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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 $2 \times 10^{-6}$ 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