Pedosphere **30**(4): 563–569, 2020
doi:10.1016/S1002-0160(17)60476-4
ISSN 1002-0160/CN 32-1315/P
© 2020 Soil Science Society of China
Published by Elsevier B.V. and Science Press

PEDOSPHERE

www.elsevier.com/locate/pedosphere

Microbial remediation of a pentachloronitrobenzene-contaminated soil under *Panax notoginseng*: A field experiment

Ning ZHANG[1,2], Dong GUO[3], Ye ZHU[2], Xiaomi WANG[2], Lingjia ZHU[2], Fang LIU[1], Ying TENG[2,*], Peter CHRISTIE[2], Zhengao LI[2] and Yongming LUO[2,4]

[1] *College of Resource and Environmental Engineering, Guizhou University, Guiyang 550025 (China)*
[2] *Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008 (China)*
[3] *Infinitus (China) Co., LTD, Jiangmen 529156 (China)*
[4] *Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003 (China)*

(Received September 8, 2016; revised December 9, 2016)

ABSTRACT

Pentachloronitrobenzene (PCNB) is an organochlorine fungicide that is mainly used in the prevention and control of diseases in crop seedlings. Microbial removal is used as a promising method for *in-situ* removal of many organic pesticides and pesticide residues. A short-term field experiment (1 year) was conducted to explore the potential role of a PCNB-degrading bacterial isolate, *Cupriavidus* sp. YNS-85, in the remediation of a PCNB-contaminated soil on which *Panax notoginseng* was grown. The following three treatments were used: i) control soil amended with wheat bran but without YNS-85, ii) soil with 0.15 kg m^{-2} of solid bacterial inoculum (A), and iii) soil with 0.30 kg m^{-2} of solid bacterial inoculum (B). The removal of soil PCNB during the microbial remediation was monitored using gas chromatography. Soil catalase and fluorescein diacetate (FDA) esterase activities were determined using spectrophotometry. In addition, cultivable bacteria, fungi, and actinomycetes were counted by plating serial dilutions, and the microbial biodiversity of the soil was analyzed using BIOLOG. After 1 year of *in-situ* remediation, the soil PCNB concentrations decreased significantly by 50.3% and 74.2% in treatments A and B, respectively, when compared with the uninoculated control. The soil catalase activity decreased in the presence of the bacterial isolate, the FDA esterase activity decreased in treatment A, but increased in treatment B. No significant changes in plant biomass, diversity of the soil microbial community, or physicochemical properties of the soil were observed between the control and inoculated groups ($P < 0.05$). The results indicate that *Cupriavidus* sp. YNS-85 is a potential candidate for the remediation of PCNB-contaminated soils under *P. notoginseng*.

Key Words: *Cupriavidus* sp. YNS-85, enzyme activities, *in-situ* remediation, PCNB removal, soil

Citation: Zhang N, Guo D, Zhu Y, Wang X M, Zhu L J, Liu F, Teng Y, Christie P, Li Z G, Luo Y M. 2020. Microbial remediation of a pentachloronitrobenzene-contaminated soil under *Panax notoginseng*: A field experiment. *Pedosphere*. **30**(4): 563–569.

INTRODUCTION

Pentachloronitrobenzene (PCNB) is an organochlorine fungicide that is mainly used in the prevention and control of diseases in crop seedlings. Pentachloronitrobenzene is persistent in soils (half-life 5–10 months) and can be biologically accumulated and magnified through the food chain, resulting in a significant risk to human health due to its carcinogenic, teratogenic, and mutagenic potential (Shin *et al*., 2003; Wen *et al*., 2010; Tas and Pavlostathis, 2014). The value of the lethal concentration (LC50) of PCNB is 0.55 mg L^{-1} in rainbow trout and 0.1 mg L^{-1} in crapet arlequin (U.S. National Library of Medicine, 1995). Extensive use has made PCNB contamination a global environmental problem (Wang *et al*., 2015). Pentachloronitrobenzene has been used extensively as a soil fungicide, especially for the prevention of soil-borne diseases in Chinese farmlands where Chinese notoginseng (*Panax notoginseng*) is grown (Li *et al*., 2009).

Chinese notoginseng is produced mainly in Wenshan, Yunnan Province, southern China, and it is traditionally valued for its protective action against cerebral ischemia, beneficial effects on the cardiovascular system, and hemostatic action in traditional Chinese medicine (Ng, 2006). Although the use of PCNB is prohibited in many countries, its biological accumulation, persistence, and low biodegradability have led to substantial accumulation of PCNB in plants, soils and water in some ecosystems (Arora *et al*., 2012). In China, quality and technical supervision rules for the ginseng product-quality standard have set the limit for ginseng PCNB as 0.1 mg kg^{-1}. A previous study detected PCNB concentrations in *P. notoginseng* of up to hundreds of μg kg^{-1} (Leung *et al*., 2005). Therefore, there is considerable interest in the control or prevention of pesticide pollution in soils used to grow these medicinal plants.

