

Heavy metal tolerance and accumulation of *Triarrhena sacchariflora*, a large amphibious ornamental grass

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ABSTRACT

In this study, we report the tolerance and accumulation of *Triarrhena sacchariflora* to copper (Cu) and cadmium (Cd). The results show that *T. sacchariflora* had strong tolerance to Cu and Cd stress. The tolerance indexes (*T_i*) were greater than 0.5 for all treatments. The bioconcentration factors (*BCFs*) to Cu and Cd were both above 1.0. The accumulation ability of roots was stronger than that of shoots, and ranges of *BCF* to Cu and Cd in roots were 37.89–79.08 and 83.96–300.57, respectively. However, the translocation ability to Cu and Cd was weak, with more than 86% of Cu or Cd accumulated in roots, suggesting an exclusion strategy for heavy metal tolerance. The uptake efficiency (*UE*) and translocation efficiency (*TE*) to Cu and Cd increased linearly as the Cu and Cd concentration in the substrate increased. *UE* was higher than *TE*, with a maximum of 2,118.90 $\mu\text{g g}^{-1}$ root dry weight (DW) (50 mg L⁻¹ Cu) and 1,847.51 $\mu\text{g g}^{-1}$ root DW (20 mg L⁻¹ Cd), respectively. The results indicate that *T. sacchariflora* is a Cu- and Cd-tolerant non-hyperaccumulator plant, suggesting that *T. sacchariflora* could play an important role in phytoremediation in areas contaminated with Cu and Cd.

Key words | accumulation, cadmium, copper, tolerance, *Triarrhena sacchariflora*

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INTRODUCTION

Heavy metal contamination in soil and water caused by human activities, such as mining and industrial activities, is a serious problem all over the world (McLaughlin *et al.* 2000; Ikenaka *et al.* 2010). It poses a serious health hazard to humans as well as plants and animals. Phytoremediation is low cost, convenient and has a high ornamental value. Hyperaccumulators can accumulate extraordinarily high amounts of heavy metals in aerial organs, without suffering phytotoxic effects. However, they have been believed to have limited potential for phytoremediation because most of them are metal selective, can be used in their natural habitats only, and have small biomass, shallow root systems and slow growth rates, which limit the speed of metal removal (Cunningham *et al.* 1995). Nevertheless, heavy metal-tolerant non-hyperaccumulator plants exist in nature, in which metals can enter root cells where they are sequestered into root vacuoles, but not translocated to shoots, preventing them from being damaged (Lin & Aarts Mark 2012). The use of these plants in phytoremediation in areas contaminated with heavy metals would be significant.

Native plants adapt to local conditions better than introduced plants (Yoon *et al.* 2006; Antonsiewicz *et al.* 2008). Therefore, using native heavy metal-tolerant plants in

phytoremediation can be a good option. *Triarrhena sacchariflora* is a perennial herbaceous ornamental plant, belonging to Gramineae, *Triarrhena*, with a distribution in the north-east, north, northwest and east of China (Liu 1996). It is a C₄ plant with high photosynthetic efficiency and high biomass. It has a large apical panicle and well-developed root system, with a unique seasonal appearance and the ability to create a beautiful landscape. It can easily adapt to the environment due to its amphibious character and is an energy crop (reviewed by Lewandowski *et al.* 2003) due to its many beneficial attributes as an energy crop. It can be applied in environmental protection, landscaping, bioenergy, pulp and paper engineering, textile, medicine and so on. All of these outstanding characteristics of *T. sacchariflora* make it an important plant resource with great potential.

Many studies have indicated that *Phragmites australis* can tolerate high concentrations of heavy metals by reducing, accumulating and compartmentalization, and play an important role in wetland ecosystems (Ali *et al.* 2002; Weis *et al.* 2004; Bonanno 2011). *T. sacchariflora* is often associated with *P. australis*. Its ecological habitat and growth are similar to *P. australis*. Thus, we hypothesize it could tolerate a high concentration of heavy metals similar

to *P. australis*. However, related studies on the tolerance and phytoremediation of *T. sacchariflora* to heavy metals are very limited. In this study, we analyzed the tolerance and accumulation characteristics of *T. sacchariflora* to Cu and Cd, aiming to assess the ability of *T. sacchariflora* to remedy Cu- and Cd-contaminated areas.

