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Ethyl lactate-EDTA composite system enhances the remediation of the cadmium-contaminated soil by Autochthonous Willow (*Salix* × *aureo-pendula* CL 'J1011') in the lower reaches of the Yangtze River

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ABSTRACT

In order to explore a practical approach to the remediation of the cadmium (Cd)-contaminated soil in the lower reaches of the Yangtze River, we evaluated the effects of a local willow (*Salix* × *aureo-pendula* CL 'J1011') of absorbing, accumulating, and translocating Cd; and assessed the potential of chelator ethylenediaminetetraacetic acid (EDTA) in combination with ethyl lactate for enhancing the efficiency of the willow in removing Cd in two water-culture growth chamber trials and a field one. The willow showed a high tolerance to Cd in growth chamber trial 1 where the Cd concentration in the medium reached up to 25 mg L^{-1} medium, and the bioaccumulation factors (BAFs) of the shoots for Cd rose from 3.8 to 7.4 as the Cd concentration in the medium was elevated from 5 to 25 mg L^{-1} medium. In growth chamber trial 2, the average Cd removal rates in two treatments with EDTA and ethyl lactate (molar ratios of EDTA to ethyl lactate = 68/39 and 53.5/53.5, respectively) reached 0.71 mg $d^{-1}pot^{-1}$ for the duration of Day 5-8 and 0.59 mg $d^{-1}pot^{-1}$ for that of Day 8-11, which were 5- and 4-fold of their counterparts in the control, respectively. In the field trial, for the remediational duration of 45 days, three treatments—willow alone, willow combined with EDTA, and willow combined with EDTA and ethyl lactate—led to decreases in the Cd concentration in soil by 5%, 20%, and 29%, respectively; increases in that in the leaves by 14.6%, 56.7%, and 146.5%, respectively; and increases in that in the stems by 15.6%, 41.2%, and 87.4%, respectively, compared to their counterparts on Day 0. These results indicate that EDTA combined with ethyl lactate significantly enhanced the efficiency of willow in removing Cd from the soil. Therefore, a phytoextraction system consisting of the autochthonous willow, EDTA, and ethyl lactate has high potential for the remediation of the Cd-polluted soil in the lower reaches of the Yangtze River.

1. Introduction

As a widespread global problem, heavy-metal pollution of soil threatens the safety of ecosystems and human health [1]. In many parts of the world, the agricultural soil is slightly or moderately contaminated by cadmium (Cd) due to a long-term use of phosphatic fertilizers, the recycling of sewage sludge, and the precipitation of the dust from smelters [2]. To meet the urgent need for removing heavy metals from the soil, a variety of soil remediation methods have been established. Phytoremediation is an in-situ remediation technique employing green plants to remove pollutants from the environment or to render them harmless [3]. Phytoremediation has become a promising alternative to the conventional methods [4,5], for it appears to be economically valuable, environmentally friendly, and esthetically attractive [6,7].

In the last decade, increasing attention has been paid to optimize the method of phytoremediation and to find the most suitable plants; the effectiveness of phytoremediation is dependent on an adequate yield of suitable plants and the efficient transfer of metals from their roots to shoots. Hyperaccumulating plants have drawn much attention because of their ability to accumulate and tolerate high levels of heavy metals [8,9]; however, their potential for the application to bioremediation is limited by their slow growth and low biomass [10,11]. Therefore, the more recent researches on phytoextraction have being focused on the plants that can achieve high biomass, although they accumulate lower levels of heavy metals [12,13]. Out of them, willow displays the characteristics suitable for phytoremediation as follows: growing fast, propagating easily, rooting deeply, adapting to a wide range of climatic conditions, and tolerating the temporarily waterlogged environment [6,14,15]. The potential of willow for phytoremediation has been established well in pot and growth chamber experiments [12,16], but not in field conditions. There is little information about the potential of autochthonous willows for remediating Cd-polluted soils in the

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most regions of China, especially in the lower reaches of the Yangtze River where the Cd concentration in soil exceeds the stipulated standard.

