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Phytoextraction of Endosulfan a Remediation Technique

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Abstract Endosulfan is a cyclodiene insecticide used all over the world for the control of various insect pests on variety of food and non crop products. Despite judicious use endosulfan has been detected in atmosphere, soil, water, sediment, surface water rain water and food stuffs, which is of concern. In view of the above the use of mustard and maize plants as potential phytoremediation inputs have been evaluated. The potential of mustard (brassica campestris Linn.) and maize (Zea Maize) to remove a organochlorine pesticide endosulfan was investigated. The disappearance rate constants of endosulfan from soil were 0.03684, 0.23490 and 0.17272 day^{-1} for unplanted treatment, planted with mustard and maize, respectively, which implied that plant uptake and phytoextraction with maize and mustard contributed 47.2% and 34.5%, respectively and other degradation processes took up 38.7% and 35.9%, respectively to the removal of the applied endosulfan from soil. The accumulated endosulfan decreased by 55%–91% in soil after growing the crop plants in soil, suggesting that plant uptake and phytoextraction might be the dominant process for endosulfan removal by the plant. This plant might be utilized as an efficient, economical and ecological alternative to accelerate the removal and degradation of agro-industrial wastewater polluted with endosulfan.

Keywords α endosulfan · β endosulfan · Phytoextraction · Remediation · Mustard · Maize

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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 (Macek and Kas 2000; Susarla et al. 2002, Xia et al. 2003). The use of plants to degrade, assimilate, metabolize, or detoxify contaminants is cost-effective and ecologically sound. Water hyacinth (Eichhornia crassipes Solms), due to its fast growth and large biogas production (Singhal and Rai 2003), has potential to cleanup various wastewaters. Inorganic contaminants such as nitrate, ammonium and soluble phosphorus (Reddy 2003), heavy metals (Muramoto and Oki 1983) can be removed efficiently by water hyacinth through uptake and accumulation. Organic pollutants such as phenols (Zhu et al. 1999) also can be absorbed, but whether those kinds of organic contaminants removed through uptake or an enhancement of mineralization due to the microbial consortia associated with the root surface is rarely studied and reported. Endosulfan (1, 2, 3, 4, 7, 7-hexachlorobicyclo-2, 2, 1-heptene-2,3-bis-hydroxy methane-5,6 sulfite) is a cyclodiene insecticide used all over the world for the control of various insect pests on variety of food and non crop products. Endosulfan is a mixture of two isomers alpha and beta (Fig. 1) in the ratio 7:3, both are toxic to aquatic animals (Schnoor et al. 1995). Because of its broad spectrum of activity, endosulfan is popular with the farmers in India and large areas of cultivation use endosulfan either as foliar application or as granular broadcast. Supervised field trials on various crops like vegetables, pulses and oilseeds have revealed that residues of endosulfan are below the Codex Maximum Tolerance limit of 2 mg/kg (Jayshree and Vasudevan 2007; Mukherjee and Gopal 1994). Despite judicious use endosulfan has been detected in atmosphere, soil, water, sediment, and surface water rain water and food stuffs (Mukherjee and Gopal

1998). Endosulfan is hydrophobic and persists in soil for more than 1 year. Endosulfan could be degraded by attack on the sulfite group by oxidation and or by hydrolysis to form the toxic endosulfan sulfate and non toxic diol, respectively (Fig. 1), (Kwon et al. 2002). Degradation of endosulfan by use of microbes is reported by (Mukherjee and Gopal 1996). Remediation of contaminated soil can be achieved by using microbes or by other cultural techniques such as soil solarization (Mukherjee et al. 2007). The use of weed like water hyacinth has also been successfully used for remediation of ethion (Xia and Xiangjuan 2006). The physiochemical parameters of endosulfan like low water solubility (0.322 mg/L, 25°C) and moderately high octanol/water partition coefficient (3.55–0.362), coupled with high persistence of endosulfan, phytoremediation of endosulfan might be a alternative remediation technique from contaminated soil or wastewater. The objective of the present study was to investigate the disappearance rates of endosulfan in unplanted and planted soil to determine the phytoremediation potential of mustard and maize for pesticide removal and assess the primary processes involved in the removal of endosulfan contaminated soil.

