ISSN 1001-0742
CN 11-2629/X

2012
Volume 24
Number 6

JOURNAL OF

ENVIRONMENTAL SCIENCES

ELSEVIER
Science Press

Sponsored by
Research Center for Eco-Environmental Sciences
Chinese Academy of Sciences

JOURNAL OF ENVIRONMENTAL SCIENCES

(http://www.jesc.ac.cn)

Aims and scope

Journal of Environmental Sciences is an international academic journal supervised by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The journal publishes original, peer-reviewed innovative research and valuable findings in environmental sciences. The types of articles published are research article, critical review, rapid communications, and special issues.

The scope of the journal embraces the treatment processes for natural groundwater, municipal, agricultural and industrial water and wastewaters; physical and chemical methods for limitation of pollutants emission into the atmospheric environment; chemical and biological and phytoremediation of contaminated soil; fate and transport of pollutants in environments; toxicological effects of terrorist chemical release on the natural environment and human health; development of environmental catalysts and materials.

For subscription to electronic edition

Elsevier is responsible for subscription of the journal. Please subscribe to the journal via http://www.elsevier.com/locate/jes.

For subscription to print edition

China: Please contact the customer service, Science Press, 16 Donghuangchenggen North Street, Beijing 100717, China. Tel: +86-10-64017032; E-mail: journal@mail.sciencep.com, or the local post office throughout China (domestic postcode: 2-580).

Outside China: Please order the journal from the Elsevier Customer Service Department at the Regional Sales Office nearest you.

Submission declaration

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere. The submission should be approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If the manuscript accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Submission declaration

Submission of the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere. The publication should be approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If the manuscript accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Editorial

Authors should submit manuscript online at http://www.jesc.ac.cn. In case of queries, please contact editorial office, Tel: +86-10-62920553, E-mail: jesc@263.net, jesc@rcees.ac.cn. Instruction to authors is available at http://www.jesc.ac.cn.

Copyright

ISSN 1001–0742 **Journal of Environmental Sciences** Vol. 24 No. 6 2012

CONTENTS

Serial parameter: CN 11-2629/X*1989*m*188*en*P*22*2012-6

Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 2012, 24(6) 1036–1046
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Combined remediation of DDT congeners and cadmium in soil by *Sphingobacterium* sp. D-6 and *Sedum alfredii* Hance

Hua Fang[1,2], Wei Zhou[1], Zhengya Cao[1], Feifan Tang[1], Dandan Wang[1], Kailin Liu[1], Xiangwei Wu[1], Xiao'e Yang[3], Yongge Sun[4,5], Yunlong Yu[1,2,*]

1. Department of Plant Protection, College of Agriculture & Biotechnology, Zhejiang University, Hangzhou 310058, China. E-mail: agri@zju.edu.cn
2. Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Ministry of Agriculture, Hangzhou 310058, China
3. MOE Key Lab of Environment Remediation & Ecosystem Health, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China
4. Department of Earth Science, Zhejiang University, Hangzhou 310027, China
5. State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China

Received 03 August 2011; revised 04 November 2011; accepted 27 December 2011

Abstract

Combined pollution of 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) and cadmium (Cd) in agricultural soils is of great concern because they present serious risk to food security and human health. In order to develop a cost-effective and safe method for the removal of DDTs and Cd in soil, combined remediation of DDTs and Cd in soil by *Sphingobacterium* sp. D-6 and the hyperaccumulator, *Sedum alfredii* Hance was investigated. After treatment for 210 days, the degradation half-lives of DDTs in soils treated by strain D-6 decreased by 8.1% to 68.0% compared with those in the controls. The inoculation of strain D-6 into soil decreased the uptake of DDTs by pak choi and *S. alfredii*. The shoots/roots ratios of *S. alfredii* for the Cd accumulation ranged from 12.32 to 21.75. The Cd concentration in soil decreased to 65.8%–71.8% for *S. alfredii* treatment and 14.1%–58.2% for *S. alfredii* and strain D-6 combined treatment, respectively, compared with that in the control. The population size of the DDTs-degrading strain, Simpson index ($1/D$) and soil respiratory rate decreased in the early stage of treatment and then gradually increased, ultimately recovering to or exceeding the initial level. The results indicated that synchronous incorporation of strain D-6 and *S. alfredii* into soil was found to significantly ($p \leqslant 0.05$) enhance the degradation of DDTs in soil and the hyperaccumulation of Cd in *S. alfredii*. It was concluded that strain D-6 and *S. alfredii* could be used successfully to control DDTs and Cd in contaminated soil.

Key words: bioremediation; cadmium; *Sphingobacterium* sp.; *Sedum alfredii* Hance; 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane
DOI: 10.1016/S1001-0742(11)60895-4

Introduction

1,1,1-Trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) was widely used as an active insecticide for agricultural purposes and public health in China from the 1940s until the 1980s (Foght et al., 2001). DDT and its two major metabolites, 1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene (DDE) and 1,1-dichloro-2,2-bis (4-chlorophenyl) ethane (DDD), are of concern because of their high persistence, toxicity and bioaccumulation. Some studies reported that the concentrations of DDT, DDD, and DDE (DDTs) in soils ranged from under detection limit to 120 μg/kg dw (mean value: 7.8 μg/kg dw), 0.40 to 2350 μg/kg dw (mean value: 63.6 μg/kg dw) and 0.52 to 414 μg/kg dw (mean value: 20.1 μg/kg dw) around/in Beijing Guanting Reservoir, Haihe Plain and Pearl River Delta, China, respectively (Li et al., 2006; Tao et al., 2008; Hu et al., 2010). Cadmium (Cd) is one of the most toxic heavy metals in the environment and has been released into agricultural soils by the disposal of municipal and industrial wastes, the application of metal-containing sewage sludge and fertilisers, atmospheric deposition and the discharge of wastewater on land (Zhou, 2003). Cd contamination has been reported to be a major constraint on food safety and agricultural land quality in China. The mean soil Cd concentrations were 1.698 and 0.741 mg/kg in topsoils (0–20 cm) and subsoils (20–40 cm) in the Shenyang Zhangshi irrigation area (Sun et al., 2006), 0.04 mg/kg in Guangdong Province (Zhang et al., 2011), 0.8 mg/kg in Hangzhou (Zhang and Ke, 2004), 0.35 mg/kg in Shanghai (Hu et al., 2004) and 1.152 mg/kg in a wastewater-irrigated field in Tianjin (Wu and Cao, 2010). In fact, an increasing number of studies have found combined contamination of DDTs and Cd in soil, sediment and foodstuffs (Gaw et al., 2008; Yang et al., 2008; Huang et al., 2011). Gaw et al. (2006) reported that Cd (< 0.1 to 1.5 mg/kg) and DDTs (< 0.03 to 34.5 mg/kg) residues were detected in horticultural

* Corresponding author. E-mail: ylyu@zju.edu.cn

and grazing soils from three regions of New Zealand. In comparison with contamination by a single pollutant, the combined pollution of DDTs and Cd may pose a more serious risk to food safety and human health through the food chain (Rignell-Hydbom et al., 2009). Therefore, it is necessary to develop an integrated remediation system to remove both DDTs and Cd residues.

Bioaugmentation of natural flora with specialized DDTs-degrading microbes (e.g., *Alcaligenes eutrophus* A5, *Serratia marcescens* DT-1P, *Pseudomonas fluorescens*, *Bacillus* sp. BHD-4, brown-rot and white-rot fungi) has been considered to be a cost-effective, safe and promising method (Purnomo et al., 2008). Similarly, phytoremediation by use of hyperaccumulators (e.g., *Sedum alfredii* Hance, *Thlaspi caerulescens* and *Solanum nigrum* L.) to accumulate Cd has been also considered as a potentially cost-effective and green alternative to traditional physical and chemical treatments, such as *ex-situ* excavation, landfill, incineration, thermal treatment, supercritical fluid extraction and sulphuric acid treatment (Krämer, 2005). Single pollutant bioremediation of DDT or Cd in soil has been well studied (Zhang et al., 2009; Li et al., 2010; Luo et al., 2011; Purnomo et al., 2011a, 2011b). Zhao and Yi (2010) reported remediation of DDT-contaminated soil using laccase from white rot fungi. Yang et al. (2004a) were the first to find that *S. alfredii* Hance has an extraordinary ability to tolerate and hyperaccumulate Cd. Long et al. (2009) reported significant hyperaccumulation of Cd in *S. alfredii* growing natively on an old lead/zinc mining site. Yang et al. (2004b) also reported the accumulation of Cd in *S. alfredii* at different Cd/Zn complex levels. However, little information is available on the combined remediation of DDTs and Cd in vegetable soil.

