poplar (UP) and leaning poplar (LP) to Cd stress in the soil, though with significant inhibition from Cd application. LP demonstrated significantly lower height and basal diameter growth than UP. Cd concentrations in different parts of both UP and LP increased with the increase of Cd in the soil and it followed a general order as Bark > Leaf > Root > Stem. Different parts of poplar had average bio-concentration factor (BCF) ranges between 0.08 to 2.36, and average translocation factor (TF) between 0.67 to 7.92, indicating a big difference of phytoextraction ability among the parts, among the treatments, and between LP and UP. Average Cd concentration, BCF, and TF for each part of LP were higher than that of UP, but the difference was not significant. Significantly higher Cd concentration was found in the tension zone of stem wood than that in the opposite zone for LP.

*Keywords:* Cd; Phytoextraction; Poplar 69; Upright growth; Leaning growth; Dendroremediation

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### INTRODUCTION

With rapid industrialization, heavy metal pollution has become a serious threat to the environment in many countries (González-Oreja *et al.* 2008; Zhang *et al.* 2018). The heavy metal cadmium enters the environment through mining and smelting of metalliferous ores, industrial processes, heating systems, urban transportation, and phosphate fertilizer, and is considered one of the most prevalent toxic pollutants to plants, animals, and human beings (McLaughlin *et al.* 1999; Han *et al.* 2002; Escarré *et al.* 2011; Mahar *et al.* 2016). Unlike organic matter, heavy metals are inherently non-biodegradable, and thus they can only be removed from the environment through physical, chemical, or biological methods or be limited from transport by immobilization using chemical or microbiological methods (Ali *et al.* 2013).

Phytoremediation, the use of plants to remove pollutants from the environment or make them stabilized or less toxic, has increasingly been examined as a practical, cost-effective, and eco-friendly technology (Pulford and Watson 2003; Li *et al.* 2012). Dendroremediation, the use of trees in phytoremediation, is of great potential for the phytoextraction and phytostabilization of heavy metal polluted soil, because of their specific characteristics such as fast rate of growth, the ability to grow on nutrient-poor soil, and deep root system (González-Oreja *et al.* 2008; Zeng *et al.* 2019).

Poplar, one of the most cultivated fast-growing species in the world, is easy to breed and has strong adaptability. Though inferior to some herbaceous plants with hyperaccumulation ability, such as *Thlaspi caerulescens* and *Arabidopsis halleri*, poplar demonstrates relatively good phytoextraction ability of heavy metals (Zhuang *et al.* 2007;

Fuksová *et al.* 2009; Antony *et al.* 2012; Pilipović *et al.* 2019; Kataweteetham *et al.* 2020). With the deep root system and a great amount of leaf mass, the poplar tree has strong transpiration and is an excellent drawer of water and elements from the whole profile of the soil. The wood and bark of poplar are supposed to be important sinks for heavy metals, where heavy metals are accumulated and immobilized with additional tissue being formed each growing season (Pulford and Watson 2003). From the perspective of wood utilization, heavy metal enriched wood also contributes to the improvement of some wood properties. Moreover, poplar's complete genome facilitates the later regulation of gene expression to improve the formation of poplar heavy metal-enriched wood and the ability of wood to accumulate heavy metals (Parrotta *et al.* 2015; Romè *et al.* 2015). Therefore, planting poplar is considered as a green and efficient heavy metal pollution control technology with the most potential and application prospects in the future (Azzarello *et al.* 2011; Thakur *et al.* 2016).

Tension wood is an abnormal wood tissue of a trunk or branch of a broad-leaved tree that is formed *via* inclining plantation and whose anatomical and physicommechanical properties are formed in an attempt to restore it to its original position while being bended under external forces (Fisher and Stevenson 1981; Archer 1987). The structure of tension wood of poplar is quite different from its normal wood. Tension wood has one more layer in the secondary cell wall layer, which is a gelatinous layer with high content of cellulose, regularly arranged with the angle close to zero, leaving plenty of micro spaces (10 to 30 nm) among the microfibril bundles (Fang *et al.* 2008; Liu and Liu 2011; Chang *et al.* 2015). These micropores could be the proper sites for the absorption and accumulation of heavy metal ions. Therefore, in the transpiration process, inorganic salts would be easily transported through the vessels of tension wood from roots to tree crowns and the gelatinous layer is supposed to help the enrichment of heavy metals in wood tissue. With the special structural characters of tension wood, it should be interesting to study the dendroremediation of leaning growing trees. However, no such study has ever been conducted.

In this study, the authors compared the one-year-old upright growing and leaning growing poplars through pot experiments in their growth, Cd accumulation in different parts of trees, and their extraction ability.

## EXPERIMENTAL

### Materials

The experiment was conducted in the experimental yard of Anhui Agricultural University, Hefei, China (117°14'46"E, 31°51'52"N). The soil used in this experiment was a mixture of local topsoil and organic nutrient soil, with the mixing ratio being 1:1. The soil pH of the mixture was 6.91, and the contents of organic matter, available N, available P, available K, and available S, were 73.10 g/kg, 1.82 g/kg, 133.8 mg/kg, 346 mg/kg, and 80.9 mg/kg, respectively. The soil nutrients were well-balanced and can meet the requirement of poplar growth. The background contents of heavy metals Cd, Cu, and Zn were 0.15, 22.68, and 37.43 mg/kg, respectively, much lower than the risk screening standards for agricultural land as per GB 15618 (2018) and were similar as the background values of the elements in this region (CNEMC and SEPA 1990; GB 15618 2018).