Compared to the conventional phytoextraction methods, chelator-assisted phytoextraction has higher efficiency in extracting metals from the polluted soil, for chelators can solubilize target metals from soil [17] to facilitate their uptake by the roots of plants and translocation from the roots to the shoots [18]. Ethylenediaminetetraacetic acid (EDTA) has become the most widely used chelator for phytoextraction due to its high extraction efficiency [19]. On the other hand, a large-scale EDTA-assisted phytoextraction may produce adverse effects on the environment because EDTA is non-biodegradable and severely inhibits plant growth, and its application increases the possibility of heavy metals leaching into groundwater [20,21]. As a potential substitute for EDTA, [S, S]-ethylenediaminedisuccinic acid (EDDS) has a strong chelating ability and high biodegradability, but its costliness restricted its application [22]. Therefore, it is necessary and urgent to find out other appropriate additives to replace EDTA and other non-biodegradable chelating agents or reduce their dosages in phytoremediation. In this connection, ethyl lactate has been recognized as a "green solvent" owing to its numerous attractive properties including strong solvency power, low toxicity, and high biodegradability [23,24]. Previously, we also reported that a combination of EDDS and ethyl lactate resulted in higher efficiency in extracting copper (Cu) than EDDS alone in soil washing experiments [25].

The present study employed two water-culture growth chamber trials and a field one to achieve the following aims: (1) to evaluate *Salix* × *aureo-pendula* CL 'J1011'—a native willow growing fast and producing high biomass—for its tolerance to Cd and potential for the phytoextraction of Cd, and (2) to assess the impact of ethyl lactate on the EDTA-assisted phytoextraction of Cd by the willow.

2. Materials and methods

2.1. Growth chamber trials

2.1.1. Materials

Ethyl lactate (>99%, guaranteed reagent grade) was purchased from Lacaster Chemical Co., England; and the salts and other chemicals (analytical reagent grade) were from Nanjing Chemical Reagents Co., Ltd., Nanjing, China. Hoagland solution was prepared using the method of Hoagland and Arnon [26]. A stock solution of Cd (1000 mM) was prepared by dissolving 30.9 mg $Cd(NO_3)_2 \cdot 4\,H_2O$ in 100 ml of double-distilled water; it was diluted to a series of Cd solutions (54–266 mM). The cuttings of willow used in all experiments of Cd uptake were male clones from an adult hybrid willow (*Salix* × *aureo-pendula* CL 'J1011'), having a uniform size—approximately 20 cm in length by 2 cm in diameter. Cuttings were allowed to root hydroponically in Hoagland's nutrient solution until their fine roots developed for about 2 weeks.

2.1.2. Willow cuttings exposed to Cd and treated with EDTA or its combination with ethyl lactate

Two sets of beakers were used as containers for cuttings in two growth chamber trials, each beaker containing 1500 ml of Hoagland medium. In growth chamber trial 1, Cd solution was directly added into the medium. The final concentrations of Cd in the medium reached 53.5, 107, 214, and 266 μM (5, 10, 20, and 25.0 mg L^{-1} medium) in four different treatments, respectively; the control contained the medium and cuttings, but no Cd. Growth chamber trial 2 consisted of three treatments in three different molar ratios of EDTA to ethyl lactate (107/0 for Treatment 1, 68/39 for Treatment 2, and 53.5/53.5 for Treatment 3) and a control containing the medium with Cd and cuttings, but without additives. Each treatment contained 107 μM of additives and 53.5 μM of Cd. For evaluating the parallelism of plant growth in different beakers prior to treatments, EDTA and ethyl lactate were not added into the medium until cuttings had been exposed to Cd for 5 days. Each treatment or control had three replicates, and each replicate included five healthy cuttings.

