



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.)

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ABSTRACT

Combining technologies offer a great potential to phytoremediate contaminated soils. As sequestration occurs, pollutants availability decline and organic amendments could counterbalance that situation. This work studies the potential of willow plants to phytoremediate soil containing *p,p'*-DDT (101.3 ng g⁻¹) and *p,p'*-DDE (381.4 ng g⁻¹) residues. The effect of root exudates, Tween 80 and citric and oxalic acids on DDTs desorption and availability from soil was tested together with the plant uptake and translocation. Treatments increased the *p,p'*-DDE/*p,p'*-DDT ratio when compared with control (water) soil. Watering with carboxylic acids led to a significant enhancement of the quantities of *p,p'*-DDT and *p,p'*-DDE desorbed from soil that was related with an increase of organic carbon in solution. Willow plants accumulated DDTs under all treatments although plants watered with carboxylic acids showed the highest leaves translocation factor for both *p,p'*-DDT and *p,p'*-DDE. Results indicate that the addition of carboxylic acids enhanced DDTs bioavailability which further increases plant uptake and translocation. The effect of surfactants on the soil–plant systems needs to be better assessed for this particular soil and plant species. The enhancement of soluble organic carbon is crucial at the moment of evaluating DDTs release from soil as well as to establish cleaning strategies.

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1. Introduction

The introduction in agriculture of the organochlorine insecticide *p,p'*-DDT led to important benefits regarding crop production and the elimination of some disease related vectors [1]. However, due to its physicochemical characteristics, high hydrophobicity, bioaccumulation, biomagnification on food chain, and persistence, its use was forbidden in most countries in the early eighties with the unique exception of malaria control [2]. In 2004, most organochlorine pesticides (OCPs) as well as other organic compounds such as polychlorinated biphenyls and polycyclic aromatic hydrocarbons were included in the dirty dozen lists of Persistent Organic Pollutants (POPs) that are regulated by the Stockholm Convention [3]. These compounds are of environmental concern due to their recalcitrance in soils and sediments, global distribution, and toxicity [4,5]. OCPs have been applied onto soil for crop protection until they were banned. Due to their properties, they could

bind strongly to organic matter and become sequestered within the soil matrix leading to persistence several years after their last usage. The occurrence of legacy pesticides on soils represents a risk for the environment since they can migrate through the profile; be uptaken by biota and transported to aquatic systems by erosion. Thus, for a sustainable management of contaminated soils it is necessary to reduce the load of recalcitrant pollutants since their availability could be modified by plant growing, irrigation or rain-dry cycles [6]. Remediation techniques include soil removal, chemical treatment or biological degradation by inoculation with specific microorganisms. Since these treatments are destructive and expensive, new technologies are being developed. Phytoremediation constitutes an emergent technique that uses the vegetation abilities to remove organic or inorganic contaminants from natural media (soil, water or sediments) [7,8]. For organic compounds it considers all the processes related to plant uptake, translocation and metabolism that lead to diminishing pollutant concentration. Thus, OCPs may be phytoremediated by (a) degradation in the root-zone through the activity of exuded enzymes or rhizospheric organisms; (b) plant uptake followed by translocation and metabolism or accumulation within plant tissues; (c) volatilization of parental compounds or their metabolites [9,10]. The relevance of each route will depend on plant species and

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pesticide characteristics. Among plant species, trees account for the majority of global biomass [11] and are dominant constituents of several ecosystem types. The wood of stems and the lipophilic cuticles of their leaves provide storage compartments and live cells, especially near the cambium and phloem, may also provide an area for rapid metabolic degradation of anthropogenic chemicals [10]. Willow trees have certain characteristics i.e. large biomass yield, extended and deep roots apparatus, high transpiration rate, that make them interesting for phytoremediation purposes [11]. Particularly, some species have been deeply studied for phytoremediating inorganic compounds [8]. Moreover, willow trees are often planted to stabilize riverbanks and lakes providing mechanical stabilization of slopes, minimizing soil erosion, decreasing the level of suspended solids in adjacent waterways and providing a better habitat for microorganisms that participate in purification processes.

In addition, the combined use of plants and agents that increase compounds availability has been proposed for improving phytoremediation strategies. Carboxylic acids, such as citric and oxalic acids, in soils have enhanced the uptake and translocation of aged *p,p'*-DDE residues in plants of the *Cucurbitaceae* family [12]. Moreover, several studies have looked into the use of surfactant-enhanced phytoremediation [13,14]. Their amphiphilic characteristics facilitate the release of organic compounds from the sorbed phase, increasing their aqueous concentrations and bioavailability [15–17].

