Ecological Engineering 71 (2014) 501–508

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Assessment of tolerance and efficiency of crop species in the phytoremediation of DDT polluted soils

Francesca M. Mitton[a], Karina S.B. Miglioranza[a], Mariana Gonzalez[a,*], Valeria M. Shimabukuro[a], José M. Monserrat[b]

[a] Laboratorio de Ecotoxicología y Contaminación Ambiental, Instituto de Investigaciones Marinas y Costeras (IIMyC), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata (UNMdP)—Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Dean Funes 3350, Mar del Plata 7600, Argentina

[b] Universidade Federal de Rio Grande—FURG, Instituto de Ciências Biológicas (ICB), Rio Grande, RS, Brazil

ARTICLE INFO

Article history:
Received 14 February 2014
Received in revised form 13 June 2014
Accepted 20 July 2014

Keywords:
Soil pollution
Phytoremediation
DDTs
Lipid peroxidation

ABSTRACT

Soil contamination by organic compounds is of great concern worldwide since it could act as a major environmental repository. The success of phytoremediation is conditioned by two main factors: the availability and toxicity of the compound to be remediated, and the plant species ability to incorporate or degrade the contaminants. This study aimed to assess the potential of different plants species for remediation of aged DDTs residues in soil. Tomato, sunflower, soybean and alfalfa plants were grown in contaminated soils (*p,p′*-DDE 455.3, *p,p′*-DDT 63.5 ng g^{-1} dry weight) for 15 and 60 days. Phytoremediation was evaluated by studying the pollutant dynamics in the soil–plant system and lipid peroxidation (LPO) as an effect biomarker. Results showed that soil DDTs levels were diminished after 60 days of plant growth, due to the combined effect of DDTs uptake by roots and rhizospheric degradation. The relative accumulation of each compound was dependent on soil levels (*p,p′*-DDE > *p,p′*-DDT > *p,p′*-DDD) and all species presented root >aerial accumulation pattern, evidencing DDTs translocation. Tomato plants were the most effective in the enhancement of pollutants bioavailability in the rizospheric soil. Plants growth induced physicochemical changes in soil and those are evidenced by increasing dehydrogenase activity and DDTs metabolism, mainly in soybean and tomato soil fractions. Root bioconcentration factors >1 were observed in 15 days tomato and alfalfa plants. Growth dilution effect was observed in roots of all species at 60 days, except soybean. DDTs uptake nor affected tomato and alfalfa plants growth neither caused oxidative stress. Considering the accumulation potential, tolerance (expressed as no evident phytotoxicity effects) and interaction with soil matrix in terms of metabolism and availability, tomato plants seems to be the best phytoremediator candidates for aged soil DDTs residues.

1. Introduction

Soil contamination by persistent organic pollutants (POPs) is a widespread environmental problem. The organochlorine insecticide *p,p′*-DDT [2,2-bis(chlorophenyl)-1,1,1-trichloroethane] has been used worldwide for several decades to control arthropod disease vectors and agricultural pests. Due to the effects induced by *p,p′*-DDT on wildlife and human health (Turusov et al., 2002), it was banned for agricultural purposes, being only employed for malaria disease vectors control (Foght et al., 2001). As *p,p′*-DDT is a lipophilic compound it remains adsorbed to soil particles, leading to half-lives of up to 30 years in this matrix. Although *p,p′*-DDT can be microbiologically or abiotically degraded to *p,p′*-DDD [1,1-dichloro-2,2-bis(chlorophenyl)ethane] or *p,p′*-DDE [1,1-dichloro-2,2-bis(chlorophenyl)ethylene], these metabolites are also hydrophobic and persistent compounds (Aislabie and Lloyd-Jones, 1995). Much of the concern over these compounds is related to their toxicity (mutagenicity, estrogenicity, and/or carcinogenic effects) and biomagnifications through aquatic and terrestrial food chains (Kelly and Gobas, 2001; Kidd et al., 2001).

DDTs residues in soil still represent a continuous source of pollution to the environment (Gong et al., 2004; Miglioranza et al., 2013), and levels above soil quality guidelines have been reported

* Corresponding author. Tel.: +54 223 4751107ext297; fax: +54 223 4753150.
E-mail addresses: franchimitton@gmail.com (F.M. Mitton), mariana.gonzalez@conicet.gov.ar (M. Gonzalez).

http://dx.doi.org/10.1016/j.ecoleng.2014.07.069
0925-8574/

worldwide (Zhou et al., 2013). Thus, actions toward environmental pollution prevention should focus on the reduction of DDT and its metabolites soil levels.

Traditional remediation procedures include soil removal, chemical treatment or biological degradation (Xia et al., 2012). However, most of these techniques are destructive and expensive, so there is a need to develop more environmentally friendly options. Phytoremediation constitutes an emergent technology that uses the vegetation abilities to remove organic or inorganic contaminants from soil, water or sediments (Alkorta and Garbisu, 2001). Phytoremediation occur by several pathways, plants can degrade organic pollutants through enzymatic activities in a process called phytodegradation. Once in plants tissue, pollutants can be accumulated (phytoaccumulation) or volatilized (phytovolatilization). Moreover, organic pollutants can be biodegraded by the rhizospheric community (rhizodegradation). All these processes can occur simultaneously, leading to a more effective phytoremediation (Pilon-Smit, 2005). Compound characteristics, plant species and environmental conditions should be considered as key factors in phytoremediation studies since it could determine which of the remediation pathway will predominate (Schnoor et al., 1995; Kvesitadze et al., 2006). Direct plant uptake of organic contaminants is primarily limited by the compound availability and uptake mechanisms. Soil conditions (pH, organic carbon, water content, texture) (Ryan et al., 1988) and plant physiology (MacFarlane et al., 1990) might influence on those processes. Plant uptake and translocation will be depend on plant physiology, root morphology and biochemical composition (Trapp, 1995). Additionally, root exudates vary through plant life cycle modifying soil pollutants availability (White et al., 2006).

Moreover, phytoremediation might be limited due to the toxic effect of contaminant on plant metabolism, growth or on rhizospheric communities (Chaudhry et al., 2005; Susarla et al., 2002). However, other variables can be measured, including biochemical changes related to oxidative stress. It is known that several organic compounds might increase the generation of reactive oxygen species (ROS) and this enhancement might generate oxidative damage, including membrane lipid peroxidation, enzyme inactivation and DNA breakage (Valavanidis et al., 2006; Monserrat et al., 2007; Ramirez-Sandoval et al., 2011). These biochemical responses can be detected earlier than growth and developmental effects being useful as toxicity markers. Therefore, the study of these biochemical parameters could be useful to determine the most efficient plant species for phytoremediation purposes. Taking this statement into account, the aim of this work was to assess the potential of different plant species for remediation of aged DDTs residues in soil by studying the pollutant dynamics in the soil–plant system and lipid peroxidation as a toxicity biomarker.

