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Bioremediation of Cd-DDT co-contaminated soil using the Cd-hyperaccumulator *Sedum alfredii* and DDT-degrading microbes

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HIGHLIGHTS

- Planting of *S. alfredii* is an effective technique for phytoextraction of Cd and DDs.
- Soil inoculation with *Pseudomonas* sp. DDT-1 increases root biomass of *S. alfredii*.
- Soil inoculation with *Pseudomonas* sp. DDT-1 improves the removal efficiency of DDs.
- The plant-microbe strategy is promising for remediation of Cd-DDT co-contaminated soil.

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ABSTRACT

The development of an integrated strategy for the remediation of soil co-contaminated by heavy metals and persistent organic pollutants is a major research priority for the decontamination of soil slated for use in agricultural production. The objective of this study was to develop a bioremediation strategy for fields co-contaminated with cadmium (Cd), dichlorodiphenyltrichloroethane (DDT), and its metabolites 1, 1-dichloro-2, 2-bis (4-chlorophenyl) ethylene (DDE) and 1, 1-dichloro-2, 2-bis (4-chlorophenyl) ethane (DDD) (DDT, DDE, and DDD are collectively called DDs) using an identified Cd-hyperaccumulator plant *Sedum alfredii* (*SA*) and DDT-degrading microbes (DDT-1). Initially, inoculation with DDT-1 was shown to increase *SA* root biomass in a pot experiment. When *SA* was applied together with DDT-1, the levels of Cd and DDs in the co-contaminated soil decreased by 32.1–40.3% and 33.9–37.6%, respectively, in a pot experiment over 18 months compared to 3.25% and 3.76% decreases in soil Cd and DDs, respectively, in unplanted, untreated controls. A subsequent field study (18-month duration) in which the levels of Cd and DDs decreased by 31.1% and 53.6%, respectively, confirmed the beneficial results of this approach. This study demonstrates that the integrated bioremediation strategy is effective for the remediation of Cd-DDs co-contaminated soils.

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1. Introduction

Heavy metals and persistent organic pollutants (POPs) are often coexisted and recognized as two major chemical families that cause soil pollution in China [1,2]. Cadmium (Cd) and 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) are of particular concern due to their persistence; potentially carcinogenic, mutagenic, and teratogenic properties; and their ubiquitous occurrence in the environment [3–5]. Cd and DDT or its metabolites 1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene (DDE) and 1,1-dichloro-2,2-bis (4-chlorophenyl) ethane (DDD) are common and important co-contaminants in Chinese agricultural soils [1,4]. Therefore, it is critical to develop efficient and cost-effective approaches to simultaneously remove multiple contaminants from co-contaminated soils.

The remediation of soils co-contaminated with metals and organics is a complex problem because the required chemical processes and remediation technologies are different for each group of pollutants [6]. Phytoremediation, which utilizes hyperaccumulating plants to extract heavy metals from contaminated soils, is a

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cost-effective and environmentally friendly approach compared to physical and chemical remediation techniques [7,8]. The feasibility of phytoremediation for soil contaminated by multiple organic and inorganic contaminants has recently been investigated [1,9–11]. Hyperaccumulating plants are valuable for the phytoextraction of metals in contaminated soils [12]. *Sedum alfredii* (*SA*) has recently been identified as a Zn and Cd co-hyperaccumulator plant species that is native to China [13]. Because of its favorable characteristics, including fast growth, high biomass, and asexual reproduction [14], this plant species is ideal for the phytoremediation of sites polluted by multiple heavy metals [15,16]. The hyperaccumulating characteristics of *SA* have been studied extensively using pot experiments and hydroponics [9,17–19]. A small number of field evaluations of the effectiveness of hyperaccumulators in remediation of Cd- or DDT-contaminated soils have been performed [10,20–22]. The roots of *SA* can excrete high levels of dissolved organic matter that complex and detoxify heavy metals in the rhizosphere; this excretion of dissolved organic matter could be beneficial to DDT-degrading microorganisms in the soil [23]. However, few studies have reported the use of metal hyperaccumulators to improve the removal of organic and inorganic pollutants from co-contaminated soils [9,24].

The rapid degradation of DDT, DDD, and DDE by certain bacteria and fungi has been shown to occur via mineralization or co-metabolism under aerobic and anaerobic conditions [25]. Thus, microbial detoxification is considered a cost-effective, safe and promising method for the removal of DDT residues from the environment [26,27]. Microorganisms capable of degrading DDT or its metabolites DDD and DDE include *Fusarium solani* [26], *Alcaligenes eutrophus* A5 [28], *Boletus edulis* [29], *Serratia marcescens* DT-1P [30], *Pseudomonas fluorescens* [31], *Cladosporium* sp. AJR3 18501 [32], *Ralstonia eutropha* A5 [33], *Pseudomonas acidovorans* M3GY [34], *Terrabacter* sp. DDE-1 [35], *Shewanella decolorationis* S12 [36], *Sphingobacterium* sp. D6 [37], *Phanerochaete chrysosporium* [38], and twelve species of brown-rot fungi [39]. However, minimal information is available on the removal or detoxification of DDT, DDD and DDE from field soils.

In our previous studies (unpublished data), a new bacterial strain, *Pseudomonas* sp. DDT-1, was shown to be capable of utilizing DDT as its sole carbon and energy source. However, there is currently a dearth of information on using the combination of rhizodegradation and phytoextraction. To our knowledge, the plant-microbial remediation of Cd-DDT co-contaminated soils has not been previously reported. Therefore, the objective of this study was to develop novel strategies for the bioremediation of Cd-DDT co-contaminated soils using *SA* in conjunction with DDT-degrading microbes.

