



Leaching Behaviour and Enhanced Phytoextraction of Additives for Cadmium-Contaminated Soil by *Pennisetum* sp.

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Abstract

The leaching behavior of five additives, including citric acid (CA), wood vinegar (WV), 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP), polyaspartic acid (PASP) and FeCl₃, was investigated to evaluate the possibility of enhanced phytoextraction of *Pennisetum* sp. from cadmium-contaminated soil. FeCl₃ and CA have the highest leaching potential due to the ability that could convert large amounts of mobile fractions of Cd. The pot experiment showed that HEDP, WV, and PASP treatments could not only significantly increase the biomass of *Pennisetum* sp., but also maintain high uptake capacity of Cd by activating the stable fractions. HEDP has the highest Cd extraction efficiency and metal extraction ratio (MER) value. The phytoremediation efficiency could be improved mainly by increasing the biomass of the tolerant shoots, and *Pennisetum* sp. seems to have the maximum potential of phytoextraction to Cd with HEDP which could achieve a higher phytoextraction effect than Cd-hyperaccumulator.

Keywords Additives · Leaching behavior · Phytoextraction · Cadmium · *Pennisetum* sp.

Soils are subject to different levels of pollution arising from industrial sources and other human activities (Begum et al. 2013). The low-cost, plant-based phytoextraction technique has often been described as a promising technique to remediate contaminated soils by heavy metals (Evangelou et al. 2007). Preferably, plants for phytoextraction should have the following characteristics: (i) tolerant to high levels of metals; (ii) reasonable accumulation of high levels of the metal in their above-ground parts. (iii) produce high biomass in the field (Alkorta et al. 2004).

In the last few years, an important number of plants described as “hyperaccumulators” have been identified to remediate metal-polluted soils. However, hyperaccumulators are, in general, relatively small, have slow rates of biomass production, and lack any established cultivation (Allica et al. 2008). Therefore, nowadays, fast-growing, high biomass crop plant species that accumulate moderate levels of metals in their shoots are widely being tested for their phytoextraction potential.

Pennisetum sp. has been proposed as a suitable phytoremediation plant (Zhang et al. 2014). The use of *Pennisetum* sp. for the remediation of metal contaminated soils appears encouraging due to their high-yield, high-biomass, high resistance to heavy metals, long vegetative season, resprouting capacity after the harvest of above-ground biomass, and high economic and recycling value for the sustainable phytoremediation process. *Pennisetum* sp. had shown potential in the phytoextraction of soil polluted with metals such as Cu, Cd when biomass was considered (Cui et al. 2016). The efficiency of a phytoextraction process also depends on soil factors such as metal mobility and soil metal phytoavailability. However, most heavy metals have low bioavailability in soils, limiting the broad application of tolerant plants in phytoextraction (Zhang et al. 2018).

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Different additives, such as EDTA (ethylene diamine tetraacetic acid), DTPA (diethylenetriaminepentaacetic acid), have been used to enhance the bioavailability of metals in contaminated soils (Afshan et al. 2015; Sun et al. 2009). It should be noticed that lots of synthetic chelating agents such as EDTA are non-biodegradable and can cause groundwater contamination due to uncontrolled leaching in the soil. Compared to EDTA, citric acid (CA) is advantageous in the use of chelate assisted phytoextraction for it is biodegradable and can be rapidly degraded to carbon dioxide and water (Huang et al. 1998). Wood vinegar (WV) has been widely used by farmers as environmentally foliar fertilizer to improve crop yields and quality. Polyaspartic acid (PASP) and 1-hydroxy ethylidene-1, 1-diphosphonic acid (HEDP) have been widely used as plant growth promoters (Wang et al. 2013). Besides, PASP is a new type of macromolecule-chelating agent, which possesses several carboxylic groups and is capable of coordinating and forming complexes with different heavy metals (Roque et al. 2004). HEDP is the most common phosphonate, which is cheap, low toxicity, biodegradable and easy to operate, and phosphonates are anthropogenic complexing agents containing one or more C–PO(OH)₂ groups (Nowack 2003) which are used in numerous technical applications as chelating agents. FeCl₃ is a useful heavy metal chelate for soil restoration based on its high extraction efficiency, cost-effectiveness, and relatively low environmental impact (Makino et al. 2008).

The application of these additives in *Pennisetum* sp. could assist in the remediation of polluted sites as well as increase the biomass and phytoextraction efficiency. However, to the best of our knowledge, enhanced phytoextraction of Cd by *Pennisetum* sp. with additives has not been investigated in previous studies. Therefore, we conducted the leaching and pot experiment to (i) evaluate the leaching efficiency of Cd by additives and the conversion of different fractions of Cd in the soil. (ii) Assess the effects of additives on plant biomass and uptake capacity of Cd. (iii) Evaluate the enhanced phytoextraction potential of *Pennisetum* sp. combined with plant biomass. (iv) Provide a theoretical basis for the screening of additives and explore the enhanced mechanism.

Materials and Methods

CA, WV, PASP, HEDP, and FeCl₃ were provided by Xilong Chemical Co., Ltd. (Guangdong, China), and all other chemicals and reagents used in this experiment were of analytical reagent grade. Plant seeds were purchased from Import Grass Species Shop in Shanghai.