Materials and Methods

Endosulfan technical mixture of $\alpha + \beta$ in the ratio 2:1, mp 88°C and 98.7% pure) was obtained gratis from Excel India Ltd (Mumbai, India). It was recrystallized from methanol to obtain the analytical standard. NMR and IR spectroscopy confirmed the identity of the compound. Acetone, dichloromethane and hexane were procured from Merck India Ltd., were glass distilled before use.

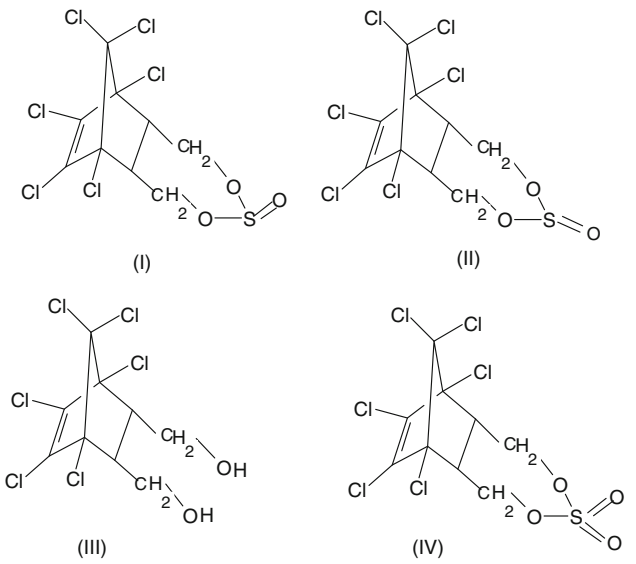

Fig. 1 Structure of α-Endosulfan (**I**) β-endosulfan (**II**), endosulfan diol (**III**) and endosulfan sulfate (**IV**)

The analysis was carried out on Varian GLC (model No. CP-3800) fitted with an auto sampler (PTV10790 and electron capture detector. The column used was a CP-Sil 5 (30 m × 0.25 mm × 0.25 μ). The column temperature was maintained at 170°C hold for 2 min @ 3°C/min raise to 210°C and @ 30°C/min 260°C hold for 5 min while the injector port and the detector were set at 250 and 300°C, respectively. The carrier gas nitrogen flow was maintained at 2 mL min^{-1} and make up flow was 27 mL min^{-1}. The retention time of α-endosulfan, β-endosulfan and endosulfan sulfate were 8.57, 10.38 and 12.38 min, respectively. The limit of detection was 0.001 µg/mL for α-endosulfan and β-endosulfan and 0.05 µg/mL for endosulfan sulfate.

Soil samples (50 g) from research fields of Indian Agricultural Research Institute, New Delhi (were no application of pesticides is made) were collected and filled in polyethylene terephthalate (PET) containers. The soil was maintained at field capacity moisture level of 15.8% for 7 days. Maize seeds (variety, Ganga Safed-2) and mustard seeds (variety Pusa Bold) were soaked in petri dishes and maintained under moisture conditions for germination. Soil samples in the PET containers were fortified with a standard solution of endosulfan ($\alpha + \beta$) at 100 µg/g level and mixed thoroughly. Germinated plants of similar shape and size (weight of each plant, 2–3 g wet mass) were selected and each plant was transplanted and inserted in an upright position in PET containers containing treated soil. Three replicates were set up for each treatment. All the treatments were placed into a growth chamber with a temperature of 25 ± 1°C, with a 14-h day (light intensity 1,400 lx) and 10-h night. The field capacity moisture was maintained by adding water to compensate for water lost through plant transpiration and evaporation during the incubation period. Samples of mustard and maize plant (root and shoot) and soil were taken on day 0, 3, 5, 7 and 10 day. The samples were extracted and processed separately for the determination of endosulfan. Two sets of control experiment were laid, untreated soil in which maize and mustard seedlings were grown. Recovery experiments were carried out by fortification of untreated soil samples (20 g), mustard and maize leaf samples (20 g) at with standard mixture of α-endosulfan, β-endosulfan and endosulfan sulfate at 1 and 0.5 µg level. The metabolite of endosulfan (I), endosulfan diol (III) was prepared in the laboratory to serve as authentic standard sample for quantification (Mukherjee and Mittal 2005). The plant samples (root and shoot 2 g) and soil (50 g) were transferred to a conical flask, to which acetone (20 mL) was added and shaken vigorously for 15 min in a horizontal shaker. The solvent was filtered through anhydrous sodium sulfate and stored. The process was repeated again with