Bioremediation is an emerging strategy in which plants and bacteria are used for the rehabilitation of polluted sites. Although bioaccumulation of PCNB has been noted in alfalfa (Li and Yang, 2013), phytoremediation is often

*Corresponding author. E-mail: yteng@issas.ac.cn.

regarded as slow and incomplete, and it may be impeded by the low bioavailability of PCNB (Teng *et al.*, 2015). In addition, the environmental fate, and even the bioremediation process, is influenced by certain abiotic and biotic effects (Gorontzy *et al.*, 1994). Any bioaugmentation strategies require a deep understanding of the ecology of contaminant degradation because each contaminated site is unique with regard to potential hazards for ecosystems and human health (Vogt and Richnow, 2013). The introduction of indigenous microorganisms in bioremediation might address some of the weaknesses of this technique. The diverse metabolic capacities of microbes make them valuable tools for the restoration of contaminated sites. However, the microbial transformation of PCNB is mainly focused on fungi (*e.g.*, *Fusarium* spp., *Sporothrix cyanescens, Mucor racemosus*, and white rot fungi) and actinomycetes (*e.g.*, *Streptomyces aureofaciens*) (Lièvremont *et al.*, 1998; Arora *et al.*, 2012). Only a few bacterial species, including a *Labrys* strain, capable of PCNB degradation, have been found (Li *et al.*, 2011), and the degradation capacity of both fungal and bacterial strains has been confirmed in only laboratory studies (Liu *et al.*, 2011; Li and Yang, 2013). Little information is available about whether they would be effective in PCNB degradation under field conditions. Soil enzyme activities and microbial biodiversity have also not been explored during the biodegradation of PCNB in the field. It is necessary to select suitable candidate organisms for the removal of PCNB (Lièvremont *et al.*, 1998), particularly in field studies because laboratory conditions cannot simulate the more complicated conditions in the field.

A PCNB-degrading bacterium, *Cupriavidus* sp. YNS-85, was isolated in our laboratory. The main goal of the present study was to determine whether this isolate could degrade PCNB in soil under *P. notoginseng*. Moreover, the activity and biodiversity profiles of soil enzymes were assayed after 1 year of *in-situ* bioremediation. Soil physicochemical properties and plant biomass were also evaluated during the biodegradation of PCNB. The results of this study should contribute to our understanding of the bioremediation potential of the bacterial isolate in PCNB-contaminated fields cultivated with *P. notoginseng*.

MATERIALS AND METHODS

Site description and soil properties

The *P. notoginseng* test area is located in the southeast of Yunnan Province, South China, and our field site is located at 23°52′83″ N, 104°32′40″ E. Each experimental plot was 10 m^2 (2 m × 5 m), with a mound height of 30 cm; the study was conducted from December 2014 to December 2015.

The test soil type at the field site is a ferruginous soil according to the World Reference Base for Soil Resources system. The average PCNB concentration and physicochemical properties of the experimental soil were determined using standard methods (Li *et al.*, 2011) and were as follows: PCNB 3.95 mg kg^{-1}, pH 6.9, total organic matter 27.3 g kg^{-1}, cation exchange capacity (CEC) 18.93 cmol kg^{-1}, total N 0.79 g kg^{-1}, total P 0.3 g kg^{-1}, total K 4.37 g kg^{-1}, hydrolyzable N 95 mg kg^{-1}, exchangeable P 0.7 mg kg^{-1}, and exchangeable K 100 mg kg^{-1}.

Experimental design and sampling

Solid bacterial inoculum was prepared by mixing a liquid suspension (optical density at 600 nm, 1.0) of *Cupriavidus* sp. YNS-85 with dry wheat bran at a mass ratio of 2:3. The control soil received wheat bran without the bacterial suspension. Two experimental treatments, named A and B, received 0.15 and 0.30 kg m^{-2} (about 8×10^{11} and 16×10^{11} colony-forming units (CFU) kg^{-1}) of the soild bacterial inoculum agent, respectively. Full-grown seeds that were 6–8 cm length and 1 cm in diameter were transplanted at the test site, with each plant occupying an area of 5 cm × 6 cm. The collection of a soil sample for each treatment was replicated five times. The surface soil (5–15 cm) was collected using the five-point sampling method and mixed thoroughly. The fresh soil samples were stored at −80 °C before the test analyses. In addition, 10 plants were collected randomly from each treatment field. Whole plants containing the taproot and fibrous roots were rinsed with deionized water to remove the soil residue. After blow-drying, the fresh weight, height, and root length of the plants were determined immediately. The dry weight was then measured after oven drying at 65 °C.

Analysis of PCNB in soil

The soil samples were freeze-dried and sieved through a 0.25-mm mesh. Extraction of PCNB was performed according the methods described by Sun *et al.* (2007) and Wang *et al.* (2015). Five grams of soil was weighed into a 60-mL glass centrifugal tube with 20 mL of a methylene chloride/hexane solution (volume:volume, 1:1) and soaked overnight. The solution was ultrasonically extracted for 30 min and then centrifuged at 2 500 r min^{-1} for 3 min. Extraction and centrifugation were repeated twice. Three samples of the collected supernatant were mixed thoroughly and then evaporated to 2 mL. The prepared solution was passed through a compound silica gel column composed of silica gel, neutral alumina, and anhydrous sodium sulfate (weight:weight:weight, 2:2:1), leached using 30 mL of a methylene chloride/hexane mixed solvent (volume:volume, 1:1), and concentrated to 1 mL with a rotary evaporator. After adding 5 mL of chromatographically pure n-hexane, the solution was concentrated to near dryness and then diluted to 1 mL with n-hexane for gas chromatography analysis.

