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Wu, N. Y., Zhang, S. Z., Huang, H. L., Shan, X. Q., Christie, P., & Wang, Y. S. (2008). DDT uptake by arbuscular mycorrhizal alfalfa and depletion in soil as influenced by soil application of a non-ionic surfactant. *Environmental Pollution*, 151(3), 569-575. <https://doi.org/10.1016/j.envpol.2007.04.005>

Published in:
Environmental Pollution

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DDT uptake by arbuscular mycorrhizal alfalfa and depletion in soil as influenced by soil application of a non-ionic surfactant

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Received 13 November 2006; received in revised form 30 March 2007; accepted 8 April 2007

Combined colonization of alfalfa roots by an arbuscular mycorrhizal fungus and addition of non-ionic surfactant to the soil promoted root and shoot uptake and soil dissipation of DDT.

Abstract

A greenhouse pot experiment was conducted to investigate the colonization of alfalfa roots by the arbuscular mycorrhizal (AM) fungus *Glomus etunicatum* and application of the non-ionic surfactant Triton X-100 on DDT uptake by alfalfa and depletion in soil. Mycorrhizal colonization led to an increase in the accumulation of DDT in roots but a decrease in shoots. The combination of AM inoculation and Triton X-100 application enhanced DDT uptake by both the roots and shoots. Application of Triton X-100 gave much lower residual concentrations of DDT in the bulk soil than in the rhizosphere soil or in the bulk soil without Triton X-100. AM colonization significantly increased bacterial and fungal counts and dehydrogenase activity in the rhizosphere soil. The combined AM inoculation of plants and soil application of surfactant may have potential as a biotechnological approach for the decontamination of soil polluted with DDT.

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Keywords: Phytoremediation; Alfalfa; DDT; *Glomus etunicatum*; Triton X-100

1. Introduction

Contamination of soils by persistent organic pollutants (POPs) is a widespread environmental problem and their removal from soil has become a major concern. As a result, remediation methodologies are of significant interest and considerable attention has been focused on phytoremediation due to its convenience, cost-effectiveness and environmental acceptability (Suresh and Ravishankar, 2004).

The organochloride insecticide dichlorodiphenyltrichloroethane (DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane)

was used throughout the world for several decades to control arthropod disease vectors and agricultural pests. Soil DDT contamination levels up to 7.5 mg kg⁻¹ have been reported (Kantachote et al., 2004). Although its use in agriculture was banned in China in 1983, unfortunately, it appears that its residues and metabolites DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane) and DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) are still widely distributed in soils. China still has two production plants producing DDT as an intermediate for dicofol production and control of malaria (Li et al., 2006). There is a wealth of information demonstrating that POPs such as DDT have low availability/mobility because of their sequestration or weathering in soil. Nonetheless, there have been some greenhouse and field-scale studies of weathered DDT or DDE uptake in a range of plants with accumulation

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concentrations at the mg kg^{-1} level (Lunney et al., 2004; White, 2002; White et al., 2006). Extensive information exists on the negative effects of DDT and its metabolites on the environment and on human health (Vieira et al., 2001; Binelli and Provini, 2003).

Arbuscular mycorrhizal (AM) fungi are ubiquitous in terrestrial ecosystems, forming symbiotic associations with roots of the majority of plant species (Smith and Read, 1997). The ubiquity of AM fungi, their capacity to enhance the tolerance of host plants to organic contaminants, and their interactions with soil microorganisms in the rhizosphere have led to recent studies on their interactions with organic pollutants in the context of phytoremediation (Joner and Leyval, 2003a; Huang et al., 2007). Research has established that AM fungi can enhance the growth of several plant species in soil with high polycyclic aromatic hydrocarbon (PAH) concentrations (Leyval and Binet, 1998). Experimental evidence has been obtained for the impact of AM on the uptake and dissipation of some organic pollutants in soil such as PAHs and atrazine (Joner and Leyval, 2003b; Huang et al., 2007). These studies indicated that AM fungi conferred some benefit on phytoremediation of soils contaminated with organic pollutants.

