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THE POWER OF MICROBES: ENVIRONMENTAL SOLUTIONS FOR PESTICIDE BIOREMEDIATION

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Abstract: Bioremediation, utilizing microbial consortia, emerges as a promising approach for mitigating pesticide contamination. Through the degradation capabilities of diverse microorganisms, bioremediation offers a sustainable and environmentally friendly solution for cleaning up pesticide pollutants. This paper explores the potential of microbial consortia in bioremediation strategies, elucidating their effectiveness, mechanisms, and applications in restoring contaminated environments.

Keywords: Bioremediation, Microbial consortia, Pesticide contamination, Environmental cleanup, Sustainable remediation

INTRODUCTION

In India, the production of pesticides started in 1952 with the establishment of a plant for production of benzene hexachloride (BHC) at Rishra near Calcutta followed by two units for manufacturing DDT {1,1,1-trichloro-2,2bis(4-chlorophenyl) ethane} by Hindustan Insecticides Ltd. Now, India is the second largest manufacturer of pesticides in Asia and ranks twelfth globally (CLI, 2002) There has been a steady growth in the production of technical grade pesticides from 5,000 metric tonnes in 1958 to 102,240 metric tonnes in 1998 (Saiyed et al., 1999). The trend is rising continuously in the manufacturing and formulation sectors of pesticide industry.

The waste generated by the pesticide industry has become a disposal problem. This is and will continue to be an environmental problem unless proper treatment technology is developed and transferred to the industry (Fulekar, 2005a). At present, the pesticide waste is being treated by physico-chemical methods which are not efficient and effective. As a result, pesticide residue remains in the soil-water environment causing toxicity to the biota and thereby entering into the food chain (CFTRI, 2003). The World Health Organization (WHO) data show that only 2 - 3% of applied chemical pesticides are effectively used for preventing, controlling and killing pests, while the rest remains in the soil (EPA, 2005). Therefore, the surface soil containing residual pesticides causes toxicity in the surrounding environment. Further, recent advances in bioremediation for the treatment of pesticide wastes as well as effluent by using different treatment technologies are essential for pesticide industry.

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The waste generated during pesticide manufacturing is very complex, containing chemical compounds used for manufacturing and the residuals generated during manufacturing/formulation process (EPA, 2005).

The most commonly used pesticides taken for the experimental study are chlorpyrifos, cypermethrin, fenvalerate, and trichlopyr butoxyethyl ester. Chlorpyrifos {0,0diethyl0-(3,5,6-trichloro-2-pyridyl) phosphorothioate} is an organophosphate insecticide used for agricultural and domestic use. Chlorpyrifos is found to be persisting moderately in soil (Exttoxnet, 1996). Cypermethrin {alphacyano-3-phenoxybenzyl-3-(2,2-dichloro-vinyl)-2,2-dimethylcyclopropane-carboxylate} is a synthetic, pyrethroid insecticide used for protection against wide range of pests. Cypermethrin is a pure racemic mixture consisting of eight stereoisomers (USDA, 1995). Fenvalerate {alphacyano-3-phenoxybenzyl-2-(4-chlorophenyl)-3-methylbutyrate} is a potent insecticide that is being used from 1976. It is a racemic mixture of four optical isomers and belongs to synthetic pyrethroid class of pesticides (WHO, 1990). Approximately 1000 tonnes per year of fenvalerate are used worldwide. It is employed in agriculture, insect control at homes; garden, on cattle and for commercial purposes. Trichlopyr butoxyethyl ester (TBEE) is a pyridinebased herbicide used for control of woody and broadleaf plants in forests, industrial lands, and parks. TBEE has the tendency to strongly adsorb to soil and organic particle and is relatively immobile (Ganapathy, 1997).

In the present study the commonly used pesticides have been taken for bioremediation under controlled environmental conditions. The surface soil treatment unit has been designed to develop the techniques for bioremediation of surface soil containing pesticides by monitoring and maintaining environmental parameters under simulated conditions. This pilot scale laboratory technique will be effective for bioremediation of pesticides in soil as well as for treatment of pesticide effluents.

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

Parameter	Soil	Cow dung slurry
pH	7.6	7.4
Moisture	4.5 %	--
Alkalinity /100gms	0.6meq	1.2meq
Dissolved Oxygen	6 mg/kg	9 mg/l
Temperature	26 °C	28 °C
Cation Exchange Capacity /100gms	108meq	---
% Organic Carbon	1.08	0.34
Phosphorus	0.25 mg/kg	0.78 mg/l
Kjeldahl Nitrogen	2100 mg/kg	8.6 mg/l
Sulphate	2.5 mg/kg	26 mg/l
Calcium	8727 mg/kg	8.6 mg/l

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Chloride	1930 mg/kg	6 mg/l
Potassium	344 mg/kg	161 mg/l
Sodium	423 mg/kg	92.8 mg/l
Magnesium	15440 mg/kg	147 mg/l
COD	220 mg/kg	200 mg/l
BOD	4 mg/kg	8 mg/l

MATERIALS AND METHODS

Chemical

Technical grade chlorpyrifos, cypermethrin, fenvalerate and trichlorpyr butoxyethyl ester (TBEE) was procured from AIMCO Pesticides, Maharashtra, India.

Soil

Alluvial soil was collected from a field located at Palghar in the periphery of Mumbai area for the experimental study. Soil was airdried, ground and passed through a 2mm pore size sieve and was stored in sealed containers at room temperature. Soil organic carbon, cation exchange capacity and other physico- chemical parameters were analyzed as shown in Table 1 (Jackson, 1973; APHA, 1995). Soil microbial status was also analyzed (Table 2).

