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Short Communication

The phytoremediation potential of bioenergy crop *Ricinus communis* for DDTs and cadmium co-contaminated soil

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ABSTRACT

Cadmium (Cd) and dichlorodiphenyltrichloroethane (DDT) or its metabolite residues are frequently detected in agricultural soils and food, posing a threat to human health. The objective of this study was to compare the ability of 23 genotypes of *Ricinus communis* in mobilizing and uptake of Cd and DDTs (*p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDD and *p,p′*-DDE) in the co-contaminated soil. The plant genotypes varied largely in the uptake and accumulation of DDTs and Cd, with mean concentrations of 0.37, 0.43 and 70.51 for DDTs, and 1.22, 2.27 and 37.63 mg kg^{-1} dw for Cd in leaf, stem and root, respectively. The total uptake of DDTs and Cd varied from 83.1 to 267.8 and 66.0 to 155.1 μg per pot, respectively. These results indicate that *R. communis* has great potential for removing DDTs and Cd from contaminated soils attributed to its fast growth, high biomass, strong absorption and accumulation for both DDTs and Cd.

1. Introduction

Pollution control has become a great challenge in the 21st century due to a dramatic increase in pollutants resulting from human activities (Larue et al., 2010). Among all, organic chemicals such as persistent organic pollutants (POPs) and heavy metals are recognized as two major chemical families that cause soil pollution (Belden et al., 2004; Xia et al., 2009). DDT has accumulated in soil and river sediments as a result of historic insecticide use against pests and mosquitoes (Lunney et al., 2004). Cadmium is a heavy metal toxic to organisms, and is the most widespread pollution heavy metal in soils. Low exposure concentrations of Cd and DDT may affect low bone mineral density and increase the risk of vertebral fractures (Rignell-Hydbom et al., 2009). Phytoremediation offers a promising approach for sustainable management of polluted soils. The role of plants or root exudates in DDTs phytoextraction and biodegradation has attracted extensive studies (Lunney et al., 2004; Mo et al., 2008; White, 2002; White et al., 2003, 2005). The primary ingredients of technical grade DDT are *p,p′*-DDT and *o,p′*-DDT, but DDD and DDE compounds are present in the mixture as byproduct of manufacturing processes (Lunney et al., 2004). Due to high persistence and toxicity, the EPA has classified DDT, DDD and DDE as priority pollutants for control. Although many interesting studies on DDE uptake by vascular plants such as alfalfa, ryegrass, pole bean, *Cucurbita* and *Cucumis* have been undertaken under field conditions (White, 2001, 2002; White et al., 2003, 2005), very few information is available on the accumulation of DDTs by other plant species. Therefore, it is necessary to identify more plant species with potential for the cleanup of chemical residues like DDTs.

Castor (*Ricinus communis*) species belongs to Euphorbiaceae family, a fast growing C3 plant, native to tropical Africa. It is an industrial crop because of its oil quality and quantity for plant-based industries for making eco-friendly paints and coatings used in chemical industry (Rajkumar and Freitas, 2008). Castor attracted attention because of its ability to grow in heavily polluted soil together with its capacity for metal ion accumulation and fast growth rate (Rajkumar and Freitas, 2008; Shi and Cai, 2009). In addition, castor is an industrial crop with multiple non-food uses and an excellent rotation and companion crop. It has economic advantage as a cash crop in modern agriculture along with remediation of heavy metal contaminated soils (Rajkumar and Freitas, 2008; Vamerali et al., 2010). However, there is no information

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available about *R. communis* in accumulating DDTs and Cd in metals/organic co-contaminated soil systems.

This study was attempted to investigate the phytoremediation of residual DDTs and Cd in co-contaminated agricultural soil using castor genotypes through pot-culture experiments, and to identify castor genotypes with greater ability in accumulating DDTs and Cd for phytoremediation use with great bio-energy value.