2.1.3. Plant growth and collection of water samples

Both sets of beakers were kept in a plant growth chamber under the controlled conditions: 25 ± 2 °C and 12 h daylight (light intensity approximately 500 mmol m^{-2} s^{-1} for growth). For growth chamber trial 1, water samples (approximately 10 ml per sample) were collected on the day before exposure to Cd (Day 0) and 1, 2, 3, 4, 5, 9, 13, and 15 day(s) after the exposure, respectively; for growth chamber trial 2, they were done on the day before exposure to Cd (Day 0) and 5, 8, and 11 days after the exposure, respectively. After exposed to Cd for 15 days in growth chamber trial 1 and 11 days in growth chamber trial 2, respectively, willow plants were harvested and dissected into the leaves and stems—two parts of the shoots—and the roots; they were weighed, washed, and dried. The Cd contents of these tissues were determined as described in the Section 2.3.

For growth chamber trial 1, the relative dry yields (RDYs) and bioaccumulation factors (BAFs) of the leaves, stems, and roots of willow (S. × *aureo-pendula* CL. 1011), respectively, were calculated. The RDYs was calculated according to the following formula: RDYs = W (treatment)/W (control), where W (control) and W (treatment) are the mean dry weights of leaves, stems, or roots grown in the medium without Cd (control) and in the medium with Cd (treatment), respectively. The BAFs were calculated according to the equation of Lunney et al. [27]. In growth chamber trial 2, Cd removal rate, q (mg d^{-1} pot^{-1}), was determined by measuring Cd concentrations in the aqueous phase before and after willow was cultivated for several days [28] and using the formula: $q = (C_0 - C)\,V/D$, where C_0 and C are the initial and equilibrium (final) Cd concentrations (mg L^{-1}), respectively, V is the volume of the solution (L), and D is the duration of willow cultivation (day).

2.2. Field trial

The soil in the experimental field was silty clay with a pH of 8.3 ± 0.1, containing 6.7% organic matter and 46.2 cmol kg^{-1} CEC (cation exchange capacity). The topsoil (0–20 cm in depth) of the field was spiked with $Cd(NO_3)_2$ whose final concentration in the soil reached about 7 mg kg^{-1} soil. The Cd concentrations in the topsoil of each treatment and of the control were measured prior to experimentation and found to be 7.05 ± 0.73 mg kg^{-1}. The experimental field was about 20 m^2 in area and divided into 20 foursquare grids with PVC clapboards. In April 2008, cuttings of the willow (*Salix* × *aureo-pendula* CL'J1011') were planted in rows with the distance of 35 cm between them and that of 30 cm between the cuttings, and about 100 cuttings in total were planted in the experimental field. The routine field management included irrigation during dry summer periods, weeding, and fertilization.

After willow naturally grew for four months, the experimental grids were divided into four groups: the soil without willow, EDTA, or ethyl lactate (Control) and three treatments—the soil with willow only (Willow), that with willow and EDTA (Willow-EDTA), and that with willow and a combination of EDTA and ethyl lactate (Willow-EDTA-ethyl lactate). Each group consisted of three replicates. In Willow-EDTA, the molar ratio of EDTA to Cd in the topsoil was 2:1; in Willow-EDTA-ethyl lactate, the molar ratio of EDTA to ethyl lactate was 82:46. The total molar dosage of EDTA and ethyl lactate in Willow-EDTA was the same with that in Willow-EDTA-ethyl lactate, which could be calculated by using the following data:

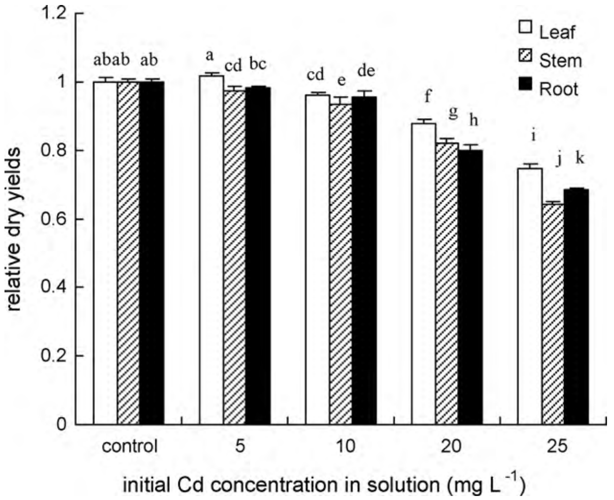

Fig. 1. Relative dry yields (RDYs) of the leaves, stems, and roots of willow, respectively, in growth chamber trial 1. Values are means of triplicates ± SD; those with the same letters are not significantly different according to one–way ANOVA test ($p < 0.05$).