The POPs in soil usually exhibit limited bioavailability to both microorganisms and plants due to their strong affinity to the soil matrix, especially soil organic matter (Chen et al., 2005). Phytoremediation of soils contaminated with POPs is thus hindered by sorption of the pollutants to soil organic matter and their low aqueous solubility (Boldrin et al., 1993; Stucki and Alexander, 1987). Solubilization agents such as surfactants have been added to soil to enhance the release of POPs from the sorbed phase and, thereby, to increase their aqueous concentrations and bioavailability (Zhou and Zhu, 2005). Although addition of surfactants has been explored in the cleanup of contaminated soils (Zhu et al., 2003), there are few reports of their application in phytoremediation. We hypothesized that the combined application of AM fungi and surfactants to be an effective strategy for soil phytoremediation owing to the enhanced mobility of POPs in the soil due to the surfactants, together with increased degradation and plant uptake of the POPs as well as improved plant establishment due to mycorrhizal colonization.

In the present study we investigated the effects of AM fungal inoculation and the combined effects of AM fungal inoculation and soil application of the surfactant Triton X-100 on DDT uptake by alfalfa, dissipation of DDT and its metabolites and the enzyme activities and microbial populations in a soil which was artificially contaminated with various levels of DDT. The aim was to evaluate the potential of AM inoculation and surfactant application to soil for the phytoremediation of POPs.

2. Materials and methods

2.1. Experimental design

A pot experiment was conducted in which non-mycorrhizal and mycorrhizal plants with no soil application of surfactant and AM-inoculated plants

grown in soil amended with the non-ionic surfactant Triton X-100 was compared. The soil was spiked with high purity DDT (98% purity, Aldrich Chemical) dissolved in hexane to give application rates of 0.0, 2.5, 5.0 and 10.0 mg kg^{-1} and 10 mL Triton X-100 was added to each pot as a 0.065 g mL^{-1} solution to reach 0.1% (w/w) in the soil. The treatments were set up in triplicate.

2.2. Soil preparation

A loamy soil was collected from the surface (0–15 cm depth) of an experimental field at Beijing Academy of Agriculture and Forest Sciences. The selected characteristics are as follows: silt, 45%; clay, 23%; sand, 32%; pH, 7.74 (1:2, soil/water); organic matter, 2.7%; NaHCO_3 -extractable P, 3.9 mg kg^{-1} ; C, 1.95%; N, 0.24%; cation exchange capacity, $25.6 \text{ cmol kg}^{-1}$; and an initial DDT concentration of 0.13 mg kg^{-1} . Soil cation exchange capacity was analyzed using the method of Rhodes (1982). Other soil characteristics were determined following the methods described by Srivastava (1992). The soil was air-dried, ground and passed through a 2-mm nylon sieve. It was then mixed with sand (1–2 mm) in a ratio of 1:1 (w/w) to produce the growth medium. The soil mixture (henceforth referred to as the soil) was sterilized by γ -radiation (10 kGy, 10 MeV γ -rays) to inactivate AM fungi and received mineral nutrients at rates of 30 mg P (KH_2PO_4), 60 mg N (NH_4NO_3), and 67 mg K (K_2SO_4) kg^{-1} soil. It was then artificially spiked with DDT with thorough mixing. The soils were allowed to dry in a fume hood for 3 days. During this period the soil samples were shaken three times a day in order to mix the compounds with the soil thoroughly and to increase the hexane volatilization rate. Finally, the soils were shaken, homogenized and incubated for 4 weeks at room temperature. We left a plate in the growth chamber with some resin inside as adsorbent to test the volatilization of DDT.

2.3. Inoculum and host plants

Inoculum of the AM fungus *Glomus etunicatum* (BGC USA01) was propagated for 10 weeks in pot culture on broomcorn (*Sorghum vulgare* Pers.) plants grown in a loamy soil in a greenhouse. The inoculum, which was air-dried and passed through a 2-mm sieve, consisted of spores, mycelium, sandy soil and root fragments containing approximately 350 spores g^{-1} soil (dry weight soil basis).