Spiking of soil

Experimental soil was treated with solvent acetone containing pesticides separately (chlorpyrifos, cypermethrin, fenvalerate and TBEE). In the treatment procedure, 25 ml of acetone containing pesticide 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 sub sample was mixed with the remaining 75% (750g) of the soil sample. All samples were thoroughly mixed with a metal spatula (Brinch et al., 2002). Soil was spiked to reach final concentrations of pesticides at 25, 50 and 100 mg/kg dry soil.

Biomass

Fresh cow-dung was collected from cattle shed. Cow-dung slurry in the ratio of 1:10 with distilled water was taken as a source of microbial biomass. Cow-dung slurry biomass was maintained by microbial biomass. Cow-dung slurry biomass was maintained by continuous aeration at the rate of 0.9 - 1.2 m³/h and by addition of one dose of nutrient - glucose (C₆H₁₂O₆)-150 mg/l, potassium dihydrogen phosphate (KH₂PO₄)-80 mg/l and ammonium sulphate {(NH₄)₂SO₄} -80 mg/l. The cow-dung slurry biomass was activated for a period of three days. Cow-dung slurry was characterized for physico-chemical and microbial status (Tables 1 and 2) (APHA 1995).

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

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Parameters	Soil	Cow-dung
Total viable count/g	1,920	65 x 10 ⁹
Total coliform count /g	760	189 x 10 ⁷
Total Yeast and Mould count/g	320	72 x 10 ³
<i>Pseudomonas</i> count/g	<30	59 x 10 ³
<i>Actinomyces</i> count/g	1,340	83 x 10 ⁴
<i>E.coli</i> count/g	Absent	23,600
Anaerobic bacterial count	<30	<30
Thermophilic bacterial count	560	790
Anaerobic spore count	Nil	Nil
Thermophilic spore count	Nil	Nil
Anaerobic thermophilic spore count	Nil	Nil
<i>Salmonella</i>	Absent	Absent
<i>S.aureus</i>	Present	Absent
<i>Shigella</i>	Absent	Absent
<i>Fecal streptococcus</i>	Absent	Present
<i>Flavobacterium</i>	Present	Absent
<i>Alcaligen</i>	Present	Absent
<i>Bacillus</i>	Present	Absent
<i>Streptococcus</i>	Absent	Present
<i>Sarcina</i>	Absent	Present
<i>Serratia</i>	Present	Absent
<i>Nocardia</i>	Absent	Present
<i>Mucor spp.</i>	Present	Present
<i>Phizopus stolonifer</i>	Present	Present
<i>Aspergillus</i>	Present	Present
<i>Penicillium</i>	Present	Present

Table 3. Parameters monitored and maintained during bioremediation of pesticides in surface soil treatment unit

Parameter	Range
C:N:P	100:10:1
pH	6.5 – 8.0
Temperature	25 – 28°C
Moisture	60 – 80%
Dissolved Oxygen	10 – 12 mg/kg

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Microbial Growth

Present

Experimental set up

A surface soil treatment unit was designed and fabricated (22 x 10 x 6 cm) (Figure 1). The soil (1 kg) spiked with 25, 50 and 100 mg/kg pesticide 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 pesticide was also run in parallel to make the comparisons. Bioremediation of the pesticide were carried out in triplicates. 0.05% Tween 80 was added to the soil as a surfactant to prevent adsorption of pesticide to soil particles. The aerobic condition was maintained by supplying symmetric air with the help of an electric air pump. Bioremediation conditions like moisture, temperature, dissolved oxygen, pH, nutrients (C, N, P) were monitored and maintained in the surface soil treatment unit (Table 3). Frequent mixing was done to allow uniform distribution of oxygen and nutrients. During the experiment for a time period of one week, soil sampling was done every day for a 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). Microbial growth was checked and monitored by streaking the serial dilution of soil sample on a nutrient agar plate (APHA, 1995).

Extraction

Soil samples drawn every day (10 g) were dried for pesticide extraction using 200ml acetone in a soxhlet extraction assembly (EPA, 2003). The 200 ml soxhlet extract was concentrated with a rotary evaporator to 10ml. Appropriate dilutions of the sample extract were then analyzed with a Hewlett – Packard GC-MS. Percentage recovery of pesticide (chlorpyrifos, cypermethrin, fenvalerate and TBEE) from soil was found to be around 65%.

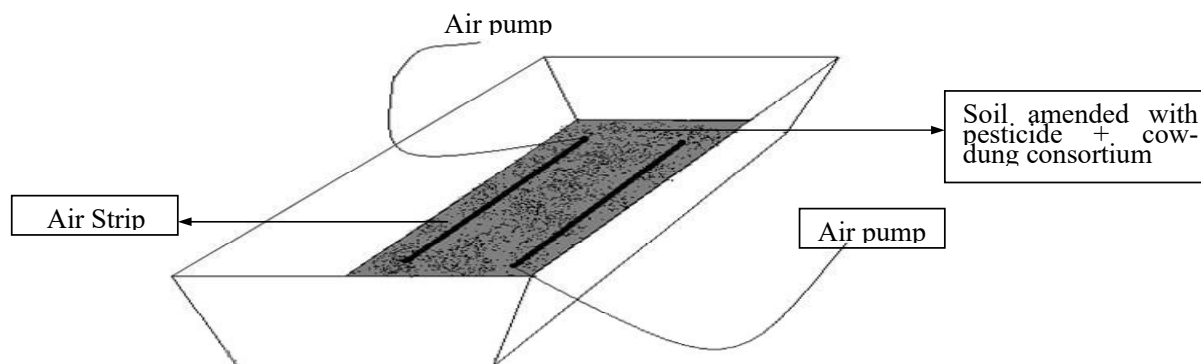


Figure 1. Schematic diagram of surface soil treatment unit (SSTU).