MATERIALS AND METHODS

Plant material

The wild seeds of *T. sacchariflora* were collected from the forest center of Dafeng in Jiangsu province, China. In the spring of 2010, healthy and plump seeds were selected and sterilized in 1% NaClO for 10 min. Then they were sown in pots and cultivated in the greenhouse of Nanjing Forestry University. In the middle of July, healthy seedlings of a similar size (40–45 cm) were selected and washed, then transferred into 1 L conical flasks and cultivated in Hoagland pancebrin. The formula for pancebrin is shown in Table 1. After 15 days, seedlings of similar size were selected and transplanted into conical flasks filled with 1/4 Hoagland nutrient solution, then treated with Cu and Cd accordingly.

Experimental design

Cu as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and Cd as $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ were added to the solution, respectively. There were five levels of Cu: 0 (control), 5, 10, 20, and 50 mg L^{-1} (Cu^{2+}) and five levels of Cd: 0 (control), 1, 5, 10, and 20 mg L^{-1} (Cd^{2+}). Each treatment had three replicates with 10 uniform plants for each replicate. Plants grew in 1 L of treatment solution at a temperature of 25 °C and with 12 h light each day. The treatment solution was replaced every 5 days. pH was measured every other day and adjusted with 0.1 mol L^{-1} sodium hydroxide (NaOH) or hydrochloric acid (HCl) to keep it within 5.5–6.0.

Table 1 | The formula of pancebrin

Reagent	Concentration (mmol/L)	Reagent	Concentration (mmol/L)
$\text{Ca}(\text{NO}_3)_2$	4	H_3BO_3	4.5×10^{-2}
KNO_3	5	CuSO_4	8×10^{-4}
KH_2PO_4	1	ZnSO_4	1.5×10^{-3}
MgSO_4	1	Na_2MoO_4	9×10^{-5}
EDTA-Fe	5×10^{-2}	MnSO_4	3.6×10^{-3}

Cu and Cd concentration determination

After 15 days, harvested plants were washed with tap water, then rinsed with deionized water and dried with filter papers. The plants were carefully divided into roots and shoots. The roots were immersed in 20 mol L^{-1} EDTA-Na for 20 min to remove metal ions adhering to the roots' surface. The samples were oven-dried at 105 °C for 30 min and then at 85 °C to constant weight. Dry weights of each sample were weighed.

The concentrations of Cu and Cd in plants were determined by inductively coupled plasma (ICP, OPTIMA-4300DV, PerkinElmer, USA). The heavy metal concentration was calculated as: $C = P \times V \times KTS / m$, where C represents the heavy metal concentration of the plant (mg kg^{-1}), P represents the sample concentration measured by ICP (mg L^{-1}), V represents the constant volume of the sample liquid (25 mL), KTS represents the divided multiples and m represents the weight of the dried samples (g).

Data analysis

Several indexes were calculated to assess *T. sacchariflora* tolerance and accumulation to Cu and Cd.

The tolerance index (TI) is based on the biomass of the plant (Shi et al. 2011) and was calculated as:

$$TI = B_t / B_c$$

where B_t (g) represents the treatment biomass and B_c (g) represents the control biomass. A higher value represents higher tolerance to metal.

The bioconcentration factor (BCF) represents the ability of the plant to absorb metal from the substrate and translocate it to above-ground tissues (Liu et al. 2008). The BCF was calculated as:

$$BCF = C_{\text{plant tissue}} / C_{\text{substrate}}$$

where $C_{\text{plant tissue}}$ (mg kg^{-1}) represents the metal concentration in plant tissue and $C_{\text{substrate}}$ (mg kg^{-1}) represents the metal concentration in the substrate.

The translocation factor (TF) estimates the ability of the plant to translocate metal from roots to shoots (Shi et al. 2011), and the TF was calculated as:

$$TF = C_{\text{shoots}} / C_{\text{roots}}$$

where C_{shoots} (mg kg^{-1}) represents the metal concentration accumulated in shoots and C_{roots} (mg kg^{-1}) represents the metal concentration accumulated in roots.

Uptake efficiency (UE) estimates the efficiency of the plant to absorb metal from the substrate. The UE was calculated as:

$$UE = A_{\text{plant}}/B_{\text{roots}}$$

where A_{plant} (g) represents the metal accumulated in the plant and B_{roots} (g) represents the biomass of the roots.

Translocation efficiency (TE) estimates the efficiency of the plant to translocate metal from the substrate. The TE was calculated as:

$$TE = A_{\text{shoots}}/B_{\text{roots}}$$

where A_{shoots} (g) represents the metal accumulated in shoots and B_{roots} (g) represents the biomass of the roots.