Alfalfa seeds (*Medicago sativa* L.) were purchased from the Chinese Academy of Agricultural Sciences, Beijing, China. They were surface sterilized in a 10% (v/v) solution of hydrogen peroxide for 10 min, rinsed with sterile distilled water and pre-germinated on moist filter paper overnight and were then ready for sowing.

2.4. Pot experiment

Each pot received 650 g of incubated soil and was equilibrated in a growth chamber for 4 d at 70% of water holding capacity. Seven pre-germinated alfalfa seeds were sown in each pot and thinned to four seedlings after growth for 7 d. Mycorrhizal treatments received 50 g of the fungal inoculum by mixing with about 200 g of soil and then placing the mixture in the middle layer of the plant pots. In the non-mycorrhizal treatments the inoculum was replaced by an equivalent amount of sterilized soil–sand mixture. For the treatments with surfactant, Triton X-100 was watered into the soil after sowing to give a concentration of 0.1% (w/w). The pots were positioned randomly in the growth chamber and re-randomized every 2 days. The experiment was conducted in a controlled-environment growth chamber with a photoperiod of 14 h at a light intensity of $250 \mu\text{mol m}^{-2} \text{ s}^{-1}$ provided by supplementary illumination. The day/night temperature regime was $25^\circ\text{C}/20^\circ\text{C}$ and the relative humidity was maintained at 70%. Distilled water was added as required to maintain soil moisture content at 70% of water holding capacity by regular weighing. Nitrogen fertilizer (as NH_4NO_3) was added to each pot 30 and 45 d after sowing to provide a total of 120 mg N per pot.

2.5. Sample preparation

Plants were harvested after growth for 60 d. Pots were left unwatered for 2 d prior to harvest. Shoots and roots were harvested separately. Any part of the plant above the soil surface was considered as shoot material and the part below the soil surface was included in the roots. Root fragments were collected by sieving the soil and adding them to the root samples. Roots were first carefully washed with tap water to remove any adhering soil particles. Then roots and shoots were rinsed thoroughly with distilled water, blotted dry and weighed. Soils were then sampled from each pot. Bulk soil was collected by gently crushing the soil and shaking the roots. The soil that adhered to the root system was obtained by shaking the roots and operationally defined as rhizosphere soil (Lynch, 1990). For enumeration of bacteria and fungi, rhizosphere soil sub-samples were stored at 4 °C for microbial assays and were performed within a week. A portion of fresh root sub-sample was taken from each treatment for the determination of the proportion of root length colonized by the AM fungus. Soil samples and the remainder of the plant samples were freeze-dried, weighed, and stored at 4 °C. Some freeze-dried plant samples were weighed, oven dried at 105 °C for 24 h and re-weighed, and the differences between the oven dry weights and the freeze-dried weights were found to be negligible.

2.6. Adsorption of DDT on roots

Adsorption of DDT on roots was carried out in batch equilibration sorption experiments. Three milligram of freeze-dried roots from both inoculated and uninoculated treatments, from the non-spiked soil, were placed in 50-mL glass centrifuge tubes containing 35 mL of distilled water saturated with $7 \mu\text{g L}^{-1}$ DDT. They were then sealed with Teflon-lined screw caps and incubated for 0.5, 1.5, 5, 10, 24, and 48 h on a reciprocating shaker at 25 °C. Thirty milliliter of the sample solutions were taken at different time intervals after centrifuging for 20 min at 4000 rpm and extracted with 1 mL hexane in preparation for DDT analysis. The adsorption experiment was carried out in triplicate and the results were expressed as percentage of DDT adsorbed on roots.

