Potential Phytoremediation of Soil Cadmium and Zinc by Diverse Ornamental and Energy Grasses

Xincun Hou,[a] Wenjun Teng,[a] Yanxia Hu,[a] Zhichen Yang,[a] Cui Li,[a] John Scullion,[b] Qiang Guo,[a] and Ruilun Zheng [a,*]

The potential of 32 frequently studied ornamental and/or energy grasses and two cadmium/zinc hyperaccumulators for phytoextraction and phytostabilization was compared by their growth in a historically contaminated soil over a three-month pot experiment. Shoot and root biomasses varied by factors of 14.2 and 62.7, respectively. Mainly due to their large biomass, Napier grass (*Pennisetum purpureum* 'Purple') and variegated giant reed (*Arundo donax* var. *versicolor*) accumulated cadmium and zinc contents in shoots up to 109.3% and 55.4% higher, respectively, than those in the cadmium/zinc hyperaccumulators, despite their lower metal concentrations. *Pennisetum purpureum* 'Purple' accumulated the most zinc and the third highest cadmium in roots. Bioconcentration factors of cadmium in roots were greater than 1 for 19 grasses. The present study demonstrated that many of these grasses may be suitable for phytostabilization of soil cadmium. *Arundo donax* var. *versicolor* exhibited the most potential for phytoextraction of soil zinc, whereas *Pennisetum purpureum* 'Purple' was best for phytoextraction and phytostabilization of cadmium and phytostabilization of zinc. Ornamental/energy grasses may have greater potentials for soil remediation than hyperaccumulators, especially given their utility and eco-economic benefits. The considerable variation in their performance emphases the value of screening to select the most effective candidates.

Keywords: Hyperaccumulator; Heavy metal; Bioconcentration factor; Phytostabilization; Phytoextraction

*Contact information: a: Research & Development Center for Grasses and Environment, Beijing Academy of Agriculture and Forestry Sciences, Beijing, 100097 China; b: Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Wales, SY23 3DA UK; *Corresponding author: rlzheng@163.com*

INTRODUCTION

Soil contamination by heavy metals is a global problem of great current interest. More than 5 million sites with an area of 20 million hectares have been contaminated by various toxic metal(loid)s globally (He *et al*. 2015; Liu *et al*. 2018). Among the toxic metal(loid)s, cadmium (Cd) is more easily taken up by crops and into the food chain due to its high mobility in soil; it is the most common toxic element (Khan *et al*. 2015; Zhao *et al*. 2015; O'Connor *et al*. 2018). Cadmium is considered a potential human carcinogen by the International Agency for Research on Cancer of the World Health Organization. Excessive Cd exposure could result in renal dysfunction, bone lesions, teratogenic effects, and other diseases (Nordberg *et al*. 2002; Zhang *et al*. 2014). Therefore, Cd is considered a critical toxic metal threatening food safety and human health (Zhao *et al*. 2015; Shahid *et al*. 2016; Huang *et al*. 2017; Rizwan *et al*. 2017). Zinc (Zn) is a common co-occurring element of Cd, also frequently reported in connection to soil contamination (Lorenz *et al*. 1997; Stephan *et al*. 2008; He *et al*. 2015).

Many remediation techniques for Cd/Zn contaminated soils have been developed (He *et al*. 2015; Liu *et al*. 2018). Among these techniques, phytoremediation is favoured due to its advantages such as the improvement of soil physicochemical and biological properties, lower cost, and easy accessibility (Sarwar *et al*. 2017; Chen *et al*. 2019). Phytoremediation mainly involves removing or stabilizing heavy metals from/in soil using green plants, processes termed phytoextraction or phytostabilization, respectively. For phytoextraction, many Cd/Zn hyperaccumulators have been reported (Reeves *et al*. 2017). However, these hyperaccumulators are usually habitat limited, slow-growing with small biomass and have shallow root systems. In addition they are hard to cultivate, making hyperaccumulators difficult to utilise in practice. Regarding phytostabilization, the mobility of heavy metals in soils can be effectively reduced *via* root adsorption/absorption, rhizosphere complexation, and soil and water conservation by plants to mitigate the ecological risks of contaminated sites (Erakhrumen *et al*. 2007; Chen *et al*. 2018).

Some non-hyperaccumulators such as ornamental or energy grasses have been evaluated for their heavy metal phytoextraction or phytostabilization potential because of their larger biomass, better adaptability, perennation, higher stress tolerance, use in landscape restoration, considerable metal accumulation, and utility for biofuel and absorbent production (Gong *et al*. 2018; Pogrzeba *et al*. 2018). Papazoglou *et al.* (2005) reported that physiological indices including biomass, plant height, and photosynthetic rates were unaffected in giant reed (*Arundo donax*, AD) when DTPA-extractable concentrations of Cd and nickel both exceeded 350 mg/kg. The shoot Cd concentration reached up to 106 mg/kg for switchgrass when soil Cd concentration was 61.4 mg/kg (Chen *et al*. 2011). For Cd the amount accumulated in shoots of hybrid pennisetum (*Pennisetum americanum* × *P. purpureum*, PAP) could reach 0.62 mg per plant when grown in soil with a Cd concentration of 8 mg/kg (Zhang *et al*. 2010), in comparison to 0.24 mg accumulated in shoots of the Cd/Zn hyperaccumulator Alpine penny-cress (*Noccaea caerulescens*, NC) at 20.3 mg Cd/kg in soil (Perronnet *et al*. 2003). The amount of Zn accumulation in shoots of AD and Amur Silvergrass (*Miscanthus sacchariflorus*, MS) could reach 15 and 7.1 mg per plant, respectively, after the two grasses were grown for 60 days in soil with up to 2000 mg/kg Zn addition (Li *et al*. 2014). In addition to phytoextraction, ornamental or energy crops have the potential to stabilize soil Cd with root exudates or *via* their robust root systems (Guo *et al*. 2017; Song *et al*. 2018). Several grass species stabilize soil Cd, with large root biomass and high Cd quantities found in roots (Golda and Korzeniowska 2016; Phusantisampan *et al*. 2016). However, there have been few studies comparing ornamental or energy grasses with a hyperaccumulator for phytoremediation potential and ranking their capacity for phytoextraction or phytostabilization when grown under the same conditions. Assessing the capacity of grasses to remediate Cd/Zn contaminated soil in comparison to hyperaccumulator species would provide information about the feasibility of using ornamental or energy grasses for soil remediation, in particular given their wider economic and ecological benefits.

The present study investigated 32 species/cultivars of ornamental and/or energy grasses covering four genera for phytoremediation (phytoextraction or phytostabilization) of a historically Cd and Zn contaminated soil. Two Cd/Zn hyperaccumulators were also included in the study for comparison. It was predicted that the high biomass of some grasses compared with the hyperaccumulators would compensate for their lower metal concentrations and that some grasses would therefore be as, or more effective, as the hyperaccumulators in soil remediation. The findings could also inform the selection of suitable grasses for phytoremediation in practice, the development of good grass cultivars for this purpose, and further research into mechanisms underlying remediation potential.

EXPERIMENTAL

Materials

The soil was collected from a lead-zinc mining area from Zhuzhou city, Hunan province, China (27°52.412'N, 113°4.193'E) and was historically heavily contaminated mainly by Cd and Zn. The cadmium, Zn, and lead (Pb) concentrations in the soil were 160.3, 6.3, and 0.71 times respectively, the screening values of the risk control standard for soil contamination of agricultural land in China (GB/T 15618 2018). After air drying, the soil was ground and passed through a 2 mm-sieve. Peat was used for plant cultivation before plants were transferred to contaminated soil. The physicochemical properties and heavy metal concentrations of the soil and peat are listed in Table 1.

Table 1. Physicochemical Properties of Soil and Peat Used

Sample	pH	TOC (%)	TN (%)	CEC (cmol/kg)	Cd* (mg/kg)	Zn (mg/kg)	Pb (mg/kg)
Soil	7.2	1.67	0.13	20.7	48.1	1575	84.6
Peat	5.1	35.7	2.4	—	0.17	30.2	17.5

* Risk screening values of Cd, Zn and Pb in Risk Control Standard for Soil Contamination of Agricultural Land in China (GB15618-2018) are 0.3, 250 and 120 mg/kg, respectively, at soil pH 6.5-7.5

A total of 34 herbaceous plants including 32 different species/cultivars of ornamental and/or energy grasses covering four genera and two Cd/Zn hyperaccumulators were collected. The common names, Latin names, and abbreviations are listed in Table 2. *Pennisetum* grasses included 13 species/cultivars. The PSI, PAP, PP, and PPP species/cultivars are characterized by large biomass and are mainly usable as energy grasses; the other nine species/cultivars are often used for ornamental purposes. The eight cultivars of *Panicum virgatum* and nine species/cultivars of *Miscanthus* investigated are mainly considered as energy grasses but are often applied in landscape restoration. All 32 grasses are routinely planted and managed for energy production, landscape improvement, or soil and water conservation. *Noccaea caerulescens* and ILC are considered as Cd and Zn hyperaccumulators and Cd hyperaccumulators, respectively (McGrath and Zhao 2003; Han *et al*. 2007; Guo *et al*. 2017; Reeves *et al*. 2017).

Plant Cultivation

The Cd hyperaccumulator ILC was propagated through tiller separation, and the 32 grasses were grown from cuttings. Regarding the use of cuttings, uniform rhizomes each with one intact bud were selected, and stalks were clipped with pruning shears to a uniform size (7-cm height) (Fig. S1). For tiller separation, ramets were clipped to get uniform seedlings (7-cm height, 4-cm root length) after stock plants were divided, and planted into peat (Fig. S2). All ramets and rhizomes were cultivated in the prepared peat and irrigated with deionized water regularly. All rhizomes or ramets were planted on the same day.

Table 2. Plant Materials Used in the Present Study

No.	Common name	Latin Name	Abbreviation	Origin
1	Alpine penny-cress	*Noccaea caerulescens*	NC	Ganges, Southern France
2	Chinese Small Iris	*Iris lactea* var. *chinensis*	ILC	Zhaosu county, YIli prefecture, Xinjiang province, China
3	Napier grass	*Pennisetum purpureum* ‘Purple’	PPP	Guangdong province, China
4	Napier grass	*P. purpureum*	PP	Guangdong province, China
5	King grass	*P. sinese*	PSI	Introduced from Colombia in 2004
6	Hybrid pennisetum	*P. americanum* × *P. purpureum*	PAP	Introduced from Colombia in 2004
7	Changsui fountain grass	*P. alopecuroides* ‘Changsui’	PAC	Bred by RDCGE*
8	Liren fountain grass	*P. alopecuroides* ‘Liren’	PAL	Bred by RDCGE
9	Ziguang fountain grass	*P. alopecuroides* ‘Ziguang’	PAZ	Bred by RDCGE
10	Baijian fountain grass	*P. alopecuroides* ‘Baijian’	PAB	Bred by RDCGE
11	Aizhu fountain grass	*P. alopecuroides* ‘Aizhu’	PAA	Bred by RDCGE
12	Rubrum fountain grass	*P. setaceum* ‘Rubrum’	PSR	Yunnan province, China
13	Feathertop fountain grass	*P. villosum* R. Br. ex Fresen.	PVR	Introduced from Spain in 2003
14	Oriental fountain grass	*P. orientale*	PO	Introduced from Canada in 2005
15	Crimson fountain grass	*P. setaceum*	PSE	Introduced from Italy in 2007
16	Blackwell switchgrass	*Panicum virgatum* ‘Blackwell’	PVB	Northern Oklahoma 37°, USA (U*)
17	Cave in rock switchgrass	*P. virgatum* ‘Cave in rock’	PVC	South Illinois 38°, USA (U)
18	Forestburg switchgrass	*P. virgatum* ‘Forestburg’	PVF	South Dakota 44°, USA (U)
19	New York switchgrass	*P. virgatum* ‘New York’	PVN	New York 40°, USA (L)
20	Alamo switchgrass	*P. virgatum* ‘Alamo’	PVA	South Texas 28°, USA (L)
21	Kanlow switchgrass	*P. virgatum* ‘Kanlow’	PVK	Central Oklahoma 35°, USA (L)
22	Pathfinder switchgrass	*P. virgatum* ‘Pathfinder’	PVP	Nebraska/Kansas 40°, USA (U)
23	Trailblazer switchgrass	*P. virgatum* ‘Trailblazer’	PVT	Nebraska 40°, USA (U)
24	Changxu Miscanthus	*Miscanthus sinensis* ‘Changxu'	MSC	Bred by RDCGE
25	Xianxu Miscanthus	*M. sinensis* ‘Xianxu’	MSX	Bred by RDCGE
26	Hongsui Miscanthus	*M. sinensis* 'Hongsui'	MSH	Bred by RDCGE
27	Mihua Miscanthus	*M. sinensis* 'Mihua'	MSM	Bred by RDCGE
28	Gold Bar Miscanthus	*M. sinensis* ‘Gold Bar’	MSG	Bred by RDCGE
29	Zebrinus Miscanthus	*M. sinensis* ‘Zebrinus’	MSZ	Introduced from Canada in 2006
30	Yaku Jima Miscanthus	*M. sinensis* ‘Yaku Jima’	MSY	Introduced from Canada in 2006
31	Giant Miscanthus	*M. giganteus*	MG	Introduced from Germany in 2006
32	Amur Silvergrass	*M. sacchariflorus*	MS	Shanxi province, China
33	Giant Reed	*Arundo donax*	AD	Jiangsu province, China
34	Variegated Giant Reed	*A. donax* var. *versicolor*	ADV	Taiwan province, China

* “RDCGE” represent Research & Development Center for Grasses and Environment; “U” and “L” represent upland and lowland ecotypes of switchgrass, respectively

After 3 weeks growth, uniform seedlings were selected and washed thoroughly with deionized water to remove attached particles (Fig. S3). Afterward, these seedlings were transplanted into the contaminated soils and clipped to 7-cm height again.