In this study, a strain of *Sphingobacterium* sp., strain D-6, previously isolated from DDTs-contaminated soil (Fang et al., 2010) and capable of utilising DDTs as its sole carbon and energy source, was used to detoxify DDT residues in soil. *S. alfredii*, a Cd-hyperaccumulator that is native to China (Yang et al., 2004b), was used to accumulate Cd. The objectives of this study were the following: (1) to explore the feasibility of strain D-6 as an inoculum to degrade or detoxify DDTs; (2) to determine the accumulation potential of Cd in *S. alfredii*; and (3) to examine the combined bioremediation efficiency of strain D-6 and *S. alfredii* for DDTs and Cd in soil. This could be useful for the improvement of soil quality.

1 Materials and methods

1.1 Chemicals

Standards of p,p'-DDT (purity $\geqslant$ 99.5%), o,p'-DDT (purity $\geqslant$ 98%), p,p'-DDD (purity $\geqslant$ 98%) and p,p'-DDE (purity $\geqslant$ 96.0%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Anhydrous sodium sulphate, *n*-hexane, and acetone of analytical grade were purchased from Dafang Chemical Co., Hangzhou, China.

1.2 Soils

Soil samples used in this study were collected from a vegetable field (640 m^2) located in Cixi, Zhejiang, China. The field soils had been heavily polluted due to the extensive and indiscriminate use of DDT prior to its banning in China and are currently used to grow vegetables. Soil samples taken from the top layer (0–15 cm) were air-dried at room temperature, mixed thoroughly and sieved (2 mm sieve) to remove stones and debris. Soil properties were as follows: organic matter content, 0.50%; total nitrogen, 8.99%; total P, 1.30%; total K, 6.41%; and pH 5.76. The background concentration of DDTs was measured to be 0.35 mg/kg (p,p'-DDT, 0.035 mg/kg; o,p'-DDT, 0.055 mg/kg; p,p'-DDD, 0.060 mg/kg; p,p'-DDE, 0.200 mg/kg) and the Cd concentration was 0.30 mg/kg.

1.3 Microorganism and *S. alfredii*

The strain D-6, isolated earlier from the DDT-contaminated soil and capable of degrading DDTs, was used in these experiments. The 16S rRNA gene sequences from strain D-6 have been deposited in the NCBI GenBank database under the accession number EU927288. The *S. alfredii* plants, collected from an old mining area, were obtained from the College of Environmental & Resource Sciences, Zhejiang University, China. The *S. alfredii* seedlings were precultured in the greenhouse prior to the experiments.

1.4 Inoculum preparation

The strain D-6 was cultured in 250 mL Erlenmeyer flasks containing 150 mL of LB medium at 30°C and shaken at 150 r/min on a rotary shaker. During the exponential phase (1 day), the cell pellets were harvested by centrifugation (10,000 $\times g$, 10 min), immediately washed 3 times with 20 mL of NaH_2PO_4-Na_2HPO_4 buffer (0.1 mol/L, pH 7.0), and finally suspended in the same phosphate buffer as the inoculant.

1.5 Soil treatment and sampling

To determine the ability of strain D-6 to degrade DDTs in soil, except for the background controls, the soil samples (1 kg dry weight) were treated with a mixed standard solution of DDTs (p,p'-DDT:o,p'-DDT:p,p'-DDD:p,p'-DDE, 1:1:1:1, m/m) in *n*-hexane to give three concentration levels of 4.0 mg DDTs/kg dry soil (p,p'-DDT, 1 mg/kg; o,p'-DDT, 1 mg/kg; p,p'-DDD, 1 mg/kg; p,p'-DDE, 1 mg/kg), 20.0 mg DDTs/kg dry soil (p,p'-DDT, 5 mg/kg; o,p'-DDT, 5 mg/kg; p,p'-DDD, 5 mg/kg; p,p'-DDE, 5 mg/kg) and 40.0 mg DDTs/kg of dry soil (p,p'-DDT, 10 mg/kg; o,p'-DDT, 10 mg/kg; p,p'-DDD, 10 mg/kg; p,p'-DDE, 10 mg/kg), respectively, according to the reported environmentally related concentrations in soils (Gaw et al., 2006; Li et al., 2006; Tao et al., 2008; Hu et al., 2010). The soil samples were left for 1 hr on a laminar flow bench for solvent evaporation, inoculated with the strain D-6 preparation to give an initial inoculum density of 1.0×10^6 CFU/g, mixed thoroughly with a plastic spoon and passed through a 2-mm mesh

to ensure the uniform distribution of the added DDTs and the inoculum, and subsequently transferred into a plastic container. Thereafter, five *S. alfredii* seedlings with healthy, equal-sized stems were planted into the soil in the plastic container. All treatments began with ten pak choi seeds, and five pak choi seedlings were retained after the germination of these seeds. The soil moisture content was constantly maintained at 60% of the water holding capacity by periodic additions of distilled water. All treatments were conducted in triplicate and placed in a greenhouse at (25 ± 1)°C. This study included sixteen treatments: background (BG), BG+*S. alfredii* Hance (SAH), BG + *Sphingobacterium* sp. D-6 (SB), BG+SB+SAH, 1.0 mg/kg DDT (1DDT), 1DDT+SAH, 1DDT+SB, 1DDT+SB+SAH, 5.0 mg/kg DDT (5DDT), 5DDT+SAH, 5DDT+SB, 5DDT+SB+SAH, 10.0 mg/kg DDT (10DDT), 10DDT+SAH, 10DDT+SB, and 10DDT+SB+SAH.

At the time intervals of 0, 30, 60, 90, 150 and 210 days, soil samples (20 g) were taken from each treatment to measure the DDTs and Cd concentrations, the DDTs-degrading strain population size, the substrate utilisation patterns of the soil microbial community and soil respiratory rate. Due to its short growth duration, five pak choi plants were harvested for measurement of the DDTs and Cd concentrations after treatment for 90 days. As a perennial herbaceous plant, five *S. alfredii* plants were collected to determine the DDTs and Cd concentrations at the end of the experiment (210 days), and the *S. alfredii* roots were washed with deionized water and immersed in 20 mmol/L Na_2-EDTA for 15 min to remove Cd residues adhering to the root surfaces.

1.6 Enumeration of the DDTs-degrading strain

The mineral salts medium, used for counting colonies of the DDTs-degrading strain, was composed of $MgSO_4{\cdot}7H_2O$ (0.4 g), $FeSO_4{\cdot}7H_2O$ (0.002 g), K_2HPO_4 (0.2 g), $(NH_4)_2SO_4$(0.2 g), $CaSO_4$ (0.08 g) and DDTs (0.001 g) in 1000 mL distilled water at pH 7.0. The number of DDT-degrading bacteria was estimated using the most-probable-number (MPN) technique (Nanjing Institute of Soil, Chinese Academy of Sciences, 1985). MPN procedure included: Soil samples (1 g dry weight equivalent) were suspended in 90 mL of sterile water on a shaker for 1 hr at 150 r/min and (25 ± 1)°C, and then settled for 30 min, and a portion of supernatant was then 10-fold serially diluted to 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} in sterile water based on the expected population contained within the soil sample. One-milliliter aliquots of each dilution step were transferred to MPN tubes containing 9 mL of sterile mineral salt medium supplemented with 1 mg/L of DDTs as the sole source of carbon and energy. For each soil sample, three replicate MPN assays were set up with three replicate MPN tubes for each dilution step. All tubes were incubated on a shaker for 7 days at 150 r/min and (30 ± 1)°C, and then the number of positive growth response may be counted by the visible turbidity in tubes. Subsequently, MPN values may be obtained from MPN tables.

1.7 Substrate utilisation patterns and soil respiration activity

Substrate utilisation patterns of the soil microbial community during the combined remediation were examined using Biolog ECO microplate™ plates (Biolog Inc., Hayward, USA) according to the method of Fang et al. (2009). Soil respiratory activity was estimated from the differences in CO_2 concentration between air passing through the measurement cells and bypassing reference air using an infrared gas analyser (ADC 2250, ADC Bioscientific Ltd., Hoddesdon, England) connected to a multipoint universal switching gas handling unit (GHU, ADC Bioscientific Ltd., Hoddesdon, England) according to the method of Boberg et al. (2008).

1.8 Measurement of DDTs residues from soil and plant

DDTs residues in soil were extracted according to the method of Fang et al. (2010). To determine the concentration of DDTs in these or *S. alfredii*, the sample (10 g) was weighed into a glass jar and 80 mL of *n*-hexane and 80 g of anhydrous sodium sulphate were added and then homogenised at 10, 000 r/min for 3 min. The mixture was decanted and filtered through a 7 cm Buchner funnel, and then the filter cake was washed successively 3 times with 25 mL of *n*-hexane. The extracts were collected in a 250 mL flat-bottomed flask, concentrated to approximately 1 mL on a vacuum rotary evaporator, immediately concentrated nearly to dryness under a gentle nitrogen flow, and finally dissolved in 5 mL of *n*-hexane for determination.

The analyses of DDTs concentrations were performed using an Agilent 6890N gas chromatography system (Agilent Technologies, USA), equipped with an electron capture detector (μ-ECD) and a DB-1701 silica capillary column (30 m×0.32 mm×0.25 μm, Agilent Technologies, USA), according to the method of Fang et al. (2010).