All data were subject to analyses of variance, regression analysis, and Duncan's tests for multiple comparisons of means using SPSS 13.0.

RESULTS AND DISCUSSION

Plants' growth and their tolerance to Cu and Cd

When the concentration of Cu and Cd was $\leq 10 \text{ mg L}^{-1}$, all of the plants grew well. The plants grew well in a week under 20 mg L^{-1} Cu stress and showed mild toxic symptoms with the extension in time. When the concentration of Cu was 50 mg L^{-1} and the concentration of Cd was 20 mg L^{-1} , the old leaves gradually showed toxic symptoms as treatment time went on.

Plants' tolerance to heavy metals is the basis of phytoremediation. Figure 1 shows that all the TI of *T. sacchariflora* to Cu and Cd were greater than 0.5. The

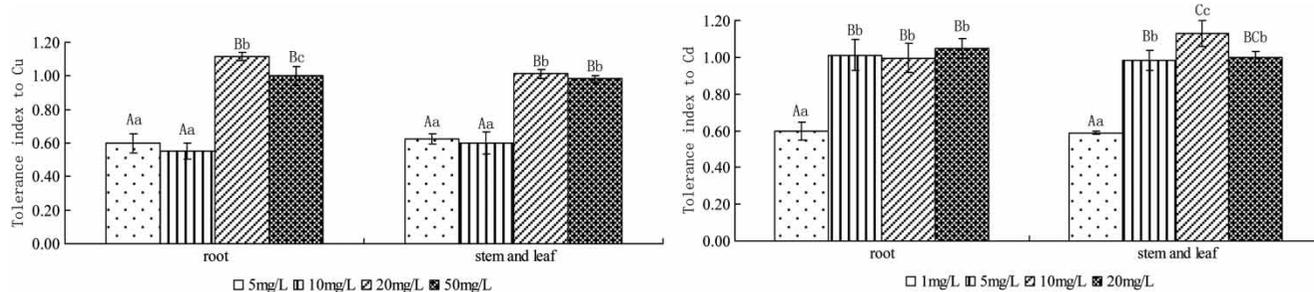


Figure 1 | Tolerance index of *T. sacchariflora* to Cu and Cd. Note: Values are means of three replicates \pm S.D. The different uppercase letters indicate significant difference at $p < 0.01$ and the different lowercase letters indicate significant difference at $p < 0.05$, whereas the same letters indicate no significant difference. The same applies to Figures 2 and 3.

ranges of TI to Cu and Cd were 0.55–1.12 and 0.59–1.13, respectively. Growth disorder causing reduction in biomass yields are commonly observed in plants subjected to high metal levels (Singh & Agrawal 2007). In this study, the biomass yields of the plant were not reduced under high Cu and Cd concentrations, resulting in either being significantly higher than the control check (CK) ($p < 0.05$) or having no significant difference with CK ($p > 0.05$). A moderate concentration of heavy metals promoted growth, such as the biomass of roots (20 mg L^{-1} Cu) and the biomass of stems and leaves (10 mg L^{-1} Cd), which were significantly higher than CK ($p < 0.05$). In contrast, the plant growth was restrained under the low Cu and Cd concentrations, and the biomass was significantly lower than CK ($p < 0.05$). Some hyperaccumulators not only have higher tolerance to higher concentrations of heavy metals, but also promote growth and biomass under moderate concentrations of heavy metal stress. However, their growth may be restrained under lower concentrations of heavy metal stress (Kupper et al. 2001). Based on the growth measurement used in the present study, these findings indicate that *T. sacchariflora* had a stronger tolerance to high concentrations of Cu and Cd.

Accumulation and distribution of Cu and Cd in plants

Table 2 shows that Cu in roots increased significantly with the increase of Cu concentration ($p < 0.01$). Cu in stems and leaves also increased with the increase of Cu concentration, except for the concentration of 20 mg L^{-1} . Cu in roots, stems, and leaves and Cu in solution was positively correlated ($r = 0.975, 0.918, p < 0.01$). Cd in roots, stems, and leaves increased dramatically with the increase of Cd concentration ($p < 0.01$), and there was a significant positive correlation between them ($r = 0.958, 0.972, p < 0.01$).

