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CADMIUM ACCUMULATION AND ITS EFFECTS ON GROWTH AND GAS EXCHANGE IN FOUR *POPULUS* CULTIVARS

JIEGANG GU[1], LIWANG QI[3], WUSHENG JIANG[2], AND DONGHUA LIU[1*]

[1]*Department of Biology, College of Chemistry and Life Sciences, Tianjin Normal University, Tianjin 300387, P.R. China*
[2]*Library, Tianjin Normal University, Tianjin 300387, P.R. China*
[3]*Laboratory of Cell Biology, Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, P.R. China*

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The effects of different concentrations of Cd^{2+} (10, 50 and 100 μM) on the growth of four *Populus* cultivars (*Populus* sp. cv Zhonglin No. 46, *P.* sp. cv Langfang No. 4, *P.* sp. cv Qingyang, *P.* sp. cv Xiaoyeyang) and Cd^{2+} ion uptake were investigated. Cd accumulation in roots, shoot stems, young leaves and mature leaves, and bark and wood of cutting stems was analyzed using ICP-AES. Leaf gas exchange and photosynthetic parameters (net photosynthetic rate, stomatal conductance, transpiration rate) were measured in the first fully expanded leaf from the top shoot at the end of the treatment period. The lower Cd concentration (10 μM) had no inhibitory effect on root growth in the four cultivars. Root growth was significantly inhibited at 100 μM Cd. Among the investigated cultivars, *P.* sp. cv Qingyang showed stronger inhibition of root growth. Cd accumulation increased significantly with increasing Cd concentration and with time in all organs of the *Populus* cultivars. Cadmium concentrated mainly in the roots, and was higher there than in aerial parts. Cd concentrations were significantly higher in bark than in wood. Under 50 μM and 100 μM Cd stress, Cd content in shoot stems was highest in *P.* sp. cv Langfang No. 4, followed by *P.* sp. cv Xiaoyeyang and *P.* sp. cv Zhonglin No. 46. The potential of the cultivars for phytoremediation of Cd is briefly discussed.

Key words: *Populus*; Cd, net photosynthetic rate, stomatal conductance, phytoremediation.

INTRODUCTION

Cadmium is a highly toxic heavy metal which can be absorbed readily by crops and accumulated in the human body through the food chain. Cleansing soil of heavy metals using traditional technologies such as excavation and chemical leaching of metals is expensive. Phytoremediation, the use of plants to remove contaminants (phytoextraction) or to stabilize the soil (phytostabilization), offers a low-cost alternative that retains the integrity of the soil and is visually unobtrusive (Pulford and Dickinson, 2005). Early phytoremediation studies used hyperaccumulator species, which are commonly grown on metalliferous soils and are able to complete their life cycle without any symptoms of metal phytotoxicity. Hyperaccumulator plants offer a real potential to extract Ni, Zn, Cu, As, Co, and possibly Pb (Baker and Brooks, 1989; McGrath et al., 1998; Lombi et al., 2001; Keeling et al., 2003). Some trees may be useful for uptake and volatilization of Hg and Se (Banuelos et al., 1999). The uptake and accumulation of pollutants vary from plant to plant, and also from species to species within a genus. With respect to individual metals, hyperaccumulation limits versus plant dry weight have been reported to be 100 mg kg^{-1} for Cd, 1000 mg kg^{-1} for Pb and 10,000 mg kg^{-1} for Zn (Baker and Brooks, 1989).

The ideal plant for metal phytoextraction should be highly productive in biomass and should assimilate and translocate to shoots a significant part of the metals of concern. Additional favorable characteristics are fast growth, easy propagation, and a deep rooting system. Some tree species, mainly willows (*Salix*) and poplars or cottonwood (*Populus*), exhibit these traits and are currently used in phytoremediation programs (Pulford and Dickinson, 2005). Studying more than 150 willow clones, Greger and Landberg (1999) observed large variation in their tolerance to Cd. A similar conclusion may be drawn from the work of Punshon and Dickinson (1999) and Vyslouzilova et al. (2003).

