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Research article

# Residues of endosulfan in surface and subsurface agricultural soil and its bioremediation

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ABSTRACT

The persistence of many hydrophobic pesticides has been reported by various workers in various soil environments and its bioremediation is a major concern due to less bioavailability. In the present study, the pesticide residues in the surface and subsurface soil in an area of intense agricultural activity in Pakkam Village of Thiruvallur District, Tamilnadu, India, and its bioremediation using a novel bacterial consortium was investigated. Surface (0–15 cm) and subsurface soils (15–30 cm and 30–40 cm) were sampled, and pesticides in different layers of the soil were analyzed. Alpha endosulfan and beta endosulfan concentrations ranged from 1.42 to 3.4 mg/g and 1.28–3.1 mg/g in the surface soil, 0.6–1.4 mg/g and 0.3–0.6 mg/g in the subsurface soil (15–30 cm), and 0.9–1.5 mg/g and 0.34–1.3 mg/g in the subsurface soil (30–40 cm) respectively. Residues of other persistent pesticides were also detected in minor concentrations. These soil layers were subjected to bioremediation using a novel bacterial consortium under a simulated soil profile condition in a soil reactor. The complete removal of alpha and beta endosulfan was observed over 25 days. Residues of endosulfate were also detected during bioremediation, which was subsequently degraded on the 30th day. This study revealed the existence of endosulfan in the surface and subsurface soils and also proved that the removal of such a ubiquitous pesticide in the surface and subsurface environment can be achieved in the field by bioaugumenting a biosurfactant-producing bacterial consortium that degrades pesticides.



## 1. Introduction

The occurrence of pesticide residues in various environments is primarily the result of the intensive use of pesticides in agriculture, pesticide industries (point source), atmospheric fall out, agricultural runoff, and industrial discharges. Among these pesticides, endosulfan is a new chemical on the Stockholm Convention on Persistent Organic Pollutants list. Endosulfan is an organochlorine pesticide under the Cyclodiene subgroup and belongs to the class of organochlorine insecticides. It was one of the leading chemicals used against a broad spectrum of insects and mites in agriculture and allied sectors (Harikrishnan and Usha, 2012). Commercial formulation of endosulfan (1,2,3,4,7,7-hexachlorobicyclo-2,2,1-heptene-2,3-bishydroxy methane-5,6-sulfite) is a mixture of stereo isomers of α and β endosulfan in the ratio 7:3 (Kataoka and Takagi, 2013). Its water solubility is 0.33 mg/L, and its half-life is more than a hundred years.

Residues of persistent organic pollutants such as endosulfan, DDT and HCH were detected in industrial, urban and agricultural soils of many countries (Eun et al., 2014; Ssebugere et al., 2010; Wang et al., 2007). Studies of major water resources in India report the occurrence of Endosulfan residues, which proves its persistence (Puneeta et al., 2011; Bhattacharya et al., 2003; Rao, 2006). Endosulfan and its metabolites varied from 0.8 mg/kg to 6.39 mg/kg and 2 mg/kg to 11.2 mg/kg in the soil of Thiruvallur District of Tamilnadu, India (Jayashree and Vasudevan, 2007a). Major reason for the persistence of endosulfan in the environment is its less water solubility and is not bioavailable for microbial degradation. Biodegradation of such less water soluble compounds in soil can be enhanced by amending synthetic or biological surfactants (Laha et al., 2009), but the usage of the former is restricted due to its toxicity and the later due to its cost. This study explores a method for bioremediation of pesticide contaminated soil to overcome this limitation of biosurfactant.

Many studies were conducted on the biodegradation of endosulfan and its biochemical pathway in an aqueous system, but there are fewer studies on endosulfan and endosulfate degradation in

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surface and subsurface soil and most of them focus on its biodegradation in a culture media (Madhu and Dileep, 2014; Niti and Bharathi, 2013; Mohit Kumar et al., 2008; Ngangbamand Dileep, 2010; Giri and Rai, 2012). Many microbes are capable of utilizing endosulfan and other pesticides in optimized laboratory conditions where they show higher efficiency, but under field conditions, there is a considerable reduction in degradation. A primary reason for this finding is that many pesticides are less bioavailable because of their solubility and adsorption to organic matter and clay particles (Kumar and Philip, 2006), and microbes are also affected by the toxicity of other pesticides and organic compounds. A study addressing those limitations in the field is required for the successful bioremediation of pesticide-contaminated sites. In the present study, pesticide residues in soil at three different depths collected from agricultural land in Pakkam Village in Thiruvallur District of Tamilnadu, India, were analyzed, and the bioremediation of pesticide-contaminated soil at different depths under a simulated soil profile condition was assessed in a glass reactor by bioaugumenting a biosurfactant-producing bacterial consortium.