2. Materials and methods

2.1. Plant material and soil characterization

Seedlings of the hyperaccumulator *SA* were originally obtained from a former Pb/Zn mining area in Zhejiang Province, China, and grown in non-contaminated soil for several generations to minimize the internal metal contents. Uniform and healthy shoots were selected and cultivated in a basal nutrient solution [40]. The soil used in the pot experiment (~400 kg) was collected from the upper layer (0–20 cm depth) of an agricultural field in Chiqi City, Zhejiang Province, China. This sampling site was co-contaminated with heavy metals and DDs derived from industrial activities, and the historical use of DDT in cotton production, respectively. The soil was air-dried at 25 °C, ground to pass through a 2 mm sieve, and stored in a plastic bag at −80 °C prior to use. Selected physical and chemical characteristics of the soil are shown in Table 1. The soil texture, pH_{water}, organic matter, cation exchange capacity (CEC), total nitrogen (N), available N, total phosphorus (P), available P, and available potassium (K) were determined according to standard methods [41]. Concentrations of Pb, Zn, Cu, and Cd were determined using ICP-MS (Agilent 7500a, Agilent, USA) after digestion of the soil with $HNO_3/HClO_4/HF$ (5:1:1 v/v/v) [41]. Concentrations of the following organochlorine pesticides (OCPs) were measured using USEPA method 8081B (described below) [42]: 1, 1-dichloro-2, 2-bis (*p*-chlorophenyl) ethylene (*p,p′*-DDE); 1, 1-dichloro-2, 2-bis (*p*-chlorophenyl) ethane (*p,p′*-DDD); 1, 1, 1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl) ethane (*o,p′*-DDT); and 1, 1, 1-trichloro-2, 2-bis (*p*-chlorophenyl) ethane (*p,p′*-DDT) (DDs = *p,p′*-DDE + *p,p′*-DDD + *o,p′*-DDT + *p,p′*-DDT); hexachlorocyclohexanes ($\sum$HCHs = α-HCH, β-HCH, γ-HCH and δ-HCH); $\sum$CHLs (sum of heptachlor, heptachlor epoxide, *cis*-chlordane, *trans*-chlordane, and *trans*-nonachlor); endosulfa (sum of α-endosulfan and β-endosulfan); hexachlorobenzene; other OCPs (sum of aldrin, dieldrin and endrin).

Table 1
Selected physical and chemical characteristics of the soil used in this study.

Soil parameters	Measured value
Soil texture	Light loam soil
pH_{water}	7.5 ± 0.07[a]
Organic matter ($g\,kg^{-1}$)	7.8 ± 0.3
Cation exchange capacity ($cmolc\,kg^{-1}$)	14.6 ± 0.8
Total nitrogen ($g\,kg^{-1}$)	1.5 ± 0.1
Available nitrogen ($mg\,kg^{-1}$)	123.8 ± 3.2
Total phosphorus ($g\,kg^{-1}$)	1.3 ± 0.1
Available phosphorus ($mg\,kg^{-1}$)	32.7 ± 2.4
Available potassium ($mg\,kg^{-1}$)	105.2 ± 3.4
Total Pb ($mg\,kg^{-1}$)	27.6 ± 1.3
Total Zn ($mg\,kg^{-1}$)	5.2 ± 0.3
Total Cu ($mg\,kg^{-1}$)	15.2 ± 1.1
Total Cd ($mg\,kg^{-1}$)	0.9 ± 0.04
DDs ($mg\,kg^{-1}$)[b]	0.7 ± 0.03
$\sum$HCHs ($ng\,g^{-1}$)[c]	6.2 ± 4.1
$\sum$CHLs ($ng\,g^{-1}$)[d]	5.5 ± 2.7
Endosulfa ($ng\,g^{-1}$)[e]	3.2 ± 1.7
Hexachlorobenzene ($ng\,g^{-1}$)	3.0 ± 1.8
Other OCPs ($ng\,g^{-1}$)[f]	3.9 ± 2.2

[a] Mean ± standard deviation.
[b] DDs; sum of *p,p′*-DDE, *p,p′*-DDD, *o,p′*-DDT and *p,p′*-DDT.
[c] $\sum$HCHs; sum of α-HCH, β-HCH, γ-HCH and δ-HCH.
[d] $\sum$CHLs; sum of heptachlor, heptachlor epoxide, *cis*-chlordane, *trans*-chlordane, and *trans*-nonachlor.
[e] Endosulfa; sum of α-endosulfan and β-endosulfan.
[f] Other OCPs; are sum of aldrin, dieldrin and endrin.

2.2. Inoculum preparation

The DDT-degrading bacterial strain DDT-1 was isolated from a DDT-contaminated soil (4.3 ± 1.2 $\mu g\,g^{-1}$) sample collected from an area situated near a pesticide manufacturing factory in Zhejiang province, China, as described by Fang et al. [37]. The taxonomy of *Pseudomonas* sp. DDT-1 was confirmed using 16S rDNA sequence analysis (GenBank accession number: JN157618). The isolate was grown in lysogeny broth (LB) medium, maintained in 40% glycerol, and stored at 0 °C. The isolate DDT-1 was cultured in 500 ml Erlenmeyer flasks containing 250 ml of LB medium at 30 °C and 150 rpm on a rotary shaker. During the exponential phase (24 h), cell pellets were harvested by centrifugation (8000 × *g*, 10 min), immediately washed three times with 30 ml of phosphate buffer (pH 7.0), and resuspended in additional phosphate buffer. Bacterial concentrations were determined using the most-probable-number procedure of Gronewold and Wolpert [43].