Analytical procedures

Soil sample extract was analyzed by Gas chromatographic/mass spectroscopy (GC-MS) (Hewlett Packard GC-MS instrument Model No. G1800A) for pesticides and its intermediates. The instrument is equipped with electron ionization detector. Conditions maintained for the quantitative and qualitative analyses were: oven temperature – 100°C, Injection temperature – 250°C, detector temperature – 280°C.

RESULTS

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The surface soil contamination with pesticides is a common environmental problem posed by pesticide manufacturing and formulation units. The recent advances in bioremediation using microbial technology would prove to be an effective treatment technique for pesticides like cypermethrin, fenvalerate, chlorpyrifos and TBEE. In the present study, surface soil treatment unit (SSTU) (Figure 1) has been designed wherein, technical grade pesticide cypermethrin, fenvalerate, chlorpyrifos and TBEE were 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 physico chemical characteristics of cow-dung slurry and soil were carried out and are presented in Table 1. The data indicates presence of organic carbon, nitrogen, phosphorus, sulphate, calcium, chloride, sodium, potassium and magnesium in cow-dung slurry and soil. The microbial characterization of soil and cow-dung is presented in Table 2. The data indicates the presence of bacteria, fungi and actinomycetes in soil as well as in cowdung 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 pesticides. The bioremediation conditions like pH, moisture, temperature, dissolved oxygen and nutrient level (C: N: P) are presented in Table 3.

Chlorpyrifos

The concentration of chlorpyrifos and its intermediates during the bioremediation of 25, 50 and 100 mg/kg chlorpyrifos amended soil is estimated and presented in Figure 2. The analyses carried out on GC-MS showed that chlorpyrifos was rapidly hydrolyzed to 3,5,6 trichloro2-pyridinol (TCP) in 25 and 50 mg/kg chlorpyrifos amended soil while in 100 mg/kg chlorpyrifos amended soil it was present till the 3rd day of the experiment. Residue analyses showed that the most persistent intermediates extracted were benzyl pyridine and TCP. In the surface soil treatment unit containing 25 mg/kg chlorpyrifos spiked soil, during the eight treatment days, we found that TCP was detected in soil for 4 days and benzyl pyridine for 6 days and then potentially further metabolized into other simpler compounds. In the case of 50 mg/kg chlorpyrifos amended soil, the study showed that TCP was detected in the soil for a period of 6 days and very low concentrations of benzyl pyridine were found in the soil till the 5th day. In the case of 100 mg/l chlorpyrifos amended soil, the data indicates that both TCP and benzyl pyridine were present in the soil till the end of the experimental study.

Cypermethrin

The concentration of cypermethrin and its intermediates during the bioremediation experiment at the three concentrations in soil treated with activated cow-dung slurry is presented in Figure 3. The quantitative and qualitative analysis carried out on GC-MS showed that cypermethrin was hydrolyzed to 3-phenoxy benzaldehyde and 3phenoxy-benzyl alcohol.

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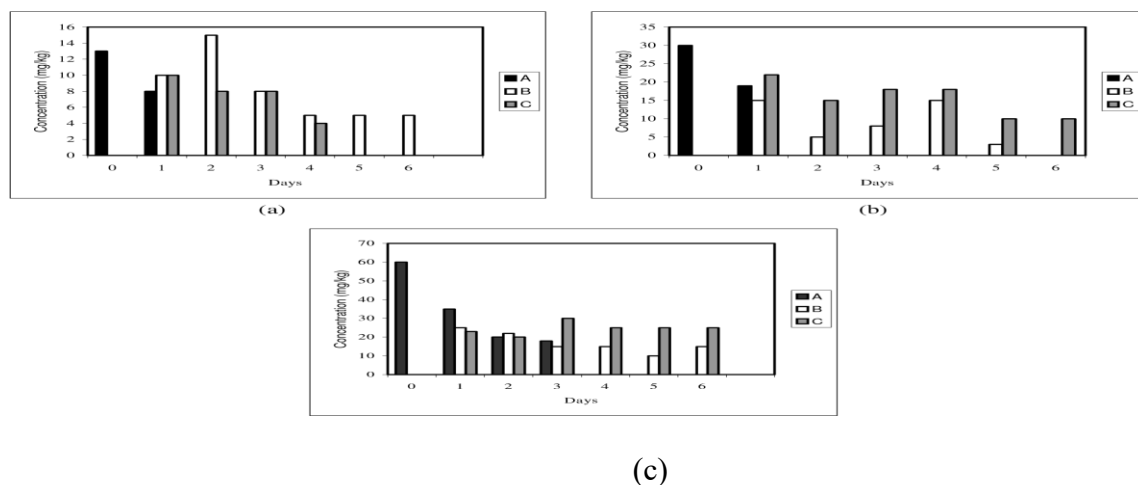


Figure 2. Concentration of intermediates found during the bioremediation of chlorpyrifos amended soil (a) 25 mg/l chlorpyrifos amended soil (b) 50 mg/l chlorpyrifos amended soil (c) 100 mg/l chlorpyrifos amended soil

where A = Chlorpyrifos, B = Benzyl pyridine and C = TCP.