1.9 Determination of Cd in soil and plants

The soil, pak choi or *S. alfredii* samples (0.2 g dry weight) were digested with a concentrated acid mixture (HNO_3/$HClO_4$, 5:1, *V*/*V*) at 200–220°C. The Cd concentrations in the digested solution were analysed by inductively coupled plasma mass spectroscopy (ICP-MS) (Agilent 7500a, USA). The operating conditions were as follows: RF power 1190 V, sampling depth 7.6 mm, carrier gas flow rate 1.08 L/min, peristaltic pump flow rate 0.1 r/sec, Torch-H 1.1 mm, Torch-V 0.2 mm, extract 120.1 V, Einzel 79 V, omega 5.9 V, peristaltic pump uptake speed 0.1 r/sec, peristaltic pump uptake time 60 sec, peristaltic pump stabilisation time 60 sec, integration time 60 sec and 3 repetitions.

1.10 Recovery study

To confirm the validity of the DDTs extraction method, recovery studies were carried out at spiking levels of 0.1, 1, 10 mg/kg in soil and 0.01, 0.1, 1 mg/L in pak choi. Each recovery assay and blank sample was extracted and analyzed as described above. All treatments were conducted in triplicate. The recoveries of DDTs ranged

from 91.2% to 102.9% with relative standard deviation (RSD) ⩽ 6.9% in soil and 98.2%–103.8% with RSD ⩽ 6.9% in pak choi. These data indicate that the extraction method was satisfactory for analysis of DDTs residues.

1.11 Statistical analysis

The means and standard errors of the data were calculated using Excel (Microsoft, USA). The kinetics data of DDTs degradation were obtained using a first-order model. Soil microbial diversity was calculated as the Simpson index ($1/D$):

$$D = \sum \frac{n_i\,(n_i - 1)}{N\,(N - 1)} \quad (1)$$

where, n_i refers to absorbance value, N is total absorbance resulting from different treatments of all wells. One-way analysis of variance (ANOVA) was conducted to determine the significance of the difference between the different treatments using SPSS 11.5 for Windows (SPSS Inc., USA).

2 Results

2.1 Biodegradation of DDTs and development of the DDT-degrading strain, microbial functional diversity and soil respiration

The degradation of DDTs in soil was subject to pseudo first-order kinetics (Fig. 1). As shown in Table 1, the degradation half lives (DT_{50}) of p, p'-DDT in BG+SAH, BG+SB and BG+SB+SAH significantly ($p \leqslant 0.05$) decreased to 89.6%, 60.5% and 51.2%, respectively, of those in BG, after treatment for 210 days. A similar trend was observed for o, p'-DDT, p, p'-DDD, and p, p'-DDE in all treatments. The degradation half-lives of DDTs in the soils inoculated with strain D-6 decreased by 8.1% to 68.0% compared with those in the control. After treatment for 210 days, the final residual amounts of DDTs in the soils were measured to be 0.148, 0.130, 0.094, 0.067 mg in BG, BG+SAH, BG+SB, BG+SB+SAH treatments, 1.404, 1.062, 0.675, 0.441 mg in 1DDT, 1DDT+SAH, 1DDT+SB, 1DDT+SB+SAH treatments, 9.198, 7.677, 5.166, 3.204 mg in 5DDT, 5DDT+SAH, 5DDT+SB, 5DDT+SB+SAH treatments, and 22.068, 18.063, 11.961, 8.955 mg in 10DDT, 10DDT+SAH, 10DDT+SB, 10DDT+SB+SAH treatments, respectively. The ANOVA analyses showed that the final residual amounts of DDTs in the inoculated treatments significantly

Table 1 Kinetic data for the degradation of DDTs in the soil resulting from different treatments after 210 days

DDT	Treatment	Final residues (mg/kg)	DT_{50} (day)	Treatment	Final residues (mg/kg)	DT_{50} (day)
p,*p*′-DDT	BG	0.015	161.2 a	1DDT	0.35	141.4 a
	BG+SAH	0.013	144.4 b	1DDT+SAH	0.19	100.4 b
	BG+SB	0.008	97.6 c	1DDT+SB	0.10	68.6 c
	BG+SB+SAH	0.006	82.5 d	1DDT+SB+SAH	0.06	56.3 d
	5DDT	2.69	216.6 a	10DDT	5.96	277.2 a
	5DDT+SAH	2.23	192.5 b	10DDT+SAH	4.97	231.0 b
	5DDT+SB	1.04	96.3 c	10DDT+SB	2.38	113.6 c
	5DDT+SB+SAH	0.56	69.3 d	10DDT+SB+SAH	1.86	94.9 d
o,*p*′-DDT	BG	0.021	165.0 a	1DDT	0.32	144.4 a
	BG+SAH	0.018	144.4 b	1DDT+SAH	0.24	117.5 b
	BG+SB	0.011	99.0 c	1DDT+SB	0.12	72.2 c
	BG+SB+SAH	0.008	84.5 d	1DDT+SB+SAH	0.05	51.0 d
	5DDT	2.21	173.3 a	10DDT	5.85	266.5 a
	5DDT+SAH	1.94	144.4 b	10DDT+SAH	4.93	203.8 b
	5DDT+SB	1.21	101.9 c	10DDT+SB	3.01	121.6 c
	5DDT+SB+SAH	0.61	68.6 d	10DDT+SB+SAH	2.01	92.4 d
p,*p*′-DDD	BG	0.028	192.5 a	1DDT	0.31	121.6 a
	BG+SAH	0.023	150.7 b	1DDT+SAH	0.25	101.9 b
	BG+SB	0.016	111.8 c	1DDT+SB	0.14	75.3 c
	BG+SB+SAH	0.011	91.2 d	1DDT+SB+SAH	0.10	64.8 d
	5DDT	2.08	177.7 a	10DDT	5.53	256.7 a
	5DDT+SAH	1.31	113.6 b	10DDT+SAH	4.17	177.7 b
	5DDT+SB	1.11	106.6 c	10DDT+SB	2.71	121.6 c
	5DDT+SB+SAH	0.60	75.3 d	10DDT+SB+SAH	2.03	101.9 d
p,*p*′-DDE	BG	0.10	203.8 a	1DDT	0.58	288.8 a
	BG+SAH	0.09	187.3 b	1DDT+SAH	0.50	239.0 b
	BG+SB	0.07	144.4 c	1DDT+SB	0.39	182.4 c
	BG+SB+SAH	0.05	115.5 d	1DDT+SB+SAH	0.28	123.8 d
	5DDT	3.24	346.5 a	10DDT	7.18	407.7 a
	5DDT+SAH	3.05	288.8 b	10DDT+SAH	6.60	346.5 b
	5DDT+SB	2.38	203.8 c	10DDT+SB	5.19	239.0 c
	5DDT+SB+SAH	1.79	150.7 d	10DDT+SB+SAH	4.05	173.3 d

BG: background; SAH: *S. alfredii* Hance; SB: *Sphingobacterium* sp. D-6; 1DDT: 1 mg/kg DDTs; 5DDT: 5 mg/kg DDTs; 10DDT: 10 mg/kg DDTs. Data followed by a different letter in the same column are significantly different ($p \leqslant 0.05$).
DT_{50}: degradation half live for DDTs, DT_{50} was calculated by the first-order function ($C = C_0 \times e^{-kt}$, $DT_{50} = (\ln 2)/k$.