2. Material and methods

2.1. Soil

Typical Patagonian soils were obtained from a typical apple and peach field settled in Villa Regina city in the upper valley of the Rio Negro watershed (S 39°04.9′14″ W 67°02.9′59″). Soils belongs to the Aridisols order (Soil Survey Staff, 1999), with 2.7% of organic carbon, 14.1% of sand, 62.9 of silt and 23% of clay and are highly DDTs polluted (455. 3 and 63.5 ng g^{-1} dry weight of *p,p′*-DDE, and *p,p′*-DDT, respectively) (Gonzalez et al., 2010). Soil was air-dried until constant weight and ground to obtain a homogeneous matrix and kept at 4 °C before conducting the experiment.

2.2. Plant growth

Seeds of tomato (10), sunflower (10), soybean (10), and alfalfa (100) were placed in rectangular pots of 6000 cm^3 covered with aluminum foil and containing 1000 g of Villa Regina dry soil under greenhouse conditions (10–26 °C, light:dark 14:10 h). Three planted pots were established for each species and time period. After 2 days of plant emerging, the number of individuals per pot was reduced to 2 by extracting those plants grown close to the border of the pot or near to other plant. Unplanted pots (Un) were also established. All pots (planted and unplanted) were weeded on demand and weekly watered with tap water.

2.3. Soil and plant sampling

Destructive harvest was performed at 15 (first period) and 60 days (second period) after germination to study the influence of life stage on pesticide uptake and lipid peroxidation levels. Two or three plants were harvested per pot and period. Root, stem and leaves obtained from each pot were composite and analyzed as a single pool. Within pots, three separated soil fractions that varied in the influence exerted by the plant root were defined according to White (2001). Bulk soil samples (BS), that has no contact with plant roots, were taken from the top of individual planted pots. The near-root soil (NRS) was operationally defined as the soil that was under root influence and it settled within the volume occupied by them. The rhizosphere soil (Ri) was defined as the soil that remained attached to the roots and needs to be mechanically removed was obtained by washing roots with distilled water and centrifugation of water-Ri solution at 840 × *g* for 10 min at room temperature (this procedure was selected to preserve fine roots during rhizosphere extraction). Additionally, soil samples from Un soils were obtained at 15 and 60 days.

Plant and soil sub-samples were air dried, kept at room temperature or frozen (−80 °C) until analysis.

2.4. DDTs extraction and purification

All solvents were residue analysis quality and other reagents were obtained from Merck Co. (Darmstadt, Germany).

DDTs were extracted according to Metcalfe and Metcalfe (1997), with modifications of Miglioranza et al. (2003). Sub-samples of 5 g of dry soil and 3 g of wet weight plant tissues were homogenized with sodium sulfate and spiked with 20 ng of PCB #103 as internal standard; they were Soxhlet extracted (8 h) with a mixture of hexane–dichloromethane (50:50), and then concentrated under vacuum and nitrogen flow to a final volume of 2 mL. For plant extracts, lipid percentage was calculated after removing them by gel permeation chromatography in Bio Beads S-X3 (200–400 mesh size, Bio Rads Laboratory, Hercules, CA, USA), and dried under vacuum and nitrogen flow to constant weight. Clean up of all extracts containing pesticides was performed by silica gel chromatography and extracts were concentrated to 1 mL and kept in sealed vials at −20 °C prior to chromatographic analysis.

2.5. Chromatographic determination

DDTs (p,p′DDE, p,p′-DDT and p,p′-DDD) were identified and quantified using a Gas Chromatograph (with autosampler) Shimadzu 17-A gas equipped with a ^{63}Ni Electron Capture Detector (GC-ECD) and a capillary column coated with SPB-5 [(5%phenyl)-methyl polysiloxane, 30 m × 0.25 mm i.d. × 0.25 μm film thickness; Supelco Inc.]. One microliter was splitless injected at 275 °C. The ECD temperature was 290 °C. The oven temperature program was: start at 100 °C and held for 1 min, followed by an increase of

5 °C min^{-1} up to 150 °C, held for 1 min, then 1.5 °C min^{-1} up to 240 °C, and then 10 °C min^{-1} up to 300 °C for 10 min. Ultra-high purity Helium was used as carrier gas (1.5 mL min^{-1}) and nitrogen as make-up gas (Miglioranza et al., 2003). The standard solutions used for identification and quantification of single compounds were a Standard Pesticide Mixture of organochlorine pesticides from Ultra Scientific, RI, USA and PCB #103 from Accustandard Absolute Standards, INC, CT, USA. Retention times of each compound were confirmed by running solution of single compounds from Dr. Ehrenstorfer, Augsburg, Germany, with purity ≥96%.

2.6. Quality control and assurance

Laboratory and instrumental blanks analyzed throughout the procedure indicate that there were not contaminants or interference on samples during laboratory handling. Single compounds recoveries, calculated by spiking matrix and surrogate recovery, were greater than 90%. Instrumental detection limits (DL) for DDTs were calculated according to Keith et al. (1983) and were <0.2 ng mL^{-1}, method detection limits were <0.033 ng g^{-1}.

2.7. Dehydrogenase activity determination

Soil dehydrogenase activity (DHA) analysis was used to assess the microbiological activity on soil fraction (Wu et al., 2008; Nosalewicz and Nosalewicz, 2011) differing in their proximity to roots (BS and NRS) from plants grown on Villa Regina soil.

One gram of soil samples was incubated, in triplicate, during 24 h at 25 °C and darkness with 0.2 mL of 0.4% 2-*p*-iodophenyl-3 *p*-nitrophenyl-5 tetrazolium chloride (INT) as a substrate. The iodonitrotetrazolium formazan (INTF) formed was measured spectrophotometrically at 490 nm (Trevors, 1984; García et al., 1997).

2.8. Soil pH and humidity

Soil pH was measured in soil/deionized water suspension 1/2.5 (w/v). Water content was determined by constant-weight drying in an oven at 110 °C.

2.8.1. Lipid peroxidation

The thiobarbituric acid reactive substances method (TBARs) (Khan and Panda, 2008) was used to estimate lipid peroxidation (LPO) in plants grown in Villa Regina soil (DDT polluted). Additionally, LPO control was established by grown plants in non-DDT polluted soil. Tissues were homogenized (1:5) in trichloroacetic acid (TCA) 0.1%. Extract (41.2 μL) was added to a reaction mixture made with 150 μL of 20% acetic acid, 150 μL of thiobarbituric acid (0.8%), 50 μL of Milli Q water and 20 μL of sodium dodecyl sulfate (SDS, 8.1%). Samples were heated at 95 °C for 30 min and after cooling for 10 min, 100 μL of Milli Q water and 500 μL of n-butanol were added. The organic phase (150 μL) was obtained by centrifugation at 3000 × *g* at 15 °C for 10 min, and the fluorescence was registered in a microplate reader (excitation: 515 nm, emission: 553 nm). Tetramethoxypropane (TMP, Across Organics) was employed as an external standard and LPO levels were expressed as nanomoles of TMP per gram of wet tissue.