2.7. Assessment of AM colonization of alfalfa roots

The proportion of total root length colonized by the AM fungus was assessed by cutting a random sub-sample of 1 g fresh roots into 0.5- to 1.0-cm-long segments. Root segments were cleaned in 10% KOH for 10 min at 90 °C in a water bath, rinsed in water, and then stained with 0.1% Trypan blue for 3–5 min at 90 °C in a water bath. Mycorrhizal colonization was determined by the grid line intersect method (Giovannetti and Mosse, 1980).

2.8. DDT analysis

All soil samples were subjected to Soxhlet extraction. One gram of soil was placed in a cellulose extraction thimble with an equivalent quantity of anhydrous sodium sulphate and extracted with 100 mL of hexane/acetone mixture (1:1, v/v) for 24 h. A cleanup step prior to analysis was mandatory in order to improve detection and quantification limits and to extend the column lifetime. The cleanup was done using a modification of the method of Gong et al. (2004). Briefly, the sample extract was transferred to a separating funnel and cleaned with 6 mL of concentrated sulfuric acid three times, then cleaned three times with 30 mL 2% Na_2SO_4 aqueous solution and dried by passing through a column packed with 2 g of anhydrous Na_2SO_4 . Finally, it was concentrated and ready for DDT analysis.

The same Soxhlet extraction was conducted on the tissue samples after they were chopped with pruning shears and ground up using a mortar and pestle, by which we obtained the contents of DDT inside the roots plus the DDT absorbed on the root surface. In order to detect the DDT fraction on the root surface, a sequential extraction was carried out before the Soxhlet extraction using a modification of the method of Chen et al. (2007). Three grams of mycorrhizal and non-mycorrhizal fresh roots from soil spiked with 10 mg kg^{-1} DDT were washed with distilled water and placed in 50 mL glass centrifuge tubes with Teflon-lined screw caps. They were then extracted with 20 mL of 0.01 mol L^{-1} CaCl_2 for 2 h and the bulk solution then filtered. The filtrate was extracted twice

with 20 mL hexane and the extracts were concentrated in a rotary evaporator. The CaCl_2 fraction represented DDT dissolved in the aqueous solution within the apparent free space of the roots. The fraction representing the DDT strongly adsorbed on the root surface was extracted with 20 mL hexane for 2 h. The hexane solution was then purified using the same method as that used for soils and plant tissues. Following the hexane extraction, the root tissues were freeze-dried, ground and subjected to Soxhlet extraction.

Extracts were analyzed using an Agilent 6890 GC equipped with a ^{63}Ni electron capture detector and a capillary column ($30 \text{ m} \times 0.25 \mu\text{m} \times 0.32 \text{ mm}$). The column oven was programmed from an initial temperature of 80 °C to 240 °C at a rate of $20 \text{ }^\circ\text{C min}^{-1}$, held for 1 min, and then ramped at a rate of $8 \text{ }^\circ\text{C min}^{-1}$ to 280 °C with a final hold time of 1 min. The detector and injector were maintained at 280 and 220 °C, respectively, and the injector was in the splitless mode. Nitrogen was the carrier gas at 1 mL min^{-1} . Injection volume was 1 μL in *n*-hexane. Recoveries for the extraction procedure and GC analysis were tested by spiking a proportion of DDT into the soil and the ground plant materials which were then incubated for 60 d. The recoveries averaged 90.3% with an RSD < 4.5% ($n = 5$).

2.9. Microbial enumeration and determination of enzyme activities

Culturable bacterial and fungal counts were made using the plate count method. Microbial counts were determined by means of three replicate rhizosphere soil samples. Plate counts of bacteria and fungi were estimated from 10^{-6} and 10^{-3} dilutions plated on beef extract peptone and Martin's medium which were incubated at 30 °C for 2 d and 5 d, respectively. After incubation the numbers of bacterial cells and fungi were counted.