2.Methods

2.1. Experimental design

The aged DDTs contaminated soil was collected from the surface layer (0–20 cm) of soils in Cixi county of Zhejiang Province, China, which had been polluted due to extensive and indiscriminate use of DDT for cotton production before it was banned several decades ago. Soil samples were air-dried and grounded to pass a 2-mm nylon sieve prior to use. The soil was classified as sandy loam (82% sand, 14% silt, and 4% clay) with 0.35 mg kg^{-1} of DDTs, and 0.42 mg kg^{-1} of total Cd. The soils were spiked with DDTs and Cd at the rate of 1.7 and 2.8 mg kg^{-1} soil, respectively, 1.5 kg of the treated soil was placed in each plastic pot. The moisture of soils in the pot was adjusted with distilled water to 60% of water holding capacity and maintained at this moisture level for one month incubation at natural temperature and light in field before planting *R. communis* seeds obtained from Oil Crop Research Institute, Chinese Academy of Agricultural Sciences, Wuhan, China, were sowed directly into the soil. To simulate field conditions, the plants were cultivated under open field of agricultural experimental station of Zhejiang University, China and no fertilizers were added. There were three replications for each genotype of the castor plant and unplanted pots as control. Pots were watered daily to maintain 65% of water-holding capacity with minimal leaching. Two weeks after germination, the seedlings were thinned to two per pot. Plants were harvested for analysis after 2 months of growth.

2.2. Soil DDTs extraction procedure

Soil samples were collected from the pots immediately after plant harvest, thoroughly homogenized, freeze-dried and sieved to pass a 100-mesh sieve prior to analysis. DDTs extraction and cleanup were performed according to USEPA method 3550C (Ultrasonic extraction) and 3620C (Florisil cleanup) with minor modifications. Briefly, 5-g soil was placed in each glass vials with a Teflon cap, soaked with 15 ml hexane/acetone (1:1 v/v) overnight, ultrasonic extraction for 1 h with ultrasonic instrument (Ishine, China). After each extraction, separation was accomplished by centrifuging at 3300*g* force for 5 min. Supernatant samples were cleaned up by using a glass chromatographic column loaded with 1 cm height of anhydrous Na_2SO_4; 13 cm height of Florisil was suspended in *n*-hexane and then 1 cm height of anhydrous Na_2SO_4 from bottom to top. The DDTs sample was eluted with 30 ml of acetone/hexane (22:125 v/v) three times and then carefully concentrated near to dry in a rotary vacuum evaporator (BÜCHI Rotavapor, Germany) at 38 °C. Afterwards, 2 ml of chromatography grade hexane was added in rotary steam bottle, mixed and filtered through 0.22-μm organic phase membrane and samples were sealed in vials for analysis.

2.3. Plant DDTs extraction procedure

Root, stem, and leaves of harvested castor plants were thoroughly rinsed with tap water to remove attached soil particles, and then carefully washed with ultra pure water. The freeze-dry plant tissues were weighed immediately, and ground into powder of less than 0.25 mm with an agate ball mill (Retsch RS100, Germany) prior to analysis. DDTs extraction of plant sample (about 0.5 g of roots and 1 g of shoots) was the same as that for soil (using 15 ml hexane/acetone (4:1 v/v)). Cleanup procedure for plant samples included sulfonation (EPA 3660B) and Florisil column chromatography (EPA 3620C) in sequence to remove photosynthetic pigments, lipids and other co-extractants.

2.4. DDTs analysis and quality control

DDTs concentrations in soil or plant extracts were determined using GC-μECD (Agilent 7890A, USA) with a capillary column (J&W 123-7732, 30 m × 320 μm × 0.25 μm) and an auto injector system according to USEPA method 8081B (2007). DDTs were identified by retention time against standards and quantified using peak area integration. Standard samples of *p,p′*-DDT, *o,p′*-DDT, *p,p′*-DDD and *p,p′*-DDE (purity >99.5%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). External standards of DDT and DDD were prepared in hexane and standard curve-fit linear line. Recovery of spiked DDTs was 95.6% (±3.5) for soil samples and 96.2% (±4.1) for plant samples.

