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Cadmium stabilization with nursery stocks through transplantation: A new approach to phytoremediation

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ABSTRACT

Disposal of heavy metal contaminated biomass after phytoremediation is still unfeasible. This paper presents a viable phyto-extraction approach in which metals in contaminated soils are stabilized by nursery stocks before transplantation for greening. In this respect, two pot-experiments are reported comparing seven nursery stocks species exposed to different Cd levels. The first experiment revealed that Cd was mainly stabilized in the roots of all species studied. Greater amounts of Cd were accumulated in the epidermis than cortex plus stele. *Cupressus Blue Ice* showed greatest tolerance to the 100 and 200 mg kg^{-1} Cd stresses. The second experiment additionally evaluated the possible risk of Cd release after transplanting the Cd treated plants into uncontaminated soil. After 120 days of transplantation, the relatively trace amounts of Cd in the roots of *Euonymus japonicus*, *Pittosporum tobira* and *C. Blue Ice* had either been partially transferred into the shoots or released into the soil. The highest Cd concentration increase in bulk soil (0.428 mg kg^{-1}), however, was much lower than the environmental quality standard for soils of China (1 mg kg^{-1}). The potential effectiveness of this technique in the use of Cd-contaminated soil and further investigation needed in the field trials were also evaluated.

1. Introduction

The technique of phytostabilization can be defined as the establishment of a vegetative cover by woody species on contaminated soils to minimize the mobility of heave metals in polluted soils [1–3]. It is based on the high potential of woody plant species to stabilize heavy metals by their accumulation, adsorption or precipitation within the root zone [4,5]. Furthermore, some woody plants have vast root systems and can promote physical stabilization of the substrate in which they are growing, especially on sloping ground, thereby reducing the dispersion of metal-contaminated particles and the migration of contaminants into surface and ground waters [1–3]. Compared with phytoextraction, which may result in serious hazard by the accumulation of heavy metals and the risk of transfer to the food chain [6–8], phytostabilization is a more feasible approach for the long-term management of contaminated areas. Over the past decade, therefore, this technique has been successfully adopted in environments affected by mine tailings [1,9] and afforested land [5]. Phytostabilization, however, also has a disadvantage. Since its purpose is to stabilize metals rather than extract them, the technique cannot be used for cleaning contaminated soils [3], let alone for remediating the widespread moderately contaminated arable lands as those in China.

With the advance of urbanization in China, afforesting nursery stocks for urban or road greening has become big business [10], so that nursery stock cultivation has developed into a major agricultural practice in recent years. Currently, because of the high cost of using containers, a large number of nursery stocks are planted directly on cropland and out-planted for marketing. This pattern of cultivation utilizing fertile arable soils inevitably imposes restrictions on agricultural production in a country with a large population with insufficient land resources.

Vast areas of contaminated arable soils in China (estimated 20 million ha) [11] are in need of remediation. We therefore propose that cultivating nursery stocks be diverted to these contaminated soils for 1 or 2 years before transplanting for urban or road greening. By the conventional replacement of the woody plants, we suggest that this technique not only resolves the problem of reusing contaminated land, but also presents a low risk means of disposal of contaminated biomass, compared with previous approaches [3,4]. Most importantly of course, large areas of fertile land can be released for crop production.

To implement this strategy, nursery stocks should be from a metal-tolerant species. In fact, after being screened over a long period, nursery stocks can quickly acclimate to the poor transplanting environments, such as the roadside, which are characterized

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by low water potential and nutrient availability. Although nursery stocks are not especially selected for heavy metal tolerance, many woody species have high metal-resistance potentials, e.g., *Quercus ilex* (Holm oak) [5,12], *Salix* (willow) [8,13–20], *Betula* (birch) [21], *Populus* (poplar) [22–24], and *Acer* (maple)[12]. All have ability to translocate trace metals from roots to shoots which is species-specific. For example, there is increasing evidence that *Salix* species are particularly able to accumulate considerable amounts of Cd in their leaves [8,13–20], and that Holm oak shows a high Cd retention capacity in fine roots and low rate of Cd translocation to the leaves [5]. Thus, to minimize the potential risk of recontamination by defoliation, the tolerance and accumulation of metals in the root system needs to be considered for the nursery stocks.

Another important issue is to evaluate the environmental risk and economic benefits of transplanting. Although there are many reports on the screening of woody plants for use in phytoremediation [4], especially of willow [8,13–20], the disposal of contaminated biomass following phytoremediation has not been extensively addressed [3,4,6,7,25]. Various strategies for the disposal of harvested biomass (i.e., composting, compaction, incineration, ashing, pyrolysis, direct disposal, liquid extraction) have been described previously [26,27]. However, all of these methods are pre-treatment steps, and significant amounts of contaminated biomass still remain after each process [26,27]. Furthermore, farmers have not been found willing to pay for phytoremediation which they consider as government responsibility [28].

Thus, this study conducted with seven nursery stocks species, commonly used for urban greening in China, attempts to (1) explore Cd tolerance and Cd accumulation patterns of these plants, and screen desirable plants that can be transplanted from Cd-contaminated sites; and (2) evaluate the environmental risk of the nursery stocks after transplanting. This study tries to draw researchers' attention to feasible strategies for dealing with hazardous biomass after phytoremediation. The ultimate aim is the successful promotion of this new technique for remediation of heavy metal-contaminated soils.