### *Plantation and Cd treatments*

Branch cuttings from the new branches of naturally growing poplar 69 trees (*Populus deltoides* Bartr. cv. 'Lux' (I-69/55)) in an experimental forest near the university campus were used in the experiment. This clone was originally introduced from Italy to China about 40

years ago. Thirty cuttings with 15 cm in length and in good and uniform growth conditions were planted in 30 pots in March 2018. The size of each pot was 33 cm in diameter and 38 cm in height. After 2 months of natural growth, Cd(NO<sub>3</sub>)<sub>2</sub> solution was applied to each pot according to the pre-designed concentrations of Cd. A total of four treatments were included, which were 5, 20, 50, and 100 mg/kg, namely C1, C2, C3, and C4, respectively, plus the control group C0, where no extra Cd was added. For each treatment, the authors also set two growth patterns, *i.e.*, normal upright growth and artificial leaning (45°) growth. Three repetitions were set for every Cd treatment and growth pattern. In the beginning, the pots were placed in a greenhouse where the sunshine and ventilation were both not good. Therefore, the saplings grew slowly during the period from March to June. At the beginning of July, the pots were moved to a well-lit and well-ventilated place with a transparent cover to avoid the influence of a rainstorm. The pots of artificial leaning groups were then set inclined by 45° angle and each sapling was fixed by a bamboo pole. The pots were placed in a random distribution to avoid the influence of the position. The experimental location was far from the industrial district and traffic highway, where the influence of air pollution was negligible.

#### *Measurement and management*

Monthly measurements of poplar tree growth in terms of height and basal diameter were taken from August to November at the end of each month. The height of the tree was measured from the part near the soil surface in the pot to the top of the tree. The circumference was measured on the trunk at the height 5 cm from the soil surface in the pot with a measuring tape, whereby the basal diameter was calculated. The poplars were watered depending on their needs and evenly for each pot, about every other day during the summertime, and to avoid over-watering and Cd leaching.

#### *Sampling, pretreatment, and analysis*

The harvest of the 30 pots of both upright poplar (UP) and leaning poplar (LP) under all five treatments took place at the beginning of December. However, leaf samples were collected before the leaves began to fall in November. Then, in the harvest, the whole tree was pulled out to sample the root, stem, and bark. No branches grew out for the one-year-old poplar trees. The trunk was sampled at three heights, *i.e.*, the basal, middle, and top parts along the trunk, for stem wood and bark. For the trunk of LP, also at the three heights, stem and bark samples were taken in the two zones, *i.e.*, the tension zone (TZ), which is located at the upper part of the trunk of LP, and the opposite zone (OZ), which is located at the lower part of the trunk of LP.

Root samples were first rinsed with tap water to get rid of the attached soil, and then all tree samples were rinsed three times with deionized water. All rinsed tree samples of root, stem, bark, and leaf were oven-dried at 60 °C for approximately 24 h to constant weight. The temperature was set to this value to prevent Cd volatilizing at higher temperatures. The dried samples were ground to obtain a homogeneous powder with a metal-free mill and stored in sealed plastic bags until analysis.

Metal concentrations of Cd in plant samples were determined by graphite furnace atomic absorption spectrophotometer (TAS990 GF, Beijing PERSEE General Instrument Co. Ltd, Beijing, China).

#### *Calculation and data processing*

Bio-concentration factor (BCF) and translocation factor (TF) are the factors to determine the translocation ability of plants to accumulate heavy metals or metalloids (Wu *et al.* 2010). The BCF was calculated as the ratio of the heavy metal/metalloid concentration in plant tissues to that in the soil, *i.e.*, Eq. 1,

$$\text{BCF} = C_{\text{plant tissue}} / C_{\text{soil}} \quad (1)$$

where  $C_{\text{plant tissue}}$  is the concentration of an element in plant tissue (mg/kg) and  $C_{\text{soil}}$  is the concentration of the element in the soil (mg/kg).

The TF was calculated as the ratio of an element concentration in the plant's aerial part to that in the plant root, as Eq. 2,

$$\text{TF} = C_{\text{aerial part}} / C_{\text{root}} \quad (2)$$

where  $C_{\text{aerial part}}$  is the concentration of an element in plant's aerial part (mg/kg) and  $C_{\text{root}}$  is the concentration of the element in plant's root (mg/kg).

