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Cypermethrin biodegradation in contaminated surface soil - A laboratory technique

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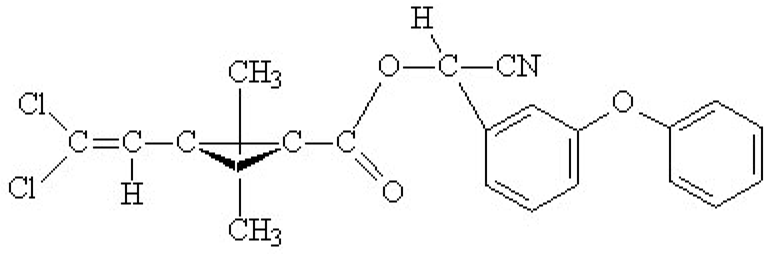
ABSTRACT

The surface soil contamination with pesticides is a persistent environmental problem in the pesticide manufacturing and formulation industries. The present physico – chemical and biological treatment is not efficient & effective to remove the pesticides from the contaminated soil. The microbial degradation would be an effective treatment technology for the remediation of pesticides. In the present study, a laboratory technique has been developed for cypermethrin biodegradation. The varying concentrations of cypermethrin i.e. 25ppm, 50ppm and 100 ppm were taken in surface soil treatment unit and bioremediation conditions were monitored and maintained. The biodegradation of cypermethrin has resulted into the formation of intermediates such as 3-phenoxy benzaldehyde, 3-phenoxy benzyl alcohol and 3-phenoxy benzoic acid. These compounds are less toxic than the parent compound and in the long run would be mineralized into nutrient & biomass. The present treatment technology would prove to be an effective technique for treatment of surface soil contaminated with pesticides. This technology would be useful to the pesticide industry for treatment of the pesticide wastes.

Key words: Cypermethrin, Bioremediation, Surface Soil treatment Unit, Cow -dung, Biomass.

INTRODUCTION

Cypermethrin [alpha-cyano-3-phenoxybenzyl-3-(2,2-dichloro-vinyl)-2,2-dimethylcyclopropanecarboxylate] is a synthetic, pyrethroid insecticide used for protection against wide range of pests. Cypermethrin is a pure racemic mixture consisting of eight stereoisomers. Cypermethrin manufacturing and formulation industries are treating effluents and wastes by physico – chemical methods, which are insuffienct & ineffective, thus the residual cypermethrin finds its way into the environment and food chain. It is found to be moderately persistent in soil with a typical half-life in aerobic soil ranging from 6 – 20 days (USDA, 1995 & Extoxnet, 1996).



Chemical structure of Cypermethrin

The bioremediation using microbial consortium will be a beneficial technique for degradation of cypermethrin into less toxic compounds. Microbes play a significant role in the degradation of cypermethrin. Hydrolysis and photolysis are the major reactions responsible for the degradation of cypermethrin in soil. Principal degradation route of cypermethrin, found to occur in soil is through hydrolysis of the ester linkage (DeeAn Jones, CA). Studies done by Tallur et. al shows that a bacterium capable of utilizing pyrethroid pesticide cypermethrin as sole source of carbon was isolated from soil and was identified as a *Micrococcus sp*. The organism degraded cypermethrin by hydrolysis of ester linkage leading to loss of insecticidal activity. In another investigation, microbial transformation of technical cypermethrin in aerobic batch enrichment cultures using microbial enrichments was done containing *Pseudomonas fluorescens*, *Achromobacter* sp. and *Bacillus cereus. The microbial population was able* to transform cypermethrin with a half-life of 7 to 14 days at a concentration of 50 mg/liter in the presence of Tween 80, leading to formation of 3-phenoxybenzoic acid as a major transformation products (Maloney et.al. 1988).

In the present experimental study, a surface soil treatment unit has been designed to study bioremediation of cypermethrin in the soil by using cow dung biomass containing bacteria, fungi and actinomycetes. The aim of the study is to investigate the degradation potential of activated cow dung biomass towards cypermethrin and its intermediates. The bioremediation under aerobic conditions by activated cow dung biomass under controlled conditions will prove to be an effective technique for biodegradation of cypermethrin.

