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Potential use of edible crops in the phytoremediation of endosulfan residues in soil

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HIGHLIGHTS

- Endosulfan uptake and translocation present vegetable interspecific variations.
- The biomarker lipid peroxidation correlated with the endosulfan accumulation.
- Sunflower is the best suitable for the phytoremediation of endosulfan in soils.

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ABSTRACT

Endosulfan is a persistent and toxic organochlorine pesticide of banned or restricted use in several countries. It has been found in soil, water, and air and is bioaccumulated and magnified in ecosystems. Phytoremediation is a technology that promises effective and inexpensive cleanup of contaminated hazardous sites. The potential use of tomato, sunflower, soybean and alfalfa species to remove endosulfan from soil was investigated. All species were seeded and grown in endosulfan-spiked soils (8000 ng g^{-1} dry weight) for 15 and 60 days. The phytoremediation potential was evaluated by studying the endosulfan levels and distribution in the soil-plant system, including the evaluation of soil dehydrogenase activity and toxic effects on plants. Plant endosulfan uptake leads to lower insecticide levels in the rhizosphere with regards to bulk soil or near root soil at 15 days of growth. Furthermore, plant growth-induced physical-chemical changes in soil were evidenced by differences in soil dehydrogenase activity and endosulfan metabolism. Sunflower showed differences in the uptake and distribution of endosulfan with regard to the other species, with a distribution pesticide pattern of aerial tissues > roots at 15 days of growth. Moreover, at 60 days, sunflower presented the highest pesticide levels in roots and leaves along with the highest phytoextraction capacity. Lipid peroxidation levels correlated positively with endosulfan accumulation, reflecting the negative effect of this insecticide on plant tissues. Considering biomass production and accumulation potential, in conjunction with the reduction of soil pesticide levels, sunflower plants seem to be the best phytoremediation candidate for endosulfan residues in soils.

1. Introduction

Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9a-hexahydro-6, 9-methano-2, 3, 4-benzodioxyanthiepin-3-oxide) represents the last organochlorine pesticide broadly being used in worldwide agriculture. It was commonly applied on fruits, cotton, vegetables, tobacco, sugarcane, and tea for the control of tsetse flies, mites, home garden pests, and cabbage worms, as well as its use as a wood preservative (Rice et al., 1997; Antonious et al., 1998). In 2001, the Agency for Toxic Substances and Disease Registry

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(ATSDR) enlisted endosulfan as a persistent toxic pollutant, and in 2011 it was declared a persistent organic pollutant (POP) by the Stockholm Convention (UNEP/POPS/POPRC, 2008). However, endosulfan is still considered an environmental concern because the POP convention relaxed the ban on endosulfan use for a few crop–pest complexes with a five-year phase-out period (UNEP/POPS/POPRC, 2008). Technical-grade endosulfan consists of a mixture containing 95% of two diastereoisomers, known as α-endosulfan and β-endosulfan, in ratios varying from 2:1 to 7:3 (Kennedy et al., 2001). Endosulfan isomers can be transformed to the more hydrophilic endosulfan diol, lactone and hydroxy ether metabolites, but the main degradation product formed through biological transformation is endosulfan sulfate (Goswami et al., 2009).

The persistence of endosulfan in soil and water environments has been reported under different conditions (Singh and Singh, 2014). Thus, endosulfan residues might be still found in soil samples, representing a source of pollution to the environment despite the recent laws banning its use (Jia et al., 2010). Much of the concern over these compounds is related to their toxicity and biomagnification through aquatic and terrestrial food chains (Kelly and Gobas, 2001). Thus, strategies for endosulfan removal from the environment should be studied to develop remediation techniques.

Phytoremediation might be used to remove organic contaminants, including organochlorine pesticides, from soil based on several plant mechanisms or plant–microbe interactions (Gerhardt et al., 2009). The plant species intended for use in phytoremediation should grow well in pesticide-contaminated soils, because several reports indicate that organochlorine pesticides are toxic to several plant species (Sharada et al., 1999; Perez et al., 2008). Additionally, to show the toxic effects on plant growth that will limit phytoremediation success (Susarla et al., 2002), other variables, including biochemical responses, can occur and influence the pollutant uptake or metabolism. Several organic compounds are known to increase the generation of reactive oxygen species (ROS), leading to oxidative damage, including membrane lipid peroxidation (LPO). As a result, an increase in LPO levels due to plant exposure to 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) and endosulfan was reported for several plant species (Mitton et al., 2014; Ramirez Sandoval et al., 2011) indicating the utility of this biochemical response as a toxicity indicator.