After 15 days of growth, Cu in plants under all treatments was much higher than CK, with 18.26, 38.54, 45.88, and 92.33 times as much as CK in roots and 12.12, 33.09, 20.62, and

Table 2 | Cu and Cd concentrations in different parts/tissues, *BCF* and *TF* to Cu and Cd

Treatment (mg L ⁻¹)	Concentration (mg kg ⁻¹)		<i>BCF</i>				
	Root	Stem and leaf	Root	Stem and leaf	Plant	<i>TF</i>	
Cu	0	20.52 ± 1.77 ^{Aa}	2.35 ± 0.83 ^{Aa}	–	–	–	–
	5	374.75 ± 32.01 ^{Bb}	28.48 ± 2.65 ^{Bb}	74.95 ± 6.40 ^{Aa}	5.70 ± 0.53 ^{Aa}	32.44 ± 1.75 ^{Aa}	0.12 ± 0.01 ^{Aa}
	10	790.83 ± 48.15 ^{Cc}	77.75 ± 5.35 ^{Cd}	79.08 ± 4.82 ^{Aa}	7.78 ± 0.54 ^{Bb}	34.78 ± 2.14 ^{Aa}	0.16 ± 0.02 ^{Bb}
	20	941.5 ± 11.17 ^{Dd}	48.45 ± 6.04 ^{Bc}	47.08 ± 0.56 ^{Bb}	2.42 ± 0.30 ^{Cc}	21.24 ± 0.78 ^{Bb}	0.07 ± 0.01 ^{Cc}
	50	1,894.67 ± 55.86 ^{Ee}	150.13 ± 15.83 ^{De}	37.89 ± 1.12 ^{Bc}	3.00 ± 0.317 ^{Cc}	17.02 ± 1.10 ^{Cc}	0.12 ± 0.02 ^{Aa}
Cd	0	0.00 ± 0.00 ^{Aa}	0.00 ± 0.00 ^{Aa}	–	–	–	–
	1	300.57 ± 6.88 ^{Bb}	26.20 ± 1.03 ^{Bb}	300.57 ± 6.88 ^{Aa}	26.20 ± 1.03 ^{Aa}	136.33 ± 7.58 ^{Aa}	0.13 ± 0.01 ^{Aa}
	5	755.83 ± 11.86 ^{Cc}	39.00 ± 1.92 ^{Bc}	151.17 ± 2.37 ^{Bb}	7.80 ± 0.38 ^{Bb}	65.72 ± 2.35 ^{Bb}	0.08 ± 0.01 ^{Bc}
	10	1,265.33 ± 112.96 ^{Dd}	78.97 ± 4.03 ^{Cd}	126.53 ± 11.30 ^{Cc}	7.90 ± 0.40 ^{Bb}	51.39 ± 1.84 ^{Cc}	0.11 ± 0.01 ^{Ab}
	20	1,679.17 ± 23.53 ^{Ee}	116.30 ± 10.49 ^{De}	83.96 ± 1.18 ^{Dd}	5.82 ± 0.52 ^{Cc}	37.72 ± 1.13 ^{Dd}	0.10 ± 0.01 ^{ABb}

Note: Values are means of three replicates ±S.D. The values in the same row followed by different uppercase letters indicate significant difference at $p < 0.01$ and by different lowercase letters indicate significant difference at $p < 0.05$, whereas the same letters indicate no significant difference.

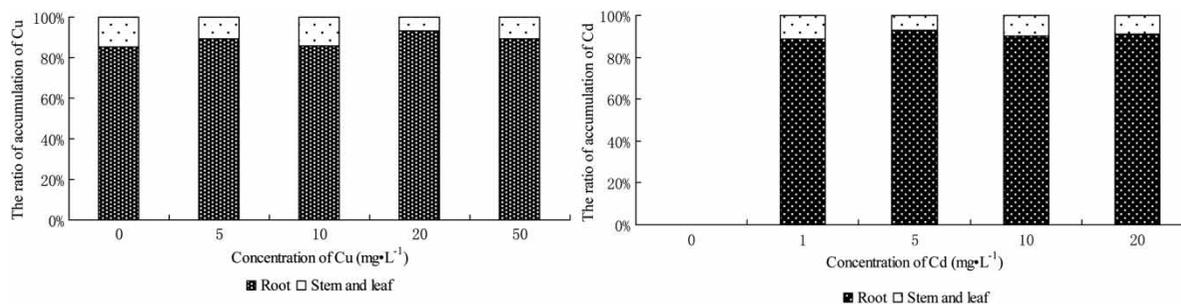
63.89 times as much as CK in stems and leaves, for the four concentrations of Cu treatment, respectively. Cu is essential to plant growth, but it has toxic effects when shoots or leaves accumulate Cu in concentrations exceeding 20 µg g⁻¹ (Borkert *et al.* 1998). Cardwell *et al.* (2002) investigated 15 aquatic species in southeast Queensland, Australia, and found that the highest Cu concentration in leaves of emergent plants was 34 µg g⁻¹ and 1,571 µg g⁻¹ in roots. In this study, several Cu values in roots and leaves were higher than the concentrations mentioned above.