The pre-contaminated soil for extraction procedure was sandy soil obtained from the surface (0–20 cm depth) of an arable field in Nanjing. This soil was artificially contaminated with Cd as Cd(NO₃)₂·4H₂O. After metals were

added, soils were equilibrated for 20 days and subjected to five saturation cycles with deionized water and air-dried, and then the concentrations of Cd in the contaminated soil were measured. Artificially contaminated soils were rated in three levels, 0.63 ppm (lightly-contaminated), 1.24 ppm (moderately-contaminated), and 1.81 ppm (heavily-contaminated), respectively. The field-contaminated soils for pot experiment were collected from the agricultural area in Yixing (Nanjing City, China). All soils were air-dried, crushed and passed through a 2-mm diameter sieve, and thoroughly mixed prior to experiments (Makino et al. 2008). The physicochemical parameters of soils were measured in Table 1.

The comparison of the Cd-extraction effect with five different additives was carried out in 50 mL polyethylene tubes. The concentrations of four additives (CA, PASP, HEDP and FeCl₃) were 10, 20, 50 mM, and the concentrations of WV were 0.5%, 1.0%, 2.5% (w/v), respectively. The extraction experiments were conducted with the ratio of solid (mass) to solution (volume) at 5:1. The soil sample (5 g) in triplicate was suspended in a 25 mL extracting solution and agitated at 25°C with 180 rpm for 6 h, subsequently centrifuged at 4000 rpm for 10 min to obtain the supernatant. Then the residue was continuously rinsed with 25 mL water and centrifuged. The concentration of Cd in supernatant mixture filtered through a 0.45 μm membrane was then measured.

In pot experiment, the field-contaminated soils after pretreatment were packed into microcosms (1500 g per microcosm) with an inner diameter of 15 cm and a depth of 12 cm. The sand and gravel on the screen were more than 0.15 mm to help drain and avoid soil loss. 20 Seeds were soaked in distilled water and then spread in each pot. Five seedlings with the growth height of about 10 cm were retained in each pot after 3 weeks of seed emergence. Biomass was measured regularly using analytical balance. A volume of 240 mL of deionized water was added each week to ensure soil WCH of 80%. Plants were cultivated in a greenhouse with natural sunlight under controlled conditions of 60% relative humidity and 25°C. Each pot had 8-in. plate on the bottom to avoid cross-contamination. After 42 days of emergence, aqueous additives applications were applied every 7 days for three times in triplicate during the experiment. The potted plants were harvested 14 days after the treatment for further analyses.

To compare and analyze the change of the heavy metal fraction within the soils before and after washing, 1.00 g soil sample sieved through a 2-mm nylon was analyzed using the modified Community Bureau of Reference (BCR) sequential extraction procedure (Table S1 and Text S1) to determine metal concentrations in different fractions (Nemati et al. 2009). A BCR certified reference material (GBW07437, CNAC) was subjected to the BCR protocol, and the Cd recovery observed was higher than 85% in all steps, indicating satisfactory recovery of the fractionation process.

Table 1 Selected physicochemical properties of the pre-contaminated soil

	pH	SOM (g kg ⁻¹)	CEC (cmol kg ⁻¹)	Available K (mg kg ⁻¹)	Total N (g kg ⁻¹)	Total P (g kg ⁻¹)	Total K (g kg ⁻¹)	Cd (mg kg ⁻¹)	Clay (%)	Silt (%)	Sand (%)
Pre-contaminated soil	5.19	22.11	13.45	47.51	0.23	0.42	9.82	0.13	8.61	28.15	56.44
Field-contaminated soil	6.28	25.67	6.23	26.81	0.16	0.36	5.42	9.32	15.13	32.21	52.37

The total concentrations of Cd in samples were determined using an atomic absorption spectrophotometer (Zhang et al. 2010). Prior to measuring, plants were cleaned with deionized water, and dried in an oven at 105°C for 20 min then at 75°C to constant weight; then, roots and shoots were manually pulverized in liquid nitrogen and ground through 0.15 mm sieve. Each 0.50 g of dried biomass in triplicate added 6.00 mL of HNO₃ (70 wt%) and 2.00 mL of hydrogen peroxide (H₂O₂ 30 wt%). A microwave oven was programmed to perform plant biomass digestion. The digestion process of rhizosphere soils and BCR residues before Cd concentration measurement were described in Text S2.

The content of Cd in standard soil materials (SRM 2710a, Montana I Soils, NIST) was 0.086 ± 0.023 ppm, and the content of Cd in standard plant materials (GBW07602, Shrub Branches, CNAC) was 0.32 ± 0.07 ppm. Standard reference materials were also analyzed as part of the QA/QC protocol, and the relative standard deviation of each heavy metal was less than 10%. Recovery rates of 95% ± 5% were obtained by using a series of GSS series of standard samples and a series of GSV standard samples and the blank in each batch of soil samples.

Calculations and significance of translocation factor [TF (%) = metal concentration in shoot/metal concentration in root (ppm) * 100] was adopted from Trotta et al. (2006). Tolerance index [TI = biomass on a soil enriched (g)/biomass on a control soil (g)] was adopted from Baker et al. (1994). Bio-concentration factor (BCF), the ratio of the metal concentration in the different plant organs to the metal concentration in the soil, was adopted from Tu et al. (2002). Metal extraction ratio (MER), the ratio of metal accumulation in the shoots to that in soil, was adopted from Mertens et al. (2005). PEN is defined that the number of plants required to extract 1.00 g of metal in consideration of the biomass of the shoots (Garcia et al. 2004).

