Endosulfan remediation using aquatic and terrestrial plant species

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ABSTRACT

Soil and water pollution due to hydrophobic organic pollutants is a serious environmental problem. The natural degradation of pesticides consumes time, so the processes that accelerate the decontamination of the affected environment are significant. Phytoremediation is an emerging technology which promises effective and inexpensive cleanup of hazardous waste sites contaminated with metals, hydrocarbons or pesticides. Plants can interact with hazardous organic compounds through degradation or accumulation. The potential of aquatic plant species Giant salvinia (*Salvinia molesta*), and the terrestrial plant species, Spinach (*Spinacia oleracea*) and Tomato (*Solanum lycopersicum*), to remove persistent organochlorine pesticide endosulfan from contaminated water and soil respectively were investigated. Within 30 days of observation in the experimental plot, a percentage removal of 98% (with an initial concentration of 123 µg/L endosulfan) was observed with *Salvinia molesta* species. Thus *Salvinia molesta* proved to be the best variety among the different plant species selected for the study. Among the selected terrestrial plant species, Spinach and Tomato, percentage removal of endosulfan was found to be higher with Tomato. On day 21, complete removal of pesticide (with an initial concentration of 140 µg/Kg endosulfan) occurred in the soil in which phytoremediation was done with Tomato while Spinach took about 28 days for complete removal of endosulfan. Isomers of endosulfan (endosulfan-alpha and endosulfan-beta) and also endosulfan sulphate were determined during the analysis of the samples. Phytoremediation thus proved to be an efficient, economical and ecological alternative to accelerate the removal of endosulfan from water and soil.

Keywords: endosulfan, organochlorine pesticide, phytoremediation, tomato, spinach

INTRODUCTION

Phytoremediation is an emerging technology that is rapidly gaining interest and promises effective and inexpensive cleanup of hazardous waste sites contaminated with metals, hydrocarbons, pesticides, and chlorinated solvents. Phytoremediation encompasses an array of plant-associated processes known to mitigate contaminants from soil, sediment, and water [1,2]. Plants can interact with hazardous organic compounds through degradation or accumulation [3,4]. Within a plant, the contaminant may be adsorbed on a cell surface or accumulated in the cell. The estimated half-lives for the combined toxic residues (endosulfan plus endosulfan sulfate) range from 9 months to 6 years [5]. Phytoremediation takes advantage of the natural processes of plants. These processes include water and chemical uptake, metabolism within the plant, exudate release into the soil that leads to contaminant loss and the physical and biochemical impacts of plant roots [6]. Water hyacinth has been successfully used for remediation of waste water and ethion from ground water [7,8].

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The potential of aquatic plant species Giant salvinia (*Salvinia molesta*), and the terrestrial plant species, Spinach (*Spinacia oleracea*) and Tomato (*Solanum lycopersicum*), to remove organochlorine pesticide endosulfan from contaminated water and soil respectively were investigated. The objective of the work was to study the endosulfan remediation efficiency with selected plants and plant-pesticide uptake, as well as the behavior of pesticide translocation in the plant organs.

MATERIALS AND METHODS

Reagents and standards

Technical grade endosulfan (α+ β-endosulfan) and endosulfan sulphate of 99.5 % purity was obtained from from Merck (Germany) to compare and quantify the sample concentrations. All the reagents used were of analytical grade.

Phytoremediation using selected plant species

The effectiveness of free floating aquatic plant species such as Giant salvinia (*Salvinia molesta*) and Water spangles (*Salvinia minima*) and also submerged aquatic species Hydrilla (*Hydrilla verticillata*) to remediate endosulfan contaminated water was investigated. The phytoremediation by *Salvinia molesta* in water was carried out using water sample artificially spiked with 123 μg/L endosulfan and a portion of the spiked samples were removed at different intervals (0, 3, 7, 14, 21 and 28 days) and analyzed for total recoverable endosulfan.