2.9. Statistical analysis

Pesticide residues data are expressed in ng g^{-1} on a dry weight basis (dw). Values of pesticides, LPO and DHA represent the mean of three independent extractions and quantification of different soils or plant tissues. Statistical analysis was performed using Infostat Software Package (Grupo InfoStat, 2008). Non-parametric ANOVA Friedman tests or a t-paired test for dependent samples were applied to assess differences among plant tissues or soil fractions within species at 15 and 60 days of growth. One way ANOVA tests followed by Tukey analysis or a Student *t* test was used to test differences among species. When parametric requirements (normality and variance homogeneity) were not fulfilled a Kruskal Wallis test followed by a pair comparison was used. The significance level was set at $\alpha = 0.05$ (Zar, 1984).

3. Results and discussion

3.1. Soils

3.1.1. Unplanted pots

Unplanted pots (Un) were established for determining the influence of wetting on DDTs soil content throughout the exposition period when plants are not present. Soil DDTs levels increased from 518 ng g^{-1} dw (day 1) to 1143 and 823 ng g^{-1} dw at 15 and 60 days, respectively. Pollutant enhancement by watering regimen in this soil was previously reported by Mitton et al. (2012) and this behavior was also informed for other soils and compounds (White et al., 1998; Kottler et al., 2001). The reason for the changes associated with the additions and losses of water from soil are uncertain. It has been suggested that soil organic matter becomes fragmented and more porous and the mesh structure disintegrates upon drying (Kottler et al., 2001; White, 2001), leading to pollutants release from soil matrix.

Additionally, microorganism catabolic activity accounts for the removal of hydrophobic organic contaminants from soil (Semple et al., 2003). The initial soil (*p,p′*-DDE + *p,p′*-DDD)/*p,p′*-DDT) ratio was 7.18 and increased up to 8.4 and 9.6 in Un soils at 15 days and 60 days, respectively. A detailed analysis of DDTs levels in those soils showed that the relations of DDT, DDE and DDD in the initial soil to Un soil were 1.7, 2 and 3.2 at 15 days and 1.1, 1.5 and 1.4 at 60 days, respectively. These results, showed the enhancement of DDTs availability followed by metabolism triggered by watering regimen.

3.1.2. Planted soil (polluted soil)

When comparing BS samples from planted and Un soil samples, the joined effect of plant growth and soil wetting can be assessed. At 15 days, the lowest levels were found in BS for *p,p′*-DDT and *p,p′*-DDD in soybean pots and for *p,p′*-DDD in tomato pots (Table 1). These results were in agreement with the higher DHA in BS of these species (Fig. 1), indicating that microbial activity was influenced by the plant presence at 15 days. The subsequent plant growth induced both chemical and physical changes in soil leading to an increase of BS/Un ratio for *p,p′*-DDD in all species that was also related with a DHA enhancement (Table 1).

The NRS showed similar *p,p′*-DDT and *p,p′*-DDE levels than BS and Un soil but lower *p,p′*-DDD levels at 15 days of growth (Table 1). Plant growth changed the NRS/BS ratios of *p,p′*-DDT and *p,p′*-DDE being higher than 1 for all species after 60 days except for soybean that showed a markedly reduction on *p,p′*-DDT levels (Table 1). At 15 days DHA was dependent on plant species (Fig. 1). Tomato and soybean showed lower DHA in NRS than in BS, while sunflower and alfalfa showed the inverse pattern. At 60 days, tomato and alfalfa plants changed the DHA pattern, with higher values in NRS.

Changes in DDTs levels and DHA in the soil fractions might be linked to variations on microbial communities due to different kind and quantity of root exudates (Ziegler et al., 2013). Results showed that at 15 days the higher microbial activity could be responsible of the *p,p′*-DDD metabolism in tomato and soybean BS under anaerobic conditions. So, DDA (bis(4′-chlorophenyl) acetate) or DDMU (1-chloro-2,3-bis-(4′-chlorophenyl) ethylene) might be

Table 1
BS/Un, NRS/BS and Ri/BS ratios for DDTs in tomato, sunflower, soybean and alfalfa cultures at 15 and 60 days. Un: unplanted soil; BS: bulk soil; NRS: near root soil; Ri: Rhizosphere.

	Compound	BS/Un		NRS/BS		Ri/BS	
		15	60	15	60	15	60
Tomato	*p,p′*-DDT	0.9	0.7	1.1	1.5	2.5	1.5
	p,p′-DDE	0.8	0.9	1.1	1.1	2.4	1.0
	p,p′-DDD	0.5	3.7	0.8	0.5	5.1	0.4
Sunflower	*p,p′*-DDT	1.2	0.7	0.9	1.5	1.3	1.5
	p,p′-DDE	1.2	1.0	0.7	1.1	1.2	1.0
	p,p′-DDD	1.1	3.5	0.4	0.7	2.0	0.6
Soybean	*p,p′*-DDT	0.6	3.8	1.4	0.3	2.1	0.2
	p,p′-DDE	0.9	1.2	1.0	0.9	1.4	0.7
	p,p′-DDD	0.4	4.2	0.5	0.5	3.6	0.5
Alfalfa	*p,p′*-DDT	0.9	0.8	0.9	1.9	1.3	1.4
	p,p′-DDE	0.9	0.9	0.9	1.9	1.5	1.1
	p,p′-DDD	0.8	2.4	0.0	0.9	4.0	0.7