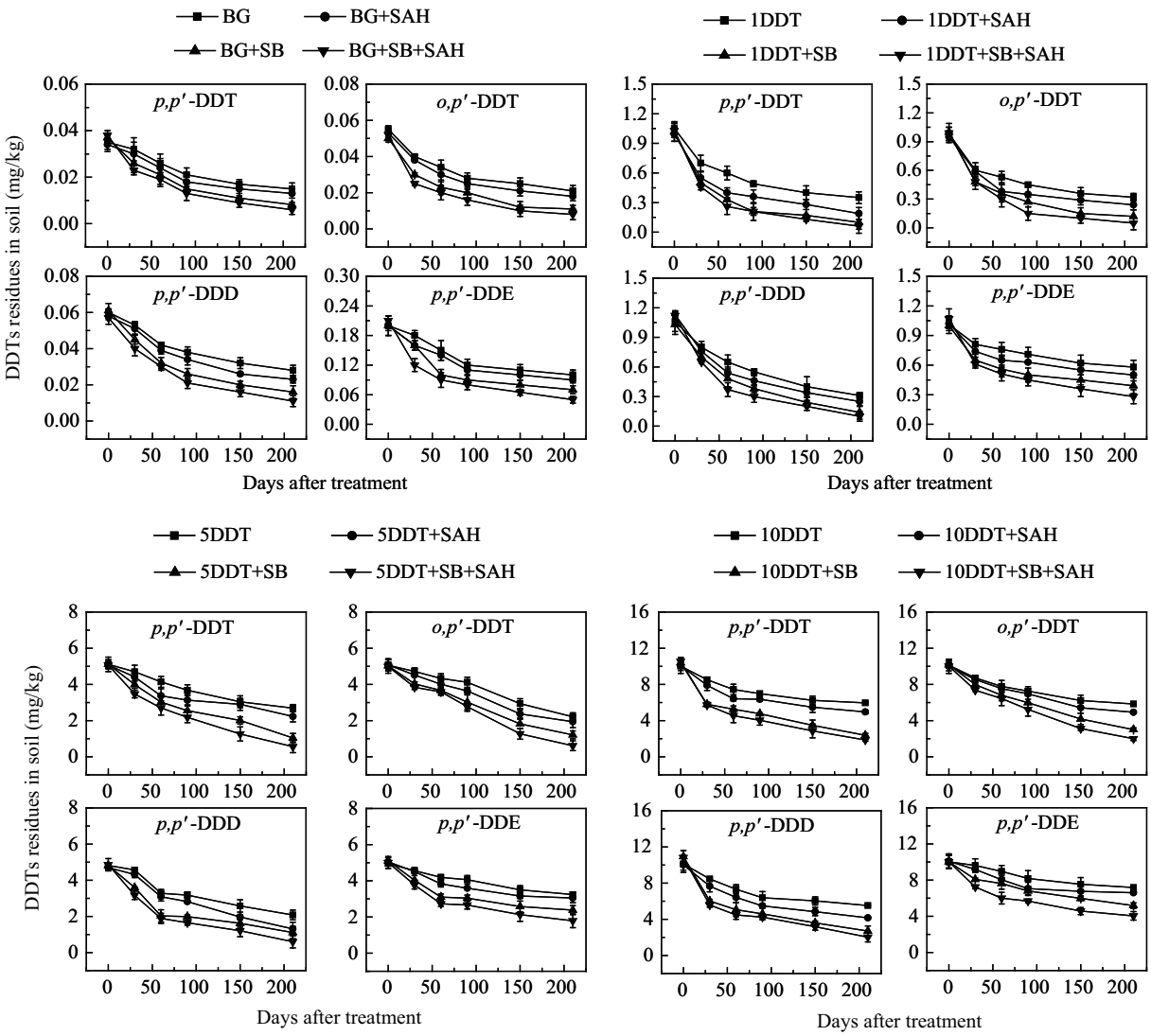

Fig. 1 Degradation of DDTs at various levels in soil.

($p \leqslant 0.05$) decreased to 31.4% to 45.3% of those in the uninoculated treatments. The results indicated that strain D-6 clearly accelerated the degradation of DDTs in the soils.

As shown in Fig. 2, when compared with the initial inoculation density, the DDTs-degrading strain population size in BG+SB and BG+SB+SAH significantly ($p \leqslant 0.05$) decreased to 16.7% and 25.0% at 30 days and 9.2% and 12.5% at 60 days, respectively. Ninety days after inoculation, the population size of the DDTs-degrading strain gradually increased, it then recovered to the initial inoculation level after 150 days and exceeded the inoculation density by 8.3% (BG+SB) and 20.8% (BG+SB+SAH) after 210 days. A similar but more rapid change was observed in the treatment of 1DDT+SB and 1DDT+SB+SAH, 5DDT+SB and 5DDT+SB+SAH, 10DDT+SB and 10DDT+SB+SAH.

The Simpson index ($1/D$) and soil respiration are customarily used to assess the dominant population and overall activity of soil microorganisms (Gomez et al., 2006). As shown in Fig. 3, after 0, 30, 60, 90, 150 and 210 days of treatment, the $1/D$ was respectively 1.13, 1.13, 1.17, 1.16, 1.19, 1.16 times higher in BG+SAH, 3.68, 1.42, 1.28, 1.28, 1.45, 1.38 times higher in BG+SB, and 3.56, 1.39, 1.30, 1.33, 1.51, 1.44 times higher in BG+SB+SAH in comparison with BG. The ANOVA analysis confirmed that the $1/D$ in BG+SAH, BG+SB and BG+SB+SAH was significantly ($p \leqslant 0.05$) higher than that in BG, suggesting that strain D-6 and *S. alfredii* rhizosphere could synergistically increase populations of the dominant soil microorganisms. A similar trend was also observed for soil respiratory rate in all treatments (Fig. 3).

2.2 Uptake of DDTs by pak choi and *S. alfredii*

The uptake of DDTs in the soil by pak choi and *S. alfredii* is shown in Table 2. After treatments for 90 days, the amounts of DDTs taken up by the five pak choi plants from the soils were measured to be 2.77, 2.08, 1.92, 1.62 μg in BG, BG+SAH, BG+SB, BG+SB+SAH treatments, 4.99, 3.43, 2.81, 1.75 μg in 1DDT, 1DDT+SAH, 1DDT+SB, 1DDT+SB+SAH treatments, 8.00, 5.26, 3.20, 2.53 μg in 5DDT, 5DDT+SAH, 5DDT+SB, 5DDT+SB+SAH treatments, and 14.06, 6.59, 4.73, 3.21 μg in 10DDT, 10DDT+SAH, 10DDT+SB, 10DDT+SB+SAH treatments, respectively. After treatments for 210 days, the quantities of DDTs taken up by

Table 2 Uptake of DDTs in the soil by pak choi after 90 days and by *S. alfredii* after 210 days (unit: mg/kg)

Plant	Treatment	*p,p′*-DDT	*o,p*-DDT	*p,p′*-DDD	*p,p′*-DDE	DDTs
Pak choi	BG	0.061 ± 0.034 a	0.040 ± 0.004 a	0.010 ± 0.003 a	0.064 ± 0.016 a	0.175 ± 0.021 a
	BG+SAH	0.044 ± 0.013 bc	0.026 ± 0.006 b	0.005 ± 0.002 b	0.049 ± 0.006 b	0.124 ± 0.015 b
	BG+SB	0.041 ± 0.018 bc	0.025 ± 0.011 b	0.005 ± 0.001 b	0.048 ± 0.033 b	0.119 ± 0.016 b
	BG+SB+SAH	0.031 ± 0.023 c	0.019 ± 0.005 b	0.004 ± 0.001 b	0.042 ± 0.021 b	0.096 ± 0.018 b
	1DDT	0.074 ± 0.006 a	0.051 ± 0.014 a	0.023 ± 0.004 a	0.162 ± 0.022 a	0.310 ± 0.042 a
	1DDT+SAH	0.053 ± 0.020 b	0.040 ± 0.026 a	0.016 ± 0.003 ab	0.099 ± 0.018 b	0.208 ± 0.039 b
	1DDT+SB	0.048 ± 0.007 bc	0.029 ± 0.013 b	0.011 ± 0.006 b	0.084 ± 0.007 b	0.172 ± 0.034 b
	1DDT+SB+SAH	0.035 ± 0.008 c	0.023 ± 0.004 b	0.010 ± 0.004 b	0.037 ± 0.009 c	0.105 ± 0.015 c
	5DDT	0.118 ± 0.007 a	0.107 ± 0.032 a	0.110 ± 0.030 a	0.188 ± 0.106 a	0.523 ± 0.031 a
	5DDT+SAH	0.057 ± 0.017 b	0.070 ± 0.011 b	0.057 ± 0.016 b	0.147 ± 0.024 b	0.331 ± 0.040 b
	5DDT+SB	0.050 ± 0.010 b	0.033 ± 0.005 c	0.033 ± 0.007 c	0.088 ± 0.066 b	0.204 ± 0.023 c
	5DDT+SB+SAH	0.037 ± 0.009 c	0.031 ± 0.014 c	0.021 ± 0.005 c	0.068 ± 0.021 b	0.157 ± 0.019 c
	10DDT	0.250 ± 0.076 a	0.175 ± 0.054 a	0.194 ± 0.050 a	0.312 ± 0.107 a	0.931 ± 0.060 a
	10DDT+SAH	0.075 ± 0.019 b	0.080 ± 0.015 b	0.099 ± 0.101 b	0.170 ± 0.079 a	0.424 ± 0.045 b
	10DDT+SB	0.064 ± 0.011 bc	0.069 ± 0.008 bc	0.038 ± 0.034 c	0.136 ± 0.044 b	0.307 ± 0.044 c
	10DDT+SB+SAH	0.054 ± 0.052 c	0.051 ± 0.022 c	0.025 ± 0.007 c	0.071 ± 0.014 c	0.201 ± 0.017 c
S. alfredii	BG+SAH	0.021 ± 0.010 a	0.012 ± 0.002 a	0.003 ± 0.001 a	0.026 ± 0.003 a	0.062 ± 0.009 a
	BG+SB+SAH	0.014 ± 0.003 b	0.010 ± 0.002 a	0.002 ± 0.001 a	0.019 ± 0.005 b	0.045 ± 0.008 b
	1DDT+SAH	0.025 ± 0.008 a	0.018 ± 0.004 a	0.010 ± 0.002 a	0.047 ± 0.011 a	0.100 ± 0.014 a
	1DDT+SB+SAH	0.016 ± 0.003 b	0.011 ± 0.003 a	0.006 ± 0.002 a	0.015 ± 0.004 b	0.048 ± 0.006 b
	5DDT+SAH	0.030 ± 0.009 a	0.033 ± 0.006 a	0.028 ± 0.005 a	0.081 ± 0.016 a	0.172 ± 0.027 a
	5DDT+SB+SAH	0.023 ± 0.005 a	0.014 ± 0.003 b	0.012 ± 0.002 b	0.043 ± 0.013 b	0.092 ± 0.013 b
	10DDT+SAH	0.039 ± 0.011 a	0.043 ± 0.015 a	0.044 ± 0.010 a	0.089 ± 0.023 a	0.215 ± 0.020 a
	10DDT+SB+SAH	0.022 ± 0.006 b	0.020 ± 0.004 b	0.014 ± 0.002 b	0.038 ± 0.012 b	0.094 ± 0.011 b

Data followed by a different letter in the same column are significantly different ($p \leqslant 0.05$).