2.5. Cadmium analysis

Sub-samples of plant were digested with 5 ml of concentrated HNO_3 and 1 ml $HClO_4$ in closed Teflon vessels until the solution was clear at 170 °C, and soil samples were digested with 5 ml concentrated HNO_3, 1 ml HF, and 1 ml of $HClO_4$ at 180 °C. Cadmium concentrations in the digested samples were determined using inductively coupled plasma optical emission spectrometer (Thermo Scientific ICAP 6000 series, USA).

2.6. Data analysis

Statistical analyses were carried out by ANOVA tests and means were compared by the LSD test ($p < 0.05$) using the statistics analysis system (SAS, version 8.0). Graphical work was performed using Origin v.8.

3. Results and discussion

3.1. Plant growth and biomass

After grown for two months in pots under field conditions, the average heights of plants were 54 cm, and total dry weight was 8.3 g per pot. Significant ($p < 0.05$) differences in root and shoot biomass were observed among the 23 genotypes (Fig. 1). The roots of castor spread to the entire soil core in the pots during the experimental period, and the average root weight accounted for 28% of the total biomass. Castor has a massive root system, which penetrates soil depth to several meters, much deeper than other herbaceous plants. Root is the first part of plants to be exposed to heavy metals or POPs in contaminated soil, lush root increased contact with soil contaminants and more pollutants can been absorbed by the plants. This is the most interesting advantage of castor over other plants for polluted soil remediation.

3.2. DDTs and Cd concentration in plants

Significant ($p < 0.05$) differences in Cd and DDTs concentrations were observed among the 23 tested genotypes of castor (Fig. 2). Across all the genotypes, concentrations of Cd and DDTs were considerably higher in roots than in shoots, which agrees with previous reports on spinach, alfalfa, ryegrass, pole bean, teosinte, maize and tall fescue (Lunney et al., 2004; Mo et al., 2008; White,

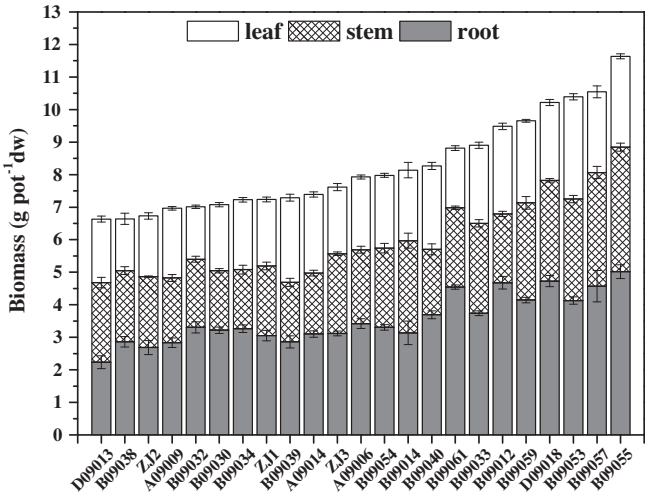

Fig. 1. The differences in leaf, stem, and root dry biomass of 23 genotypes of *Ricinus communis* after 2 months of growth in Cd and DDTs co-contaminated soil.

2001; White et al., 2005) as well as some energy crops (Shi and Cai, 2009), but is different from the distribution of DDTs in pigeon pea, zucchini, and pumpkin (Lunney et al., 2004; White, 2001; White et al., 2005).

The mean concentrations of DDTs in leaf, stem and root were 0.37, 0.43 and 70.51 mg kg^{-1} dw, respectively, which was higher than those found in the shoot of alfalfa, ryegrass, maize or tall fescue (Lunney et al., 2004; Mo et al., 2008), but lower than those in zucchini and pumpkin grown in high DDT-contaminated soils (Lunney et al., 2004). In this study, castor root was found to have a higher DDTs concentration, as compared to zucchini and pumpkin (Lunney et al., 2004), or alfalfa (Mo et al., 2008). The initial concentration of DDTs in soils was 1.7 mg kg^{-1}, 15 times lower than that reported by Mo et al. (2008). This result indicates that the castor plant has an exceptional capacity to accumulate DDTs in its developed root system. The mean Cd concentrations in the leaf, stem and root of castor were 1.22, 2.27 and 37.63 mg kg^{-1} dw, respectively. Shi and Cai (2009) have found that *R. communis* is more tolerant than other energy crops at spiked levels of 50–200 mg Cd kg^{-1} soil in a pot experiment. This result implies that castor species might be a Cd-excluder plant tolerant to high Cd stress.