The main objective of this study was to evaluate the endosulfan phytoremediation potential of different crops species. For this purpose, plant uptake, soil levels and rhizospheric enhancement were evaluated in conjunction with LPO as a toxicity biomarker.

2. Material and methods

2.1. Soil

Soils representative of the Argentinean Pampa (association of typical argiudols and udifluvent) were sampled from a soybean field near La Dulce village in the Río Quequén Grande watershed (S 38° 11.7′ 29″ W 59° 08.8′ 36″). Surface soils had a total pesticide level (endosulfan, DDTs, HCHs, heptachlors, dieldrin and chlordanes) lower than 2×10^{-6} mg g^{-1}, and comprised 1.9% organic carbon, 60.7% sand, 31.8% silt and 7.3% clay (Gonzalez et al., 2010). The soil samples were air-dried until constant weight, ground to obtain a homogeneous matrix and maintained at 4 °C before conducting the experiment. Each soil sample was spiked by adding technical endosulfan (Master R, Chemiplant S.A. 35%) dissolved in acetone to achieve a final concentration of 10 μg g^{-1}. After the solvent evaporated, the spiked soil was shaken for 30 days until a homogeneous distribution of pesticides was achieved. Then, the sample was maintained for one week at room temperature, avoiding light effects or evaporation processes, before it was used in the phytoremediation experiments. The pesticide residues were analyzed immediately before initiating the experiments to evaluate the homogeneous distribution of the spiked soil.

2.2. Plant growth

Seeds of tomato (10), sunflower (10), soybean (10), and alfalfa (50) were placed in rectangular pots measuring 6000 cm^3, covered with aluminum foil and containing 1000 g of spiked dry soil under greenhouse conditions (10–26 °C, light:dark 14:10 h). Three planted pots were established for each species and time period. Unplanted pots (Un) were also employed during the experiments. All pots (planted and unplanted) were weeded on demand and watered weekly with tap water.

2.3. Soil and plant sampling

To study the influence of life stage on pesticide uptake and LPO, a destructive harvest was performed at 15 (first period) and 60 days (second period) after germination (appearance of the first true leaves). Two or three plants were harvested per pot and period. Roots, stems and leaves obtained from each pot were pooled and analyzed as a single sample. Plant subsamples were immediately frozen and maintained at −80 °C until analysis.

Within each pot, three separated soil fractions were defined according to White (2001) in relation to the influence exerted by the plant root. Bulk soil (BS) that had no contact with plant roots was taken from the top of individual planted pots. The near-root soil (NRS) was operationally defined as the soil that was under root influence. The NRS settled within the volume occupied by the roots. The rhizosphere soil (Ri) was defined as the soil that remained attached to the roots and required mechanical removal. The Ri was obtained by washing the roots with distilled water and a 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. Soil samples were maintained frozen (−80 °C) until analysis.

2.4. Dehydrogenase activity determination

Soil dehydrogenase activity (DHA) analysis was used to assess the microbiological activity in the soil samples (Wu and Brookes, 2005), differing in their proximity to roots (BS and NRS), from plants grown on endosulfan-polluted soils. One gram of each soil sample was incubated, in triplicate, for 24 h at 25 °C, in 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 a 490 nm (Trevors, 1984; García et al., 1997).

2.5. Soil pH and humidity

The soil subsamples were air dried at room temperature until they achieved a constant weight. The soil pH was measured in a soil/deionized water suspension of 1/2.5 (w/v). The water content was determined by constant-weight drying in an oven at 110 °C.

2.6. Endosulfan extraction and purification

All solvents of residue analysis quality and other reagents were obtained from Merck Co. (Darmstadt, Germany). The endosulfan (α-Endosulfan + β-Endosulfan + Endosulfan sulfate) was extracted according to Metcalfe and Metcalfe (1997), with the modifications

of Miglioranza et al. (2003). Subsamples of 5 g of air-dried soil and 3 g of wet plant tissues were homogenized with sodium sulfate and spiked with 20 ng of PCB #103 as a surrogate standard; these were Soxhlet extracted (8 h) with a mixture of hexane–dichloromethane (50:50), then concentrated using a vacuum pump, and finally concentrated to 2-mL volume under nitrogen flow. The lipid percentage was calculated after removing the plant extracts by gel permeation chromatography in Bio Beads S-X3 (200–400 mesh size, Bio-Rad Laboratories, Hercules, CA, USA), and then drying the extracts under vacuum and nitrogen flow to a constant weight. The cleaning of all extracts containing pesticides was performed by silica gel chromatography, concentrated to 1 mL and maintained in sealed vials at −20 °C prior to gas chromatography analysis.

