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Potential of different species for use in removal of DDT from the contaminated soils

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ABSTRACT

Dichlorodiphenyltrichloroethane (DDT) and its main metabolites, *p,p′*-DDD and *p,p′*-DDE (DDTs in this study included DDT, DDD and DDE), are frequently detected in agricultural soils even though its usage in agriculture was banned in 1980s or earlier. In this study, eleven plants including eight maize (*Zea mays*) cultivars and three forage species (alfalfa, ryegrass and teosinte) widely cultivated in China were grown in the soils spiked with DDTs to investigate their potential for removal of DDT from the contaminated soils. The plants varied largely in their ability to accumulate and translocate DDTs, with the bioconcentration factor (BCF; DDT concentration ratio of the plant tissues to the soils) ranging from 0.014 to 0.25 and the translocation factor (TF; DDT concentration ratio of the shoots to the roots) varying from 0.35 (*Zea mays* cv Chaotian-23) to 0.76 (*Zea mays* spp. mexicana). The amount of DDT phytoextraction ranged from 3.89 μg (ryegrass) to 27.0 μg (teosinte) and accounted for <0.1% of the total initial DDTs spiked in the soils. After 70 d, the removal rates reached 47.1–70.3% of the total initial DDTs spiked in the soils with plants while that was only 15.4% in the soils without plant. Moreover, the higher removal rates of DDTs occurred at the first 20 d of experiment, and then the removal rate decreased with time. The highest amount of DDTs phytoextracted was observed in teosinte, followed by *Zea mays* spp. mexicana, but the highest removal rate of DDTs was found in maize (*Zea mays* cv Jinhai-6). Even though phytoextraction is not the main removal process for DDTs, the plant species especially *Zea mays* cv Jinhai-6 showed high potential for removing DDTs from the contaminated soils.

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1. Introduction

Dichlorodiphenyltrichloroethane (DDT) was one of the most widely used pesticides in agriculture in many countries until the 1970s. Estimates showed that China produced at least 435,200 tonnes of DDT and its main metabolites [1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene, *p,p′*-DDE; 1,1-dichloro-2,2-bis(4-chlorophenyl)-ethane, *p,p′*-DDD] from the 1950s to May 1983 when their production was banned, accounting for 20% of the global production (Hua and Shan, 1996). Part of DDT was released into the environment during the past decades and this trend continues, because of its usage as an anti-malaria agent or of an impurity in other pesticides such as dicofol (Wong et al., 2005).

DDT and its two metabolites are classified as persistent organic pollutants (POPs) and their pollution is of worldwide concern due to their persistence in the environment, bioaccumulation, and negative effects on soil microbial, plant, animal life and humans (Katsoyiannis and Samara, 2004; Turusov et al., 2002). Recently, high levels of DDT and its main metabolites have been frequently detected in various media such as soils, water, sediments and plants in China, as well as in other countries (Waliszewski et al., 2004; Chau, 2005; Nakata et al., 2005; Kurt-Karakus et al., 2006; Li et al., 2006; Katsoyiannis and Samara, 2007; Cai et al., 2008). Finding of higher DDT concentrations in the cultivated soils than in the non-cultivated ones reveals either that DDT was used in the past or that is currently illegally used for agricultural reasons (Chau, 2005). DDT residues in agricultural soils are of great concern due to the uptake of DDT by plants, accumulation in food chain, and re-emission from soils to the atmosphere. Thus, the removal of DDT and its metabolites from the agricultural environment has intrigued many scientists in recent years.

Phytoremediation is an *in situ* and cost-effective technology in which the plants are applied to extract, directly or indirectly degrade or remove organic and inorganic contaminants from the contaminated natural media, including POPs such as DDT and its metabolites (Cunningham and Ow, 1996; Tang, 2005). Literature review, however, showed that few publications were available on phytoremediation of DDT in spite of its widespread occurrence (Garrison et al., 2000; Lunney et al., 2004). Garrison et al. (2000) reported biotransformation of DDT by plant cultures. White et al.