2.3. Pot experiment

Approximately 1.0 kg of air-dried soil was placed in individual pots (15 cm height × 15 cm diameter). The soil in all pots received 80, 50, and 100 mg kg^{-1} of N, P, and K, respectively (as NH_4NO_3, KH_2PO_4, and KCl). The main treatments included two Cd levels: (i) without Cd spiking (Cd present at the existing contaminant levels in the soil), and (ii) spiked with 2.5 mg kg^{-1} Cd (as $CdNO_3 \cdot 4H_2O$). The measured total Cd concentrations in the soils in treatments (i) and (ii) were 0.895 (Cd_{low}) and 3.225 mg kg^{-1} (Cd_{high}) soil, respectively. The DDs concentration was 0.715 mg kg^{-1} soil. The treated soils were subsequently incubated for 30 days at 25 °C under natural light with five cycles of saturation with distilled water every 6 days followed by air-drying. The sub-treatments included the following: (i) an unplanted, untreated control (CTRL); (ii) soil planted with *SA* (*SA*); (iii) soil inoculated with the DDT-degrading strain DDT-1 (M); and (iv) soil planted with *SA* and inoculated with strain DDT-1 (*SA* + M). For treatments (iii) and (iv), the inoculation level of strain DDT-1 was approximately 1.2×10^7 colony-forming units (cfu) g^{-1} in the soil prior to *SA* planting. Eight uniform seedlings of *SA* grown for three weeks in a basic nutrient solution [40] were planted per pot with three replicate pots per treatment. The soil moisture content was maintained at 60% of the field holding capacity (24.5%, w/w) by adding deionized water every three days for 180 days. Plants were grown in a glasshouse with natural light and a day/night temperature cycle of 26/20 °C and humidity cycle of 70/80%.

After six months, all plants were uprooted, and soil particles were manually removed. Roots and shoots were divided, and plant materials were rinsed under tap water for 5 min, washed with MilliQ-water for 2 min, freeze-dried for 72 h in a Lyophlock 12 Freeze Dry System (Labconco, Kansas, USA), weighed, and crushed to a fine powder of less than 0.25 mm particle size in a ball grinder (Retsch, MM301, Haan, Germany).

Soil samples were collected from each pot after harvesting, freeze-dried for 48 h, and ground in an agate mortar to pass through a 100-mesh nylon sieve for subsequent analysis. All plant and soil samples were stored thereafter in sealed plastic bags at −80 °C until analysis.

2.4. Field experiment

A field experiment was conducted at the Cd-DDs co-contaminated agricultural site in Chiqi City, China, where the soil samples for the pot experiments were collected. The treatments (with four replicates) included the following: (i) an unplanted, untreated control (CTRL); (ii) planted with *SA* (*SA*); (iii) inoculated with DDT-1 (M); and (iv) planted with *SA* and inoculated with DDT-1 (*SA* + M). Each plot was 2.5 × 4 m surrounded by a 0.4 m buffer zone. The composite soil samples for each plot (0–20 cm depth) were well-mixed prior to Cd and DDs analysis. The concentrations of Cd and DDs in the soil were 0.70 and 0.55 mg kg^{-1} soil, respectively.

One week before transplanting *SA*, each plot received 1 kg of compound fertilizer (equivalent to 150, 150, and 150 kg of N, P, and K per hectare, respectively). Soil moisture content was maintained at approximately 80% (w/w) of the field holding capacity and checked gravimetrically every week by drying sub-samples at 105 °C in a forced-air oven to a constant weight. Six days later, a suspension of strain DDT-1 (6 l) with 0.5% glucose was sprayed using a knapsack sprayer at a dose of 150 l ha^{-1} in the (iii) and (iv) treatment plots only, and the inoculation level of strain DDT-1 was approximately 1.0×10^6 cfu g^{-1} in the upper 10 cm of soil. The remaining plots received only water. One day later (February 28th, 2008), uniform seedlings of *SA* were transplanted at 15 × 20 cm spacing. Plant tops were harvested and weighed at 7 months, 13 months, and 18 months. Soil (0–20 cm depth) was also sampled from all of the plots. The concentrations of Cd and DDs in plant and soil samples were analyzed as described below.

2.5. Soil and plant analysis and quality control

2.5.1. Cd analysis and quality control

Sub-samples of plant materials (100 mg) were digested using 4 ml of concentrated HNO_3 and 1 ml $HClO_4$ at 170 °C in closed Teflon vessels until the solution was clear.

A sequential extraction procedure [44] was used to determine the exchangeable, carbonate-bound, oxide-bound, organic-bound, and residual Cd fractions to assess the fractionation of Cd in the soil. The total soil Cd was equal to the sum of these five fractions. Cd concentrations in these extracts were determined using ICP-MS (Agilent 7500a, Agilent, USA).

Certified reference materials for soils (GBW07409, Center of Standard Materials of China) and plants (GBW10014, Center of Standard Materials of China) were included in the analyses. The recoveries (100 ± 9%) for Cd were within the certified limits for the reference materials.