Fenvalerate

The degradation of fenvalerate and detection of intermediate metabolites are presented in Figure 4. The compounds such as 4-chloro- α (1-methylethyl) benzene acetic acid and α -cyano-3-phenoxybenzyl alcohol were found to be the principal intermediates of fenvalerate degradation. After duration of one week, at 100 mg/kg concentration, fenvalerate was still detected in the soil. However, at 50 and 25 mg/kg, fenvalerate was found completely metabolised into its intermediates by the action of microorganisms.

Trichlopyr butoxyethyl ester (TBEE)

The concentration of TBEE and its intermediates during the course of bioremediation of TBEE contaminated surface soil at 25, 50 and 100 mg/kg were studied. It is evident from the GC-MS data that TBEE was rapidly broken down into trichlopyr acid via hydrolysis of the ester functional moiety (Figure 5). The compounds trichlopyr acid and 3,5,6 trichloro pyridinol were found to be the principal metabolites of TBEE biodegradation. In the treatment unit containing 25, 50 and 100 mg/kg TBEE contaminated soil respectively, results suggest that; TBEE has been converted into trichlopyr acid within 24 h. In 100 mg/kg TBEE contaminated soil trichlopyr acid and 3,5,6 trichloropyridinol (TCP) were found throughout the eight days of the experiment.

COD and BOD

The chemical oxygen demand (COD) concentration studied during the bioremediation of each pesticide in the SSTU under controlled environmental conditions overall showed very little variation among treatments and pesticide concentration (Figure 6). The percentage decrease in COD measured during the bioremediation of chlorpyrifos showed 63.4% reduction in the COD for 25 mg/kg chlorpyrifos amended soil, 56.2% COD reduction for 50 mg/kg chlorpyrifos amended soil, 48.7% COD reduction in the case of 100 mg/kg chlorpyrifos

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amended soil while in control soil the percentage COD decrease was around 68%. Similar results for reduction in COD concentration have been found during bioremediation of cypermethrin, fenvalerate and TBEE at varying concentrations in surface soil treatment unit (Table 4). Figure 7 shows variation in Biological Oxygen Demand during Bioremediation of pesticide-amended soil in Surface soil treatment unit at varying concentration. The percentage increase in Biological Oxygen Demand (BOD) found during the bioremediation of each pesticide at varying concentration is presented in Table 5. Again biological oxygen demand was very similar among the different pesticide treatments.

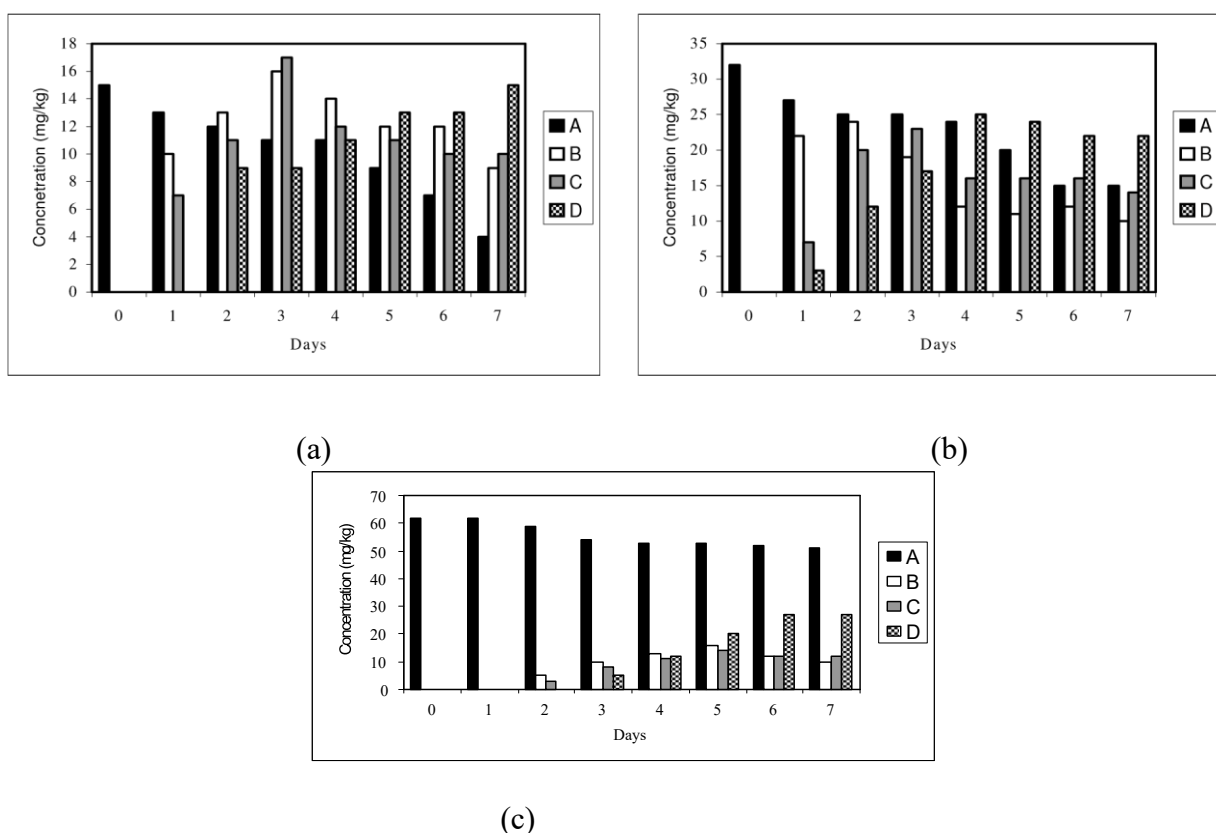


Figure 3. Concentration of intermediates found during the bioremediation of Cypermethrin amended soil (a) 25 mg/l Cypermethrin amended soil (b) 50 mg/l Cypermethrin amended soil (c) 100 mg/l Cypermethrin amended soil where A = Cypermethrin, B = 3-phenoxy benzaldehyde, C = 3-phenoxy benzyl alcohol and D = 3-phenoxy benzoic acid.