Quantities and qualities of pesticides used in Argentina vary with the productive system. Patagonian agriculture is almost exclusively based on fruit and wine production concentrated mainly on the Rio Negro watershed. The historical use of pesticides is reflected on the occurrence of OCPs in surface soils, sediments, macrophytes and fish from the Negro River basin with a clear predominance of the metabolite *p,p'*-DDE [17–19]. Soil profiles showed DDTs distribution up to 30 cm and values were above soil quality guidelines [17]. The main willow specie in the area corresponds to *Salix humboldtiana*, Wild variety, which is found as a prolific species across the riverbank [20]. Moreover, this specie acts on fruit fields as wind barriers and the wood is used to build wooden apple boxes. Thus, willows constitute a good candidate for soil remediation purposes in the region.

In order to cope with this problem, the aim of the present study was to assess the phytoremediation of aged DDTs residues in soils by willow trees considering the impact of organic amendments on this process. Thus, the effect of willow root exudates, a non-ionic surfactant (Tween 80) and carboxylic acids (citrate and oxalate) on DDTs desorption from soil, together with plant uptake, metabolism and translocation were considered.

2. Materials and methods

2.1. Soil

The soil was sampled between trees from a typical apple and peach field settled in Villa Regina city in the Upper Valley of the Rio Negro basin (S 39°04.9'14", W 67°02.9'59"). The soil is classified as an Aridisol and has 2.7% organic carbon, 14.1% sand, 62.9% silt and 23% clay.

2.2. Plant growth

Rectangular pots of 6000 cm³ (20 cm × 20 cm × 15 cm) covered with aluminum foil were filled with approximately 1200 g of dry Villa Regina soil. Willows (*S. humboldtiana*) were taken from those grown naturally in the vicinity of the University campus (Mar del Plata, Argentina). Tree cuttings (30 cm length and 1.5–2 cm

diameter) were removed from a mature tree. Cuts were grown in tap water in a greenhouse at temperature of 10–26 °C under natural sunlight (light:dark cycle 14:10 h) until the development of roots (20–30 cm length) and 20–40 leaves with a total weight ranging between 60 and 80 g. After this 30-day period of growth cuts were planted into the pots. Tap water where cuts were grown was kept as a source of root exudates. Root exudates are known to be a mixture of carbohydrates (glucose, xylose, mannitol, maltose, oligosaccharides); amino acids (glutamate, isoleucine, methionine, tryptophan); aromatic compounds (benzoate, phenols, l-carvone, limonene, p-cymene) and organic acids (acetate, citrate, malate, propionate, oxalate) among other compounds [21]. Moreover, root exudates have been reported to contain organic acids at concentrations of 0.05 and 0.1 M [22].

Plants were grown in a greenhouse (under similar conditions of temperature and light:dark cycle as those of cuts) under four regimes, one treated with tap water (control) and three treatments prepared in tap water (a) organic acids (0.05 M sodium citrate and oxalate); (b) Tween 80 at 9.2 mg L⁻¹ (corresponding to 0.5 critical micelle concentration, cmc) and (c) root exudates. All treatment solutions were adjusted to pH 6.8–7 by adding up to 4 drops of a 1 N HCl solution. One plant was put in each pot and three pots were established for each treatment. Unplanted control pots were also established for each regime. All pots (planted and unplanted) were weekly watered with 50 mL tap water or treatment solution or additionally watered when necessary. All solutions were added below the aluminum foil.

2.3. Soil and plant sampling

After 40 days of plant growth a destructive harvest was performed. Unplanted control soil samples were collected from each treatment. Bulk soil samples (BS) that have no contact with plant roots, were taken from the top of individual pots. The near-root soil (NRS) was operationally defined as the soil that was under root influence and settled within the volume occupied by them. The rhizosphere (Ri) was defined as the soil that remained attached to the roots at harvest and needed to be removed. Then, Ri was obtained by washing roots with distilled water and centrifugation of water-Ri solution at 840 × g. All soil fractions were air dried and ground to ensure homogeneous texture. In order to assess the pesticide water-Ri partition during rhizosphere soil extraction, the root washing water was filtered and kept at 4 °C until OCPs analysis.

In addition to roots, stems and leaves were also washed to remove attached soil particles. Plants and soils were kept in a freezer at –20 °C until OCPs analysis.

2.4. Desorption assays

Desorption studies were carried out with BS and NRS from all treatments and control pots. Soil was air dried, crushed and sieved through a 2 mm sieve. Desorption was measured using batch duplicated experiments. Soil (5 g) and MilliQ water as desorption solution (20 mL) were shaken in centrifuge tubes end-over-end for 24 h at 20 °C, then centrifuged at 1720 × g for 15 min. The supernatant was analyzed for pesticide residues and data were expressed as ng L⁻¹.