2.5.2. Soil DDs extraction procedure

The DDs extraction and purification procedures were performed according to USEPA method 3550 C (Ultrasonic extraction) and 3620 C (Florisil cleanup) with minor modifications. Briefly, 10 g of soil was placed in each glass vial, covered with a Teflon cap, saturated with 50 ml of hexane/acetone (1:1 v/v) overnight, shaken in the dark for 1 h at room temperature and 180 rpm on a rotary shaker, and extracted for 30 min using an ultrasonic instrument (Ishine, China). After each extraction, separation was accomplished by centrifugation at 3000 × *g* for 5 min. The supernatants were further prepared using a glass chromatographic column loaded from bottom to top as follows: 1 cm (height) of anhydrous Na_2SO_4; 13 cm of Florisil suspended in *n*-hexane; and 1 cm of anhydrous Na_2SO_4. The DDs sample was eluted with 30 ml of acetone/hexane (22:125 v/v) three times and then carefully concentrated to near dryness in a rotary vacuum evaporator (Büchi Rotavapor, Germany) at 38 °C. Next, 2 ml of chromatography-grade hexane was added to a rotary steam bottle and mixed and filtered through a 0.22 μm organic phase membrane; the samples were then sealed in vials for analysis.

2.5.3. Plant DDs extraction procedure

DDs were extracted from the plant samples (approximately 0.5 g of roots and 2 g of shoots) according to the same procedure as for soil with the exception of using 50 ml hexane/acetone (4:1 v/v). The DDs purification procedure for the plant samples included sequential sulfonation (USEPA 3660B) and Florisil column chromatography (USEPA 3620 C) to remove photosynthetic pigments, lipids and other co-extractants.

2.5.4. DDs analysis and quality control

DDs concentrations in soil or plant extracts were determined using GC-μECD (Agilent 7890A, USA) with a capillary column (J&W 123-7732, 30 m × 0.25 mm × 0.25 μm, Agilent, USA) and an auto injector system according to USEPA method 8081B [42]. The GC operating conditions were as follows: injector, 250 °C; detector, 300 °C; initial oven temperature, 160 °C, ramped 10 °C min^{-1} to 240 °C and held for 5 min; carrier gas: ultrahigh-purity nitrogen, flow 4.85 ml min^{-1}. DDs were identified by comparing the retention times to those of the standards and were quantified using peak area integration. Standard samples of *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDD and *p,p′*-DDE (purity >99.5%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). External standards of DDT, DDD and DDE

were prepared in hexane and analyzed to obtain a standard linear regression. The recovery of spiked DDs was 96.2% (±4.5) in soil samples and 94.9% (±4.2) in plant samples.

2.5.5. Data analysis

The translocation factor (TF) is defined as the ratio of the pollutant concentration in the shoots to the concentration in the roots. The bioaccumulation factor (BAF) is the ratio of the pollutant concentration in the plant to the concentration in soil. The phytoextraction ratio (PR) is the total amount of pollutant in the plant versus that in the soil. Total pollutant accumulation per plant organ was calculated by multiplying the tissue concentration of pollutant with the root or shoot dry weight. The removal efficiency (RE) is the percent change in concentration of the pollutant in the soil after and before treatment [45,46].

The significance of the treatments was evaluated using the SPSS v.13.0 software package (SPSS Inc., Chicago, IL, USA). A one-way analysis of variance (ANOVA) followed by a Tukey's multiple comparison test (at P <0.05) was employed.

3. Results and discussion

3.1. Pot experiment

3.1.1. Growth of S. alfredii

Soil inoculation using DDT-1 increased the *SA* root biomass but had no significant effect on the shoot biomass at both Cd levels (Fig. 1). The stimulation of *SA* root growth by strain DDT-1 could be due to inoculation-induced variations in the rhizobacterial community structure within the soil [47]. The shoot biomass was significantly (P <0.05) greater (32.9%) in the Cd_{high} soil than in the Cd_{low} soil regardless of DDT-1 inoculation. This enhanced growth in high-Cd soil is similar to previously reported results showing that *SA* thrived in Cd-contaminated soils [13,48]. The results from this study indicate that *SA* can adapt easily to Cd in soils, and it is practical and feasible to remediate Cd-contaminated soil by planting *SA*.

3.1.2. Cd and DDs concentrations in S. alfredii

Cadmium concentrations in roots, stems, and leaves were greater in the Cd_{high} treatment than in the Cd_{low} treatment (Fig. 2). The inoculation of the soil with DDT-1 had no significant effect on the Cd concentrations in the roots, stems, and leaves of *SA* at both soil Cd levels as compared to those without DDT-1 inoculation. (Fig. 2). DDT-1 inoculation corresponded to a decrease in the DDs concentrations in *SA* with the exceptions of *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD in the roots and *p,p′*-DDD in the stems (Fig. 3 and supplementary material Fig. S1).

The DDs amounts in roots and shoots (stems and leaves) of *SA* grown in potted soil without DDT-1 inoculation were 4.6 and 43.1 μg, respectively. The DDs values for roots and shoots of plants grown in soil with DDT-1 inoculation were 6.5 and 24.7 μg, respectively. The incorporation of strain DDT-1 into the soil increased the total amount of DDs in the roots while decreased the DDs levels in the shoots. This result is in agreement with other study in which *Glomus etunicatum* respectively increased and decreased the levels of DDs accumulation in alfalfa roots and shoots [49]. This is mainly due to DDT-1 inoculation enhancing the removal of DDs from soil, and increasing *SA* root biomass that could, in turn, enhance the sequestration, adsorption, and relative bioaccessibility of DDs in roots, thus reducing the translocation of DDs from root to shoot [49]. However, elucidation of any direct effect needs to be addressed by further work.