Statistical analysis was performed by using the SPSS version 16.0 for Windows software package (SPSS, Chicago, IL, USA). Summarized results were presented as the treatment means (± standard deviation, SD) from three replicate measurements of three independent experiments. One-way ANOVA was used to assess the significance of differences between means. Comparisons among means were performed using LSD test at the 5% level. Pearson's correlation coefficients between indexes about Cd accumulation in *Pennisetum* sp. were calculated based on linear correlation between two variables ($p < 0.05$ or < 0.01).

Results and Discussion

The leaching efficiency of five activators on Cd increased with the concentration of additives at three different polluted conditions (Fig. 1). This was consistent with the

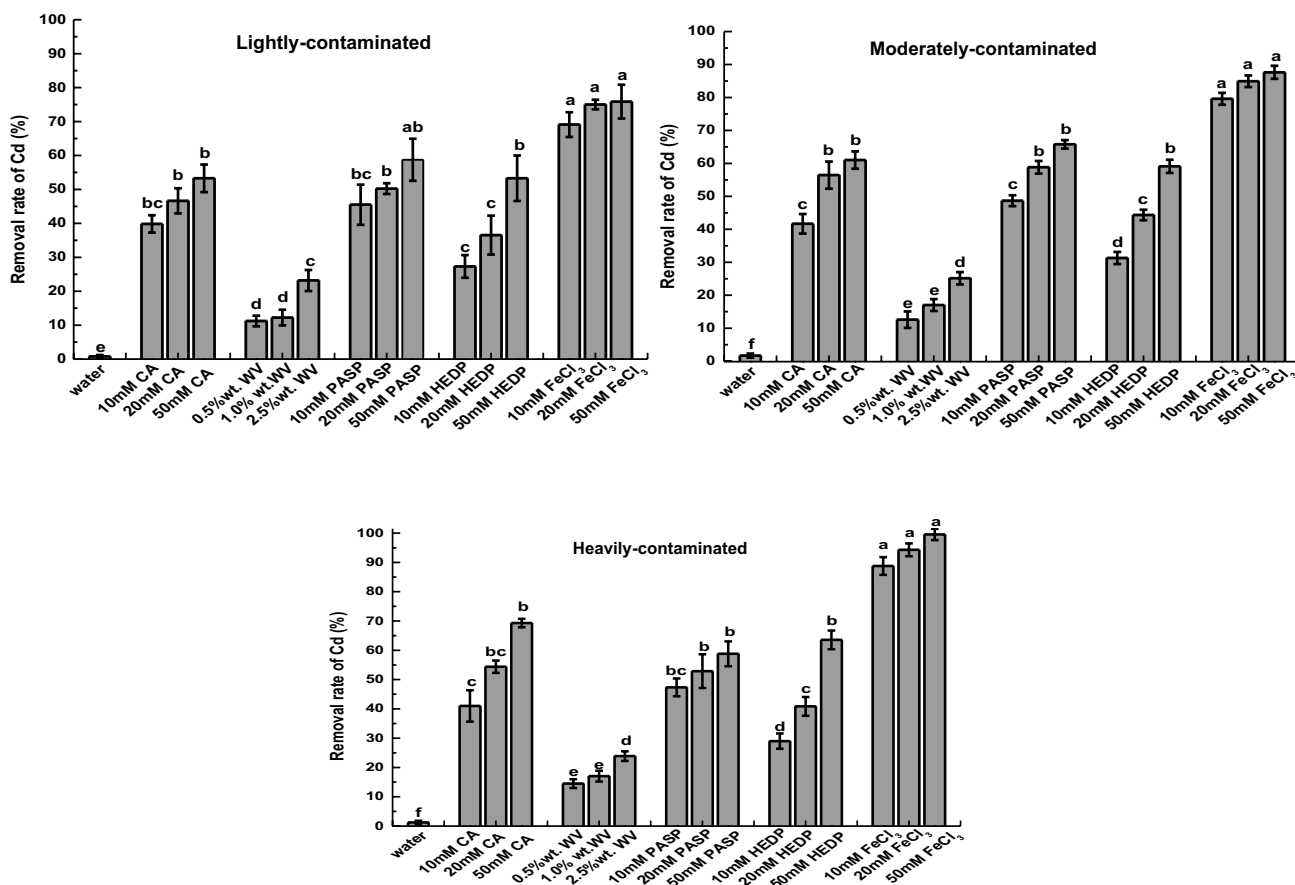


Fig. 1 Leaching rate of Cd in soils under light, moderate, and heavy Cd-contaminated conditions treated with different concentrations of chelators (CA, WV, PASP, HEDP, and FeCl₃). Error bars represent

the standard errors of the means (n=3). Bars marked by the same letter(s) are not significantly different at p<0.05 by Duncan’s new multiple range test

result of Wang et al. (2016) that removal efficiencies for Cd increased significantly with an increase GLDA (glutamic acid diacetic acid, tetrasodium salt) concentrations from 1.00 to 50.00 mM. It indicated that higher concentrations of chelating agent could bind to more heavy metal ions, directly promoting metal ion–ligand complexation reaction to move in the direction leading to chelate formation (Wu et al. 2015), which increase the removal ability.

The removal efficiency of HEDP was most significant with increasing concentration. The removal efficiency of HEDP was from 27.32% to 53.37% with light contaminated soils and increased approximately twofold (Fig. 1). It might be explained by the high concentration of Cd in the soil enhancing phosphonates coordination of more than one sigma electron-pair donor group from the same ligand to the same central atom (Nowack 2003). Similar leaching efficiency under three polluted conditions were listed in descending order as follows: FeCl₃ > PASP > CA > HEDP > WV (Fig. 1). WV showed the lowest removal efficiency of Cd ranging from 12.6% to 25.2% with moderately contaminated soil (Fig. 1). The

low pH of the soil might result in limited ability of WV to increase heavy metal activity by increasing soil acidity. CA and PASP were more effective and stable in extracting Cd at three different polluted conditions, ranging from 39.83% to 69.35% and 45.51% to 65.84%, respectively (Fig. 1).