*e-mail: donghua@mail.zlnet.com.cn

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Seventeen poplar clones were evaluated in an investigation by Laureysens et al. (2004a). Obviously, optimization of the potential of *Salix* and *Populus* for Cd extraction depends on the choice of appropriate genotypes or hybrids that combine traits such as high Cd accumulation in shoots, sufficient Cd tolerance, and high biomass productivity. Short-rotation coppice of willow and poplar has shown particular promise as a renewable energy crop having the ability to accumulate higher levels of Cd (Robinson et al., 2000; Hammer et al., 2003; Laureysens et al., 2004b). Moreover, several studies have shown willow (*Salix* spp.) to accumulate high levels of heavy metals, although with significant clonal variation in uptake (e.g., Nielsen, 1994; Aronsson and Perttu, 2001; Dickinson, 2006). However, literature on heavy metal accumulation within poplar is less extensive.

Plants can withstand heavy-metal accumulation until the metal reaches the toxicity threshold in the tissue. Generally, a high amount of Cd in plants reduces growth in roots and shoots, and causes leaf rolling and chlorosis (Sanitr di Toppi and Gabbrielli, 1999). At low levels of metal contamination, visual symptoms of phytotoxicity may be less pronounced or even absent. Taken up by roots, Cd^{2+} can induce deficiencies and imbalances of mineral nutrients. It can reduce nutrient uptake and translocation through competition (Clemens, 2006). Cadmium may affect photosynthesis at different levels, including stomatal conductance, Calvin cycle enzyme activity, photosynthetic pigments, thylakoid ultrastructure, and electron transport activity (Krupa and Baszynski, 1995; Vassilev and Yordanov, 1997), but some aspects remain unclear.

The aims in this study were (1) to evaluate the cadmium accumulation capacity of four selected *Populus* cultivars treated with different concentrations of Cd, (2) to determine the amounts of Cd allocated to the different organs of the four cultivars, (3) to assess their tolerance as judged by growth responses and photosynthetic performance, and (4) to evaluate their potential for phytoremediation of Cd-contaminated soils.

MATERIALS AND METHODS

PLANT MATERIAL AND GROWTH CONDITIONS

The four poplar cultivars, *Populus*. sp. cv Zhonglin No. 46, *P.* sp. cv Langfang No. 4, *P.* sp. cv Qingyang and *P.* sp. cv Xiaoyeyang, were provided by the Chinese Academy of Forestry, Beijing, P.R. China. Woody cuttings (25 cm long) from year-old shoots were rooted in vermiculite for a month. Then they were selected for uniformity of roots and new shoots, and transferred to full-strength Hoagland nutrient solution spiked with different concentrations of Cd (0, 10, 50, 100 μM) and grown for 4 weeks. Cadmium was provided as cadmium chloride ($CdCl_2$). The nutrient solution consisted of 0.75 mM K_2SO_4, 0.65 mM $MgSO_4$, 0.01 mM KCl, 0.25 mM KH_2PO_4, 2 mM $Ca(NO_3)_2$, 100 μM FeEDTA, 10 μM H_3BO_3, 1 μM $MnSO_4$, 0.1 μM $CuSO_4$, 0.05 μM $(NH_4)_6Mo_7O_4$ and 1 μM $ZnSO_4$, adjusted to pH 5.5. The experiments were conducted in a greenhouse under a 14 h photoperiod at 26/18°C (day/night) and 65–75% humidity. The solutions were constantly aerated and replaced every week. Root and shoot length was measured before the solutions were replaced. Any visible symptoms of Cd toxicity in the leaves and roots were noted. All treatments were done in five replicates.

ANALYSIS OF CD AND GAS EXCHANGE

Leaf gas exchange and photosynthetic parameter measurements

Leaf gas exchange and photosynthetic parameters (net photosynthetic rate, stomatal conductance, transpiration rate) were measured in the first fully expanded leaf from the top shoot at the end of the treatment period, using an Li–6400 (LI-COR Corp., U.S.A.) apparatus. The measurements were performed between 11:00 a.m. and 12:00 p.m. under the greenhouse conditions described above.

Sampling procedure and Cd determination

At the end of the experiment, roots and shoots from each treatment were harvested from the cuttings. After removal of necrotic and putrid tissue, the roots were rinsed in tap water and deionized water to remove traces of nutrients and Cd ions from the root surfaces. The plants were divided into roots, new shoots (stems, young leaves, mature leaves) and cutting stems (bark and wood). Then they were dried in a forced-air oven for 2 days at 45°C, followed by 3 days at 80°C and overnight at 105°C, and ground with a cutting mill (IKA-Werke GMBH & CO.KG, Germany). Cd content was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Leeman Labs Inc., New Hampshire, U.S.A.) after wet-ashing (Duan, 2003).

Analysis of variance (ANOVA) using Sigma statistical software (Jandel Scientific Corporation) was performed. Tests of equality of means used the t-test. Statistical significance was assumed at $p < 0.05$.