In the gas chromatography analysis, the prepared sample solutions were determined using a Model 7890 gas chromato-

graph (Agilent Technologies, Santa Clara, USA) equipped with an electron capture detector and a chromatographic column (DB-35 MS). The general operating conditions were as follows: sample injection volume 1.0 μL, flow rate of carrier gas (N_2) 1.0 mL min^{-1}, injector temperature 260 °C, and detector temperature 310 °C. The column temperature was controlled at 80 °C initially for 0.5 min, increased to 210 °C at a rate of 40 °C min^{-1} and held for 0.5 min, increased to 230 °C at a rate of 6 °C min^{-1} and held for 1 min, and finally increased to 280 °C at a rate of 40 °C min^{-1} and held for 0.5 min.

Quality control comprising blanks, parallel spiked-matrix samples, and PCNB recoveries was set up during sample analysis. The average recovery of the PCNB standard sample (10 μg g^{-1}) was 92.0%. The relative standard deviation of this method was 1.7%, and the limit of detection was 1.3–4.7 μg kg^{-1}. The PCNB standard (purity 98.1%) was obtained from Dr. Ehrenstorfer GmbH, Augsburg, Germany. Qualitative and quantitative analyses were performed on the basis of the retention time and peak area of the target, respectively.

Soil enzyme assays

Soil catalase activity was assayed according to the method of Rodríguez-Kábana and Truelove (1982). Subsamples (5 g) of the air-dried soil were sieved (1 mm), placed in a 150-mL conical flask with 40 mL of distilled water and 5 mL of 0.3% hydrogen peroxide, and shaken at 120 r min^{-1} for 30 min. A 5 mL aliquot of 1.5 mol L^{-1} vitriol was added to stop the reaction, and the solution was then filtered. Twenty-five milliliters of the supernatant were titrated with 0.002 mol L^{-1} potassium permanganate. The volume of potassium permanganate used to evaluate the catalase activity was measured. Blanks without soil samples were included. The soil fluorescein diacetate (FDA) hydrolytic activity was assayed according to Tu *et al.* (2011). Sieved 2-mm soil (5 g) was placed in a 50-mL conical flask with 15 mL of 60 mmol L^{-1} potassium dihydrogen phosphate buffer (pH 7.6) and 0.2 mL of FDA stock solution. The mixed suspension was incubated at 30 °C and shaken at 200 r min^{-1} for 20 min. Fifteen milliliters of dichloromethane/methanol (1:1 volume:volume) were added and thoroughly mixed to stop the reaction. The suspension was then transferred to a 50-mL centrifuge tube, centrifuged at 2 000 r min^{-1} for 3 min, and filtered. The absorbance of the supernatant was measured at 490 nm by using a spectrophotometer. The FDA esterase activity was evaluated using calibration standard graphs.

Cultivable microbial counts and community structure

The counts of cultivable bacteria, fungi, and actinomycetes were conducted using the spread plate method. Ten grams of soil (after conversion by moisture content) were added into 100 mL of sterile water and shaken at 180 r min^{-1} for 15 min. Ten-fold serial dilutions were made, and the suspensions of bacteria, fungi, and actinomycetes were finally diluted to 10^{-4}, 10^{-1}, and 10^{-3}, respectively. Each dilution was repeated three times. The bacteria were quantified on yeast-peptone-glucose agar and incubated at 37 °C. The fungi and actinomycetes were quantified on potato-dextrose and Gause agar, respectively, and incubated at 30 °C. The microbial counts were expressed as average CFU g^{-1} dry soil on an oven-dry basis.

The BIOLOG community level physiological profiles of the different soil microbial communities were determined using BIOLOG ECO plates (BIOLOG Inc., Hayward, USA) (Garau *et al.*, 2012; Liu *et al.*, 2012). Briefly, 10 g of fresh soil was added to 100 mL of distilled water and shaken at 250 r min^{-1} for 10 min. Ten-fold serial dilutions were performed, and 150 μL of the final 10^{-3} dilution was inoculated into the BIOLOG ECO plates for incubation at 25 °C for 7 d. The color development in each well was measured at 590 nm by using a microplate reader at regular 12-h intervals.

Statistical analysis

All data in triplicate were processed using Microsoft Excel 2007 and are expressed as mean ± standard deviation values. All statistical analyses were performed using the SPSS 17.0 software package. The chemical and enzymatic data were analyzed with one-way analysis of variance, and pairs of mean values were compared using Duncan's multiple range test at 5% significance level.