The accumulation ability to Cd was even stronger, for after the 15-day growth, Cd accumulation in plants under all treatments was also much higher than CK, with 112.57, 283.08, 473.91, and 628.90 times as much as CK in roots and 47.64, 70.91, 143.58, and 211.45 times as much as CK in stems and leaves, for the four concentrations of Cd treatment, respectively. The range of Cd concentrations found in contaminated plants was 5–30 µg g⁻¹ (Kabata-Pendias & Pendias 2000). In this study, the measured Cd concentration accumulated in plants was mostly higher than these values, especially in the roots.

The roots of *T. sacchariflora* showed strong bioaccumulation capacity, and the range of *BCFs* for Cu and Cd were 37.89–79.08 and 83.96–300.57, respectively. The bioaccumulation capacity of stems and leaves was smaller than that of roots, while *BCFs* were still higher than 1.0. The range of *BCFs* for Cu and Cd of the plant (including roots, stems, and leaves) was 17.02–34.78 and 37.72–136.33, respectively. All of the above indicated that *T. sacchariflora* had a strong ability to accumulate Cu and Cd, but the bioaccumulation capacity weakened with the increase of Cu and Cd concentration.

However, the translocation factors (*TFs*) of *T. sacchariflora* under all treatments were lower than 1.0, indicating the limited ability of transferring Cu and Cd from roots to shoots.

The Cu and Cd accumulated by *T. sacchariflora* were mostly in roots, with Cu taking up 86.04–93.41% of the total accumulation and Cd taking up 88.52–92.92% (Figure 2). The greatest mean concentrations of heavy metals were found in the below-ground plant parts of *P. australis* during the spring season and the average standing

**Figure 2** | The ratio of accumulation of Cu and Cd in different parts.

stock of heavy metals was higher in the below-ground than in the above-ground phytomass (Grisey *et al.* 2012). The accumulation and translocation characteristics of *T. sacchariflora* were related to its own growth strategies. Cu and Cd accumulated in *T. sacchariflora* were mostly accumulated in root tissues, suggesting an exclusion strategy for heavy metals' tolerance. As an amphibious and large herbaceous plant, *T. sacchariflora* possesses rich root systems. There is an obvious retention effect of roots to Cu and Cd.

The hyperaccumulators were defined with the following characteristics. (1) The content of metal elements in plants should exceed the critical value, such as for a Cu-hyperaccumulator or a Cd-hyperaccumulator, the Cu and Cd should be higher than 1,000 and 100 mg kg⁻¹, respectively (Baker & Brooks 1989). (2) *BCF* is higher than 1.0 (Zu *et al.* 2004). (3) *TF* is higher than 1.0 (Zu *et al.* 2004). In this study, the maximal Cu concentration in the shoots was 150.13 mg kg⁻¹, while the maximal Cu concentration in the roots was 1,894.67 mg kg⁻¹ which exceeded 1,000 mg kg⁻¹. The accumulation of Cd in the shoots was 116.3 mg kg⁻¹ when the Cd concentration in solution was 20 mg L⁻¹, which reached 100 mg kg⁻¹ as the critical value of a Cd-hyperaccumulator. In addition, the Cd accumulation in the roots under all Cd treatments exceeded 100 mg kg⁻¹. Furthermore, *BCF* to Cu and Cd is higher than 1.0, indicating its large bioaccumulation capacity to Cu and Cd. However, *TFs* to Cu and Cd were lower than 1.0, and more than 86% of Cu and Cd was distributed in the roots.

The tested materials were *T. sacchariflora* seedlings, about 6-month-old, and the accumulation of Cu and Cd would increase for older seedlings. The biomass of

T. sacchariflora is large and generally reaches its maximum after two to three years. The biomass in shoots is up to 19,000–22,000 kg DW hm⁻² in the second growing season (Lewandowski *et al.* 2003). Therefore, although *T. sacchariflora* is not a hyperaccumulator, the total accumulation of Cu and Cd is still considerable.