RESULTS

MACROSCOPIC EFFECTS OF CD ON ROOT AND SHOOT GROWTH

The effects of Cd on root growth varied with the Cd concentration and the cultivar (Fig. 1). The lower Cd

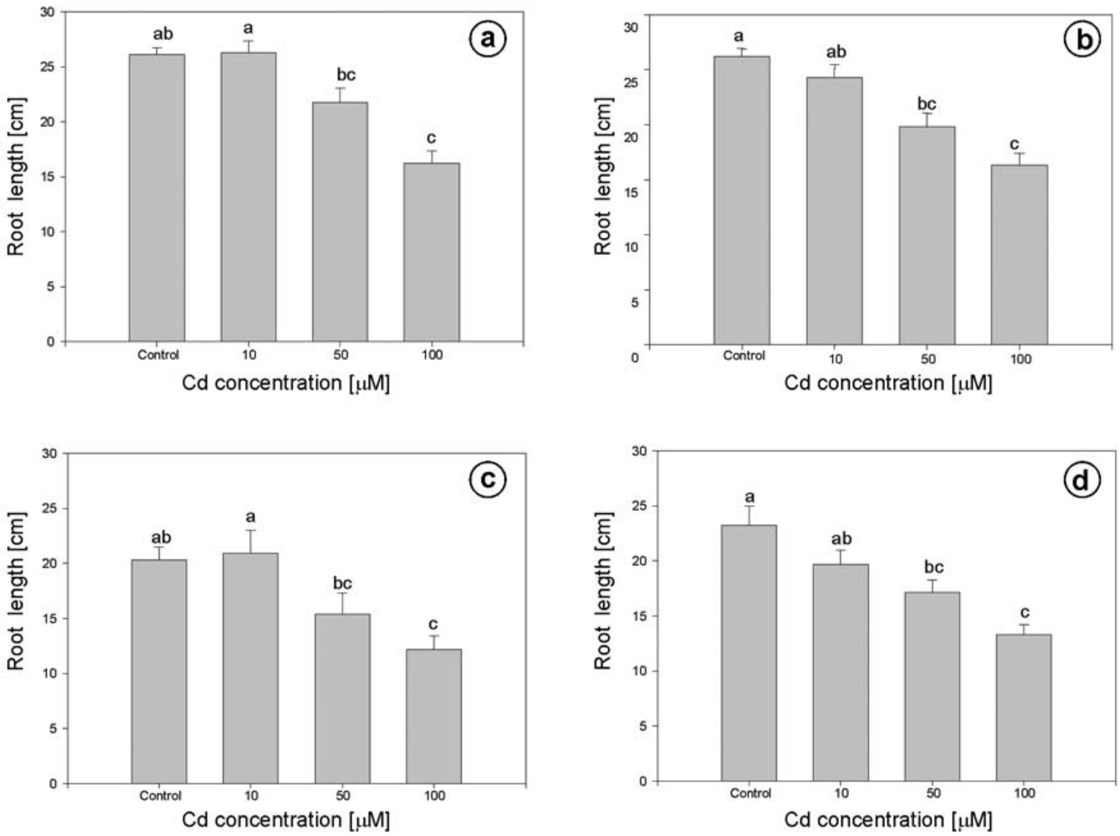

Fig. 1. Effects of different Cd concentrations on root growth of *P.* sp. cv Zhonglin No. 46 (**a**), *P.* sp. cv Langfang No. 4 (**b**), *P.* sp. cv Xiaoyeyang (**c**) and *P.* sp. cv Qingyang (**d**). Vertical bars denote SE (n = 5). Columns denoted by different letters indicate values significantly differing at $p < 0.05$ by one-way ANOVA.

concentration (10 μM) did not inhibit the root growth of the cultivars as compared with the control. Root growth was inhibited significantly at 100 μM Cd. Among the four cultivars, *P.* sp. cv Qingyang showed the strongest inhibition of root growth: versus the control, 26.2% reduction of root length in the 50 μM treatment and 42.9% in the 100 μM treatment.

In the 50 μM and 100 μM Cd treatments, shoots of *P.* sp. cv Zhonglin No. 46 showed no reduction of cumulative length versus the control (Fig. 2a). Cadmium exerted a marked inhibitory effect on shoot elongation in *P.* sp. cv Langfang No. 4 and *P.* sp. cv Qingyang Cd after four weeks of cultivation in those treatments (Fig. 2b,d). *P.* sp. cv Xiaoyeyang exposed to 100 μM Cd showed significant reduction of cumulative shoot length versus the control (Fig. 2c).