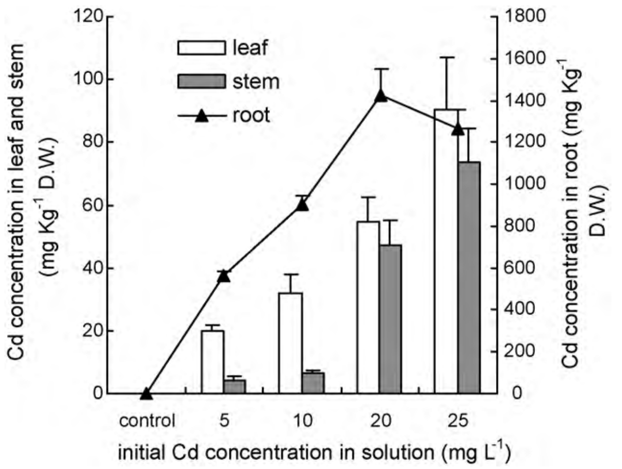

Fig. 2. Cd concentrations in the leaves, stems, and roots of willow in growth chamber trial 1. Values are means of triplicates ± SD.

the soil depth = 20 cm, the Cd concentration in soil = 7 mg kg^{-1}, and the soil bulk density = 1.4×10^3 kg m^{-3}. The leaves and stems of willow as well as the topsoil of each grid were sampled every half month, and willow was harvested 45 days after treatments (the end of the growing season).

2.3. Analyses of plant and soil samples

Soil samples were weighed and dried. The leaves and stems were weighed, washed, and dried. After dried in an oven at 65 ± 5 °C for 1 week, the tissues were finely crushed, and those with accurate weights were digested by HNO_3 as previously described [29]. The Cd contents of plant and soil samples were determined using an atomic absorption spectrophotometer (Thermo, USA) with a detection limit of 0.004 ppm.

2.4. Statistical analysis

Data are given as means ± standard deviations (SD). One-way ANOVA (Duncan HSD) was used for the analysis of variance ($p < 0.05$) of the data.

3. Results

3.1. Effect of Cd on willow growth in growth chamber trial 1

As shown in Fig. 1, the application of Cd to the medium at all concentrations except 5 mg L^{-1} medium resulted in significantly lower relative dry yields (DRYs) of the leaves, stems, and roots in comparison with their counterparts in the control ($p < 0.05$). However, the application of Cd even at its highest concentration only led to decreases in dry weight by 25.5%, 35.7%, and 31.6% for the leaves, stems, and roots, respectively. This result suggested that willow had a high tolerance to Cd.

3.2. Bioaccumulation and distribution of Cd in willow in growth chamber trial 1

As Cd concentration in the medium was increased from 5 to 25 mg L^{-1} medium, Cd concentrations in the leaves, stems, and roots of willow all rose greatly (Fig. 2). On the other side, Cd concentration in the medium appeared to be negatively correlated with the bioaccumulation factor (BAF) of the roots for Cd, but not closely correlated with that of the shoots (consisting of leaves and stems) (Table 1). The BAFs of the shoots for Cd (the sum of the BAFs of the leaves and stems) increased from 3.8 to 7.4 as Cd concentration in the medium was elevated from 5 to 25 mg L^{-1} medium. In terms of the BAFs of the two tissues of the shoots, corresponding to every Cd concentration in the medium, the BAF of the leaves was higher than that of the stems. In addition, the BAF of the roots decreased from 112 to 51 as Cd concentration in the medium was increased from 5 to 25 mg L^{-1} medium. Corresponding to each Cd concentration in the medium, the BAF of the roots was much higher than that of the shoots, indicating that Cd was still mainly deposited in the roots.