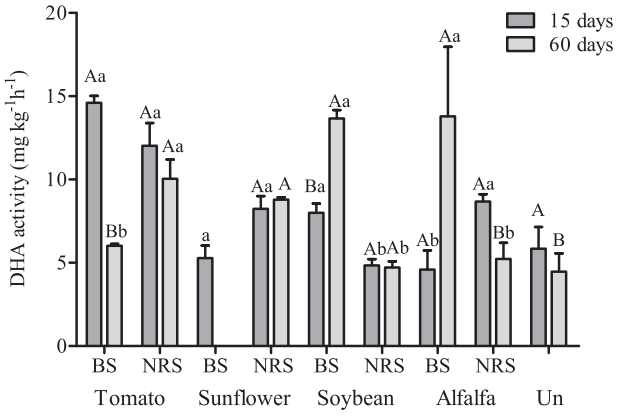

Fig. 1. Dehydrogenase activity (DHA, mg kg^{-1} h^{-1} as mean + 1 standard error) in unplanted soil (Un), bulk soil (BS) and near root soil (NRS) of tomato, sunflower, soybean and alfalfa plants at 15 and 60 days of growth in Villa Regina soil. *Upper case letters indicate statistically significant differences ($p < 0.05$) between stages for each soil fraction; lower case letter indicate statistically significant differences ($p < 0.05$) between NRS and BS within each growth stage.*

formedalthough these compounds were not analyzed in this work (Heberer and Dunnbier, 1999; Gao et al., 2010; Purnomo et al., 2010). Moreover, several factors affect the soil enzymatic activity such as humidity, pH, and oxygen availability and it is known that plant growth can modify these parameters (Wolińska and Bennicelli, 2010). Water content ranged between 30 and 35% and pH values ranged between 7.7 and 8.8 in BS and between 7.9 and 7.7 in Un soils at 15 and 60 days. The optimum pH for DHA was reported between 6.6 and 9.5, with very low activity below or above these values (Trevors, 1984). In this study, pH or soil humidity did not explain differences found on DHA. Oxygen diffusion rate is usually considered to be the most critical proximal regulator of microbial activities ,(Wolińska and Stępniewska 2011) and the deshydrogenases are sensitive enzymes that indirectly depend on the soil aeration status (Wolińska and Bennicelli, 2010). In this sense, DHA was higher in BS at 60 days (Fig. 1) and it is known that DHA increase under anaerobic conditions. Plant growth and metabolism would probably decrease the O_2 soil levels leading to higher DHA and favoring the anaerobic metabolism of *p,p′*-DDT and therefore increasing *p,p′*-DDD levels in BS. Moreover, the NRS by definition is a transition soil fraction under the influence of root plant and those factors that can affect DHA might be quite variable explaining the lack of a clear pattern.

The NRS/BS and Ri/BS ratios showed an increase of *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD concentration in Ri from 15 days (Table 1). The *p,p′*-DDT levels in Ri ranged between 150.5 and 269.5 ng g^{-1} dry weight for alfalfa and tomato, respectively, and *p,p′*-DDE levels between 1248.5 and 2032.8 ng g^{-1} dry weight for soybean and tomato, respectively. Although at 15 days the "rizhospheric effect" increased the pesticide availability in this soil compartment it was dependent on the specie. Previous studies showed the DDTs availability enhancement by root exudates, that are rich in carboxylic acids, among other compounds such as carbohydrates and amino acids in rhizospheric soil (White et al., 2003; Rentz et al., 2005; Mitton et al., 2012). Moreover, this process was related with basic pH values (8–10). In this study, BS and NRS from tomato showed higher pH values (8.8 and 8.3 respectively) than the remaining species which ranged between 7 to 8, suggesting that this species had high carboxylic acids content on their root exudates. It is known that carboxylic acids might stimulate alkalinity release in soil due to the decarboxilation by soil microorganism and cations release by disruption of soil structure (Rukshana et al., 2012).

At 60 days, the Ri/BS relationship was lower than at 15 days (Table 1). This time-dependent decrease of DDTs levels in Ri might be explained by the continuous DDT uptake by plant roots which was also accomplished by an increased pesticide availability. In this sense, other authors reported changes in pesticide levels in different soil fractions. Gonzalez et al. (2003) found a decrease in *p,p′*-DDT and *p,p′*-DDE concentration in Ri relative to BS after 60 days growth in tomato plants. White et al. (2001) reported a decrease of *p,p′*-DDE levels in Ri of pumpkin and spinach grown during 80 days, suggesting plant-facilitated mobilization and/or degradation of *p,p′*-DDE residues. Moreover a similar pattern for *p,p′*-DDE in the soil fractions of alfalfa, rye, and bean was reported (White, 2000).

3.2. Plant uptake and tissue distribution pattern

DDTs levels in tomato, sunflower, soybean, and alfalfa tissues grown for 15 and 60 days are shown in Fig. 2. At both stages, all species accumulated more DDTs in the root than in aerial parts, independently of the lipid content, indicating that other factors are involved on root uptake from soil. The relative accumulation of each compound was dependent on soil levels following the pattern *p,p′*-DDE > *p,p′*-DDT > *p,p′*-DDD ($p < 0.05$). However, the relation between parental compound and metabolites varied with plant species and growth period. Since the biochemical characteristics of plants significantly affect the uptake of organic chemicals, the type and quantity of lipids may have a significant effect on the storage capacity of high lipophilic compounds such as DDTs (Simonich and Hites, 1994; Whitfield et al., 2008). Lipid percentage ranged between 0.1–0.6 in roots and 0.1–0.8 in aerial tissues (Table 2). At 15 days, tomato plants showed the higher *p,p′*-DDT

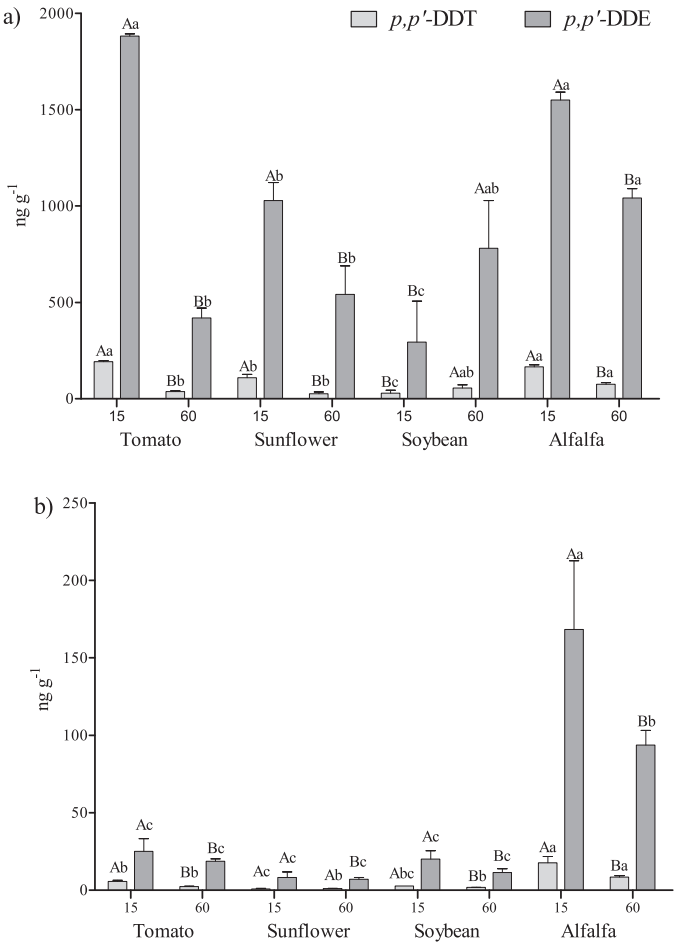

Fig. 2. DDTs concentration (ng g^{-1} dw) in roots (a) and aerial tissues (b) of tomato, sunflower, soybean and alfalfa plants 15 and 60 days of growth. *Upper case letters indicate statistically significant differences ($p < 0.05$) in DDTs compounds between stages within each species and tissue; lower case letters indicate statistically significant differences ($p < 0.05$) for DDTs among species for one tissue and stage.*