2. Materials and methods

2.1. Pot experiment 1

The soil used for the pot experiments was collected from coastal grey soil at 0–20 cm depth in Hangzhou, Zhejiang Province, China, with a pH of 7.31, organic matter 5.33 g kg^{-1}, total-N 1.14 g kg^{-1}, Olsen-P 3.45 mg kg^{-1}, and NH_4AC extractable-K 67.3 mg kg^{-1}. The soil was air-dried, crushed to pass through a 2 mm sieve and mixed well with slow release fertilizer at the rate of 20 g kg^{-1} (APEX, J.R. Simplot Co., Ltd., USA). To avoid heterogenous distribution of Cd in the experimental soil, 228 g of $CdCl_2{\cdot}2.5H_2O$ were dissolved with 3000 ml of distilled water and then mixed thoroughly with 5 kg of soil. This soil with 20,000 mg kg^{-1} Cd was air-dried, passed through a 2 mm sieve, and then mixed thoroughly and appropriately with non-Cd contaminated soil to produce three concentrations of soil Cd. In all there were four treatments each with 4 replicates as follows: CK (no Cd added), 50 mg kg^{-1} Cd, 100 mg kg^{-1} Cd and 200 mg kg^{-1} Cd.

Seven one-year-old nursery stocks, i.e., *Acer plamatum* (*A. plamatum*, a species of maple, leaves turn brilliant shades of scarlet in autumn), *Ligustrum vicaryi* (*L. vicaryi*, a multi-stemmed shrub with golden yellow leaves), *Aucuba japonica* (*A. japonica*, a spotted laurel, leaves speckled in yellow), *Euonymus japonicus* (*E. japonicus*, a species of spindle, leaves 4–5 cm long with broad white margin), *Buxus megistophylla* (*B. megistophylla*, an evergreen shrub with grooved and glabrous leaves), *Pittosporum tobira* (*P. tobira*, an evergreen shrub, leaves are oval in shape with edges that curl under, leathery, darker and shinier on the upper surfaces), and *Cupressus Blue Ice* (*C. Blue Ice*, a steeple-shaped cypress with compact and beautiful blue-green foliage), were obtained from Zhejiang Senhe Seeds Co. Ltd., China. Uniform-sized seedlings of species were planted directly into plastic pots (13 cm in width × 12 cm in height) filled with 2 kg of pretreated soil. The soil was irrigated with distilled water daily to keep soil moisture at approximately 70% field water holding capacity. The experiment was conducted in a greenhouse where the daily photoperiod was 12 h and the maximum temperature was 30 °C, while the daily minimum temperature at night was adjusted to 20 °C. Seedlings from each treatment were harvested after 84 days. The harvested plants were separated into three parts (leaf, stem and root). After washing thoroughly with distilled water, the epidermal tissue was carefully scraped from the taproot, the remainder being considered as cortex plus stele.

2.2. Pot experiment 2

This experiment aimed to examine whether Cd was released into soil from Cd-treated roots after transplantation. In this case, experiment 1 was repeated with *E. japonicus*, *P. tobira* and *C. Blue Ice* with each treatment replicated 8 times. After 84 days growth, roots of the three species of each treatment were washed thoroughly with distilled water to remove adhering soil particles. For each treatment, four of the replicates were separated into three parts (leaf, stem and root) and oven-dried for determination of total Cd content, while the other four replicates were immediately transplanted into plastic pots (13 cm in width × 12 cm in height) filled with 2 kg of non-Cd-contaminated soil. The soil was irrigated with distilled water daily to keep moisture at approximately 70% field water holding capacity. The experiment was conducted in a greenhouse where the daily photoperiod was 12 h and the maximum temperature was 30 °C, the daily minimum temperature at night was adjusted to 20 °C. Plants were grown for 120 days before harvest and separated into three parts (leaf, stem and root). Rhizosphere soils and roots were separated from the bulk soil by hand: the soil particles adhering closely to the roots (up to 2.0 mm around the root) were considered to be rhizosphere soil [29]. The bulk and rhizosphere soils were sieved (<2 mm) for analysis of Cd concentration.

2.3. Cadmium analyses

The harvested parts of plants in experiments 1 and 2 were washed thoroughly with distilled water and then oven-dried for 72 h at 70 °C. Samples were weighed, and ground to pass through a 2 mm sieve for analyzing the Cd concentration. Dried samples (up to 0.1 g DW) of the studied plants were digested with 10 ml of nitric acid at 160 °C for 24 h. Soil samples were collected from pot experiment 2 at harvest to determine Cd in the bulk and rhizosphere soils. Soil samples (0.2 g) were digested with 1 ml of distilled water, 1 ml of HCl and 4 ml HNO_3 in a microwave oven (CEM MARS-240, USA). After digestion and dilution of plant and soil samples, Cd was analyzed by an atomic absorption spectrophotometer (AAS-800; PerkinElmer; USA; with a lowest detectable limit of 0.2 (g kg^{-1}).