3.3. Effects of EDTA and ethyl lactate on willow extracting Cd in growth chamber trial 2

After exposed to Cd for 5 days, cuttings in the control and all treatments displayed similar Cd removal rates (Fig. 3). For both of the duration of Day 5-8 and of Day 8-11, the Cd removal rates in Treatment 1 (molar ratio of EDTA to ethyl lactate = 107/0) were higher than those in the control, indicating that EDTA facilitated willow extracting Cd from the medium. Moreover, for the duration of Day 5-8 and of Day 8-11, both Treatments 2 and 3 (molar ratios of EDTA to ethyl lactate = 68/39 and 53.5/53.5, respectively) resulted in significantly higher Cd removal rates by willow in comparison to Treatment 1 (molar ratio of EDTA to ethyl lactate = 107/0) ($p < 0.05$). This suggested that the addition of ethyl lactate produced a significantly stronger effect of increasing the EDTA-assisted Cd removal rate by willow than the same mole of EDTA. The average Cd removal rates in Treatments 2 and 3 reached 0.71 mg d^{-1}pot^{-1} for Day 5-8 and 0.59 mg d^{-1}pot^{-1} for Day 8-11, which were 5- and 4-fold of their counterparts in the control, respectively.

Fig. 4 shows the variations in Cd concentrations in the leaves, stems, and roots of harvested willow *S.* × *aureo-pendula CL.* 1011 in different treatments. The application of EDTA or its combination

Table 1
Bioaccumulation factors (BAFs) for Cd of the leaves, stems, and roots of willow, respectively, in growth chamber trial 1.

Treatment (mg L^{-1})	Leaf	Stem	Root
5	3.2±0.39	0.64±0.19	112±6.9
10	3.5±0.31	0.70±0.12	90±2.5
20	2.8±0.37	2.4±0.34	71±2.6
25	4.1±0.21	3.3±0.18	51±1.6

Values are means of triplicates ± SD.

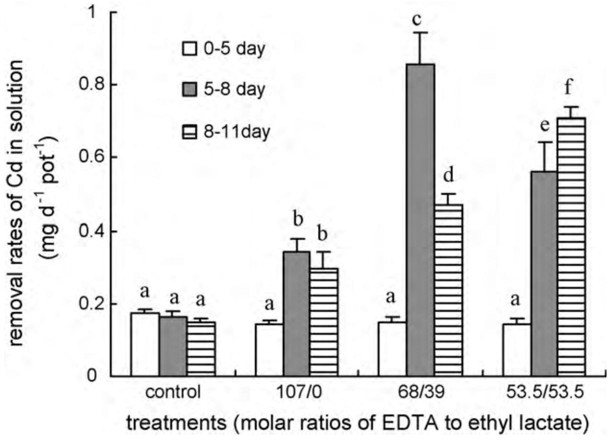

Fig. 3. Cd removal rates by willow in treatments with EDTA and ethyl lactate in different ratios in growth chamber trial 2. Values are means of triplicates ± SD; those with the same letters are not significantly different according to one–way ANOVA test ($p < 0.05$).

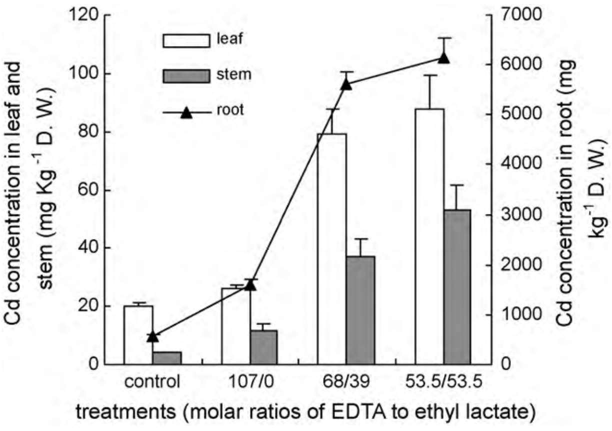

Fig. 4. Effects of different ratios of EDTA to ethyl lactate on Cd concentrations in the leaves, stems, and roots of willow. Values are means of triplicates ± SD.