levels in root despite its lower lipid content, while for aerial tissues, lipids correlated with DDTs accumulation in tomato, soybean and sunflower plants. Root tissues showed a growth dilution effect leading to lower *p*,*p*′-DDT levels in 60 days plants, except soybean. However, root lipid percentage was not affected by growth indicating that besides its hydrophobic characteristic, other process are involved on the DDTs accumulation in this organ. In aerial tissues no differences were found for DDTs levels between stages, suggesting the continuous DDTs transference from root.

The (*p*,*p*′-DDE + *p*,*p*′-DDD)/*p*,*p*′-DDT ratio was lower in aerial tissues than in roots at both stages, except for sunflower. These results suggest that accumulation or translocation is dependent on plants species and growth stage.

Table 2
Lipid percentage in roots and aerial tissues of tomato, sunflower, soybean and alfalfa plants at 15 and 60 days of growth.

Specie	Lipid (%)		
		15	60
Tomato	Root	0.17	0.28
	Aerial	0.84	0.34
Sunflower	Root	0.49	0.60
	Aerial	0.28	0.19
Soybean	Root	0.10	0.16
	Aerial	0.57	0.16
Alfalfa	Root	0.37	0.30
	Aerial	0.33	0.74

3.2.1. Root–soil interface

Root bioconcentration factor (RBCF) was calculated by determining the dry-weight ratio of DDTs concentration in root to that in BS of the corresponding pot (Table 3). At 15 days, tomato and alfalfa bioconcentrated DDTs from soil (RBCF > 1). Except for tomato, *p*,*p*′-DDD RBCF were higher than *p*,*p*′-DDT and *p*,*p*′-DDE RBCF. Considering the result obtained in Ri, the high *p*,*p*′-DDD and *p*,*p*′-DDT availability leads to a high bioconcentration. This result showed the root exudates effect in the soil–root interface. Particularly, despite the high *p*,*p*′-DDD availability in Ri, tomato plants did not bioconcentrate this compound. At 60 days alfalfa plants bioconcentrated *p*,*p*′-DDT in roots (RBCF > 1) while tomato plants diminished all RBCF. Sunflower and soybean RBCF did not vary between 15 and 60 days. These results agree with the growth-dilution effect because all of them, except soybean, showed lower DDTs concentration in roots growth at 60 days (Fig. 2). In this sense, this lower root bioconcentration in older plants was previously reported (Gonzalez et al., 2003). However when the biomass and DDTs concentration are considered together all species increased DDTs burden at 60 days with soybean rising up to 34 times (data not shown). So, root pesticide uptake might be a continuous process and the extent of the uptake depends on the plant species (Gonzalez et al., 2003).

Except for 15 days alfalfa plants, the (*p*,*p*′-DDE + *p*,*p*′-DDD)/*p*,*p*′-DDT ratio in roots of all species and stages was higher than in Ri suggesting the *p*,*p*′-DDT metabolism in roots or preferential uptake from soil.

3.2.2. Root to aerial route

Water and solutes are transported upward from the root into other plant parts through the xylem. This flux is driven by the water potential gradient, created throughout the plant during transpiration. The combination of solubility of chemicals in water and the solubility within the cell membrane, which is rich in lipids, determines the chemicals movement into roots and subsequent transport to the plant shoot. The less polar the compound, the more retain on lipid fraction, being less mobile across the endodermis (Trapp, 1995). Depending on the species and compound, the uptake and translocation lead to different plant tissue distribution pattern. Translocation was evaluated by the ratio of contaminant concentration in aerial tissues to that in roots, named aerial translocation factor (ATF), and as the ratio of contaminant concentration in the leaves to that in the stem (leaf translocation factor, LTF, Table 3). DDTs translocation was observed in all species at both stages despite it was low (AFT < 1), mainly due to the high lipophilicity of these compounds (Kow: 4.9–6.9). Although, alfalfa showed the higher AFT, it is difficult to attribute these values to the translocation, due to their small size and its proximity to the soil. In the remaining species, the ATF increased, indicating the continuous DDTs translocation throughout life cycle associated with plant growth. Consequently, the DDTs burden in 60 days plants was up to 8 times greater than in 15 days plants (data not shown). The root–stem–leaves pathway was evaluated in 60 days plants through the LTF and values agree with the soil uptake route with low transference to aerial tissues. However, high LTF were found for *p*,*p*′-DDD in tomato plants. The higher transpiration rate of tomato plants could explain the higher DDTs translocation (Murano et al., 2010). Although translocation is highly dependent on plant transpiration, other mechanisms have been proposed for high lipophilic compounds such as dieldrin and *p*,*p*′-DDT in some plant species (Whitfield et al., 2008; Chhikara et al., 2010; Murano et al., 2010). In this sense, some studies demonstrated the presence of a lipid

Table 3
DDTs root bioconcentration factors (RBCF), aerial translocation factor (ATF), and leaves translocation factors (LTF) of tomato, sunflower, soybean and alfalfa plants, growth at 15 and 60 days. LTF was only calculated for 60 days plants. * *p,p′*-DDD values in root and aerial tissues were below detection limit; ** stems and leaves were not discriminated in any stage.

Species	Compound	RBCF		ATF		LTF
		15	60	15	60	60
Tomato	*p,p′*-DDT	1.79	0.70	0.03	0.09	0.28
	p,p′-DDE	2.23	0.59	0.01	0.03	0.26
	p,p′-DDD	*	0.24	*	0.07	1.85
Sunflower	*p,p′*-DDT	0.77	0.50	0.01	0.06	0.46
	p,p′-DDE	0.85	0.74	0.01	0.03	0.78
	p,p′-DDD	0.99	1.05	0.00	0.11	0.26
Soybean	*p,p′*-DDT	0.39	0.19	0.02	0.13	0.63
	p,p′-DDE	0.33	0.85	0.02	0.09	0.52
	p,p′-DDD	0.76	0.54	0.07	0.18	0.53
Alfalfa	*p,p′*-DDT	1.46	1.21	0.11	0.11	**
	p,p′-DDE	1.66	1.61	0.11	0.09	**
	p,p′-DDD	1.74	1.05	0.06	0.05	**