Two terrestrial plant species, Spinach (*Spinacia oleracea*) and Tomato (*Solanum lycopersicum*) were selected for the investigation of the pesticide removal from soil. Medium growing plants with extensive rooting system were selected for the study. Triplicate plant chambers were used and half month old seedlings were transplanted into rectangular shaped growth chambers, which were then placed in a controlled–temperature greenhouse. Water was added daily to adjust the soil to appropriate moisture content. Natural light was used for the green house study. Soil required for the study was collected from the experimental plot of Centre for Water Resources Development and Management (CWRDM), Kozhikode. The soil samples were collected from the experimental plot and subjected to chemical analysis as per standard methods [9,10]. Soil collected did not have any previous exposure to pesticides .The soil selected for the study was sampled and characterized. An amount of 3.5 kg of soil taken in each experimental tray was spiked with 140 μg/Kg of endosulfan and the selected terrestrial plants were grown in the phytoremediation areas. Control experiments without the plant species were also laid to study natural degradation of the pesticide. The soil samples were collected from the experimental site at regular intervals and analyzed for endosulfan after 0, 7, 14, 21 and 28 days.

The aquatic and terrestrial plants used for remediation were also extracted and the concentration of endosulfan was determined. All the samples were extracted and processed separately following standard methods [11,12]. The experiments were carried out in triplicate and the average values were taken. Isomers of endosulfan (endosulfan-alpha, endosulfan-beta) and endosulfan sulphate were determined during the analysis of the samples.

Extraction of pesticide residues

For the extraction of the pesticide residues from water, liquid-liquid extraction method was adopted. Extraction of pesticides from soil required a more polar solvent than hexane or dichloromethane alone. Hence a mixed extracting solvent with added acetone was used. Organics were extracted by

shaking with a solvent mixture of chromatographic grade n-hexane and acetone. Extraction of pesticide residues were carried out following standard methods with some modifications. For extraction of water samples, one litre water sample was taken into a separating flask. It was mixed with 30 g of NaCl and 50 ml of n-hexane. Sample was shaken well and hexane layer was separated. This process was repeated thrice and hexane portions were pooled together. The co extractives were removed from the concentrated extract on an alumina column overlaid with 1g anhydrous sodium sulphate to remove any remaining water molecules. The extract was concentrated to around 10 ml on Rotor evaporator. The concentrated extract was transferred to air-tight, amber coloured GC vials and stored at 4°C until analysis [11,12].

For the extraction of pesticide residues from plants, samples were placed in glass tubes, homogenized twice with 4 ml of ethyl acetate. An additional solvent (2 ml) was utilized each time for washing. The homogenized samples were centrifuged for 10 min at 4600 rev. /min, the extract was transferred to another tube and concentrated to 1 ml. Clean-up was accomplished by passing the extract through a column containing a small amount of glass wool at the base and 3.5 g of aluminium oxide with a thin layer of anhydrous sodium sulfate lying on top. A hexane–ethyl acetate (80:20, v/v) mixture (10 ml) was used to elute the pesticides from the column. Finally, the extracts were concentrated to an appropriate volume (2–10 ml) and analyzed by GC–ECD [13].

Analysis of pesticide residues

After processing the samples through the different extraction steps, the final concentrated and cleaned up sample was analyzed using Gas Chromatograph using Electron Capture Detector which is specific and highly sensitive for halogenated compounds. A Varian make CP-3800 Gas Chromatograph equipped with Ni^{63} ECD electron capture detector was used to analyze the pesticides. One microlitre volume of each extract was injected into the injection port using the micro syringe. WCOT fused silica capillary column of length 30m , 0.32 mm internal diameter, 0.25 µm film thickness was fitted and standard temperature programs were used. Nitrogen (99.999% purity) was used as the carrier gas and the gas inlet pressure was 80 psi corresponding to a flow rate of 2 ml min^{-1}. The temperature for injector and detector were 250 and 300°C, respectively. The temperature column was programmed from 130 (hold 1 min) to 200°C at 5°C (hold 10 min) and then from 200 to 232°C at 1°C $min.^{-1}$ [14].The chromatograms were recorded and integrated using Star Workstation software. The pesticides detected were compared with that of the standards. Accuracy within-day and between-day precision were assessed using QC samples at three concentration levels of 50, 100 and 200 µg/L. The samples were all run in triplicate (n = 3) on three different days and the RSD and relative error (RE) were calculated for each. Acceptable precision here was considered to be an RSD of <5%. The overall accuracy was assessed by subtracting the theoretical concentration of each QC sample from the mean concentration determined from the three days of analyses. Detection (LOD) and quantitation (LOQ) limits were calculated relative to the values for the blank at the retention times of the analytes (10 injections).