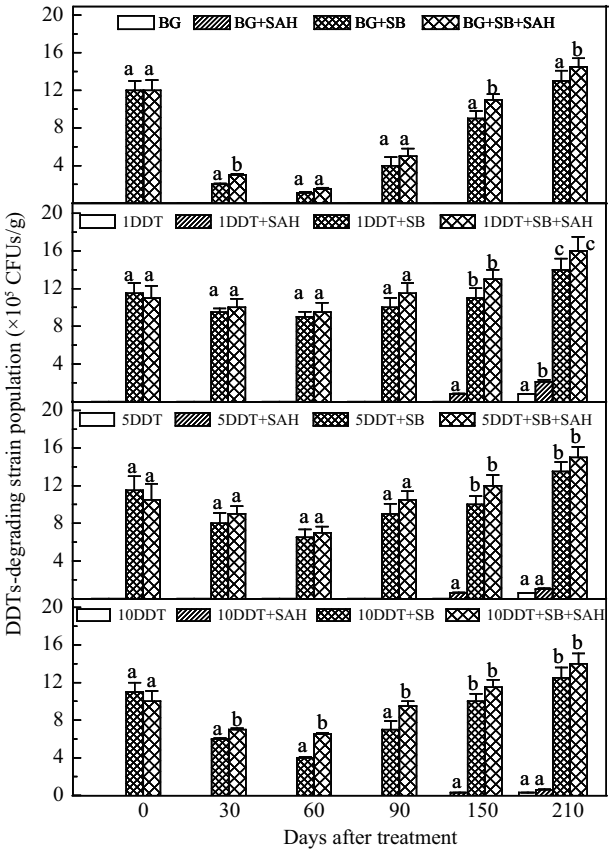

Fig. 2 Dynamics of the DDTs-degrading strain population during the bioremediation. The different letters above the columns at each sampling time indicate that the values are significantly different ($p \leqslant 0.05$).

the five *S. alfredii* plants from the soils were 0.98 and 0.75 μg in BG+SAH and BG+SB+SAH treatments, 1.62 and 0.81 μg in 1DDT+SAH and 1DDT+SB+SAH treatments, 2.77 and 1.52 μg in 5DDT+SAH and 5DDT+SB+SAH treatments, and 3.52 and 1.57 μg in 10DDT+SAH and 10DDT+SB+SAH treatments, respectively. The ANOVA analyses showed that the uptake quantities of DDTs in the inoculated treatments significantly ($p \leqslant 0.05$) decreased to 22.8% to 58.5% by pak choi and 44.6% to 76.5% by *S. alfredii*, respectively, compared to those in the uninoculated treatments. The results indicated that the inoculation of strain D-6 into soil decreased the uptake of DDTs by pak choi and *S. alfredii*, furthermore, the uptake amounts of DDTs by *S. alfredii* were clearly lower than those by pak choi.

2.3 Accumulation of Cd

Prior to the experiments, the Cd amount in soils (1 kg dry weight) was measured to be 0.300 mg. As shown in Tables 3 and 4, the Cd amounts in the BG+SAH, 1DDT+SAH, 5DDT+SAH and 10DDT+SAH treatments were measured to be 96.70, 90.25, 64.25 and 55.25 μg in five *S. alfredii* shoots, 4.65, 4.15, 3.65 and 3.30 μg in five *S. alfredii* roots, 2.90, 3.10, 3.15 and 3.70 μg in five pak choi plants, and 172.80, 182.7, 184.5 and 188.1 μg in the remaining soils. The ANOVA analyses showed that the Cd amounts in the BG+SAH treatment were 1.07, 1.51, 1.75 times higher in the *S. alfredii* shoots, 1.12, 1.27, 1.41 times higher in the *S. alfredii* roots, 0.94, 0.92, 0.78 times lower in the pak choi, and 0.95, 0.94, 0.92 times lower in the soils, respectively, than those in the 1DDT+SAH, 5DDT+SAH and 10DDT+SAH treatments. The results indicated that the addition of DDTs decreased the Cd accumulation in *S. alfredii* and pak choi. The amounts of Cd in the BG+SB+SAH, 1DDT+SB+SAH, 5DDT+SB+SAH and 10DDT+SB+SAH treatments were 110.25, 133.25, 192.5 and 234.55 μg in five *S. alfredii* shoots, 8.95, 9.25, 12.00 and 13.65 μg in five *S. alfredii* roots, 3.90, 4.20, 4.65 and 4.95 μg in five pak choi plants, and 153.00, 128.70, 80.10 and 36.90 μg in the remained soils. The ANOVA

Table 3 Cadmium accumulation in *S. alfredii* after treatment for 210 days

Treatment	SAH shoots			SAH roots			Cd accumulation ratio (shoots/roots)
	Dry biomass (g/plant)	Cd concentration (μg/g)	Cd accumulation (μg/plant)	Dry biomass (g/plant)	Cd concentration (μg/g)	Cd accumulation (μg/plant)	
BG+SAH	0.470	41.15 ± 0.70 a*	19.34 ± 0.33 a	0.055	16.88 ± 1.98 a	0.93 ± 0.11 a	20.80
BG+SB+SAH	0.496	44.45 ± 0.95 b	22.05 ± 0.47 b	0.064	28.00 ± 1.04 b	1.79 ± 0.07 b	12.32
1DDT+SAH	0.483	37.35 ± 0.80 a	18.05 ± 0.39 a	0.057	14.57 ± 1.12 a	0.83 ± 0.06 a	21.75
1DDT+SB+SAH	0.532	50.10 ± 1.10 b	26.65 ± 0.59 b	0.061	30.34 ± 1.52 b	1.85 ± 0.09 b	14.41
5DDT+SAH	0.465	27.60 ± 0.50 a	12.85 ± 0.23 a	0.054	13.51 ± 0.58 a	0.73 ± 0.03 a	17.60
5DDT+SB+SAH	0.611	63.05 ± 1.95 b	38.50 ± 1.19 b	0.068	35.30 ± 2.39 b	2.40 ± 0.16 b	16.04
10DDT+SAH	0.461	24.00 ± 1.20 a	11.05 ± 0.55 a	0.052	12.70 ± 0.50 a	0.66 ± 0.03 a	16.74
10DDT+SB+SAH	0.698	67.20 ± 2.90 b	46.91 ± 2.02 b	0.069	39.52 ± 2.53 b	2.73 ± 0.17 b	17.18

* Data followed by a different letter in the same column are significantly different ($p \leqslant 0.05$).