The technical grade Cypermethrin is taken for the bioremediation study. The activated cow – dung was used as a source of biomass for the degradation of cypermethrin under controlled environmental conditions. The experimental set up has been designed by fabricating a Surface Soil Treatment Unit (22cm x 10cm x 6cm). Alluvial soil was collected for the experiment from Palghar located near Mumbai region and was air-dried and sieved through 2mm mesh. The soil and cow dung were characterized for physico – chemical parameters and microorganisms like bacteria, fungi & actinomycetes. Cypermethrin was amended in the soil at 25ppm, 50ppm and 100ppm concentration respectively (Brinch et al., 2002). The cypermethrin-amended soil (1kg) was taken in the Surface Soil treatment Unit and activated cow – dung slurry biomass was added at the ratio of 1:1. Symmetric air was supplied to maintain the aerobic bioremediation conditions. The environmental parameters were monitored during bioremediation over a period of time. The soil samples (10gms) at a frequency of 24 hours over period of eight days were removed and air-dried at room temperature. Cypermethrin and its intermediated were extracted from soil sample through soxhlet extraction assembly. The soxhlet extract was analyzed for cypermethrin degradation and its intermediates using GC - MS Soil extract samples were analyzed in the Sophisticated Analytical Instrumentation Facility (SAIF), IIT Mumbai.

RESULTS & DISCUSSION

The surface soil contamination with Cypermethrin is a common environmental problem found near the 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. In the present study, soil surface treatment unit (SSTU) has been designed wherein, technical grade cypermethrin was amended in alluvial soil at three different concentrations viz. 25ppm, 50ppm & 100ppm and bioremediation of cypermethrin is carried out using activated cow dung biomass. The physico – chemical characterization of cow dung biomass and soil were carried out and are presented in Table No 1. The data shows presence of inorganic nutrients such as organic carbon, nitrogen, phosphorus, sulphate, calcium, chloride, sodium, potassium & magnesium in cow – dung and soil that served as a good nutrient source for the growth of microorganisms. The data shows presence of diverse microbial community in soil as well as in cow dung. The presence of nutrients as well as microorganisms in cow – dung and soil has been found to have great influence on the bioremediation of cypermethrin. The bioremediation conditions like pH, moisture, temperature, dissolved oxygen and nutrient level (C: N: P) were monitored and maintained in surface soil treatment unit (Table 2).

During bioremediation, degradation pattern of cypermethrin and its intermediates in 25ppm, 50ppm and 100 ppm cypermethrin amended soil was studied. The concentration of cypermethrin and its intermediates during the bioremediation at varied concentration in soil is estimated and presented in figure 1. The quantitative and qualitative analysis carried out on GC – MS shows that cypermethrin was hydrolyzed to 3 – phenoxy benzaldehyde and 3 – phenoxy benzyl alcohol. Investigations done by DeeAn Jones, California, USA also demonstrates that hydrolysis of the ester linkage in cypermethrin is the primary route of biodegration. Another study done by Tallur et al. demonstrates that *micrococcus sp*. isolated from soil-utilized cypermethrin as a sole source of carbon leading to hydrolysis of ester linkage to yield 3-phenoxybenzoate. During the present investigations it has been found that cypermethrin has degraded upto73.3% in 25ppm cypermethrin amended soil, 53.2% in 50ppm cypermethirn amended soil and 17.7% in 100ppm cypermethrin amended soil respectively (Fig 1). A novel study done by Maloney et al. also shows that microbial consortium can transform cypermethrin with a half-life of 7 to 14 days at a concentration of 50ppm in the presence of Tween 80. In another experiment, biodegradation of Cypermethrin was investigated using *Pseudomonas* in activated sludge under aerobic conditions. Cypermethrin at 20ppm was found to mineralize completely over a period of 48h at ambient temperature (Jilani et al. 2006).

