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BIOREMEDIATION OF CHLORPYRIFOS IN SURFACE SOIL TREATMENT UNIT USING MICROBIAL CONSORTIUM

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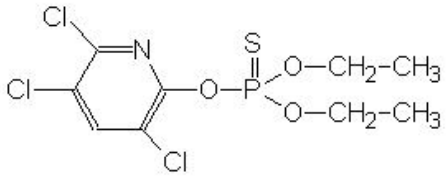
ABSTRACT

In the present study, surface soil treatment unit (SSTU) has been designed wherein alluvial soil (1kg) spiked with 25, 50 and 100 mg/l chlorpyrifos respectively was taken for bioremediation using cow – dung consortium as biomass. The ability of activated cow dung slurry consortium to degrade chlorpyrifos at varying concentration was investigated under controlled environmental conditions. The experimental finding shows that chlorpyrifos was rapidly hydrolyzed into TCP in each surface soil treatment unit at varying concentrations. 3,5,6 trichloro-2-pyridinol (TCP) and benzo pyridine were found to be the most persistent intermediates remaining in the soil during bioremediation. In surface soil treatment unit containing 25 mg/l and 50mg/l soil spiked with chlorpyrifos, TCP and benzo pyridine were disintegrated into 3 – methyl phenol, chloromethylethyl benzene, alpha hydroxy benzene acetic acid within a duration of 8 days, whereas in 100 mg/l spiked concentration, TCP and benzo pyridine were found persistent. The finding shows that bioremediation of chlorpyrifos was proportional to the percentage decrease in COD. These results highlight the potential of cow dung slurry consortium for bioremediation of soil contaminated with chlorpyrifos in a surface soil treatment unit.

Keyword: Bioremediation, chlorpyrifos, surface soil treatment unit, microbial consortium.

INTRODUCTION

Chlorpyrifos {O, O-diethyl-O- (3,5,6-trichloro-2-pyridyl) phospothorothioate} is an organophosphate insecticide used for agricultural and domestic use. The manufacturing and formulation of chlorpyrifos generates wastes containing toxic levels of chlorpyrifos. Due to ineffective and insufficient treatment and disposal facilities chlorpyrifos was found persisting in the residue even after physico-chemical treatment of effluent (TERI, 2003), which creates environmental hazards in soil and aquatic ecosystem and further causes toxicity by entering into the food chain. The study carried out in India shows that chlorpyrifos was present in 100% of the environmental samples, and in 75% of these samples; chlorpyrifos residues exceeded the recommended limit by 3.9 to 7.6 times (C.F.T.R.I, 2003). Chlorpyrifos is found to be persisting moderately in soil. The large variation in half-life of chlorpyrifos (10 – 141 days) has been attributed to variation in environmental factors like pH, temperature, moisture, organic carbon and pesticide formulation (Getzin, 1981). Chlorpyrifos is found to adsorb strongly to soil particles and it is not readily soluble in water (Extoxnet, 1996). Reports from the Environmental Protection Agency suggests that a wide range of water and terrestrial ecosystems may be contaminated with chlorpyrifos (EPA, 1997).



Structure of Chlorpyrifos

The bioremediation of chlorpyrifos using microbial consortium will be a beneficial technique for degradation of chlorpyrifos wastes into less toxic compounds. Microorganisms play an important role in intermediate degradation and subsequent mineralization of pesticides. Microbial degradation of a given pesticide may be of cometabolic, incidental nature or may be linked with energy production or nutrient procurement and thus support the growth of degrading consortium (IUPAC report on pesticides, 1997). The principle reactions involved in the biodegradation of chlorpyrifos are hydrolysis, oxidation, reduction, alkylation and dealkylation (Singh *et al*., 1999). A novel study done by Singh *et al*., 2003 on kinetics of chlorpyrifos degradation in five United Kingdom soil varying in pH from 4.7 to 8.4 suggested that dissipation of chlorpyrifos was mediated by the co – metabolic activities of soil microorganisms. Studies done by Yang *et al.* (2005) found that *Alcaligenes faecalis* utilized chlorpyrifos as the sole carbon and energy source in liquid media and was found to have the same degradation ability when applied to soil. In another