Table 4. Percentage reduction in COD of pesticides at varying concentrations during bioremediation in SSTU.

Concentration Pesticide	25 mg/kg	50 mg/kg	100 mg/kg	Control
Chlorpyrifos	63.4%	56.2%	48.7%	68%
Cypermethrin	61.5%	56.0%	49.5%	63.7%.
Fenvalerate	63%	57.4%	48.2%	65.6%.

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TBEE	61.8%	55.5%	50.3%	64.5%
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DISCUSSION

The indiscriminate use of pesticides in agriculture has resulted into contamination of soil-water environment leading to toxicity in the biota. The remediation of (Table 2) that activated cow-dung slurry and soil contains robust mixed community of microorganisms like bacteria, fungi and actinomycetes, which was found effective in biodegradation of pesticide amended soil (Fulekar, 2005a).

The presence of high concentration of nutrients in cowdung slurry and soil (Table 1) further enhanced microbial activities in surface soil treatment unit (SSTU). The bioremediation conditions pH (6.5 – 8.0), C:N:P ratio (100 : 10 : 1), DO (10 – 12 mg/l), moisture (60 – 80%) and temperature (25 – 28°C) have been monitored and maintained during the bioremediation of each pesticide at varying concentrations.

During bioremediation, it was found that chlorpyrifos was rapidly hydrolyzed to 3,5,6 trichloro-2-pyridinol (TCP) at all concentrations studied (Figure 2). Report on *Enterobacter* strain isolated from soil showed that the bacterium had strong phosphotriesterase (OPH) activity and it hydrolyzed a 35 mg/l concentration of chlorpyrifos within 24h in liquid culture media (Singh et al., 2004). 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; Exttoxnet, 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) that degradation of chlorpyrifos was rapid in alkaline soils with pH 7.7 and 8.4.

During the study, it was found that TCP and benzyl pyridine were the most persistent intermediates. Studies carried out by Baskaran et al. (2003) also state that primary metabolite TCP persist for longer duration in soil. In the present study, the surface soil treatment unit containing 100 mg/kg chlorpyrifos amended soil (Figure 2), TCP and benzyl pyridine was partially degraded and found accumulated and persistent till the 8th day of the experiment, whereas in 50 mg/kg and 25 mg/kg chlorpyrifos amended soil TCP and benzyl pyridine were completely disintegrated into simpler compounds which would be mineralized further into nutrient, biomass and inorganic on sufficient acclimatization.

Investigations done by DeeAn Jones (1995) demonstrates that hydrolysis of the ester linkage in cypermethrin is the primary route of biodegradation. The quantitative and qualitative analysis carried out on GC-MS during the course of bioremediation shows that cypermethrin was hydrolyzed to 3-phenoxy benzaldehyde and 3-phenoxy benzyl alcohol (Figure 3). This is in agreement with the studies done by Tallur et al. (2007), that *Micrococcus* sp. isolated from soil, utilized cypermethrin as a sole source of carbon leading to hydrolysis of

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ester linkage to yield 3phenoxybenzoate. A novel study done by Maloney et al. (1988) also showed that microbial consortium can transform cypermethrin with a half-life of 7 to 14 days at a concentration of 50 mg/l in the presence of Tween 80.

The GC-MS analytical data for fenvalerate suggest that the compound was rapidly broken down via cleavage at the ester functional moiety (Figure 4). Hydroxylation of fenvalerate has also been found to take place, which is followed by ester and ether cleavage and subsequently with oxidation and hydrolysis of conjugates. The compounds such as 4-chloro-alpha (1-methylethyl) benzene acetic acid and alpha-cyano-3-phenoxybenzyl alcohol were found to be the principal intermediates of fenvalerate degradation. Previous study shows that *Bacillus cereus*, *Pseudomonas fluorescens* and *Achro-mobacter* sp were able to transform fenvalerate in presence of tween 80 within 5 days (Maloney et al., 1988). The present bioremediation study showed that the parent compound fenvalerate has been degraded mainly into principal intermediates 4-chloro-alpha (1-methylethyl) benzene acetic acid and alpha-cyano-3-phenoxybenzyl alcohol due to the ester cleavage.

In the case of TBEE amended surface soil, the GC-MS quantitative analysis showed that TBEE was rapidly broken down into trichlopyr acid via hydrolysis of the ester functional moiety. It was observed that hydrolysis and reduction reactions were the principal mechanisms occurring during the course of bioremediation of TBEE in surface soil treatment unit. The compounds trichlopyr acid and 3,5,6-trichloro-pyridinol were found to be the principal metabolites of TBEE biodegradation (Figure 5).

In the treatment unit containing 25, 50 and 100 mg/kg TBEE contaminated soil respectively; TBEE has been biotransformed into trichlopyr acid within 24 h. Studies done by Bidlack (1978) also state that TBEE disintegrates rapidly into trichlopyr acid by virtue of hydrolysis with a half-life of three hours. Studies carried out by Baskaran et al. (2003) also stated that primary metabolite TCP persist for longer in soil. Research data showed that TCP will eventually convert to CO₂ (Ghassemi et al., 1981; Cryer, 1993).