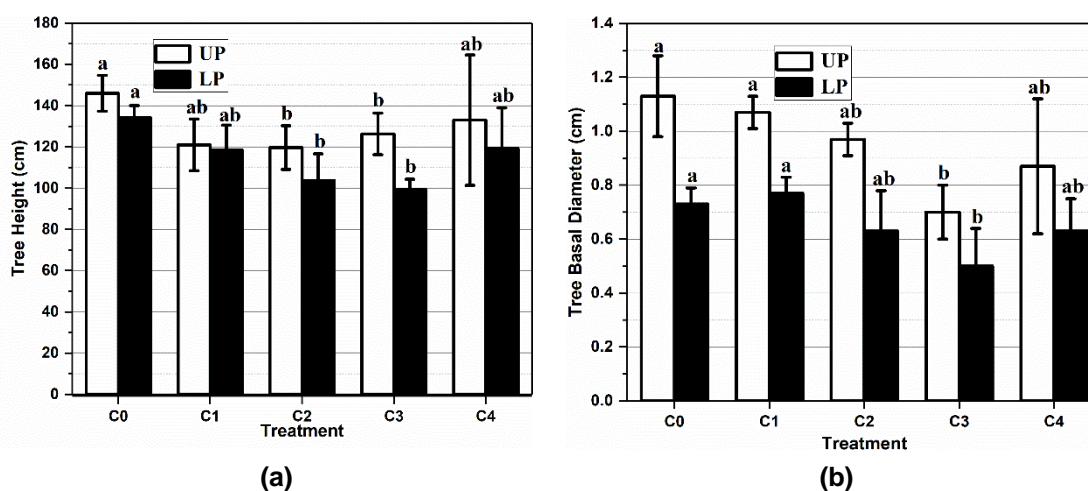
All data calculations and tables were performed in Excel 2013 (Microsoft, Redmond, Washington, USA), and figures were drawn using Origin Pro2017 (Originlab, 2017, Northampton, Massachusetts, USA). Multi-way analysis of variance (ANOVA) and the least significant difference test was applied to identify significant differences between the means in SPSS 19.0 (IBM, Armonk, New York, USA).

## RESULTS AND DISCUSSION

### Poplar Growth

No visual phytotoxicity symptoms, such as necrotic spots on leaves, were observed on the poplar trees during the whole growing period and at harvest. Tree heights and basal diameters of both UP and LP at all treatments increased with time, indicating that poplar trees in both upright and leaning growth patterns can grow under Cd stress.

The height and basal diameter of poplar trees were significantly influenced by Cd addition, with that in the control group (C0) being higher than other treatments at harvest (in December) for both UP and LP (Fig. 1 (a) and (b)), with the exception of the basal diameter of LP at C1. The result indicated the inhibition of tree growth by Cd stress in the soil, which is consistent with the findings described by Wang and Jia (2010). The heights of UP at harvest at C1, C2, C3, and C4 were decreased 15.53%, 16.35%, 12.22%, and 8.07% compared to that at C0, respectively, while that of LP decreased 10.71%, 20.53%, 23.44%, and 10.04%, respectively.



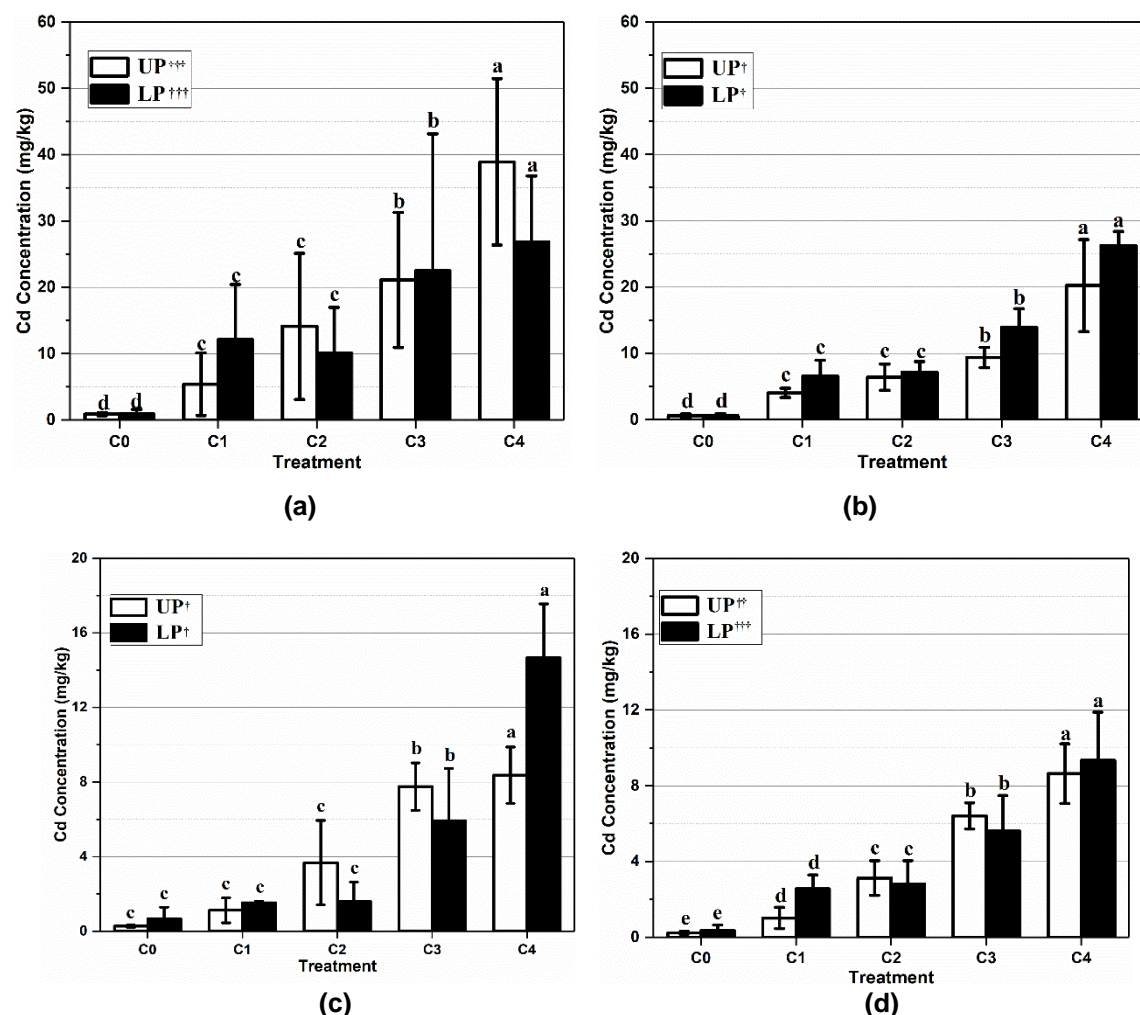
**Fig. 1.** Tree heights and basal diameter of UP and LP at harvest under different concentrations of Cd treatment: (a) Tree height; (b) Tree basal diameter; [Note: Average increment of tree height and basal diameter of UP and LP of three repetitions with the error bar as standard deviation ( $n = 3$ ). The different letters (a,b,ab) are significantly different based on the least significant difference test ( $p < 0.05$ )]