3.1.3. Translocation factors (TF), bioaccumulation factors (BAF) and phytoextraction ratio (PR) for Cd and DDs

DDT-1 inoculation had no significant effect on the TF, BAF, or PR of Cd in *SA* (Table 2). The mean values of TF, BAF and PR of Cd were 2.08, 54.6 and 0.39, respectively, in the low-Cd soil, and 1.65, 28.2 and 0.25 in the Cd-spiked soil. Previous studies have reported similar results showing that the TF of *SA* for Cd varied from 3.36 to 4.43 and the BAF of *SA* for Cd ranged from 7.35 to 38 [50,51]. These observations are consistent with the characteristics of Cd hyperaccumulators [7].

Soil inoculation with DDT-1 decreased the values of TF, BAF, PR of *SA* for DDs (Table 2). The TF, BAF, PR values of *SA* decreased by 0.15- to 1.2-fold with the treatment. Lower TF, BAF, PR values for DDs were observed in the treatments of inoculation with DDT-1 because DDT-1 inoculation increased the overall amount of DDs removed from the soil and decreased the extent of DDs accumulation in the shoots. For *SA* plants with or without DDT-1 inoculation, the TF values were >15 for *o,p′*-DDT and >1.0 for *p,p′*-DDD but were <1.0 for *p,p′*-DDT and *p,p′*-DDE. The TF value was <0.1 for *p,p′*-DDE, which is much lower than the corresponding values (0.4–1.2) for *Cucurbita pepo* ssp. *pepo* [46,52]. This result implies that *p,p′*-DDE absorbed by *SA* plants was retained in the roots with only a small portion translocated to the shoots. The BAF values were <5.0 for *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE, which are lower than those previously observed in pumpkin (>8) and zucchini (>20) for *p,p′*-DDE [53]. However, higher BAF values (>29) were observed for *o,p′*-DDT, which suggests that *SA* plants may be particularly well adapted to accumulate *o,p′*-DDT. The shoot PR values for *o,p′*-DDT were 0.21–0.51 and varied from 0.004 to 0.04 for *p,p′*-DDE, *p,p′*-DDD, and *p,p′*-DDT (Table 2). These results are indicative of relatively low levels of DDs phytoextraction by *SA*, with the exception of *o,p′*-DDT.

3.1.4. The removal of Cd and DDs from the soil

3.1.4.1. Cd removal from the soil. Planting *SA* alone corresponded to Cd removal percentages of 32 and 39% for the low-Cd and spiked Cd levels, respectively (Fig. 4). The presence of DDT-1 microbes alone had no effect on Cd removal. Planting *SA* (with or without DDT-1 microbes) decreased the concentrations of all forms of Cd in the soil relative to those in soil without plants (Table 3). The exchangeable, carbonate-bound, oxide-bound, organic-bound, and residual Cd fractions were reduced by 25.5–39.1%, 35.2–40.4%, 10.3–33.4%, 24.4–36.4%, and 21.1–28.4%, respectively in the low and high Cd soils. This result suggests that *SA* plants can efficiently take up Cd from the resistant (i.e., oxide-bound, organic-bound, and residual) fractions, as well as the bioavailable (i.e., exchangeable and carbonate-bound) fractions. This uptake capability equaled that of the pot-grown hyperaccumulator *T. caerulescens* [54]. Our results confirm the previous finding that hyperaccumulating plants may be able to mobilize insoluble Cd in the soil [55,56].

3.1.4.2. The removal of DDs from the soil. *SA* with DDT-1 inoculation decreased the concentrations of *p,p′*-DDE, *o,p′*-DDT, *p,p′*-DDD, *p,p′*-DDT and DDs in the soil by 27.5, 39.4, 51.9, 34.0 and 33.3%, respectively, relative to uninoculated soil (Table 4 and Fig. 4). Furthermore, in the *SA* + M treatment, it was estimated that there was 256 μg of DDs dissipation in the soil, including a larger amount of DDs (198 μg) degradation in the soil, and a much smaller amount of DDs (31.2 μg) accumulation in the *SA* plant, with only 6.5 μg and 24.7 μg DDs in the roots and shoots, respectively. In our study, *Pseudomonas* sp. DDT-1 capable of utilizing DDs as its sole source of carbon and energy was isolated and purified (data not presented). The results show that the incorporation of strain DDT-1 into the soil enhances the degradation of DDs, thereby indicating that strain DDT-1 has the potential to significantly reduce DDs concentrations in a cost-effective manner and is a promising candidate for the bioremediation of soils contaminated by DDs. A similar study by

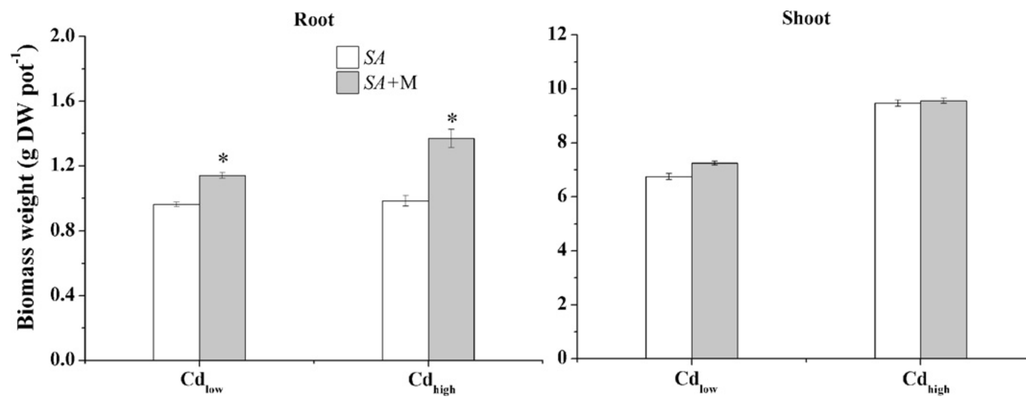

Fig. 1. Plant biomass of *Sedum alfredii* (*SA*) after 180 d growth in a Cd-DDs co-contaminated pot soil with low (Cd_{low} = 0.895 mg kg^{-1}) and high Cd (Cd_{high} = 3.225 mg kg^{-1}) concentrations, with 0.715 mg kg^{-1} DDs. M = *Pseudomonas* sp. DDT-1. * represent significant at P <0.05.

