Volume : 2 | Issue : 8 | Aug 2013 • ISSN No 2277 - 8160

Research Paper | Microbiology

Bioremediation of Endosulfan in Surface Soil Treatment Using Microbial Consortia

Arunkumar. S — Dept. of Botany and Microbiology, A.V.V.M. Sri. Pushpam College (Autonomous), Poondi – 613 503, Tamilnadu, India

Chandrasekaran. R — Dept. of Botany and Microbiology, A.V.V.M. Sri. Pushpam College (Autonomous), Poondi – 613 503, Tamilnadu, India.

ABSTRACT *Endosulfan is an organochlorine pesticide used throughout the world for higher agricultural production. Long term effect on ecosystem may increase the concentration of toxicant in organism towards the top of the food chain increases. The purpose of this study to investigate the ability of activated cow dung slurry to degrade endosulfan. In the present study, surface soil treatment unit (SSTU) has been designed wherein, technical grade pesticide endosulfan was amended separately in alluvial soil at three different concentrations viz. 25, 50 and 100 mg/kg and bioremediation is carried out using activated cow-dung biomass. COD and BOD as indicators of bioremediation were also monitored during the course of experiment. Among this study maximum percentage of endosulfan degradation recorded at 5th treatment samples compared than other sampling days. The percentage of endosulfan degradation straightly increases if the time increases. The presence of nutrients as well as microorganisms in cow-dung and soil has been found to have great influence on the bioremediation of endosulfan. These results indicated that cow dung slurry contains microbes that can be potentially used for bioremediation in endosulfan contaminated environments.*

KEYWORDS: Endosulfan, Cow dung, Bioremediation, Microbial consortia

INTRODUCTION

In developing countries like India where the economy depends largely on agricultural products, one cannot afford to loose the harvest to pests. In India, 15–20% of the total harvest is destroyed by pests resulting in uncontrolled use of pesticides by the Indian farmers. India is the largest consumer of pesticides in South Asian countries where maximum (44.5%) consumption of the total pesticides is by cotton crop (Agnihotri, 1999). Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water, bottom sediments, and food. Long term effect on ecosystem may increase the concentration of toxicant in organism towards the top of the food chain increases (Samanta et al. 2002).

Endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9 ahexahydro- 6,9 -methano- 2,3,4- benzodioxathiepin-3-oxide, CAS No. 115-29-7) is an organochlorine pesticide used throughout the world for higher agricultural production. Technical endosulfan consists of α and β isomers (7:3) and is extensively used for the protection of cotton, tea, sugarcane, vegetables, and fruit crops. India the world's largest user of endosulfan, and a major producer with three companies—Excel Crop Care, H.I.L., and Coromandal Fertilizers—producing 4,500 tonnes annually for domestic use and another 4,000 tonnes for export (Indian Chemical Council, 2009). Nitrogen fixation, which is required for the growth of higher plants, is hindered by pesticides in soil. Reduction of this symbiotic chemical signaling result in reduced nitrogen fixation and thus reduced crop yields (Rusty Rockets, 2007). Root nodule formation in these plants saves the world economy $10 billion in synthetic nitrogen fertilizer every year (Fox et al., 2007).

These fertilizers are costly and tend to result in soil hardening and fertility decline. Cow dung have considerable potential for biodegradation and biotransformation of petroleum product, further contribute to plant production and biogeochemical processes (Adegunlayu *et al*. 2007). There have been number of studies for cost-effective and environmentally friendly method of hydrocarbon removal from oil-contaminated sites has intensified efforts towards animal manure application to oil polluted sites (Amadi and De Bari 1992, Obire and Akinde 2004, Akiakwo *et al*. 2005). Therefore an attempt has been made to examine the cow dung as a source of endosulfan utilizing microorganisms to clean environmental contaminant. The present study was concentrated on bioremediation of endosulfan in surface soil treatment using microbial consortia.

MATERIALS AND METHODS

Soil sample collection

Soil used for the bioremediation of endosulfan in surface soil treatment using microbial consortia was obtained from a cotton cropping farm near Thanjavur, Tamilnadu, India.