2.5. Organic carbon (OC) content of soil fractions and desorption solutions

In order to assess if organic amendments modified the carbon content of soils or desorption solutions, dissolved organic carbon (DOC) in the supernatants from batch tests and OC in unplanted soil, BS and NRS were determined (duplicated) by spectrometric

measurement (590 nm) after wet C oxidation with potassium dichromate [23].

2.6. DDTs extraction and purification

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

2.6.1. Soils and plant tissues

DDTs (*p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD) were extracted according to Metcalfe and Metcalfe [24], with modifications of Miglioranza et al. [25]. Subsamples of 10 g dry soil and 3 g wet weight plant tissues were homogenized with sodium sulfate and spiked with 20 ng 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. Lipid percentage was calculated after removing them from plant extracts by gel permeation chromatography using Bio Beads S-X3 (200–400 mesh, 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, then concentrated to 1 mL and kept in sealed vials at -20°C until chromatographic analyses.

2.6.2. Water samples

Aqueous solution from desorption studies as well as those from Ri washing were liquid–liquid extracted according to Gonzalez et al. [26]. Solutions were spiked with 20 ng PCB #103 as internal standard in acetone and extraction was performed by adding a mixture of dichloromethane:hexane (2:1), which was shaken vigorously for 2 h in a horizontal shaker. The bottle was kept at 4°C undisturbed to separate the two layers. The organic layer was transferred to a glass balloon and evaporated to 2 mL for further clean up using silica gel chromatography. Extracts were concentrated to 1 mL and kept in sealed vials at -20°C prior to GC analyses.

2.7. Chromatographic determination

DDTs (*p,p'*-DDE, *p,p'*-DDT and *p,p'*-DDD) were identified and quantified using a Gas Chromatograph (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 \times 0.25 mm i.d. \times 0.25 μm film thickness; Supelco Inc.]. One μl 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 $5^{\circ}\text{C min}^{-1}$ up to 150°C , held for 1 min, then $1.5^{\circ}\text{C min}^{-1}$ up to 240°C , and then $10^{\circ}\text{C min}^{-1}$ 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 [24]. The standard solution used for identification and quantification of single compounds was a pesticide mixture from Ultra Scientific, RI, USA and PCB #103 from Accustandard Absolute Standards, Inc., CT, USA.

Table 2

DDTs concentration (ng g^{-1} dry weight) in initial soil (In) and unplanted pots watered with water (Co), root exudates (Re), carboxylic acids (Ca) and Tween 80 (Tw 80). Within rows, values followed by the same letter are not significantly different (at 0.05 level) using ANOVA Friedman test.

Compound	Watering solution				
	In	Co	Re	Ca	Tw 80
<i>p,p'</i> -DDT	101.3 ± 13.4^c	86.6 ± 6.1^c	$69.8 \pm 9.9^{a, b}$	56.7 ± 6.4^a	$74.7 \pm 15^{b, c}$
<i>p,p'</i> -DDE	381.4 ± 50.4^a	440.1 ± 47.5^a	407.5 ± 75.2^a	422.5 ± 66.6^a	457.3 ± 83.8^a
<i>p,p'</i> -DDD	$0.4 \pm 4 \times 10^{-2} a$	$0.7 \pm 5 \times 10^{-2} b$	3.5 ± 0.1^c	$5.3 \pm 4 \times 10^{-2} e$	4.1 ± 0.6^d

Table 1

Total biomass and length (root + aerial tissues) in plant watered with water (Co), root exudates (Re), carboxylic acids (Ca) and Tween 80 (Tw 80) at the beginning (Initial) and at the end of the experiment (Final). Within row (treatment) values followed by the same letter are not significantly different (at 0.05 level) using paired *t*-test.

Watering solution	Total length (cm)		Total weight (g)	
	Initial	Final	Initial	Final
Co	59.7 ± 10.6^a	69.0 ± 9.7^a	76.6 ± 12.4^a	69.0 ± 6.0^a
Re	57.3 ± 13.3^a	49.8 ± 5.3^a	66.3 ± 4.8^a	62.1 ± 5.3^a
Ca	54.7 ± 1.9^a	55.5 ± 5.8^a	67.1 ± 14.2^a	57.9 ± 13.3^a
Tw 80	54.5 ± 5.9^a	55.3 ± 4.6^a	70.4 ± 21.6^a	64.9 ± 18.7^a

2.8. Quality control and assurance

Laboratory and instrumental blanks analyzed throughout the procedure indicate that there was not contamination or interference on samples during laboratory handling. Recoveries, calculated by spiked matrixes, were greater than 90%. Detection limits (DL) for DDTs, according to Keith et al. [27], were $<0.2\text{ ng g}^{-1}$.