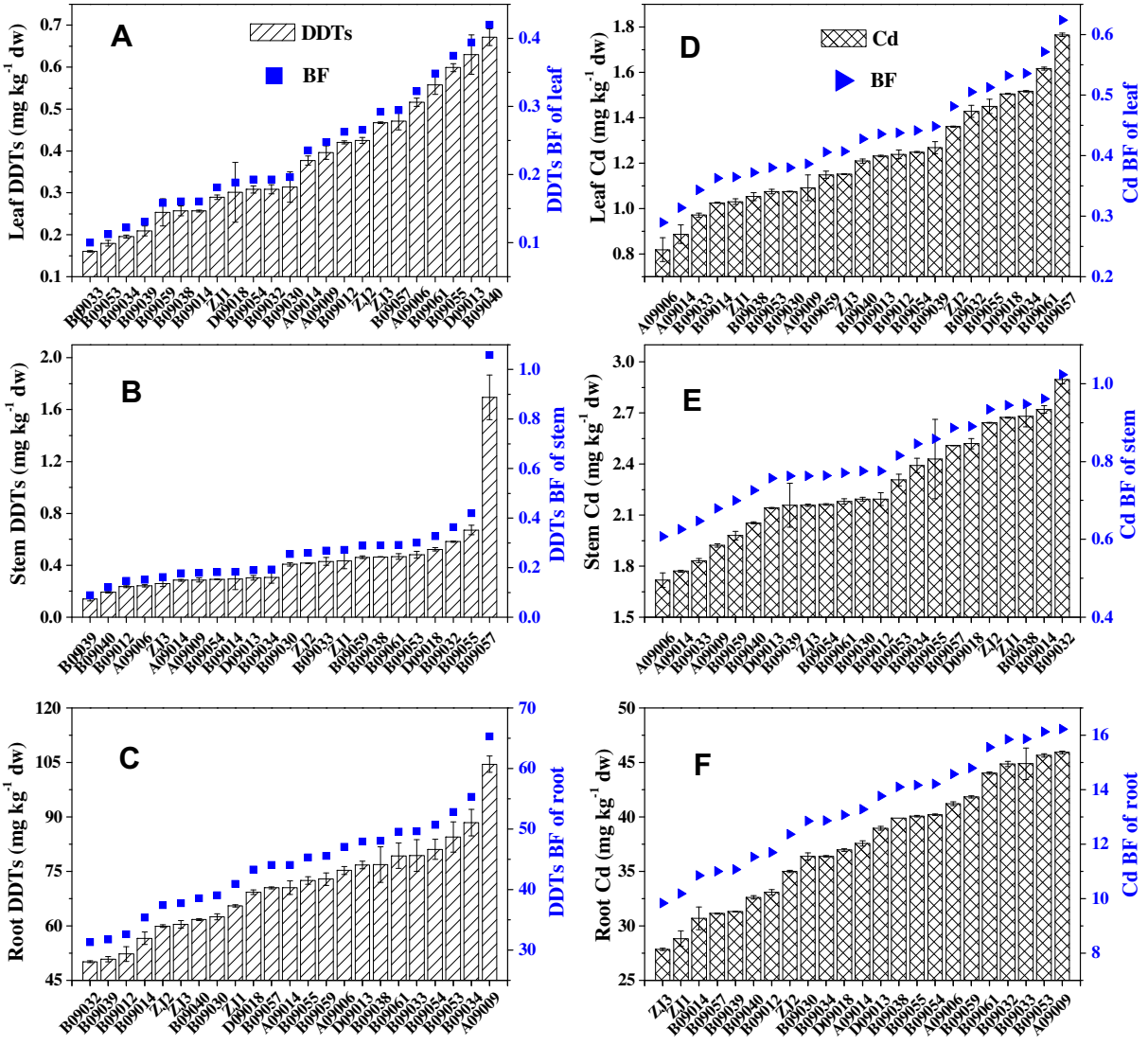

Fig. 2. Concentrations and bioconcentration factors (BF) of DDTs (A–C) or Cd (D–F) in leaf, stem, and root of 23 genotypes of *Ricinus communis*.