2.7. Chromatographic determination

Endosulfans were analyzed according to Miglioranza et al. (2003), using a Shimadzu GC-ECD 17A, equipped with a fused-silica capillary column of 30 m, SPB-5 (0.25 mm i.d., 0.25 μm film thickness; Supelco, Sigma–Aldrich, USA). The initial oven temperature was 100 °C, held for 1 min, followed by increases 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, held for 3 min. The inject port was set at 275 °C, and the detector was set at 300 °C. The carrier gas was ultra-high purity helium (1.5 mL min^{-1}). Quantification was performed using a standard purchased from Ultra Scientific (North Kingstown, RI, USA).

2.8. Quality control and assurance

Laboratory and instrumental blanks analyzed throughout the procedure indicate that there were no contaminants or interferences of the samples during laboratory handling. Single compound recoveries, calculated by a spiking matrix and surrogate recovery, were greater than 90%. Instrumental detection limits (DL; 0.1 ng mL^{-1}) for endosulfan were calculated according to Keith et al. (1983), and the method detection limits were <0.033 ng g^{-1}.

2.9. Lipid peroxidation

The thiobarbituric acid-reactive substances (TBARS) method, according to Khan and Panda (2008), with modifications by Mitton et al. (2014), was used to estimate lipid peroxidation (LPO) in plants grown in spiked and control (non-spiked) soils. Tissues were homogenized (1:5) in 0.1% trichloroacetic acid (TCA). An extract (41.2 μL) was added to a reaction mixture consisting of 150 μL of 20% acetic acid, 150 μL of 0.8% thiobarbituric acid, 50 μL of Milli Q water and 20 μL of 8.1% sodium dodecyl sulfate (SDS). The 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: 520 nm; emission: 580 nm). Tetramethoxypropane (TMP, Across Organics) was employed as an external standard and the LPO levels were expressed as nanomoles of TMP per gram of wet tissue.

2.10. Statistical analysis

The pesticide residue data were expressed in ng g^{-1} on a dry weight basis (dw). The values of the pesticides, LPO and DHA represent the mean of three independent extractions and the quantification of different soil fractions or plant tissues. Statistical analysis was performed using Infostat Software Package (Grupo InfoStat, 2008). A non-parametric ANOVA Friedman test or t-paired test for dependent samples was applied to assess the differences among plant tissues or soil fractions within species at 15 and 60 days of growth. Significant differences among species were assessed by a factorial ANOVA test, considering the treatments (treated and control) and times (15 and 60 days) as factors, followed by Tukey analysis. The significance level was set at $\alpha = 0.05$ (Zar, 1984). Normality and variance homogeneity were verified using Shapiro–Wilks test and analyzing the residual plots, respectively, before using ANOVA analysis.

3. Results and discussion

3.1. Soil

3.1.1. DHA and total endosulfan levels

Soil function and characteristics may influence a pesticide's availability, metabolism and, ultimately, its uptake and translocation by plants. As a result, plant growth will also influence soil processes. Among other parameters used to characterize the soil system, dehydrogenase enzymes, which occur intracellularly in all living microbial cells and are linked with microbial respiratory processes (Bolton et al., 1985), could be used as an indicator of the overall microbial activity of soils. Spiked soils (SS) exhibited higher dehydrogenase activity (DHA) than non-spiked soils (NSS, Table 1). Several studies reported either DHA inhibition or stimulatory effects in the presence of pesticides (Singh and Singh, 2005). Some microbial groups are capable of using applied pesticides as a source of energy and nutrients to multiply, whereas the pesticide may be toxic to other groups (Johnsen et al., 2001). As a result, microbial communities with a high metabolic capacity for using organic pollutants as carbon and energy sources might be favored. The analysis of spiked planted soils showed that the DHA activity in BS and NRS plants at 15 days of growth was higher than at 60 days, in all species, except tomato NRS (Table 1). This depletion of DHA activity was also in agreement with a reduction in the total endosulfan levels for all species and soil fractions, except in soybean BS. Additionally, the pH values in BS and NRS were higher at 60 days than at 15 days of growth (except in sunflower plants). Plant growth is known to modify these soil parameters (Yan et al., 1996), because plant growth might lead to the decarboxylation of organic anions present in root exudates by microorganisms. The increase in soil pH and pesticide metabolism with plant age was previously reported for the same species growing in DDT-polluted soils (Mitton et al., 2014), however, it was associated with increments in DHA activity. Thus, the dynamics in the root–soil system seem to depend on both soil and pesticide characteristics.