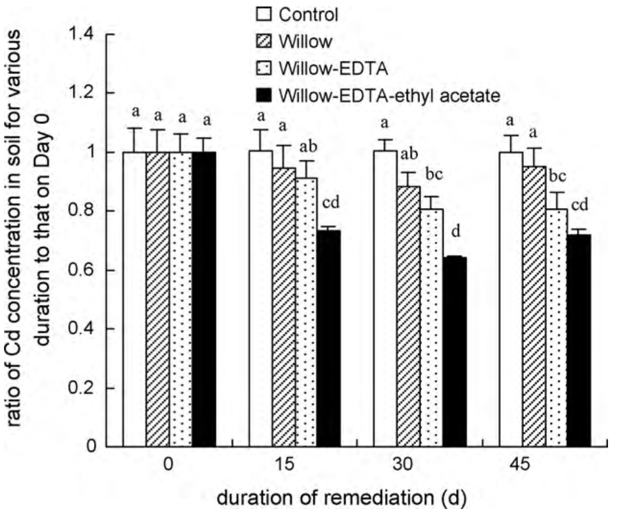

Fig. 5. Cd concentrations in soils in different treatments for various remediational duration in a field trial. Initial Cd concentrations (mg kg^{-1}D.W. ± SD) in soils of the control, Willow, Willow-EDTA, and Willow-EDTA-ethyl lactate were 6.34 ± 0.57, 5.09 ± 1.34, 5.14 ± 1.14, and 5.67 ± 0.20, respectively. Values are means of triplicates ± SD; those with the same letters are not significantly different according to one–way ANOVA test ($p < 0.05$).

with ethyl lactate to the medium apparently increased Cd concentrations in the leaves, stems, and roots. Treatments 2 and 3 (molar ratios of EDTA to ethyl lactate = 68/39 and 53.5/53.5, respectively) demonstrated significantly higher effectiveness in enhancing the accumulation of Cd in all of these tissues than Treatment 1 (molar ratio of EDTA to ethyl lactate = 107/0) ($p < 0.05$); particularly, in Treatment 3, Cd concentrations in the leaves, stems, and roots reached 87.8, 52.9, and 6161 mg kg^{-1}, respectively, which were 4-,13- and 11-fold of those in the control. These data indicated that compared to EDTA alone, its combination with ethyl lactate produced a much stronger effect of improving the accumulation of Cd in all of the three tissues of willow. Moreover, in all treatments, Cd concentrations in the roots were remarkably higher than those in the shoots ($p < 0.05$), suggesting that these treatments stimulated Cd uptake by the roots more effectively than Cd translocation from the roots to the shoots.

3.4. Effects of EDTA and ethyl acetate on the phytoextraction of Cd by willow in field

Fig. 5 shows the relationship between the remediational duration of different treatments and the Cd concentration in the field soil. For all of the remediational duration, treatment Willow led to lower Cd concentrations in soil compared to those in the control, but their differences were insignificant ($p > 0.05$). As shown in Table 2, the Cd concentration in the shoots rose with increased duration of treatment Willow: for that of 45 days, the Cd concentrations in the leaves and stems increased by 14.6% and 15.6%, respectively, compared to their counterparts on Day 0; however, these increases were still insignificant ($p > 0.05$). These results suggested that treatment Willow only produced a weak effect of the remediation of Cd in soil.

Treatment Willow-EDTA led to significantly lower ($p < 0.05$) Cd concentrations in soil for all remediational duration except for 15 days compared to those in the control (Fig. 5). Furthermore, compared to treatment Willow, Willow-EDTA also led to significantly higher ($p < 0.05$) Cd concentrations in the leaves and stems after the remediation for 45 days (Table 2). For three of the remedi-

Table 2
Cd concentrations in the leaves and stems of willow for different remediational duration relative to those on Day 0.