The inhibitory effect of heavy metal Cd on poplar tree height was not proportional to the concentrations of Cd in soil, as had been observed in a previous study by Redovnikovic *et al.* (2017). Similar results could be found for basal diameter for UP at all treatments and LP at C2, C3, and C4 treatments. The basal diameter of LP at C1 was even slightly larger than that at C0, probably indicating some promotion effect on basal diameter growth at low Cd treatment. Both heights and basal diameter of UP trees at harvest were larger than that of LP for all treatments (Fig. 1 (a) and (b)), indicating the inhibitory effect of leaning plantation on poplar growth. The differences of height between UP and LP were 7.24%, 1.96%, 11.88%, 19.10%, and 9.23%, respectively, for the treatments from C0 to C4, and that of basal diameter were 23.12%, 14.38%, 16.56%, 20.98%, and 18.75%, respectively, indicating greater inhibition on basal diameter.

## Cd Concentration In Poplar Trees

### *Cd concentration in the main parts of poplar trees*

Figure 2 presents Cd concentration in the root, stem, bark, and leaf of UP and LP under different Cd treatments.

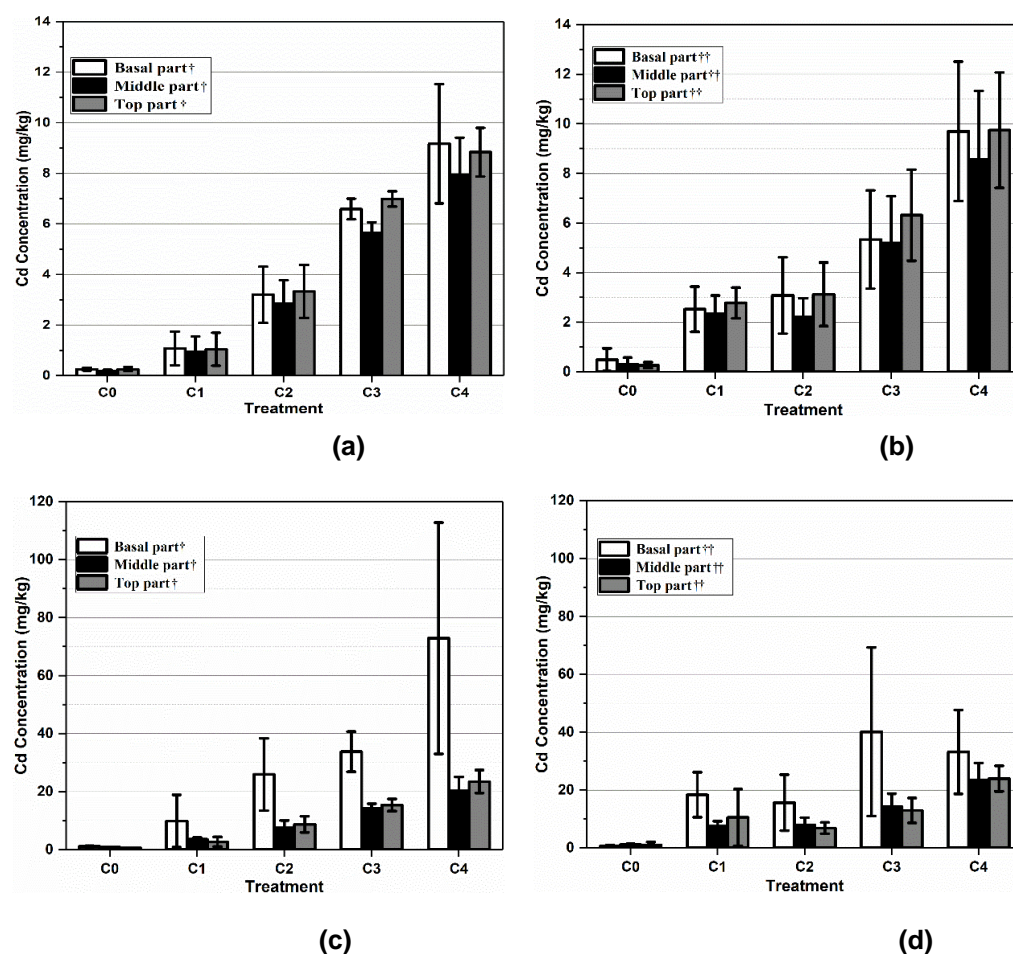


**Fig. 2.** Cd concentration in different parts of poplar trees: (a) Bark; (b) Leaf; (c) Root; (d) Stem; [Note: † Average concentration of Cd in leaf or root of three repetitions with the error bar as standard deviation ( $n = 3$ ). †† Average concentration of Cd in the bark or stem at three heights of three repetitions of UP with the error bar as standard deviation ( $n = 9$ ). ††† Average concentration of Cd in the bark or stem in the two zones (tension zone vs opposite zone) at three heights of three repetitions of LP with the error bar as standard deviation ( $n = 18$ ). The different letters (a, b, c, d, and e) are significantly different based on the least significant difference test ( $p < 0.05$ )]