RESULTS AND DISCUSSION

PCNB removal

The soil contents of PCNB 1 year after the bioaugmentation treatments are presented in Fig. 1. The mean PCNB contents in the control and treatments A and B after the application of solid bacterial inoculum were 2.607 ± 0.227,

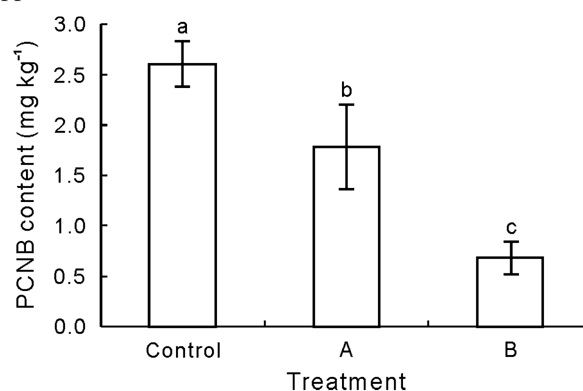

Fig. 1 Removal of pentachloronitrobenzene (PCNB) as affected by *Cupriavidus* sp. YNS-85. Control = soil amended with wheat bran but not *Cupriavidus* sp. YNS-85; A = soil with 0.15 kg m^{-2} of solid bacterial inoculum of YNS-85; B = soil with 0.30 kg m^{-2} of solid bacterial inoculum of YNS-85. Values are means ± standard deviations (n = 5). Bars with different lowercase letters are significantly different (P < 0.05).

1.778 ± 0.420, and 0.682 ± 0.162 mg kg^{-1}, respectively. Treatments A and B, in which 31.8% and 76.7% of PCNB was degraded, showed significantly lower PCNB contents than the control ($P < 0.05$). This indicates that the application of solid bacterial inoculum significantly enhanced the removal of PCNB, and with larger amount of inoculum, more PCNB was removed. The PCNB content in the control was significantly lower ($P < 0.05$) than the initial content in the soil (4.167 ± 0.43 mg kg^{-1}). This loss may be attributed to natural attenuation (Bento *et al*., 2005; Huang *et al*., 2007) by biodegradation, sorption, volatilization, and transformation (Gomes *et al*., 2013).

Cupriavidus sp. strain YNS-85 was isolated from a long-term PCNB-polluted field cultivated with *P. notoginseng*. This isolate has been confirmed to be able to degrade PCNB under laboratory conditions. The biodegradation efficiency was 79.4% within 7 d in solution, and 37.8% PCNB was removed from the soil 30 d after inoculation. The results of the field experiment also indicate a substantial degradation effect by the bacterium. In the laboratory experiment, metabolism of PCNB by *Cupriavidus* sp. strain YNS-85 was analyzed, and pentachloroaniline (PCA) was found to be one of the metabolites. In addition, the isolate enhanced the accumulation of its intermediate PCA, together with the removal of PCNB from spiked soil (data unpublished). However, 201 intermediates of pollutants are likely to possess higher toxicity than their parent compounds (Tas and Pavlostathis, 2014). A previous study has shown that the toxicity of PCA is lower than that of PCNB (Torres *et al*., 1996). The metabolism of PCNB by the *Cupriavidus* strain in the field experiment may have been similar to that in the laboratory, but this requires further study.

Microbial degradation has been proposed as a strategy to enhance the remediation of soils contaminated with organics (Juhasz and Naidu, 2000). Bacterial remediation of PCNB has also been demonstrated in other studies. *Nocardioides* sp. PD653 was found to require additional nutritional factors to multiply (Takagi *et al*., 2009), while *Alcaligenes* sp. PCNB-2, *Labrys portucalensis* PCNB-21, and *Arthrobacter nicotianae* DH19 used PCNB as the sole carbon and energy source, with a degradation rate of 83%–90% (Shin *et al*., 2003; Li *et al*., 2011; Wang *et al*., 2015). Furthermore, Li *et al*. (2011) found that the degradation of PCNB by *L. portucalensis* PCNB-21 in sterile soils was more rapid than that in non-sterile soils, indicating that the remediation efficiency of microbial agents might be constrained by the indigenous soil microflora. Some studies have shown that determination of ferrous ion concentration in soil can also look for microbial effects on PCNB degradation (Hakala *et al*., 2007). The indigenous bacteria *Klebsiella* spp. have a better effect than the non-indigenous *Pseudomonas* spp. on the repair of tributyltin (Abubakar *et al*., 2016), which shows the advantage of indigenous microbial remediation.

Soil physicochemical properties and growth of P. notoginseng

Differences in soil physicochemical properties among the three treatments are shown in Table I. Numerous differences in soil properties were observed between the control and initial values, and this is consistent with the results of Chen and Xu (2005) and Zeng *et al*. (2009). The total and available N contents were slightly higher at the higher amount of the inoculum (0.30 kg m^{-2}). Previous studies have found that microbes can change the chemical properties of the soil (Yu *et al*., 2011). In our study, total P and available K contents were a little higher in the inoculated plots than in the control plot, and organic matter, alkali-hydrolyzable N, and available K contents increased with the higher amount of the inoculum. However, these differences are not significant.