After four weeks, *P.* sp. cv Xiaoyeyang showed no obvious toxicity symptoms in leaves under any Cd treatment. At 100 μM Cd, chlorosis and flecks showed in young leaves of *P.* sp. cv Qingyang (Fig. 3a), and chlorosis and necroses in *P.* sp. cv Zhonglin No. 46 (Fig. 3b) and *P.* sp. cv Langfang No. 4 (Fig. 3c,d).

CD UPTAKE AND ACCUMULATION

Table 1 shows Cd accumulation in different organs of *Populus* cultivars. The Cd content of the roots, stems, young leaves and mature leaves, and cutting stem bark and wood in the four *Populus* cultivars increased significantly with the Cd concentration.

On average the Cd content in roots treated with 10, 50 and 100 μM Cd was about 35, 111 and 179 times the control levels, respectively. In the 100 μM Cd treatment, the Cd concentrations in roots of *P.* sp. cv Xiaoyeyang (3070.74±79.59 μg/g DW) and *P.* sp. cv Qingyang (2977.88±182.89 μg/g DW) were higher than in the other two cultivars. In the 10 μM Cd treatment, *P.* sp. cv Langfang No. 4 roots accumulated 720.27±3.48 μg/g DW. In the 50 μM Cd treatment, the roots of *P.* sp. cv Qingyang accumulated 2100.18±103.88 μg/g DW. The data indicate that Cd accumulated mainly in the roots, in higher amounts than in the aerial parts of the plants (Tab. 1).

This investigation also assessed Cd content in cutting stems and new shoots of the four *Populus* cultivars. Cd concentrations were significantly higher in bark than in wood. On average, the Cd con-