2.9. Statistical analysis

The data reported in this work are expressed in ng g^{-1} on a dry weight basis. Results from each treatment represent the mean of three independent extractions and quantifications of different soil, water or plant samples. Statistical analyses were performed using Infostat Software Package [28]. Nonparametric ANOVA Friedman tests followed by a *t*-paired test for dependent samples were applied to assess differences among treatments as well as differences among tissues or soil fractions within treatments. The significance level was set at $\alpha = 0.05$ [29].

3. Results and discussion

Plant total length and biomass at the beginning and at the end of the experiment are displayed in Table 1. Treatments had no significant effect on final individual plant growth suggesting that neither amendments nor growing conditions had phytotoxic effects.

3.1. Effect of soil amendments on DDTs concentrations in soils

3.1.1. Unplanted pots

Unplanted control pots were established in all treatments for determining the influence of the amendments on DDTs concentration in soils. All pots including control, showed depletion on *p,p'*-DDT concentration with respect to the initial soil with significant differences ($p < 0.05$) from control in those watered with root exudates and carboxylic acids (Table 2). Conversely, concentration of *p,p'*-DDD increased under all regimes. The initial soil (*p,p'*-DDE + *p,p'*-DDD)/*p,p'*-DDT ratio was 3.8 and increased up to 5.1, 5.9, 7.5 and 6.2 for control, root exudates, carboxylic acids and Tween 80, respectively. These increased metabolite levels in soils after wetting would respond to the concurrent findings of the increased microorganism activity and pollutants availability after dry-wetting cycles [30] in addition to their ability to

Table 3

BS/CS, NRS/BS and Ri/BS ratios in planted pots watered with water (Co), root exudates (Re), carboxylic acids (Ca) and Tween 80 (Tw 80). CS: control soil; BS: bulk soil; NRS: near root soil; Ri: rhizosphere.

Watering solution	Compound	BS/CS	NRS/BS	Ri/BS
Co	<i>p,p'</i> -DDT	1.6	0.9	1.4
	<i>p,p'</i> -DDE	1.4	0.9	1.2
	<i>p,p'</i> -DDD	0.9	0.6	1.1
Re	<i>p,p'</i> -DDT	1.5	1.3	1.6
	<i>p,p'</i> -DDE	1.2	1.2	1.3
	<i>p,p'</i> -DDD	0.2	0.7	0.7
Ca	<i>p,p'</i> -DDT	1.7	1.3	1.2
	<i>p,p'</i> -DDE	1.2	1.1	1.0
	<i>p,p'</i> -DDD	0.1	1.0	1.3
Tw 80	<i>p,p'</i> -DDT	2.2	1.4	1.1
	<i>p,p'</i> -DDE	1.2	1.2	0.9
	<i>p,p'</i> -DDD	0.3	0.7	0.5

degrade organic compounds [31–33]. It is known that metabolism of *p,p'*-DDT under aerobic conditions mainly leads to *p,p'*-DDE, thus explaining the intrinsic condition of this contaminated soil by intensive and past use of *p,p'*-DDT in the cultivars. However, the occurrence of the main anaerobic metabolite of *p,p'*-DDT, *p,p'*-DDD, at higher levels compared with the initial soil can be related with pot irrigation, that may have caused saturated conditions. Similarly, other agricultural soils with predominance of *p,p'*-DDE residues also have *p,p'*-DDD metabolites [34]. Moreover, from the metabolite parental ratio it seems that Tween 80 and organic acids increase pesticide metabolism. These amendments may induce the growing of specific *p,p'*-DDT degrader-microorganisms or facilitate the co-metabolism of these compounds.

3.1.2. Planted pots

In all treatments the BS showed higher levels of *p,p'*-DDT and *p,p'*-DDE than the corresponding unplanted pot, a fact reflected by BS/CS ratios >1 (Table 3). Plant growth induces both chemical and physical changes in soil that result in a plant-based increase of pesticide availability. On the other hand, the ratio BS/CS for *p,p'*-DDD ranged between 0.05 and 0.29 for amended pots, while it was about 1 in the control pot. The growth of willow plants helps keeping an aerobic environment reducing the anaerobic metabolism of *p,p'*-DDT that was observed in the unplanted pots. Regarding differences among BS, NRS and Ri levels from Table 3 it can be seen that under all regimes (control and amendments) the Ri was enriched in DDTs residues (Ri/BS ratio >1). The effect of amendments on the root–soil system is clearly observed when analyzing NRS/BS ratios. For all compounds NRS/BS ratios were similar to Ri/BS ratios, indicating that NRS would behave as Ri under the different treatments.

Table 4

Organic carbon content of soil fraction and desorption solution and *p,p'*-DDT and *p,p'*-DDE desorption from soil of unplanted and planted pots watered with water (Co), root exudates (Re), carboxylic acids (Ca) and Tween 80 (Tw 80). Un: unplanted pots; BS: bulk soil; NRS: near root soil. Within columns, values followed by the same letter are not significantly different (at 0.05 level) using ANOVA Friedman test.