Ten plants were collected randomly from each treatment plot to determine whether supplementation with the bacterial isolate would hinder the growth of *P. notoginseng*. The results (Table II) show that the survival rate of the plants in treatment A was higher (69%) than that in treatment B (38%), indicating that a certain amount of solid inoculum had the potential to promote the survival of *P. notoginseng*, but an excess had the opposite effect. *Cupriavidus* sp. has been demonstrated to promote plant growth (Pereira *et al*., 2015). However, previous studies have shown that some plant species can accumulate organic pollutants (Tu *et al*., 2011). The survival rate of *Cupriavidus* sp. in treatment B was lower, and the PCNB content was lower. This indicates

TABLE I

Soil physicochemical properties under different treatments after 1 year of the field experiment

Treatment[a]	Organic matter	Total			Hydrolyzable N	Exchangeable		CEC[b]
		N	P	K		P	K	
	g kg^{-1} dry soil				mg kg^{-1} dry soil			cmol kg^{-1}
Control	10.77 ± 1.0[c]	0.66 ± 0.04	0.63 ± 0.14	21.28 ± 1.03	42.63 ± 9.93	22.07 ± 6.16	226 ± 46.99	11.1 ± 0.86
A	10.24 ± 0.8	0.55 ± 0.05	0.91 ± 0.13	21.72 ± 1.20	43.37 ± 5.45	22.67 ± 5.94	280 ± 50.47	10.8 ± 0.65
B	12.18 ± 1.6	0.69 ± 0.03	0.70 ± 0.08	20.46 ± 0.19	49.25 ± 3.68	22.66 ± 2.67	285 ± 40.18	11.5 ± 0.40

[a] Control = soil amended with wheat bran but without *Cupriavidus* sp. YNS-85; A = soil with 0.15 kg m^{-2} of solid bacterial inoculum of YNS-85; B = soil with 0.30 kg m^{-2} of solid bacterial inoculum of YNS-85.
[b] Cation exchange capacity.
[c] Means ± standard deviations (n = 3).

TABLE II

Bioindicators of growth of *Panax notoginseng* under different treatments after 1 year of the field experiment

Treatment[a]	Survival rate	Fibrous root length	Spore length	Root length	Root diameter	Height	Fresh weight	Dry weight
		cm					g plant^{-1}	
Control	57%	8.3 ± 6.1[b]	2.7 ± 0.6	3.4 ± 0.6	2.1 ± 0.3	26.9 ± 8.6	26.6 ± 10.0	7.3 ± 2.5
A	69%	9.5 ± 6.6	2.5 ± 0.6	3.3 ± 1.3	2.0 ± 0.3	24.6 ± 8.6	20.1 ± 8.5	6.1 ± 2.3
B	38%	9.6 ± 6.0	3.0 ± 0.8	2.8 ± 1.2	2.1 ± 0.3	25.2 ± 6.5	17.0 ± 8.6	5.5 ± 2.2

[a] Control = soil amended with wheat bran but without *Cupriavidus* sp. YNS-85; A = soil with 0.15 kg m^{-2} of solid bacterial inoculum of YNS-85; B = soil with 0.30 kg m^{-2} of solid bacterial inoculum of YNS-85.
[b] Means ± standard deviations (n = 10).

that the decline in PCNB can be ascribed to the application of the bacterial agent and not to uptake by *P. notoginseng*.

Soil FDA esterase and catalase activities

Soil enzymes that catalyze a wide range of soil biological processes have been widely considered as effective indicators of soil "function" and general microbial activities (Killham and Staddon, 2002). Soil FDA esterase and catalase activities can represent hydrolysis and oxidation-reduction levels. Soil FDA esterase and catalase activities under different treatments are shown in Fig. 2. The FDA esterase activities in treatments A and B were significantly lower than those in the control. This indicates that the application of the solid inoculum significantly decreased the activity of FDA esterase in the soil. The disparity of the effects of the solid inoculum can be observed in the soil redox level and overall microbial activity. Numerous studies on the effects of pesticides on soil enzyme activity have been conducted (Gianfreda *et al.*, 1995; Sannino and Gianfreda, 2001). The amount of pesticide processed is the same; mean soil redox levels decline with the addition of bacterial inoculum, and overall microbial activity may decline as a result of bacterial inoculum application. Soil catalase activity shows a significant correlation with organic C content in the soil and can be used to determine soil biomass (Stępniewska *et al.*, 2009). Although the soil catalase activity in treatment A decreased after 1 year of microbial remediation, it was not significantly different from that in the control. The soil catalase activity in treatment B was significantly lower than those in the control and treatment A. This may be due to the larger amount of bacterial inoculum negatively affecting other microorganisms; therefore, treatment A had the more appropriate application rate.