2.4. Tolerance index

Cd tolerance of the plant species was calculated as the tolerance index (T_i) which provides the percentage of dry biomass (g per plant) of Cd-treated plant (*DWt*) minus control (*DWc*) plant over control plant according to the following equation:

$$\frac{DWt - DWc}{DWc} \times 100\% = T_i$$

Table 1
Dry weight (g) of leaf, stem and root of seven nursery stock species in control treatment over an 84-day experimental period.

	Dry weight (g plant^{-1})						
	A. plamatum	*L. vicaryi*	*A. japonica*	*E. japonicus*	*B. megistophylla*	*P. tobira*	*C. Blue Ice*
Leaf	2.81 ± 0.46	4.99 ± 0.74	4.18 ± 0.67	1.92 ± 0.42	4.59 ± 0.78	2.77 ± 0.47	1.92 ± 0.33
Stem	4.49 ± 0.78	2.53 ± 0.38	4.33 ± 0.56	0.79 ± 0.18	5.06 ± 1.36	2.37 ± 0.79	3.57 ± 0.79
Roots	2.18 ± 0.44	5.31 ± 0.89	5.62 ± 1.47	1.67 ± 0.43	6.91 ± 1.04	2.94 ± 0.57	3.37 ± 0.78
Total	9.47 ± 1.56	12.8 ± 2.06	14.1 ± 3.78	4.38 ± 0.29	16.6 ± 3.23	8.09 ± 2.56	8.68 ± 1.42

2.5. Statistical analysis

All experimental data shown in tables and figures were examined statistically by an analysis of variance. Means of four replicates were subjected to Duncan's New Multiple Range Test at a 0.05 probability level using SPSS software.

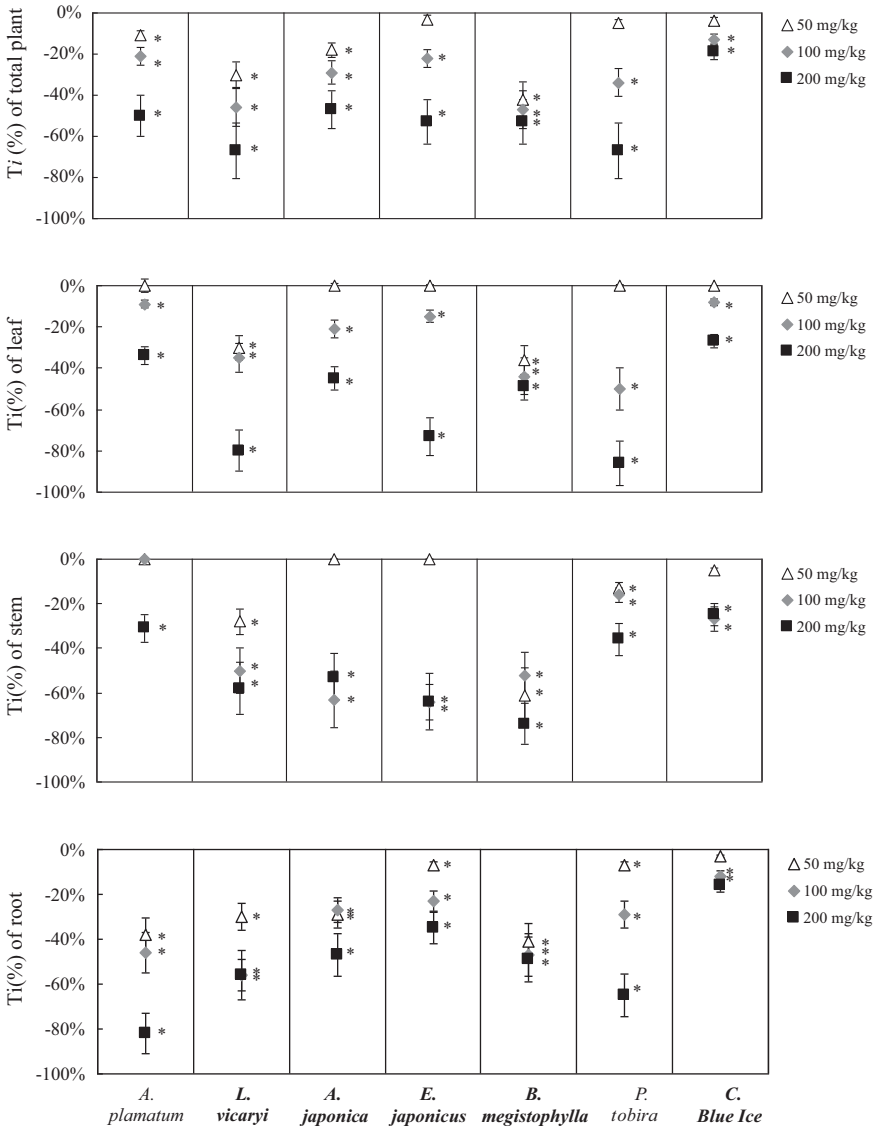
3. Results

3.1. Plant growth

Plant biomass is an important parameter for evaluation of total Cd accumulation in phytostabilization. The dry weight biomass of the leaves, stems, root parts and total plant for the control seedlings was determined over the experimental period (84 days) (Table 1). Among the seven studied plants, *B. megistophylla* had the largest total biomass of 16.6 g, followed by *A. japonica*, *L. vicaryi*, *A. plamatum*, *C. Blue Ice*, *P. tobira*, and *E. japonicus* with the lowest biomass of 4.38 g. Root biomass was generally higher than the biomass of stems and leaves, except for *A. plamatum* and *E. japonicus* (Table 1).