Watering solution	Soil fraction	Organic carbon		Desorption (ng g ⁻¹)	
		Soil (%)	Desorption solution (mg mL ⁻¹)	<i>p,p'</i> -DDT	<i>p,p'</i> -DDE
Co	Un	3.0	5.5	1.3 ^d	4.6 ^b
	BS	2.9	6.7	1.5 ^d	5.5 ^b
	NRS	3.1	7.4	1.0 ^d	3.7 ^b
Re	Un	3.0	9.4	1.1 ^d	5.3 ^b
	BS	2.9	8.4	2.2 ^d	8.2 ^b
	NRS	2.6	7.4	1.3 ^d	4.6 ^b
Ca	Un	3.1	27.5	2.3 ^c	16.1 ^a
	BS	2.8	17.5	3.6 ^c	14.7 ^a
	NRS	2.9	16.3	3.6 ^c	12.8 ^a
Tw 80	Un	2.9	9.9	0.7 ^e	4.0 ^b
	BS	2.8	7.5	1.1 ^e	4.0 ^b
	NRS	2.8	7.3	1.0 ^d	3.1 ^b

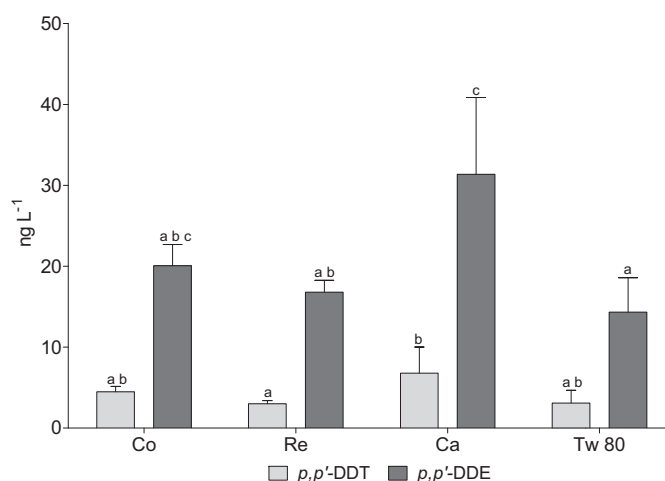


Fig. 1. Concentration of DDTs (ng g⁻¹) in water obtained during the extraction of rhizospheric soil from plants watered with water (Co), root exudates (Re), carboxylic acids (Ca) and Tween 80 (Tw 80). Values followed by the same letter indicate no statistically significant differences (ANOVA Friedman test at 0.05 level) among treatments for each individual compound.

The findings of plant-induced alterations in the *p,p'*-DDT and *p,p'*-DDE levels in soil fractions differing in their proximity to plant roots agree with results from other plant species [35,36].

The increase in availability mediated by plants is a dynamic process; from our results it can be seen that as the contaminant is released from its sequestered state in the BS, the residue may be temporarily accumulated on NRS and Ri soil until it is metabolized or plant uptaken.

3.2. Effect of amendments on soil water partition of DDTs: water from rhizosphere extraction and desorption studies

Fig. 1 shows the levels of DDTs in water obtained from root washing during the rhizosphere extraction. Although under all treatments DDTs were detected, water from treatments with carboxylic acids showed the highest *p,p'*-DDE and *p,p'*-DDT levels. These results showed the fast solubilization of compounds from fresh Ri and the role of organic acids on this process. Thus, soil watering may lead pesticide lixiviation as well as facilitate plant uptake by roots.

Table 4 shows the relative desorption from unplanted pots, BS and NRS. Pesticide desorption followed the pattern *p,p'*-DDE > *p,p'*-DDT > *p,p'*-DDD in agreement with the initial pesticide pattern distribution of soils. A significant enhancement of desorption was

observed for unplanted soil, BS and NRS from pots watered with carboxylic acids as observed in rhizosphere waters. Otherwise Tween 80 amendment caused a depletion on *p,p'*-DDT desorption in unplanted soil and BS. Gonzalez et al. [17] showed that the magnitude of pesticide desorption is dependent on surfactant and pesticide concentration. They found that treatment of Villa Regina soil with Tween 80 at 2 cmc lead to higher desorption than at 10 cmc. In this work, surfactant concentration was used at sub cmc levels in order to avoid plant toxicity and high surfactant loads in soil and desorption. Thus it could be hypothesized that *p,p'*-DDT desorption by Tween 80 in this soil may be dependent on surfactant concentration with an optimal level at about 2 cmc.