Seed propagation was used for NC. After soaking in 30% H_2O_2 solution for 30 min and thorough washing with deionized water, the seeds were placed in petri dish with moist filter paper at 25 °C in the dark for 2 weeks (Fig. S4). Then, the germinated seeds were transferred to vermiculite for further cultivation, maintaining moisture *via* deionized water irrigation. After cotyledons were totally developed, they were watered with a nutrient solution. Seedlings were transplanted to peat and irrigated with deionized water after growing in vermiculite for 5 months. After 3 weeks' growth in peat, uniform seedlings were selected, washed and transplanted into contaminated soils on the same day as other herbaceous plants.

After being mixed thoroughly with fertilizers to avoid any nutrient limitation, the soil was potted (1.3 kg per pot: top/bottom diameter 15 cm/11 cm, depth 13 cm). Fertilizer inputs consisted of 200 mg nitrogen, 150 mg phosphorus, and 60 mg magnesium as urea, KH_2PO_4, and $MgSO_4{\cdot}7H_2O$, respectively, per kilogram soil (Fig. S5). One seedling was planted in contaminated soil per pot. Each plant species/cultivar was replicated four times. Pots were arranged in a randomized complete block design in a greenhouse with ambient lighting, 70% to 80% relative humidity and with 20 to 35°C/15 to 20°C day/night time temperatures. Deionized water was added according to regular weighing to hold soil moisture content at 60 to 70% of the water holding capacity.

After 3 months' growth, plant shoots were cut above soil and roots were removed carefully from soils by gentle sieving. Shoots and roots were washed thoroughly with deionized water, dried at 105°C for 0.5 h then at 70°C for 48 h, and then weighed. Roots were scanned into WinRhizo software (V5.0, Regent Instruments, Quebec, Canada) for the estimation of root length (RL) and root surface area (RSA) before drying (Zheng *et al.* 2013).

Analytical Methods

The physicochemical properties of soil and peat used were analyzed as described by Lu (2000). The pH values of soil and peat were measured in suspensions of solid substrate and water at a ratio of 1:2.5 using a pH meter. Total organic carbon and total nitrogen in soil and peat were determined using potassium dichromate oxidation and potassium sulfate oxidation-ultraviolet spectrophotometry methods. The cation exchange capacity of soil was analyzed using the ammonium acetate method. Soil and peat samples were finely ground (< 0.149 mm) then digested with concentrated HCl-HNO_3-$HClO_4$ (3:1:1) to assay total metal concentrations (Zheng *et al.* 2013).

Dried shoot and root samples were ground and digested using concentrated HNO_3 in a microwave digestion system (Guo *et al*. 2017). Cadmium and Zn in digestion solutions were analyzed using inductively coupled plasma mass spectroscopy (ICP-MS, Agilent 7500, Santa Clara, CA, USA). Blanks and certified reference materials (CRM) GBW07603 (bush twigs and leaves) and GBW07401 (soils) were included for quality assurance. The recovery ratios for reference samples ranged from 87 to 112%.

Data Analysis

The translocation factor (TF), calculated as the ratio of metal concentration in plant shoot to that in root, describes the movement of metals from root to shoot with implications for phytoextraction potential (Zheng *et al*. 2017). The bioconcentration factor of shoots (BCFS) and of roots (BCFR), defined as the ratios of metal concentrations in plant shoots and roots to that in soil respectively, indicate the potential of a plant for metal accumulation in shoot and root respectively at a given level of metal contamination (McGrath and Zhao 2003; Zheng *et al*. 2017). Metal amounts accumulated in plant shoot and root were equal to the products of their metal concentration and biomass. Uptake efficiency (UE) was calculated as the ratio of the metal amount in the whole plant, including shoot and root, to root dry weight.

All data that met the normal distribution condition were analyzed with one-way analysis of variance. The least significant difference test ($p < 0.05$) was used for mean comparisons for data that met the conditions of homogeneous variance, while Tamhane's T2 test was used if equal variance of the data was not assumed. Data with a non-normal distribution were analyzed with the Kruskal-Wallis test. Pearson and Spearman analysis was conducted to examine the relationships between Cd and Zn in shoot and root concentrations, TFs and UEs. These tests were also used to study relationships between Cd and Zn accumulation and rooting parameters. Multiple linear regressions using a stepwise method were employed to examine relationships between Cd/Zn concentrations in shoot or root and the rooting parameters. All statistical analysis was performed using SPSS 22.0 software (IBM, Armonk, New York, USA).

RESULTS AND DISCUSSION

Plant Growth

After 3 months' growth in the contaminated soil, the 32 grasses did not exhibit visual toxicity symptoms except for MSY, which ceased growth after two weeks. Among the other grasses, the six with the largest shoot dry weight (SW) were PSI, PAP, PP, PPP, ADV, and AD, whose SW was 10.7 to 14.2 times that of PAB with the smallest SW ($p < 0.05$) (Fig. 1a). The three grasses with the largest root dry weight (RW) were PVR, PP and PSI, 45.9 to 62.7 times that of NC with the smallest RW ($p < 0.05$) (Fig. 1b).

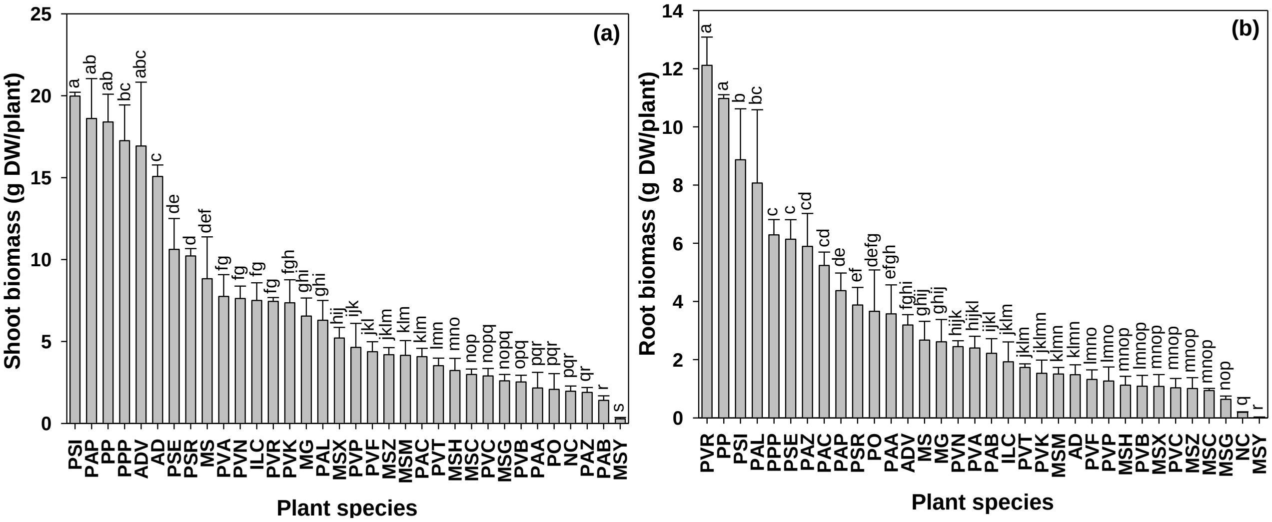

Fig. 1. Shoot (a) and root (b) dry weight of 32 grasses and 2 hyperaccumulators (mean ± SD, n = 4). Means with a common letter above columns do not differ at $p < 0.05$.

Considering findings within each genus or species, for *Pennisetum* there were slight differences in SW between PSI, PAP, PP, and PPP, yet RW differences were larger in the order PP > PSI > PPP > PAP with a 2.5 times range difference ($p < 0.05$). Among the other nine species/cultivars, PSE had the largest SW, 7.5 times that of PAB with the lowest SW ($p < 0.05$). The RW of PAB was 81.7% less than that of PVR ($p < 0.05$) (Fig. 1b). For *Panicum virgatum*, the eight cultivars had high variability, the largest PVA being 3.1 times the SW of the smallest. *P. virgatum* 'New York' had the largest RW 2.4 times that of PVC the smallest ($p < 0.05$). Biomass of the three lowland cultivars (PVA, PVK, PVN) was generally greater than that of the five upland cultivars (Fig. 1a, b). Among nine species/cultivars of *Miscanthus*, excluding MSY, SW of MS was largest following by MG, and MSG had the smallest SW. Shoot dry weight of MS was 3.4 times that of MSG. RW varied by a factor of 4.2 and the sequence of RW was similar to that of shoots. Biomass of *M. sinensis* was generally smaller than that of MG and MS (Fig. 1a, b). There was no significant difference in SW between AD and ADV, yet RW for ADV was 2.2 times that for AD ($p < 0.05$). *Iris lactea* var. *chinensis* and NC had an intermediate and a small biomass respectively, relative to all herbaceous plants (Fig. 1).

Cd and Zn Concentrations for Various Plants

The shoot Cd concentration of NC (475 mg/kg) was far greater (10.0 to 297 times) that of the 32 grasses (1.6 to 47.6 mg/kg) ($p < 0.05$). *Iris lactea* var. *chinensis* had the second highest shoot Cd concentration statistically similar to that of PPP and 1.3 to 33.1 times of the other 31 grasses ($p < 0.05$). The two grasses with highest shoot Cd concentrations were PPP and PAP, 25.1 to 29.8 times of that for PAC with the lowest Cd concentration ($p < 0.05$) (Fig. 2a). *M. sinensis* 'Yaku Jima' that showed distinct toxicity symptoms had the highest Cd concentration in roots, followed by NC and MSX. The lowest root Cd concentration found was for PO, followed by PAZ and PAL. Excluding MSY, root Cd concentrations varied by a factor of 12.3 among grasses (Fig. 2b).