3.2. Tolerance index (T_i)

The tolerance index (T_i) as described above provides a quantitative method for expression of Cd tolerance of plants. In the



Asterisks indicate values that differ significantly from the control at $P < 0.05$.

Fig. 1. Tolerance index (*Ti*) of seven nursery stock species following 84-day Cd exposure.

Table 2
Cd concentrations in leaf, stem and root of seven nursery stock species following 84-day Cd exposure.

	Cd treatment (mg kg^{-1})	Cd concentration (mg kg^{-1})						
		A. plamatum	*L. vicaryi*	*A. japonica*	*E. japonicus*	*B. megistophylla*	*P. tobira*	*C. Blue Ice*
Leaf	0	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
	50	15.3 ± 2.9 c	18.4 ± 9.2 b	6.8 ± 4.0 b	8.1 ± 4.4 b	5.2 ± 1.9 b	85.8 ± 12 b	0.4 ± 0.1 b
	100	58.5 ± 5.0 b	14.9 ± 2.0 b	23.1 ± 2.0 a	7.0 ± 2.4 b	4.0 ± 1.3 b	192.6 ± 25.1 a	6.8 ± 2.7 a
	200	199.2 ± 25.4 a	54.2 ± 8.5 a	29.4 ± 4.8 a	19.4 ± 10.8 a	15.0 ± 4.8 a	215.9 ± 16.6 a	6.6 ± 1.0 a
Stem	0	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 d
	50	8.1 ± 3.1 b	11.5 ± 3.5 c	0.8 ± 0.1 c	0.2 ± 0.2 c	0.5 ± 0.1 c	30.0 ± 5.3 b	2.9 ± 1.0 c
	100	10.9 ± 4.1 b	31.9 ± 4.5 b	3.9 ± 2.0 b	39.7 ± 7.0 b	9.2 ± 2.9 b	49.0 ± 8.9 a	38.6 ± 8.1 b
	200	31.1 ± 5.6 a	123.4 ± 21.6 a	59.9 ± 13.5 a	145.4 ± 8.7 a	102.0 ± 6.3 a	59.9 ± 10.5 a	104.4 ± 6.8 a
Root	0	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d
	50	703 ± 90 c	419 ± 83 c	225 ± 18 b	625 ± 49 c	403 ± 121 c	332 ± 83 b	215 ± 19 c
	100	1504 ± 174 b	722 ± 148 b	1492 ± 169 a	1864 ± 139 b	828 ± 142 b	726 ± 106 a	981 ± 72 b
	200	4376 ± 135 a	2081 ± 229 a	1530 ± 178 a	3120 ± 485 a	1633 ± 344 a	810 ± 174 a	1876 ± 196 a
Total plant	0	0.0 ± 0.0 d	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d
	50	118 ± 23 c	182 ± 38 b	74 ± 16 b	203 ± 47 c	125 ± 30 c	135 ± 28 c	86 ± 18 c
	100	238 ± 45 b	262 ± 48 b	688 ± 101 a	755 ± 179 b	270 ± 47 b	290 ± 31 b	412 ± 32 b
	200	411 ± 119 a	1160 ± 207 a	646 ± 189 a	1843 ± 328 a	616 ± 112 a	471 ± 47 a	964 ± 186 a
Ratio of leaf to root	0	0.022	0.044	0.030	0.013	0.013	0.258	0.002
	50	0.039	0.021	0.015	0.004	0.005	0.265	0.007
	100	0.046	0.026	0.019	0.006	0.009	0.267	0.004
	200	0.022	0.044	0.030	0.013	0.013	0.258	0.002

Means followed by different letters within the same column are significantly different at $P < 0.05$ according to Duncan's New Multiple Range Test at the 5% probability level.

treatment with 50 mg kg^{-1} Cd, T_i values for *L. vicaryi* and *B. megistophylla* were −30% and −42%, followed by *A. plamatum* and *A. japonica* with T_i of −11% and −18%, respectively. *E. japonicus*, *P. tobira* and *C. Blue Ice* were relatively tolerant species with T_i of −3%, −5% and −4%, respectively (Fig. 1). When soil Cd increased to 100 mg kg^{-1}, T_is of all the studied plants were below −20% except *C. Blue Ice* with a T_i of −13%. In the treatment with 200 mg kg^{-1} Cd, the T_i of *C. Blue Ice* fell to −19%, while T_i values of all other plants were below −50%.

In the treatment with 50 mg kg^{-1} Cd, inhibitory effects were found mainly in roots rather than in stems or in leaves. For example, supplying the plants with 50 mg kg^{-1} Cd did not suppress stem and leaf growth in *A. plamatum* and *A. japonica* with a T_i of 0% but dramatically decreased root growth with T_is of −38% and −29%, respectively. Similar phenomena were also found in *E. japonicus*, *P. tobira* and *C. Blue Ice*. However, in the treatments with 100 and 200 mg kg^{-1} Cd, leaf and stem biomass was decreased more significantly than root biomass. For example, the leaf T_i value of *E. japonicus* was decreased to −73%, but the root T_i value was only lowered to −35% in the treatment with 200 mg kg^{-1} Cd. Similar phenomena were noted in *L. vicaryi*, *P. tobira* and *C. Blue Ice*.