Contamination protocols

The dried and sieved soil was transferred from storage, rewetted to a moisture content of 15% (dry weight), and incubated at 20°C. The effects of artificial contamination were tested on dried soils that had been rewetted and incubated for 5 days prior to the contamination procedure. In the partial treatment protocol, 500 μl of solvent or contaminant is added to a 25% fraction (5 g) of the soil sample and the flasks are closed for 5 min to let the solvent disperse. There after the solvent is evaporated for 16 h, and the subsample is mixed with the remaining 75% (15 g) of the soil sample (Ulla *et al.,* 2002). All mixings were performed thoroughly in each separate flask for 1 min with a metal spatula.

Soil preparation

In the treatment procedure, 25ml of acetone containing endosulfan was added to 25% of the soil sample (250 g), the flasks were closed for 5 min to let the solvent disperse. Thereafter the solvent is evaporated for 16 h at room temperature and the subsample was mixed with the reaming 75% (750g) of the soil sample. All samples were thoroughly mixed with a metal spatula. Soil was spiked to reach final concentrations of endosulfan at 25, 50 and 100 mg/kg dry soil.

Experimental set up

The soil (1 kg) spiked with 25, 50 and 100 mg/kg endosulfan respectively, was taken in the treatment unit and mixed thoroughly with activated cow-dung slurry biomass (1 liter) using mechanical stirring. A control unit, without microbial inoculam was also maintaining. 0.05% Tween 80 was added to the soil as a surfactant to prevent adsorption of pesticide to soil particles.

Experimental design

Normal soil + Cow dung slurry
25% contaminated soil + Cow dung slurry
50% contaminated soil + Cow dung slurry
100% contaminated soil + Cow dung slurry

During the experiment for a time period of one week, soil sampling was done every day for a time period of one week. Chemical and biological oxygen demand (COD, BOD) as indicators of bioremediation were also monitored during the course of experiment (APHA, 1995).

Extraction (EPA, 2003)

Soil samples drawn every day (10 g) were dried for pesticide extraction using 200 ml acetone in a soxhlet extraction assembly. The 200ml soxhlet was concentrated with a rotary evaporator to 10 ml.

Estimation of Endosulfan (Chand Pasha and Badiadka Narayana, 2008)

10 ml of soil extract was taken in a clean 100 ml conical flask and add 5 ml of acid reagent (15.2 g of p-toluene sulfonic acid in 50 ml of isopropanol, to this 10 ml water was added) and 1 ml of alcoholic potassium hydroxide (2%) solution and kept in hot water bath at 90ºC for 10 min. After 10 min. add 10 ml absorbance solution (Sulfur dioxide) mixed well and pH was adjusted to 4±0.2. Then add 1ml 0.1% thionin and made upto 25 ml using distilled water and incubate at room temperature for 10 min. then measure the absorbance of the content at 600 nm. Black also maintains using distilled water. The results obtained in the present investigation were subject to statistical analysis like Mean and Standard Deviation (SD) by Zar (1984).

RESULTS

The physico chemical and microbial characteristics of cow-dung slurry and soil were carried out and are presented in Table – 1 and Table – 2 a,b. The analyzed data were indicates the presence of bacteria and fungi in soil as well as in cow dung slurry. The presence of nutrients as well as microorganisms in cow-dung and soil has been found to have great influence on the bioremediation of endosulfan. The maximum viable microorganisms present in cow dung slurry compare than soil sample. Similarly maximum viable fungi presented in cow dung slurry.

In the present study, surface soil treatment unit (SSTU) has been designed wherein, technical grade pesticide endosulfan was amended separately in alluvial soil at three different concentrations viz. 25, 50 and 100 mg/kg and bioremediation is carried out using activated cow-dung biomass. The endosulfan degradation percentage is calculated at different time interval. Such as 0-5 days the results were presented in Fig - 1. Among this study maximum percentage of endosulfan recorded at 5th treatment samples compared than other sampling days. The percentage of endosulfan degradation straightly increases if the time increases.

The BOD measured during the bioremediation of each pesticide showed some variation in concentration due to the growth and proliferation of prominent microorganisms in the presence of high nutrient availability of cow-dung slurry and soil under simulated conditions. The COD monitored during bioremediation showed that the reduction in COD concentration was directly proportional to the degradation of the parent compound into its intermediates or less harmful compounds with increasing period of time (Fig – 2).

Fig - 3 shows variation in Biological Oxygen Demand during Bioremediation of endosulfan amended soil in Surface soil treatment unit at varying concentration. The percentage increase in Biological Oxygen Demand (BOD) found during the bioremediation of endosulfan at varying concentration is presented in fig - 1.