Treatments	Duration of remediation (d)			
	0	15	30	45
Leaf				
Willow	100±21.5a	105.3±18.3a	108.1±8.8a	114.6±15.7a
Willow-EDTA	100±22.8a	128.6±13.5ab	152.6±11.9bc	156.7±14.3c
Willow-EDTA-ethyl lactate	100±21.8a	125.7±7.03ab	237.2±9.95d	246.5±9.35d
Stem				
Willow	100±23.3a	103.3±18.5ab	112.7±8.6ab	115.6±9.5ab
Willow-EDTA	100±21.5a	122.2±14.4abc	128.3±11.7bcd	141.2±9.0cd
Willow-EDTA-ethyl lactate	100±12.9a	151.6±9.6d	194.8±4.7e	187.4±7.11e

Values are means of triplicates ± SD; those with the same letters are not significantly different according to one–way ANOVA test ($p < 0.05$).

ational duration of Willow-EDTA, the Cd concentrations in soil decreased by 9%-20%, and those in the leaves and stems increased by 28.6-56.7% and 22.2-41.2%, respectively, in comparison with their counterparts on Day 0.

Compared to Willow-EDTA, treatment Willow-EDTA-ethyl lactate (molar ratio of EDTA to ethyl lactate = 82:46) led to significantly lower ($p < 0.05$) Cd concentrations in soil for all remediational duration (Fig. 5); and significantly higher ($p < 0.05$) Cd concentrations in the leaves for all remediational duration except for 15 days, and significantly higher ($p < 0.05$) Cd concentrations in the stems for all remediational duration (Table 2). For all of the remediational duration, Willow-EDTA-ethyl lactate led to decreases in Cd concentrations in soil by 26%-36% and increases in those in the leaves and stems by 25.74-146.5% and 51.6-94.8%, respectively, in comparison with their counterparts on Day 0.

4. Discussion

4.1. Potential of willow for the phytoremediation of Cd

The suitability of a plant for phytoremediation depends on its characteristics in the following aspects: (1) tolerance to heavy metals; (2) size, growth rate, and rooting depth; (3) accumulation of heavy metals in its above-ground parts; and (4) climatic adaptation and pest resistance [6,7,14]. In this study, we investigated two of these aspects of the willow (*Salix* × *aureo-pendula* CL 'J1011') regarding their impact on its effectiveness in the remediation of Cd.

In growth chamber trial 1, willow revealed a high tolerance to Cd (up to 25 mg L^{-1} medium); it was able to survive through the whole experimental period even as Cd concentration was increased to 25 mg L^{-1} medium. In the field trial, willow was also capable of growing and reproducing in the soil with an impermissibly high Cd concentration. These results suggest that it is practical and feasible to remediate the soil containing a high Cd concentration by planting the willow (*Salix* × *aureo-pendula* CL 'J1011').

Regarding the distribution of Cd in the above-ground parts of willow, Cd concentrations in the leaves were higher than those in the stems (Table 1 and Fig. 2), indicating that Cd was translocated from the stems to leaves. This result is consistent with those of willow clones [30] and seven clones of *Salix spp*. reported by Vysloužilová et al. [31]. On the other side, Cd was still mainly deposited in the roots, for the BAFs of the roots for Cd were much higher than those of the shoots (Table 1 and Fig. 2). This is due to the difference between the higher integral uptake rates of Cd by willow and its lower translocation rates from the roots to the shoots [32]; Zhang et al. also considered the roots as a barrier to the translocation of metals to the shoots [33]. Nevertheless, increasing the Cd concentration in the medium did not result in a decrease in the BAFs of the shoots (3.8-7.4) (Table 1), implying that willow has a stable ability to accumulate Cd in the shoots. Considering that the Cd accumulated in the shoots can be removed when they are cut from the adult hybrid willow as new male clones, i.e. cuttings, planting the willow (*Salix* × *aureo-pendula* CL 'J1011') appears to be a practical and effective approach to the remediation of the Cd-contaminated soil.