FeCl₃ showed the highest removal efficiency of Cd compared with other additives, which could remove 69.1% to 75.9% cadmium with light-contaminated soil. With the increase of the pollution level, the removal efficiency of FeCl₃ also increased to 88.8% to 99.5% (Fig. 1). The possible reason might be that the low pH of extraction because of the proton release and the generation of hydroxides as well as the formation of Cd–Cl complex inhibited re-absorption of the extracted Cd to adsorption sites on the surface of the soil particles, so as to enhance Cd extraction of FeCl₃ (Qin et al. 2004; Makino et al. 2008). However, the effect of the increasing concentration of FeCl₃ on the removal efficiency was not significant. This also indicated that low concentration of FeCl₃ had efficiency and economic potential to achieve the same removal rate as high concentration of FeCl₃ with heavily contaminated soil of Cd.

The distribution and conversion of the metals studied into leachable fractions (L) and the three fractions of BCR sequential extraction (F1 + F2 + F3), as well as residual fraction (R), are shown in Fig. 2. Based on the data of different treatments (except CK) in Fig. 2, Pearson's correlation coefficients was calculated in Fig. 3, and also indicate significant correlations between: L and F1 ($r = -0.957^{**}$; $p < 0.01$); L and F2 ($r = -0.906^{**}$; $p < 0.01$); L and F3 ($r = -0.905^{**}$; $p < 0.01$); L and R ($r = -0.841^{**}$; $p < 0.01$). The Cd of each fraction in the soil was converted to leachable fraction, and the conversion rate indicated reduction of fractions, due to the effect of the washing treatment. It also indicated favorable tendency of each fraction of Cd converted to the leachable fraction was different as the leaching rate increases, decreasing in the order: exchangeable and acid-soluble fraction (F1) > reducible fraction (F2) > oxidizing fraction (F3) > R. However, the orders of conversion amount were: F1 > F2 > R > F3.

In Fig. 2, the higher conversion ability of F1 and F2 than the other fractions were found in FeCl₃ and CA. The conversion rate of F1 in FeCl₃ and CA were the highest, ranging from 85.91% to 91.84% and 38.42% to 70.73%, respectively. The potential reason might be the low pH caused by the hydrolysis of FeCl₃ and the application of CA (Makino et al. 2006) increased the conversion rate of F1. F1 includes weakly adsorbed metal species and those that are trapped on the soil surface by relatively weak electrostatic interactions and that can be released by the ion exchange process. This fraction is susceptible to changes in pH, and would be easily released into environment dangerously (Nemati et al. 2009). Therefore, FeCl₃ and CA were effective additives to promote conversion of the mobile fractions (F1 and F2).

However, the conversion rate of F1 in WV, HEDP and PASP was the lowest compared with other fractions, and these three additives had higher conversion rates in F3. F3 might be released through complexation or bioaccumulation in various forms of organic matter such as living organism, organic coatings on inorganic particles and debris (Kennedy et al. 1997). This fraction is not considered to be highly mobile or available, so it is more stable and less harmful than F1 and F2. It may be the case therefore that the extraction efficiency of FeCl₃ and CA was higher than WV, HEDP, and PASP.

The high yield of plant biomass is the primary condition for successful phytoremediation of tolerant plants. According to Fig. 4, all additives except FeCl₃ can promote the growth of plant biomass. HEDP had the highest promoting effect on biomass, which had increased by 43.15% compared to CK. It might be ascribed to that HEDP as an organic phosphate fertilizer can promote the formation of trace elements in the soil, and the soluble chelate is absorbed by plants (Steber and Wierich 1986), thereby stimulating plant growth and increasing biomass. CA could adjust the pH of soil, improve the absorption of iron by plant and reduce the soil salt injury, thus promote the biomass growth of plant (Huang et al. 1998). WV and PASP are widely used as foliar fertilizers and plant growth promoters (Wang et al. 2013). The biomass under FeCl₃ treatment was the lowest, with a reduction of 10.7%, and the potential reason is that Cd–Cl complexes formed by the ion exchange between Fe and Cd led to more toxicity and reduced biomass (Sun et al. 2008).

Pearson's correlation coefficients (Table 2) also indicate significant correlations between: root biomass and shoot biomass ($r = 0.972^{**}$, $p < 0.01$). This suggested that the