Table 4
Lipid peroxidation (LPO) in roots and aerial tissues of 15 and 60 days tomato, sunflower, soybean and alfalfa plants. LPO was expressed as nanomoles of TMP (tetramethoxypropane) per gram of wet tissue, estimated by the TBARs (thiobarbituric acid reactive substances) method. *Upper case letters indicate statistically significant differences ($p < 0.05$), between unexposed and exposed plants within each tissue, for each species; lower case letters indicate statistically significant differences ($p < 0.05$) between stages within each tissue.*

Species		LPO			
		15 days		60 days	
		Unexposed	Exposed	Unexposed	Exposed
Tomato	Root	14.99 ± 17.7^{Aa}	24.32 ± 26.1^{Aa}	5.43 ± 1.5^{Aa}	14.52 ± 8.9^{Aa}
	Aerial	10.01 ± 4^{Aa}	13.44 ± 8.4^{Aa}	13.96 ± 9.3^{Aa}	25.08 ± 13.3^{Aa}
Sunflower	Root	33.27 ± 4.2^{Aa}	14.96 ± 6.0^{Ba}	1.79 ± 1.0^{Ab}	2.13 ± 0.3^{Aa}
	Aerial	29.39 ± 5.7^{Aa}	8.02 ± 2.5^{Bb}	19.15 ± 3.4^{Aa}	24.2 ± 1^{Aa}
Soybean	Root	60.92 ± 13.3^{Aa}	14.00 ± 6.8^{Ba}	10.06 ± 2.0^{Ab}	11.44 ± 5.7^{Aa}
	Aerial	116.02 ± 22.6^{Aa}	37.87 ± 4.5^{Ba}	39.93 ± 3.3^{Bb}	65.43 ± 13^{Aa}
Alfalfa	Root	50.99 ± 18.8^{Aa}	31.76 ± 7.1^{Aa}	32.49 ± 6.9^{Aa}	65.78 ± 38.6^{Aa}
	Aerial	104.76 ± 35.4^{Aa}	84.04 ± 12.9^{Aa}	70.93 ± 25.1^{Aa}	139 ± 26.8^{Aa}

transfer protein (LTP), proteins which contain a hydrophobic cavity, in phloem sap of tomato plants (Maldonado et al., 2002; Mitton et al., 2009). This kind of proteins can be related to DDTs translocation ability in plants and more research is required to determine the mechanisms involved in DDTs translocation.

3.3. Plant responses and toxicity

Phytotoxicity of a xenobiotic is indicated by the stress responses that can be molecular, physiological and/or morphological. Any factor altering growth or metabolism, will also affect the phytoremediation process. It is known that organic contaminants could inhibit plant growth (Zhang et al., 2011; Ahammed et al., 2012). In the current study, polluted soils had no statistically significant effect on tomato plant growth. A biomass reduction of about 37% in root and 49% in aerial was observed in 15 days tomato plants from polluted soils but no differences were found for length. Moreover at 60 days plants were recovered from this effect observed at early growth stages (data not shown).

Sunflower plants showed 70% reduction of root length and 35% lower aerial biomass ($p < 0.05$) when exposed to DDTs for 15 days and, similarly to tomato, no difference was observed between exposed or non exposed plants after 60 days. For soybean plants an inverse pattern was found with an increase of aerial length at 15 days and a significant reduction (45%) after 60 days exposure. At 15 days, exposed alfalfa plants showed higher root and aerial biomass ($p < 0.05$) than unexposed plants, but a trend toward biomass reduction with growth was observed at 60 days.

On the other hand, the role of oxidative stress in the phytotoxicity of several organic chemicals has been documented (Macek et al., 2000; Susarla et al., 2002) and correlation between morphological signals of phytotoxicity and oxidative damage was observed (Bhattacharjee, 2005). Lipid peroxidation (LPO) might be a good biomarker of oxidative damage and the results, estimated by the TBARs method are shown in Table 4. Differences in LPO levels between young and old plants are linked to the development of antioxidant defenses with growth (Kuk et al., 2003, 2006). In this sense, the studied species were in line with this statement under non-exposed conditions. However, LPO response to DDT exposure was species dependent. Tomato and alfalfa plants which showed the highest pesticide levels did not show differences in LPO compared with unexposed plants. Conversely, for sunflower and soybean, at 15 days, roots and aerial LPO was lower in exposed than in unexposed plants, suggesting the trigger of antioxidant responses at early stages, thus reducing LPO levels. However, at 60 days this defense might be overcome in aerial soybean tissues, as LPO content was higher in exposed plants (Table 4).

Thus, the studied species had different susceptibility and growth response to DDTs exposure. These results indicate that tomato and alfalfa plants are not being affected by DDTs uptake, in terms of growth and LPO and, moreover, they showed to be the best DDTs accumulators.

4. Conclusion

This study demonstrated the differential uptake, translocation and effect on final DDTs level and availability in soil by the studied species. On the basis of its higher accumulation potential, no evident phytotoxicity effects and enhancement of soil microbial activity, tomato plants figure out as the best phytoremediation

candidate. The mechanism involved on reduction of soil DDTs levels by tomato plants are not only related to compounds uptake and translocation either also with *in situ* metabolism in soil matrix. This result is of remarkable concern since biodegradation is the most desirable remediation pathway for highly recalcitrant compounds since solve the problems linked to plant management after pollutant uptake. Moreover, it will allow the use of edible species and the remediation of soils devoted to food production. So, further efforts will be required to understand the DDTs metabolism in the root soil interface of tomato plants considering molecular approaches to gain more insight in the knowledge about microbial density, activity and diversity as well on the induction of genes encoding enzymes involved in the DDTs degradation process. On the other hand, the formation of DDT by-product such as DDA and DDMU are also of environmental concern so their monitoring is recommended during phytoremediation studies.

Acknowledgments

This work was funded by grants from Universidad Nacional de Mar del Plata (EXA 516/10-EXA 609/12) and ANPCyT (PICT-07/410). This work is part of the Ph.D. Thesis of the first author (Mitton, F. M).

References

Ahammed, G.J., Wang, M.M., Zhou, Y.H., Xia, X.J., Mao, W.H., Shi, K., Yu, J.Q., 2012. The growth, photosynthesis and antioxidant defense responses of five vegetable crops to phenanthrene stress. Ecotoxicol. Environ. Saf. 80, 132–139.

Aislabie, J., Lloyd-Jones, G., 1995. A review of bacterial degradation of pesticides. Aust. J. Soil Res. 33, 925–942.

Alkorta, I., Garbisu, C., 2001. Phytoremediation of organic contaminants in soils. Bioresour. Technol. 79, 273–276.

Bhattacharjee, S., 2005. Reactive oxygen species and oxidative burst: roles in stress, senescence and signal transduction in plants. Curr. Sci. 89, 7–10.