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(2005, 2006) investigated phytoextraction of *p,p′*-DDE, and found that accumulation and translocation of DDT or DDE by plants were somewhat species-specific or cultivar-specific (Lunney et al., 2004). But until now, no literature reported which plant has the greatest potential for phytoremediation of DDT in soils. Further research should be done in this direction.

The objectives of the present study were to investigate the uptake and translocation of DDT in soil-plant system, and to compare the potential of various plant species and cultivars endemic to China for removal of DDT from the contaminated soils.

2. Materials and methods

2.1. Chemicals and materials

In order to compare the phytoextraction potential of different species and cultivars for DDT, two plant species were selected, including maize (*Zea mays*) and forage. The forage species included alfalfa (*Medicago sativa*), ryegrass (*Lolium multiflorum*), and teosinte (*Zea mays* ssp. *parviglumis*) which may have potential for phytoremediation of PAHs (Fan et al., 2007) or DDT (Lunney et al., 2004). Eight representative cultivars of maize were selected from the cultivars grown widely in China, namely, Jinhai-6 (MV1), Wanqin (MV2), *Zea mays* spp. *mexicana* (MV3), Huanong-1 (MV4). Huahai (MV5), Huidan (MV6), Baiyunuo (MV7) and Chaotian-23 (MV8). The latter was sweet maize, while the others were feed maize. All the plant seeds for experiment were obtained from Guangdong Academy of Agricultural Sciences, China.

The DDTs (DDTs in this study stands for *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD) of analytical grade for pot trial were obtained from Taigu Chemical Factory of Tianjin, China.

A composite stock standard solution of DDTs (100 mg/l) for analysis was used, containing *p,p′*-DDE, *p,p′*-DDD and *p,p′*-DDT. The working standard solutions were prepared by diluting appropriate volumes of the stock standard solution. 2,4,5,6-Tetrachloro-*m*-xylene (TC*m*X) and pentachloronitrobenzene were used as surrogate and internal standard solution, respectively. These standards were purchased from J&K Chemical Limited Co. (Beilinwei, Beijing, China). Another standard solution, *o,p′*-DDT, was purchased from China's Research Center of Standard Material.

Analytical grade organic solvents including *n*-hexane, dichloromethane (DCM), methanol and acetone were redistilled prior to use. Silica gel (100–180 mesh, Guangzhou Chemical Reagent Co.) was Soxhlet-extracted with DCM and methanol, respectively, for 12 h, dried at 130–140 °C for 4 h before use. Neutral alumina (80–100 mesh, Guangzhou Chemical Reagent Co.) was activated at 250 °C, for 12 h. Anhydrous sodium sulfate was dried at 250 °C for 4 h and stored in a sealed desiccator. These materials were purchased from Guangzhou Chemical Reagent Co., China.

All the glassware were soaked in K_2CrO_4–H_2SO_4 solution for 30 min, washed with tap water and redistilled water and then were dried at 250 °C for 2 h.

2.2. Experimental design

The experiment was carried out in the glasshouse of South China Agricultural University, Guangzhou, China. The soil was paddy soil with 24.9 g/kg dry weight (d.w.) of organic matter, 1.02 g/kg (d.w.) of total N, 0.92 g/kg (d.w.) of total P, 19.35 g/kg (d.w.) of total K, 78.8 mg/kg (d.w.) of available N, 29.0 (d.w.) mg/kg of available P, 6.28 of pH and 310 g/kg (d.w.) of water-holding capacity. The soil was air-dried, crushed, mixed thoroughly and passed through a 5-mm sieve. There were 5.0 kg of soils in each pot. Prior to cultivation, the soil was fertilized 0.20 g/kg N, 0.15 g/kg P and 0.15 g/kg K with urea, superphosphate and potassium chloride, respectively.