It could be found that Cd concentration in different parts of poplar trees under both growth patterns increased with the treatments from C0 to C4, *i.e.*, with the increase of Cd concentrations in the soil. This result was consistent with many other studies on the poplar dendroremediation of heavy metals (Robinson *et al.* 2000; Laureysens *et al.* 2004; Madejón *et al.* 2004; Algreen *et al.* 2013; Redovniković *et al.* 2017).

The Cd concentration in the bark and leaf were much higher than that in the root and stem, for each treatment and each growth pattern (Fig. 2). Between bark and leaf, the bark demonstrated higher Cd concentration than the leaf, and between root and stem, the root demonstrated higher Cd concentration than the stem for each treatment and each growth pattern. This result was similar with many studies in that wood contained the least among the various parts, but different from them in that they either exhibited highest levels either in leaves or in the roots, not in the barks (Laureysens *et al.* 2004; Unterbrunner *et al.* 2007; He *et al.* 2013; Pilipović *et al.* 2019). The difference in the conclusion of experiments might be related to the species and age of poplars used in the experiment, cultivation pattern, site conditions, as well as Cd stress concentration and time (Romè *et al.* 2015).

Through comparison between the two growth patterns, LP had higher Cd concentration than UP at C1 and C4 for root and stem, at C1 and C3 for the bark, and all Cd addition treatments for the leaf (Fig. 2). It was worth noting that the LP trees contained more Cd than UP in all four parts at C1 treatment. The LP had a higher average Cd concentration of all treatments than UP in the root, stem, and leaf, but not in the bark.



**Fig. 3.** Cd concentration in the stems and barks of poplar trees along the height: (a) Stem of UP; (b) Stem of LP; (c) Bark of UP; (d) Bark of LP. Note: † Average concentration of Cd in stem or bark of a certain height of UP of three repetitions with the error bar as standard deviation ( $n = 3$ ). †† Average content of Cd in the two zones of stem of a certain height of LP of three repetitions with the error bar as standard deviation ( $n = 6$ )

### *Vertical distribution of Cd in the trunk parts of poplar trees*

Figure 3 compares Cd concentration in the stem and bark of UP and LP along the three trunk heights, *i.e.*, basal, middle, and top. The Cd contents in the bark were accordingly much higher than that in the stem for all treatments and both growth patterns along the height. It was also found that Cd concentration in the stem and bark increased from C0 to C4 at each height and for each growth pattern. With the exception of LP at C0, Cd concentration in the middle stem were slightly lower than that in the top and basal stem for both growth patterns (Fig. 3 (a) and (b)). Additionally, with the exception of LP at C0, Cd concentration in the bark demonstrated much higher Cd concentration in the basal than that in the middle and top (Fig. 3 (c) and (d)). This result was different from the vertical distribution of Cd along the trunk of *Salix*, where Cd increased significantly with height (Sander and Ericsson 1998). In trees, various atypical distributions of an element were observed due to different mechanisms of transport of metals and metalloids (Toppi and Gabbriellini 1999).

By comparing between the two growth patterns, the authors found that at the treatments of C1 and C4, Cd concentration in the stem at all three heights of LP were higher than that of UP, while it was opposite for the treatments of C2 and C3. The authors also found that Cd concentration in the bark at all three heights of LP were much higher than that of UP at C1 treatment, but it was not always the case for other treatments. Thus, Cd concentration in both stem and bark of LP were higher than that of UP at C1 treatment, along the three trunk heights.

### *Cd concentration in the two trunk zones of LP*

Table 1 compares Cd content in the stem and bark of TZ and OZ of LP trunks. The authors also found that Cd concentration in both zones generally increased from C0 to C4, with an insignificant difference between C1 and C2. For the stem, TZ demonstrated higher Cd concentration than OZ, except C2. For the bark, TZ had lower Cd concentration than OZ at C1, C2, and C4 and it was opposite at C3 and C0, with great standard deviations for both cases (Table 1).

**Table 1.** Cd Concentration in the Stem and Bark Parts of the Two Trunk Zones of LP Under Different Cd Treatments (mg/kg)

Treatment	Stem		Bark	
	Tension Zone <sup>†</sup>	Opposite Zone <sup>†</sup>	Tension Zone <sup>†</sup>	Opposite Zone <sup>†</sup>
C0	0.42 ± 0.36 <sup>d</sup>	0.28 ± 0.27 <sup>d</sup>	1.08 ± 0.86 <sup>c</sup>	0.81 ± 0.39 <sup>c</sup>
C1	2.91 ± 0.67 <sup>c</sup>	2.19 ± 0.66 <sup>c</sup>	10.52 ± 6.91 <sup>b</sup>	13.73 ± 9.66 <sup>b</sup>
C2	2.77 ± 1.21 <sup>c</sup>	2.85 ± 1.34 <sup>c</sup>	9.78 ± 7.66 <sup>b</sup>	10.49 ± 6.38 <sup>b</sup>
C3	6.56 ± 1.99 <sup>b</sup>	4.67 ± 1.19 <sup>b</sup>	25.18 ± 24.99 <sup>a</sup>	19.73 ± 16.38 <sup>a</sup>
C4	9.63 ± 2.33 <sup>a</sup>	9.04 ± 2.86 <sup>a</sup>	26.80 ± 8.56 <sup>a</sup>	26.96 ± 11.68 <sup>a</sup>

Note: † Average concentration of Cd with standard deviation (n =9). The same letter within columns is not significantly different according to the least significant difference test (p<0.05).