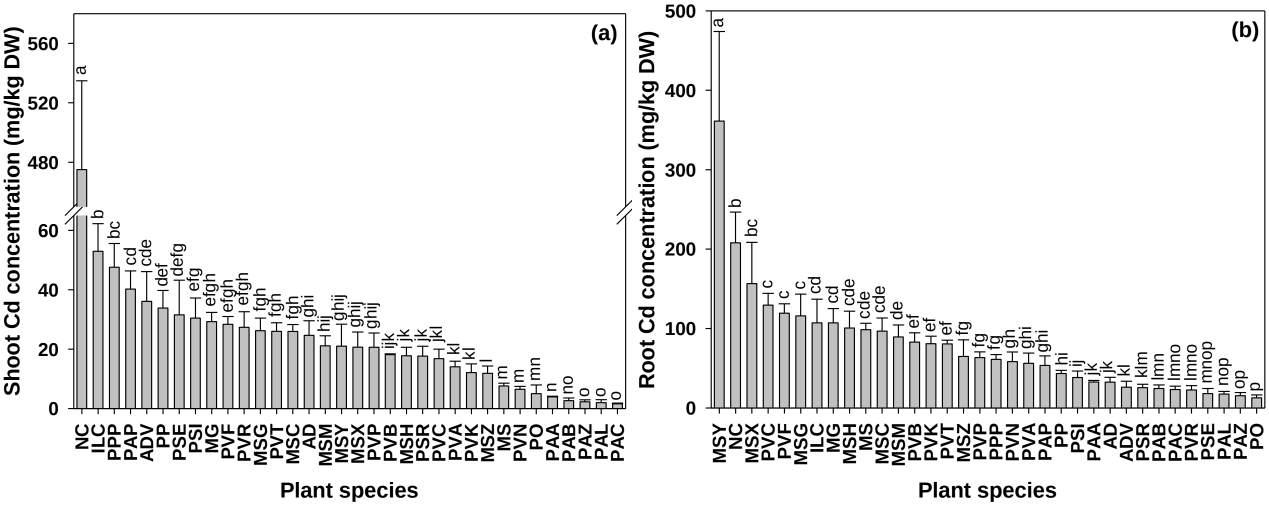

Fig. 2. Cadmium concentration in shoot (a) and root (b) of 32 grasses and 2 hyperaccumulators (mean ± SD, n = 4). Means with a common letter above columns do not differ at $p < 0.05$.

Considering species/cultivars of each genus/species, shoot Cd concentrations for *Pennisetum*, *Miscanthus*, and *Panicum virgatum* varied by factors of 29.8, 3.9 and 4.4 ($p < 0.05$) respectively, and with PPP, MG, PVF having the highest shoot Cd concentrations

(Fig. 2a). Root Cd concentrations among species/cultivars of *Pennisetum*, *Miscanthus*, and *Panicum virgatum* varied by factors of 4.8, 5.6, and 2.3, respectively ($p < 0.05$), with PPP, MSY, and PVC having the highest concentration. There was no significant difference in root Cd concentrations between AD and ADV, but there was a higher shoot Cd concentration in ADV ($p < 0.05$). Root Cd concentrations of the thirteen species/cultivars of *Pennisetum* genus were generally low in the ranking of the grasses. The lowland cultivars (PVA, PVK, PVN) had generally lower shoot Cd concentrations than the upland cultivars for *Panicum virgatum*. Root Cd concentrations of the nine species/cultivars of *Miscanthus* generally ranked high among the grasses (Fig. 2b).

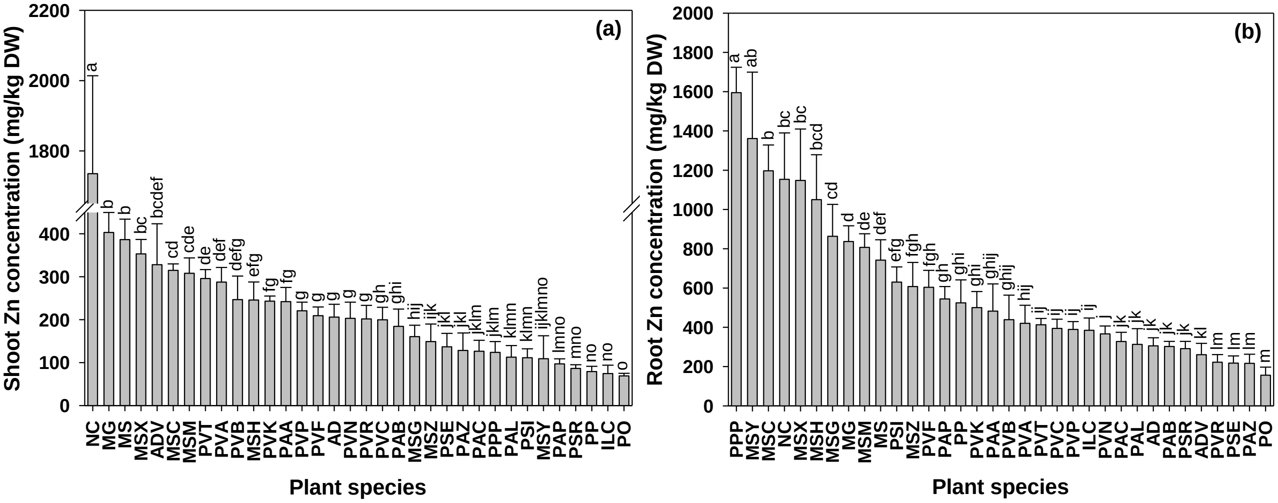

Fig. 3. Zinc concentration in shoot (a) and root (b) of 32 grasses and 2 hyperaccumulators (mean ± SD, n = 4). Means with a common letter above columns do not differ at $p < 0.05$.

Among grasses, MG had the highest shoot Zn concentration, followed by MS and MSX, with PO having the lowest concentration. Shoot Zn concentration of MG was 5.9 times that of PO. Shoot Zn concentration of NC was far higher than in the grasses, 4.3 times that of MG ($p < 0.05$). Though it had high shoot Cd concentration, ILC had the second lowest shoot Zn concentration (Fig. 3a). Root Zn concentrations varied by a factor of 11.2 among the grasses. The highest root Zn concentration was found in PPP, followed by MSY and MSC. *P. orientale* had the lowest Zn concentration in roots (Fig. 3b).

Within each genus/species, shoot Zn concentrations for *Pennisetum*, *Miscanthus*, and *Panicum virgatum* varied by factors of 3.5, 3.7, and 1.5 respectively ($p < 0.05$), with PAA, MG, PVT having the highest Zn concentrations in shoots (Fig. 3a). The root Zn concentrations within each genus/species for *Pennisetum*, *Miscanthus*, and *Panicum virgatum* varied by factors of 10.2, 2.2, and 1.7 respectively ($p < 0.05$), with PPP, MSY, and PVF having the highest Zn concentrations (Fig. 3b). There was no significant difference in root Zn concentrations between ADV and AD, though shoot Zn concentration of ADV was 59.5% higher than that of AD ($p < 0.05$). Root Zn concentrations of the nine species/cultivars of *Miscanthus* were generally high in the ranking of the grasses (Fig. 3b).

Cd and Zn Amounts Accumulated in Various Plants

Total Cd accumulations in shoots of NC, PPP, and PAP did not differ significantly ($p < 0.05$) and were larger than those of the other grasses. Grasses such as

PPP, PAP, PP, PSI, and ADV accumulated 49.9% to 109.3% more Cd than the hyperaccumulator ILC. Shoot Cd contents varied markedly among all 34 plants by a factor of 318, and among the grasses by a factor of 281 (Fig. 4a). The largest Cd amount in roots was accumulated by PP, followed by PSI and PPP. Notably, the second lowest root Cd accumulation was in NC. Cadmium accumulated in roots of PP was 12.0 times that of NC ($p < 0.05$) (Fig. 4b).

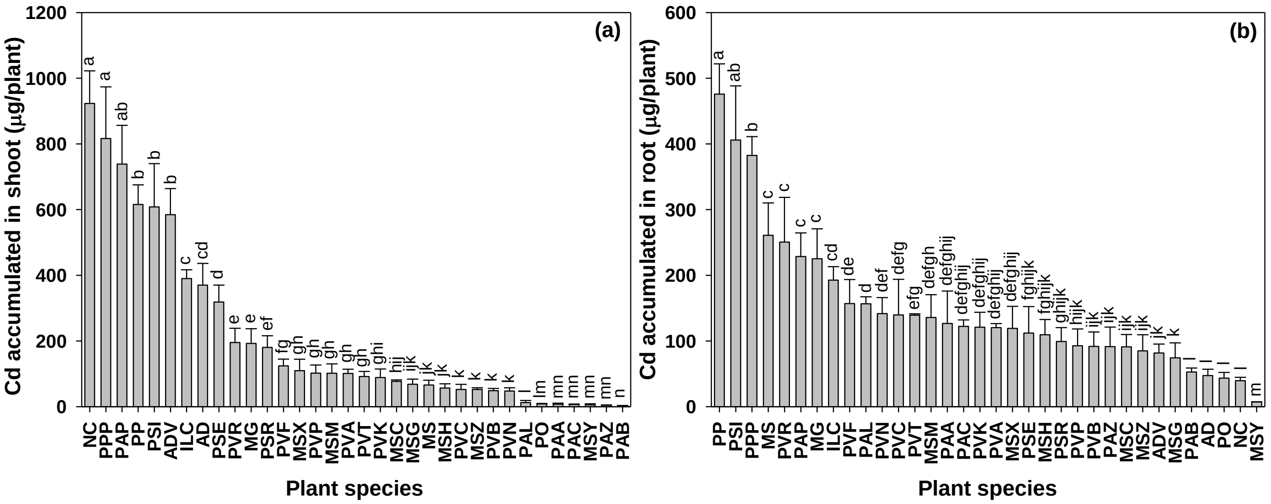

Fig. 4. Cadmium amount in shoot (a) and root (b) of 32 grasses and 2 hyperaccumulators (mean ± SD, n = 4). Means with a common letter above columns do not differ at $p < 0.05$.

Considering species/cultivars of each genus/species, shoot Cd amounts for *Pennisetum*, *Miscanthus*, and *Panicum virgatum* varied by factors of 281, 33.5 and 2.6, respectively ($p < 0.05$), with PPP, MG, PVF having the highest Cd amounts in shoots. Root Cd amounts in species/cultivars of each genus/species of *Pennisetum*, *Miscanthus*, and *Panicum virgatum* varied by factors of 10.9, 36.1, and 1.7 ($p < 0.05$), respectively, with PP, MS, PVF having the highest Cd amounts in root. Total Cd amounts in shoots and roots of ADV were 57.9% and 72.5% greater than those of AD respectively ($p < 0.05$). Shoot Cd amounts of ADV and AD ranked high among the grasses but root Cd amounts ranked lowly.

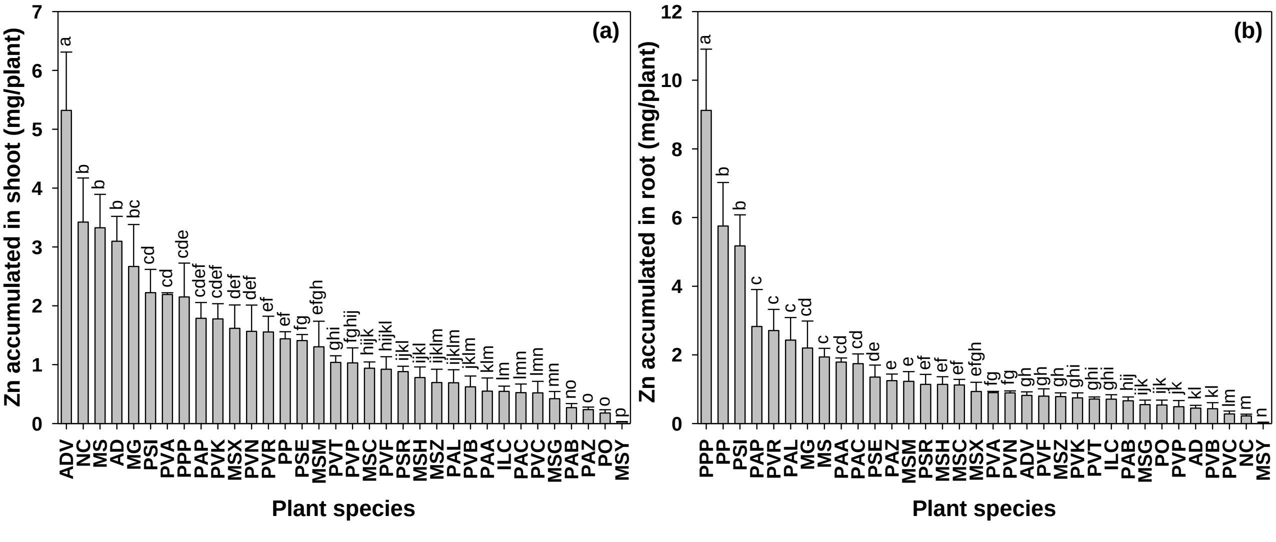

Fig. 5. Zinc amount accumulated in shoot (a) and root (b) of 32 grasses and 2 hyperaccumulators (mean ± SD, n = 4). Means with a common letter above columns do not differ at $p < 0.05$.