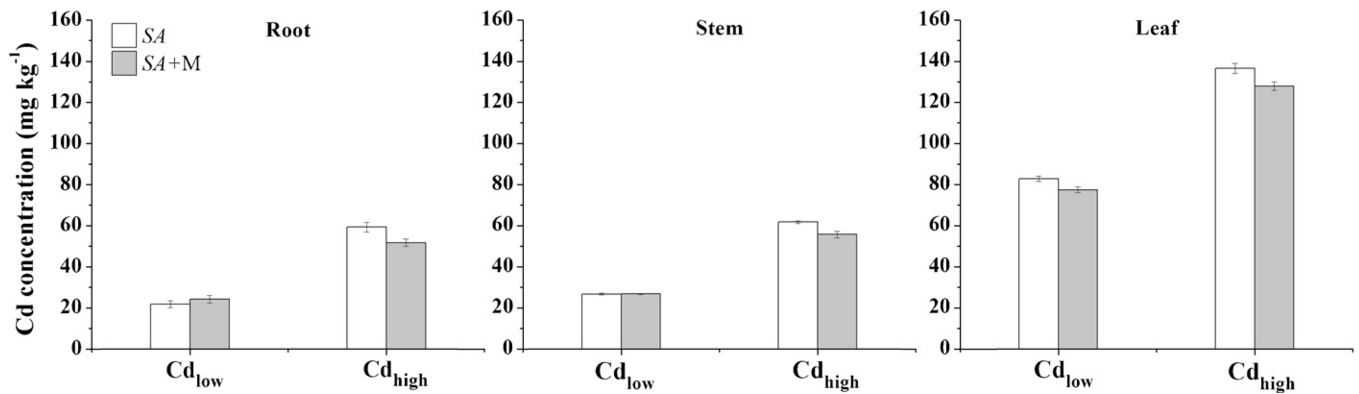

Fig. 2. Cd concentrations of *Sedum alfredii* (*SA*) after 180 d growth in a Cd-DDs co-contaminated pot soil with low (Cd_{low} = 0.895 mg kg^{-1}) and high Cd (Cd_{high} = 3.225 mg kg^{-1}) concentrations, with 0.715 mg kg^{-1} DDs. M = *Pseudomonas* sp. DDT-1.

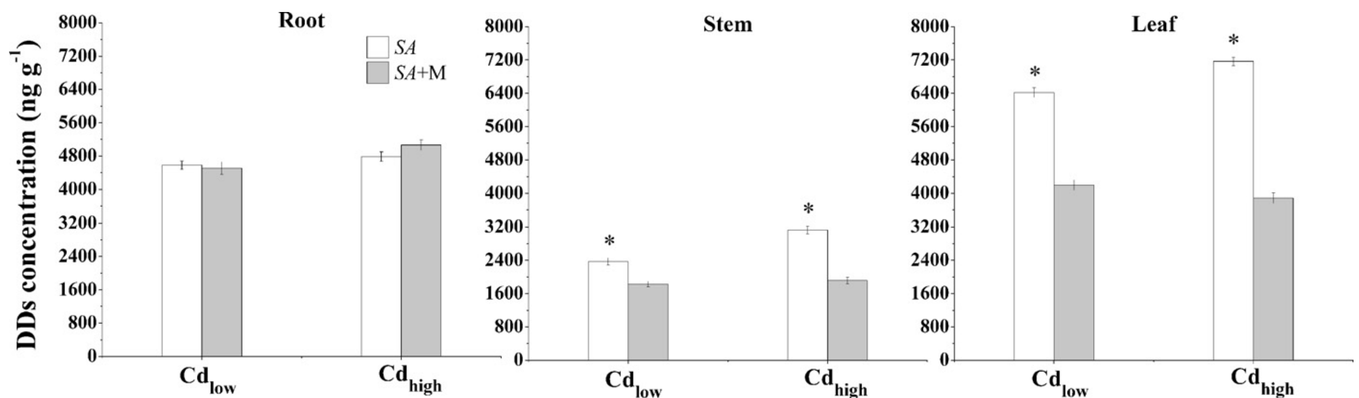

Fig. 3. Concentrations of DDs in root, stem, and leaf of *Sedum alfredii* (*SA*) after 180 d growth in a Cd-DDs co-contaminated pot soil with low (Cd_{low} = 0.895 mg kg^{-1}) and high Cd (Cd_{high} = 3.225 mg kg^{-1}) concentrations, with 0.715 mg kg^{-1} DDs. M = *Pseudomonas* sp. DDT-1. * represent significant at P <0.05.

Table 2
Translocation factor (TF), bioaccumulation factor (BAF) and phytoextraction ratio (PR) of DDs and Cd in *Sedum alfredii* (*SA*).