Table 1
Translocation factor (TF) of DDTs and Cd for different castor (*Ricinus communis*) genotypes.

Variety name	DDTs		Cd	
	TF_{leaf}	TF_{stem}	TF_{leaf}	TF_{stem}
A09006	0.0068 bc	0.0032 g	0.0199 de	0.0417 f
A09009	0.0038 e	0.0027 g	0.0238 d	0.0418 f
A09014	0.0053 cd	0.0040 f	0.0236 d	0.0471 e
B09012	0.0080 b	0.0045 f	0.0374 c	0.0663 c
B09014	0.0045 d	0.0052 e	0.0334 c	0.0886 a
B09030	0.0050 d	0.0065 de	0.0296 d	0.0603 d
B09032	0.0062 c	0.0116 b	0.0318 c	0.0645 c
B09033	0.0020 f	0.0054 e	0.0216 d	0.0408 f
B09034	0.0022 f	0.0035 fg	0.0417 b	0.0657 c
B09038	0.0033 e	0.0060 de	0.0264 d	0.0672 c
B09039	0.0041 de	0.0028 g	0.0405 b	0.0689 b
B09040	0.0109 a	0.0031 g	0.0371 c	0.0630 c
B09053	0.0021 f	0.0057 de	0.0235 d	0.0505 e
B09054	0.0038 e	0.0036 f	0.0311 cd	0.0538 de
B09055	0.0083 b	0.0093 bc	0.0362 c	0.0606 d
B09057	0.0067 c	0.0240 a	0.0567 a	0.0806 b
B09059	0.0035 e	0.0063 d	0.0274 cd	0.0473 e
B09061	0.0070 bc	0.0059 de	0.0367 bc	0.0495 e
D09013	0.0082 b	0.0039 f	0.0316 c	0.0550 d
D09018	0.0043 d	0.0076 c	0.0407 b	0.0682 b
ZJ1	0.0044 d	0.0067 cd	0.0357 c	0.0927
ZJ2	0.0071 bc	0.0069 cd	0.0389 c	0.0755 b
ZJ3	0.0077 b	0.0043 f	0.0414 b	0.0775 b

Translocation factor (TF) expressed as the DDTs or Cd concentration ratio of the shoots to the roots. TF_{leaf} is leaf bioconcentration factor and TF_{stem} is stem bioconcentration factor.

3.3. Genotypic difference in DDTs and Cd uptake

The bioconcentration factor (BCFs) of castor genotypes for DDTs varied from 0.10 to 0.42 in leaf, 0.09 to 1.06 in stem, and 31.34 to 65.33 in root (Fig. 2). These values were different from those reported for maize, forage species, alfalfa and ryegrass (Lunney et al., 2004; Mo et al., 2008) or lettuce and radish grown in contaminated horticultural soils (Gaw et al., 2008). The castor plant had a much higher root BCFs values (31–65) than other tested plants including tall fescue and alfalfa (with a root BCF about 1.2), and zucchini, pumpkin (with a root BCF < 1.0) (Lunney et al., 2004). The average BCFs of castor genotypes for Cd was 0.43, 0.80 and 13.30 in leaf, stem and root, respectively, higher than the values reported by Shi and Cai (2009). These results confirmed that the castor plant has an exceptional capacity for the accumulation of DDTs, particularly in root when grown in contaminated soils.