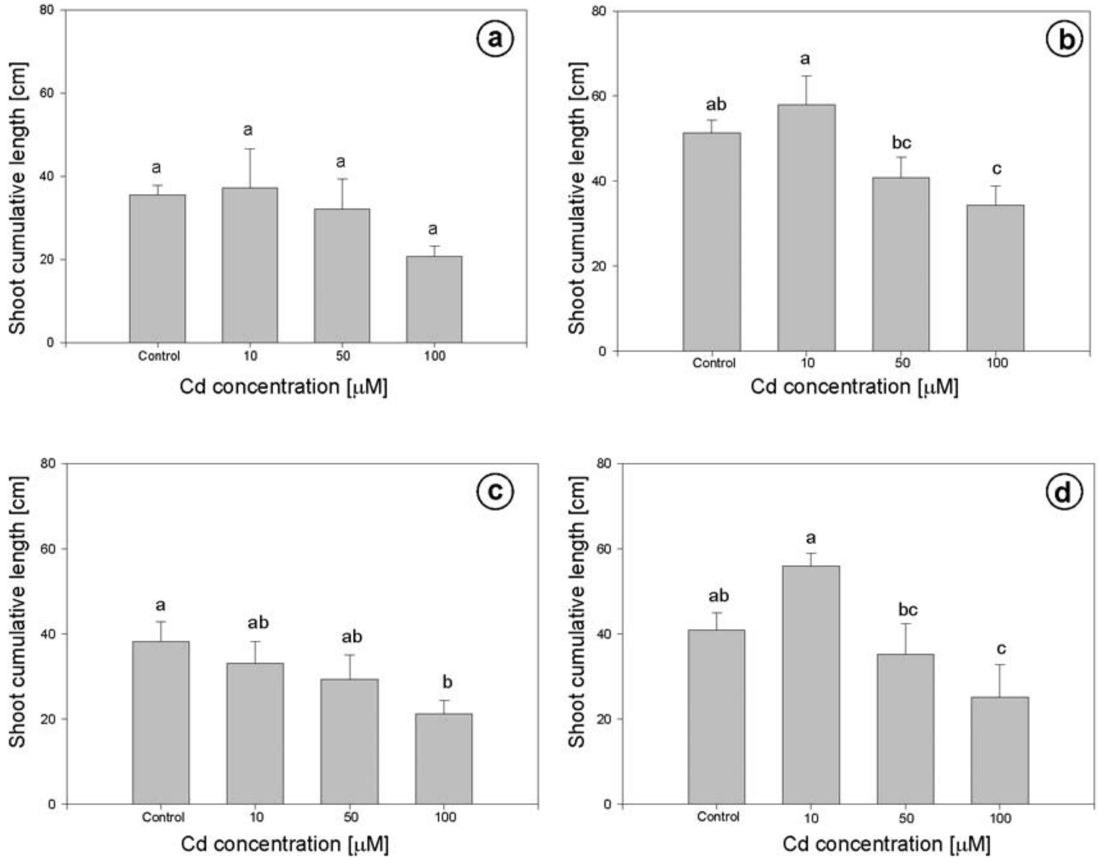

Fig. 2. Effects of different Cd concentrations on shoot growth of *P.* sp. cv Zhonglin No. 46 (**a**), *P.* sp. cv Langfang No. 4 (**b**), *P.* sp. cv Xiaoyeyang (**c**) and *P.* sp. cv Qingyang (**d**). Vertical bars denote SE (n = 5). Columns denoted by different letters indicate values significantly differing at $p < 0.05$ by one-way ANOVA.

centration of *P.* sp. cv Zhonglin No. 46 and *P.* sp. cv Langfang No. 4 in bark was ten times the concentration in wood. In bark of cutting stems, Cd content was highest in *P.* sp. cv Xiaoyeyang and *P.* sp. cv Langfang No. 4.

In the 50 μM and 100 μM Cd treatments, Cd content in shoot stems was highest in *P.* sp. cv Langfang No. 4, followed by *P.* sp. cv Xiaoyeyang, *P.* sp. cv Zhonglin No. 46, and lastly *P.* sp. cv Qingyang.

In all cultivars the Cd concentrations were significantly higher in young leaves than in mature leaves. In the 50 μM and 100 μM Cd treatments, Cd levels in leaves (both young and mature) were higher in *P.* sp. cv Langfang No. 4 than in the other three cultivars. *P.* sp. cv Qingyang accumulated only 107.29±0.69 μg/g DW and 132.26±2.61d μg/g DW Cd in younger leaves and 33.22±0.80 μg/g DW and 42.86±0.28 Cd in mature leaves, respectively.

CD EFFECT ON FOLIAGE GAS EXCHANGE AND PHOTOSYNTHESIS

Figure 4 shows changes in the net photosynthesis rate in the foliage of all cultivars at various Cd concentrations. In the 10 μM Cd treatment, no significant inhibitory effect versus the control was found in any of the cultivars, but such an effect was evident in the 50 μM and 100 μM Cd treatments: at the two doses, respectively, the net photosynthesis rate in foliage of *P.* sp. cv Zhonglin No. 46 declined to 78.4% and 27.4% of the control values, in *P.* sp. cv Langfang No. 4 to 72.9% and 32.2%, in *P.* sp. cv Qingyang to 38.7% and 27.8%, and in *P.* sp. cv Xiaoyeyang to 51.7% and 32.1%. Stomatal conductance (Fig. 5) and transpiration rates (Fig. 6) exhibited similar trends.

DISCUSSION

Inhibition of biomass production in plants undergoing heavy metal stress is a widely observed effect in phytotoxicity studies. All *Populus* cultivars exposed to 10 μM Cd in the present investigation were found to be tolerant to Cd, with no significant reduction of cumulative shoot length and root length versus the control. This result agrees with the finding of Pilipovic et al. (2005) that cultivars of *Populus*