3.1.2. Endosulfan levels and isomer distribution

Unplanted pots (Un). Pots without plants were established to determine the influence of watering on the endosulfan dynamics in soil. An analysis of the total endosulfan concentration (α-endosulfan + β-endosulfan + endosulfan sulfate) showed lower levels of all compounds after 15 and 60 days of soil watering (Fig. 1). Because the pots were covered with aluminum foil to avoid volatilization, these results might indicate a decline from endosulfan degradation to other metabolites not analyzed or from percolation with the irrigation water.

Additionally, the time dependent decrease was due to a reduction in the α-isomer at 15 days, followed by a reduction of the β– isomer at 60 days, with a concomitantly increment of the sulfate metabolite (Fig. 1). These results suggest that endosulfan sulfate is primarily formed from α-isomer and, as time passed, β– isomer also began to degrade the sulfate metabolite. Moreover, this behavior is in agreement with the β-endosulfan structure configuration of chlorines that favors physical (soil adsorption) and

Table 1
Values of pH, endosulfan content (α-endosulfan + β-endosulfan + endosulfan sulfate, ng g^{-1} dry weight) and dehydrogenase activity (DHA; mg kg^{-1} h^{-1}) in initial non spiked soil (NSS); initial spiked soil (SS), bulk soil (BS) and near root soil (NRS) of tomato, sunflower, soybean and alfalfa plants at 15 and 60 days of growth in spiked soils.

Specie	Days	Soil fraction	pH	Endosulfan (ng g^{-1} dw)	DHA (mg kg^{-1} h^{-1})
	Initial	NSS	7.5 ± 0.3	1.18 ± 0.25	1.64 ± 0.1
		SS	7.7 ± 0.4	7959 ± 493	6.61 ± 0.4
Tomato	15	BS	7.5 ± 0.4	5366 ± 290**	5.36 ± 0.7**
		NRS	8.2 ± 0.1	3234 ± 891**	4.23 ± 1.4
	60	BS	8.3 ± 0.2**	4733 ± 309	4.83 ± 0.4
		NRS	8.3 ± 0.1**	2469 ± 194	4.77 ± 0.1**
Sunflower	15	BS	7.9 ± 0.4	5514 ± 1159**	4.81 ± 0.5**
		NRS	8.1 ± 0.5	3480 ± 2475**	5.18 ± 0.6**
	60	BS	7.7 ± 0.2	3984 ± 743	3.97 ± 0.1
		NRS	7.7 ± 0.3	321 ± 30	3.60 ± 0.04
Soybean	15	BS	7.5 ± 0.2	3076 ± 494	*
		NRS	7.7 ± 0.1	4975 ± 976**	5.47 ± 0.3**
	60	BS	8.1 ± 0.2**	3669 ± 101**	5.28 ± 0.6
		NRS	7.9 ± 0.1**	3543 ± 465	3.60 ± 0.1
Alfalfa	15	BS	7.3 ± 0.3	3376 ± 581**	6.17 ± 0.4**
		NRS	7.7 ± 0.1	5299 ± 965**	6.68 ± 0.4**
	60	BS	8.4 ± 0.2**	2203 ± 331	4.82 ± 0.5
		NRS	8.2 ± 0.2**	3055 ± 632	6.04 ± 0.4

*Non determined DHA.
**Indicate statistically significant differences between stage of plant growth (15 and 60 days) within each species and soil fraction (BS and NRS) ($p \leq 0.05$).

metabolic stability and storage in biological media (Singh and Singh, 2014) and affords its resistance to hydrolysis and enzymatic degradation (Loibner et al., 1998).

Planted pots. Similar to unplanted pots, all soil fractions (BS, NRS and Ri) demonstrated a reduction in endosulfan levels with respect to the initial soils (Figs. 1 and 2). However, at 15 days of plants growth, the BS showed higher endosulfan levels than the corresponding Un soil (Figs. 1 and 2), demonstrating that plant presence might prevent pesticide loss from leaching processes because roots

Table 2
Length and biomass percentages (%) of tomato, sunflower, soybean and alfalfa roots and aerial tissues of plants grown in spiked soils respect to plants grown in non spiked soils for 15 and 60 days.

Specie	Tissue	Length		Biomass	
		15 days	60 days	15 days	60 days
Tomato	Root	80	121	14*	34*
	Aerial	101	111	50*	59*
Sunflower	Root	21*	27*	41*	11*
	Aerial	78*	86*	20*	73*
Soybean	Root	109	65*	22*	13*
	Aerial	189*	27*	40*	23*
Alfalfa	Root	91	50*	66	33*
	Aerial	127*	45*	1*	153*

*Indicate statistically significant differences between plants grown in spiked soils or in non spiked soils ($p \leq 0.05$).