DISCUSSION

The data indicates presence of organic carbon, nitrogen, phosphorus, sulphate, calcium, chloride, sodium, potassium and magnesium in cow-dung slurry and soil. The higher nutrient availability and larger microbial population of the cow-dung slurry and soil-pesticide mix was found to affect bioremediation of pesticides under controlled environmental conditions. This is in agreement with the finding that animal-derived lagoon effluents are a good source of inorganic nutrients and organic matter and they have an impact on the degradation and transport of soil-applied pesticides (Huang *et al.,* 2000).

In the present investigation the presence of nutrients as well as microorganisms in cow-dung and soil has been found to have great influence on the bioremediation of pesticides. Research studies compiled and documented showed that adaptability of microorganisms during bioremediation releases enzymes, which metabolizes wide spectrum of anthropogenic chemicals (Fulekar, 2005b). Investigations done on United Kingdom and Australian soil for chlorpyrifos degradation by soil microbial community also showed TCP as the primary intermediate of chlorpyrifos (Singh *et al.,* 2003; Extoxnet, 1996). The degradation rate of chlorpyrifos was found increasing with increase in pH, in particular at alkaline conditions. This is in agreement with the finding of Singh *et al.* (2003).

Table - 1
Physico-Chemical characteristics of soil and cow dung slurry

S. No.	Physico-Chemical Parameter	Soil	Cow dung slurry
1	pH	7.70±0.20	7.23±0.34
2	Moisture (%)	8.95±0.09	-
3	Dissolved Oxygen (mg/kg)	6.87±0.41	6.4±0.21
4	Temperature (ºC)	27±1.00	29±1.50
5	Cation Exchange Capacity/100g	89meq	-
6	% Organic Carbon	1.04±0.01	1.02±0.10
7	Phosphorus (mg/kg)	9.6±0.29	10.1±0.70
8	Kjeldahl Nitrogen (mg/kg)	72.36±5.73	81.99±0.13
9	Sulphate (mg/kg)	3.0±0.02	2.5±0.24
10	Calcium (mg/kg)	115.43±3.29	112.22±1.14
11	Chloride (mg/kg)	184.34±11.57	212.70±2.32
12	Potassium (mg/kg)	98.20±2.06	100.10±2.74
13	Sodium (mg/kg)	24.30±0.38	2.80±0.90
14	Magnesium (mg/kg)	43.40±0.84	6.36±0.56

Values are expressed (Mean± Standard Deviation)

Table - 2
Microbial characteristics of soil and cow dung slurry
a. Bacteria

S. No.	Microbial characteristics	Soil			Cow dung slurry		
		10^{-4}	10^{-5}	10^{-6}	10^{-4}	10^{-5}	10^{-6}
1	Total viable count /g	67×10^{5}	44×10^{6}	36×10^{7}	201×10^{5}	129×10^{6}	98×10^{7}

b. Fungi

S. No.	Microbial characteristics	Soil			Cow dung slurry		
		10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
1	Total Fungal count /g	59×10^{2}	7×10^{3}	3×10^{4}	38×10^{2}	12×10^{3}	8×10^{4}

Fig – 1
Endosulfan degradation using microbial consortia

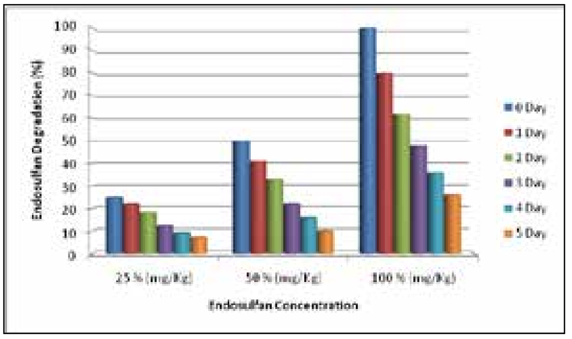


Fig – 2
Variation in COD during bioremediation of endosulfan amended soil in surface soil treatment unit