Table. - 1: Physico – Chemical characteristics of soil & cow dung biomass

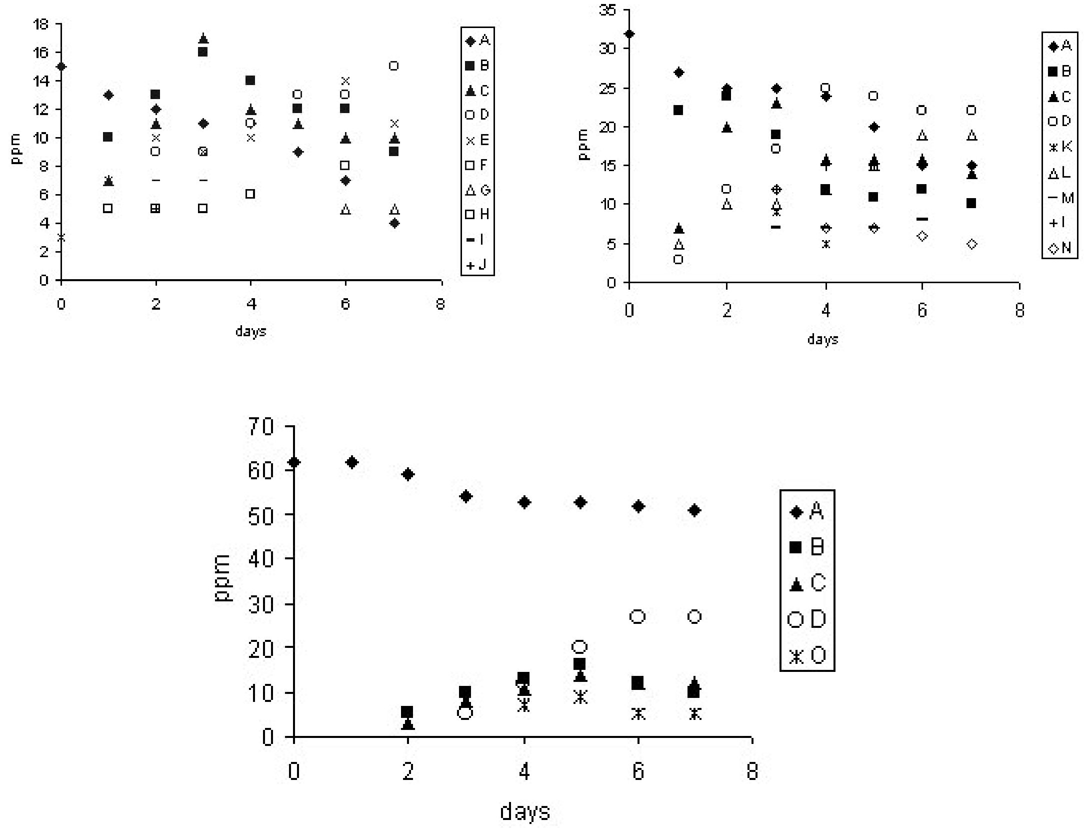
Parameter	Soil	Cow Dung Slurry
pH	7.6	7.4
Moisture	4.5 %	-
Alkalinity /100gms	0.6meq	1.2meq
Dissolved Oxygen	6 ppm	9 ppm
Temperature	26 ^{0}C	28 ^{0}C
Cation Exchange Capacity /100gms	108meq	-
% Organic Carbon	1.08	0.34
Phosphorus	0.25ppm	0.78ppm
Kjeldahl Nitrogen	2100ppm	8.6ppm
Sulphate	2.5ppm	26ppm
Calcium	8727ppm	8.6ppm
Chloride	1930ppm	6ppm
Potassium	344ppm	161ppm
Sodium	423ppm	92.8ppm
Magnesium	15440ppm	147ppm
COD	220ppm	200ppm
BOD	470ppm	880ppm

Table. - 2: Parameters monitored & maintained during bioremediation of cypermethrin in surface soil treatment unit

Parameter	Range
C:N:P	100:10:1
pH	6.5 – 8.0
Temperature	25 – 28 ^{o}C
Moisture	60 – 80%
Dissolved Oxygen	10 – 12 ppm
Microbial Growth	Present

Bioremediation data illustrates that the most persistent intermediates were found to be 3 – phenoxy benzyl alcohol and 3 – phenoxy benzoic acid in all the three concentrations of cypermethrin-amended soil. In the surface soil treatment unit containing 25ppm and 50 ppm cypermethrin amended soil, presence of benzene derivatives like 2,4 bis (1,1 dimethyl ethyl) phenol, chloromethyl ethyl benzene and 2,4 bis (methyl ethyl) benzene has been found which is unlike in the case of 100-ppm cypermethrin amended soil (Fig 1).

Variation in COD of cypermethrin-amended soil during the bioremediation in a surface



A – Cypermethrin
B – 3-phenoxy benzyldehyde
C – 3-phenoxy benzyl alcohol
D – 3-phenoxy benzoic acid
E – 2,4 bis (1,1 dimethyl ethyl) phenol
F – chloromethyl ethyl benzene
G – 2,4 bis (methylethyl) benzene
H – 3,5 cyclohexadiene, 1,2 dione
I – 2-(methylthio) benzothiazole
J – 5,7,7,7 tetrachloro heptene
K – cyclopropanoic acid
L – 3(4 – methoxy phenyl), 2- propenoicacid
M- methyl carbazole
N – phenol, 4,4'methylenebis
O – 2(methyl benzothiazole)