### **Phytoextraction Ability**

#### *BCF*

The BCF represents the ability of element migration in the soil-plant system and is an evaluation index reflecting the capacity of plants to absorb and transfer heavy metals or metalloids into their bodies. The higher the factor, the stronger the ability of absorbing elements in the plant.

BCFs of all parts ranged between 0.08 and 2.36 (Tables 2, 3), which fell in the same range as in other studies on poplar but were at a relatively low level, compared with the hyperaccumulators (Zhuang *et al.* 2007; Algreen *et al.* 2013; Redovniković *et al.* 2017). BCFs generally declined from C1 to C4, with the exception of the leaf of UP at C4 and the root of LP. This result was consistent with the conclusion of the study by Algreen *et al.* (2013) and Zhang *et al.* (2013) that BCF values decreased with the increase of Cd concentration in soil. However, it was different from the study of poplars on purple soil and alluvial soil with low Cd treatments from 0 to 1.5 mg/kg, where an increase of BCF with the increase of Cd concentration was reported (Wu *et al.* 2010). For both growth patterns, bark and leaf had higher BCFs than that of stem and root. The BCF of the root of LP at C2 was rather low, which might be due to the strong transport of Cd from the underground part to the aerial parts. C1 demonstrated much higher BCFs than all other three treatments, for each part and the two growth patterns, especially for LP (Table 2 and 3).

**Table 2.** BCF of Different Parts of UP Under Different Cd Treatments

Treatment	Upright Poplar			
	Root†	Stem††	Bark††	Leaf†
C1	0.22 ± 0.13 <sup>a</sup>	0.20 ± 0.18 <sup>a</sup>	1.05 ± 1.11 <sup>a</sup>	0.79 ± 0.13 <sup>a</sup>
C2	0.18 ± 0.11 <sup>b</sup>	0.16 ± 0.05 <sup>b</sup>	0.70 ± 0.55 <sup>b</sup>	0.32 ± 0.10 <sup>b</sup>
C3	0.15 ± 0.03 <sup>b</sup>	0.13 ± 0.01 <sup>b</sup>	0.42 ± 0.20 <sup>b</sup>	0.19 ± 0.03 <sup>b</sup>
C4	0.08 ± 0.02 <sup>b</sup>	0.09 ± 0.02 <sup>b</sup>	0.39 ± 0.33 <sup>b</sup>	0.20 ± 0.07 <sup>b</sup>
Average	0.16	0.15	0.64	0.38

Note: † Average BCF in leaf or root of three repetitions with standard deviation (n = 3). †† Average BCF in the bark or stem at three heights of three repetitions of UP with standard deviation (n = 9). The same letter within columns are not significantly different according to the least significant difference test (p < 0.05).

**Table 3.** BCF of Different Parts of LP under Different Cd Treatments

Treatment	Leaning Poplar			
	Root†	Stem†††	Bark†††	Leaf†
C1	0.30 ± 0.02 <sup>a</sup>	0.50 ± 0.14 <sup>a</sup>	2.36 ± 1.62 <sup>a</sup>	1.28 ± 0.46 <sup>a</sup>
C2	0.08 ± 0.05 <sup>b</sup>	0.14 ± 0.06 <sup>b</sup>	0.50 ± 0.34 <sup>b</sup>	0.36 ± 0.08 <sup>b</sup>
C3	0.12 ± 0.06 <sup>b</sup>	0.11 ± 0.04 <sup>b</sup>	0.45 ± 0.41 <sup>b</sup>	0.28 ± 0.06 <sup>b</sup>
C4	0.15 ± 0.03 <sup>b</sup>	0.09 ± 0.03 <sup>b</sup>	0.27 ± 0.10 <sup>b</sup>	0.26 ± 0.02 <sup>b</sup>
Average	0.16	0.21	0.9	0.55

Note: † Average BCF in leaf or root of three repetitions with standard deviation (n = 3). ††† Average BCF in the bark or stem in the two zones (tension zone vs opposite zone) at three heights of three repetitions of LP with standard deviation (n = 18). The same letter within columns are not significantly different according to the least significant difference test (p < 0.05).

By comparing the BCFs between the two growth patterns, it could be found that LP demonstrated higher average absorbing ability than UP for the aerial parts (Tables 2 and 3). The BCF range of UP was between 0.08 and 1.01, with that of the stem being between 0.09 and 0.2 (Table 2), while that of LP was between 0.08 and 2.36, with that of the stem being between 0.09 and 0.5 (Table 3). It was especially worth noting that, BCFs of the 4 parts of LP had much higher BCFs than UP at C1 treatment. This result showed that LP demonstrated higher accumulation ability of Cd from the soil than UP, especially at lower Cd treatment.



*TF*

The TF has been used to evaluate the ability of plants to translocate and accumulate heavy metals or metalloids from the underground part (root) to the above-ground parts. The higher the TF, the stronger the transport capacity of heavy metals from roots to the aerial parts.