Chhikara, S., Paulose, B., White, J.C., Dhankher, O.P., 2010. Understanding the physiological and molecular mechanism of persistent organic pollutant uptake and detoxification in cucurbit species (Zucchini and Squash). Environ. Sci. Technol. 44, 7295–7301.

Chaudhry, Q., Blom-Zandstra, M., Gupta, S., Joner, E.J., 2005. Utilizing the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. Environ. Sci. Pollut. Res. Int. 12, 34–48.

Foght, J., April, T., Bigga, K., Aislabie, J., 2001. Bioremediation of DDT-contaminated soils: a review. Biorem. J. 53, 225–246.

Gao, J., Ellis, L.B.M., Wackett, L.P., 2010. The University of Minnesota Biocatalysis/Biodegradation Database: improving public access. Nucleic Acids Res. 38, D488–D491, URL: ⟨http://www.umbbd.msi.umn.edu/⟩.

García, C., Hernández, T., Costa, F., 1997. Potential use of dehydrogenase activity as an index of microbial activity in degraded soils. Commun. Soil Sci. Plant Anal. 28, 123–134.

Gong, Z.M., Tao, S., Xu, F.L., Liu, W.X., Cui, Y.H., Cao, J., Wang, X.J., Shen, W.R., Zhang, W.J., Qing, B.P., Sun, R., 2004. Level and distribution of DDT in surface soils from Tianjin, China. Chemosphere 54, 1247–1253.

Gonzalez, M., Miglioranza, K.S.B., Aizpún, J.E., Isla, F.I., Peña, A., 2010. Assessing pesticide leaching and desorption in soils with different agricultural activities from Argentina (Pampa and Patagonia). Chemosphere 81, 351–358.

Gonzalez, M., Miglioranza, K.S.B., Moreno, J.E., Moreno, V.J., 2003. Occurrence and distribution of organochlorine pesticides (OCPs) in tomato (*Lycopersicon esculentum*) crops from organic production. J. Agric. Food Chem. 51, 1353–1359.

Grupo InfoStat, 2008. InfoStat Professional, Versión 1.1. Manual del Usuario, Facultad de Ciencias Agropecuarias. Universidad Nacional de Córdoba, Argentina.

Heberer, T., Dunnbier, U., 1999. DDT metabolite bis(chlorophenyl) acetic acid: the neglected environmental contaminant. Environ. Sci. Technol. 33, 2346–2351.

Keith, L.H., Crumett, W., Wentler, G., 1983. Principles of environmental analysis. Anal. Chem. 55, 2210–2218.

Kelly, B.C., Gobas, F.A.P.C., 2001. Bioaccumulation of persistent organic pollutants in lichen-caribou-wolf food chains of Canada's central and western arctic. Environ. Sci. Technol. 35, 325–334.

Khan, M.H., Panda, S.K., 2008. Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physiol. Plant 30, 81–89.

Kidd, K., Bootsma, H., Hesslein, R., Muir, D.C.G., Hecky, R., 2001. Biomagnification of DDT through the benthic and pelagic food webs of Lake Malawi, East Africa: importance of trophic level and carbon source. Environ. Sci. Technol. 35, 14–20.

Kottler, B.D., White, J., Kelsey, J.W., 2001. Influence of soil moisture on the sequestration of organic compounds in soil. Chemosphere 42, 893–898.

Kuk, Y.I., Shin, J.S., Chung, J.S., Kwon, O.D., Kim, D.K., Han, O., Guh, J.O., 2003. Differential tolerances, their mechanism on the response of tobacco (*Nicotiana tabacum* L.) leaves of varying ages to protoporphyrinogen oxidase-inhibiting herbicides and paraquat. Korean J. Weed Sci. 23, 100–111.

Kuk, Y.I., Shin, J.S., Jung, H.I., Guh, J.O., Jung, S., Burgos, N.R., 2006. Mechanism of tolerance to paraquat in cucumber leaves of various ages. Weed Sci. 54, 6–15.

Kvesitadze, G., Khatisashvili, G., Sadunishvili, T., Ramsden, J.J., 2006. In: George Kvesitadze, G., Khatisashvili, G., Sadunishvili, T., Ramsden, J.J. (Eds.), The ecological importance of plants for contaminated environments. Biochemical Mechanisms of Detoxification in Higher Plant, Basis for Phytoremediation, New York, NY, USA, pp. 167–209.

Macek, T., Macková, M., Ká, J., 2000. Exploitation of plants for the removal of organics in environmental remediation. Biotechnol. Adv. 18, 23–34.

MacFarlane, J.C., Pfleeger, T., Fletcher, J., 1990. Effect, uptake and distribution of nitrobenzene in several terrestrial plants. Environ. Toxicol. Chem. 9, 513–520.

Maldonado, A.M., Doerner, P., Dixon, R.A., Lamb, C.J., Cameron, R.K., 2002. A putative lipid transfer protein involved in systemic resistance signaling in *Arabidopsis*. Nature 419, 399–403.

Metcalfe, T.L., Metcalfe, C.D., 1997. The trophodynamics of PCBs including mono and non-ortho congeners in the food web of north-central Lake Ontario. Sci. Total Environ. 201, 245–272.

Miglioranza, K.S.B., Aizpún, J.E., Moreno, V.J., 2003. Dynamics of organochlorine pesticides in soils from a SE region of Argentina. Environ. Toxicol. Chem. 22, 712–717.

Miglioranza, K.S.B., Gonzalez, M., Ondarza, P.M., Shimabukuro, V.M., Isla, F.I., Fillmann, G., Aizpún, J.E., Moreno, V.J., 2013. Assessment of Argentinean Patagonia pollution: PBDEs, OCPs and PCBs in different matrices from the Río Negro basin. Sci. Total Environ. 452-453, 275–285.

Mitton, F.M., Pinedo, M.L., de la Canal, L., 2009. Phloem sap of tomato plants contains a DIR1 putative ortholog. J. Plant Physiol. 166, 543–547.

Mitton, F.M., Gonzalez, M., Peña, A., Miglioranza, K.S.B., 2012. Effects of amendments on soil availability and phytoremediation potential of aged *p,p′*-DDT, *p,p′*-DDE and *p,p*-DDD residues by willow plants (*Salix* sp.). J. Hazard. Mater. 203–204, 62–68.

Monserrat, J.M., Martínez, P.E., Geracitano, L., Amado, L.L., Martins, C., Martinez, G., Pinho, G., Lopes, L., Chaves, I.S., Ferreira-Cravo, M., Ventura-Lima, J., Bianchini, A., 2007. Pollution biomarkers in estuarine animals: critical review and new perspectives. Comp. Biochem. Physiol. C: Pharmacol. Toxicol. Endocrinol. 146, 221–234.