Rhizosphere dehydrogenase activity was assayed using a modification of the method of Dick et al. (1996). Six grams of rhizosphere soil were thoroughly mixed with 0.06 g CaCO_3 and three replicate samples were placed in test tubes and 3 mL of 1% 2, 3, 5-triphenyltetrazolium chloride (TTC) and 2.5 mL distilled water were added. Samples were incubated at 37 °C for 24 h and then 10 mL methanol added to each tube and the samples were vortexed. The soil suspensions were then filtered and the filtrates were diluted with methanol to 50 mL. The color's intensity was measured at 485 nm using a spectrophotometer. Dehydrogenase activities in the samples were calculated using calibration curves prepared from 2, 5, 10, and 20 and 30 μg triphenyl formazan (TPF) mL^{-1} standards. Results are presented as mg TPF g^{-1} soil.

2.10. Data analysis

Data were subjected to two-way analysis of variance using the SPSS version 10.0 software package to determine the significance of soil DDT concentration and AM inoculation with or without the surfactant as sources of variation. Comparisons between means were carried out using Duncan's multiple range test at a significance level of $p < 0.05$.

3. Results

3.1. Root colonization and plant biomass

Table 1 displays the mycorrhizal colonization and biomass of alfalfa after growth for 60 d. No mycorrhizal colonization was observed in the roots of uninoculated plants. Mycorrhizal colonization of the roots of inoculated plants decreased from 70.3 to 49.7% with increasing DDT concentration from 0 to 10 mg kg^{-1} . Mycorrhizal root colonization varied from 66.2 to 49.1% for the treatments that included Triton X-100. Mycorrhizal plants had higher shoot yields and lower root yields than non-mycorrhizal controls with only one exception when DDT was applied at 10 mg kg^{-1} . DDT application generally increased shoot and root biomass and application of Triton

Table 1
Mean shoot and root dry matter yield (freeze-dried basis) and proportion of root length colonized by the AM fungus after cultivation of mycorrhizal alfalfa with or without surfactant and non-mycorrhizal controls in soil containing different levels of added DDT^a (mean $\pm$ SE, $n = 3$)

Initial DDT addition (mg kg ⁻¹)	Mycorrhizal status/surfactant addition	Shoot weight (g pot ⁻¹)	Root weight (g pot ⁻¹)	Mycorrhizal colonization ^b (%)
0.0	Non-mycorrhizal	0.91 $\pm$ 0.04g	0.70 $\pm$ 0.03c	0.0
	Mycorrhizal	1.16 $\pm$ 0.07bd	0.65 $\pm$ 0.06fh	70.3 $\pm$ 1.29a
	Mycorrhiza + surfactant	0.82 $\pm$ 0.03gh	0.56 $\pm$ 0.01g	66.2 $\pm$ 0.83b
2.5	Non-mycorrhizal	1.16 $\pm$ 0.09bcdef	0.74 $\pm$ 0.10d	0.0
	Mycorrhizal	1.29 $\pm$ 0.01abde	0.66 $\pm$ 0.09eh	68.5 $\pm$ 0.57a
	Mycorrhiza + surfactant	1.05 $\pm$ 0.05efg	0.62 $\pm$ 0.02bcdefgh	59.2 $\pm$ 3.03c
5.0	Non-mycorrhizal	1.14 $\pm$ 0.12de	1.06 $\pm$ 0.06a	0.0
	Mycorrhizal	1.34 $\pm$ 0.11ac	0.63 $\pm$ 0.09f	52.7 $\pm$ 2.52d
	Mycorrhiza + surfactant	1.37 $\pm$ 0.08ab	0.84 $\pm$ 0.13b	51.3 $\pm$ 3.27d
10.0	Non-mycorrhizal	1.23 $\pm$ 0.04abdef	0.82 $\pm$ 0.03bcd	0.0
	Mycorrhizal	1.39 $\pm$ 0.04a	0.85 $\pm$ 0.06abcde	49.5 $\pm$ 2.53d
	Mycorrhiza + surfactant	1.00 $\pm$ 0.05fh	0.79 $\pm$ 0.06c	49.2 $\pm$ 1.22d
Significance of DDT level		***	***	***
Mycorrhizal status/surfactant		***	***	***
DDT $\times$ mycorrhizal status/surfactant		*	***	***

*Significant by analysis of variance at $p < 0.05$.