3.3. Cd uptake and Cd release from roots

3.3.1. Cd concentrations

Cd concentrations in the studied plants increased proportionally to the increase of Cd applied (Table 2). In the treatment with 50 mg kg^{-1} Cd, Cd concentrations in the seven nursery stocks tested ranged from 74 (*A. japonica*) to 203 (*E. japonicus*) mg kg^{-1}. When the Cd treatment level rose to 100 mg kg^{-1}, Cd concentrations ranged from 238 (*A. plamatum*) to 755 (*E. japonicus*) mg kg^{-1}. Furthermore, in the treatment with 200 mg kg^{-1} Cd, Cd concentrations ranged from 481 (*A. plamatum*) to 1843 (*E. japonicus*) mg kg^{-1}.

The accumulation of Cd was more pronounced in roots than in leaves and stems in all plant species studied. For example, in the treatment with 200 mg kg^{-1} Cd, Cd concentrations in the roots of the studied plants were all higher than 1000 mg kg^{-1} except for *P. tobira* with 810 mg kg^{-1}; while Cd concentrations in leaves and stems were all less than 220 and 150 mg kg^{-1}. Cd concentration ratios of leaves to roots were all lower than 0.05 except for *P. tobira* with the value of 0.27, indicating that more Cd was accumulated in roots than in leaves and stems regardless of plant species tested.

Generally, in the roots of studied species, Cd accumulation was considerably higher in the epidermis than in the cortex plus stele in the Cd treatments with 50, 100 and 200 mg kg^{-1} (Table 3). Interestingly, we observed a trend of decrease in Cd concentration ratios of epidermis over cortex plus stele (E/CS) proportional to the increase in Cd levels. For example, the E/CS ratios of *A. plamatum* in the treatment with 50, 100 and 200 mg kg^{-1} Cd were 12.9, 7.3 and 4.2, respectively. This result indicates that the root epidermis is an important sink site for Cd, and the stabilizing capacity tends to be saturated by enhanced Cd exposure. *C. Blue Ice* had the highest ratios of E/CS with values of 29.5, 37.1 and 14.1 in the treatments with 50, 100 and 200 mg kg^{-1} Cd, respectively.

3.3.2. Total Cd content

Total Cd content of the three tested species, namely *E. japonicus*, *P. tobira* and *C. Blue Ice*, is shown in Table 4. For the 50 mg kg^{-1} Cd treatment, the greatest total Cd content was noted in *P. tobira* with 1266 (g plant^{-1}, followed by *E. japonicus* and *C. Blue Ice*. In the higher treatments, however, with 100 and 200 mg kg^{-1} Cd, *C. Blue Ice* accumulated the greatest total Cd contents with 3023 and 5525 (g plant^{-1}, respectively. In *P. tobira*, Cd content was even lower in the 200 mg kg^{-1} Cd treatment (1047 (g plant^{-1}) than in the 100 mg kg^{-1} Cd treatment (1905 (g plant^{-1}), because of the drastic decrease in biomass in the higher Cd treatment as shown in Fig. 1. All of the three studied species showed a high Cd retention capacity in the roots. In the treatment with 50 mg kg^{-1} Cd, total Cd contents in roots were 893, 907 and 707 mg plant^{-1} in *E. japonicus*, *P. tobira* and *C. Blue Ice*, which represented 97.8%, 71.6% and 98.6% of the total Cd content in plants, respectively (Table 4). Similar patterns of distribution were also observed in the treatments with 100 and 200 mg kg^{-1} Cd.

The total Cd content ratio of plant over soil (relative uptake ‰) was calculated for each Cd treatment (Table 4). In the treatment with 50 mg kg^{-1} Cd, the highest relative uptake was found in *P. tobira* with value of 12.74‰, followed by *C. Blue Ice* and *E. japonicus*, while in the treatments with 100 and 200 mg kg^{-1} Cd, the highest relative uptake was observed in *C. Blue Ice* (15.1‰ and 13.64‰).

Table 3
Cd concentrations in epidermis and in cortex plus stele of roots of seven nursery stock species following 84-day Cd exposure.

Cd treatment (mg kg^{-1})		Cd concentration (mg kg^{-1})						
		A. plamatum	*L. vicaryi*	*A. japonica*	*E. japonicus*	*B. megistophylla*	*P. tobira*	*C. Blue Ice*
50	Epidermis (E)	1380 ± 337	2450 ± 436	1341 ± 391	3308 ± 650	1146 ± 210	1206 ± 341	2481 ± 520
	Cortex plus stele (CS)	107 ± 26	295 ± 70	75 ± 38	270 ± 84	167 ± 40	239 ± 64	84 ± 31
	Ratio of E/CS	12.9	8.3	17.9	12.3	6.9	5.0	29.5
100	Epidermis	2791 ± 545	3680 ± 638	4201 ± 722	5369 ± 789	4580 ± 914	2874 ± 643	4976 ± 695
	Cortex plus stele	380 ± 66	432 ± 58	381 ± 49	360 ± 84	436 ± 88	678 ± 74	134 ± 38
	Ratio of E/CS	7.3	8.5	11.0	14.9	10.5	4.2	37.1
200	Epidermis	5380 ± 1380	4250 ± 1235	5319 ± 1048	7320 ± 934	6425 ± 1260	3640 ± 715	6452 ± 825
	Cortex plus stele	1286 ± 257	870 ± 262	1079 ± 21	1035 ± 389	1586 ± 406	1230 ± 370	459 ± 74
	Ratio of E/CS	4.2	4.9	4.9	7.1	4.1	3.0	14.1

Table 4
Total Cd content in plant parts and relative uptake in three nursery stock species following 84-day Cd exposure.

