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Bioaccumulation of Endosulfan from Contaminated Sediment by *Vallisneria spiralis*

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Endosulfan ($C_9H_6Cl_6O_3S$) is a non-persistent organochlorine insecticide that is used widely in agriculture to control invertebrate pests. It is a cyclodiene ester pesticide with two isomers (α and β). These have half-lives of only a few days in water but the toxic biological metabolite, endosulfan sulfate, has an aqueous half-life of several weeks (Peterson and Batley 1993). It is a hydrophobic compound that can be sorbed to soil and sediment, having in this situation a longer environmental half-life (Rao and Murty 1980). Batley and Peterson (Leonard et al. 1999) have ranked it between pesticides with highest potential for impact on the riverine environment. Although the longer persistence of endosulfan in soil suggests that field runoff during storm events may be the major source of endosulfan in fish kills (Leonard et al., 1999). In an artificial streams study Hose et al. (2002) found a NOEC value for benthic macroinvertebrates of 6.14 μg/L with interstitial water that corresponded with a nominal spiked concentration of 2 mg endosulfan/Kg. The authors found significant changes in the abundance of several macroinvertebrate taxa which could result in significant effects on macroinvertebrates populations and communities.

Macrophytes can be an important link between xenobiotics and other trophic levels. Desy et al. (2002) found that macrophyte-associated freshwater invertebrates bioconcentrate cadmium (Cd) from their main food source that were macrophytes and associated peryphyton. No significant correlations were obtained between Cd in these invertebrates and water or sediment concentrations. Instead organism Cd levels were very tightly linked to Cd concentrations in macrophytes.

There are not many works about bioaccumulation of organic compounds by macrophytes. The purpose of this study was to learn about the possibility for macrophytes to bioaccumulate endosulfan, in the lab, from spiked sediments, and to describe bioaccumulation model and kinetic parameters for *Vallisneria spiralis*. This species is a rooted macrophyte frequently find in freshwater environment at Buenos Aires province, Argentina. The research is to know if macrophytes have a potential to transfers endosulfan to higher trophic levels and if they could work as environmental reservoir of cyclodiene compounds.

MATERIALS AND METHODS

Technical grade endosulfan (98% purity) was obtained from Bayer Argentina. All solvents and chemicals used in the analytical determinations were of chromatographic

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quality.

The freshwater rooted macrophyte *Vallisneria spiralis* (Hydrocatitaceae) was used in this study. This is a native species that is very abundant in freshwater environments in Buenos Aires Province (Cabrera 1954). Plants were maintained for three weeks in a pool with a mix of sand and natural unpolluted sediment. Young plants were placed in 500 mL glass beakers containing 300 mg of sediment contaminated with Endosulfan. Beakers with the same amount of uncontaminated sediment were the controls. The containers were set up in glass aquaria, which were then filled with clean water. Each aquarium had a capacity of 45 L (50 cm length x 30 cm width x 30 cm height) and contained five beakers with two young plants in each experiment. The experiment was carried out under a L/D photoperiod of 14/10 hr at a light intensity of 2400 lux, 22 °C +/- 1°C in a controlled environment chamber. Each glass aquarium was aerated. The experiment was duplicated.

The sediment used in this study was taken from a natural unpolluted stream. It was dried at 105 °C overnight, ground, and then passed through three sieves of 2.3, 1.2 and 1 mm mesh respectively, in order to obtain a homogeneous matrix. The characterization of the sediment was done by the method of Bouzoukis (Carter 1993). Textural analysis revealed a clay, sand and silt content of 17.02, 25.5 and 57.41 %, respectively. The total organic carbon (TOC) was measured according to Carter (1993). Physico-chemical parameters were measuring according to the Standard Methods (APHA 1998).

Sediment was contaminated at a concentration of 100 mg of technical grade endosulfan/kg. Bioconcentration experiments were carried out following the recommendations described by US EPA (2000) and Connell (1990). The period of bioconcentration experiments, including the uptake and elimination periods, was five days; estimated taking in account $log\ k_{ow}$ of endosulfan. The times of sample extraction in each period were selected following a geometric series. After the uptake or exposure period of two days, the plants were transferred to glass beakers containing uncontaminated sediment and placed under the same conditions as those described above. The elimination of the insecticide from the plants was followed for three days. When plants were sampled, sediment around the roots was carefully washed away in order to leave intact the plants and avoid damage to the roots. Plants were dried with absorbent paper in order to remove excess water, and fresh mass was determined for each plant sample. Lyophilisation of the plants for 30 hours was performed and dry mass was determined.