20 mL acetone. The procedure was repeated two more times. The combined filtrate was centrifuged and then filtered. The filtrate was concentrated under rota vapor, transferred to a separatory funnel. Saline water 2% (50 mL) was added to it and the pesticide exchanged into hexane (3 × 30 mL). The combined organic layer was dried and evaporated under reduced pressure to remove all the traces of hexane. The sample was made up in hexane (10 mL) and analyzed by GLC using a Varian instrument. Soil samples were extracted with acetone in a horizontal shaker and filtered. The extract was concentrated and partitioned into dichloromethane, the organic solvent evaporated completely under vacuum and the concentrate made up in hexane for GLC analysis.

Results and Discussion

The percent recovery of α-endosulfan, β-endosulfan and endosulfan sulfate from soil and plant ranged from 89.76 to 96.35. Initial concentration of endosulfan in unplanted soil, soil planted with mustard and maize were 98.9, 92.9 and 94.5 mg/kg, respectively. Endosulfan disappeared from soil by 10 day by 46.7, 15.0 and 8.0% in untreated, followed by soil planted with maize and mustard, respectively. The major metabolite of endosulfan was detected on day 3 and peaked in day 5 samples in all the three treatments (Table 1). The uptake of endosulfan by mustard and maize is presented in Table 2. The initial uptake of endosulfan by mustard and maize was 49.2 and 47.2% by day 3, which increased to 52% by day 5 in mustard and slowly decreased to 16.2% by day 10. The initial loss of endosulfan applied soil was also quantified (Table 3). The data indicates that in untreated soil the loss was gradual from 92.7% to 46.7%, during a period of 10 day which may be attributed to soil characteristics whereas in treatments with mustard and maize the percent loss was 39.1%–8.0% and 41.9%–15.0%, respectively. The disappearance rate constants of endosulfan from soil were 0.03684, 0.23490 and 0.17272 day^{-1} for unplanted treatment, planted with mustard and maize, respectively, which implied that plant uptake and phytoextraction with maize and mustard contributed 47.2% and 34.5%, respectively and other degradation processes took up 38.7% and 35.9%, respectively to the removal of the applied endosulfan from soil. The accumulated endosulfan decreased by 55%–91% in soil after growing the crop plants in soil, suggesting that plant uptake and phytodegradation might be the dominant process for ethion removal by the plant. This plant might be utilized as an efficient, economical and ecological alternative to accelerate the removal and degradation of agro-industrial wastewater polluted with ethion. This is only a preliminary investigation and further work in pots and micro-plots need to be carried out. Also in this study edible plant was used as a source of remediation. It will be appropriate to use non edible plants as a source for phytoremediation technique. However the use of barley crop plants as a source of phytoremediation has been reported

Table 1 Concentration (mg/kg) of endosulfan in soil

Treatment	Days	Average residues (mg/kg)			
		α-endo	β-endo	Sulfate	Total
Control	0	73.4	25.5		98.9
Maize		72.4	20.5		92.9
Mustard		64.5	30.0		94.5
Control	1	64.5	27.2		91.7
Maize		35.1	14.9		50.0
Mustard		34.8	17.2		52.0
Control	3	50.1	20.7	10.5	81.3
Maize		20.0	8.0	8.0	36.0
Mustard		16.0	8.0	10.0	34.0
Control	5	32.5	15.5	18.5	66.5
Maize		17.0	5.0	9.0	32.0
Mustard		12.3	6.2	3.4	21.9
Control	7	31.4	25.6		57.0
Maize		14.0	5.0		19.0
Mustard		10.7	3.2		13.9
Control	10	26.1	20.1		46.2
Maize		10	4.0		14.0
Mustard		6.4	1.2		7.6

Table 2 Concentration (mg/kg) of endosulfan in plant

Days	Mustard					Maize				% Uptake
	α-endo	β-endo	Sulfate	Total	% Uptake	α-endo	β-endo	Sulfate	Total	
3	41.3	5.21	–	46.51	49.2	34.5	9.43	–	43.93	47.2
5	30.45	4.22	4.54	39.21	52.0	24.87	6.89	5.78	37.54	40.4
7	24.11	2.33	–	26.44	27.9	14.56	4.77	–	19.33	20.8
10	14.23	1.12	–	15.35	16.2	4.27	0.88	–	5.15	5.5