Regarding Zn amounts in shoots, it was largest for ADV, followed by NC, MS, and AD. Shoot Zn amount in ADV was 55.4% greater than that of NC ($p < 0.05$). *M. sinensis* ‘Yaku Jima’ accumulated the smallest amount of Zn in shoots, followed by PO and PAZ. Shoot Zn amounts varied by a factor of 29.9 between ADV and PO (Fig. 5a). *Pennisetum purpureum* ‘Purple’ accumulated the largest Zn amount in roots, followed by PP, PSI, and PAP. The smallest amount of Zn in roots was accumulated by MSY, followed by NC and PVC. Root Zn accumulated by PPP was 41.1 times of that by NC ($p < 0.05$) (Fig. 5b).

Regarding species/cultivars of each genus/species, shoot Zn amounts for *Pennisetum*, *Miscanthus*, and *Panicum virgatum* varied by factors of 12.1, 122, and 4.2 ($p < 0.05$), with PPP, MS, PVA having the highest Zn amounts in shoots (Fig. 5a). Root Zn amounts in species/cultivars for Pennisetum, *Miscanthus*, and *Panicum virgatum* varied by factors of 16.8, 77.1, and 3.2 ($p < 0.05$), with PPP, MG, PVA having the highest Zn amounts in roots (Fig. 5b). Both ADV and AD accumulated high amounts of Zn in shoots but not in roots. Zinc amounts in shoots and roots of ADV were 71.8% and 82.9% greater than those of AD, respectively ($p < 0.05$). Zinc amounts accumulated in roots of species/cultivars of *Pennisetum* ranked high (1.1 to 9.1 mg/plant) except for PAB and PO (0.54 to 0.66 mg/plant) among the grasses. Lowland cultivars of *Panicum virgatum* (PVA, PVK, PVN) accumulated more Zn in shoots than upland ones in general.

BCFS, BCFR, TF, and UE of Cd and Zn for Various Plants

Only NC had a BCFS of Cd markedly greater than 1. The BCFSs of Cd for ILC and PPP were slightly higher than 1. *Noccaea caerulescens* had a BCFS 9.0 and 9.5 times those of ILC and PPP respectively. Bioconcentration factors of shoots varied by a factor of 31.2 among the grasses (Fig. 6a). The BCFSs of Cd for various species/cultivars of each of the genus/species of *Pennisetum*, *Miscanthus*, *Panicum virgatum*, and *Arundo donax* ranged from 0.03 to 1.04, 0.25 to 0.61, 0.13 to 0.59, and 0.51 to 0.75, respectively. There were 19 grasses whose BCFRs were larger than 1. *Noccaea caerulescens* had the highest BCFR (4.3), 3.9 times that of the PAP BCFR (1.1) ($p < 0.05$) (Fig. 6b). The bioconcentration factors of roots of Cd for various species/cultivars of *Pennisetum*, *Miscanthus*, *Panicum virgatum*, and *Arundo donax* ranged from 0.27 to 1.27, 1.35 to 7.51, 1.17 to 2.69, and 0.54 to 0.68, with 15.4%, 100%, 100%, and 0% of species/cultivars having a BCFR exceeding 1 respectively.

Among the plants tested, only NC had a BCFS of Zn (1.1) exceeded 1, the grasses having low BCFSs (0.04 to 0.26). The Cd hyperaccumulator ILC had the second lowest BCFS for Zn. Only PPP had a Zn BCFR (1.01) higher than 1, with the others (0.10 to 0.86) less than 1 (Fig. 7).

The TFs of Cd for NC, PSE, ADV and PVR were larger than 1, and not significantly different; that for NC was 1.4 to 38.7 times those for the grasses ($p < 0.05$). The TF of Cd for ILC was lower than 1. *M. sinensis* ‘Yaku Jima’, which showed severe toxicity symptoms had the lowest TF, followed by PAC and PAL. Cadmium TFs of species/cultivars for *Pennisetum*, *Miscanthus*, *Panicum virgatum*, and *Arundo donax* varied from 0.07 to 1.72, 0.06 to 0.28, 0.13 to 0.32, and 0.76 to 1.38 respectively. For Zn, only NC and ADV had TFs larger than 1, and these TFs did not differ significantly. Zinc TF for MSY was the smallest followed by PPP, then PP. The TFs of Zn among the 34 plants varied by a factor of 25.0 and among the 32 grasses by 20.6 (Table 3). Zinc TFs of various species/cultivars for *Pennisetum*, *Miscanthus*, *Panicum virgatum,* and *Arundo donax* ranged from 0.08 to 0.92, 0.06 to 0.52, 0.35 to 0.72 and 0.67 to 1.3, respectively. *Miscanthus* and *Arundo donax* ranked low and high in TFs among grasses respectively, for both Cd and Zn.

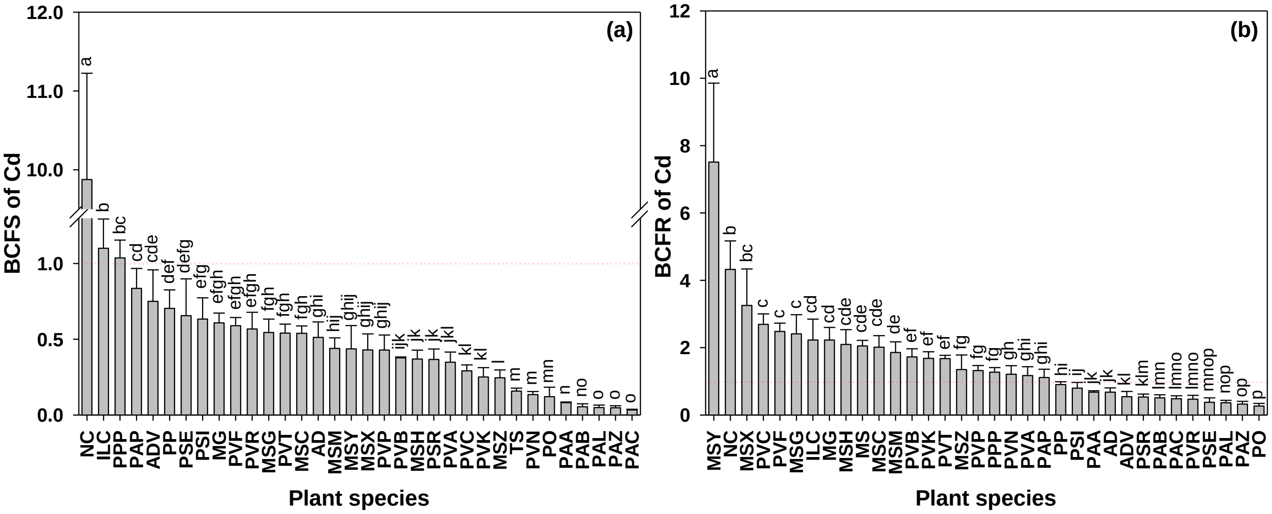

Fig. 6. The BCFS (a) and BCFR (b) of Cd for 32 grasses and 2 hyperaccumulators (mean ± SD, n = 4). Means with a common letter above columns do not differ at $p < 0.05$.

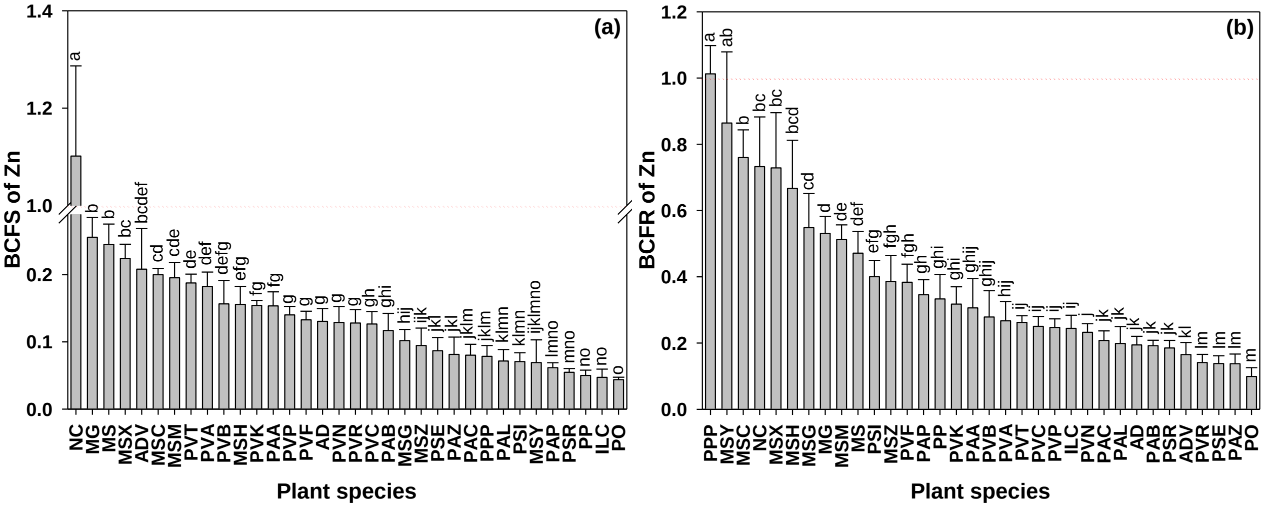

Fig. 7. The BCFS (a) and BCFR (b) of Cd for 32 grasses and 2 hyperaccumulators (mean ± SD, n = 4). Means with a common letter above columns do not differ at $p < 0.05$.

Uptake efficiencies (UEs) were calculated to compare the Cd/Zn uptake capacity of plant roots based on Cd/Zn amount accumulated in the whole plant. The UEs of Cd and Zn for NC were respectively 10.7 to 327 and 6.9 to 96.6 times those of the grasses ($p < 0.05$) (Table 3). *Iris lactea* var. *chinensis* had the third largest UE of Cd after NC and MSY, 21.5 times that of PO ($p < 0.05$), and had an intermediate UE for Zn. Among the grasses, PO had the lowest UE for Cd and Zn. For the UE of Zn, MSX had the highest value of the remaining grasses, 13.8 times that of PO ($p < 0.05$).

In order to ascertain whether Cd and Zn behave in a similar fashion in uptake by roots and translocation from root to shoot, correlations of shoot concentrations, root concentrations, UE and TF between Cd and Zn were analyzed; this analysis also had the potential to provide some basis for proposing mechanisms for the variations observed. Among the 32 grasses, root Cd concentrations were positively correlated to root Zn concentrations ($p < 0.0001$) (Fig. 8b), but this was not the case for shoots (Fig. 8a). The UEs of Cd were positive correlated to those of Zn ($p < 0.0001$) (Fig. 8c) but their TFs were not (Fig. 8c, d).