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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	---	9 mg/l
Temperature	26 ^{0}C	28 ^{0}C
Cation Exchange Capacity /100gms	108meq	---
% Organic Carbon	1.08	0.34
Phosphorus	0.25mg/l	0.78mg/l
Kjeldahl Nitrogen	2100mg/l	8.6mg/l
Sulphate	2.5mg/l	26mg/l
Calcium	8727mg/l	8.6mg/l
Chloride	1930mg/l	6mg/l
Potassium	344mg/l	161mg/l
Sodium	423mg/l	92.8mg/l
Magnesium	15440mg/l	147mg/l
COD	220mg/l	200mg/l
BOD	4mg/l	8mg/l

study, the ability of *Enterobacter asburiae* was investigated to mineralize Chlorpyrifos under different culture conditions and was found to utilize the compound as sole carbon and phosphorus source. The study demonstrated that *Enterobacter asburiae* hydrolyzed chlorpyrifos to diethylphosphorothioate (DETP) and 3,5,6 – trichloro – 2-pyridinol (TCP) (Singh *et al*., 2004). Various opd genes have been isolated from different microorganisms from different geographical regions, some of which have been shown to hydrolyze chlorpyrifos (Horne *et al*., 2002).

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

MATERIALS AND METHODS

Chemical: Technical grade chlorpyrifos 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 air-dried, 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).

Table 2. Parameters monitored and maintained during bioremediation of chlorpyrifos 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 mg/l
Microbial Growth	Present

Spiking of Soil: Experimental soil was treated with solvent acetone containing chlorpyrifos. In the treatment procedure, 25 ml of acetone containing chlorpyrifos was added to 25 % of the soil sample (250g), the flasks were closed for 5 mins to let the solvent disperse. Thereafter the solvent is evaporated for 16hrs 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

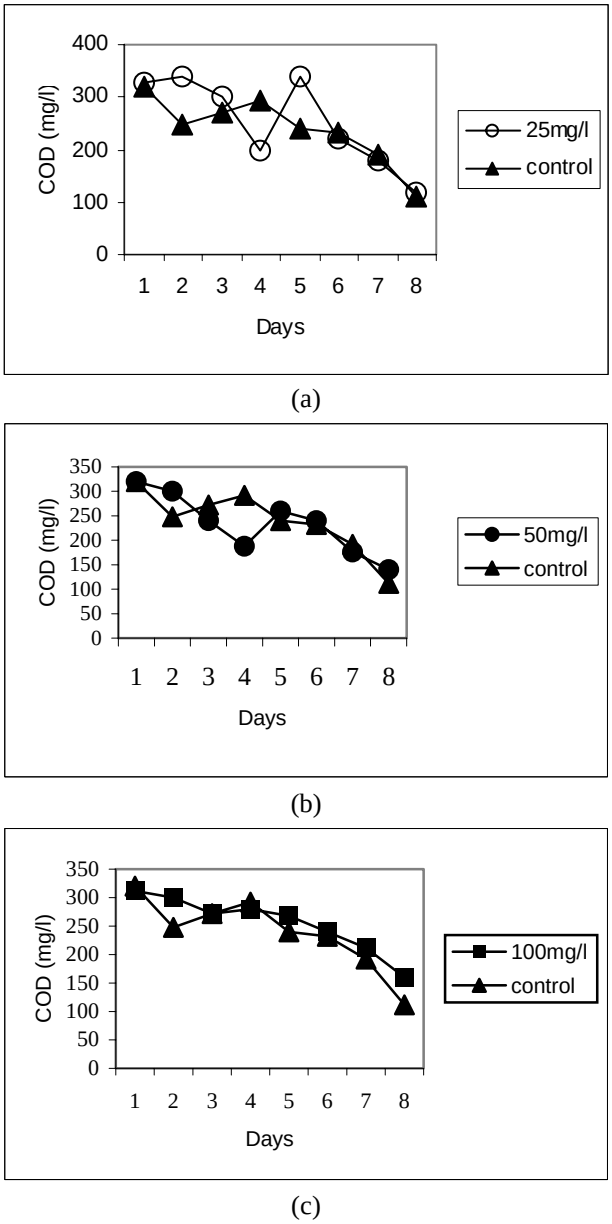


Fig. 1. Variation in COD during Bioremediation of Chlorpyrifos amended soil in Surface soil treatment unit: (a) 25mg/l initial concentration (b) 50mg/l initial concentration (c) 100 mg/l initial concentration.