Murano, H., Otani, T., Seike, N., Sakai, M., 2010. Dieldrin uptake and translocation in plants growing in hydroponic medium. Environ. Toxicol. Chem. 29, 142–148.

Nosalewicz, A., Nosalewicz, M., 2011. Effect of soil compaction on dehydrogenase activity in bulk and rhizosphere. Int. Agrophys. 25, 47–51.

Pilon-Smit, E., 2005. Phytoremediation. Annu. Rev. Plant Biol. 56, 15–39.

Purnomo, A.S., Mori, T., Kamei, I., Nishii, T., Kondo, R., 2010. Application of mushroom waste medium from *Pleurotus ostreatus* for bioremediation of DDT-contaminated soil. Int. Biodeterior. Biodegrad. 64, 397–402.

Ramirez-Sandoval, M., Melchor-Partida, G.N., Muñiz-Hernández, S., Girón-Perez, M.I., Rojas-García, A.E., Medina-Díaz, I.M., Robledo-Marenco, M.L., Velázquez-Fernández, J.B., 2011. Phytoremediatory effect and growth of two species of *Ocinum* in endosulfan polluted soil. J. Hazard. Mater. 192, 388–392.

Rentz, J.A., Alvarez, P.J.J., Schnoor, J.L., 2005. Benzo [*a*]pyrene *co*-metabolism in the presence of plant root extracts and exudates: implications for phytoremediation. Environ. Pollut. 136, 477–484.

Ryan, J.A., Bell, R.M., Davidson, J.M., O'Connor, G.A., 1988. Plant uptake of non-ionic organic chemicals from soils. Chemosphere 17, 2299–2323.

Rukshana, F., Butterly, C.R., Baldock, J.A., Xu, J.M., Tang, C., 2012. Model organic compounds differ in priming effects on alkalinity release in soils through carbon and nitrogen mineralisation. Soil Biol. Biochem. 51, 35–43.

Schnoor, J.L., Licht, L.A., McCutcheon, S.C., Lee, W.N., Carreira, L.H., 1995. Phytoremediation of organic and nutrient contaminants. Environ. Sci. Technol. 29, 318–323.

Semple, K.T., Morriss, W.W.J., Paton, G.I., 2003. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. Eur. J. Soil Sci. 54, 809–818.

Simonich, S.L., Hites, R.A., 1994. Vegetation–atmosphere partitioning of polycyclic aromatic hydrocarbons. Environ. Sci. Technol. 28, 939–943.

Soil Survey Staff, 1999. Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys. In: Agric. Handbook, second ed. Soil Survey Staff, Washington, DC: U.S, 869pp.

Susarla, S., Medina, V.F., McCutcheon, S.C., 2002. Pytoremediation: an ecological solution to organic chemical contamination. Ecol. Eng. 1818, 647–658.

Trapp, S., 1995. In: Trapp, S., Mc Farlane, C. (Eds.), Model for uptake of xenobiotics into plants. Plant Contamination—Modeling and Simulation of Organic Chemical Processes, Boca Raton, FL, USA, pp. 107–151.

Trevors, J., 1984. Effect of substrate concentration, inorganic nitrogen, O_2 concentration, temperature and pH on dehydrogenase activity in soil. Plant Soil 77, 285–293.

Turusov, V., Rakitsky, V., Tomatis, L., 2002. Dichlorodiphenyltrichloroethane (DDT): ubiquity, persistence, and risks. Environ. Health Perspect. 110, 125–128.

Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Saf. 64, 178–189.

White, J.C., Quiñones-Rivera, A., Alexander, M., 1998. Effect of wetting and drying on the bioavailability of organic compounds sequestered in soil. Environ. Toxicol. Chem. 17, 2378–2382.

White, J.C., 2000. Phytoremediation of weathered *p,p′*-DDE residues in soil. Int. J. Phytorem. 2, 133–144.
White, J.C., 2001. Plant-facilitated mobilization and translocation of weathered 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene (*p,p′*-DDE) from an agricultural soil. Environ. Toxicol. Chem. 20, 2047–2052.
White, J.C., Incorvia Mattina, M.J., Lee, W.Y., Eitzer, B.D., Iannucci-Berger, W., 2003. Role of organic acids in enhancing the desorption and uptake of weathered *p,p*-DDE by *Cucurbita pepo*. Environ. Pollut. 124, 71–80.
White, J.C., Parrish, Z.D., Gent, M.P.N., Ianucci-Berger, W., Eitzer, B.D., Isleyend, M., Incorvia Mattina, M., 2006. Soil amendments, plant age, and intecropping impact *p,p′*-DDE bioavailability to Cucurbita pepo. J. Environ. Qual. 35, 992–1000.
Whitfield, A.M.L., Rutter, A., Reimer, K.J., Zeeb, B.A., 2008. The effects of repeated planting, planting density, and specific transfer pathways on PCB uptake by *Cucurbita pepo* grown in field conditions. Sci. Total Environ. 405, 14–25.
Wolińska, A., Bennicelli, R., 2010. Dehydrogenase activity response to soil reoxidation process described as varied condition of water potential, air porosity and oxygen availability. Pol. J. Environ. Stud. 19, 651–657.
Wolińska, A., Stępniewska, Z., 2011. Microorganisms abundance and dehydrogenase activity as a consequence of soil reoxidation process. In: Miransari, M. (Ed.), Soil Tillage & Microbial Activities. Research Singpost, Kerala, India, pp. 111–143.
Wu, N., Zhang, S., Huang, H., Shan, X., Christie, P., Wang, Y., 2008. DDT uptake by arbuscular mycorrhizal alfalfa and depletion in soil as influenced by soil application of a non-ionic surfactant. Environ. Pollut. 151, 569–575.
Xia, C., Ma, X., Liu, S., Fan, P., 2012. Studies on remediation of DDT-contaminated soil and dechlorination of DDT. Procedia Environ. Sci. 16, 289–292.
Zar, J.H., 1984. Biostatistical Analysis, second ed. Prentice-Hall, Englewood Cliffs, NJ.
Zhang, B., Chu, G., Wei, C., Ye, J., Li, Z., Liang, Y., 2011. The growth and antioxidant defense responses of wheat seedlings to methoate stress. Pestic. Biochem. Physiol. 100, 273–279.
Zhou, Q., Wang, J., Meng, B., Cheng, J., Lin, G., Chen, J., Zheng, D., Yu, Y., 2013. Distribution and sources of organochlorine pesticides in agricultural soils from central China. Ecotoxicol. Environ. Saf. 93, 163–170.
Ziegler, M., Engel, M., Welzl, G., Schloter, M., 2013. Development of a simple root model to study the effects of single exudates on the development of bacterial community structure. J. Microbiol. Methods 94, 30–36.