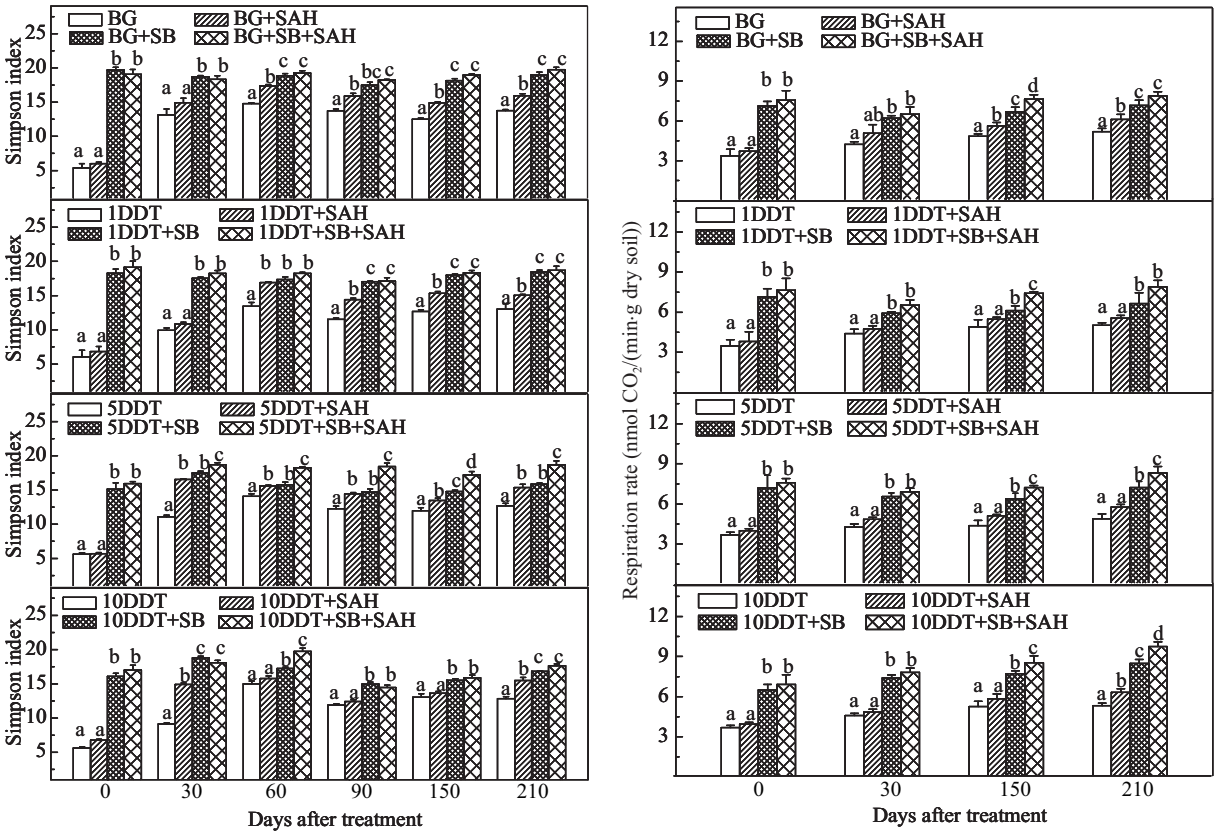

Fig. 3 Variation of Simpson index and soil respiration rate during the bioremediation. The different letters above the columns at each sampling time indicate that the values are significantly different ($p \leqslant 0.05$).

analyses showed that the Cd concentrations in the *S. alfredii* treatments inoculated with strain D-6 were 1.14, 1.48, 3.00, 4.25 times higher in the *S. alfredii* shoots, 1.92, 2.23, 3.29, 4.14 times higher in the *S. alfredii* roots, 1.34, 1.35, 1.48, 1.34 times higher in the pak choi, 0.89, 0.70, 0.43, 0.20 times lower in the soils, respectively, than those in the corresponding uninoculated treatments. The results indicated that the inoculation with strain D-6 increased the Cd accumulation in *S. alfredii* and pak choi. In this study, the ratios of Cd accumulation in the shoots and roots of *S. alfredii* ranged from 12.32 to 21.75 (Table 3), indicating that Cd was primarily accumulated in the shoots of *S. alfredii*.

As shown in Table 4, soil Cd concentrations in the BG+SAH, 1DDT+SAH, 5DDT+SAH and 10DDT+SAH treatments decreased respectively to 65.8%, 70.7%, 70.6% and 71.8% of those in the corresponding treatments without *S. alfredii*, indicating that *S. alfredii* could clearly accumulate Cd in soil. Meanwhile, soil Cd concentrations in the BG+SB+SAH, 1DDT+SB+SAH, 5DDT+SB+SAH and 10DDT+SB+SAH treatments decreased to 58.2%, 49.8%, 30.7% and 14.1%, respectively, of those in the corresponding uninoculated treatments, suggesting that the inoculation of strain D-6 into soil enhanced the accumulation of Cd in *S. alfredii*.

3 Discussion

In the early stage of treatment (60 days), the population size of the DDTs-degrading strain decreased gradually (Fig. 2), suggesting that strain D-6 may have an adaptation process in soil. However, no obvious variation of the Simp-

Table 4 Cadmium accumulation in soil and pak choi after treatment for 90 days

Treatment	Cd accumulation in soil (mg/kg dry soil)	Cd accumulation in pak choi		
		Dry biomass (g/plant)	Cd concentration (μg/g)	Cd accumulation (μg/plant)
BG	0.292 ± 0.027 a	3.16	0.16 ± 0.01 a	0.51 ± 0.03 a
BG+SB	0.285 ± 0.011 a	3.35	0.20 ± 0.03 a	0.67 ± 0.10 a
BG+SAH	0.192 ± 0.021 b	3.23	0.18 ± 0.01 a	0.58 ± 0.03 a
BG+SB+SAH	0.170 ± 0.015 b	3.37	0.23 ± 0.02 a	0.78 ± 0.07 b
1DDT	0.287 ± 0.031 a	3.22	0.15 ± 0.01 a	0.48 ± 0.03 a
1DDT+SB	0.283 ± 0.019 a	3.30	0.22 ± 0.03 ab	0.73 ± 0.10 b
1DDT+SAH	0.203 ± 0.015 b	3.27	0.19 ± 0.02 a	0.62 ± 0.07 b
1DDT+SB+SAH	0.143 ± 0.012 c	3.34	0.25 ± 0.03 b	0.84 ± 0.10 b
5DDT	0.290 ± 0.022 a	3.06	0.18 ± 0.01 a	0.55 ± 0.03 a
5DDT+SB	0.276 ± 0.026 a	3.18	0.22 ± 0.01 a	0.70 ± 0.03 a
5DDT+SAH	0.205 ± 0.018 b	3.14	0.20 ± 0.02 a	0.63 ± 0.06 a
5DDT+SB+SAH	0.089 ± 0.009 c	3.22	0.29 ± 0.05 b	0.93 ± 0.16 b
10DDT	0.291 ± 0.028 a	3.02	0.15 ± 0.02 a	0.45 ± 0.06 a
10DDT+SB	0.270 ± 0.020 a	3.11	0.28 ± 0.03 bc	0.87 ± 0.09 b
10DDT+SAH	0.209 ± 0.023 b	3.08	0.24 ± 0.02 b	0.74 ± 0.06 b
10DDT+SB+SAH	0.041 ± 0.017 c	3.19	0.31 ± 0.04 c	0.99 ± 0.13 b

Data followed by a different letter in the same column are significantly different ($p \leqslant 0.05$).

son index and soil respiration rate were observed in the inoculated treatments, while significant increases were observed in the uninoculated treatments (Fig. 3). This may be attributed to be growth of pak choi and *S. alfredii* and substantial proliferation of their rhizosphere microorganisms. After 90 days of treatment, the DDTs-degrading strain population size gradually increased, ultimately recovering to or exceeding the initial level, and the Simpson index and soil respiration rate increased slightly. This may be because the DDTs-degrading strain adapted to the DDTs due to selective pressure and then proliferated substantially. Throughout the experiments, the DDTs-degrading strain population size in BG+SB+SAH was higher than that in BG+SB, which may be because the *S. alfredii* rhizosphere resulted in higher numbers and activity of the DDTs-degrading strain capable of utilising the root exudates as carbon and energy sources. A similar phenomenon was found by Abou-Shanab et al. (2003), who showed that plant exudates might contribute to the activity of soil microflora. Megharaj et al. (2003) also reported that microbial activity in the rhizosphere of plants was several orders of magnitude greater than that in the bulk soil. Some studies have reported that phenolic root exudates and terpenes may foster the activity of the rhizosphere bacteria that degrade DDTs by inducing enzymes or providing co-metabolic growth substrates (Fletcher and Hegde, 1995).

The increase of DDTs-degrading strain biomass resulted in the accelerated degradation of DDTs (Fig. 1 and Table 1). Similar to this result, genetically modified bacteria with DDTs-degrading abilities, such as *Pseudomonas acidovorans* M3GY, may prove useful in the bioremediation of DDTs (Golovleva et al., 1988). Purnomo et al. (2011b) reported that *Gloeophyllum trabeum* remediated about 64% of the initial DDT in historically contaminated soil and was considered as the most promising brown-rot fungi for use in the bioremediation of DDT-contaminated soil. Zhao and Yi (2010) showed that laccase extract from white rot fungi was an efficient and safe agent for bioremediation of DDT-contaminated soil. Reductive dechlorination of DDT and *meta*-ring cleavage of metabolites in soil by *Ralstonia eutropha* A5, *Alcaligenes* sp. JB1 and *Pseudomonas aeruginosa* 640X were also found (Nadeau et al., 1998). However, a contrasting study was reported by Safferman et al. (1995), who showed that *Phanerochaete sordida* was not successful in remediating DDT-contaminated soil.

Meanwhile, as shown in Table 2, the increase of the DDTs-degrading strain population size obviously decreased the uptake amounts of DDTs by pak choi and *S. alfredii*. This may have resulted from the degradation of most of the DDTs residues by strain D-6. Additionally, the uptake of DDTs residues was more rapid by pak choi than that by *S. alfredii*. This may be attributed to differences between the plant species (Suresh et al., 2005).