In order to estimate possible adverse effects of endosulfan in the bioconcentration experiment, growth performance of the plants was checked by determining the initial size of plants, compared to their final size by counting the number of leaves, number of roots, total biomass in terms of fresh mass and Chlorophyll "a" content corrected for pheofitines. This last parameter was performed by a fluorometer (Turner 700) equipped with special excitation and emission filters according to the method developed by Welschmeyer (1994). After the uptake period they were transferred to uncontaminated sediment. After the three day elimination period, plants were left in contact with the sediment for seven more days. This completed a total depuration period of ten days. At this time, the same growth measurements mentioned above were performed (see also Biernacki et al. 1997).

Plant tissues and sediments were extracted using the soxhlet extraction method according to US EPA (3540C-1996). Water samples were extracted by the liquid-liquid extraction method using hexane. US EPA method 3620B (1996) was applied as a clean-up procedure using a chromatographic column (12 mm id x 370 mm length) filled with activated Florisil. Extracts were concentrated by rotavapor and N_2 to 2 mL and analyzed for endosulfan using a Shimadzu gas chromatograph, model GC17A, with electron capture detector (GC-ECD) following the general description of the US EPA method 8270C (1996). Reconfirmation and identification of endosulfan metabolites were performed using a gas chromatograph (Shimadzu GCMS/QP5050A) equipped with mass Spectrometer. Pesticide concentrations were expressed as milligrams per kilogram of dry sediment (ppm) with a detection limit of 0.001 ppm. The lipid content of the plant roots and leaves was estimated, using lyophilized tissues via hexane extraction.

Data from the bioaccumulation experiment were fitted by non-linear regression analysis using the Gauss Newton estimation method. The loss function was: (observed values – predicted values)^2. Correlation indices of this function were obtained by linear regression analysis. Somatic indexes and chlorophyll "a" content were evaluated by one-way analysis of variance followed by a Dunnett's test to compare treatment with controls plants (Sparks 2000).

RESULTS AND DISCUSSION

Neither endosulfan (α and β) nor endosulfan sulphate were found in leave tissues. Data for roots, from the uptake and depuration phases of this experiment are shown in Figure 1. Physico-chemical parameters measured in sediment eluate are shown in Table 1. Experimental data fit well with a two-compartment model incorporating plant and sediment. The recoveries of endosulfan from spiked sediments and spiked plants ranged from 83 – 105 % and 89 – 107 %, respectively.

According to this approach, it is assumed that no transformation of chemical occurred during the exposure phase; it is also assumed that the macrophytes-sediment exchange can be described by a first order reaction:

$$dCm/dt = k_1 C_s - k_2 C_m \qquad 1$$

Where, C_s = chemical concentration in the sediment; C_m = chemical concentration in the macrophyte.

As C_s is >> C_m it is considered a constant at time t and after integration of equation 1 gives:

$$C_m = (k_1/k_2)\, C_s\, (1 - e^{-k2t}) \text{ model for uptake} \qquad 2$$

Macrophyte bioconcentration increased with time (t) until $C_m = (k_1/k_2)\, C_s$ o $dC_m/dt = 0$.

The C_m/C_s or k_1/k_2 ratio is known as bioconcentration factor *BCF*.

During depuration experiments $C_s = 0$ so equation 1 becomes: $dC_m/dt = - k_2 C_m$ which after integration
becomes: $C_m = C_{mto} exp^{(-k2t)}$ *model for depuration* 3

where C_{mto} is the chemical concentration in the macrophyte at the beginning of depuration phase (*to*). We obtained, from our bioaccumulation experiment, the follow toxicokinetic parameters k_1, k_2 and *BCF* values of: 10.89, 0.11 and 99, respectively. Taking into account equations 2 and 3 the time to reach a value that is 80 percent of steady-state was 15.88 hr, and 95 percent depuration was achieved in 39.57 hr.

Table 1. Sediment physico-chemical parameters measured at the end of the bioaccumulation experiment. TOC = total organic carbon as a percentage of dry mass of the sediment (dm), nd = not detectable.