Cd treatment (mg kg^{-1})	Plant species	Cd content (μg plant^{-1})				Relative uptake ‰[a]			
		Leaf	Stem	Root	Total plant	Leaf	Stem	Root	Total plant
50	*E. japonicus*	17 ± 9	2 ± 1	893 ± 108	913 ± 231	0.17	0.02	8.93	9.13
	P. tobira	245 ± 80	114 ± 35	907 ± 221	1266 ± 310	2.45	1.14	9.07	12.74
	C. Blue Ice	1 ± 1	9 ± 3	707 ± 119	717 ± 179	0.01	0.09	7.07	7.17
100	*E. japonicus*	11 ± 4	12 ± 7	2415 ± 739	2438 ± 632	0.55	0.60	12.10	12.26
	P. tobira	267 ± 45	159 ± 43	1529 ± 306	1955 ± 434	1.33	0.80	7.65	8.78
	C. Blue Ice	3 ± 2	100 ± 18	2920 ± 470	3023 ± 351	0.02	0.50	14.66	15.10
200	*E. japonicus*	10 ± 4	42 ± 13	3448 ± 885	3500 ± 704	0.03	0.11	8.62	8.75
	P. tobira	81 ± 17	148 ± 30	818 ± 174	1047 ± 361	0.20	0.37	2.05	2.62
	C. Blue Ice	12 ± 4	166 ± 27	5347 ± 1190	5525 ± 1196	0.03	0.42	13.40	13.64

[a] Relative uptake: ratio of total Cd content of plant over soil.

Table 5
Total Cd content in plant parts and the Cd content ratio between after and before transplanted for 120 days.

Cd treatment (mg kg^{-1})	Plant species	Cd content (μg plant^{-1})				Ratio of total Cd content between after and before transplanted			
		Leaf	Stem	Root	Total plant	Leaf	Stem	Root	Total plant
50	*E. japonicus*	58 ± 12	12 ± 6	796 ± 140	866 ± 240	3.41	6.00	0.89	0.95
	P. tobira	364 ± 48	128 ± 20	668 ± 109	1160 ± 249	1.49	1.12	0.74	0.92
	C. Blue Ice	14 ± 8	53 ± 18	613 ± 160	680 ± 168	14.00	5.89	0.87	0.95
100	*E. japonicus*	83 ± 16	40 ± 16	2287 ± 375	2210 ± 376	7.55	3.33	0.86	0.91
	P. tobira	367 ± 49	231 ± 42	1189 ± 206	1787 ± 461	1.37	1.45	0.78	0.91
	C. Blue Ice	38 ± 9	264 ± 38	2420 ± 310	2722 ± 648	12.67	2.64	0.83	0.90
200	*E. japonicus*	95 ± 21	108 ± 27	2948 ± 451	3151 ± 420	9.50	2.57	0.83	0.90
	P. tobira	108 ± 32	167 ± 47	674 ± 170	949 ± 286	1.33	1.13	0.76	0.91
	C. Blue Ice	62 ± 22	340 ± 60	4360 ± 804	4769 ± 890	5.17	2.09	0.80	0.86

To examine whether Cd was released into soil from Cd-treated plant roots, the Cd content ((g plant^{-1}) of the three species (i.e., *E. japonicus*, *P. tobira* and *C. Blue Ice*) was determined after the plants were transplanted into non-Cd-contaminated soil for 120 days (Table 5). Cd contents of transplants were slightly decreased for all treatments, ranging from 5% to 14% decrease compared with the Cd content before transplanting. After transplanting, Cd content in stems and leaves of the three species tested for all Cd treatments were increased by 1.12–14.00 times, and Cd content in roots was decreased by 11–26%, suggesting that Cd in plants was transferred from roots to shoots during the 120-day growth period. The ratio of Cd transference from roots to shoots was more pronounced in *C. Blue Ice* than in *E. japonicus* and *P. tobira*.

After the three Cd-treated species, namely *E. japonicus*, *P. tobira* and *C. Blue Ice*, had been transplanted for 120 days, Cd accumulation was observed in the rhizosphere soil for all Cd treatments (Table 6). Cd concentrations in the rhizosphere soil increased proportionally to Cd treatment level, ranging from 1.24 to 1.35, 3.57 to 6.07 and 6.18 to 7.83 mg kg^{-1} in the treatments with 50, 100 and 200 mg kg^{-1} Cd, respectively. There was no significant change in Cd concentrations of bulk soil compared with the rhizosphere soil in the treatment with 50 mg kg^{-1}. Cd concentration was found to increase in the bulk soil in the treatments with 100 and 200 mg kg^{-1} Cd. The highest Cd concentration of 0.43 mg kg^{-1} the in bulk soil found in *P. tobira* treated with 200 mg kg^{-1} was much lower than the environmental quality standard for soils of China (1 mg kg^{-1}).