Table 3. TF and UE of Cd and Zn for Various Grasses and 2 Hyperaccumulators

No.	Plant species	TF		UE (mg/g root)	
		Cd	Zn	Cd	Zn
1	NC	2.34 ± 0.45 a	1.52 ± 0.15 a	5.02 ± 0.54 a	18.9 ± 3.97 a
2	ILC	0.51 ± 0.06 e	0.19 ± 0.02 rs	0.33 ± 0.10 b	0.70 ± 0.19 kmo
3	PPP	0.78 ± 0.13 d	0.08 ± 0.01 t	0.19 ± 0.03 bcde	1.82 ± 0.43 cdef
4	PAP	0.84 ± 0.25 d	0.18 ± 0.02 rs	0.23 ± 0.05 bc	1.09 ± 0.38 hijkl
5	PSI	0.80 ± 0.13 d	0.17 ± 0.02 s	0.11 ± 0.01 hij	1.09 ± 0.39 hijkl
6	PP	0.78 ± 0.09 d	0.15 ± 0.02 s	0.10 ± 0.01 ij	0.66 ± 0.12 lmo
7	PAC	0.07 ± 0.01 k	0.39 ± 0.09 klmno	0.02 ± 0.00 lm	0.43 ± 0.06 pq
8	PAL	0.07 ± 0.01 k	0.37 ± 0.05 mno	0.02 ± 0.01 lm	0.44 ± 0.13 opq
9	PSR	0.69 ± 0.06 d	0.30 ± 0.05 opq	0.07 ± 0.02 k	0.53 ± 0.06 mnop
10	PAB	0.11 ± 0.03 jk	0.60 ± 0.15 cdefghijk	0.03 ± 0.01 lm	0.41 ± 0.08 pq
11	PAA	0.12 ± 0.00 j	0.52 ± 0.10 ijkm	0.03 ± 0.00 l	0.62 ± 0.16 lmnop
12	PVR	1.22 ± 0.11 c	0.92 ± 0.13 b	0.04 ± 0.01 l	0.35 ± 0.05 qr
13	PO	0.37 ± 0.11 ef	0.53 ± 0.14 ghijklm	0.02 ± 0.00 m	0.20 ± 0.03 s
14	PSE	1.72 ± 0.15 b	0.63 ± 0.12 cdefghij	0.07 ± 0.01 k	0.45 ± 0.02 nop
15	PAZ	0.12 ± 0.03 jk	0.59 ± 0.11 cdefghij	0.02 ± 0.00 m	0.26 ± 0.06 rs
16	PVB	0.21 ± 0.03 fgh	0.53 ± 0.22 fghijklm	0.12 ± 0.03 ghi	1.09 ± 0.12 ij
17	PVC	0.13 ± 0.01 j	0.51 ± 0.05 jk	0.18 ± 0.02 def	0.97 ± 0.07 j
18	PVF	0.24 ± 0.04 fgh	0.35 ± 0.07 mnop	0.22 ± 0.02 bcd	1.32 ± 0.11 ghi
19	PVN	0.14 ± 0.04 ij	0.55 ± 0.07 efghij	0.09 ± 0.03 jk	1.01 ± 0.24 ijk
20	PVA	0.30 ± 0.06 f	0.70 ± 0.11 cefgi	0.10 ± 0.02 ijk	1.51 ± 0.43 fghi
21	PVK	0.15 ± 0.03 ij	0.49 ± 0.06 jk	0.14 ± 0.03 fg	1.7 ± 0.25 defg
22	PVP	0.32 ± 0.04 f	0.57 ± 0.01 defghij	0.13 ± 0.03 gh	1.07 ± 0.19 ij
23	PVT	0.32 ± 0.04 f	0.72 ± 0.05 cf	0.13 ± 0.01 g	1.01 ± 0.06 j
24	MSX	0.13 ± 0.02 j	0.32 ± 0.05 nop	0.25 ± 0.06 bc	2.71 ± 0.27 b
25	MSZ	0.19 ± 0.03 ghi	0.25 ± 0.06 pqr	0.11 ± 0.02 ghij	1.16 ± 0.25 hij
26	MSY	0.06 ± 0.02 k	0.06 ± 0.01 t	0.47 ± 0.09 b	2.73 ± 0.83 bcd
27	MSH	0.18 ± 0.03 hi	0.24 ± 0.03 qr	0.15 ± 0.03 efg	1.78 ± 0.37 cdef
28	MSM	0.24 ± 0.04 fgh	0.38 ± 0.01 lmn	0.15 ± 0.02 fg	1.66 ± 0.23 defgh
29	MSG	0.23 ± 0.05 fgh	0.19 ± 0.04 rs	0.22 ± 0.04 bc	1.51 ± 0.20 efgh
30	MG	0.28 ± 0.05 f	0.48 ± 0.07 jkm	0.18 ± 0.02 cdef	1.87 ± 0.20 cdef
31	MSC	0.28 ± 0.07 fg	0.27 ± 0.03 pq	0.18 ± 0.02 def	2.20 ± 0.23 c
32	MS	0.08 ± 0.01 k	0.52 ± 0.05 hijk	0.12 ± 0.01 ghi	2.00 ± 0.18 cdf
33	AD	0.76 ± 0.07 d	0.67 ± 0.02 cfg	0.29 ± 0.09 b	2.51 ± 0.76 bcdf
34	ADV	1.38 ± 0.12 c	1.26 ± 0.22 a	0.21 ± 0.03 bcd	1.93 ± 0.32 cdef

Results are expressed as mean ± SD (n = 4). Means with a common letter do not differ at $p < 0.05$.

DISCUSSION

Such an extensive comparison of the ornamental and/or energy grasses with Cd/Zn hyperaccumulators in the same growing conditions and using in-situ historically contaminated soil has not been previously reported. The grasses used covered 4 genera and included cultivars/species within these genera. Their potential for both phytoextraction and phytostabilization for Cd and Zn was compared. Substantial Cd amounts were accumulated in shoots of PPP and PAP, similar to those of the Cd/Zn hyperaccumulator NC and greater than that of Cd hyperaccumulator ILC, respectively; in shoots of ADV Zn amounts were higher than for NC. Cadmium BCFRs for 19 out of 32 grasses exceeded 1 but Zn BCFR exceeded one only for PPP. *Pennisetum purpureum* ‘Purple’ exhibited the most potential for phytoextraction of Cd and phytostabilization of both Cd and Zn. *A. donax* var. *versicolor* was the best candidate for Zn phytoextraction. There were very large variations between grasses in terms of both remediation mechanisms, underlying the value in screening for their potential and further research into the basis of this variation.

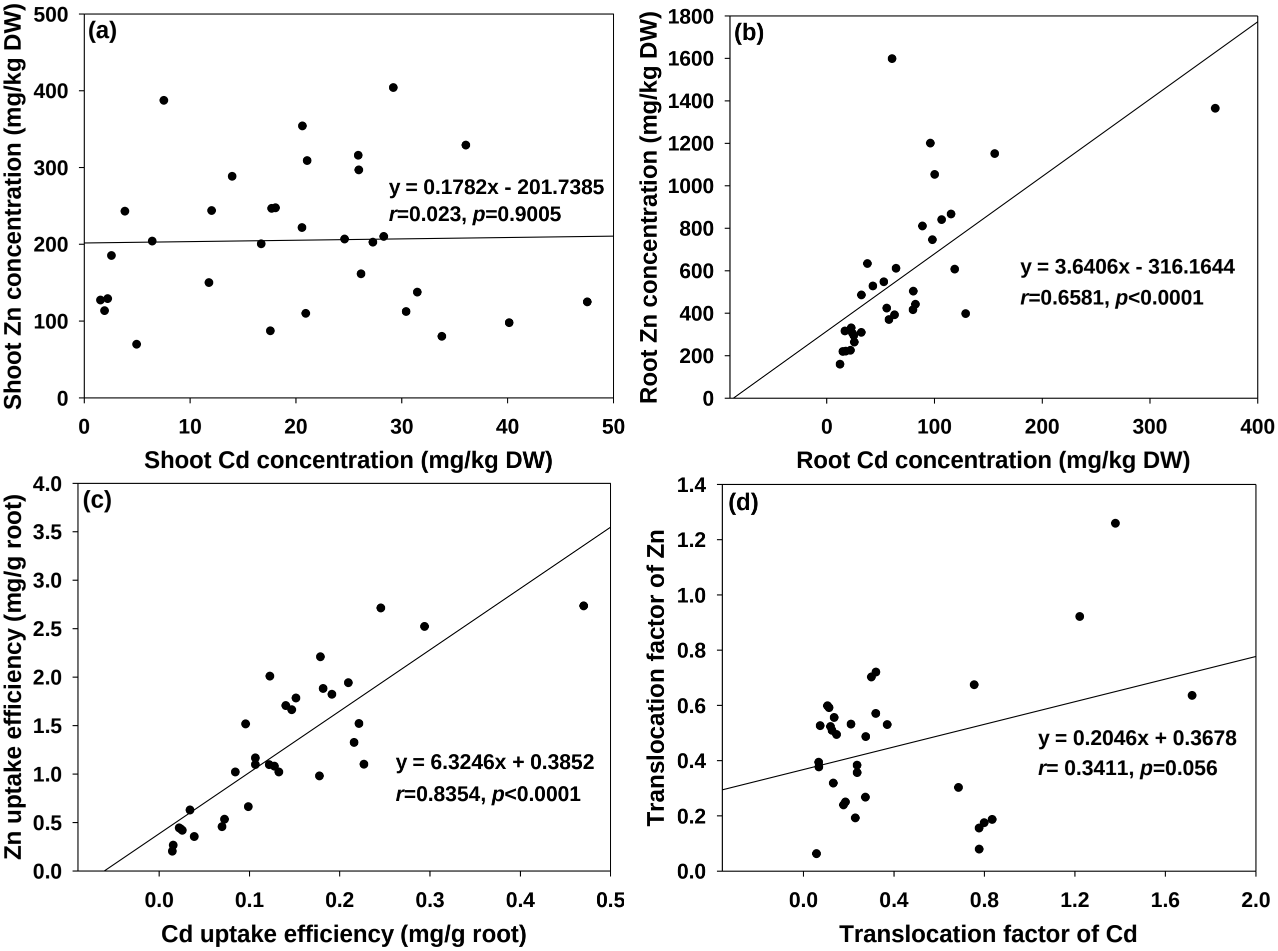

Fig. 8. Correlations between Cd and Zn as for their shoot concentrations (a), root concentrations (b), uptake efficiencies (c), and translocation factors (d) for 32 grasses.

Though shoot Cd concentrations in the *Pennisetum* spp. PPP and PAP were lower than NC and ILC, this was counter-balanced by their large SW (Figs. 1a, 2a, 4a). Similar results were obtained in other studies. For example though Cd concentrations in aerial part of *Pennisetum* sp. were lower than the Cd hyperaccumulator (*Sedum plumbizincicola*), and Cu concentrations lower than in the Cu tolerant *Elsholtzia splendens*, *Pennisetum* sp. accumulated and removed more Cd and Cu due to its much larger biomass (Xu *et al*. 2019). However, soil Cd concentration was very low (<0.42 mg/kg) and 1% hydroxyapatite was added in that study. In a study by Hu *et al.* (2018), Cd amounts in shoots and roots of *Pennisetum sinese* removed 1.56 and 0.30 mg per plant respectively when soil Cd concentration was 8 mg/kg, removal rates higher than those obtained herein even though the soil Cd contamination was greater. This apparent inconsistency may be attributed to the smaller biomass, which was probably due to higher Cd concentration in soil or limited pot soil volume in the present study (Table 1, Fig. 4), or metal availability due to differences in soil pH. It was also reported that *Pennisetum purpureum* could accumulate 0.52 and 0.58 mg Cd in shoots and roots respectively per plant without any N fertilizer or chelating agent addition (Chen *et al*. 2017). Accumulations of Cd and Zn in shoots of PAP were up to 0.62 and 8.2 mg per plant, with BCFS of 5.9 and 2.7 respectively, when Cd and Zn concentrations of soil were 8 and 600 mg/kg (Zhang *et al*. 2010). These studies did not include a hyperaccumulator for reference and a limited range of grasses were compared, but their findings are broadly in line with those of the present study.

Switchgrass can have an aboveground annual yield of up to 20 Mg/ha (Singh *et al*. 2010). Switchgrass is tolerant to Cd and suitable for its phytoextraction *in situ* due to this high yield and ease of cultivation (Reed *et al*. 2002; Chen *et al*. 2012). There were eight switchgrass cultivars investigated in the present study (Table 1). Three cultivars, ‘Alamo’, ‘Kanlow’ and ‘Blackwell’, used herein, were also investigated by Sun *et al*. (2018). Their ranking of Cd concentrations in shoot or root among the three cultivars differed from the present findings, but that of Cd accumulation in shoot and root were similar (Figs. 2 and 4). For ‘Alamo’ a higher Cd translocation factor and lower Cd concentration in shoot and root than ‘Cave-in-Rock’ was consistent with a previous study (Liu *et al*. 2016). Higher biomass and higher Cd amounts in shoots of Alamo than Blackwell was also obtained by Chen *et al.* (2011), who suggested that Alamo had greater potential than Blackwell for Cd phytoextraction.