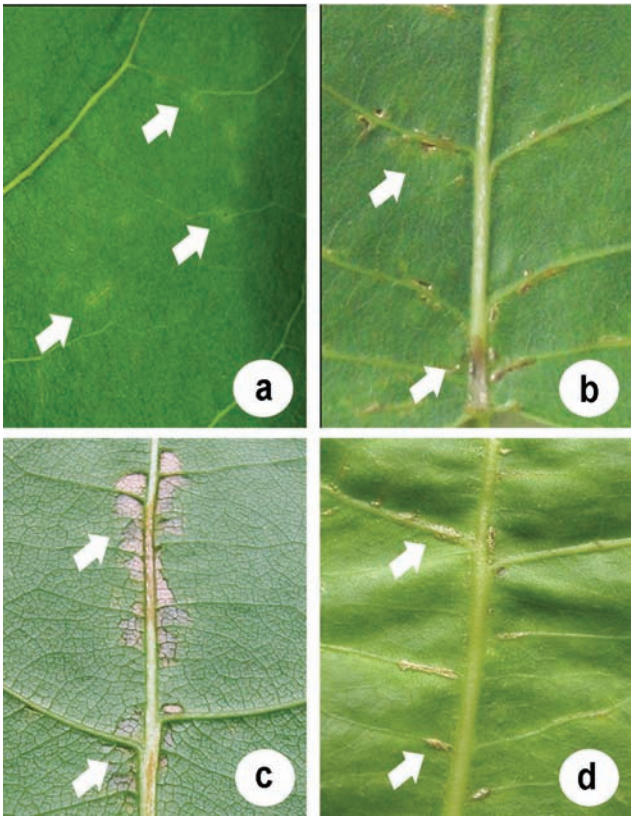

Fig. 3. Visible symptoms of Cd toxicity in leaves of *Populus* cultivars exposed to 100 μM Cd after 4 weeks of cultivation. In leaves of *P.* sp. cv Qingyang (**a**) and *P.* sp. cv Zhonglin No. 46 (**b**), patchy chlorosis developed and tiny necrotic spots spread along the veinlets. The necrotic spots developed to well-visible necroses in *P.* sp. cv Langfang No. 4 (**c,d**). Arrows indicate chlorosis.

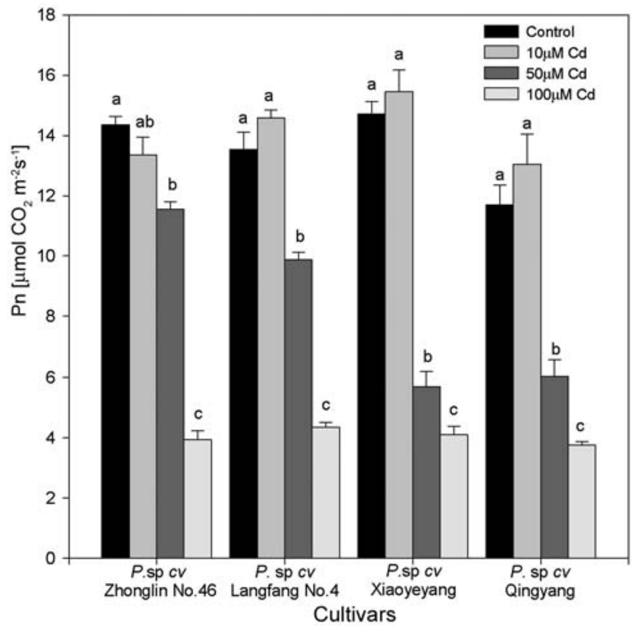

Fig. 4. Effects of different Cd concentrations on leaf net photosynthesis rate in *P.* sp. cv Langfang No. 4, *P.* sp. cv Zhonglin No. 46, *P.* sp. cv Qingyang and *P.* sp. cv Xiaoyeyang. Vertical bars denote SE (n = 5). Columns denoted by different letters indicate values significantly differing at $p < 0.05$ by one-way ANOVA.

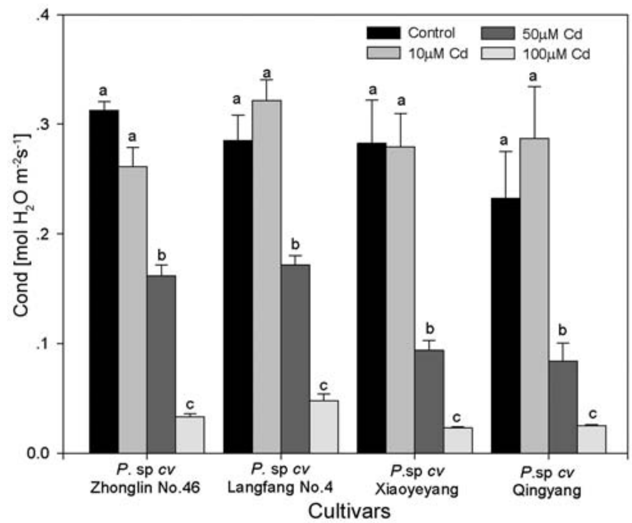

Fig. 5. Effects of different Cd concentrations on leaf stomal conductivity in *P.* sp. cv Langfang No. 4, *P.* sp. cv Zhonglin No. 46, *P.* sp. cv Qingyang and *P.* sp. cv Xiaoyeyang. Vertical bars denote SE (n = 5). Columns denoted by different letters indicate values significantly differing at $p < 0.05$ by one-way ANOVA.

showed no serious disturbance of growth in the presence of 10 μM Cd. Also in agreement with the present results, Shukla et al. (2003), Sottnikova et al. (2003) and Cosio et al. (2005) showed that at high Cd concentrations (50 μM and 100 μM Cd) roots clearly suffered the effects of Cd toxicity more than shoots.