In addition, the inoculation and proliferation with the DDTs-degrading strain enhanced the Cd accumulation in *S. alfredii*, furthermore, this accumulation gradually increased with the increasing concentrations of DDTs added to soils. This may be because strain D-6, capable of utilising DDTs as a source of carbon and energy, proliferated substantially and then increased the bioavailability of Cd to *S. alfredii*. Some studies found that soil microorganisms played a key role in altering heavy metal bioavailability (immobilization, solubilization) by releasing specific compounds that form complexes with Cd, by localized acidification of the environment and by changing heavy metal speciation (Chanmugathas and Bollag, 1987; Giller et al., 1998; Megharaj et al., 2003; Wu et al., 2006; Jing et al., 2007). Leyval et al. (1993) reported that inoculation of the bacterium *Agrobacterium* sp. increased the uptake of Cd in the pine rhizosphere. Jing et al. (2007) reported that microbial populations had the potential to enhance phytoremediation processes by affecting heavy metal mobility and availability to the plant through release of chelating agents, acidification, phosphate solubilization and redox changes. Abou-Shanab et al. (2003) showed that *Sphingomonas macrogoltabidus*, *Microbacterium liquefaciens* and *Microbacterium arabinogalactanolyticum* increased Ni accumulation in *Alyssum murale* by 17%, 24% and 32.4%, respectively, compared with the uninoculated controls. In the uninoculated treatments, however,

the Cd accumulation in *S. alfredii* decreased with the increasing concentrations of DDTs added to soils. This may be due to adverse impacts by DDTs on indigenous microorganisms including the rhizosphere microorganisms of *S. alfredii* and pak choi, resulting in a decrease of Cd bioavailability. In this study, Cd was mainly accumulated in the shoots of *S. alfredii*. A similar result was reported by Yang et al. (2004b), who showed that the concentration of Cd in the different parts of *S. alfredii* was highest in the leaves, then in the stems, and lowest in the roots. Long et al. (2009) also reported that the shoot/root ratios of *S. alfredii* for the hyperaccumulation of Cd ranged from 3.36 to 4.43.

In this study, Cd was also accumulated in pak choi. Similar to this result, Sheng and Xia (2006) reported that *Brassica napus* could accumulate Cd from soil. Verma et al. (2007) reported that Cd accumulated in radishes (*Raphanus sativus*), carrots (*Daucos carota*), spinach (*Spinacia oleracea*) and cabbage (*Brassica oleracea*). Moreover, significant differences were observed in the accumulation and distribution of trace elements among plant species and among cultivars within species (Grant et al., 2008). Liu et al. (2010) reported that there was a significant difference on average in the accumulated Cd concentrations under three Cd concentrations treatments (1.0, 2.5 and 5.0 mg/kg) with concentrations of 0.88, 4.45 and 7.76 mg/kg, respectively, among 40 Chinese cabbage cultivars.

The results obtained in this study indicate that strain D-6 accelerated the degradation of DDTs and that *S. alfredii* accumulated Cd from the soil. In this study, the remediation potential of strain D-6 associated with *S. alfredii* was higher than that in their single remediation treatment. Therefore, a synergistic bioremediation potential was observed between strain D-6 and *S. alfredii*. Synchronous use of microbial remediation and phytoremediation may be a promising method to remove DDTs and accumulate Cd. However, soil is a complex environment containing mixed populations of microorganisms with synergistic and antagonistic activities. Therefore, the efficiency of the bioaugmentation in soil was found to be largely dependent on the inoculum density, survival, competitiveness, colonisation, and physical diffusion capacity of the introduced strain D-6 (Purnomo et al., 2011a). In view of this, multiple inoculations are likely to be required in a field study (Morgan and Watkinson, 1989). Meanwhile, the efficiency of phytoremediation depends on the physiological and biochemical characteristics of the hyperaccumulators, such as the growth rate, biomass, ease of harvest, rhizosphere environment, and the tolerance and accumulation of a range of heavy metals in the shoots (Yang et al., 2004b). Additionally, soil traits (pH, aeration, salinity, cation exchange capacity, organic matter content, clay content, and redox potential) and environmental conditions (temperature, humidity and dissolved oxygen) altering the bioavailability of DDTs also played a key role in the bioremediation process (Baczynski et al., 2010). Thus, understanding how these factors affect the biodegradation of DDTs permits the rational design of treatments and amendments to enhance bioremediation *in situ* or *ex situ* (Foght et al., 2001). Wenzel (2009) reported that limited bioavailability of a pollutant may be overcome by the design of plant-microbial consortia that are capable of mobilising metals by modification of the rhizosphere pH and ligand exudation, or enhancing the bioavailability of organic pollutants by the release of biosurfactants. In summary, the successful remediation of pesticides in combination with heavy metal pollution may need a complex set of environmental conditions in addition to the survival and substantial proliferation of the degrading strain and the fast growth of the hyperaccumulators.

4 Conclusions

The combined remediation of strain D-6 and *S. alfredii* significantly accelerated the degradation of DDTs and enhanced the accumulation of Cd in *S. alfredii*. During the remediation process, the DDTs-degrading strain population decreased during the early stage of inoculation and, thereafter, gradually increased, recovering to or exceeding the initial inoculation level. A similar trend was also observed for the Simpson index ($1/D$) and the soil respiratory rate in all treatments. The results indicate that strain D-6 and *S. alfredii* can be used synchronously and successfully for the removal or detoxification of DDTs residues and the hyperaccumulation of Cd in *S. alfredii*.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 20907040, 20877068), the National High Technology Research and Development Program (863) of China (No. 2009AA06Z316), the Public Science and Technology Research Funds Projects of the Ministry of Environmental Protection of China (No. 201109018), the Major State Basic Research Development Program of China (No. 2009CB119000), the Scientific Research Fund of Zhejiang Provincial Education Department of China (No. Y200908848).

References

Abou-Shanab R A, Angle J S, Delorme T A, Chaney R L, van Berkum P, Moawad H et al., 2003. Rhizobacterial effects on nickel extraction from soil and uptake by *Alyssum murale*. *New Phytologist*, 158(1): 219–224.

Baczynski T P, Pleissner D, Grotenhuis T, 2010. Anaerobic biodegradation of organochlorine pesticides in contaminated soil-significance of temperature and availability. *Chemosphere*, 78(1): 22–28.

Boberg J, Finlay R D, Stenlid J, Näsholm T, Lindahl B D, 2008. Glucose and ammonium additions affect needle decomposition and carbon allocation by the litter degrading fungus *Mycena epipterygia*. *Soil Biology & Biochemistry*, 40(4): 995–999.

Chanmugathas P, Bollag J M, 1987. Microbial role in immobilization and subsequent mobilization of cadmium in soil suspensions. *Soil Science Society of America Journal*, 51(5): 1184–1191.

Fang H, Dong B, Yan H, Tang F F, Yu Y L, 2010. Characterization of a bacterial strain capable of degrading DDT congeners and its use in bioremediation of contaminated soil. *Journal of Hazardous Materials*, 184(1-3): 281–289.

Fang H, Yu Y L, Wang X G, Chu X Q, Yang X E, 2009. Persistence of the herbicide butachlor in soil after repeated applications and its effects on soil microbial functional diversity. *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes*, 44(2): 123–129.

Fletcher J S, Hegde R S, 1995. Release of phenols by perennial plant roots and their potential importance in bioremediation. *Chemosphere*, 31(4): 3009–3016.

Foght J, April T, Biggar K, Aislabie J, 2001. Bioremediation of DDT-contaminated soils: a review. *Bioremediation Journal*, 5(3): 225–246.

Gaw S K, Kim N D, Northcott G L, Wilkins A L, Robinson G, 2008. Uptake of ΣDDT, arsenic, cadmium, copper, and lead by lettuce and radish grown in contaminated horticultural soils. *Journal of Agricultural and Food Chemistry*, 56(15): 6584–6593.

Gaw S K, Wilkins A L, Kim N D, Palmer G T, Robinson P, 2006. Trace element and ∑DDT concentrations in horticultural soils from the Tasman, Waikato and Auckland regions of New Zealand. *Science of the Total Environment*, 355(1-3): 31–47.

Giller K E, Witter E, Mcgrath S P, 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. *Soil Biology & Biochemistry*, 30(10-11): 1389–1414.

Golovleva L A, Pertsova R N, Boronin A M, Travkin V N, Kozlovsky S A, 1988. Kelthane degradation by genetically engineered *Pseudomonas aeruginosa* BS827 in a soil ecosystem. *Applied and Environmental Microbiology*, 54(6): 1587–1590.

Gomez E, Ferreras L, Toresani S, 2006. Soil bacterial functional diversity as influenced by organic amendment application. *Bioresource Technology*, 97(13): 1484–1489.

Grant C A, Clarke J M, Duguid S, Chaney R L, 2008. Selection and breeding of plant cultivars to minimize cadmium accumulation. *Science of the Total Environment*, 390(2-3): 301–310.

Hu W Y, Lu Y L, Wang T Y, Luo W, Shi Y J, Giesy J P et al., 2010. Spatial variability and temporal trends of HCH and DDT in soils around Beijing Guanting Reservoir, China. *Environmental Geochemistry and Health*, 32(5): 441–449.