There are large differences in uptake capacity and tolerance to Cd/Zn between different species of *Miscanthus* (Pidlisnyuk *et al*. 2014; Barbosa *et al*. 2015; Zhang *et al*. 2015; Guo *et al*. 2016). Cadmium concentrations in shoots and roots for three *Miscanthus* spp. varied by factors of 6.7 and 3.0 respectively; *M. sinensis* had the highest and intermediate concentrations of Cd in root and shoot, respectively, and MS had the lowest Cd concentrations in both shoot and root, among the three species (Guo *et al*. 2016). In the present study, shoot concentration, root concentration, and TF of Cd in the eight *Miscanthus* species/cultivars varied in the ranges 11.8–29.3 mg/kg, 64.9–361.3 mg/kg, and 0.06–0.28, respectively, at a soil total Cd 48.1 mg/kg. In another study investigating MS, its shoot concentration, root concentration and TF of Cd varied in the ranges 0.92–18.36 mg/kg, 2.34–64.77 mg/kg, and 0.50–0.29, respectively, at soil total Cd concentrations from 1 to 100 mg/kg (Zhang *et al*. 2015), consistent with results reported here. In addition, reported tolerance of *Miscanthus* spp. to heavy metals varied in the literature (Pidlisnyuk *et al*. 2014; Guo *et al*. 2016). Three *Miscanthus* spp. exhibited different growth and physiological responses after treated with 0–200 μmol/L Cd in solutions. In the present study, only MSY showed marked sensitivity to Cd/Zn contamination. The MS tolerance to and low accumulation of Cd may in part be due to Cd induced malate secretion (Guo *et al*. 2016, 2017). Results by Zhang *et al.* (2015) also suggested that MS had a strong ability to tolerate Cd but a poor ability to translocate Cd from root to shoot (Pidlisnyuk *et al*. 2014). Regarding Zn, it was reported that the aerial biomass and Zn accumulation of MG was much higher than *M. sinensis*, consistent with results herein, although the reported higher shoot Zn concentration and TF for *M. sinensis* than for MG differ from the present results (Fig. 3, Table 3) (Barbosa *et al*. 2015). The low TF of *M. sinensis* obtained by Lee *et al*. (2014) is similar to findings herein, indicating that *M. sinensis* mainly accumulated Cd and Zn in roots. The high Zn concentrations in shoots of MS were consistent with the results by Li *et al*. (2014).

Arundo donax is highly tolerant to heavy metals. It has a large biomass and a fast growth rate even under abiotic stresses. This species mainly accumulated heavy metals in belowground structures with BCFR and TF values lower than 1 when planted in contaminated municipal sludge, landfill soils, and mine sites (Papazoglou *et al*. 2005; Guo and Miao 2010; Nsanganwimana *et al*. 2014). In addition to being highly tolerant to heavy metals such as Zn and Pb, AD can accumulate high Zn concentrations (Barbosa *et al*. 2015), as in the present study. In contrast, here ADV, which has been rarely evaluated for phytoremediation, had high shoot concentrations, high accumulation and TF (>1) of Cd and Zn in comparison to AD (Figs. 2, 3, 4, 5, Table 3). *A. donax* var. *versicolor* exhibited a substantial capacity for phytoextraction of soil Cd and Zn.

Iris lacteal, considered a Cd hyperaccumulator, is reported to be highly tolerant to Cd and have a potential capacity to phytostabilize and phytoextract soil Cd (Han *et al*. 2007; Guo *et al*. 2017). In a nutrient solution with Cd concentration of 0 to 50 mg/L for 21 days, ILC had Cd concentrations in shoots and roots up to 218 and 2714 mg/kg respectively, without exhibiting any toxicity symptom or growth reduction (Guo *et al*. 2017). *Iris lactea* had higher Cd concentration in shoots than in the grasses in the present study, indicating its strong phytoextractive capacity, though shoot Cd concentration was less than the 100 mg/kg threshold of hyperaccumulator status (Fig. 2a). The presently observed Cd TF of less than one is consistent with other studies (Han *et al*. 2007; Guo *et al*. 2017). It is notable however that ILC had low Zn concentrations in shoots and roots, highlighting the difficulties associated with phytoremediation of soils with multi-element contamination.

Plants with BCFSs and TFs >1 are usually considered suitable for phytoextraction (McGrath and Zhao 2003; Liu *et al*. 2018). However, fast growing non-hyperaccumulators that have large biomass are well-adapted to stresses and have practical cropping characteristics may be more suitable for phytoextraction, since their larger aerial biomass can more than compensate for the lower concentration of heavy metals. In the present study, PPP and PAP accumulated similar amounts of Cd in shoots to the hyperaccumulator NC, and more than ILC, even though both PPP and PAP had lower TF (<1) and lower BCFS (≤1) (Fig. 6a, Table 3). Likewise, ADV with low BCFS (<1) and high TF (>1) accumulated 55.4% more shoot Zn than NC. In addition, there are multiple uses for these high yielding lignocellulosic grasses, such as the production of energy (second generation ethanol and biofuels), paper pulp, building materials and adsorbents; they also have a role in soil erosion control and landscape restoration (Ververis *et al*. 2004; Pirozzi *et al*. 2010; Nasso *et al*. 2011; Gong *et al*. 2018; Pogrzeba *et al*. 2018). Thus, utilization of these grasses may promote heavy metal phytoextraction of soil whilst generating income.

Plants with a high BCFR (>1) and low TF (<1) are considered to be suitable for phytostabilization (Yoon *et al*. 2006; Cheraghi *et al*. 2011). There were 19 out of 32 grasses with BCFR>1 and TF<1 for Cd (Fig. 6b, Table 3). All species/cultivars of *Panicum virgatum* as well as *Miscanthus* having high BCFRs (>1) and low TFs (<1) suggested that the two had more potential capacity in soil Cd phytostabilization than other genera on the whole. In addition, several grasses such as PP, PSI and PVR with high root Cd accumulation but RCFRs<1 could also potentially stabilize soil Cd due to their well-developed root systems (RW, RL and RSA) (Figs. 1b, S6, Table S1).

Regarding Zn, NC with a high BCFS>1 and high TF>1 was potentially suitable for phytoextraction of Zn (Fig. 7a, Table 3). Only PPP with a high BCFR not less than one and low TF<1 had the potential for phytostabilization (Fig. 7b, Table 3). Furthermore, PPP accumulated the most Zn in roots among the cultivars/species tested (Fig. 5b). Therefore, although many of the tested ornamental and/or energy grasses in the present study had the potential to phytostabilization soil Cd combined with many of the other benefits described above, only PPP also had phytostabilization potential for soil Zn.

There were positive correlations for root concentrations and UEs per gram root between Cd and Zn, but not for shoot concentrations and TFs (Fig. 8a, b, c, d). This may imply that sorption of Cd by roots of these grasses potentially shares the channel of Zn, while the translocation of Cd with Zn from root to shoot may be more complicated. Cadmium and Zn have the same electron configuration and similar chemical properties. Thus, key transporters such as ZRT and IRT-like proteins responsible for Zn transport are also able to transport Cd (Fulekar *et al*. 2009; Jin *et al*. 2010). Cadmium could enter plant roots *via* the Zn channel and compete with Zn for binding sites on the root surface (Rizwan *et al*. 2019). Therefore, under the same conditions, it is reasonable that plants having strong sorptive capacity of Zn by root could easily sorb Cd, and vice versa. However, other processes such deposition in apoplast of root cortex, inhibition by casparian strip and endodermis wall, complexation by phytochelatins, compartmentalization in vacuole and competition for binding sites of transporters with Zn^{2+}, Ca^{2+} and Fe^{2+} may disrupt root sorption for Cd. The influence of related factors and mechanisms affecting translocation of Cd from root to shoot, including loading and unloading between phloem and xylem, and transpiration traction remain unclear (Wang *et al*. 2015). Cadmium transport is affected by complex interactions and competition between Cd and Zn for transporter binding sites in loading or unloading processes, which may explain the lack of correlation for shoot concentrations and TFs between Zn and Cd (Fig. 8c, d).

This study did not have as a primary aim the elucidation of mechanisms explaining differences in performance between grasses. Nevertheless, certain plant characteristics explained a very large proportion of the variation in this performance. The correlation matrix between Cd/Zn accumulation and root parameters showed that RL, RSA and RW were all positively correlated with Cd and Zn amounts accumulated in root and the whole plant, and with shoot Cd amount ($p < 0.01$) (Table S1). These findings indicate that rooting characteristics (RL, RSA and RW) also were likely factors influencing Cd and Zn accumulation in grasses. The greatest Cd amount accumulated in shoots of PPP among grasses may be attributed to its long RL, large RSA and RW, in addition to large SW (Figs. 1 and S6, Table S1). The highest PPP accumulation of Zn in roots among the cultivars/species tested (Fig. 5b) may also be attributable to the longest RL, large RSA, and RW (Figs. 1b and S6, Table S1). On the other hand, concentrations of Cd/Zn in shoots or roots were also key factors influencing the Cd/Zn accumulation in addition to plant parameters. Multiple linear regressions suggested that the ratio of RL to RW (RL/RW) was a significant factor favoring Cd concentrations in shoots and roots and root Zn concentrations. However, greater RW went against shoot Zn concentrations (Table S2). This may because that the increased root growth allowed more Zn to be stored within roots thereby decreasing shoot Zn concentrations.

The experimental set-up involving growing plants in pots is likely to have constrained the growth and metal accumulation of the large biomass grasses such as PPP, PAP, and ADV. Thus, although they accumulated Cd or Zn in shoots at levels similar to or larger than the smaller biomass hyperaccumulators NC and ILC, their phytoextraction potential may have been underestimated. The short-term (3 months' growth in soil) is a limitation in the present study. A further focus on a more limited range of plants with larger pots or even field plots and multiple harvests is needed; multiple harvests would indicate in particular whether annual rates of phytoextraction could be sustained. It is worth noting also that heavy metal accumulation in energy grasses grown at 100 mg Cd/kg soil could enhance biomass enzymatic saccharification and hexoses, and bioethanol yields through increasing hemicellulose and pectin contents and reducing cellulose levels (Cheng *et al*. 2018). Thus, phytoremediation of heavy metal contaminated soil using energy grasses has considerable potential to couple soil remediation with production, making better use of the contaminated land.

CONCLUSIONS

1. High yielding energy grasses such as PPP, PAP, and ADV accumulated similar or more Cd/Zn in shoots than hyperaccumulators tested, though their shoot Cd/Zn concentrations were lower. Grasses with high growth are potentially more effective than hyperaccumulators in soil remediation due to the compensation of large biomass for lower metal concentrations and also their well-developed root systems.
2. Excluding MSY and the two hyperaccumulators, biomass, Cd and Zn concentrations, and Cd and Zn amounts in shoots varied by factors of 14.2, 29.8, 5.9, 281, and 29.9, respectively. Those of roots varied by factors of 18.9, 12.3, 10.2, 10.9, and 32.5, respectively; TFs of Cd and Zn varied by factors of 24.7 and 16.3, respectively. There were also large variations within species/genus for Cd/Zn concentrations and accumulated amounts underlining the value of this screening investigation
3. Grasses PPP and ADV were the best candidates for Cd and Zn extraction, respectively. Most grasses could potentially stabilize soil Cd by root sequestration. *Panicum virgatum* as well as *Miscanthus* were more suitable for soil Cd phytostabilization compared with other grasses tested. *Pennisetum purpureum* 'Purple' is proposed as a comprehensive candidate in phytoextraction and phytostabilization for soil Cd and phytostabilization for soil Zn.
4. Regarding soils contaminated by both Cd and Zn, NC and ADV were prime candidates in phytoextraction, and PP, PSI, PPP were better for phytostabilization. The high Cd but low Zn concentrations for the Cd hyperaccumulator ILC indicates the difficulties in successful phytoremediation of soils with multi-metal contamination.
5. In addition to the considerable effectiveness in soil remediation, the utility of energy grasses in production of bioenergy, paper pulp, building materials, *etc*. means that soil remediation using these grasses can integrate remediation with economic outcomes, making the approach much easier to apply and to be accepted.