Table 3 Disappearance of endosulfan from soil

Days	% Initial loss of endosulfan		
	Control	Maize	Mustard
0	100	100	100
1	92.7	41.9	39.1
3	82.2	38.7	35.9
5	67.2	34.4	23.1
7	57.6	20.4	14.7
10	46.7	15.0	8.0

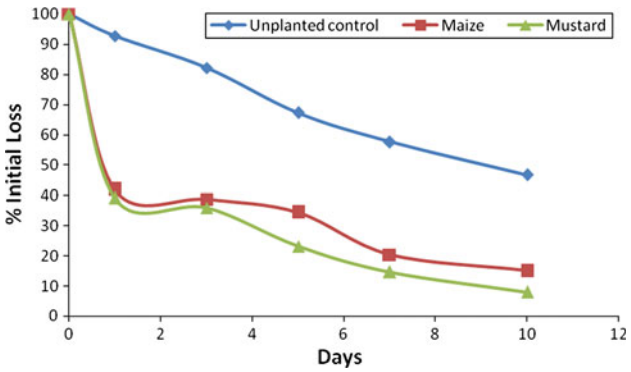

Fig. 2 Disappearance of endosulfan from soil unplanted and planted with mustard with maize

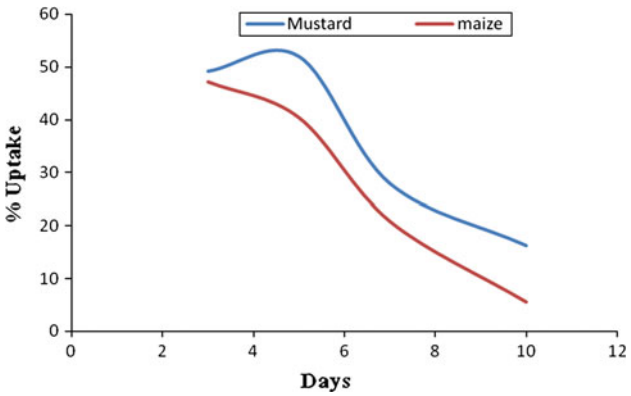

Fig. 3 Uptake of endosulfan by plant

(Briggs et al. 1982). Water hycyanith has been successfully used for remediation of waste water (Trivedy et al. 2002) and ethion from ground water (Hullong and Xiangiun 2006) and Remediation techniques have advantages and usefulness in paper and pulp industry (Yedla et al. 2002). In the present study edible plant like maize and mustard were used. The endosulfan uptake by the plant parts did not affect the morphology of the growing parts. The residues of endosulfan incorporated were in very low amounts than the prescribed limit set by Codex of 2 mg/kg. 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 containing up to 10 mg/mL endosulfan, and may decontaminate soil and also waste water polluted with endosulfan. This technique of using plants might provide an efficient, economical and ecological alternative to accelerate the removal and degradation of agro-industrial wastewater polluted with endosulfan. The plants may also be specific for endosulfan, hence further study in micro plots contaminated with pesticide needs to be carried out using a variety of plant sources (Figs. 2, 3; Table 4).

This study has will impact the environmental monitoring assessment and remediation techniques. This is only a preliminary investigation and further work in micro plots need to be carried out. Careful selection of plant and plant

Table 4 Regression and half life of endosulfan in different treatments

Treatment	Regression equation Y=	Half life (days)	Correlation coefficient (r^2)
Unplanted soil	−0.016x+1.006	18.8	0.991
Soil planted with mustard	−0.102x+1.873	3.01	0.976
Soil planted with maize	−0.075x+1.857	4.01	0.938

variety is critical, first, to ensure that the plant is appropriate for the climatic and soil conditions at the site. Plant species that are long-term competitors and survivors under adverse changing conditions normally have an advantage. Depending on the climatic and soil conditions, the plant may need resistance or tolerance to diseases, heat, cold, insects, drought, chemicals, and stress to maximize its survival rate. A screening of a wide variety of plant species need to be carried out to single out the most effect plant for phytoremediation.

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