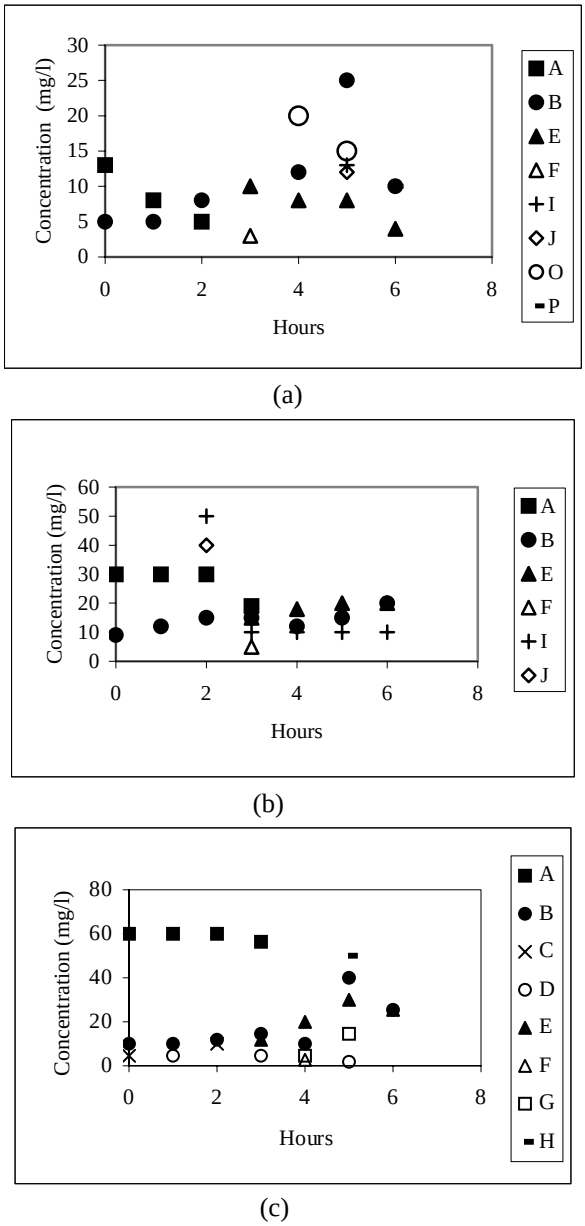


Fig. 2. Concentration of intermediates found every hour during the bioremediation of chlorpyrifos amended soil (a) 25mg/l chlorpyrifos amended soil (b) 50mg/l chlorpyrifos amended soil (c) 100 mg/l chlorpyrifos amended soil.

accordingly spiked with 25, 50 and 100 mg/l of chlorpyrifos separately and taken in surface soil treatment unit.

Biomass: Cow dung slurry in the ratio of 1:10 with distilled water was taken as a source of microbial biomass. Cow dung slurry was characterized for physico – chemical status (Table 1) (APHA, 1975). Biomass was activated & maintained by supplying air and nutrients (C, N, P).

Experimental set up: The surface soil contamination is a common problem in agriculture and waste treatment site. Therefore, a bioreactor for treatment of surface soil was specially designed and fabricated having dimensions 22cm x 10cm x 6cm. The soil (1kg) spiked with 25, 50 and100mg/l chlorpyrifos 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 chlorpyrifos was also run in parallel to make the comparisons. 0.05 % Tween 80 was added to the soil as a surfactant to prevent adsorption of

chlorpyrifos 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 2). 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 hour on the 0^{th} day (0^{th} hour to 7^{th} hour) and then every day for a period of one week. Chemical Oxygen Demand (COD) as an indicator of bioremediation was also monitored during the course of experiment. Microbial growth was checked and monitored by streaking the serial dilution of soil sample on a nutrient agar plate.

Extraction: Soil samples drawn every hour and every day (10g) was dried for chlorpyrifos 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 chlorpyrifos from soil was found to be around 65%.

Analytical Procedures: Soil sample extract was analyzed by Gas chromatography/mass spectroscopy (GC-MS) (Hewlett Packard GC-MS instrument Model No. G1800A) for chlorpyrifos and its intermediates. The instrument is equipped with electron ionization detector. Conditions maintained for the quantitative and qualitative analyses are: oven temperature – 100 ^{0}C, Injection temperature – 250 ^{0}C, detector temperature – 280 ^{0}C.