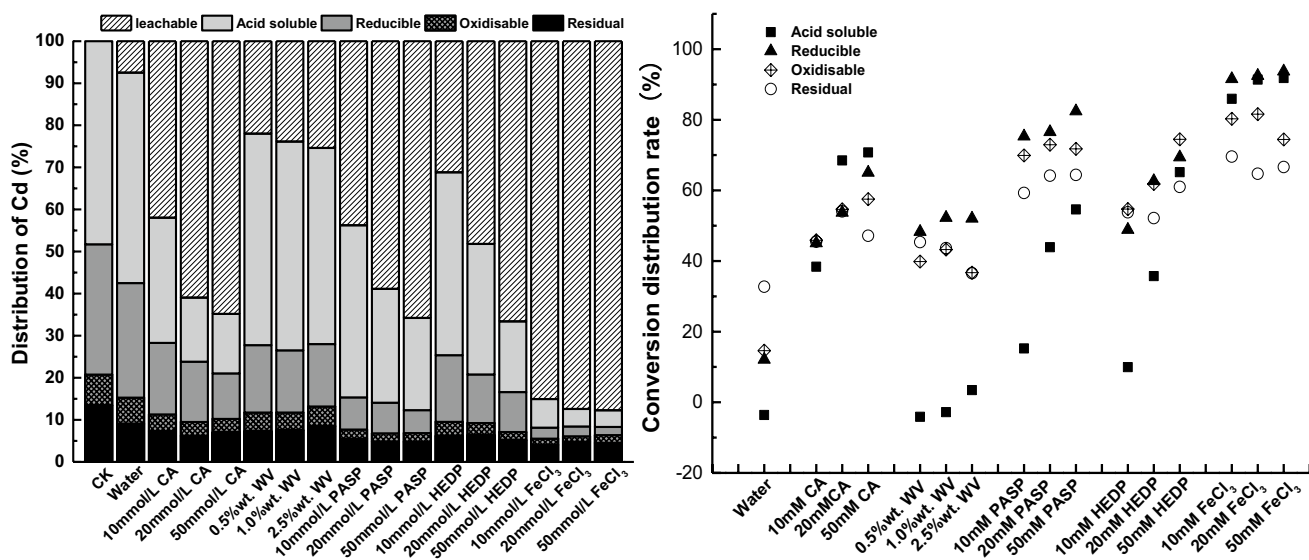


Fig. 2 BCR sequential extraction and conversion rate of different speciation of Cd in moderately-contaminated soils under different treatments. Values shown are the mean ($n = 3$)

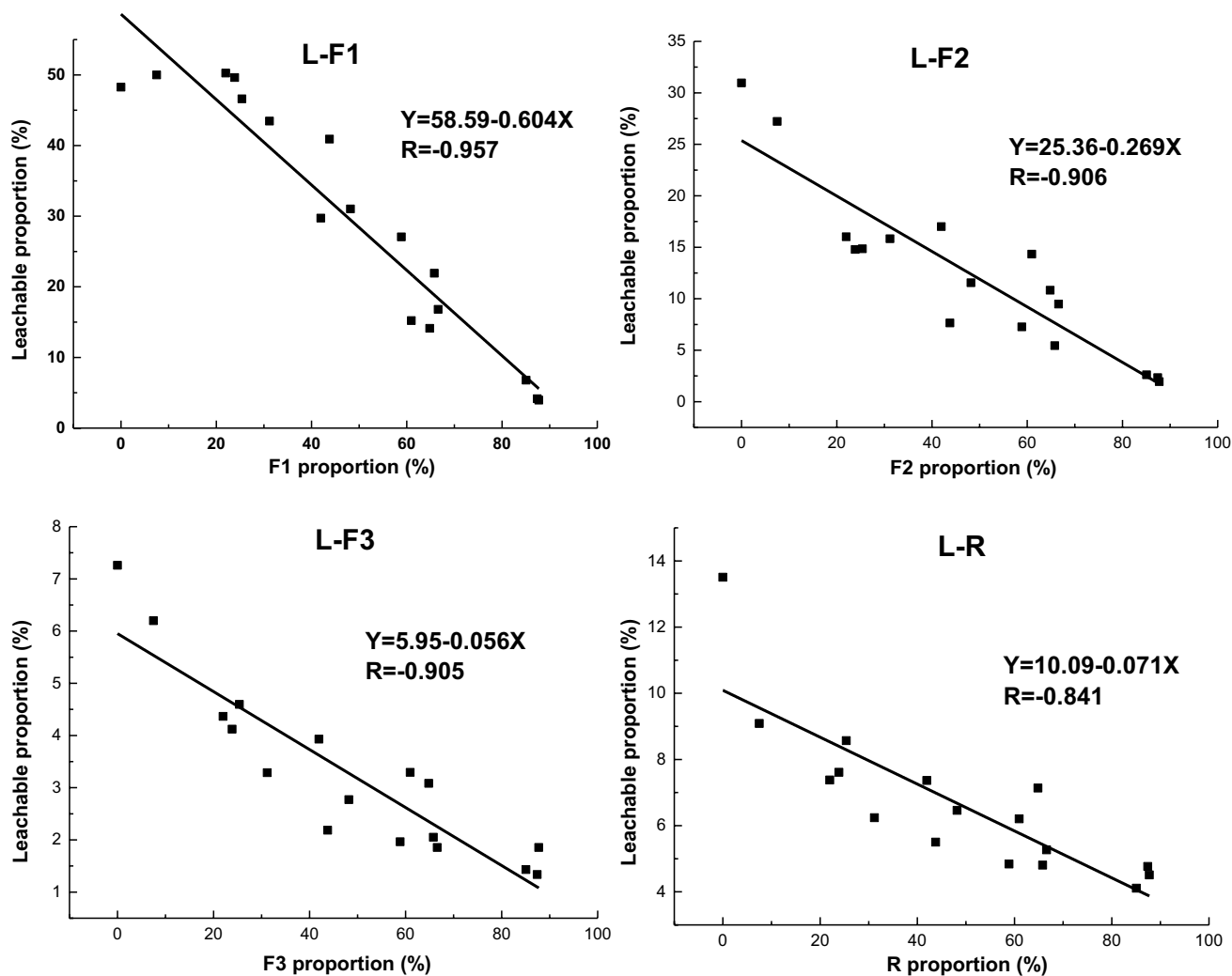


Fig. 3 Correlations of leachable fractions (L), BCR sequential extraction (F1 + F2 + F3) and residual fraction (R)

growth of the shoot part was controlled and influenced by root development. Biomass of shoot and root under different treatment is listed in same descending order as follows: HEDP > WV > PASP > CA > CK > FeCl₃.