Fig. - 1: Concentration of cypermethrin and intermediates analyzed every day during the bioremediation in surface soil treatment unit (1) 25ppm cypermethrin amended soil (2) 50ppm cypermethrin amended soil (3) 100 ppm cypermethrin amended soil.

soil treatment unit is shown in figure 2. The decrease in COD with increasing duration of bioremediation was observed. The percentage decrease in Chemical Oxygen Demand (COD) measured during the bioremediation shows 61.5% COD reduction in the case of 25ppm cypermethrin amended soil, 56.0% COD reduction for 50ppm chlorpyrifos amended soil and 49.5% reduction in the COD for 100 ppm chlorpyrifos amended soil while in control soil the percentage COD decrease was around 63.7% (Fig 2). Figure 3 shows the variation in BOD of the experimental soil. Variation in the biological oxygen demand of the soil amended with cypermethrin indicates the activity of microorganisms during bioremediation. Similarly, percentage variation in Biological Oxygen demand

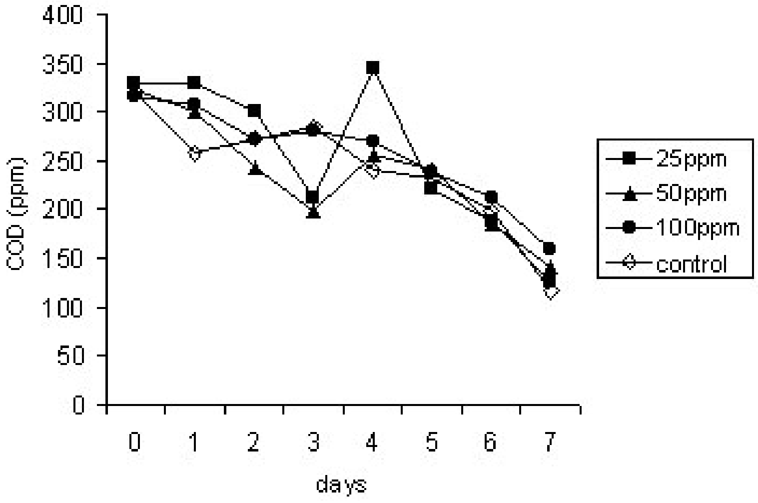


Fig. - 2: Variation in COD during Bioremediation of Cypermethrin amended soil in Surface soil treatment unit

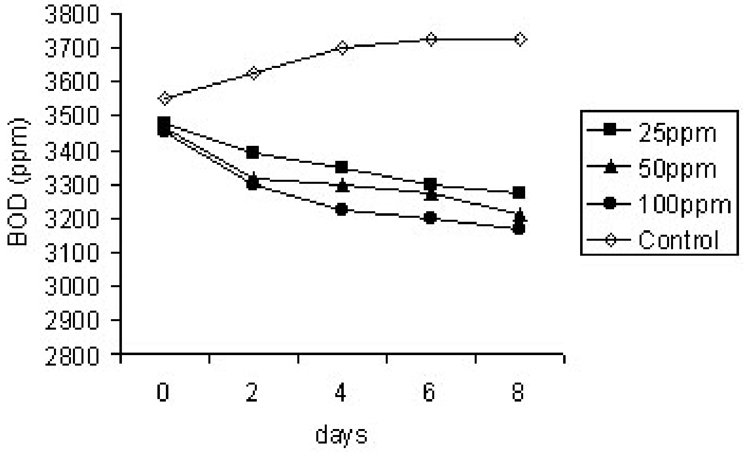


Fig. - 3: Variation in BOD during bioremediation of Cypermethrin amended soil in Surface soil treatment unit