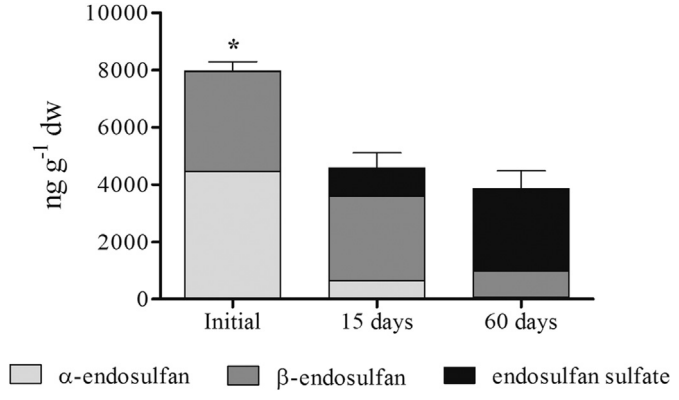

Fig. 1. Endosulfan concentration (ng g^{-1} dw) in spiked soils at the beginning (In) and in unplanted pots watered with water during 15 and 60 days. * indicate statistically significant differences ($p < 0.05$).

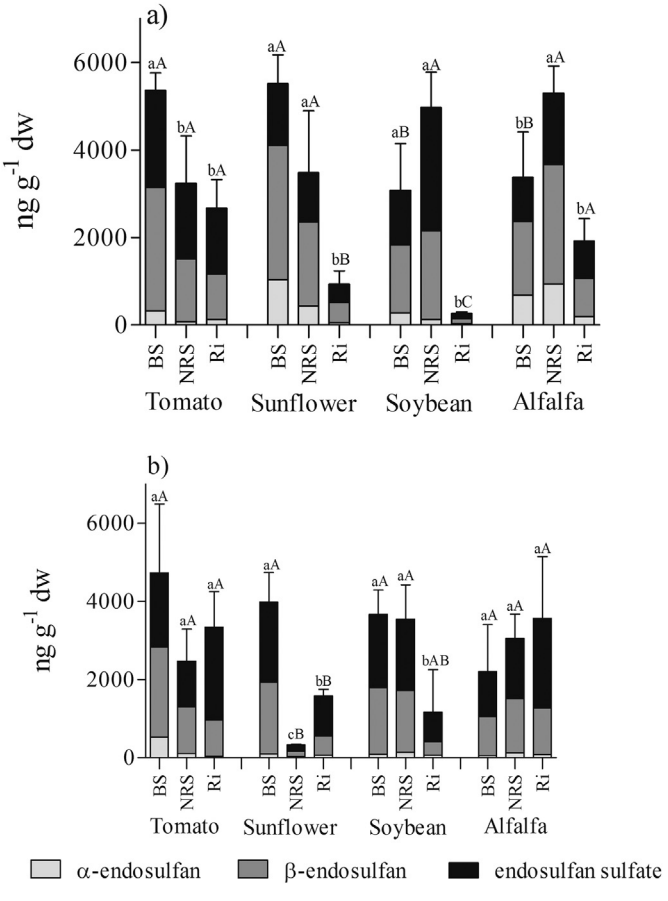

Fig. 2. Endosulfan concentration (ng g^{-1} dw) in bulk soil (BS), near root soil (NRS) and rizhosphere (Ri) of tomato, sunflower, soybean and alfalfa plants grown during 15 (a) and 60 (b) days. Lower case letters indicate statistically significant differences ($p < 0.05$)for Endosulfan among soil fraction for one specie and stage; upper case letters indicate statistically significant differences ($p < 0.05$) in Endosulfan compounds among species within each soil fraction and stage.

increase soil stability and reduce water percolation by plant uptake.

However, plants did influence the pesticide levels in soils with time, (Fig. 2) exhibiting a lower endosulfan concentration at 60 days in BS and NRS, whereas an inverse pattern was observed in Ri. Therefore, this decrease in BS and NRS endosulfan levels may be connected with pesticide plant uptake, in addition to a continuous microbial metabolism in soils. The pesticide distribution within the different soil compartments (BS, NRS and Ri) might be the result of the plant-facilitated mobilization of endosulfan. White (2001) describes this behavior as a dynamic process where as the contaminant is released from the BS, the residue may temporarily be re-adsorbed in the Ri toward the root.

The ratio of metabolite/parental (α-endosulfan + β-endosulfan/endosulfan sulfate) represents the extent of metabolism in soils, considering that the α-endosulfan metabolism rate is higher than that of β-endosulfan. In addition, β-endosulfan can be converted to α-endosulfan (Singh and Singh, 2014). The higher ratio of metabolite/parental found in BS at 15 days in relation to Un soil (Figs. 1 and 2) demonstrated the plant influence on endosulfan metabolism. Otherwise, the Ri exhibited ratios greater than 1 at 15 and 60 days. These results suggest a rhizospheric effect on endosulfan metabolism caused by an increase in microbial density and/or metabolic activity of the released root exudates (Chaudhry et al., 2005), as contaminant degradation might be directly attributed to root-driven extracellular enzymes (Gao et al., 2010). However, this rhizospheric effect at 15 and 60 days was not evidenced by the DHA activity of these soil fractions.