Tolerance index is another feature that affects the phytoremediation process, and value greater than threshold is important for phytoremediation studies (Bluskov et al. 2005). TI indicated significant relationship with shoot biomass ($r = 0.998^{**}$, $p < 0.01$) and root biomass ($r = 0.976^{**}$, $p < 0.01$) in Table 2. The potential reason might be the stimulation of tolerance mechanisms, such as powerful antioxidant defense system (Khan et al. 2009). The TI of FeCl₃ was the lowest and less than 1, having TI of shoot at 0.75, thus depicting Cd had become deleterious for *Pennisetum* sp. This finding is in agreement with earlier reports indicating toxicity of Cd–Cl has an adverse effect on TI of *Echinochloa polystachya* (Solis-Dominguez et al. 2007).

The four additives, WV, PASP, HEDP, and FeCl₃, have no significant effect on the Cd uptake of the roots of the *Pennisetum* sp., while the content of Cd in root of CA was reduced by 9.63% (Fig. 5). All the additives had a negative impact on the uptake of Cd in shoot, reducing from 21.41% to 10.17% (Fig. 5). It indicated that CA harmed the ability of the *Pennisetum* sp. to absorb and transport Cd. This result may be explained by the fact that the stress of Cd uptake by *Pennisetum* sp. was enhanced by biomass growth (Quartacci et al. 2003). In addition, the main converted fractions of CA were F1 and F2, and the conversion efficiency of less bioavailable fractions (F3 and R) was low. Therefore, it also results in that CA could not significantly increase the concentration of available and mobile forms of Cd. The other four additives do not affect the absorption capacity of Cd in the roots of *Pennisetum* sp., but also have a negative effect on the transport capacity of Cd from root to shoot. For WV,

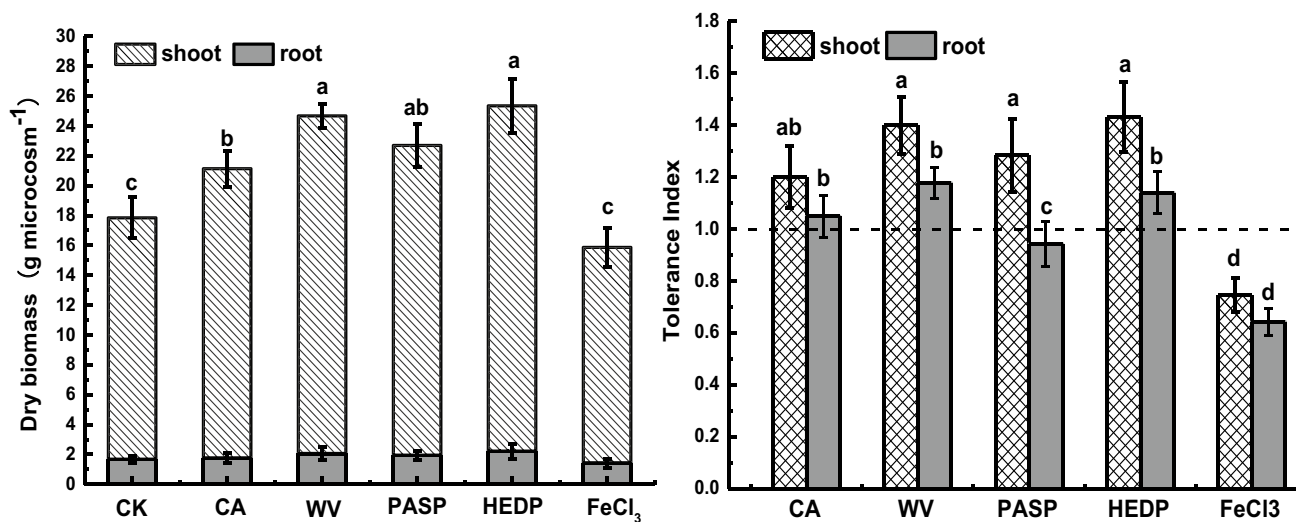


Fig. 4 Effects of the application of additives on the biomass and tolerance of shoot and root in *Pennisetum* sp. Threshold for tolerance index is 1. Error bars represent the standard errors of the means

($n=3$). Bars marked by the same letter(s) are not significantly different at $p < 0.05$ by Duncan's new multiple range test

Table 2 Pearson's correlation coefficients between some indexes about Cd accumulation in *Pennisetum* sp.