RESULTS AND DISCUSSION

Among the different aquatic plant species selected for remediating endosulfan, *Salvinia molesta* was found to be the most effective. The effectiveness of different plant species to remove endosulfan from contaminated water is indicated in figure 1. In the phytoremediation study using *Salvinia molesta*, endosulfan disappeared from water within 28 days, while 25.86 % still remained in untreated control (Table 1). In order to confirm that endosulfan removal from water occurred due to phytoremediation, endosulfan was extracted from *Salvinia molesta* and analyzed. A total concentration of 82.22µg/Kg endosulfan was detected in Salvinia.

Table 1. Percentage removal of endosulfan from water in untreated control and *Salvinia molesta*.

Time interval (days)	Control				*Salvinia molesta*			
	Endo alpha (µg/L)	Endo beta (µg/L)	Total Endo-sulfan (µg/L)	% Removal	Endo Alpha (µg/L)	Endo Beta (µg/L)	Total Endosulfan (µg/L)	% Removal
3	33.72	33.45	67.17	32.83	7.44	39.57	47.01	52.99
7	26.51	30.89	57.4	42.6	7.79	21.49	29.28	70.71
14	20.1	28.14	48.24	51.76	5.81	12.29	18.11	81.89
21	15.44	23.49	38.94	61.06	3.72	BDL	3.72	96.28
28	8.35	17.5	25.86	74.14	BDL	BDL	BDL	100

BDL: Below Detection Limit

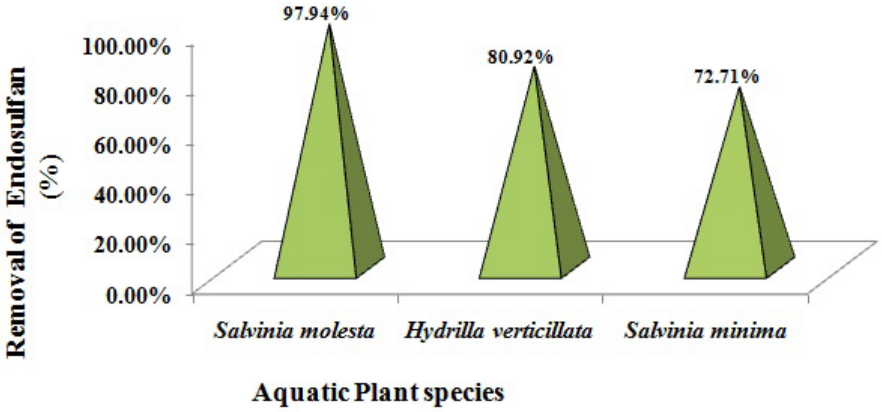

Figure 1. Comparison of endosulfan removal efficiencies of different aquatic plant species.

In the phytoremediation experiments carried out using the terrestrial plant species *Spinacia oleracea* and *Solanum lycopersicum*, the results indicated that endosulfan removal occurred at a faster rate on phytoremediation than the natural degradation process. The presence of the toxic metabolite, endosulfan sulfate residue was detected in plant tissue after exposure to endosulfan. Endosulfan was removed from contaminated soil within 21 days on phytoremediation using Tomato and within 28 days on phytoremediation using Spinach. Comparison of percentage removal of endosulfan from soil by *Spinacia oleracea* and *Solanum lycopersicum* with Control also indicated that while endosulfan was completely removed by phytoremediation within one month, nearly fifty percentage of endosulfan still remained in unplanted control. Decrease in concentration of endosulfan on phytoremediation is indicated in figure 2. Comparison of percentage removal of endosulfan on phytoremediation using *Spinacia oleracea* and *Solanum lycopersicum* with control is indicated in figure 3.