Transport of Cd from roots to shoots and leaves is a critical step for Cd phytoextraction. *Populus* has the ability to take up and accumulate Cd primarily in its roots, with lower concentrations in shoots. Many key factors affect how much Cd taken into the root reaches the vascular systems for upward transport: the activity of metal-sequestering pathways in root cells, the efficiency of radial symplastic passage, and vascular loading activity (Clemens et al., 2002). The data in Table 1 show that the stem:root Cd content ratio increased with the Cd treatment dose in *Populus*. sp. cv Zhonglin No. 46 and *P.* sp. cv Langfang No. 4. In shoot stems of *P.* sp. cv Langfang No. 4, the stem:root Cd ratio reached 0.14 at 50 μM Cd and 0.29 at 100 μM Cd. In *P.* sp. cv Xiaoyeyang and *P.* sp. cv Qingyang, however, Cd transport to shoots as measured by the stem:root

TABLE 1. Cd content in different organs of four poplar cultivars exposed to different Cd concentrations, after 4 weeks of treatment

Clone	Treatment (μM Cd)	Organ (μg/g DW)						Stem: Root Cd content ratio
		Root	Cutting stem		New shoot			
			Bark	Wood	Stem	Young leaves	Mature leaves	
P. sp cv Zhonglin No.46	Control	30.36±0.27a	5.43±0.17a	1.13±0.15a	4.31±0.49a	8.45±0.01a	15.91±0.16a	
	10	336.49±5.57b	65.84±7.35b	5.92±0.11b	38.54±0.70b	63.44±0.48b	29.31±0.03b	0.11
	50	1217.04±30.02c	256.20±14.18c	21.89±2.50c	222.7±10.81c	226.69±0.28c	91.56±0.58c	0.18
	100	1927.72±5.19d	316.97±3.57d	46.62±2.34d	418.81±18.50d	318.90±5.73d	165.20±1.55d	0.22
P. sp cv Langfang No.4	Control	10.37±0.15a	4.07±0.06a	0.19±0.16a	3.21±0.52a	5.08±0.01a	3.90±0.07a	
	10	720.27±3.48b	74.28±0.63b	7.06±0.54b	49.25±0.84b	81.32±2.88b	29.61±0.61b	0.07
	50	1940.79±3.71c	365.11±9.19c	32.61±5.58c	278.94±1.61c	369.63±0.62c	93.71±8.22c	0.14
	100	2590.43±72.76d	583.56±3.27d	55.84±7.12d	746.56±14.76d	514.08±0.55d	196.56±3.44d	0.29
P. sp cv Xiaoyeyang	Control	8.59±0.01a	17.87±0.06a	4.24±0.10a	11.18±0.05a	14.76±0.08a	16.64±0.45a	
	10	375.05±23.26b	137.02±1.80b	34.53±0.28b	94.02±0.36b	59.23±0.10b	48.82±0.35b	0.25
	50	1330.09±44.66c	335.58±6.52c	108.11±0.87c	231.69±4.94c	134.06±8.20c	71.33±1.84c	0.17
	100	3070.74±79.59d	651.57±16.52d	265.88±6.25d	479.94±8.08d	157.83±6.29d	118.56±1.35d	0.16
P. sp cv Qingyang	Control	9.84±0.08a	7.76±0.24a	1.69±0.04a	6.79±0.54a	7.64±0.15a	5.49±0.02a	
	10	622.05±40.41b	94.23±1.51b	22.50±0.41b	84.53±1.46b	98.14±0.24b	25.30±0.07b	0.14
	50	2100.18±103.88c	217.52±1.84c	37.97±2.47c	143.40±0.44c	107.29±0.69c	33.22±0.80c	0.07
	100	2977.88±182.89d	379.30±0.63d	73.85±2.42d	235.33±1.92d	132.26±2.61d	42.86±0.28d	0.08

Means followed by corresponding SE values and letters indicate significant differences between means. Values followed by different letters differ significantly at $p < 0.05$. Means ± SE, n= 3

Cd ratio declined. In *P.* sp. cv Qingyang it reached only 0.07 and 0.08 at both 50 μM and 100 μM Cd.