On the other hand, other authors have shown that Tween 80 increase diazinon availability at a wide range of concentrations (0.75 mg L^{-1} to 10 g L^{-1}) [37]. However, this result was obtained in a soil with lower clay content. Sánchez-Martín et al. [38] found that the non-ionic surfactant Triton X-100 could be adsorbed on clay minerals such as montmorillonite and illite. The adsorption of the surfactants by clay minerals depends on the nature of the surfactants, structure of the clay minerals and pesticide characteristics. Lombardi et al. [39] characterized montmorillonite as the dominant clay mineral in Rio Negro soils. Thus, the reduced desorption under Tween 80 treatment, in the soil fraction without plant influence may respond to the reported observations that soils with medium content of clay minerals reduce considerably the washing efficiency of non-ionic surfactants by sorption onto the soil matrix [17]. In addition, when considering the NRS, this depletion on *p,p'*-DDT desorption by Tween 80 seems to be counterbalanced by the influence of plant roots. These results suggest some interactions among the high hydrophobic DDTs, the surfactant Tween 80 and the clay matrix in Villa Regina soils. Thus, it could be interesting to test other non-ionic surfactants and establish which relationship exists between them and the plant presence.

It is known that carboxylic acids are involved in the release of humic or fulvic substances into soil solution [40]. Measurements of organic carbon in the soil fraction after the desorption experiment showed little variation with a trend to diminish in NRS in some cases. However, the determination of the organic carbon content in the solutions after the desorption experiment revealed that treatment with organic acids led to an increase of organic carbon in solution between 2.2 and 5 times in the different soil fractions (Table 4). These results indicate that *p,p'*-DDE and *p,p'*-DDT desorption from soils treated with carboxylic acids is clearly related with the release of organic carbon from soil, which in turn leads to a higher compound availability in soil solution.

Finally, from Fig. 1 and Table 4 it can be seen that water partition of pesticides was greater on fresh Ri than on BS and NR air dried. Soil manipulation as well as some root-water transference of pesticides during washing may explain such differences. Moreover, since desorption studies were carried out during 24 h, soil to water partition of pesticides is likely to be under equilibrium.

3.3. Effects of soil amendments on DDTs uptake and translocation by willows

3.3.1. Plant uptake and tissue distribution

Fig. 2 shows DDTs levels in willow roots, stem and leaves at the beginning and after exposure to all regimes. Growing on contaminated soils results in an effective accumulation of *p,p'*-DDT, *p,p'*-DDE and *p,p'*-DDD. Under all regimes, compounds were taken up by plant roots and the distribution pattern was roots > leaves > stem. This result accurately indicates the translocation of DDTs by willow plants since volatilization from soil to aerial plant parts had been prevented by covering soil surface with aluminum foil. Root concentration of DDTs (*p,p'*-DDT + *p,p'*-DDE + *p,p'*-DDD) ranged from 5150 ng g^{-1} (root exudates