Parameter	Sediment control	Sediment + endosulfan
Endosulfan mg/Kg	nd	69.22 (±3.25)
TOC % dm	3 (±0.55)	3.5 (±0.35)
pH	6.84 (±0.22)	6.75 (±0.25)
Conductivity μS/cm	186 (±25)	205 (±32)
Alkalinity mg $CaCO_3$/L	60 (±4.88)	58 (±4.75)
Sulphide mg/L	nd	nd
Nitrites μg/L	8.2 (±1.34)	9.7 (±0.96)
Nitrates mg/L	1.68 (±0.34)	1.53 (±0.55)
Phosphates μg/L	226	205
Ammonium mg/L	10 (±1.38)	9.5 (±2.11)

Table 2 shows plant growth and chlorophyll "a" content of treated and control plants. No statistically significant effects were recorded for any of the measured parameters. No acute effect was apparent after 48 hrs of exposure to 100 μg of endosulfan per gram of sediment. At the end of the exposure phase, the root residue (RR) in *V. spiralis* was almost 10 mg endosulfan per gram of total lipids. When plants were transferred to clean sediment they began their depuration phase without detriment to their growth. Neither endosulfan nor endosulfan sulfate were detected in the water column. Relatively fast uptake (k_1) and clearance (k_2) rates were recorded during the endosulfan bioaccumulation experiment. Perhaps a lipid partitioning process is occurring in the roots. Gobas et al. (1991) studied the bioconcentration of a series of

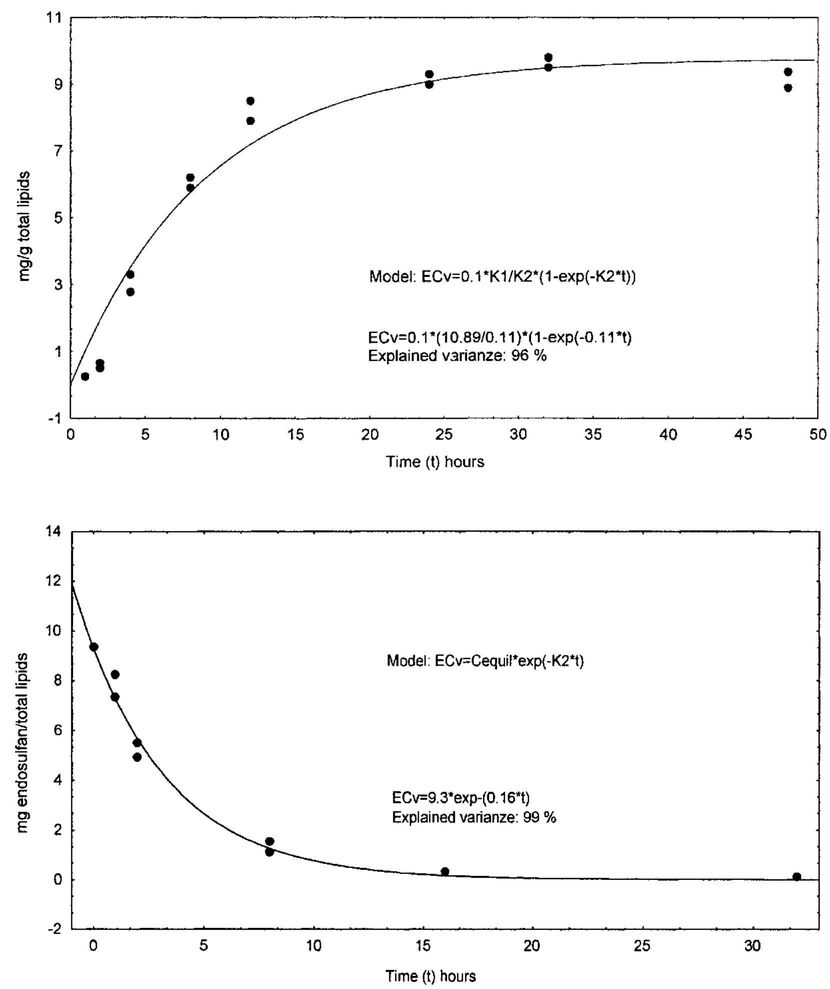

Figure 1. Root bioaccumulation of endosulfan for *Vallisneria spiralis*; data showed as total endosulfan concentration per gram of total lipids (CEv). Upper curve: uptake phase. Lower curve: depuration phase.

