A PILOT STUDY ON ROOT-PROMOTING DETOXIFICATION OF PCDD/Fs CONTAMINATIONED SOIL BY MAIZE PRHZOSPHERE

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Introduction

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) are a group of the most recalcitrant pollutants. They have caused global concerns due to their bioaccumulation through the food chain and extraordinary toxicity[1]. Soil is considered as the biggest pool of PCDD/Fs in environment. Some remediation technologies for decontamination of the POPs-polluted soils, including physical, chemical and biological methods, may have the potential to remedy soils contaminated by PCDD/Fs.

Rhizoremediation, recognized as a cost-effective and environmentally friendly method, has received much attention in the last few years. The use of rhizoremediation for removing the high hydrophobic contaminants, such as PAHs[2] and PCBs[3] has been well documented. It has found that plant (e.g., species, age of the plant, rooting density), the properties of soil and contaminants are important factors influencing the development of microbial community and the metabolic pathways in the rhizosphere[4]. In addition, biodegradation in rhizosphere is strongly related to the distance to root[5]. More rapid degradation of several xenobiotics in the rhizosphere of plant species than those of bulk soil have been reported[3]. However, the fate of PCDD/Fs in rhizosphere has not much been studied so far.

The objective of this study was to investigate the rhizosphere effect of maize plants on PCDD/Fs removal in contaminated soil and test its applicability for rhizoremediation. To evaluate the influence of soil microbial community on the removal of PCDD/Fs, the bacterial community 16S ribosomal DNA (rDNA) of the different soil types (rhizosphere soil, vicinal-rhizosphere soil and non-rhizosphere) was extracted and analyzed by polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE) technology.

Materials and methods

Experimental set up and sampling

A pot experiment of maize (Zea may L.) was set up to study the bioremediation of PCDD/Fs. Each pot was filled with 200 g homogenized soil spiked with seven indicator PCDD/Fs congeners. Pre-germinated seedlings of maize were cultivated in contaminated soil for nine weeks in a green house. Hoagland's solution was watered daily to keep the soil moisture at 60% of field capacity. Bulk non-vegetated contaminated soil was used as the control. All treatments were repeated in triplicate. At the end of the experiment, soil and roots samples were taken. Above-ground parts of maize were discarded due to their negligible contributions for the removal of PCDD/Fs[6]. The control soil was defined as non-rhizosphere soil, while the soil not adhering to root after gentle shaking of the plants was considered as vicinal-rhizosphere soil and the soil adhering to roots tightly was considered as rhizosphere soil. Finally, the roots were washed by deionized water to remove all soil particles.

PCDD/Fs analysis

PCDD/Fs were analyzed following the method described by zhang et al. (2008)[7]. Briefly, about 3 g freeze-dried soil and root samples were spiked with $^{13}C_{12}$-labelled internal standards, and then followed by Soxhlet extraction and a two-stage open column cleanup procedure. Finally, The cleaned extract was analyzed by an Autospec Ultima high resolution mass spectrometer (Micromass, UK) coupled with a Hewlett–Packard (PaloAlto, CA, USA) 6890 Plus gas chromatograph (HRGC-HRMS).

Microbial community diversities analysis by PCR-DGGE

Microbial community composition analysis mainly included three steps: total DAN extraction; the bacteria 16S rDNA (V3 region) amplification and DGGE separation analysis. Total DNA isolation from the samples was carried out based on a protocol from Zhou et al. (1996)[8], with minor modifications. 5 g soil samples was mixed with 13.5 ml of DNA extraction buffer (100 mM Tris-HCl [100 mM sodium EDTA, 100 mM sodium phosphate, 1.5 M NaCl, 1% CTAB, pH 8.0) and 100 ml of proteinase K (10 mg/ml), followed by SDS (20%) lysis and phenol/ chloroform purification. A 200bp sequence of the Bacteria 16S rDNA (V3 region) was amplified by PCR with the primers GC-338f and 519r from 2µl of extracted DAN. DGGE of the PCR products (40 µl) was performed using a BioRad electrophoresis (Dcode, Universal Mutation Ssystem, Biorad). Polyacrylamide (8%) gels varied from 30% to 60%. The running time and voltage were standardized at 5h and 200V. After electrophoresis, gels were stained for 30 min with GeneFinder™ and photographed under UV light by gel imaging system.

Results and discussion:

Concentrations and TEQs in soil

The total concentrations of PCDD/Fs in rhizosphere soil, vicinal-rhizosphere and non-rhizosphere soil were 813.0, 1141.0 and 1407.3 ng/kg d.w., respectively; corresponding values for the total TEQ values of PCDD/Fs were 239.6, 279.7 and 310.7 ng/kg d.w. As shown in Figure 1, The mean concentrations of total PCDD/F congeners in soil samples decreased in the order of rhizosphere soil > vicinal-rhizosphere > non-rhizosphere soil. This indicated that the growth of maize root can promote the dissipation of PCDD/Fs in soil and the removal effect can be related to the distance from the root.

Removal rate of PCDD/Fs in rhizosphere

Plant-microbial interactions within rhizosphere can produce beneficial effect on degradation or accumulations of organic contaminants. To evaluate the influence of maize root on the dissipation of PCDD/Fs, the removal rates of PCDD/Fs in maize rhizosphere (including rhizosphere、vicinal-rhizosphere soil) were calculated based on the relative decrement compared with those in non-rhizosphere soil. The results were given in Figure 2. The relative removal rates of the seven PCDD/F congeners in rhizosphere soil were as follows: 27/28-TCDD 68.0%; 137-TCDD 36.6%; 2378-TCDD 18.7%; 12378-PeCDD 26.7%; 123478-HxCDD 29.1%; 1234678-HpCDD 22.4%, 2378-TCDF 23.7%. The lower values of removal rates were observed in vicinal-rhizosphere soil, which ranged from 3.2% to 27.3%. Generally, the removal rates of PCDD/Fs decreased with the increase of the numbers of chlorine atoms for PCDD/F congeners.