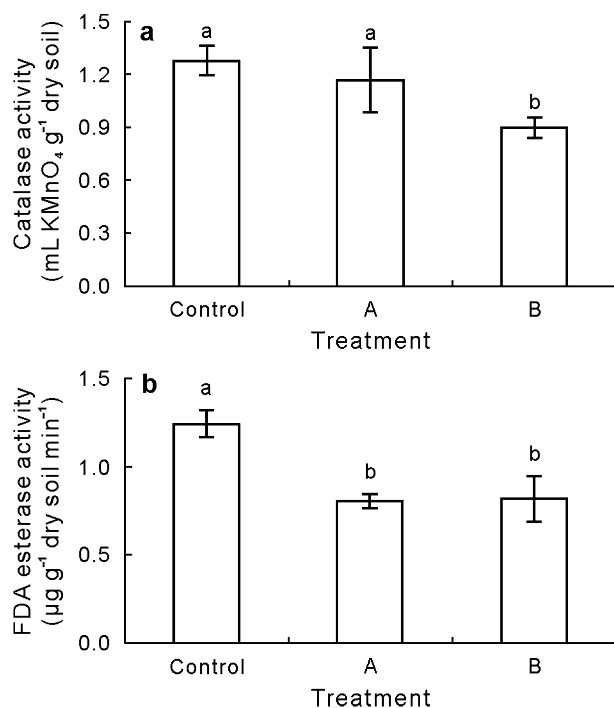

Fig. 2 Soil catalase activities (a) and fluorescein diacetate (FDA) esterase activities (b) under different treatments after 1 year of the field experiment. Control = soil amended with wheat bran but not *Cupriavidus* sp. YNS-85; A = soil with 0.15 kg m^{-2} of solid bacterial inoculum of YNS-85; B = soil with 0.30 kg m^{-2} of solid bacterial inoculum of YNS-85. Values are means ± standard deviations (n = 3). Bars with different letters are significantly different ($P < 0.05$).

Soil cultivable microbial counts and microbial biodiversity

The counts of the cultivable bacteria, fungi, and actinomycetes are shown in Table III. The bacterial count decreased with an increase in the amount of solid inoculant (Control > A > B), possibly because of competition between *Cupriavidus* sp. YNS-85 and the bacterial microflora (Fontaine and Barot, 2005). The fungal counts declined in the following sequence: B > Control > A. The sequence of the actinomycete counts was A > Control > B. Fungi have been found to cause black root rot in *P. notoginseng* in China (Mao *et al.*, 2014), and the determination of soil cultivable microbial counts can explain the impact of different quantities of the solid inoculant on the microbial counts and survival rate (Table II) associated with the fungi.

The BIOLOG method is useful for the study of the functional diversity of the microbial community (Garland and Mills, 1991). Although strain YNS-85 is an indigenous bacterium isolated from the study soil, the effects of inoculation with a large number of strain YNS-85 cells on other microorganisms require further study. The average well-color development (AWCD) can be used as an indicator of soil microbial activity in terms of functional diversity, showing

TABLE III

Cultivable microbial counts in the soil under different treatments 1 year after the field experiment

Treatment[a)]	Bacteria	Fungi	Actinomycetes
	CFU[b)] g^{-1} dry soil		
Control	$28.7 \times 10^8 \pm 2.3 \times 10^{8c)}$	$13.4 \times 10^5 \pm 0.5 \times 10^5$	$2.16 \times 10^6 \pm 0.5 \times 10^6$
A	$10.1 \times 10^8 \pm 1.1 \times 10^8$	$9.0 \times 10^5 \pm 1.4 \times 10^5$	$4.03 \times 10^6 \pm 0.5 \times 10^6$
B	$5.8 \times 10^8 \pm 0.9 \times 10^8$	$19.7 \times 10^5 \pm 1.0 \times 10^5$	$1.42 \times 10^6 \pm 0.6 \times 10^6$

[a)] Control = soil amended with wheat bran but without *Cupriavidus* sp. YNS-85; A = soil with 0.15 kg m^{-2} of solid bacterial inoculum of YNS-85; B = soil with 0.30 kg m^{-2} of solid bacterial inoculum of YNS-85.
[b)] Colony-forming units.
[c)] Means ± standard deviations (n = 3).

the effects of the solid bacterial agent used (Garland and Mills, 1991). The AWCD data from the soil samples under the three treatments are shown in Fig. 3. The AWCD values of treatment A increased rapidly after 48 h and reached 0.12 within 168 h of incubation, with a final value of 1.2; the AWCD values of the control and treatment B were only 0.8 and 0.85, respectively, at the end of the incubation period ($P < 0.05$). This indicates that an intermediate dose of the bacterial isolate may enhance microbial activity, but a large amount may have an effect similar to the control. This may be attributed to the competitive effect of an adequate input of the bacterial isolate on other microorganisms (Fontaine and Barot, 2005). Overall, the impact of strain YNS-85 on microbial biodiversity was only confirmed over 1 year of remediation, and thus, it may represent a promising treatment for the remediation of PCNB-contaminated soils.

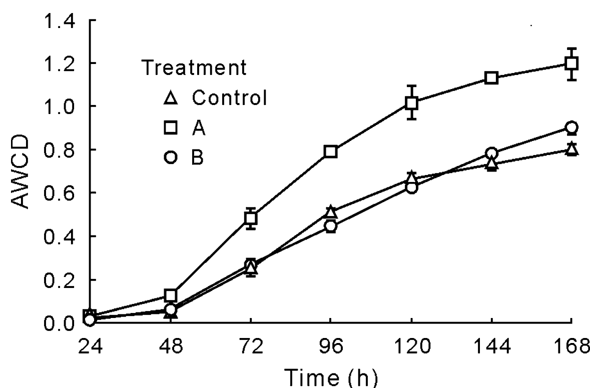

Fig. 3 Variation in average well-color development (AWCD) for soil samples from different treatments 1 year after the field experiment. Values are means ± standard deviations (n = 3). Control = soil amended with wheat bran but not *Cupriavidus* sp. YNS-85; A = soil with 0.15 kg m^{-2} of solid bacterial inoculum of YNS-85; B = soil with 0.30 kg m^{-2} of solid bacterial inoculum of YNS-85.