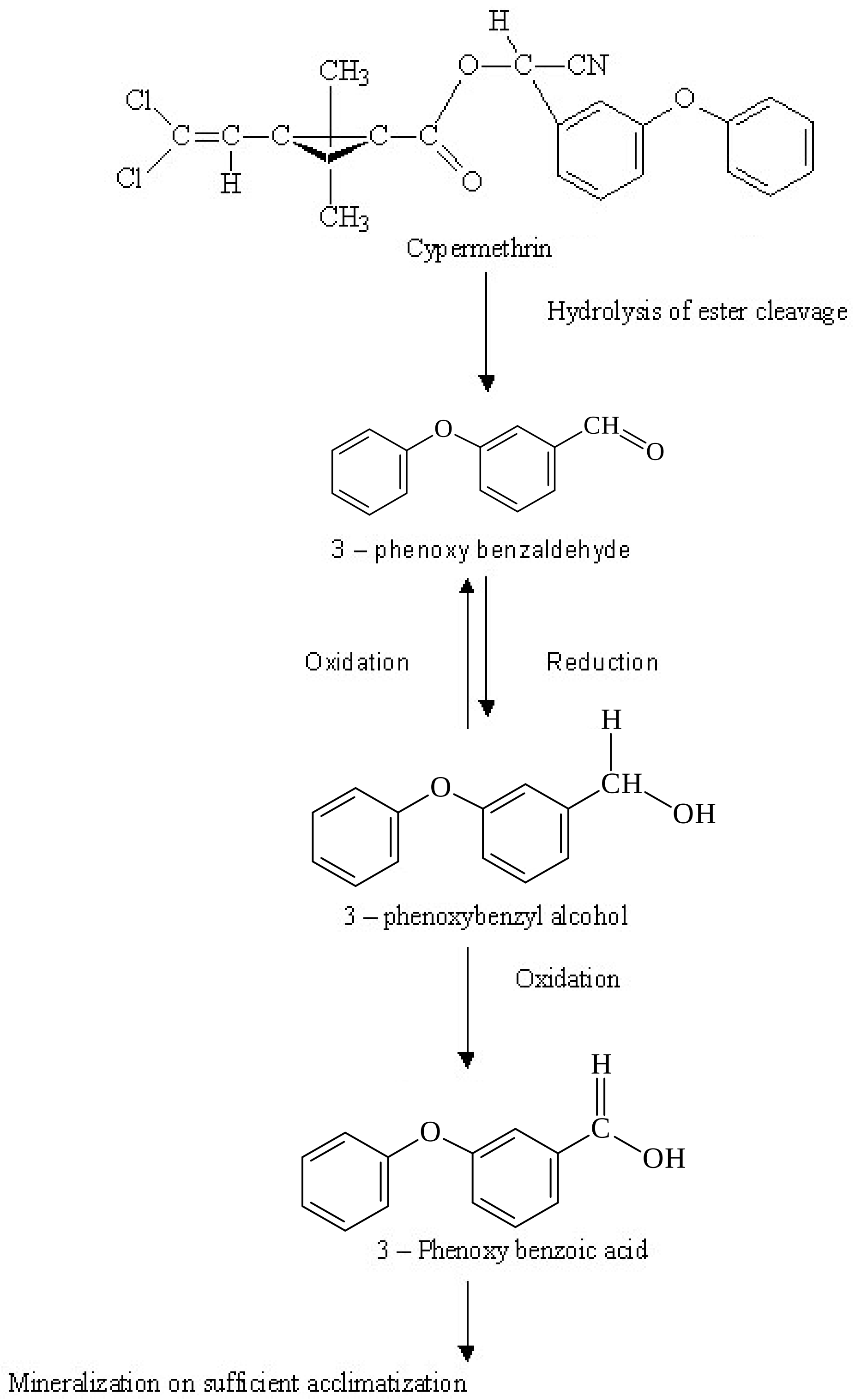


Fig. - 4: Bioremediation pathway of Cypermethrin in Surface soil treatment unit.

(BOD) measured during bioremediation shows 5.8% decrease in BOD in the case of 25ppm chlorpyrifos amended soil, 7.3% decrease in BOD in the case of 50ppm chlorpyrifos amended soil and 8.2% decrease in BOD in the case of 100ppm chlorpyrifos amended soil, while in control soil the percentage increase in BOD was 4.9% (Fig 3). Variation in percentage increase and decrease of COD and BOD in the three different cypermethrin amended soil (25ppm, 50ppm & 100ppm) as compared to the control is attributed to accumulation of intermediates like 3 – phenoxybenzyl alcohol and 3 – phenoxy benzoic acid.

The present study illustrates that due to the cumulative effect of microbial consortium present in soil and cow – dung slurry, the persistent intermediates like be 3 – phenoxy benzyl alcohol and 3 – phenoxy benzoic acid were disintegrated into simpler organic acids and other benzene derivatives which would be mineralized further into nutrient, biomass & inorganic on sufficient acclimatization. Thus, the results are explained considering the higher nutrient availability and the larger microbial population of the cow- dung slurry & soil. 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 impact on the degradation and transport of soil-applied pesticides (Huang et al., 2000). Research studies have documented that extreme adaptability of microorganisms gives them the capacity to alter enzymatic machinery to metabolize wide spectrum of xenobiotic and anthropogenic chemicals (Fulekar, M.H., 2005).

The present laboratory surface soil treatment technique with activated biomass and soil microflora would be an effective technology for bioremediation of cypermethrin.

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