Treatments		*p,p′*-DDE			*o,p′*-DDT			*p,p′*-DDD			*p,p′*-DDT			Cd		
Main	Sub	TF	BAF	PR	TF	BAF	PR	TF	BAF	PR	TF	BAF	PR	TF	BAF	PR
Cd_{low}[a]	*SA*	0.07	0.65	0.005	20.4	44.8*	0.31	3.71*	1.01*	0.007*	0.56	3.90*	0.03	2.33	56.4	0.39
	SA + M[b]	0.05	0.48	0.004	15.7	29.5	0.21	1.46	0.51	0.004	0.43	2.72	0.02	1.82	52.7	0.39
Cd_{high}	*SA*	0.09*	0.75*	0.007*	22.2	53.2*	0.51*	2.93*	1.29*	0.012*	0.52*	4.10*	0.04*	1.61	29.5	0.24
	SA + M	0.04	0.41	0.004	18.9	29.1	0.28	1.01	0.53	0.005	0.30	2.11	0.02	1.68	26.9	0.26

* Significantly different means (P <0.05; t-test) between the treatment without and with DDT-1 microbes inoculation, by each Cd rate.
[a] Cd_{low} = 0.895 mg kg^{-1} Cd; Cd_{high} = 3.225 mg kg^{-1} Cd.
[b] M = *Pseudomonas* sp. DDT-1.

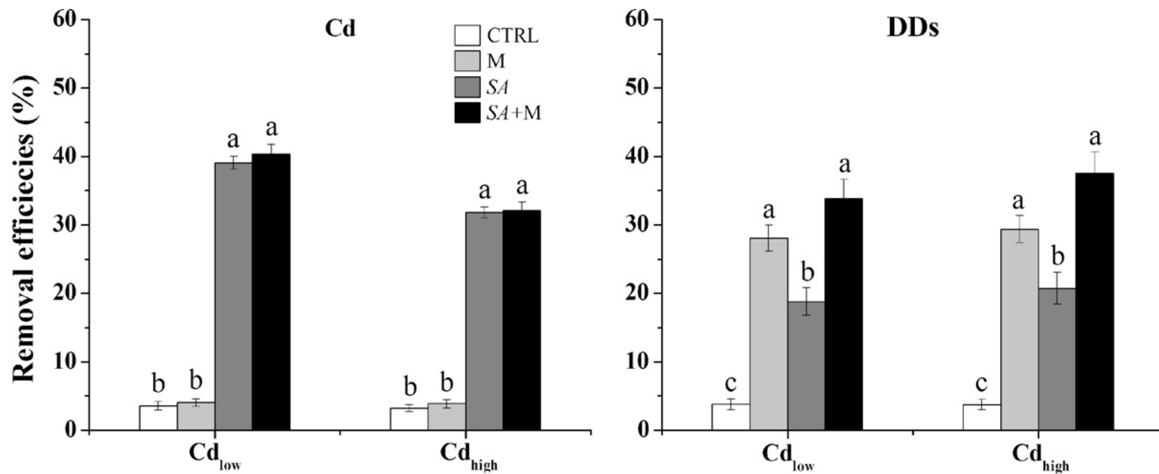

Fig. 4. Removal efficiencies of Cd and DDs after 180 d growth of *Sedum alfredii* (*SA*) in a Cd-DDs co-contaminated pot soil with low (Cd_{low} = 0.895 mg kg^{-1}) and high Cd (Cd_{high} = 3.225 mg kg^{-1}) concentrations, with 0.715 mg kg^{-1} DDs. CTRL = control; M = *Pseudomonas* sp. DDT-1. Different letters indicate significant differences (P <0.05) among treatments, by each Cd rate.

Table 3
Concentrations of soil Cd (mg kg^{-1}) in different fractions measured at the end of the pot experiment (180 d).

Treatments		Cd concentrations in different fractions (mg kg^{-1})					Total (mg kg^{-1})
Main	Sub	Exchangeable	Carbonates-bound	Oxides-bound	Organic-bound	Residual	
Cd_{low} [b]	Control	0.381a [a]	0.148a	0.037a	0.008a	0.278a	0.853a
	SA	0.222b	0.082b	0.035a	0.004b	0.205b	0.547b
	SA + M [c]	0.229b	0.083b	0.031a	0.004b	0.189b	0.536b
Cd_{high}	Control	1.377a	0.819a	0.514a	0.035a	0.376a	3.120a
	SA	1.023b	0.519b	0.342b	0.024b	0.291b	2.199b
	SA + M	0.994b	0.530b	0.339b	0.023b	0.301b	2.187b

[a] Means followed by the same letter, by the main treatments, in each column are not significantly different (P <0.05).
[b] Cd_{low} = 0.895 mg kg^{-1} Cd; Cd_{high} = 3.225 mg kg^{-1} Cd.
[c] *SA* = *Sedum alfredii*; M = *Pseudomonas* sp. DDT-1.

Table 4
Concentrations of DDs (mg kg^{-1}) in the soil received different treatments measured at the end of the pot experiment (180 d).

Treatments		DDs (mg kg^{-1})				
Main	Sub	*p,p′*-DDE	*o,p′*-DDT	*p,p′*-DDD	*p,p′*-DDT	DDs [b]
Cd_{low} and Cd_{high} [c]	Control	0.338a [a]	0.071a	0.077a	0.203a	0.688a
	M	0.264b	0.049b	0.044c	0.148bc	0.505c
	SA	0.305a	0.047b	0.056b	0.165b	0.573b
	SA + M [d]	0.245b	0.044b	0.037c	0.133c	0.459c

[a] All concentrations are means across Cd levels and replications. Means followed by the same letter in each column are not significantly different (P <0.05).
[b] DDs = *p,p′*-DDE + *p,p′*-DDD + *o,p′*-DDT + *p,p′*-DDT.
[c] Cd_{low} = 0.895 mg kg^{-1} Cd; Cd_{high} = 3.225 mg kg^{-1} Cd.
[d] *SA* = *Sedum alfredii*; M = *Pseudomonas* sp. DDT-1.