***Significant by analysis of variance at $p < 0.001$.

^a Values followed by the same letter within a column are not significantly different according to Duncan's multiple range test at the 5% level.

^b Colonization of NM plants was 0%, it was therefore excluded from statistical analysis of effects of DDT/surfactant on mycorrhizal colonization.

X-100 decreased them. Although these yield effects were statistically significant they were numerically small.

3.2. Accumulation of DDT in plants

DDT accumulation in alfalfa is shown in Fig. 1. Roots accumulated much more DDT than did shoots. DDT concentrations in both shoots and roots increased markedly with increasing DDT application rate to soil irrespective of inoculation treatment. AM inoculated roots accumulated consistently more DDT than non-mycorrhizal roots. Addition of Triton X-100 led to increased DDT concentrations in roots. Mycorrhizal plants showed lower shoot DDT accumulation than non-mycorrhizal controls, but application of Triton X-100 to the soil significantly elevated DDT accumulation in the shoots ($p < 0.05$).

3.3. Dissipation of DDT in soils

Negligible changes in the DDT concentration in the spiked soils were detected after aging for 4 weeks. After harvest, only DDT was detected in the bulk soil samples but the two metabolites DDD and DDE were detected in the rhizosphere soils from all treatments (Fig. 2). However, the concentrations were very low and no clear differences in concentrations could be detected among the different treatments. The DDT concentrations in soil decreased markedly compared with the initial levels and this effect was more pronounced in the rhizosphere soil than in the bulk soil. A 66.8–95.4% reduction of the initially added DDT was observed in the rhizosphere soils compared to 13.1–64.8% in the bulk soils. An interesting observation was that in the presence of Triton X-100 the DDT residual concentrations were much lower in the bulk soils than in the

rhizosphere soils as well as in bulk soils without Triton X-100 application. For instance, when 10.0 mg kg⁻¹ of DDT was added to the soil, the DDT concentration decreased to 27.3% of the original level in the bulk soil compared to 87.8% in the bulk soil in the absence of Triton X-100 and 47.3% in the rhizosphere soil in the presence of Triton X-100.

3.4. DDT adsorption on roots

DDT sorption on mycorrhizal and non-mycorrhizal roots increased rapidly during the first 5 min and then remained constant (Fig. 3). A maximum of up to 79.6% of DDT was sorbed on mycorrhizal roots compared to 80.3% on non-mycorrhizal roots. DDT sorption on non-mycorrhizal and on mycorrhizal roots was not significantly different ($p > 0.05$).

3.5. Microbial assays and dehydrogenase activities

Soil microbial counts and dehydrogenase activities in rhizosphere soil varied among the treatments (Table 2). Total culturable bacteria and fungi varied from 1.0×10^7 to 3.4×10^7 cells g⁻¹ and 2.8×10^7 to 4.4×10^7 cells g⁻¹. The mean bacterial numbers were at least three orders of magnitude higher than the fungal counts. AM inoculation significantly increased bacterial and fungal counts. Dehydrogenase activity changed in a similar way to the counts of soil microorganisms.

4. Discussion

A major finding of the present study is the impact that AM fungal colonization and Triton X-100 had on the accumulation of DDT in alfalfa roots and shoots. We hypothesized that such

Fig. 1. DDT concentrations in alfalfa (a) shoot and (b) root tissues by Soxhlet extraction. Data are means of three replicates and on a dry matter (freeze-dried) basis. Non-mycorrhizal (□); Mycorrhizal (▨); Mycorrhiza + surfactant (■). Bars, standard errors. Values followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% level.

