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Application of acclimated sewage sludge as a bio-augmentation/bio-stimulation strategy for remediating chlorpyrifos contamination in soil with/without cadmium

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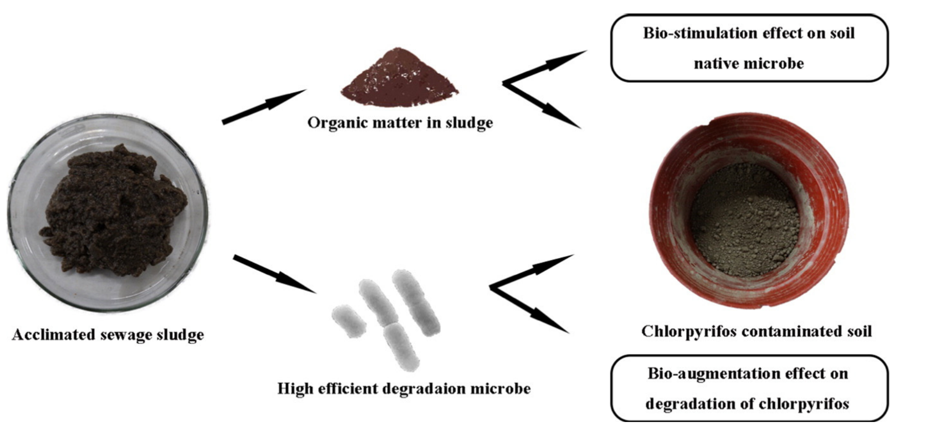
HIGHLIGHTS

- Acclimated sewage sludge efficiently enhanced the dissipation of chlorpyrifos in soil.
- The degradation efficiency of chlorpyrifos was not significantly affected by Cd.
- The application of ASS greatly influenced the bacterial community structure in soil.
- The presence of acclimated sewage sludge enhanced the soil biochemical quality.
- The soil fertility improved after remediation.

GRAPHICAL ABSTRACT



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ABSTRACT

This experiment was performed to investigate the effects of acclimated sewage sludge (ASS) and sterilized ASS on the fates of chlorpyrifos (CP) in soil with or without cadmium (Cd), as well as the improvement of soil biochemical properties. Results showed that both ASS and sterilized ASS could significantly promote CP dissipation, and the groups with ASS had the highest efficiency on CP removal, whose degradation rates reached 71.3%–85.9% at the 30th day (40.4%–50.2% higher than non-sludge groups). Besides, the degradation rate of CP was not severely influenced by the existence of Cd, and the population of soil microorganism dramatically increased after adding sludge. The soil enzyme activities (dehydrogenase, acid phosphatase and FDA hydrolase activities) ranked from high to low were as follows: groups with sterilized ASS > groups with ASS > groups without sludge. Simultaneously, 16S rRNA gene sequencing revealed that ASS changed bacterial community structure and diversity in soil. In addition, alkali-hydrolyzable nitrogen and Olsen- phosphorus increased after application of sludge, indicating that the addition of ASS (or sterilized ASS) could effectively improve soil fertility.

1. Introduction

Chlorpyrifos [$C_9H_{11}C_{l3}NO_3PS$ or *O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is a broad-spectrum systemic organophosphorus insecticide widely applied for pest control in agriculture(Tejada et al., 2011). Chlorpyrifos (CP) is persistent and stable in soil, which is difficult to dissipate by nature. The half-lives of CP ranged from 7 days to 120 days, and its degradation in soil depended upon the soil type(Getzin, 1981a). A review by Singh and Walker (2006) demonstrated CP could be degraded by bacterial and fungi through catabolic and co-metabolic mechanisms. During the process of CP degradation, TCP is the most frequently detected intermediate (Getzin, 1981b).

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Due to high toxicity, TCP was listed as persistent and mobile pollutant in 2001 by US Environmental Protection Agency(Peng et al., 2013). TCP has anti-microbial properties, which prevents the proliferation of chlorpyrifos degrading microorganisms (Singh and Walker, 2006). The accumulation of TCP in soil can cause difficulty in microbe generation, hence hinder the degradation of CP or TCP.

Bio-stimulation agent can improve the activity of microorganisms, thereby promoting CP passivation in polluted soil (Aceves-Diez et al., 2015). Organic amendment was one frequently used bio-stimulation agent which was rich in nutrients that could be easily assimilated by soil microorganisms, thereby alleviating the inhibitory effects of residual CP on soil microbes (Kadian et al., 2012). Sewage sludge (SS) is a by-product from citizen wastewater treatments, which can be used as soil amendments to stimulate soil microbial activity due to its rich organic matters, nutrition N and P (Fernandes et al., 2005). In addition, SS may enhance the bioremediation of hydrocarbon-polluted soils by bringing in microbe consortium, which may have high degradation ability due to the acclimation of organic pollutants in wastewater (Wang et al., 2016). Some reports suggested that the acclimation of activated SS by particular organics could promote pollutant tolerance and degradation ability of microorganisms in sludge, enhancing its utilization value in the remediation of organics contaminated soil (Lin et al., 2014; Wang et al., 2016; Yang et al., 2012). In this study, acclimated sewage sludge (ASS) by simulated wastewater contaminated with CP was utilized to investigate its potential for soil remediation.

As heavy metal pollution becomes one main pollution type, soil pollution tends to mix with two or more pollutants (Zhou et al., 2015). However, researches on the co-presence of organophosphorus pesticides and heavy metal in soil as well as the influence of heavy metal on the remediation of CP pollution are still scarce. Thus, it is meaningful to consider the existence of heavy metal in the remediation of CP.