3.2. Vegetation analysis

3.2.1. Plant growth

Xenobiotics might alter growth and/or metabolism in plants and these effects will also affect the phytoremediation process. The results of length and biomass in roots and aerial tissues of tomato, sunflower, soybean and alfalfa plants grown in non-spiked and spiked soils are shown in Table 3. A length reduction in root and aerial tissues was observed in sunflower plants from polluted soils at 15 days of growth. On the contrary, soybean plants exhibited an aerial length increase for the same time period. Otherwise, at 60 days, a root and aerial length reduction was observed in sunflower-, soybean- and alfalfa-exposed plants. The results showed a biomass reduction in all exposed plants at both stages of growth (15 and 60 days), except in alfalfa roots after 15 days. An interesting result was that the length of the tomato plants was not affected by endosulfan, whereas endosulfan was associated with a reduction in the biomass at both stages of growth. On the contrary, aerial tissues of alfalfa plants presented an inverse relationship between length and biomass indicating that the growth in length is at the expense of biomass. Several authors reported that pesticide presence affects different plant processes that ultimately influence plant growth. Endosulfan affects cell division in root meristems of *Biden laevis* hydroponically grown at concentrations of 0.01–5 μl g^{-1} (Perez et al., 2008). Chouychai (2012) also showed that the endosulfan and lindane occurrence in soils decreased seedling growth in *Brassica chinensis*.

3.2.2. Endosulfan content in plant tissues

All the species accumulated endosulfan isomers (α + β) and the

Table 3
Endosulfan root bioconcentration factors (RBCF), stem translocation factor (STF) and leaves translocation factors (LTF) of tomato, sunflower, soybean and alfalfa plants, grown for 15 and 60 days.

Specie	Compound	RBCF		STF		LTF	
		15	60	15	60	15	60
Tomato	α-Endosulfan	2.0	0.7	0.5	0.3	0.3	0.09
	β-Endosulfan	1.8	1.5	0.2	0.4	0.09	0.05
	Endosulfan sulfate	4.9	6.2	0.8	0.7	0.03	0.04
Sunflower	α-Endosulfan	0.8	104.6	2.1	0.03	0.05	0.6
	β-Endosulfan	0.7	21.7	1.4	0.01	0.08	0.8
	Endosulfan sulfate	1.4	20.1	1.2	0.3	2.4	
				0.02			
Soybean	α-Endosulfan	1.8	2.3	0.7	0.2	0.25	1.5
	β-Endosulfan	3.4	3.3	0.1	0.02	0.3	0.2
	Endosulfan sulfate	7.5	9.0	0.3	0.2	0.1	0.04
Alfalfa	α-Endosulfan	3.2	5.7	0.3	0.05	*	*
	β-Endosulfan	4.8	6.3	0.3	0.07		
	Endosulfan sulfate	7.7	13.1	0.5	0.07		

*Stems and leaves were not discriminated in any stage.