Aerobic bioremediation was carried out in SSTU using continuous symmetric aeration with the help of electric air pump. 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. Previous research studies also reported that COD is a direct indicator of bioremediation (Singh and Fulekar, 2007). The physico-chemical parameter as indicated in Table 3 were also monitored and maintained for the bioremediation of chlorpyrifos, cypermethrin, fenvalerate and TBEE under controlled conditions in SSTU as a simulated pilot scale study.

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). Research studies compiled and documented showed that adaptability of microorganisms during bioremediation releases enzymes, which metabolizes wide spectrum of anthropogenic chemicals (Fulekar, 2005b). The present

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surface soil treatment technique used for bioremediation of pesticides using activated cow-dung and soil microflora would be an effective treatment technology for other group of pesticides and its effluents.

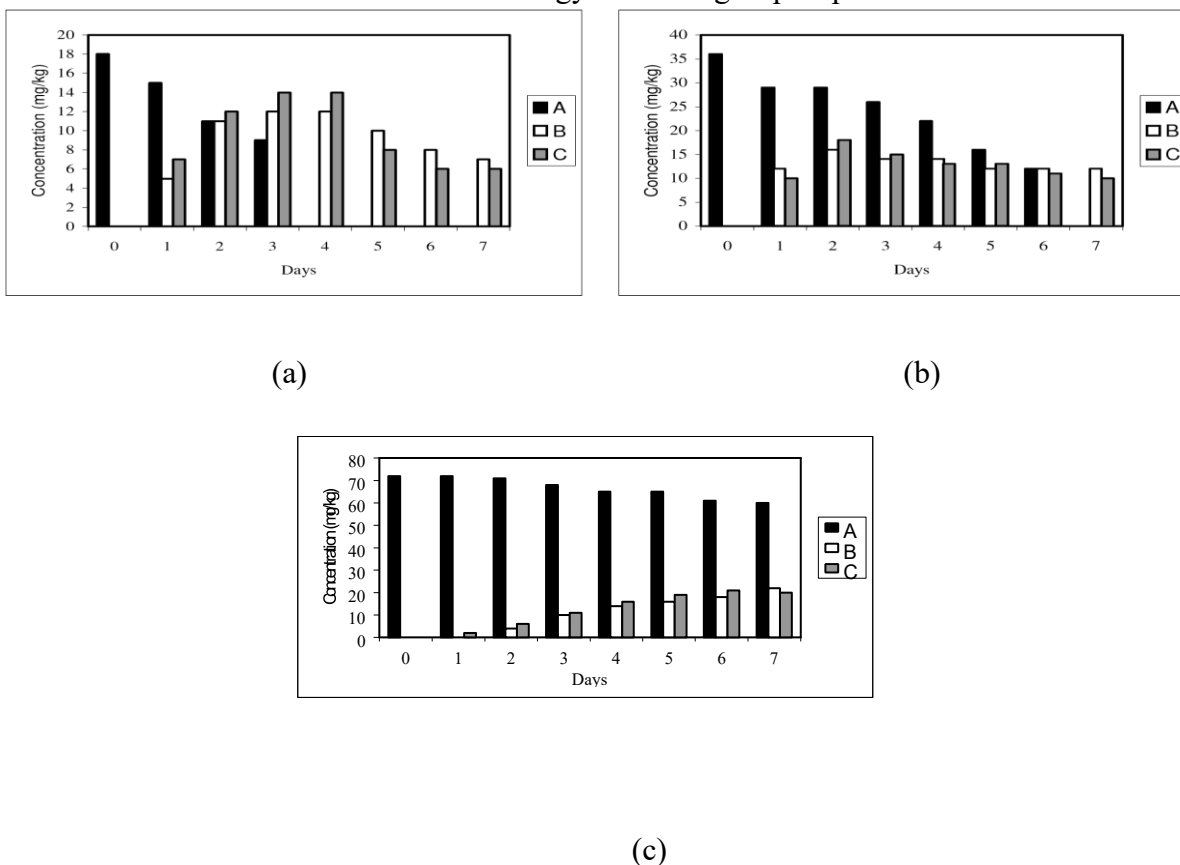


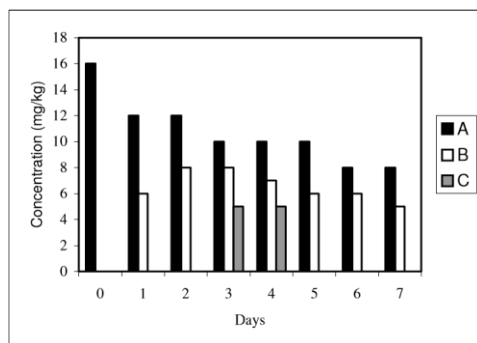
Figure 4. Concentration of intermediates found during the bioremediation of Fenvalerate amended soil (a) 25 mg/l Fenvalerate amended soil (b) 50 mg/l Fenvalerate amended soil (c) 100 mg/l Fenvalerate amended soil where A = fenvalerate, B = 4-chloro-alpha (1-methylethyl) benzene acetic acid and C = alpha-cyano-3-phenoxybenzyl alcohol.

Table 5. Percentage increase in BOD of pesticides at varying concentrations during bioremediation in SSTU.