CONCLUSIONS

Currently, microbial removal is used as a promising method for *in-situ* removal of many organic pesticides and pesticide residues. The data from this field experiment showed that the soil PCNB content decreased after application of the solid inoculum of *Cupriavidus* sp. YNS-85, indicating that this isolate played an important role in the removal of PCNB in soil under *P. notoginseng*. The plant survival rate, soil enzyme activity, soil cultivable microbial counts, and microbial community structure indicate that 0.15 kg m^{-2} is a more suitable application rate of the solid inoculant than 0.30 kg m^{-2}. This study demonstrates the potential for field application of the solid inoculant of *Cupriavidus* sp. YNS-85 for the remediation of PCNB-contaminated soils. However, the relationship between the bacterium agent and fungus, whether *P. notoginseng* can bioaccumulate PCNB, and how the degradation byproducts migrate need to be studied further under field conditions.

ACKNOWLEDGEMENT

This research was funded by grants from the Major Science and Technique Programs of Yunnan Province, China (No. 2016ZF001) and the Science and Technique Programs of Yunnan Province, China (No. 2017IB038).

REFERENCES

Abubakar A, Mustafa M B, Johari W L W, Zulkifli S Z, Yusuff F B M. 2016. Tributyltin (TBT) tolerance of indigenous and non-indigenous bacterial species. *Water Air Soil Pollut*. **227**: 258.

Arora P K, Sasikala C, Ramana C V. 2012. Degradation of chlorinated nitroaromatic compounds. *Appl Microbiol Biotechnol*. **93**: 2265–2277.

Bento F M, Camargo F A O, Okeke B C, Frankenberger W T. 2005. Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresour Technol*. **96**: 1049–1055.

Chen C R, Xu Z H. 2005. Soil carbon and nitrogen pools and microbial properties in a 6-year-old slash pine plantation of subtropical Australia: Impacts of harvest residue management. *Forest Ecol Manag*. **206**: 237–247.

Fontaine S, Barot S. 2005. Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. *Ecol Lett*. **8**: 1075–1087.

Garau G, Castaldi P, Deiana S, Campus P, Mazza A, Deiana P, Pais A. 2012. Assessment of the use potential of edible sea urchins (*Paracentrotus lividus*) processing waste within the agricultural system: Influence on soil chemical and biological properties and bean (*Phaseolus vulgaris*) and wheat (*Triticum vulgare*) growth in an amended acidic soil. *J Environ Manage*. **109**: 12–18.

Garland J L, Mills A L. 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Appl Environ Microbiol*. **57**: 2351–2359.

Gianfreda L, Sannino F, Violante A. 1995. Pesticide effects on the activity of

free, immobilized and soil invertase. *Soil Biol Biochem*. **27**: 1201–1208.

Gomes H I, Dias-Ferreira C, Ribeiro A B. 2013. Overview of *in situ* and *ex situ* remediation technologies for PCB-contaminated soils and sediments and obstacles for full-scale application. *Sci Total Environ*. **445**-**446**: 237–260.

Gorontzy T, Drzyzga O, Kahl M W, Bruns-Nagel D, Breitung J, von Loew E, Blotevogel K H. 1994. Microbial degradation of explosives and related compounds. *Crit Rev Microbiol*. **20**: 265–284.

Hakala J A, Chin Y P, Weber E J. 2007. Influence of dissolved organic matter and Fe(II) on the abiotic reduction of pentachloronitrobenzene. *Environ Sci Technol*. **41**: 7337–7342.

Huang Y, Zhao X, Luan S J. 2007. Uptake and biodegradation of DDT by 4 ectomycorrhizal fungi. *Sci Total Environ*. **385**: 235–241.

Juhasz A L, Naidu R. 2000. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: A review of the microbial degradation of benzo[a]pyrene. *Int Biodeter Biodegr*. **45**: 57–88.

Killham K, Staddon W J, Burns R G, Dick R P. 2002. Bioindicators and sensors of soil health and the application of geostatistics. *In* Burns P G, Dick R P (eds.) Enzymes in the Environment. Marcel Dekker, New York. pp. 391-406.

Leung K S Y, Chan K, Chan C L, Lu G H. 2005. Systematic evaluation of organochlorine pesticide residues in Chinese materia medica. *Phytother Res*. **19**: 514–518.

Li J, Dong F S, Liu X G, Zheng Y Q, Yao J R, Zhang C P. 2009. Determination of pentachloronitrobenzene and its metabolites in ginseng by matrix solid-phase dispersion and GC-MS-MS. *Chromatographia*. **69**: 1113–1117.

Li R, Zheng J W, Ni B, Chen K, Yang X J, Li S P, Jiang J D. 2011. Biodegradation of pentachloronitrobenzene by *Labrys portucalensis* pcnb-21 isolated from polluted soil. *Pedosphere*. **21**: 31–36.

Li Y Y, Yang H. 2013. Bioaccumulation and degradation of pentachloronitrobenzene in *Medicago sativa*. *J Environ Manage*. **119**: 143–150.