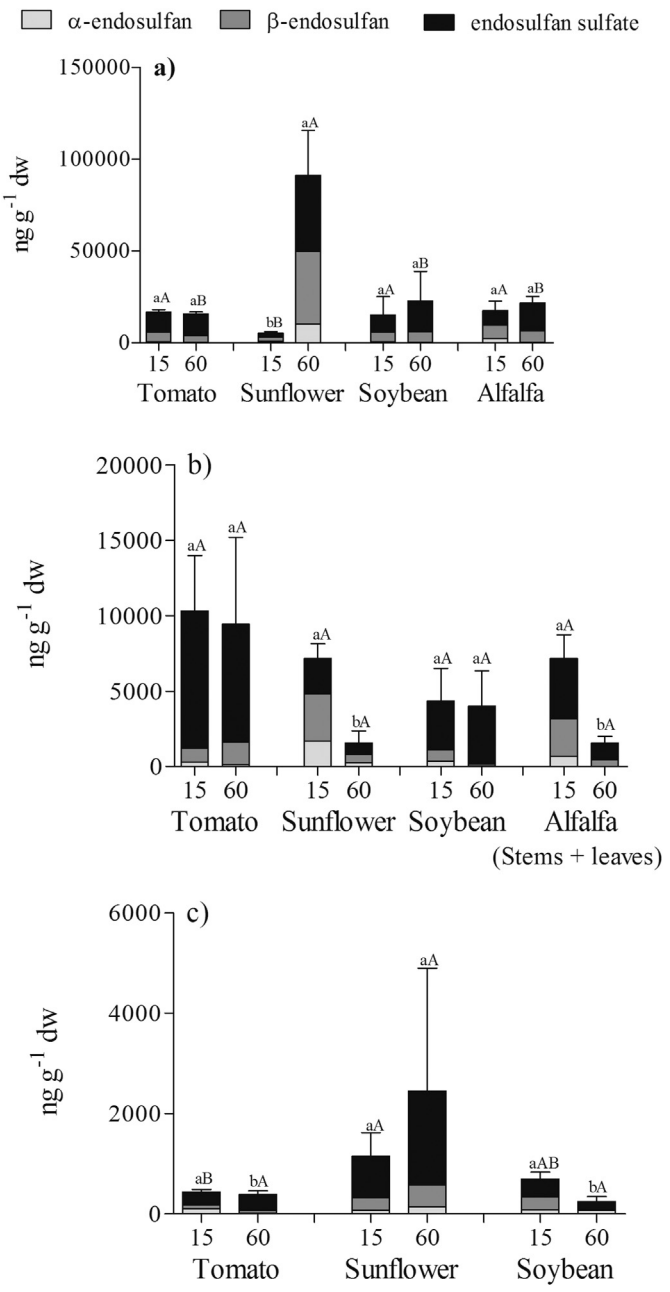

Fig. 3. Endosulfan concentration (ng g^{-1} dw) in roots (a), stems (b) and leaves (c) of tomato, sunflower, soybean and alfalfa plants at 15 and 60 days of growth. Lower case letters indicate statistically significant differences ($p < 0.05$) for Endosulfan between stages for one specie and tissue; upper case letters indicate statistically significant differences ($p < 0.05$) in Endosulfan compounds among species within each tissue and stage.

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 and stage; lower case letters indicate statistically significant differences ($p < 0.05$) between stages within each tissue.

Species		Lipid peroxidation			
		15 days		60 days	
		Unexposed	Exposed	Unexposed	Exposed
Tomato	Roots	14.99 ± 17.7^{Aa}	21.50 ± 5.6^{Aa}	5.43 ± 1.5^{Aa}	19.30 ± 9.9^{Aa}
	Aerial	10.01 ± 4^{Aa}	5.66 ± 3.1^{Aa}	13.96 ± 9.3^{Aa}	28.35 ± 4.5^{Aa}
Sunflower	Roots	33.27 ± 4.2^{Aa}	3.72 ± 1.7^{Ba}	1.79 ± 1.0^{Ab}	11.81 ± 2.7^{Aa}
	Aerial	29.39 ± 5.7^{Aa}	7.04 ± 1.1^{Ab}	19.15 ± 3.4^{Ba}	113.44 ± 53.5^{Aa}
Soybean	Roots	60.92 ± 13.3^{Aa}	13.57 ± 8.5^{Ba}	10.06 ± 2.0^{Ab}	15.29 ± 9.6^{Aa}
	Aerial	116.02 ± 22.6^{Aa}	28.64 ± 12.7^{Ba}	39.93 ± 3.3^{Ab}	46.40 ± 9.8^{Aa}
Alfalfa	Roots	50.99 ± 18.8^{Aa}	39.13 ± 9.6^{Aa}	32.49 ± 6.9^{Aa}	22.46 ± 7.1^{Aa}
	Aerial	104.76 ± 35.4^{Aa}	27.69 ± 13.3^{Ba}	70.93 ± 25.1^{Aa}	56.35 ± 11.5^{Aa}

presence of endosulfan sulfate in their tissues might be the result of both uptake and/or within-plant metabolism (Fig. 3). Plant accumulation of organic contaminants from contaminated soils, including endosulfan pesticides, is well documented (Ramirez-Sandoval et al., 2011; Singh and Singh, 2014), and pollutant accumulation in each plant tissue is known to depend on plant characteristics (plant morphology, concentration of lipids, transpiration rate) (Trapp and McFarlane, 1995; Barber et al., 2004). Accordingly, the endosulfan uptake and translocation in the studied vegetables were independent of the lipid tissue content, indicating that other factors are involved in those processes. At both stages, the endosulfan accumulation followed the pattern roots > stems > leaves, with the exception of that in sunflower plants, which, at 15 days of growth, exhibited the order stems > roots > leaves. These results accurately indicate that all species translocate endosulfan effectively, because the volatilization from soil to aerial plant parts had been prevented by covering the soil surface with aluminum foil.