This research provides direct evidence about the feasibility of ASS as a bio-augmentation/bio-stimulation amendment to remediate chlorpyrifos contamination in Cd polluted soil. To investigate the bio-augmentation and bio-stimulation effect, sterilized ASS and unsterilized ASS were both introduced. Furthermore, soil microorganism quantity, dehydrogenase activity, acid phosphatase activity and FDA hydrolase activity were presented to reflect the soil microbe activity variety after remediation. In addition, alkali-hydrolyzable nitrogen and Olsen- phosphorus were measured to determine the improvement of soil fertility.

2. Material and methods

2.1. Chemicals

CP standard was purchased from AccuStandard, Inc. (USA). TCP standard was purchased from Sigma (St. Louis, MO, USA). The 48% CP latex and analytical-grade salt of $CdCl_2$ were obtained from KeLong Chemical Reagent Factory in Chengdu, China. All other chemicals and reagents were analytical-reagent grade or higher purity.

2.2. Soil preparation

The soil originally free of CP was collected from Sichuan University, Wangjiang campus, which was selected at depth of 5–20 cm from the surface. The soil sample was air-dried, sieved through a 2 mm mesh and then analyzed their main physical and chemical properties. For simulation of Cd contaminated soil: part of sieved soil was added 0.8 g L^{-1} $CdCl_2 \cdot 2.5H_2O$ saline solution (50 mL kg^{-1}) and fully mixed with the soil, then equilibrated in a dark room for two months prior to the experiment. Before remediation, 50 mg kg^{-1} and 100 mg kg^{-1} CP contaminated soil (both in Cd polluted soil and soil free of Cd) were prepared by adding a certain amount of CP.

2.3. ASS preparation

SS obtained from Chengdu First Sewage Treatment Plant (Chengdu, China) was acclimated by simulated wastewater containing 500 mg L^{-1} sodium acetate, 500 g L^{-1} ammonium chloride, 1000 mg L^{-1} sodium chloride, 300 mg L^{-1} potassium dihydrogen phosphate, and 300 mg L^{-1} dipotassium phosphate, then kept aerating for one week to rejuvenate the sludge. Wastewater was changed every 3 days. After the sludge rejuvenation, CP was added into wastewater with initial dosage at 10 mg L^{-1}, and the dosage gradually increased and finally maintained at 250 mg L^{-1}. At the same time, the dosage of sodium acetate gradually reduced with the increase of CP concentration (Sodium acetate as carbon source in the early phase and CP as carbon source later). Acclimation process lasted for 4 weeks before application. After acclimation, the ASS was centrifuged and the sediment was preserved at 0 °C before application. Autoclaving was used to sterilize ASS samples, which dehydrate the ASS firstly, then put ASS in glass beaker autoclaving three times with 121 °C for 20 min (Trevors, 1996).

2.4. Remediation process

In this study, 18 treatments were set. All treatments were presented in Table 1. The remediation process was carried out in plastic pots with 200 g (dry mass) of contaminated soil each. The groups with sludge were mixed with 20 g of ASS or sterilized ASS. The soil moisture content was adjusted to 70% of its water holding capacity prior to incubation by adding ultrapure water. Each of treatments were carried out in triplicate and incubated for 30 days under the stable condition in biochemical incubator with temperature at 30 °C, and regular watering to keep moisture content. At the 15th day and the 30th day, the concentrations of residue CP and TCP were detected. Dehydrogenase and acid phosphatase activities were detected at the 7th, 14th, 21th, 30th day, and FDA hydrolase activities were detected at the 7th, 14th, 30th day.

2.5. Determination of residual CP and TCP

The extraction of CP and TCP was performed according to Ahmad, Peng et al. (Ahmad et al., 2012; Peng et al., 2013). For the analysis of CP and TCP in soil, 5 g of soil was shaken with 25 mL of acetonitrile: water (90:10, v/v) for 1 h and centrifuged for 5 min at 4000 rpm. The supernatant was filtered through a 0.45-μm fiber filter in preparation for HPLC analysis, 20 μL of the resulting solution was analyzed by reversed-phase HPLC (SHIMADZU, HPLC-CBM20A). The separation column (4.6 mm × 250 mm × 5 μm) for the HPLC was filled with

Table 1
Experimental treatments.

Abbreviation	Treatment description
S_1N	Natural soil sample 1
S_1S	Soil sample 1 and amended with sterilized sludge
S_1A	Soil sample 1 and amended with acclimated sludge
S_2N	Natural soil sample 2
S_2S	Soil sample 2 and amended with sterilized sludge
S_2A	Soil sample 2 and amended with acclimated sludge
$S_1N + C_1$, $S_1N + C_2$	Soil sample 1 spiked with chlorpyrifos
$S_1S + C_1$, $S_1S + C_2$	Soil sample 1 spiked with chlorpyrifos, and amended with sterilized sludge
$S_1A + C_1$, $S_1A + C_2$	Soil sample 1 spiked with chlorpyrifos, and amended with acclimated sludge
$S_2N + C_1$, $S_2N + C_2$	Soil sample 2 spiked with chlorpyrifos 1
$S_2S + C_1$, $S_2S + C_2$	Soil sample 2 spiked with chlorpyrifos and amended with sterilized sludge
$S_2A + C_1$, $S_2A + C_2$	Soil sample 2 spiked with chlorpyrifos and amended with acclimated sludge

C_1: soil samples were spiked with chlorpyrifos at the concentration of 50 mg kg^{-1}. C_2: soil samples were spiked with chlorpyrifos at the concentration of 100 mg kg^{-1}.

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