Lièvremont D, Seigle-murandi F, Benoit-guyod J L. 1998. Removal of PCNB from aqueous solution by a fungal adsorption process. *Water Res*. **32**: 3601–3606.

Liu C S, Shih K, Sun C X, Wang F. 2012. Oxidative degradation of propachlor by ferrous and copper ion activated persulfate. *Sci Total Environ*. **416**: 507–512.

Liu Y, Shen J M, Chen Z J, Yang L, Liu Y, Han Y. 2011. Effects of amorphous-zinc-silicate-catalyzed ozonation on the degradation of *p*-chloronitrobenzene in drinking water. *Appl Catal A Gen*. **403**: 112–118.

Mao Z S, Long Y J, Zhu Y Y, Zhu S S, He X H. 2014. First report of *Cylindrocarpon destructans* var. *destructans* causing black root rot of sanqi (*Panax notoginseng*) in China. *Plant Dis*. **98**: 162.

Ng T B. 2006. Pharmacological activity of Sanchi ginseng (*Panax notoginseng*). *J Pharm Pharmacol*. **58**: 1007–1019.

Pereira S I A, Pires C, Henriques I, Correia A, Magan N, Castro P M. 2015. Assessment of rhizospheric culturable bacteria of *Phragmites australis* and *Juncus effusus* from polluted sites. *J Basic Microbiol*. **55**: 1179–1190.

Rodríguez-Kábana R, Truelove B. 1982. Effects of crop rotation and fertilization on catalase activity in a soil of the southeastern United States. *Plant Soil*. **69**: 97–104.

Sannino F, Gianfreda L. 2001. Pesticide influence on soil enzymatic activities. *Chemosphere*. **45**: 417–425.

Shin S K, Kim J E, Kwon G S, Kwon J W, Oh E T, So J S, Koh S C. 2003. Isolation and identification of a pentachloronitrobenzene (PCNB) degrading bacterium *Alcaligenes xylosoxidans* PCNB-2 from agricultural soil. *J Microbiol*. **41**: 165–168.

Stępniewska Z, Wolińska A, Ziomek J. 2009. Response of soil catalase activity to chromium contamination. *J Environ Sci*. **21**: 1142–1147.

Sun N, Hao L L, Xue J, Jin H Y, Tian J G, Lin R C. 2007. Multi-residue analysis of 18 organochlorine pesticides in 10 traditional Chinese medicines by gas chromatography (GC). *J Health Sci*. **53**: 464–469.

Takagi K, Iwasaki A, Kamei I, Satsuma K, Yoshioka Y, Harada N. 2009. Aerobic mineralization of hexachlorobenzene by newly isolated pentachloronitrobenzene-degrading *Nocardioides* sp. strain PD653. *Appl Environ Microbiol*. **75**: 4452–4458.

Tas D O, Pavlostathis S G. 2014. Occurrence, toxicity, and biotransformation of pentachloronitrobenzene and chloroanilines. *Crit Rev Environ Sci Technol*. **44**: 473–518.

Teng Y, Wang X M, Li L N, Li Z G, Luo Y M. 2015. Rhizobia and their bio-partners as novel drivers for functional remediation in contaminated soils. *Front Plant Sci*. **6**: 32.

Torres R M, Grosset C, Steiman R, Alary J, Fourier J. 1996. Liquid chromatography study of degradation and metabolism of pentachloronitrobenzene by four soil micromycetes. *Chemosphere*. **33**: 683–692.

Tu C, Teng Y, Luo Y M, Sun X H, Deng S P, Li Z G, Liu W X, Xu Z H. 2011. PCB removal, soil enzyme activities, and microbial community structures during the phytoremediation by alfalfa in field soils. *J Soil Sediment*. **11**: 649–656.

U.S. National Library of Medicine. 1995. Hazardous Substances Data Bank. U.S. National Library of Medicine, Bethesda.

Vogt C, Richnow H H. 2013. Bioremediation *via in situ* microbial degradation of organic pollutants. *In* Schippers A, Glombitza F, Sand W (eds.) Geobiotechnology II. Springer, Berlin, Heidelberg. pp. 123–146.

Wang Y, Wang C, Li A, Gao J. 2015. Biodegradation of pentachloronitrobenzene by *Arthrobacter nicotianae* DH19. *Lett Appl Microbiol*. **61**: 403–410.

Wen H W, Hsieh M F, Wang Y T, Chung H P, Hsieh P C, Lin H, Chou F I. 2010. Application of gamma irradiation in ginseng for both photodegradation of pesticide pentachloronitrobenzene and microbial decontamination. *J Hazard Mater*. **176**: 280–287.

Yu X, Liu X, Zhu T H, Liu G H, Mao C. 2011. Isolation and characterization of phosphate-solubilizing bacteria from walnut and their effect on growth and phosphorus mobilization. *Biol Fert Soils*. **47**: 437–446.

Zeng D H, Hu Y L, Chang S X, Fan Z P. 2009. Land cover change effects on soil chemical and biological properties after planting Mongolian pine (*Pinus sylvestris* var. *mongolica*) in sandy lands in keerqin, northeastern China. *Plant Soil*. **317**: 121–133.