Uptake efficiency and translocation efficiency to Cu and Cd

UE to Cu and Cd, and *TE* to Cd increased linearly with the increase of Cu and Cd concentration. The relationship could be described with the equations: $y = 477.75x - 535.37$ ($R^2 = 0.9147$), $y = 475.76x - 546.62$ ($R^2 = 0.9929$) and $y = 43.448x - 49.866$ ($R^2 = 0.9629$), respectively, where y represents *UE* or *TE*, x represents the Cu or Cd concentration in solution. *TE* to Cu had an increase–decrease–increase tendency as the Cu concentration increased. *UE* was higher than *TE*, with a maximum 2,118.90 µg g⁻¹ DW (50 mg L⁻¹Cu) and 1,847.51 µg g⁻¹ DW (20 mg L⁻¹Cd), respectively. *UE* and *TE* to Cd were higher than to Cu when under the same concentration of Cu and Cd (Figure 3).

CONCLUSIONS

This study shows that *T. sacchariflora* can tolerate high concentrations of Cu and Cd without suffering adverse physiological effects, and can produce a significant amount of biomass while accumulating high concentrations

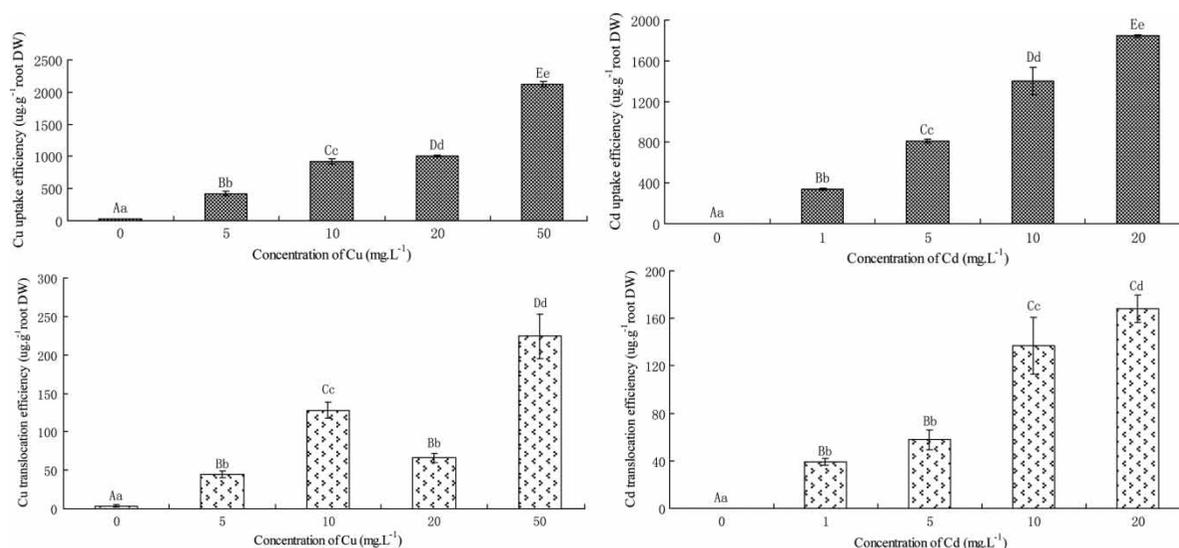


Figure 3 | The uptake efficiency and translocation efficiency of *T. sacchariflora* to Cu and Cd.

of these metals. *T. sacchariflora* is a root bioaccumulator species, showing high accumulation of Cu and Cd in roots and low translocation from roots to other tissues, suggesting inherent filter mechanisms to reduce toxic concentrations through the plant. The results indicate that *T. sacchariflora* is a Cu-tolerant and Cd-tolerant non-hyperaccumulator plant. Moreover, the significant positive correlation between Cu or Cd in plants and that in substrate suggests that *T. sacchariflora* is potentially useful for monitoring Cu and Cd contamination. Its properties, such as broad ecological amplitude, high biomass, high tolerance, retention and accumulation to Cu and Cd, make *T. sacchariflora* an effective plant for phytoremediation of Cu-contaminated and Cd-contaminated areas, minimizing the migration of Cu and Cd to reduce the risk of their entry into the food chain.

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