	TI	TF	BA-Sh	BA-R	Sh-Cont	R-Cont	Sh-Bio	R-Bio
TI	1.000							
TF	-0.539*	1.000						
BA-Sh	0.914**	-0.202	1.000					
BA-R	0.916**	-0.374	0.445	1.000				
Sh-Cont	-0.427	0.879**	-0.025	-0.111	1.000			
R-Cont	0.059	0.042	0.292	0.427	0.511*	1.000		
Sh-Bio	0.998**	-0.549*	0.908**	0.909**	-0.441	0.046	1.000	
R-Bio	0.976**	-0.411	0.966**	0.973**	-0.246	0.208	0.972**	1.000

The absolute values of the correlation coefficient is greater than 0.5 are given in bold

TI tolerance index, TF translocation factor, BA-Sh total bioaccumulation in shoot, BA-R total bioaccumulation in root, Sh-Cont Cd content in shoot, R-Cont Cd content in root, Sh-Bio shoot biomass, R-Bio root biomass

*, **Significant at $p < 0.05$ and < 0.01 , respectively

PASP and HEDP treatments, on the one hand, this is probably due to the restriction of Cd uptake by biomass growth; on the other hand, the extraction efficiency of Cd by these additives is low, so many mobile fractions with high bioavailability were still fixed in soil particles. The low conversion of F3 and R also affects the enhancement of FeCl₃ on uptake content of Cd. Moreover, as a Cd-tolerant plant, the toxicity of Cd-Cl formed by the exchange of Fe ions and Cd on *Pennisetum* sp. made the tolerance genotypes resistant to phytoextraction of Cd (He et al. 2017).

Effects of additives on BCF and TF are shown in Table 3. Bioconcentration factor (BCF) is the defining parameter in phytoremediation, providing basis on the uptake of metal, storage in the roots, and mobilization into aerial plant parts (Zayed et al. 1998). BCF can also

evaluate the potential of accumulating metals, and BCF values greater than 1 are indicative of a potential hyperaccumulator species (Zhang et al. 2002). BCFs of shoot and root under CK treatment were 3.29 and 2.64. It indicated the root of *Pennisetum* sp. had higher accumulation capacity of Cd than shoot also suggested a strong phytoremediation potential for *Pennisetum* sp. In CA treatment, the BCF value of root and shoot was significantly lower than that of CK, and the BCF value of root and shoot decreased by 9.42% and 21.59%, respectively. It is noteworthy that although the biomass was increased under WV, HEDP, and PASP treatments, the BCF values of root did not decrease significantly, and the BCF values of shoot remained above 2.2. It indicated that these additives, while promoting biomass, could activate the F3 and R (stable fractions) to

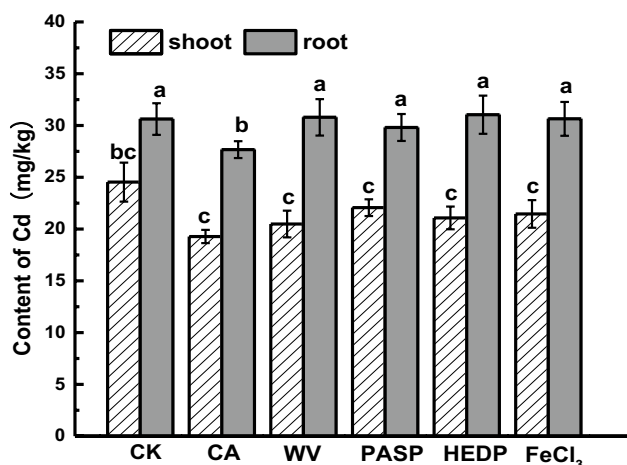


Fig. 5 The contents of Cd in the different tissues of *Pennisetum* sp. Error bars represent the standard errors of the means (n=3). Bars marked by the same letter(s) are not significantly different at $p < 0.05$ by Duncan’s new multiple range test

Table 3 Bioaccumulation coefficient (BCF) and translocation coefficient (TF) of different plant part

Treatment	BCF		TF
	Roots	Shoots	Shoots
CK	3.29 ± 0.62 ^{A,B}	2.64 ± 0.37a	0.80 ± 0.07a
CA	2.98 ± 0.24b	2.07 ± 0.21c	0.70 ± 0.12ab
WV	3.31 ± 0.36a	2.21 ± 0.18bc	0.67 ± 0.04b
PASP	3.21 ± 0.54a	2.37 ± 0.26b	0.74 ± 0.06ab
HEDP	3.34 ± 0.79a	2.27 ± 0.28b	0.68 ± 0.04b
FeCl ₃	3.29 ± 0.46a	2.31 ± 0.31b	0.70 ± 0.08ab

CK Cd-contaminated soil without application of additives, CA citric acid applied at 10 mM, WV wood vinegar applied at 1.0 wt%, PASP polyaspartic acid applied at 10 mM, HEDP 1-hydroxyethylidene-1 applied at 10 mM, FeCl₃ FeCl₃ applied at 10 mM

^AMeans ± standard errors (n = 3)

^BMeans followed by the same letter(s) within a column for a given metal element are not significantly different at $p < 0.05$, according to Duncan’s multiple range test

reduce the biomass stress on Cd uptake, thereby maintaining a high Cd extraction capacity.

Translocation coefficient (TF) is used to work out the ability of plants to translocate heavy metal from roots to harvestable parts (Zu et al. 2005), TF value greater than 0.50 indicates that the plant can transfer most of the heavy metals in root to the above-ground part, and TF value greater than 1 is an important parameter to evaluate potential of hyperaccumulators. In this study, the TF value of CK was 0.80 ± 0.07, indicating that *Pennisetum* sp. had much potential of tolerant plant rather than hyperaccumulator. TF under treatments of additives was reduced ranging from 7.50% to 16.25%. TF indicated significant relationship with Cd content in shoot

($r = 0.879^{**}$, $p < 0.01$). TF also indicated significant relationship with TI ($r = -0.539^*$, $p < 0.05$) and shoot biomass ($r = -0.549^*$, $p < 0.05$) in Table 2. This suggested that Cd was tended not to deposit on the roots, the enhancement of tolerance and shoot biomass by additives may result in negative effect of additives on TF, and heavy metal tolerant genotypes were able to restrict the upward movement and the increase of above-ground dry biomass, thus developing their tolerance (Zvobgo et al. 2018).