Table 4 demonstrates that the TFs were generally greater than or close to 1 for both upright and leaning poplars, varying between 0.67 and 7.92, thus much higher than BCFs, indicating stronger transporting capacity from poplar roots to the aboveground parts, which had also been confirmed in the study of Wu *et al.* (2010).

**Table 4.** TF of Different Parts of UP and LP Under Different Cd Treatments

Treatment	Upright Poplar			Leaning Poplar		
	Stem <sup>††</sup>	Bark <sup>††</sup>	Leaf <sup>†</sup>	Stem <sup>†††</sup>	Bark <sup>†††</sup>	Leaf <sup>†</sup>
C0	0.87 ± 0.25 <sup>c</sup>	3.43 ± 1.18 <sup>b</sup>	2.24 ± 0.42 <sup>b</sup>	0.94 ± 0.82 <sup>c</sup>	2.78 ± 3.22 <sup>b</sup>	1.80 ± 1.50 <sup>b</sup>
C1	0.88 ± 0.31 <sup>c</sup>	4.63 ± 3.00 <sup>a</sup>	4.66 ± 2.65 <sup>a</sup>	1.66 ± 0.45 <sup>b</sup>	7.92 ± 5.47 <sup>a</sup>	4.31 ± 1.59 <sup>ab</sup>
C2	0.97 ± 0.30 <sup>b</sup>	4.20 ± 2.88 <sup>ab</sup>	2.02 ± 0.75 <sup>ab</sup>	2.19 ± 1.14 <sup>a</sup>	7.25 ± 3.88 <sup>a</sup>	5.54 ± 2.44 <sup>a</sup>
C3	0.84 ± 0.16 <sup>c</sup>	2.79 ± 1.50 <sup>b</sup>	1.21 ± 0.01 <sup>b</sup>	1.05 ± 0.40 <sup>c</sup>	4.86 ± 6.31 <sup>ab</sup>	2.60 ± 0.84 <sup>b</sup>
C4	1.05 ± 0.19 <sup>a</sup>	4.81 ± 4.27 <sup>a</sup>	2.49 ± 1.03 <sup>b</sup>	0.67 ± 0.24 <sup>c</sup>	1.91 ± 0.77 <sup>b</sup>	1.84 ± 0.39 <sup>b</sup>
Average	0.92	3.97	2.52	1.3	4.94	3.22

Note: † Average TF in leaf of three repetitions with standard deviation (n = 3). †† Average BCF in the bark or stem at three heights of three repetitions of UP with standard deviation (n = 9). ††† Average BCF in the bark or stem in the two zones (tension zone vs opposite zone) at three heights of three repetitions of LP with standard deviation (n = 18). The same letter within columns are not significantly different according to the least significant difference test (p < 0.05).

With the increase of Cd concentration in the soil, the TFs of different parts of both UP and LP no longer showed a general trend of decreasing, but sometimes even increased (Table 4). This result was similar to the conclusion by Zhang *et al.* (2013), that TFs of poplar and willow varied irregularly with soil Cd concentrations (Redovniković *et al.* 2017; Wu *et al.* 2010). For poplar trees under both plantations, the TFs of the parts had the following orders: Bark > Leaf > Stem.

By comparing the TFs between the two growth patterns, it could be found that LP demonstrated higher average TFs than UP, with the TFs of the leaf, the bark, and the stem of LP being 27.8%, 24.4%, and 41.3% larger than that of UP, respectively. The TF range of UP was between 0.84 and 4.81, with that of the stem being between 0.84 and 1.05, while that of LP was between 0.67 and 7.92, with that of the stem being between 0.67 and 2.19 (Table 4). The TFs of the stem and the bark of LP were much higher than that of UP at C1, C2, C3, but not at C0 and C4. This result showed that LP demonstrated higher translocation ability of Cd from root to the trunk than UP, except the very high Cd treatment (C4) and the very low control group.

## Discussion

### *Tolerance of poplar to Cd stress*

Sapling growth has often been used to evaluate tree response to exogenous stress, with their greater sensitivity to adverse conditions than mature trees (Pulford and Watson 2003). In this study height and diameter of tree saplings were measured to investigate the influence of Cd stress, as well as leaning plantation, on poplar growth. The authors' result

demonstrated that Cd addition significantly inhibited poplar growth (Fig. 1 (a) and (b)), but the inhibitory effect was not proportional to the concentrations of Cd in soil and there might even be some promotional effect on basal diameter at low Cd treatment (Fig. 1). Both UP and LP can grow under Cd stress until at treatment with Cd addition as high as 100 mg/kg and Cd accumulation in various parts of poplars all increased with the increase of Cd addition in the soil, indicating strong Cd tolerance of poplars to heavy metal Cd and relatively considerable phytoextraction ability of poplar even at high Cd stress. The authors' previous study on poplar trees growing on the floodplain downstream a mining hill, which was polluted by Cu, Zn, Cd, and As, with Cd in the soil being approximately 10 mg/kg, showed that poplars can grow quite well at those concentration levels until 20 to 30 m, reaching their full growth (Kataweteetham *et al.* 2020). The strong tolerance ability of poplar to Cd stress makes it a promising species for the recovery of polluted regions, especially in the remediation and utilization of highly polluted land.