The stock solution of DDTs was prepared by diluting DDTs with acetone. The stock solution was mixed thoroughly with paddy soil (500 g, 2 mm) and allowed to evaporate for 48 h. The treated soil was mixed thoroughly with "clean" bulk soils (4500 g, 5 mm) to obtain the initial DDT concentration which were determined by Soxhlet extraction-gas chromatography coupled with electron capture detector (GC-ECD) as other samples. Considering the toxicity of DDTs to the plants and the concentrations used in the previous study (32.0–88.9 mg/kg d.w.) by Kiflom et al. (1999), the total initial concentration of DDTs in soils of the present study was 29.9 mg/kg (d.w.). Soils (5.0 kg) contaminated with DDTs were placed in a ceramic pot (27 × 17 cm, I.D. × height). The pot trial included 11 treatments with different species or cultivars given above and a treatment without plant (defined as Control). Immediately after being prepared, they were arranged in a completely randomized block design with five replications in a glasshouse. The soils were kept at the water-holding capacity with redistilled water for seven days and then mixed thoroughly.

Twenty seeds of maize were sown in each pot and, after germination, seedlings were manually thinned twice and three uniform seedlings were established per pot. Fifty seedlings of alfalfa or ryegrass were established to each pot. The redistilled water (200 ml) was supplied for each pot including the control treatment every day during the growth. There was no effluence from the bottom of the pots. No chemical pesticide was used. At 10, 20, 30, 50 and 70 d of growth, soil samples were carefully collected from the pot (at least 10 sites, randomly) using a 1.0 cm stainless steel sampler. The plants were harvested after 70 d of growth. The shoots (including stem and leaves) and roots of plants were carefully removed from the soils and their fresh weights were measured. The plant samples were rinsed with redistilled water, oven-dried at 55 °C and then ground (1-mm sieve). The soil samples (approximately 100 g) were air-dried, ground to pass through a 1-mm sieve, and refrigerated until analysis.

2.3. Analytical procedure, QA/QC measures and performances

Sample extraction and cleanup were performed according to USEPA methods 3550B (Ultrasonic extraction) and 3630C (Silica gel column) with modification, respectively. Dried plant (∼10 g) and soil samples (∼20 g) spiked with surrogate were extracted in triplicate with 30 ml ethyl acetate and with 30 ml acetone/DCM mixture (1:1, v/v) in sonicator (SK5200H, China) for 20 min, respectively. After each extraction, separation was accomplished by centrifuging at 3000 rpm for 5 min. The supernatant was combined and concentrated carefully to 2 ml in a rotary vacuum evaporator (Yarong, China).

The concentrated extracts were loaded on a combined column of silica gel and alumina. The glass chromatography column (25 × 1 cm I.D., Guangzhou, China), fitted with a Teflon stopcock, was packed, bottom-up, with cotton-wool (Soxhlet-extracted with DCM for 72 h before use), 3 cm alumina, 10 cm extracted silica, followed by 2 cm anhydrous sodium sulfate. DCM/*n*-hexane mixture (1:1, v/v, 50 ml) was used for elution. The collected extract was blown-down under a gentle stream of nitrogen (N_2) and diluted with *n*-hexane to an appropriate volume.

Measurements of DDTs in extract were performed following USEPA method 8081A with slight modification. The analysis was carried out by gas chromatography (GC, Hewlett–Packard 5890 Series II, Agilent Technology) coupled with electron capture detector (ECD). A HP-5 30 m × 0.32 mm I.D., 0.17 μm membrane thickness (Agilent Technology, US) was used. The GC oven temperature was raised from 150 to 280 °C at 4.0 °C/min. Nitrogen (N_2) was the carrier gas, at a flow of 2.5 ml/min. The injection was set on a splitless mode at 280 °C. The injection volume was 1.0 μl. A ^{63}Ni electron capture detector was used and its temperature was at 300 °C.

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