The Cd uptake in shoot and root of *Pennisetum* sp. were shown in Fig. 6. Higher accumulation in the aerial part that can be harvested easily than root may achieve a significant phytoextraction efficiency of heavy metals from soils (Zalewska 2012). The dominating Cd uptake by *Pennisetum* sp. was in the shoots, up to 87.75%–88.61% in the whole plant. It indicated that *Pennisetum* sp. has great potential for phytoextraction. Total Cd uptake under different treatment is listed in same descending order as follows: HEDP > WV > P ASP > CK > CA > FeCl₃. Pearson’s correlation coefficients (Table 2) also indicate significant correlations between: total bioaccumulation in shoots and shoot biomass ($r = 0.908^{**}$; $p < 0.01$), while Pearson’s correlation coefficient between total bioaccumulation in shoots and Cd content in shoot ($r = -0.025^{**}$; $p < 0.01$) was significantly uncorrelated. It indicates that the total Cd uptake of *Pennisetum* sp. was mainly through the promotion of biomass by additives rather than by enhancing the accumulation ability of Cd. The plant effective number (PEN) and the metal extraction ratio (MER) which takes the shoot biomass and the soil volume into account to be cleaned more informative (Mertens et al. 2005) have been applied to evaluate the ability to remedy contaminated soil. According to Fig. 6, the extraction ratio of Cd with different additives was 1.34%, 1.66%, 1.64%, 1.75%, and 1.11%. To remove 1.00 g of Cd from soil, more than 2520 shoots of CK would be needed when the concentration of Cd in soil, and MER of CK was 1.42%. Sun et al. (2008) also found PEN and MER of Cd in soil was 10 ppm of Cd-hyperaccumulator *Solanum nigrum* L. was 3691 and 1.07%. This suggests *Pennisetum* sp. can achieve a higher phytoextraction effect than Cd-hyperaccumulator, and enhancement of MER with HEDP was the highest as compared to CK.

In conclusion, all additives have a favorable leaching efficiency, and the concentration of additives was positively correlated with the leaching efficiency. FeCl₃ and CA have the highest leaching potential due to the ability that could convert large amounts of mobile fractions (F1 and F2). In pot experiment, CA significantly reduced the BCF values of root and shoot, while FeCl₃ significantly reduced the biomass of *Pennisetum* sp. However, HEDP, WV, and PASP could not only significantly increase the biomass of *Pennisetum* sp., but also maintain high uptake capacity (i.e., BCF and Cd concentrations in shoot) of Cd by activating

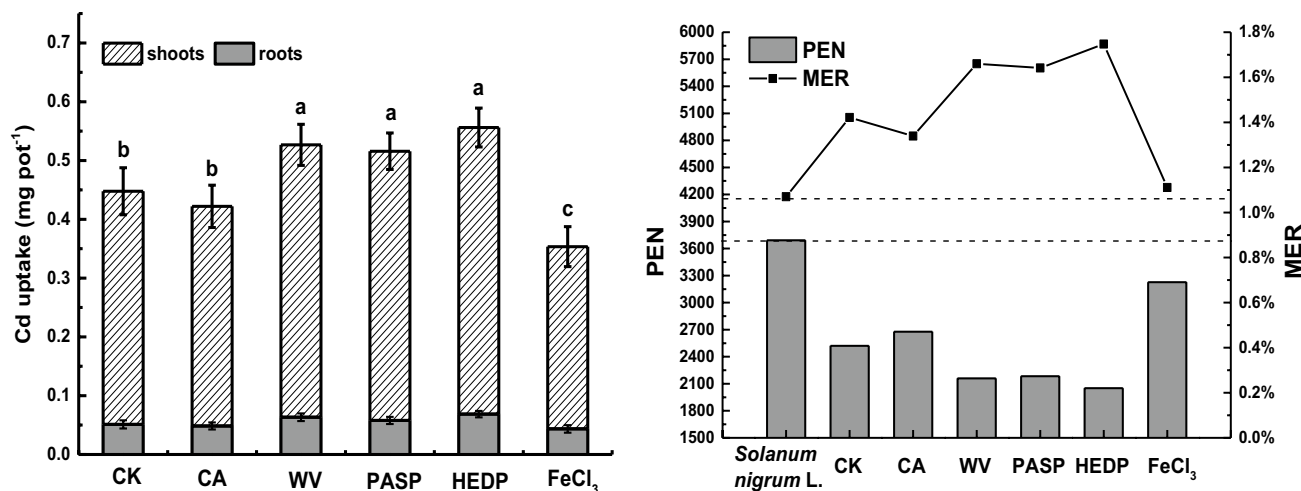


Fig. 6 Total Cd uptake, metal extraction ratio (MER) and plant effective number (PEN) of *Pennisetum* sp. under different treatments. Values shown are the mean ($n=3$). Bars marked by the same letter(s) are not significantly different at $p < 0.05$ by Duncan's new multiple range test

the stable fractions (F3 and R). The total Cd uptake under different treatment is listed in same descending order as follows: HEDP > WV > PASP > CK > CA > FeCl₃. According to MER, as a tolerant plant, the enhanced phytoextraction efficiency of *Pennisetum* sp. was higher than Cd-hyperaccumulator (*S. nigrum*). While the results were based on microcosm experiment, further work would be required to clarify the mechanisms involved for *Pennisetum* sp. and verify the phytoextraction rates under field conditions.

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