In terms of pollution control practice, considering the phytoextraction ability of poplar, its strong tolerance to heavy metals, and its considerable growth rate and biomass, poplar could be recommended as a species that can be planted in a variety of contaminated sites for pollution treatment purpose.

#### *Inhabitation of leaning plantation on poplar growth*

Although both Cd stress and leaning plantation had a significant influence on poplar growth (Table 5), leaning plantation had a more inhibitory effect on poplar growth, resulting in considerably lower height and basal diameter of LP than that of UP, especially at higher Cd treatments. In the formation of tension wood, a dissymmetrical distribution of growth stresses capable to modify the orientation of different axes in space caused the shrinkage of the gelatinous layer, resulting in high macroscopic longitudinal contraction observed in tension wood (Jourez 1997; Clair and Thibaut 2001).

#### *Comparison between UP and LP in the accumulation of Cd*

For both UP and LP, bark and leaf absorb a considerate amount of Cd than stem and root. This demonstrated strong transportation of Cd from underground parts to the top parts and the bark played important role in the transportation, probably through well-developed vessels during the transpiration process. This high transportation was confirmed by the TFs of bark and leaf, demonstrating the importance of the strong transpiration effect of broad-leaved trees, such as poplars. Besides, materials such as terpenoids, suberin, fatty acid esters, parenchyma cells, *etc.*, which are rich in the bark tissues would probably help bind the divalent ions. In addition, some amino acids in bark combine with divalent Cd ions to form metal-binding peptides (Satofuka *et al.* 2001). However, only a limited amount of Cd was accumulated in the stem wood tissues, where they are expected to be the best storage place for heavy metals because of the great biomass.

By comparing Cd accumulation of poplars between the upright and leaning plantations, LP demonstrated higher mean concentrations of Cd in various parts in general and in the trunk parts along the heights, and stronger phytoextraction ability in terms of BCF and TF than that of UP, but all did not reach a significant level (Table 5). This difference was greater in the TZ of LP, which might be due to the large number of microvoids in TZ that played an important role in the adsorption and fixation of Cd (Liu and Liu 2011; Chang *et al.* 2015). The LP at C1 treatment (5 mg/kg Cd addition) was highlighted for generally higher Cd content, BCFs, and TFs than UP and LP at other treatments, especially for the trunk parts.

**Table 5.** Multi-way ANOVA Analysis on Cd Concentration in Poplar, BCF, and TF Under Different Cd Treatments, in Different Parts, and Between the Two Growth Patterns (P value)

Factors	Basal Diameter	Cd in Parts	Cd Along Heights		Cd in Zones		BCF	TF
			Stem	Bark	Stem	Bark		
Cd treatments (T)	0.008	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.002
Parts /Heights/Zones (P)	/	< 0.001	0.105	< 0.001	0.046	0.894	< 0.001	< 0.001
Growth patterns (GP)	< 0.001	0.682	0.355	0.399	/	/	0.147	0.096
T* P	/	< 0.001	0.942	0.001	0.352	0.856	< 0.001	0.305
T * GP	0.005	0.884	0.061	0.031	/	/	0.041	0.027
P* GP	/	0.62	0.955	0.119	/	/	0.572	0.705
T * P* GP	/	0.472	1	0.018	/	/	0.186	0.359

Based on the above findings, LP had only slightly stronger Cd accumulation ability than UP for the 1-year-old poplars. However, a continuous comparison study might be worth conducting to track a longer life cycle of poplar growth under Cd stress, especially with a special focus on the C1 treatment. Further study might also be focused on cell and cell wall level to understand the mechanism of phytoextraction of Cd by poplar and on the following promotion efforts to increase phytoextraction ability and to promote the accumulation in the stem.

## CONCLUSIONS

1. The inhibitory effect of Cd addition on poplar growth was significant, and the inhibitory effect was not proportional to the concentrations of Cd in soil. Despite the inhibition effect, both upright poplar (UP) and leaning poplar (LP) can grow until at the treatment with 100 mg/kg Cd addition, indicating strong tolerance of poplar to Cd stress. Leaning plantation also inhibited tree growth in terms of height and basal diameter, especially at higher Cd addition treatments.
2. Cd concentrations in various tissues increased generally with the increase of Cd in the soil for both UP and LP. The highest Cd was accumulated in the above ground parts of transpiration, *i.e.* the bark or the leaf. Cd in the wood part of the stem was always the lowest. Cd concentrations followed a general order as Bark > Leaf > Root > Stem for the one-year-old poplars. Along the trunk height, the largest Cd content was in the top for the stem, but in the basal for the bark.
3. Different parts of poplar demonstrated average BCFs ranges between 0.08 to 2.36, and average TFs between 0.67 to 7.92, indicating the big difference in phytoextraction ability among the parts, among the treatments, and between the two plantations. Bark or leaf had the highest BCF and TF, while that of the branch and stem were the lowest, indicating poor phytoextraction ability in wood parts. BCFs declined with the increase

of Cd addition in the soil, but the change of TF was not proportional to Cd levels in the soil. At C1 treatment with low Cd addition, poplar demonstrated much higher BCF than all other three treatments, for each part, especially for LP.

4. Average Cd concentrations in the various parts in general and in the bark and stem along the heights of LP, as well as average BCF and TF for each part of LP were higher than that of UP, but the difference was not significant. Significantly higher Cd concentration was found in the TZ of stem wood than that in the OZ for LP. Thus, leaning plantation limited poplar growth but only slightly promoted Cd accumulation in poplar tissues.

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