There was no visible morphological change in plants for the treatments during the experiment, which indicated that the plant could grow well in contaminated soil and water containing endosulfan, and can decontaminate soil and also wastewater polluted with endosulfan. Presence of endosulfan residues in different parts of *Spinacia olaraecia* and *Solanum lycopersicum* was confirmed by analyzing the different parts of the plants. Distribution of endosulfan residue was found to be more concentrated in the leaves. Phytoremediation thus proved to be a cost effective and feasible method for the cleanup of soil and water contaminated with toxic persistent pesticide, endosulfan.

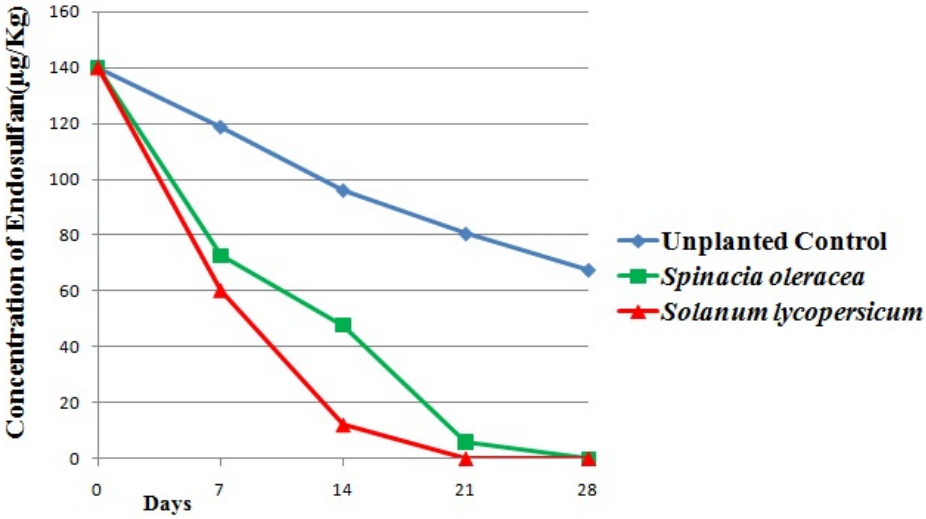

Figure 2. Concentration of endosulfan in soil in control and with *Spinacia oleracea* and *Solanum lycopersicum*.

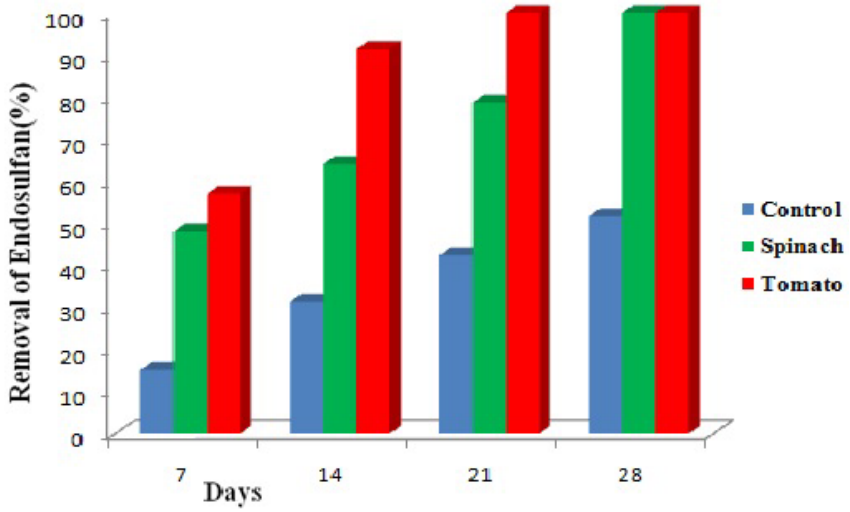

Figure 3. Comparison of percentage removal of endosulfan by *Spinacia oleracea* and *Solanum lycopersicum* with control.

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