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REFERENCES CITED

Barbosa, B., Boleo, S., Sidella, S., Costa, J., Duarte, M. P., Mendes, B., Cosentino, S. L., and Fernando, A. L. (2015). "Phytoremediation of heavy metal-contaminated soils using the perennial energy crops *Miscanthus* spp. and *Arundo donax* L.," *BioEnergy Res.* 8(4), 1500-1511. DOI: 10.1007/s12155-015-9688-9

Chen, B. C., Lai, H. Y., and Juang, K. W. (2012). "Model evaluation of plant metal content and biomass yield for the phytoextraction of heavy metals by switchgrass," *Ecotoxicol. Environ. Saf.* 80, 393-400. DOI: 10.1016/j.ecoenv.2012.04.011

Chen, B. C., Lai, H. Y., Lee, D. Y., and Juang, K. W. (2011). "Using chemical fractionation to evaluate the phytoextraction of cadmium by switchgrass from Cd-contaminated soils," *Ecotoxicology* 20(2), 409-418. DOI: 10.1007/s10646-010-0592-y

Chen, Y. H., Liu, M. J., Deng, Y. W., Zhong, F. L., Xu, B., Hu, L., Wang, M. K., and Wang, G. (2017). "Comparison of ammonium fertilizers, EDTA, and NTA on enhancing the uptake of cadmium by an energy plant, Napier grass (*Pennisetum purpureum* Schumach)," *J. Soils Sediments* 17(12), 2786-2796. DOI: 10.1007/s11368-017-1703-7

Chen, Z. Q., Liu, X., Ai, Y. W., Chen, J., Luo, X. M., Chen, J. Y., and Zhong, S. H. (2018). "Effects and mechanisms of revegetation modes on cadmium and lead pollution in artificial soil on railway rock-cut slopes," *Sci. Total Environ.* 644, 1602-1611. DOI: 10.1016/j.scitotenv.2018.06.380

Cheng, S. Z., Yu, H., Hu, M., Wu, Y., Cheng, L. L., Cai, Q. M., Tu, Y. Y., Xia, T., and Peng, L. C. (2018). "*Miscanthus* accessions distinctively accumulate cadmium for largely enhanced biomass enzymatic saccharification by increasing hemicellulose and pectin and reducing cellulose CrI and DP," *Bioresource Technol.* 263, 67-74. DOI: 10.1016/j.biortech.2018.04.031

Cheraghi, M., Lorestani, B., Khorasani, N., Yousef, N., and Karami, M. (2011). "Findings on the phytoextraction and phytostabilization of soils contaminated with heavy metals," *Biol. Trace Elem. Res.* 144(1-3), 1133-1141. DOI: 10.1007%2Fs12011-009-8359-0

Erakhrumen, A. A. (2007). "Phytoremediation: An environmentally sound technology for pollution prevention, control and remediation in developing countries," *Educ. Res. Rev.* 2(7), 151-156.

Fulekar, M. H., Singh, A., and Bhaduri, A. M. (2009). "Genetic engineering strategies for enhancing phytoremediation of heavy metals," *Afr. J. Biotechnol.* 8(4), 529-535. DOI: 10.1016/j.mrgentox.2008.11.010

GB/T 15618-2018 (2018). "Soil environmental quality risk control standard for soil contamination of agricultural land," Standardization Administration of China, Beijing, China.

Golda, S., and Korzeniowska, J. (2016). "Comparison of phytoremediation potential of three grass species in soil contaminated with cadmium," *Environ. Prot. Nat. Resour*. 27(1), 8-14. DOI: 10.1515/oszn-2016-0003

Gong, X. M., Huang, D. L., Liu, Y. G., Zeng, G. M., Wang, R. Z., Wei, J. J., Huang, C., Xu, P., Wan, J., and Zhang, C. (2018). "Pyrolysis and reutilization of plant residues after phytoremediation of heavy metals contaminated sediments: For heavy metals stabilization and dye adsorption," *Bioresource Technol*. 253, 64-71. DOI: 10.1016/j.biortech.2018.01.018

Guo, H. P., Feng, X., Hong, C. T., Chen, H. M., Zeng, F. R., Zheng, B. S., and Jiang, D. A. (2017). "Malate secretion from the root system is an important reason for higher resistance of *Miscanthus sacchariflorus* to cadmium," *Physiol. Plant.* 159(3), 340-353. DOI: 10.1111/ppl.12526

Guo, H. P., Hong, C. T., Chen, X. M., Xu, Y. X., Liu, Y., Jiang, D. A., and Zheng, B. S. (2016). "Different growth and physiological responses to cadmium of the three *Miscanthus* species," *PloS One* 11(4), 1534-1575. DOI: 10.1371/journal.pone.0153475

Guo, Q., Meng, L., Zhang, Y. N., Mao, P. C., Tian, X. X., Li, S. S., and Zhang, L. (2017). "Antioxidative systems, metal ion homeostasis and cadmium distribution in *Iris lactea* exposed to cadmium stress," *Ecotoxicol. Environ. Saf.* 139, 50-55. DOI: 10.1016/j.ecoenv.2016.12.013

Guo, Z. H., and Miao, X. F. (2010). "Growth changes and tissues anatomical characteristics of giant reed (*Arundo donax* L.) in soil contaminated with arsenic, cadmium and lead," *J. Cent. South Univ. Technol.* 17(4), 770-777. DOI: 10.1007/s11771-010-0555-8

Han, Y. L., Yuan, H. Y., Huang, S. Z., Guo, Z., Xia, B., and Gu, J. G. (2007). "Cadmium tolerance and accumulation by two species of *Iris*," *Ecotoxicology* 16(8), 557-563. DOI: 10.1007/s10646-007-0162-0

He, Z., Shentu, J., Yang, X., Baligar, V. C., Zhang, T., and Stoffella, P. J. (2015). "Heavy metal contamination of soils: Sources, indicators, and assessment," *J. Environ. Indic.* 9, 17-18.

Hu, L., Wang, R., Liu, X. L., Xu, B., Xie, T. H., Li, Y. Y, Wang, M. K., Wang, G., and Chen, Y. H. (2018). "Cadmium phytoextraction potential of king grass (*Pennisetum sinese* Roxb.) and responses of rhizosphere bacterial communities to a cadmium pollution gradient," *Environ. Sci. Pollut. Res.* 25(22), 21671-21681. DOI: 10.1007/s11356-018-2311-9

Huang, Y. Y., He, C. T., Shen, C., Guo, J. J., Mubeen, S., Yuan, J. G., and Yang, Z. Y. (2017). "Toxicity of cadmium and its health risks from leafy vegetable consumption," *Food Funct*. 8(4), 1373-1401. DOI: 10.1039/C6FO01580H

Jin, F., Wang, C., Lin, H. J., Shen, Y. O., Zhang, Z. M., Zhao, M. J., and Pan, G. T. (2010). "Heavy metal-transport proteins in plants: A review," *Chin. J. Appl. Ecol*. 21(7), 1875-1882. DOI: 10.13287/j.1001-9332.2010.0247

Khan, A., Khan, S., Khan, M. A., Qamar, Z., and Waqas, M. (2015). "The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: A review," *Environ. Sci. Pollut. Res.* 22(18), 13772-13799. DOI: 10.1007/s11356-015-4881-0

Lee, S. H., Ji, W. H., Lee, W. S., Koo, N. M., Koh, I. H., Kim, M. S., and Park, J. S. (2014). "Influence of amendments and aided phytostabilization on metal availability and mobility in Pb/Zn mine tailings," *J. Environ. Manage.* 139, 15-21. DOI: 10.1016/j.jenvman.2014.02.019

Li, C., Xiao, B., Wang, Q. H., Yao, S. H., and Wu, J. Y. (2014). "Phytoremediation of Zn- and Cr-contaminated soil using two promising energy grasses," *Water, Air, Soil Pollut.* 225, 2027. DOI: 10.1007/s11270-014-2027-5

Liu, C. H., Lou, L. Q., Deng, J. X., Li, D. J., Yuan, S. X., and Cai, Q. S. (2016). "Morph-physiological responses of two switchgrass (*Panicum virgatum* L.) cultivars to cadmium stress," *Grassl. Sci.* 62, 92-101. DOI: 10.1111/grs.12119

Liu, L. W., Li, W., Song, W. P., and Guo, M. X. (2018). "Remediation techniques for heavy metal-contaminated soils: Principles and applicability," *Sci. Total Environ.* 633, 206-219. DOI: 10.1016/j.scitotenv.2018.03.161

Lorenz, S. E., Hamon, R. E., Holm, P. E., Domingues, H. C., Sequeira, E. M., Christensen, T. H., and McGrath, S. P. (1997). "Cadmium and zinc in plants and soil solutions from contaminated soils," *Plant Soil* 189(1), 21-31. DOI: 10.1023/A:1004214923372

Lu, R. K. (2000). *Soil Agricultural Chemical Analysis Method*, China Agriculture Science and Technique Press, Beijing, China.

McGrath, S. P., and Zhao, F. J. (2003). "Phytoextraction of metals and metalloids from contaminated soils," *Curr. Opin. Biotechnol.* 14(3), 277-282. DOI: 10.1016/S0958-1669(03)00060-0

Nasso, N. N. o. D., Roncucci, N., Triana, F., Tozzini, C., and Bonari, E. (2011). "Seasonal nutrient dynamics and biomass quality of giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus* × *giganteus* Greef et Deuter) as energy crops," *Ital. J. Agron.* 6, 152-158. DOI: 10.4081/ija.2011.e24

Nordberg, G., Jin, T. Y., Bernard, A., Fierens, S., Buchet, J. P., Ye, T. T., Kong, Q. H., and Wang, H. F. (2002). "Low bone density and renal dysfunction following environmental cadmium exposure," *Ambio* 31(6), 478-481. DOI: 10.1579/0044-7447-31.6.478

Nsanganwimana, F., Marchand, L., Douay, F., and Mench, M. (2014). "*Arundo donax* L., a candidate for phytomanaging water and soils contaminated by trace elements and producing plant-based feedstock: A review," *Int. J. Phytorem.* 16(7-12), 982-1017. DOI: 10.1080/15226514.2013.810580

O'Connor, D., Peng, T., Zhang, J., Tsang, D. C. W., Alessi, D. S., Shen, Z., Bolan, N. S., and Hou, D. (2018). "Biochar application for the remediation of heavy metal polluted land: A review of *in situ* field trials," *Sci. Total Environ.* 619-620, 815-826. DOI: 10.1016/j.scitotenv.2017.11.132

Papazoglou, E. G., Karantounias, G. A., Vemmos, S. N., and Bouranis, D. L. (2005). "Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to the heavy metals Cd and Ni," *Environ. Int.* 31(2), 243-249. DOI: 10.1016/j.envint.2004.09.022

Perronnet, K., Schwartz, C., and Morel, J. L. (2003). "Distribution of cadmium and zinc in the hyperaccumulator *Thlaspi caerulescens* grown on multicontaminated soil," *Plant Soil* 249(1), 19-25. DOI: 10.1023/a:1022560711597

Phusantisampan, T., Meeinkuirt, W., Saengwilai, P., Pichtel, J., and Chaiyarat, R. (2016). "Phytostabilization potential of two ecotypes of *Vetiveria zizanioides* in cadmium-contaminated soils: Greenhouse and field experiments," *Environ Sci Pollut Res.* 23(19), 20027-20038. DOI: 10.1007/s11356-016-7229-5

Pidlisnyuk, V., Stefanovska, T., Lewis, E. E., Erickson, L. E., and Davis, L. C. (2014). "*Miscanthus* as a productive biofuel crop for phytoremediation," *Crit. Rev. Plant Sci.* 33(1), 1-19. DOI: 10.1080/07352689.2014.847616

Pirozzi, D., Yousuf, A., Fagnano, M., Mori, M., Fiorentino, N., and Impagliazzo, A. (2010). "Lipids production by yeast grown on giant reed biomass," *J. Biotechnol.* 150, 167-168. DOI: 10.1016/j.jbiotec.2010.08.434