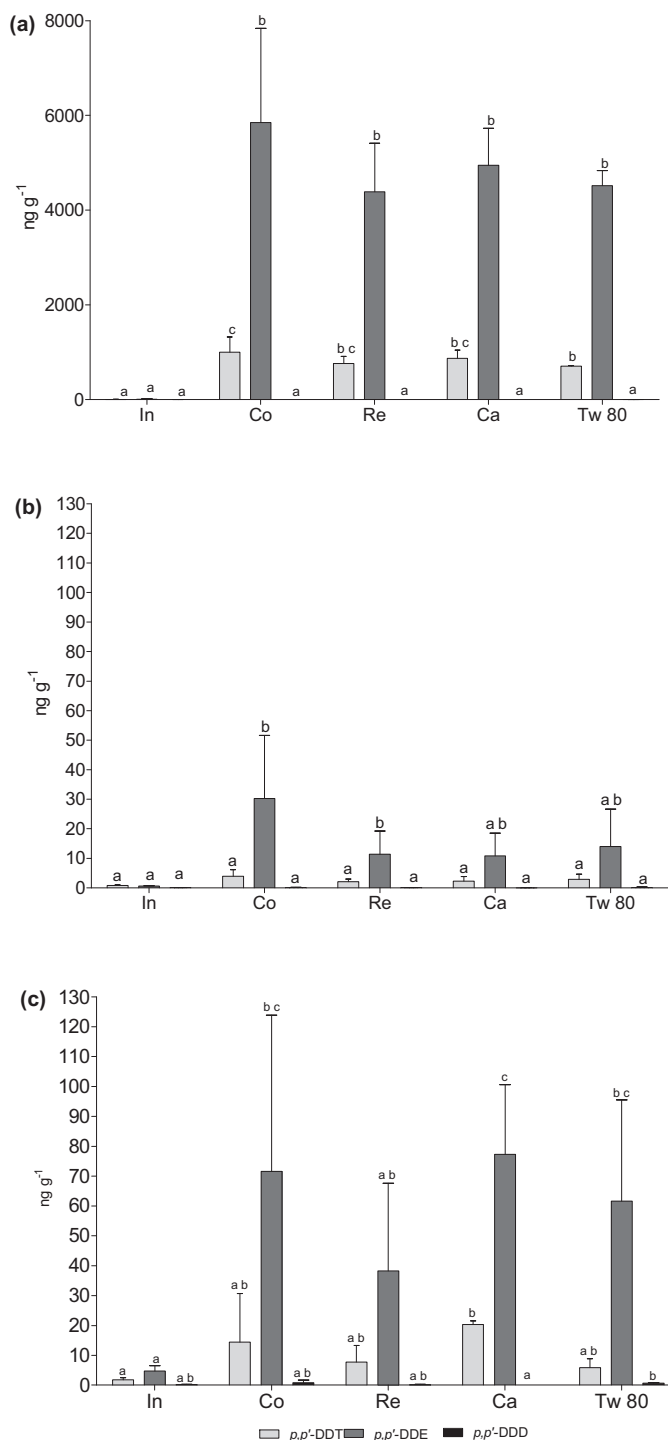


Fig. 2. DDTs concentration (ng g^{-1}) in roots (a), stems (b) and leaves (c) of willow plants at the beginning (In) and after exposure to water (Co), root exudates (Re), carboxylic acids (Ca) and Tween 80 (Tw 80). In each tissue, values followed by the same letter indicate no statistically significant differences (ANOVA Friedman test at 0.05 level) among treatments for each individual compound.

amendment) to 6854 ng g^{-1} (control-water) while for stem and leaves it varied between 13 (organic acids amendment) and 34 ng g^{-1} (control-water) and 46 (root exudates amendment) and 97 ng g^{-1} (organic acids amendment), respectively. The relative accumulation of each compound was dependent on soil levels following the pattern *p,p'*-DDE > *p,p'*-DDT > *p,p'*-DDD in all cases (ANOVA Friedman $p < 0.05$).

Table 5

Root bioconcentration factors (RBCF), stem translocation factors (STF) and leaves translocation factors (LTF) of willows watered with water (Co), root exudates (Re), carboxylic acids (Ca) and Tween 80 (Tw 80).

Watering solution	Compound	RBCF	STF	LTF
Co	<i>p,p'</i> -DDT	7.2	1×10^{-2}	2.4
	<i>p,p'</i> -DDE	9.2	1×10^{-2}	1.6
	<i>p,p'</i> -DDD	3.2	0.1	4.9
Re	<i>p,p'</i> -DDT	7.2	4×10^{-4}	2.8
	<i>p,p'</i> -DDE	9.0	3×10^{-3}	2.5
	<i>p,p'</i> -DDD	2.0	0.1	0.6
Ca	<i>p,p'</i> -DDT	8.9	4×10^{-3}	5.6
	<i>p,p'</i> -DDE	9.9	3×10^{-3}	4.7
	<i>p,p'</i> -DDD	6.1	0.1	^a
Tw 80	<i>p,p'</i> -DDT	5.9	1×10^{-2}	1.7
	<i>p,p'</i> -DDE	8.2	4×10^{-3}	3.6
	<i>p,p'</i> -DDD	2.6	0.1	3.5

^a *p,p'*-DDD values in leaves below detection limit.

The biochemical characteristics of plants are believed to significantly affect the uptake of organic chemicals [41] and numerous authors have reported that both, kind and lipid content may have a significant effect on the POPs storage capacity of plants [42,43]. However, in this study, lipids were not correlated either with treatments or with pesticide distribution. Lipid percentage ranged between 0.58 and 0.80 in roots, 1.12 and 1.38 in stems and 0.69 and 1.07 in leaves.

3.3.2. Soil to root pathway

Root bioconcentration factors (RBCFs) were calculated by determining the dry-weight ratio of DDTs concentration in root to that in BS of the corresponding pot (Table 5). Under all conditions RBCFs were >1 and being the highest values those of *p,p'*-DDE followed by those of *p,p'*-DDT and *p,p'*-DDD. Values of RBCF for *p,p'*-DDE in plants under all treatments did not differ from control values while those of *p,p'*-DDT and *p,p'*-DDD decreased and increased under Tween 80 and carboxylic acid regimes, respectively. Considering the result obtained from the metabolite/ratio in soil under those watering solutions, it can be concluded that the occurrence of *p,p'*-DDD in willow roots is a consequence of their uptake from soil rather than plant inside metabolism. This assumption is especially true for *p,p'*-DDE which constitutes the main residue in soil.