chlorinated benzenes and biphenyls in *Myriophyllum spicatum*, another submerged macrophyte species. Their study showed linear relationships between the plant-water bioconcentration factor and the octanol-water partition coefficient. It seemed that plant-water exchange was largely controlled by the chemical's tendency to partition between the lipid and aqueous phases of the plant. Lipid content for root and leaves of *V. spiralis* was measured as 7 and 3 % of lyophilised weight, respectively. The higher lipid content of roots could explain the fast partitioning to these tissues during the uptake process. The relationship between chemical uptake by roots and its subsequent translocation into the whole plant cannot be easily predicted from the physical and chemical properties of xenobiotics. Research on xenobiotic fate in plants depends largely on measurements of compound accumulation (Krstich and Schwarz 1990). These authors characterized the uptake and BCF of ^{14}C- naphtol in two varieties of fescue and concluded that the amount of chemical translocated to the shoot was directly related to the length of exposure time. Also, Ribeyre and Boudou (1990)

Table 2. Growth parameters of *Vallisneria spiralis* in control and polluted sediments.

	N° new leaves	N° new roots	FW (g)	Ca (mg/g FW)
Control Sediment	2.5 (±0.72)	35.7 (± 0.82)	10.43	405.65 (± 9.89)
Polluted S	2.1 (± 0.67)	36.3 (± 0.77)	11.97	386.35 (± 7.45)

(FW: Fresh weight; Ca: Chlorophyll "a")

found that bioaccumulation of methylmercury for two macrophyte species was related to environmental parameters such as temperature, pH, photoperiod, and light intensity. Modern pesticides are more soluble in water and have a shorter environmental half-life with respect to older pesticides like DDT or organochlorinated agrochemicals. They are characterized according the follows parameters: water solubility < 1 mg/L, Log K_{ow} > 3, and soil half-life > 30 days. Endosulfan is one of these compounds and even if it is moderately hydrophobic and persistent could be bioconcentrated by aquatic organisms. These kinds of chemicals can be found at higher concentrations in sediment and aquatic organisms in areas where they are being heavily used (Nowell et al. 1999). Endosulfan can be mobilized from contaminated soils into water bodies. There, it could be adsorbed to particulate matter, ending up in the sediment (Leonard et al. 2001). In soils of Canadian vegetables farms, concentrations of 0.013 to 14.9 mg/kg were found in silt loam soil, loamy sand and organic muck soil, the latter containing the highest levels (Gangolli 1999). Although, endosulfan is banned in at least ten countries, (including USA, UK and Colombia), during the last years (1998 to 2001) 5837 tons of endosulfan were applied in 9,078,133 ha (CASAFE 2001), of the Pampas Region (Argentina). The main crops in this area are soybean, wheat, corn and barley. From a limnological point of view, this area is characterized by very shallow ponds, called *lagunas*. They have an average depth of about 1 m, and are in permanent contact with the surrounding land due to very frequent flooding and field runoff. There, macrophytes are very abundant and are controlled by paraquat application (Di Marzio and Tortorelli 1993). In these environments amphipods, gastropods and shrimps, alternate between benthic and pleuston communities. Also, submerged aquatic vegetation, which is known to be a key structural component and regulator in ecosystems provides habitat for animal, acts as nutrient filter and stabilizes sediments of aquatic systems (Wigand et al 2000). Menone et al. (2001) found endosulfan sulfate in sediment samples and endosulfan in an associated crab. The same authors (2000) found DDT and its metabolites, γ-HCH, and several insecticides from the cyclodiene class, including endosulfan and endosulfan sulfate, were the predominant organochlorines in fish muscle tissues and the contents of the fish digestive tract. Endosulfan residue accumulation in fish showed a definite relation with the feeding habits of the fish. Omnivorous fish were highly contaminated > carnivorous > herbivorous (Gangolli 1999).

Our data have shown that *V. spiralis* could bioaccumulate endosulfan from polluted sediment. It was not translocated to the leaves after two days of exposure to the insecticide. Kinetic parameters fit with a two-compartment model. The roots/sediment exchange could be controlled by the chemical properties of the endosulfan related to its Log K_{ow} and the high lipid contents of the roots. Further studies will be performed to learn how exposure time and environmental conditions affect bioaccumulation kinetics and the translocation process.

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