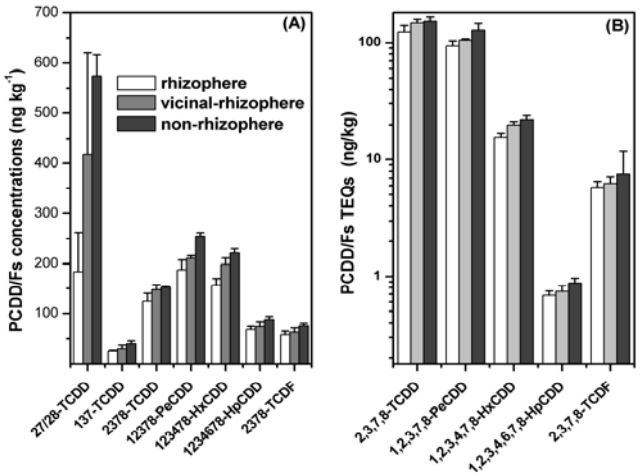

Figure 1. Concentrations (A) and TEQs (B) pattern of individual PCDD/F congeners in different soil types (rhizosphere、vicinal-rhizosphere、non-rhizosphere soil). Vertical bars represent the standard deviation.

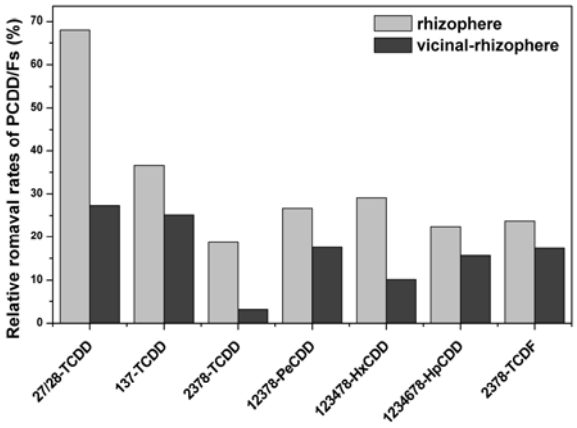

Figure 2. Relative remove rates of individual PCDD/F congeners in rhizosphre and vicinal-rhizosphere soil.

Root accumulation of PCDD/Fs

Plants promote organic contaminants dissipation by three strategies: immobilization, uptake and promotion of microbial degradation[9]. It has been proved that the root uptake of PCDD/s to aboveground biomass of maize plants is low[6]. Therefore, root accumulation and biodegradation may play an important role in the removal of PCDD/Fs in the present work.

PCDD/Fs are a group of high hydrophobic compounds (log Kow >5), and thus it is difficult for PCDD/Fs to be desorbed by the plants from soil. The root interception should be the main way that PCDD/Fs accumulated in root from soil. The root concentration factor (RCF), defined as the ratio of PCDD/Fs concentration in plant roots (ng/kg d.w.) to that in soil were calculated. The RCF values of PCDD/F congers ranged from 0.18 to 1.39 (Table 1). The RCF values of PCDD/Fs generally decreased with the increase of the numbers of chlorine atoms for PCDD/F congeners.

Analysis of microbial populations

Plant root can provide carbon substrates and nutrients, which increase the diversity of microbial community and the biomass of microbes, and thus promote the biodegradation of organic contaminants in soil. Therefore, monitoring the diversities of microbial population in rhizosphere can give more exactly interpretations for the role of microbe in the degradation of contaminants. As shown in Figure 4, The numbers of bands in rhizosphere zone soil (lanes: R1,R2, VR1, VR1, VR1) was more than those in bulk soil (lane: N). This indicated that the diversity of microbial community in rhizosphere zone soil was more abundant. However, the intensity and number of bands in R1 and R2 did not show significant differences from those in VR1 and VR2. This implied that the removal of PCDD/Fs did not only depend on the biodegradation. The adsorption/absorption of PCDD/Fs in root should also play an important role on the removal of PCDD/Fs from soil.

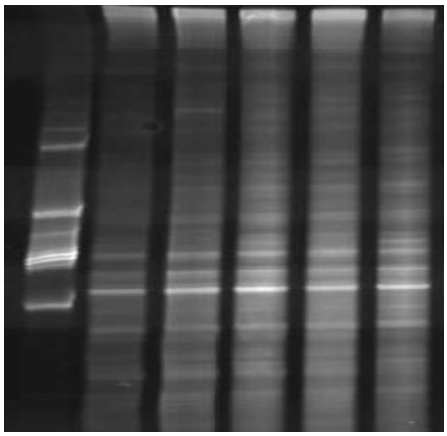

Figure 3. Diversity of microbial populations of different soil types (M = marker; R= rhizosphere soil; VR= vicinal-rhizosphere; N= non-rhizophere soil)

Table 2. The root mean concentrations and root concentration factors of individual PCDD/F congeners

	C_{root}	RCF	log*K*ow
27/28-TCDD	797.69	1.39	5.6-5.75
137-TCDD	13.25	0.33	--
2378-TCDD	34.03	0.22	6.8
12378-PeCDD	74.74	0.29	--
123478-HxCDD	38.70	0.18	7.8
1234678-HpCDD	25.49	0.29	8.0
2378-TCDF	46.90	0.62	6.1

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