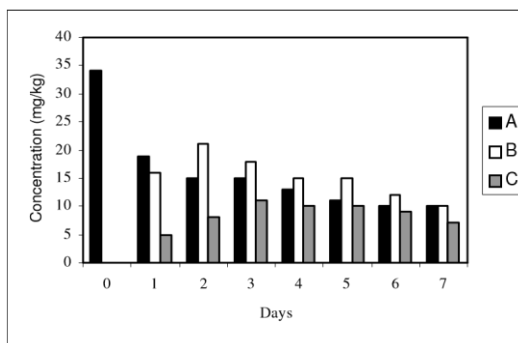
Concentration Pesticide	25 mg/kg	50 mg/kg	100 mg/kg	Control
Chlorpyrifos	22.8 %	19.76 %	17.64 %	35.21 %
Cypermethrin	16.20 %	15.38 %	7.60 %	29.86 %
Fenvalerate	25.71 %	24.50 %	18.60 %	33.30 %

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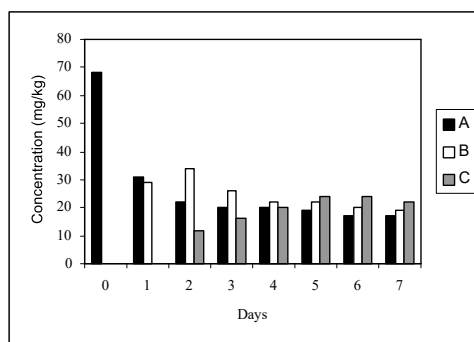
TBEE	29.76 %	21.38 %	17.14 %	34.40 %
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(a)

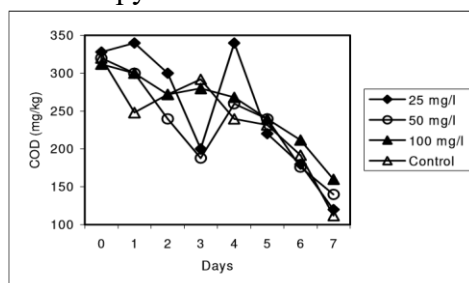


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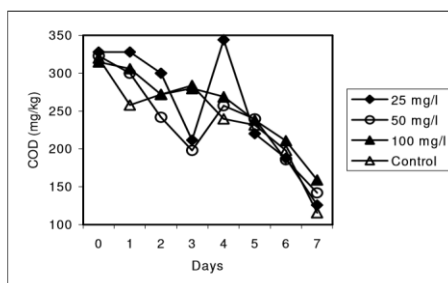


(c)

Figure 5. Concentration of intermediates found during the bioremediation of TBEE amended soil (a) 25 mg/l TBEE amended soil (b) 50 mg/l TBEE amended soil (c) 100 mg/l TBEE amended soil where A = TBEE, B = Trichlopyr acid and C = TCP.

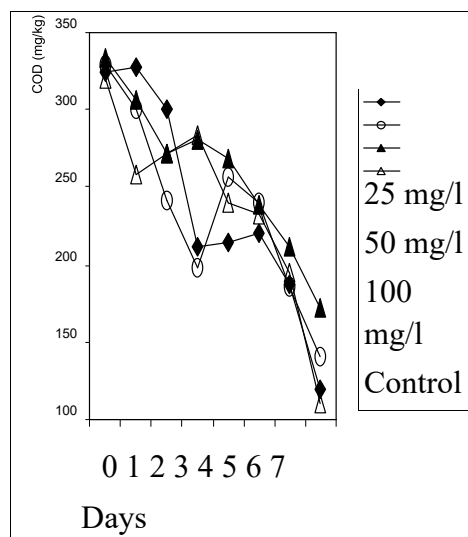


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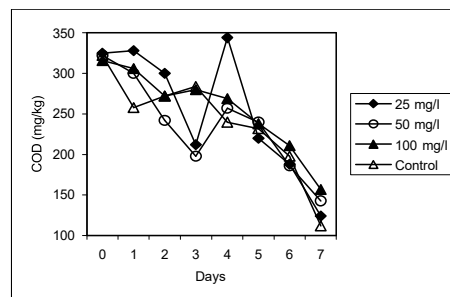


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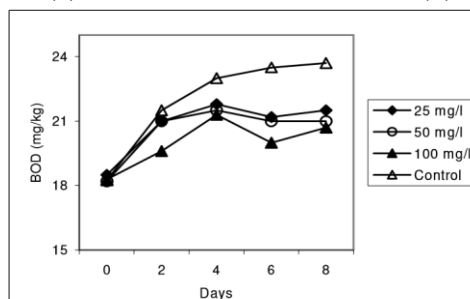
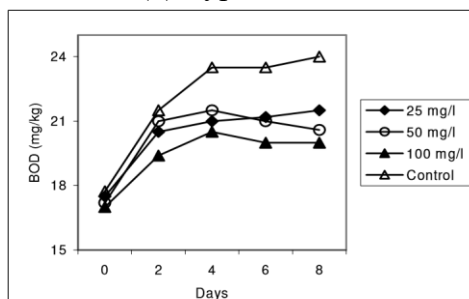


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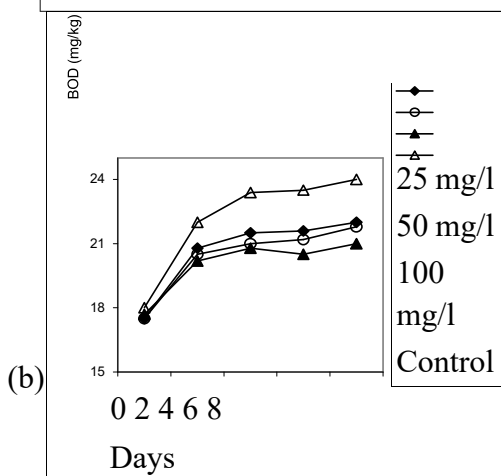