Stunting, chlorosis, necrosis and desiccation are typical toxic symptoms of Cd stress in foliage (Das et al., 1997). In this investigation, the occurrence of toxic symptoms was positively correlated with the Cd concentration in leaves. Cd levels were significantly higher in young than in old leaves of the *Populus* cultivars (Tab. 1), and all the symptoms were observed in young leaves. The younger leaves of *P.* sp. cv Qingyang accumulated less Cd, but some tiny spots and flecks were observed in individual leaves. Salt et al. (1995) found that Cd toxicity produced chlorosis in young leaves of *B. juncea*, where Cd was preferentially accumulated. Cosio et al. (2005) reported that 12 weeks of treatment with 50 μM Cd produced necrotic spots on leaves of Prayon ecotypes of *T. caerulescens*; in this case, the necroses were evenly distributed over the leaf surface.

The observed decline in the net photosynthetic rate (Fig. 4) of the foliage of the *Populus* cultivars is obviously more related to stomata constraints, such as stomatal conductance (Fig. 5) and the transpiration rate (Fig. 6) showed similar changes. With exposure to higher Cd concentrations, mesophyll impairment seems to have increased, because the net photosynthetic rates in all four *populus* showed similar declines. The reduced transpiration rate and stomatal conductance in the higher Cd treatments (50 and 100 μM) may be the result of the strong effect of Cd on guard-cell function. Perfus-Barbeoch et al. (2002) found a similar inhibiting effect in leaves of *Arabidopsis thaliana* L. Salt et al. (1995) showed that ABA-induced stomatal closure dramatically reduced Cd accumulation in shoots of *Indian mustard*. Inhibition of photosynthesis induced by Cd may also result in lower chlorophyll content. Further studies are needed to clarify this effect.

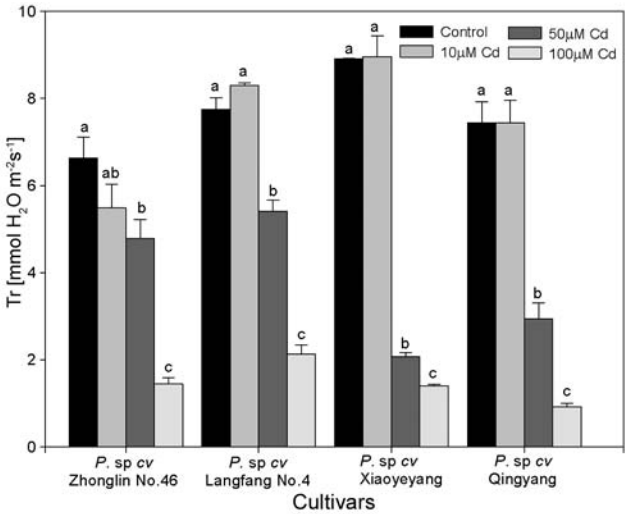

Fig. 6. Effects of different Cd concentrations on leaf transpiration rate in *P.* sp. cv Zhonglin No. 46, *P.* sp. cv Langfang No. 4, *P.* sp. cv Xiaoyeyang and *P.* sp. cv Qingyang. Vertical bars denote SE (n = 5). Columns denoted by different letters indicate values significantly differing at $p < 0.05$ by one-way ANOVA.

More Cd accumulated in bark than in wood. Cd transport within the plant can be divided into short-distance transport from the root to the vascular system, assumed to proceed by symplastic transport across the root cortex, and long-distance transport to the shoots, mainly as ions in xylem and phloem (Vassilev et al., 2002). The mobility of heavy metals in phloem plays a role in the redistribution of micronutrients and pollutants (Riesen and Feller, 2005). Li et al. (2006) suggested that gamma-glutamylcysteine or other phytochelatin pathway intermediates may be involved in long-distance transport of heavy metals in phloem as carriers of thiol peptides. The higher Cd content found in bark in this study may be the result of redistribution of Cd during long-distance transport to shoots.

All of the studied cultivars can be considered Cd-hyperaccumulators according to the currently accepted threshold level for defining hyperaccumulation, 0.01% (w/w) cadmium in shoots (Baker et al., 2000). These cultivars produce many roots and high biomass, and have a high capacity to accumulate Cd. They can play an important role in the treatment of soils stressed by Cd. *P.* sp. cv Langfang No. 4 showed higher Cd accumulation. *P.* sp. cv Qingyang showed sensitivity to Cd toxicity and a lower Cd transport ratio. These data on Cd accumulation in different organs and tissues of the investigated *Populus* cultivars may be useful in considering phytoremediation of Cd-contaminated soils in the local environment.

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