Pogrzeba, M., Rusinowski, S., and Krzyzak, J. (2018). "Macroelements and heavy metals content in energy crops cultivated on contaminated soil under different fertilization-Case studies on autumn harvest," *Environ. Sci. Pollut. Res.* 25(12), 12096-12106. DOI: 10.1007/s11356-018-1490-8

Reed, R. L., Sanderson, M. A., Allen, V. G., and Zartman, R. E. (2002). "Cadmium application and pH effects on growth and cadmium accumulation in switchgrass," *Commun. Soil Sci. Plant Anal.* 33(7-8), 1187-1203. DOI: 10.1081/css-120003881

Reeves, R. D., Baker, A. J. M., Jaffré, T., Erskine, P. D., Echevarria, G., and van der Ent, A. (2017). "A global database for plants that hyperaccumulate metal and metalloid trace elements," *New Phytol.* 218(2), 407-411. DOI: 10.1111/nph.14907

Rizwan, M., Ali, S., Adrees, M., Ibrahim, M., Tsang, D. C. W., Zia-Ur-Rehman, M., Zahir, Z. A., Rinklebe, J., Tack, F. M. G., and Ok, Y. S. (2017). "A critical review on effects, tolerance mechanisms and management of cadmium in vegetables," *Chemosphere* 182, 90-105. DOI: 10.1016/j.chemosphere.2017.05.013

Rizwan, M., Ali, S., Zia ur Rehman, M., and Maqbool, A. (2019). "A critical review on the effects of zinc at toxic levels of cadmium in plants," *Environ. Sci. Pollut. Res.* 26(7), 6279-6289. DOI: 10.1007/s11356-019-04174-6

Sarwar, N., Imran, M., Shaheen, M. R., Ishaque, W., Kamran, M. A., Matloob, A., Rehim, A., and Hussain, S. (2017). "Phytoremediation strategies for soils contaminated with heavy metals: Modifications and future perspectives," *Chemosphere* 171, 710-721. DOI: 10.1016/j.chemosphere.2016.12.116

Shahid, M., Dumat, C., Khalid, S., Niazi, N. K., and Antunes, P. M. C. (2016). "Cadmium bioavailability, uptake, toxicity and detoxification in soil-plant system," *Rev. Environ. Contam. Toxicol.* 241, 73-137. DOI: 10.1007/398_2016_8

Singh, A., Pant, D., Korres, N. E., Nizami, A. S., Prasad, S. H., and Murphy, J. D. (2010). "Key issues in life cycle assessment for ethanol production from lignocellulosic biomass: challenges and perspectives," *Bioresource Technol.* 101(13), 5003-5012. DOI: 10.1016/j.biortech.2009.11.062

Song, G., Yuan, S. X., Wen, X. H., Xie, Z. Y., Lou, L. Q., Hu, B. Y., Cai, Q. S., and Xu, B. (2018). "Transcriptome analysis of Cd-treated switchgrass root revealed novel transcripts and the importance of HSF/HSP network in switchgrass Cd tolerance," *Plant Cell Rep.* 37(11), 1485-1497. DOI: 10.1007/s00299-018-2318-1

Stephan, C. H., Courchesne, F., Hendershot, W. H., McGrath, S. P., Chaudri, A. M., Sappin-Didier, V., and Sauvé, S. (2008). "Speciation of zinc in contaminated soils," *Environ. Pollut.* 155(2), 208-216. DOI: 10.1016/j.envpol.2007.12.006

Sun, H., Xie, Y., Zheng, Y., Lin, Y., and Yang, F. (2018). "The enhancement by arbuscular mycorrhizal fungi of the Cd remediation ability and bioenergy quality-related factors of five switchgrass cultivars in Cd-contaminated soil," *Peer J.* 6(16), e4425. DOI: 10.7717/peerj.4425

Ververis, C., Georghiou, K., Christodoulakis, N., Santas, P., and Santas, R. (2004). "Fiber dimensions, lignin and cellulose content of various plant materials and their suitability for paper production," *Ind. Crops Prod.* 19(3), 245-254. DOI: 10.1016/j.indcrop.2003.10.006

Wang, X. J., Wang, W. B., Yang, L., Jin, L., Song, Y., Jiang, S. J, and Qin, L. L. (2015). "Transport pathways of cadmium (Cd) and its regulatory mechanisms in plant," *Acta Ecol. Sin.* 35(23), 7921-7929. DOI: 10.5846/stxb201404170754

Xu, L., Xing, X. Y., Liang, J. N., Peng, J. B., and Zhou, J. (2019). "*In situ* phytoremediation of copper and cadmium in a co-contaminated soil and its biological and physical effects," *RSC Adv.* 9(2), 993-1003. DOI: 10.1039/C8RA07645F

Yoon, J., Cao, X. D., Zhou, Q. X., and Ma, L. Q. (2006). "Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site," *Sci. Total Environ.* 368(2-3), 456-464. DOI: 10.1016/j.scitotenv.2006.01.016

Zhang, J., Yang, S. Y., Huang, Y. J., and Zhou, S. B. (2015). "The tolerance and accumulation of *Miscanthus sacchariflorus* (Maxim.) Benth., an energy plant species, to cadmium," *Int. J. Phytorem.* 17(6), 538-545. DOI: 10.1080/15226514.2014.922925

Zhang, W. L., Du, Y., Zhai, M. M., and Shang, Q. (2014). "Cadmium exposure and its health effects: A 19-year follow-up study of a polluted area in China," *Sci. Total Environ.* 470-471, 224-228. DOI: 10.1016/j.scitotenv.2013.09.070

Zhang, X. F., Xia, H. P., Li, Z. A., Zhuang, P., and Gao, B. (2010). "Potential of four forage grasses in remediation of Cd and Zn contaminated soils," *Bioresource Technol.* 101(6), 2063-2066. DOI: 10.1016/j.biortech.2009.11.065

Zhao, F. J., Ma, Y., Zhu, Y. G., Tang, Z., and McGrath, S. P. (2015). "Soil contamination in China: Current status and mitigation strategies," *Environ. Sci. Technol.* 49(2), 750-759. DOI: 10.1021/es5047099

Zheng, R. L., Li, C., Sun, G. X., Xie, Z. B., Chen, J., Wu, J. Y., and Wang, Q. H. (2017). "The influence of particle size and feedstock of biochar on the accumulation of Cd, Zn, Pb, and As by *Brassica chinensis* L.," *Environ. Sci. Pollut. Res.* 24, 22340-22352. DOI: 10.1007/s11356-017-9854-z

Zheng, R. L., Chen, Z., Cai, C., Wang, X., Huang, Y., Xiao, B., and Sun, G. (2013). "Effect of biochars from rice husk, bran, and straw on heavy metal uptake by pot-grown wheat seedling in a historically contaminated soil," *BioResources* 8(4), 5965-5982. DOI: 10.15376/biores.8.4.5965-5982

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APPENDIX

Fig. S1. Steps of cutting, taking *Panicum virgatum* ‘Blackwell’ as an example.

Fig. S2. Steps of tiller separation, taking *Iris lactea* var. *chinensis* as an example.

Fig. S3. After washed with deionized water, seedlings growing in peat for 3 weeks were transplanted into contaminated soils and clipped to 7-cm height, taking *Pennisetum purpureum* ‘Purple’ as an example.

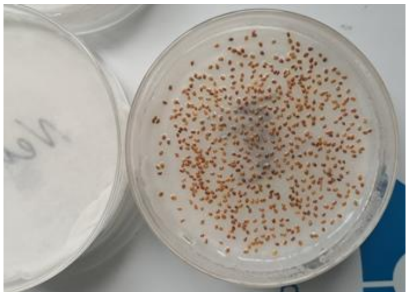

Fig. S4. Germination of *Noccaea caerulescens* seeds on moist filter paper in petri dish, keeping the water surface no more than half the diameter of the seed.

Fig. S5. Fertilizers urea, KH_2PO_4 and $MgSO_4 \cdot 7H_2O$ were dissolved in deionized water respectively, added into soil (5 mL per pot for each reagent), and then soil was homogenized thoroughly.

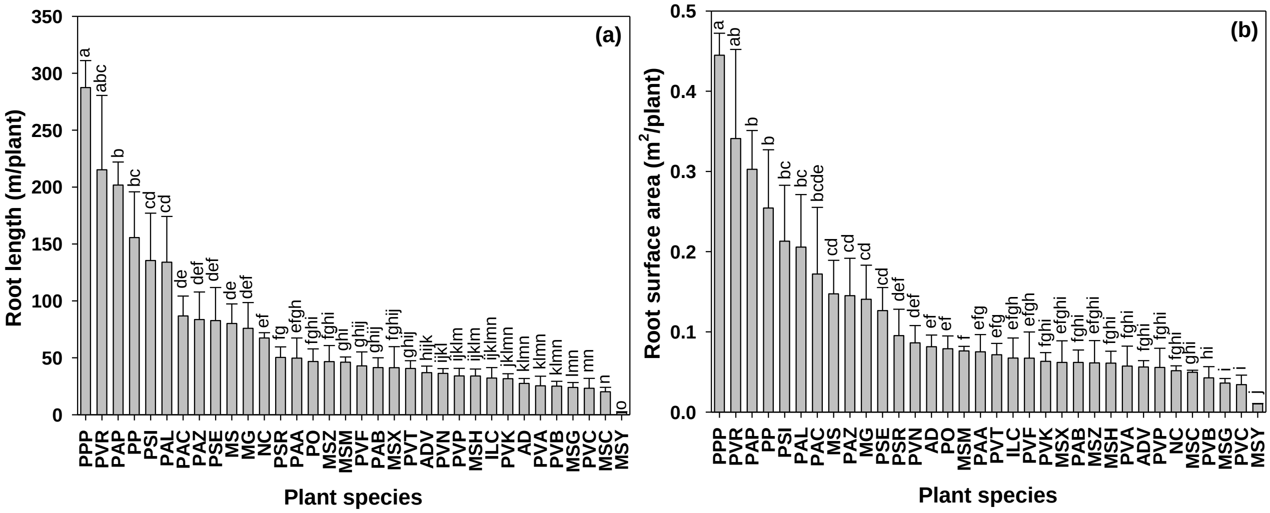

Fig. S6. Root length (a) and root surface area (b) of 32 grasses and 2 hyperaccumulators (mean ± SD, n = 4). Means with a common letter above columns do not differ at $p < 0.05$.

Table S1. Correlation Matrix (Pearson Correlation Coefficients) between Cd/Zn Accumulation in Shoot, Root and the Whole Plant and Root Parameters (n = 32) among 32 Grasses

	Cd amount accumulation			**Zn amount accumulation**		
	The whole plant	**Shoot**	**Root**	**The whole plant**	**Shoot**	**Root**
RL	0.76***	0.68***	0.76***	0.76***	0.17	0.87***
RSA	0.75***	0.67***	0.76***	0.78***	0.20	0.87***
RW	0.58**	0.46**	0.69***	0.57**	0.10	0.66***
RL/RW	-0.02	0.02	-0.1	-0.08	-0.19	0.01

** and *** indicate significance at $p < 0.01$ and $p < 0.001$, respectively; no asterisk mark indicates insignificance ($p > 0.05$).

Table S2. Multiple Linear Regressions between Cd/Zn Concentrations in Shoots and Roots ($[\text{metal}]_S$ and $[\text{metal}]_R$) and Various Predictor Factors (SW, RW, RL, RSA, RL/RW) among 32 Grasses. Based on Stepwise Regression Including only those Variables in Regression Equations that were Significant ($p < 0.05$)

Model	***r* value**	**Significance**
$[\text{Cd}]_S = 2.058 + 1.457 \cdot \text{SW} + 0.279 \cdot \text{RL/RW}$	0.733	$p < 0.001$
$[\text{Zn}]_S = 251.679 – 13.458 \cdot \text{RW}$	0.442	$p < 0.001$
$[\text{Cd}]_R = – 14.856 + 3.403 \cdot \text{RL/RW}$	0.837	$p < 0.001$
$[\text{Zn}]_R = 175.797 + 15.764 \cdot \text{RL/RW}$	0.701	$p < 0.001$