4. Discussion

4.1. Cd tolerance and potential for phytostabilization

Seven nursery stock species ranging in sensitivity and tolerance to Cd stress showed varying degrees of visible toxic symptoms after an 84 day period of Cd exposure. In the treatments at the higher levels of Cd supply (100 or 200 mg kg^{-1} Cd), the lower leaves of *A. plamatum*, *L. vicaryi* and *A. japonica* became necrotic, and the upper leaves of *L. vicaryi* and *A. japonica* showed stunting, reduced leaf size and extensive necrotic and chlorotic spotting. No obvious toxicity symptoms were observed in *C. Blue Ice*, although growth was slightly reduced in the 200 mg kg^{-1} Cd treatment. It should

Table 6
Cd concentrations of rhizosphere and bulk soil of three nursery stock species after transplanting in uncontaminated soil for 120 days.

Plant species	Cd treatment ($mg\,kg^{-1}$)	Cd concentration ($mg\,kg^{-1}$)	
		Rhizosphere soil	Bulk soil
E. japonicus	0	0.044 ± 0.037 d	0.047 ± 0.040 b
	50	1.240 ± 0.545 c	0.054 ± 0.031 b
	100	4.452 ± 1.210 b	0.242 ± 0.060 a
	200	7.830 ± 1.703 a	0.410 ± 0.258 a
P. tobira	0	0.050 ± 0.048 c	0.046 ± 0.042 b
	50	1.154 ± 0.484 b	0.048 ± 0.038 b
	100	6.070 ± 2.785 a	0.268 ± 0.161 a
	200	6.404 ± 1.508 a	0.428 ± 0.269 a
C. Blue Ice	0	0.047 ± 0.036 c	0.058 ± 0.048 b
	50	1.348 ± 0.691 b	0.057 ± 0.032 b
	100	3.569 ± 1.209 ab	0.177 ± 0.090 a
	200	6.183 ± 2.265 a	0.345 ± 0.170 a

Means followed by different letters within the same column are significantly different at $P < 0.05$ according to Duncan's New Multiple Range Test at the 5% probability level.

be emphasized that compared with previous studies [30], the Cd-contaminated soil used in the experiment was strongly toxic, even for the lowest Cd treatment ($50\,mg\,kg^{-1}$) The reason for choosing such a high concentration was that the pre-experiment studies failed to identify the limitation of tolerance of all tested plants by the absence of difference of growth between the $10\,mg\,kg^{-1}$ Cd treatment and the corresponding control (data not shown). In fact, three species, *E. japonicus*, *P. tobira* and *C. Blue Ice*, were tolerant to $50\,mg\,kg^{-1}$ Cd stress, and *C. Blue Ice* still grew well in soil contaminated with $100\,mg\,kg^{-1}$ Cd (Fig. 1).

In general, the first symptom of Cd toxicity in woody plants is an inhibition of root growth, which has been reported in the Holm oak [4]. This phenomenon was also observed in our experiments: Cd-induced suppression of root growth of *A. plamatum* and *A. japonica* prior to that of their stems and leaves in the $50\,mg\,kg^{-1}$ Cd treatment (Fig. 1). Cd inhibition of root growth might result from the higher accumulation of this metal in the root than in other plant parts [4]. Treatment with $200\,mg\,kg^{-1}$ Cd caused these woody plants to accumulate up to 810–4375 $mg\,kg^{-1}$ Cd in their roots, with ratios of Cd concentration of leaves to roots all lower than 0.05 except in *P. tobira* with a value of 0.27 (Table 2). The high capacity of roots to retain trace metals has also been reported in other woody species, such as the Holm oak [4], spruce and poplar [31].

The higher resistance in roots than in other parts of the plant may be due to a greater deposition of Cd in the epidermis (Table 3), the non-metabolic tissues of roots. As the first barrier, the epidermis can immobilize heavy metals in an inactive compartment for a period of time. Data of all species studied showed that the epidermis of roots immobilized a large amount of Cd hence restricting Cd influx into the stele (Table 3). Sorption of Cd by the epidermis might be attributed to the presence of carbohydrate compounds, including pectin with negatively charged metal-binding functional groups, as reported in fern *Lygodium japonicum* [32]. Different accumulation patterns of Cd in epidermis of the studied plant roots (Table 3) might be ascribed to the fact that carbohydrates compounds of the epidermis may be of different origin as well as expressing different degrees of methylation (methyl-esterification of the carboxyl groups) varying in ability to bind Cd [32]. In the present study, the highest ratio of Cd concentration of the epidermis over cortex plus stele was observed in *C. Blue Ice* (Table 3). This might be one of the reasons why of all the species studied, *C. Blue Ice* showed highest tolerance when exposed to $200\,mg\,kg^{-1}$ Cd.