an observation might be attributable mainly to an increase in the adsorption of DDT on colonized roots. In order to test this hypothesis we examined DDT sorption by excised root tissues and found that DDT adsorption was consistently higher on non-mycorrhizal than on mycorrhizal roots. There was no significant difference between mycorrhizal and non-mycorrhizal plants at the 5% level (Fig. 3). The adsorption experiment had some limitations due to the low solubility of DDT in water and it was not possible to elucidate the sorption behavior of DDT at high concentrations by experiment or by isotherm model prediction. We therefore proceeded to use a sequential extraction procedure with CaCl_2 and hexane to extract DDT from the fresh roots harvested from the soil spiked with 10 mg kg^{-1} DDT before Soxhlet extraction in order to clarify the impact of AM fungal colonization on the affinity of roots for DDT. The CaCl_2 extract fraction, which was considered as loosely adsorbed on root surfaces, was too low to be detected.

The hexane fraction (strongly adsorbed fraction) of DDT accounted for 5.6% and 17.2% of the total amount of DDT in mycorrhizal and non-mycorrhizal roots, respectively. This result confirms that sorption and sequestration of DDT was higher on mycorrhizal roots than on non-mycorrhizal roots and it might be ascribed to the formation of abundant extraradical mycelium (Joner et al., 2001) which has a high affinity with hydrophobic organic compounds. The combination of mycorrhizal inoculation and the surfactant Triton X-100 increased DDT accumulation in both roots and shoots. Fig. 1 shows much greater DDT accumulation in the roots in the presence of Triton X-100, especially at the highest DDT dose rate studied, which may have contributed to the increase in DDT translocation to the shoots. Another possible explanation for the enhanced accumulation of DDT in mycorrhizal roots could be an increase in the mobility and bioavailability of the contaminant in soil. It is possible that the AM fungus stimulated the mineralization of DDT in the soil via its effects on soil organisms as can be seen from the difference in bacterial counts, which in turn may have led to the changes in DDT availability. However, elucidation of any direct effect needs to be addressed by further work.

After plants' harvest the total residual concentrations of DDT in soil decreased significantly (Fig. 2). The trends following addition of Triton X-100 to the soil are of particular interest. The non-ionic surfactant greatly decreased the residual concentrations of DDT in the bulk soil while increasing the concentrations in the rhizosphere soil. Earlier studies (Kile and Chiou, 1989) have found that surfactants such as the Triton series and Brij35 significantly enhanced DDT solubility in soils owing to micelle formation. Increased solubility of DDT in soil as well as enhanced plant uptake of water facilitated by AM colonization (Morales Vela et al., 2007) may promote mass flow of DDT towards roots and sequestration in the rhizosphere, a soil zone which may promote DDT degradation and may also contain ample sites for adsorption of POPs on organic matter. Furthermore, formation of abundant extraradical mycelium by AM colonization can also benefit the sequestration of DDT in the rhizosphere zone. The rhizosphere therefore acts as a sink for DDT. This could be very important in phytoremediation because sequestration of POPs in the soil and limited bioavailability are important obstacles in the application of bioremediation methods.

Phytoremediation of organic pollutants depends on plant–microbe interactions in the rhizosphere (Joner and Leyval, 2003b). We observed a pronounced influence of the AM fungus on soil microbial abundance. It is now well established that AM fungi modify root functions (i.e. root exudation), change carbohydrate metabolism of the host plant and influence rhizosphere populations (Duponnois et al., 2005). Furthermore, AM fungi can exude substances that have a selective effect on the microbial community in rhizosphere soil and thus modify the microbial communities. Previous studies (Stelmack et al., 1999; Chen et al., 2000) have demonstrated inhibitory effects of non-ionic surfactants on bacterial growth, even under the critical micelle concentration. However, we did not find any significant

Fig. 2. Total DDT concentrations in (a) rhizosphere and (b) bulk soil. Data are means of three replicates. Non-mycorrhizal (□); Mycorrhizal (▨); Mycorrhiza + surfactant (▩). Bars, standard errors. Values followed by the same letter are not significantly different according to Duncan's multiple range test at the 5% level.