Hu X F, Wu H X, Hu X, Fang S Q, Wu C J, 2004. Impact of urbanization on Shanghai's soil environmental quality. *Pedosphere*, 14(2): 151–158.

Huang H G, Yu N, Wang L J, Gupta D K, He Z L, Wang K et al., 2011. The phytoremediation potential of bioenergy crop *Ricinus communis* for DDTs and cadmium co-contaminated soil. *Bioresource Technology*, 102(23): 11034–11038.

Jing Y D, He Z L, Yang X E, 2007. Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. *Journal of Zhejiang University Science*, B8(3): 192–207.

Krämer U, 2005. Phytoremediation: novel approaches to cleaning up polluted soils. *Current Opinion in Biotechnology*, 16(2): 133–141.

Leyval C, Surtiningsih T, Berthelin J, 1993. Mobilization of P and Cd from rock phosphates by rhizospheric microorganisms (Phosphate-dissolving bacteria and ectomycorrhizal fungi). *Phosphorus Sulfur and Silicon and the Related Elements*, 77(1-4): 133–136.

Li J, Zhang G, Qi S H, Li X D, Peng X Z, 2006. Concentrations, enantiomeric compositions, and sources of HCH, DDT and chlordane in soils from the Pearl River Delta, South China. *Science of the Total Environment*, 372(1): 215–224.

Li J H, Sun Y Y, Yin Y, Ji R, Wu J C, Wang X R et al., 2010. 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. *Journal of Hazardous Materials*, 181(1-3): 673–678.

Liu W T, Zhou Q X, An J, Sun Y B, Liu R, 2010. Variations in cadmium accumulation among Chinese cabbage cultivars and screening for Cd-safe cultivars. *Journal of Hazardous Materials*, 173(1-3): 737–743.

Long X X, Zhang Y G, Jun D, Zhou Q X, 2009. Zinc, cadmium and lead accumulation and characteristics of rhizosphere microbial population associated with hyperaccumulator *Sedum alfredii* Hance under natural conditions. *Bulletin of Environmental Contamination and Toxicology*, 82(4): 460–467.

Luo S L, Wan Y, Xiao X, Guo H J, Chen L, Xi Q et al., 2011. Isolation and characterization of endophytic bacterium LRE07 from cadmium hyperaccumulator *Solanum nigrum* L. and its potential for remediation. *Applied Microbiology and Biotechnology*, 89(5): 1637–1644.

Megharaj K V M, Sethunathan N, Naidu R, 2003. Bioavailability and toxicity of cadmium to microorganisms and their activities in soil: a review. *Advances in Environmental Research*, 8(1): 121–135.

Morgan P, Watkinson R J, 1989. Microbiological methods for the cleanup of soil and ground water contaminated with halogenated organic compounds. *FEMS Microbiology Reviews*, 5(4): 277–299.

Nadeau L J, Sayler G S, Spain J C, 1998. Oxidation of 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) by *Alcaligenes eutrophus* A5. *Archives of Microbiology*, 171(1): 44–49.

Nanjing Institute of Soil Science, Chinese Academy of Sciences, 1985. Research Methods of Soil Microorganisms. Science Press, Beijing.

Purnomo A S, Kamei I, Kondo R, 2008. Degradation of 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) by brown-rot fungi. *Journal of Bioscience and Bioengineering*, 105(6): 614–621.

Purnomo A S, Mori T, Kamei I, Kondo R, 2011a. Basic studies and applications on bioremediation of DDT: A review. *International Biodeterioration & Biodegradation*, 65(7): 921–930.

Purnomo A S, Mori T, Takagi K, Kondo R, 2011b. Bioremediation of DDT contaminated soil using brown-rot fungi. *International Biodeterioration & Biodegradation*, 65(5): 691–695.

Rignell-Hydbom A, Skerfving S, Lundh T, Lindh C H, Elmståhl S, Bjellerup P et al., 2009. Exposure to cadmium and persistent organochlorine pollutants and its association with bone mineral density and markers of bone metabolism on postmenopausal women. *Environmental Research*, 109(8): 991–996.

Safferman S I, Lamar R T, Vonderhaar S, Neogy R, Haught R C, Krishnan E R, 1995. Treatability study using *Phanerochaete sordida* for the bioremediation of DDT contaminated soil. *Toxicological & Environmental Chemistry*, 50(1-4): 237–251.

Sheng X F, Xia J J, 2006. Improvement of rape (*Brassica napus*)

plant growth and cadmium uptake by cadmium-resistant bacteria. *Chemosphere*, 64(6): 1036–1042.

Sun L N, Zhang Y H, Sun T H, Gong Z Q, Lin X, Li H B, 2006. Temporal-spatial distribution and variability of cadmium contamination in soils in Shenyang Zhangshi irrigation area, China. *Journal of Environmental Sciences*, 18(6): 1241–1246.

Suresh B, Sherkhane P D, Kale S, Eapen S, Ravishankar G A, 2005. Uptake and degradation of DDT by hairy root cultures of *Cichorium intybus* and *Brassica juncea*. *Chemosphere*, 61(9): 1288–1292.

Tao S, Liu W X, Li Y, Yang Y, Zuo Q, Li B G et al., 2008. Organochlorine pesticides contaminated surface soil as reemission source in the Haihe Plain, China. *Environmental Science and Technology*, 42(22): 8395–8400.

Verma P, George K V, Singh H V, Singh R N, 2007. Modeling cadmium accumulation in radish, carrot, spinach and cabbage. *Applied Mathematical Modelling*, 31(8): 1652–1661.

Wenzel W W, 2009. Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. *Plant and Soil*, 321(1-2): 385–408.

Wu G H, Cao S S, 2010. Mercury and cadmium contamination of irrigation water, sediment, soil and shallow groundwater in a wastewater-irrigated field in Tianjin, China. *Bulletin of Environmental Contamination and Toxicology*, 84(3): 336–341.

Wu S C, Luo Y M, Cheung K C, Wong M H, 2006. Influence of bacteria on Pb and Zn speciation, mobility and bioavailability in soil: a laboratory study. *Environmental Pollution*, 144(3): 765–773.

Yang X E, Long X X, Ye H B, He Z L, Calvert D V, Stoffella P J, 2004a. Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (*Sedum alfredii* Hance). *Plant and Soil*, 259(1-2): 181–189.

Yang X E, Ye H B, Long X X, He B, He Z L, Stoffella P J et al., 2004b. Uptake and accumulation of cadmium and zinc by *Sedum Alfredii* Hance at different Cd/Zn supply levels. *Journal of Plant Nutrition*, 27(11): 1963–1977.

Yang X L, Wang S S, Bian Y R, Chen F, Yu G F, Gu C G et al., 2008. Dicofol application resulted in high DDTs residue in cotton fields from northern Jiangsu Province, China. *Journal of Hazardous Materials*, 150(1): 92–98.

Zhang H, Dang Z, Zheng L C, Yi X Y, 2009. Remediation of soil co-contaminated with pyrene and cadmium by growing maize (*Zea mays* L.). *International Journal of Environmental Science and Technology*, 6(2): 249–258.

Zhang H H, Chen J J, Zhu L, Li F B, Wu Z F, Yu W M et al., 2011. Spatial patterns and variation of soil cadmium in Guangdong Province, China. *Journal of Geochemical Exploration*, 109(1-3): 86–91.

Zhang M K, Ke Z X, 2004. Heavy metals, phosphorus and some other elements in urban soils of Hangzhou City, China. *Pedosphere*, 14(2): 177–185.

Zhao Y C, Yi X Y, 2010. Effects of soil oxygen conditions and soil pH on remediation of DDT-contaminated soil by laccase from white rot fungi. *International Journal of Environmental Research and Public Health*, 7(4): 1612–1621.

Zhou Q X, 2003. Interaction between heavy metals and nitrogen fertilizers applied to soil-vegetable systems. *Bulletin of Environmental Contamination and Toxicology*, 71(2): 338–344.

JOURNAL OF ENVIRONMENTAL SCIENCES

Journal of Environmental Sciences (Established in 1989)
Vol. 24 No. 6 2012

Supervised by	Chinese Academy of Sciences	**Published by**	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental		Elsevier Limited, The Netherlands
	Sciences, Chinese Academy of Sciences	**Distributed by**	
Edited by	Editorial Office of Journal of	Domestic	Science Press, 16 Donghuangchenggen
	Environmental Sciences (JES)		North Street, Beijing 100717, China
	P. O. Box 2871, Beijing 100085, China		Local Post Offices through China
	Tel: 86-10-62920553; http://www.jesc.ac.cn	Foreign	Elsevier Limited
	E-mail: jesc@263.net, jesc@rcees.ac.cn		http://www.elsevier.com/locate/jes
Editor-in-chief	Hongxiao Tang	**Printed by**	Beijing Beilin Printing House, 100083, China

CN 11-2629/X **Domestic postcode: 2-580** **Domestic price per issue RMB ¥ 110.00**

ISSN 1001-0742