In the present study, three species, namely *E. japonicus*, *P. tobira* and *C. Blue Ice*, showed high tolerance in terms of tissue growth up to $50\,mg\,kg^{-1}$ Cd stress (Fig. 1) and can be recommended as promising woody plants for phytoremediation of Cd-contaminated soil. *C. Blue Ice* in particular grown in soil contaminated with 100 and $200\,mg\,kg^{-1}$ Cd showed greater tolerance than the other two species (Fig. 1). It can therefore be recommended for planting in environments severely contaminated by Cd (higher than $50\,mg\,kg^{-1}$ Cd).

4.2. Environmental risk assessment of transplantation

The capacity of woody plants such as spruce, poplar and carambola to bind heavy metals, is reported to reach its maximum after the first vegetation period [1,33]. Our results with nursery stocks also showed a high efficiency in binding-Cd in roots during the 84-day Cd treatment (Table 4). The maximum of total Cd content at 5525 ($g\,plant^{-1}$ was reached at 84 day in *C. Blue Ice* for the $200\,mg\,kg^{-1}$ treatment. This high capacity to remove Cd has to be considered in relation to the relatively short turnover of nursery stocks which in conventional practice takes only 1 or 2 years. By comparison arbor species such as willow are usually harvested after more than 6 years [28], transplanting heavy metal-stabilized nursery stocks for urban or road greening may be a more efficient way to remediate and reuse contaminated soil.

To put this into practice, it is most important that heavy metal release from the transplanted plants should be kept at as low a concentration as possible. From our findings, there is low risk of pollution to soil or human beings from the woody plant leaves for the following reasons: (1) Cd was stabilized mainly in the plant roots, as shown in *E. japonicus* and *C. Blue Ice* (Table 4); (2) the nursery stocks are usually planted in urban green space or in the green belt at the road side, far away from the food chain; and (3) the contaminated woody seedlings can be mix-planted with "clean" seedlings to further disperse the Cd levels by transplanting them together in various areas.

The risk of Cd release from contaminated woody roots was assessed in the present study. Total Cd contents of three species, namely *E. japonicus*, *P. tobira* and *C. Blue Ice*, were slightly lower at transplanting after 120 days (Table 5), compared with the Cd-polluted plants before transplantation. Some Cd was redistributed from the roots into the leaves during 120-day growth period (Table 4). Some was also released from roots (Table 5) with trace amounts being found in the rhizosphere (Table 6). The Cd increase in the rhizosphere might result from desorption of Cd from exchangeable sites of root epidermal tissues by competition with other divalent ions, such as Ca^{2+} [34]. In addition, some Cd in the peeled epidermal residues may have further contributed to the increase in Cd level in the rhizosphere during root regeneration. However, the transfer process of Cd from the rhizosphere to the bulk soil was so low during the 120-day growth period that the increase in Cd concentration in bulk soil was only detected in the 100 and 200 Cd treatments (Table 6). The maximum value of the Cd concentration of bulk soil was $0.428\,mg\,kg^{-1}$, obtained from *P. tobira* in the $200\,mg\,kg^{-1}$ Cd treatment, a value well below the environmental quality standard for soils of China ($1\,mg\,kg^{-1}$). In fact, in the case of the maximum amount of Cd (0.756 mg) released from *P. tobira* in the $200\,mg\,kg^{-1}$ Cd treatment, the risk of soil pollution by roots is thus negligible, given the high retention of Cd by the roots, the large mass of bulk soil relative to the rhizosphere soil and plant root biomass.

4.3. Potential for phytoremediation of contaminated soil

To the best of our knowledge, there are no viable risk free methods for the disposition of hazardous biomass after phytoremediation. Smelting of contaminated plant material has been

considered as a promising method because it can significantly reduce plant biomass [25–27]. However, detailed eco-toxicological and economical studies have not been investigated as to assess the industrial risks associated with utilization of such ashed materials.

In the present study, we propose for the first time a phytoextracting approach of stabilizing metals from contaminated soil by nursery stocks prior to their transplantation for greening. The pattern of high root retention and low risk after transplantation contributes to the feasibility of this technique for soil cleansing. More importantly, it is an eco-friendly technique, which would be of benefit in the production as well as providing a feasible solution to the remediation of agricultural land polluted by heavy metals.

Although this technique imposes no environmental risk in itself, the transplanted sites should be confined to avoid an increase in background pollution in the region [35]. The transplantation technique is especially unsuitable for public parks and childrens' playgrounds, because the contaminated leaves may still cause health injury (Table 4). Areas away from human habitation such as the green belt of highway are recommended as transplanting sites.

Furthermore, this is an original investigation that is limited to a pot study. Limitations of this approach in a field trial deserve further investigation. The following aspects need to be considered. (1) In the field, trees are transplanted with root balls. Although the root ball of the nursery stock is very small (about 5 g each in the present study), and peasants usually shake off the bulk soil adhering to the roots to retard the loss of topsoil, the presence of Cd in root ball must be considered in field studies. (2) Human and environmental risk can be significantly decreased if contaminated trees and the "clean" trees are planted together, but the appropriate ratio needs to be systematically evaluated. (3) The overall plant-and-transplant practice might have effect leaching of metals to the groundwater or spreading of metals through wind and water erosion. Thus, nursery practices, such as tillage and weed control, should be kept at a low a level as possible. Furthermore, a package of complementary techniques could also be considered in the future work, e.g., enhancing the pH by liming, immobilization of heavy metals by the application of soil additives, and improving soil quality by fertilization [3].

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