Translocation factor (TF) is another indicator reflecting pollutant transfer to shoots from the roots (Mo et al., 2008). The calculated DDTs TF values for different castor genotypes were generally <1.0, ranging from 0.002 to 0.0109 for leaf and from 0.0027 to 0.024 for stem (Table 1). This result implied that most DDTs absorbed by castor plant were retained in roots with a small portion being translocated to the shoots. Kiflom et al. (1999) and Mo et al. (2008) have suggested that, apart from the biological processes of DDTs entering plant roots, it is likely that some DDTs can remain adsorbed on the external root surface even though the roots are rinsed thoroughly, and consequently the DDTs concentration in the roots is overestimated. The high DDTs content in castor root might be also related to its vitality with strong stretch ability to explore the soil. The castor had a Cd TF value of 0.0333 for leaf and 0.0620 for stem, similar to the observations by Shi and Cai (2009).

The total amounts of DDTs and Cd accumulated in each plant varied from 83.1 (B09032) to 267.8 μg (B09053), and 66.04 (ZJ3) to 155.1 μg (B09053), respectively (Fig. 3). Root DDTs and Cd respectively accounted for 95.6–99.4% and 82.1–93.7% of total plant uptake, due to higher concentrations of DDTs and Cd in the roots and comparable root biomass to the shoot. The amounts of DDTs accumulated in castor were much higher than most plants reported in previous studies (Gaw et al., 2008; Kiflom et al., 1999; Lunney et al., 2004; Mo et al., 2008; White et al., 2005), but was lower than *p,p′*-DDE accumulation in pumpkin (White, 2002). This result implies that castor plant has great potential for phytoremediation of DDTs/Cd co-contaminated soils, particularly if root biomass can be harvested and removed.

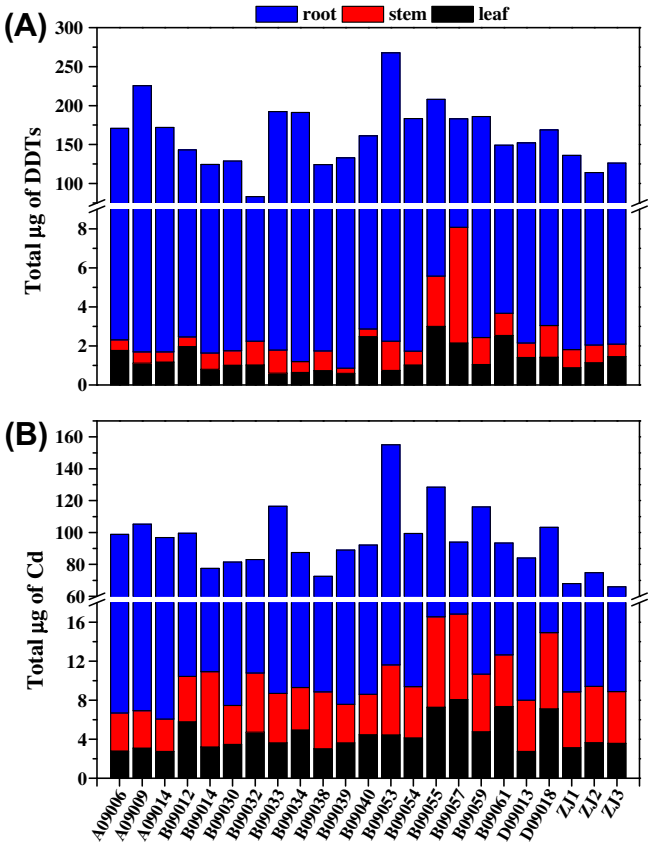

Fig. 3. Total amounts of DDTs (A) and Cd (B) in plant extracted by 23 genotypes of *Ricinus communis*.

4. Conclusion

The bio-energy crop *R. communis* can be considered as a DDTs and Cd accumulator plant species. This plant has a greater accumulation factor for these contaminants than most plants reported in literature. There is difference in the accumulation and translocation of DDTs and Cd among the different genotypes of the plant. B09053 accumulated 267.8 μg DDTs and 155.1 μg Cd per pot after two months of growth in the co-contaminated soils due to its high biomass production. Therefore, some of the castor genotypes are promising candidates for phytoremediation of DDTs/Cd co-contaminated soils.

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