According to the results of the field trial, the amount of Cd removed by the shoots reached 0.040 kg Cd/ha in the first year under the condition that willow was planted at the density of 5 plants per m^2 (100 plants cover 20 m^2 in area); for the layer of soil 20-cm in depth with an average bulk density of 1350 kg/m^3, its Cd content was about 17.1 kg Cd/ha. Based on the above data, the calculated percentage of the Cd removed by the shoots of willow reached 0.23%, which was twice that of the cuttings of the willow (*Salix viminalis Arresoe*) [14], so the amount of Cd removed by the shoots of willow could be compared with the total Cd content in the soil. Furthermore, the estimate only shows the uptake of the young willow trees in the first growing season, but their biomass will increase in the coming years [14].

4.2. Advantages of the phytoextraction system of willow-EDTA-ethyl lactate

Chemically enhanced phytoextraction has been proposed as an effective approach to the remediation of the soil polluted with heavy metals [10,18,22]. Our results in this study also demonstrate that the application of EDTA is effective in decreasing Cd concentration in soil, and increasing its accumulation in the leaves, stems, and roots of willow. The application of EDTA to the medium or soil enhanced the efficiency of willow in removing Cd (Figs. 3 and 5) mainly due to the formation of a stable Cd-EDTA complex. Theoretically, the efficiency of chelation depends on the stability constant of the formed metal-chelator complex. EDTA has a strong chemical affinity for Cd (logKs = 16.62) [34]; the formation of a stable Cd-EDTA complex in the soil treated with EDTA facilitates the solubilization of Cd there. Thus the soil has more available Cd for uptake by the roots of willow and translocation from the roots to the shoots.

The application of EDTA in combination with ethyl lactate to the medium led to significant increases in Cd removal rate by willow (Fig. 3), in Cd uptake by the roots and in Cd accumulation in the shoots (Fig. 4). Also, in the field trial, Willow-EDTA-ethyl lactate resulted in significantly lower Cd concentrations in soil in comparison with Willow-EDTA (Fig. 5). Accordingly, due to Willow-EDTA-ethyl lactate, the percent of removed Cd in the total Cd content of soil approached 0.39% (Its calculation is based on the method described in the Section 4.1). Our previous study of soil washing showed that the application of ethyl lactate enhanced the extraction efficiency of EDDS for Cu (ratio of EDDS to Cu = 2:1) mainly due to the mechanism that ethyl lactate increases the stability constant of Cu-EDDS complex [25]. For the possible mechanism by which the addition of ethyl lactate led to an increase in the extraction efficiency of EDTA for Cd in this study, we infer that its addition changes the dielectric constant of the soil washing solution, resulting in a higher stability constant of the Cd-EDTA complex. Ethyl lactate has been reported as a "green" solvent due to its numerous attractive advantages including high solvency; 100% of biodegradability; easy recycle; lower price and easy availability; and non-corrosive, non-carcinogenic, and non-ozone depleting qualities [23,24]. Therefore, incorporating eco-friendly ethyl lactate into EDTA-enhanced phytoextraction is practical and advantageous to the ecosystem, for it will remarkably lower the risk of exposing the environment to excessive non-biodegradable EDTA in a large-scale EDTA-assisted phytoremediation by substantially reducing the dosage of EDTA.

5. Conclusion

In water-culture trial 1, willow showed a high tolerance to Cd (up to 25 mg L^{-1} medium); the BAFs of the shoots for Cd attained to the range of 3.8-7.4, which did not fall with the increased Cd concentrations. In water-culture trial 2 and the field trial, the application of EDTA combined with a low dosage of ethyl lactate significantly enhanced the efficiency of willow in removing Cd from the medium and soil. Therefore, considering the characteristics of this autochthonous willow (having a high tolerance to Cd and the ability to absorb and accumulate it), economic values, and ecological benefit; and the easy harvest, a phytoextraction system consisting of this willow, EDTA, and ethyl lactate has great potential for the remediation of the Cd-polluted soil, especially in the lower reaches of the Yangtze River.

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