Fig. 3. DDT adsorbed on alfalfa roots. Results are expressed as percentage of DDT adsorbed on roots ($n = 3$). Bars, standard errors.

difference in the microbial counts ($p > 0.05$) between the mycorrhizal and mycorrhizal plus surfactant treatments. Negative effects of surfactant application might be alleviated by AM colonization.

5. Conclusions

To our knowledge, the present study is the first to report the effects of arbuscular mycorrhizal colonization combined with application of surfactant to the soil on DDT uptake by plants and DDT dissipation in the soil. AM colonization assisted in the uptake of DDT by alfalfa roots and the combined effects of AM colonization and Triton X-100 increased the accumulation of DDT in both the roots and shoots. In addition, Triton

Table 2

Mean bacterial and fungal counts (CFU/g) and soil dehydrogenase activity in rhizosphere soil^a (mean $\pm$ SE, $n = 3$)

Initial DDT addition (mg kg ⁻¹)	Mycorrhizal status/surfactant addition	Dehydrogenase (mg/kg/24 h)	Bacterial counts ($\times 10^7$)	Fungal counts ($\times 10^4$)
0.0	Non-mycorrhizal	15.3 $\pm$ 0.80i	1.42 $\pm$ 0.09f	3.64 $\pm$ 0.21cd
	Mycorrhizal	47.5 $\pm$ 1.30h	2.41 $\pm$ 0.15cd	4.20 $\pm$ 0.28abc
	Mycorrhiza + surfactant	14.8 $\pm$ 0.30i	2.22 $\pm$ 0.07d	3.92 $\pm$ 0.14c
2.5	Non-mycorrhizal	200.7 $\pm$ 2.14f	1.75 $\pm$ 0.04e	3.93 $\pm$ 0.13c
	Mycorrhizal	216.0 $\pm$ 10.23df	2.46 $\pm$ 0.1cd	4.64 $\pm$ 0.18a
	Mycorrhiza + surfactant	78.1 $\pm$ 2.73g	2.31 $\pm$ 0.07cd	4.17 $\pm$ 0.17ac
5.0	Non-mycorrhizal	216.1 $\pm$ 6.06d	2.30 $\pm$ 0.22cd	3.10 $\pm$ 0.36de
	Mycorrhizal	323.2 $\pm$ 4.79a	2.87 $\pm$ 0.05b	4.43 $\pm$ 0.13ab
	Mycorrhiza + surfactant	169.9 $\pm$ 2.91e	2.50 $\pm$ 0.43bcd	4.0 $\pm$ 0.23bc
10.0	Non-mycorrhizal	277.4 $\pm$ 5.65c	2.62 $\pm$ 0.19bc	2.67 $\pm$ 0.09e
	Mycorrhizal	307.8 $\pm$ 5.12b	3.41 $\pm$ 0.15a	3.25 $\pm$ 0.19d
	Mycorrhiza + surfactant	215.9 $\pm$ 3.56d	3.00 $\pm$ 0.16ab	2.83 $\pm$ 0.1e

Significance of

DDT level

Mycorrhizal status/surfactant

DDT $\times$ mycorrhizal status/surfactant

n.s.

n.s.

***Significant by analysis of variance at $p < 0.001$.

n.s., Not significant.

^a Values followed by the same letter within a column are not significantly different according to Duncan's multiple range test at the 5% level.

X-100 increased the mobility and thereby the transport of DDT from the bulk soil to the rhizosphere, an effect which may have promoted both plant uptake and soil degradation of DDT. The combined use of AM inoculum and surfactants may have some potential as a biotechnological approach for the decontamination of soils contaminated with organic pollutants. However, detailed and comprehensive studies on the selection of plant species, AM fungi and surfactants will be required to effective remediation methods under field conditions.

Acknowledgements

This work was funded by the National Natural Science Foundation of China (Project 20677072, 20621703) and the Hi-tech Research and Development Program of China (Project 2006AA06Z349).

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