Table 5
Biomass, concentrations of Cd and DDs (mg kg^{-1}) in the shoots of *Sedum alfredii* (*SA*) in a field trial across different sampling dates.

Sampling date	Treatments	Shoot Biomass (kg ha^{-1})	Shoot Cd (mg kg^{-1})	Shoot DDs (ng g^{-1})				
		Dry weight	Cd	*p,p′*-DDE	*o,p′*-DDT	*p,p′*-DDD	*p,p′*-DDT	DDs [a]
Sept-2008	*SA*	1490	71.6	206	3147*	101*	824*	4277*
	SA + M [b]	1610	68.4	185	2686	78	677	3626
Apr-2009	*SA*	1600	67.6	173	2854*	89*	749*	3864*
	SA + M	1780	62.3	158	2288	67	576	3089
Sept-2009	*SA*	1680	61.6	156	2527*	78*	700*	3461*
	SA + M	1850	59.3	133	2052	58	493	2736

* Significantly different means (P <0.05; *t*-test) between the treatment without and with DDT-1 microbes inoculation, by each sampling date.
[a] DDs = *p,p′*-DDE + *p,p′*-DDD + *o,p′*-DDT + *p,p′*-DDT.
[b] *SA* = *Sedum alfredii*; M = *Pseudomonas* sp. DDT-1.

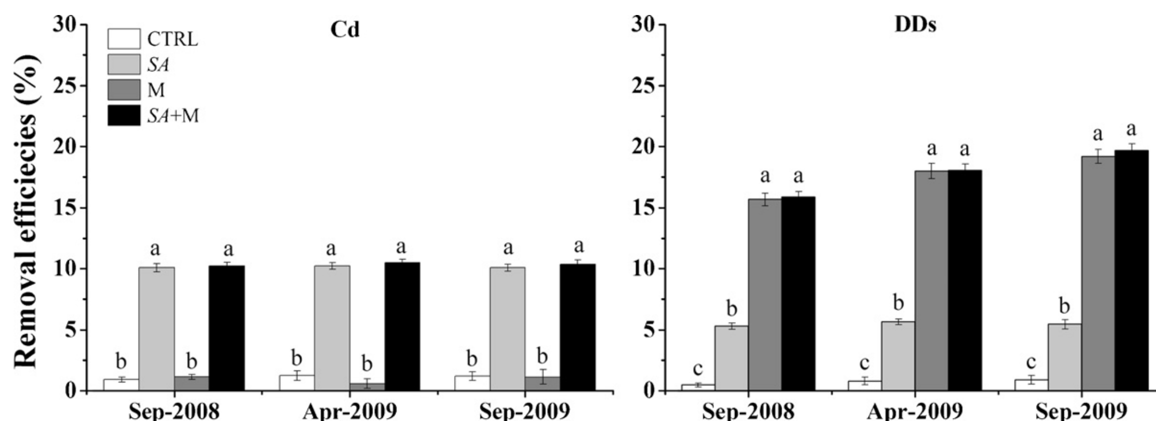

Fig. 5. Removal efficiencies of Cd and DDs by *Sedum alfredii* (*SA*) with or without DDT degrading microbes (M) across three sampling dates in a low Cd-DDs co-contaminated field soil. CTRL = control; M = *Pseudomonas* sp. DDT-1. Different letters indicate significant differences (P <0.05) among treatments, by each sampling date.

Mitra et al. [26] reported that genetically improved recombinants of the soil fungus *Fusarium solani* exhibit superior DDT degradation characteristics.

3.2. Field experiment

Inoculation using the DDT-1 strain had no significant effect on the shoot biomass yields (Table 5). In all control soils, Cd and DDs concentrations did not significantly change (~3.4% for Cd and ~2.1% for DDs) over the course of the experiment. Soil inoculation of *SA* with DDT-1 decreased the concentrations of Cd and DDs from 0.695 to 0.479 mg kg^{-1} (31.1%) and from 0.549 to 0.255 mg kg^{-1} (53.6%), respectively, over the 18-month period (Fig. 5 and supplementary material Fig. S2). The inoculation of the soil with the DDT-1 strain had no significant effect on Cd removal from soil by *SA*. The mean Cd contents in the roots and shoots of *SA* were 5.5 and 110 g ha^{-1} per cropping season, respectively (data not presented). Phytoextraction of Cd in this study was lower than that by *T. caerulescens* (540 g Cd ha^{-1}, over three years) in a field trial by Hammer and Keller [57]. Phytoextraction of Cd from soil is dependent on soil types, sources of contamination, and other environmental factors [21].

Inoculation with DDT-1 decreased the concentrations of all DDT isomers in *SA* shoots, with the exception of *p,p′*-DDE, relative to controls without DDT-1 (Table 5). The net uptake of DDs by *SA* shoots and roots were 5.45 and 0.79 g ha^{-1}, respectively (data not shown).

4. Conclusions

This study demonstrated that the planting of *SA* is an effective technique for phytoextraction of Cd from co-contaminated soils. The inoculation of strain DDT-1 to potted soils could increase the root biomass of *SA* and enhance the rhizodegradation of DDs in the soil. The results of this study indicate that the application of *SA* together with strain DDT-1 appears to be a promising approach for the bioremediation of soils co-contaminated by Cd and DDs. However, further studies on the survival, colonization, and population levels of strain DDT-1 in soil are required to develop more effective bioremediation strategies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2012.07.033.

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