## 2. Materials and methods

### 2.1. Sampling of pesticide contaminated surface and subsurface agricultural soil

Pesticide contaminated surface and subsurface agricultural soil was collected from Pakkam, a village situated in Thiruvallur district adjacent to Chennai, a city in the state of Tamilnadu, India. An area of two intense agricultural activities with different cropping patterns (A: ornamental flowers; B: rice) was selected in Pakkam village. Soil samples from different depths (0–15 cm, 15–30 cm and 30–40 cm) were collected using a core sampler. The collected soil samples from each depth were processed and analyzed as described by Jayashree and Vasudevan (2007a). Pesticide residues in the soil were analyzed by Gas Chromatograph- Mass Spectrometry as per Greeshma & Vasudevan (2015).

### 2.2. Bioremediation of pesticide-contaminated agricultural soil

Pesticide-contaminated soil in bulk amounts at different depths of soil was sampled from plot B with paddy cultivation, was dried in the shade and processed. The dried samples were bioaugumented with a biosurfactant-producing bacterial consortium consisting of three bacterial strains. The bacterial strains in the consortium were *Bordetella petrii* I GV 34 (NCBI Accession no. KJ022624), *B. petrii* II GV 36 (NCBI Accession no. KJ022625) and *Achromobacter xyloxidans* GV 47 (NCBI Accession no. KJ022626). The moisture content and pH of the soil were maintained at 7.0 and 20%, respectively. The bioremediation study was conducted in a simulated soil profile by loading the bioaugumented soil (20 Kg) at different depths into a glass reactor with suitable control.

#### 2.2.1. Experimental design

A glass reactor with a volume of 4500 $cm^3$, height of 40 cm, length of 15 cm and width of 10 cm was used for the experimental set up (Fig. 1). The reactor was equipped with two sampling ports on either side at depths of 25 cm and 35 cm from the top. Bioaugumented pesticide-contaminated soil samples from different depths of soil were loaded into the reactor. The reactor was filled with subsurface soil (30–35 cm depth) to a depth of 5 cm from the bottom of the reactor, followed by subsurface soil (20–25 cm depth) filled to 30 cm from the bottom of the reactor and then a surface soil layer was filled up to 35 cm from the reactor bottom. Samples from the reactor were extracted via the sampling port at 5-day intervals. The study was conducted for duration of 30 days with suitable control.

#### 2.2.2. Bioaugumentation of bacterial consortium

Bacterial consortium comprising of bacterial strains referred in 2.2 was inoculated in MSM Greeshma and Vasudevan (2013) spiked with 50 mg/L of endosulfan and cultivated in a bioreactor (HWS, Germany) of 2 L capacity, until a bacterial count of $10^7$ cfu/mL was reached. The culture was then harvested by centrifuging the MSM broth at 8000 rpm in a refrigerated centrifuge (Sigma). The harvested bacterial cells were then resuspended in required volume of buffered water to maintain soil moisture content of 20% in each layer. Bacterial consortium so prepared was then augumented ($10^5$ cfu/g of soil) in pesticide contaminated surface and subsurface soil separately Bioaugumented soil was mixed separately and thoroughly for uniform distribution of bacterial cells and moisture. The bioaugumented subsurface and surface soil was loaded from bottom to top in the reactor. The growth of bacteria in the reactor was examined at 5 days interval by viable plate count enumeration method.

#### 2.2.3. Quantification of pesticides

Pesticides in the soil were extracted as per Jayashree and Vasudevan (2007b) and Ghadiriand Rose (2001). The extracted samples were analyzed in a GC system -Trace GC-1000 by gas chromatograph mass spectrometer (Thermofisher Scientific, USA) analysis (Greeshma and Vasudevan, 2015).

### 2.3. Denaturing gradient gel electrophoresis and specific primer based confirmation of bioaugumented bacterial strains

The genomic DNA from the different layers of soil in the reactor were extracted and purified using a Qiagen (QiaAmp® DNA stool Mini kit) DNA isolation kit in accordance with the manufacturer's protocol (Qiagen, Germany). The DNA extract was amplified using PCR (Arulazhaghan and Vasudevan, 2009). An aliquot of 5 μL crude DNA was run in 1% agarose gel electrophoresis. Crude DNA from the soil was diluted to 25 μL in sterile water and was quantified using a UV–VIS spectrophotometer (Agilent). The DGGE was performed using a D Code Universal Detection System instrument and a gradient (former model 475, Bio-Rad) following the protocol mentioned by Arulazhaghan et al. (2010). The specific primer of the product size 907 bp and length 21 bp (FP-CGGTACCTGCAGAA-TAAGCAC &RP-GTACAAGACCCGGGAACGTAT) was designed for the bacterial strains in the consortium. The total bacterial DNA extracted from the soil was then amplified using this primer. The bacterial community analysis of the various bands detected in DGGE was carried out using the bioinformatics tool PyElph software.

### 2.4. Statistical analysis

All of the results represented in the present study were the average of the triplicate data. The standard deviation of the mean is represented as an error bar in the graph. Data obtained for the growth of bacteria in the control and bioaugumented soil and the difference in treatment were analyzed statistically using one-way ANOVA. Effect of soil depth and time on data for degradation of pesticide and growth of bacteria were analyzed by two way anova. Biodegradation of pesticides at different layers of soil was evaluated based on the half-life of degradation (Strotmann, 2000).