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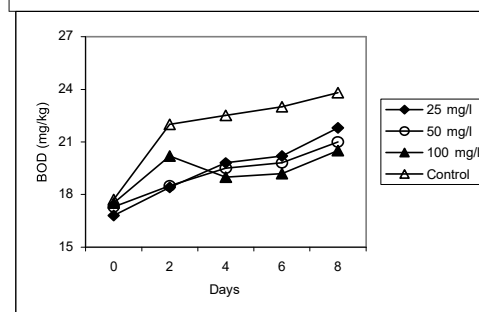
Figure 6. Variation in COD during Bioremediation of pesticide-amended soil in Surface soil treatment unit: (a) Chlorpyrifos amended soil (b) Cypermethrin amended soil (c) Fenvalerate amended soil (d) TBEE amended soil



(a)



(b)



(c)

(d)

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Figure 7. Variation in BOD during Bioremediation of pesticide-amended soil in Surface soil treatment unit: (a) Chlorpyrifos amended soil (b) Cypermethrin amended soil (c) Fenvalerate amended soil (d) TBEE amended soil

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REFERENCES

- APHA, AWWA WPCF (1995). Standard Methods for the Examination of Water and Wastewater. American Public Health Association / American Water Works Association/Water Environmental Federation, 19th Edition, Washington DC.
- Baskaran S, Kookana RS, Naidu R (2003). Contrasting behavior of chlorpyrifos and its primary metabolite TCP (3,5,6-trichloro-2pyridinol) with depth in soil profiles. *Australian J. of Soil Res.* 41(4): 749 – 760.
- BIDLACK HD (1978). The hydrolysis of triclopyr EB ester in buffered deionized water, natural water, and selected soils. DowElanco Data Package Report No. ABM-106279-E. DPR# 51566-001.
- Brinch UC, Ekelund F, Jacobsen CS (2002). Method for spiking soil samples with organic compounds. *Applied and Environ. Microbiol.* 68 (4): 1808 – 1816.
- Central Food Technological Research Institute (2003). Report of analysis of pesticide residues in soft drink samples, Mysore, India.
- Central Labor Institute (2002). Government of India, National Research Project: Survey of Process Safety, Health and Work Environment in Pesticide Industries in India, pp. 1-215.
- Cryer SA (1993). The dissipation and movement of triclopyr in a Northern U.S.A. forest ecosystem. DowElanco. Study No: PM912502. Data package Report No. ABM-143895-E. DPR# 51566-021.
- DeeAn Jones (1995). Environmental Fate of Cypermethrin. Environmental Monitoring and Pest management Branch,
- Department of Pesticide Regulation, Sacramento, California, USA. Environmental Protection Agency (2003). Soxhlet Extraction Method 3540, Test Methods, Washington, D.C.
- Environmental Protection Agency (2005). Pesticide Product databases, Washington, D.C.
- Exttoxnet (1996). Extensive Toxicology Network, Pesticide Information Profiles, www.exttoxnet.orst.edu

Original Article

- Fulekar MH (2005a). Bioremediation Technologies for Environment. *Indian Journal of Environ. Protection.* 25(4): 358 – 364. Fulekar MH (2005b). *Environmental Biotechnology.* Oxford and IBH Publishing House, New Delhi, India.
- Ganapathy C (1997). Environmental fate of Trichlopyr. *Environmental Monitoring and Pest Management Branch, Department of Pesticide Regulation, Sacramento, California, USA.*
- Ghassemi M, Fargo L, Painter P, Quinlivan S, Scofield R, Takata A (1981). Environmental fates and impacts of major forest use pesticides. U.S. EPA. Office of Pesticides and Toxic Substances. Washington D.C.
- Huang XL, Linda S, Nakatsu C (2000). Impact Of Animal Waste Lagoon Effluents On Chlorpyrifos Degradation In Soils. *J. of Environ. Toxicol. and Chem.* 9: 2864-2870.
- Jackson ML (1973). *Soil Chemical Analysis.* Prentice-Hall of India, New Delhi.
- Maloney SE, Maule A, Smith ARW (1988). Microbial transformation of the pyrethroid insecticides: Permethrin, deltamethrin, fastac, fenvalerate and fluvalinate. *Applied and Environ. Microbiology.* 54(11): 2874-2876.
- Saiyed HN, Bhatnagar VK, Kashyap R (1999). Impact of pesticide use in India. *Asian-Pacific Newsletter.* 3: 66-67.
- Singh BK, Walker A, Morgan AW, Wright DJ (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.
- Singh BK, Walker A, Morgan AW, Wright DJ (2004). Biodegradation of chlorpyrifos by *Enterobacter* strain B-14 and its use in Bioremediation of contaminated soils. *Applied and Environ. Microbiol.* 70(8):4855 – 4863.
- Singh D, Fulekar MH (2007). Bioremediation of phenol using microbial consortium bioreactor. *Innovative Romanian Food Biotech.* 1(1):30 – 35.
- Tallur PN, Megadi VB, Ninnekar HZ (2007). Biodegradation of Cypermethrin by *Micrococcus* strain CPN1. *Biodegradation.* DOI - 10.1007/s10532-007-9116-8.
- USDA (1995), Agricultural Research Service. ARS Pesticide properties. www.arsusda.gov/rsml/textfiles/cypermethrin
- World Health Organization (1990). Report on Fenvalerate. *Environmental Health Criteria, International Program on Chemical Safety.*

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