The well known hyperaccumulators zucchini and pumpkin plants of the *Cucurbitaceae* family concentrate *p,p'*-DDE in their roots at levels about 10–20 times those found in soil [36]. Conversely, species like crimson clover, mustard, hairy vetch, and rye grass are considered poor accumulators since they accumulate 2–5 times the amount of pollutant in the soil [39]. In this work willow plants can be classified as medium–high accumulators of *p,p'*-DDE since they have levels in roots 8–9 times higher than those found in soil. Moreover under amendment growing they may also be medium–high accumulators of *p,p'*-DDT.

3.3.3. Root to aerial route

DDTs levels in leaves and stems are conditioned by the pesticide characteristics and the plant translocation abilities. In some cases, pollutant uptake and translocation lead to a homogeneous distribution (leaves = stem) as was reported by Whitfield Åslund et al. [44] for PCBs in zucchini plants, while for DDTs the accumulation was higher in leaves [45]. Translocation can be evaluated by calculation of the ratio of contaminant concentration in stem to that in roots, called *stem translocation factor* (STF), or as the ratio of contaminant concentration in the leaves to that in the stem (*leaves translocation factor*, LTF). Translocation is of interest because a successful phytoremediation candidate must transfer most pollutants from roots to aerial tissues [46]. The combination of chemical solubility in water and within the lipophilic cell membrane determines its

movement into roots and subsequent transport to the plant shoot and leaves [47]. As is shown in Table 5 all treatments increased the *p,p'*-DDE translocation to leaves although no effect on RBCF was observed and conversely the *p,p'*-DDD LTFs were depleted, except for Tween 80. Under the carboxylic acid treatment, *p,p'*-DDT also had higher LTF than control plants, while those watered with Tween 80 had lower values. Translocation is highly dependent on both chemical polarity and plant transpiration. The less polar a compound is, the more it is retained on lipid material, being less mobile across the endodermis [10]. But in this work, as in other ones, *p,p'*-DDE, a highly hydrophobic compound ($\log K_{ow}$ 6.96), is being translocated and organic acids, root exudates and the non ionic surfactant Tween 80 are enhancing such mobilization to leaves. However carboxylic acids showed the highest factors and also enhanced *p,p'*-DDT ($\log K_{ow}$ 6.91) translocation. Moreover, LTF could be underestimated by processes like pesticide metabolism within plant and volatilization of compounds during transpiration [9]. White and Kottler [40] proposed that the release of low molecular weight organic acids that are frequently exuded by plants results in deconstruction of soil matrix that may subsequently increase both nutrient and pollutant availability. Those findings agree with this study, where soil amendment with citric and oxalic acids results in significantly greater quantities of DDTs being transferred to the aerial tissues of willows. Translocation seems to be assisted by organic acids that may act as pesticide carriers alone or in association with some amphiphilic molecules. Although root exudates were used in this work on the basis that their content in carboxylic acids would have an effect on pollutant availability, no analysis about composition was performed and results showed that watering with root exudates, was not as effective as organic acids alone. This would mean that the organic acid content in willow exudates after 30 days was not high enough to enhance pollutant availability or else that other compounds from the complex composition of exudates would be interfering with this process.

Interestingly, in this study we also found that Tween 80 treatment, at 0.5 cmc, not only significantly decreased pesticide concentration in the plant stem in comparison to the control treatment but also resulted in low RBCF and LTF. Considering the low aqueous availability of DDTs observed in desorption assays from soil fractions of Tween 80 treatments it seems that some soil pesticide–surfactant interactions occur and also limit plant uptake.

4. Conclusions

The findings of this study indicate that amendments affect willow phytoremediation of aged DDTs residues in soils. Bioavailability of aged pesticide residues in soil is one of the most important factors to be considered for assessing both environmental risk and phytoremediation efficiency. The results showed that willow root exudates, non-ionic surfactant (Tween 80) and carboxylic acids (citrate and oxalate) had effects on DDTs desorption from soil, plant uptake metabolism and translocation.

Treatments with organic acids enhanced the availability of DDTs and it is related to the increase of the organic carbon content in solution, leading to higher uptake and translocation by willows.

The surfactant Tween 80 presented a dual behavior. Thus results showed that this compound would increase the *p,p'*-DDT metabolism in soils, nevertheless the surfactant also interacts with clay soil preventing the pesticide transfer to the aqueous phase.

More research is needed to explain the complex interactions among pesticides, soil matrix and amendments in the used tree–soil system. Those amendments that increase pesticide availability and metabolism would help the phytoremediation of hydrophobic compounds and, in this sense willows can act as “water–pesticide pumps” due to its high transpiration rate.

However it is important to consider the potential lixiviation of pesticides which would represent a risk for groundwater contamination.

As a whole, the mechanisms by which highly sequestered residues of organic contaminants are taken up and translocated in willow plants remain unknown; however some specific issue about the role of dissolved organic carbon were addressed in this work. More studies are needed to gain some insight on stem partition and volatilization by leaves after translocation on a longer time study as well as under field conditions.

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