Endosulfan levels were similar in all species after 15 days of growth, except for sunflower plants that exhibited lower levels (Fig. 3a). Nevertheless, at 60 days, sunflower roots exhibited the highest endosulfan levels, whereas tomato plants exhibited the highest stem endosulfan levels at both growth stages (Fig. 3b). These species-specific differences might be related to differences in the transpiration rate, which is a leading factor that positively affects the uptake and translocation of organic compounds (Trapp and McFarlane, 1995). Accordingly, the higher transpiration rate of tomato plants could explain the higher endosulfan translocation (Murano et al., 2010). A comparison of the endosulfan levels in leaves showed that sunflower plants exhibited the highest pesticide concentration in both stages, being higher at 60 days. This continuous pesticide uptake by all plant species is in agreement with the depletion of the endosulfan levels of BS and NRS after 60 days of plant growth, as previously discussed.

The root bioconcentration factor (RBCF), calculated by determining the dry-weight ratio of the endosulfan concentration in roots to that in BS of the corresponding pot (Table 3), allows the potential differences in pesticide uptake ability by plants to be studied. All species were characterized by a RBCF > 1 (except for α- and β-endosulfan in sunflower plants at 15 days and α-endosulfan in tomato plants at 60 days), indicating the plants' ability to accumulate these pesticides in their roots. In addition, all species, except sunflower at 60 days, presented the highest RBCF for the endosulfan sulfate, suggesting a greater uptake of this compound or metabolism into the plant roots. A comparison of RBCF at both stages exhibited differences among species, indicating a specific variance depending on where each species is located in its life cycle.

The stem translocation factor (STF) was evaluated as the ratio of contaminant concentration in stems to that in roots, and the ratio of contaminant concentration in the leaves to that in the stems was termed leaf translocation factor (LTF, Table 3). All species presented STF < 1, except sunflower at 15 days, which presented STF of 2.09, 1.38 and 1.19 for α-endosulfan, β-endosulfan and endosulfan sulfate, respectively, indicating that endosulfan translocation is dependent on compound concentration. All species exhibited the highest STF at 15 days of growth, except for β-endosulfan in tomato. The LTF < 1 exhibited in all species indicated that endosulfan remains on plant stems at both stages (except for endosulfan sulfate in sunflower and α-endosulfan in soybean at 60 days of growth).

Finally, the phytoextraction potential of the species was assessed considering the relation between the endosulfan burden in soils and plants, expressed as a percentage. Results showed that all species except soybean increase their phytoextraction percentage at 60 days concomitantly with a decrease in soil pesticide levels. Sunflower plants presented the highest phytoextraction percentage (2.23%) followed by tomato (1.18%), soybean (0.43) and alfalfa (0.11).

3.2.3. Lipid peroxidation

Lipid peroxidation (LPO) was measured as a biomarker of oxidative damage; the results 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., 2006), and under non-exposed conditions, the studied species were in line with this statement. At 15 days of growth, with the exception of tomato roots, exposed plants presented a lower LPO in all tissues than the control plants. This result suggests that endosulfan accumulation at early stages triggers antioxidant responses that reduce LPO levels. However, at 60 days, an increase in the LPO content in all exposed plants, except alfalfa, was observed (Table 4). Thus, the continuous endosulfan uptake might affect the antioxidant system or generate reactive oxygen species that could not be afforded by the antioxidant responses induced earlier. The decrease in biomass observed in the vegetables at 60 days of growth (Table 2) is also in concordance with the effect generated by the endosulfan accumulation by plants. The highest LPO levels observed in tomato roots and sunflower plants grown for 15 and 60 days were in line with the high endosulfan accumulation. However, despite the LPO increase, sunflower plants were observed to have incorporated and translocated endosulfan efficiently.

4. Conclusion

The findings of this study indicate that tomato, sunflower, soybean and alfalfa plants are characteristically different in the uptake and translocation of endosulfan, and the subsequent biochemical effects when they are grown in endosulfan-spiked soil. The

mechanism(s) involved in reducing endosulfan levels in soil might be related to the uptake and translocation of compounds and the *in situ* metabolism in the soil matrix. Sunflower plants were determined to be the best phytoremediation candidate due to the evident decrease of soil pesticide levels facilitated by the high biomass production and uptake capacity of the plant, evidenced by its high phytoextraction percentage. In addition, lipid peroxidation, used as a biomarker of oxidative stress, correlated positively with endosulfan levels in plants. Finally, the results indicate that endosulfan exposure had negative effects on plant growth leading to oxidative stress, evidenced by the high lipid peroxidation levels observed in tomato, sunflower and soybean plant growth at 60 days. Further study will be required to understand how legacy pesticides might affect crops.

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