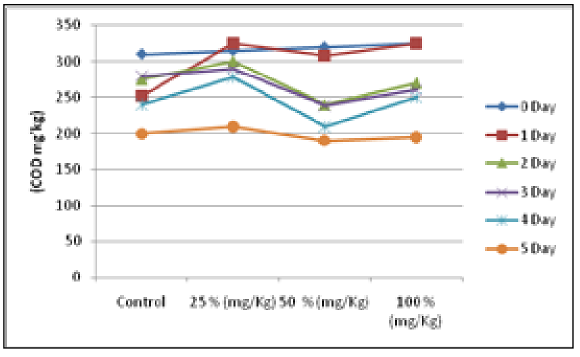
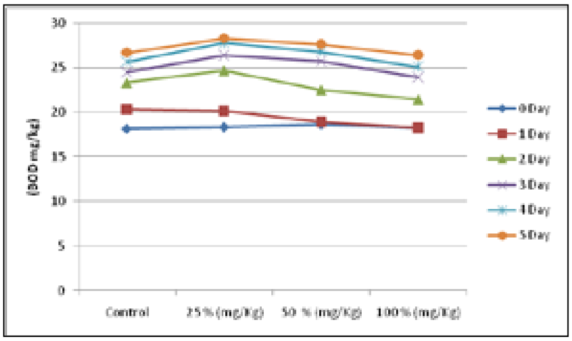


Fig – 3
Variation in BOD during bioremediation of endosulfan amended soil in surface soil treatment unit



REFERENCES

Adegunloye D V, Adetuyi F C, Akinyosoye F A and Doyeni M O. 2007. Microbial analysis of compost using cow dung as booster. Pakistan Journal of Nutrition., 6(5): 506-510. | Agnihotri, N.P., 1999. 'Pesticide Safety Evaluation and Monitoring' Division of Agricultural chemicals, Indian Agricultural Research Institute (IARI) New Delhi, p. 13. | Akiakwo M O G, Akinde S B and Dollah S A. 2005. Synopsis of available technologies for the cleanup of oil spill in a terrestrial environment. Journal of Environment Management Education., 2(2): 56-61. | Amadi A and De Bari Y. 1992. Use of poultry manure for amendment of oil polluted soils in relation to growth of maize (Zea mays L.). Environment International., 18: 521-527. | Chand Pasha and Badiadka Narayana., 2008. Spectrophotometric determination of endosulfan using thionin and methylene blue as chromogrnic reagents. Bull. Environ. Contam. Toxicol., 80: 85-89. | Extoxnet., 1996. Extensive Toxicology Network, Pesticide Information Profiles, www.extoxnet.orst.edu. | Fox, J.E, Gulledge, J, Engelhaupt, E, Burrow, M.E, and McLachlan, J.A., 2007. "Pesticides reduce symbiotic efficiency of nitrogen-fixing rhizobia and host plants". Proceedings of the National Academy of Sciences of the USA., 104 (24): 10282–10287. | Fox, J.E., Gulledge, J, Engelhaupt, E, Burrow, M.E, and McLachlan, J.A., 2007. "Pesticides reduce symbiotic efficiency of nitrogen-fixing rhizobia and host plants". Proceedings of the National Academy of Sciences of the USA., 104 (24): 10282–10287. | Fulekar, M.H., 2005. Bioremediation Technologies for Environment. Indian Journal of Environ. Protection., 25(4): 358 – 364. | Huang, X..L, Linda, S., and Nakatsu, C., 2000. Impact of Animal Waste Lagoon Effluents on Chlorpyrifos Degradation in Soils. J. of Environ. Toxicol. and Chem., 9: 2864-2870. | Indian Chemical Council (January 9, 2009). "Form for submission of information specified in Annex E". Retrieved 2009-01-29. | Obire O and Akinde S B. 2004. Poultry manure amendment of oil polluted soils for sustainable development in the Niger-Delta. Journal of Niger Environmental Society., 2(2): 138-143. | Rusty Rockets, 2007. Down On The Farm? Yields, Nutrients And Soil Quality. Scienceagogo.com. Retrieved on September 15, 2007. | Samanta, K.S, Singh, O,V., and Jain, R.K., 2002. Polycyclic aromatic hydrocarbon: environmental pollution and bioremediation. Trends in Biotechnology., 20(60): 243-248. | Singh, B.K., Walker, A., Morgan, A.W., and Wright, D.J., 2003. Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos degrading bacterium. Applied and Environ. Microbiol., 69(9): 5198 –5206. | Ulla C., Brinch., Flemming Ekelund, and Carsten S. Jacobsen., 2002. Method for Spiking Soil Samples with Organic Compounds. Applied And Environmental Microbiology., 68(4): 1808–1816. | Zar, J.H., 1984. In: Biostatistical Analysis, Englewood Cliffs, N.J. Prentice hall, Inc., 437-467. |