RESULTS AND DISCUSSION

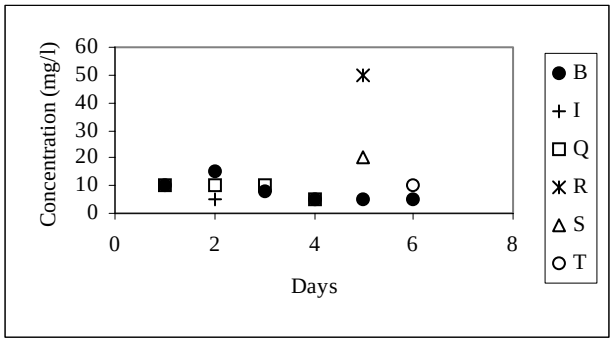
The surface soil contamination with Chlorpyrifos is a common environmental problem found near the pesticide manufacturing and formulation units. The recent advances in bioremediation using microbial technology would prove to be an effective treatment technique for pesticides like chlorpyrifos. In the present study, surface soil treatment unit (SSTU) has been designed wherein, technical grade chlorpyrifos was amended in alluvial soil at three different concentrations viz. 25mg/l, 50mg/l and 100mg/l and bioremediation of chlorpyrifos is carried out using activated cow dung consortium as biomass. The physico – chemical characteristics of cow dung slurry and soil were carried out and are presented in table 1. The data indicates high concentration of organic carbon, nitrogen, phosphorus, sulphate, calcium, chloride, sodium, potassium & magnesium in cow – dung slurry and soil which served as a good nutrient source for microorganisms. The presence of nutrients as well as microorganisms in cow – dung and soil has been found to have great influence on the bioremediation of chlorpyrifos. 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).

When the biomass is added to the Chlorpyrifos amended soil at varying concentration in different bioreactor, the microorganisms present therein experiences inhibition and shock and slowly start adapting in the contaminated environment by using carbon as a source of nutrient; thereby biodegradation of the compound begins. The carbon source being used by microorganisms is directly proportional to the COD reduction in the samples (McTernon *et al*., 1991). Variation in COD of chlorpyrifos amended soil during the bioremediation in a surface soil treatment unit is shown in figure 1. The decrease in COD with increasing duration of bioremediation was observed. The percentage decrease in Chemical Oxygen Demand (COD) measured during the bioremediation shows 48.7 % COD reduction in the case of 100mg/l chlorpyrifos amended soil, 56.2 % COD reduction for 50mg/l chlorpyrifos amended soil and 63.4 % reduction in the COD for 25 mg/l chlorpyrifos amended soil while in control soil the percentage COD decrease was around 68 %.

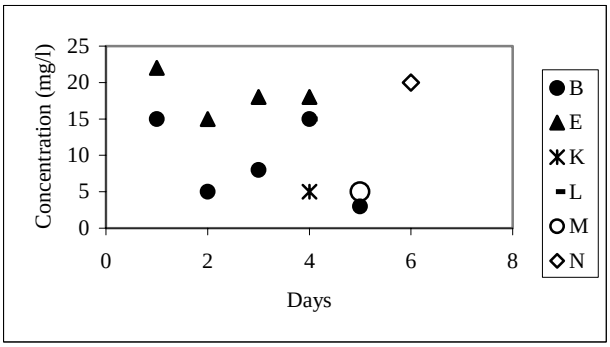
The concentration of chlorpyrifos and its intermediates during the bioremediation of 25mg/l, 50mg/l and 100mg/l chlorpyrifos amended soil is estimated and presented in Fig. 2 and 3. The GC – MS data shows that chlorpyrifos was rapidly hydrolyzed to 3,5,6-trichloro-2- pyridinol (TCP) at the 3^{rd} hour in all the three concentration of soil amended chlorpyrifos viz. 25mg/l, 50mg/l and 100mg/l. TCP is found to be the principal metabolite of chlorpyrifos (Extoxnet, 1996). 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). Another study demonstrated that ^{14}C labeled chlorpyrifos degradation in liquid culture with the help of microorganisms hydrolyzed chlorpyrifos to diethylhosphorothiote (DEPT) and 3,5,6- trichloro-2-pyridinol and utilized DEPT for growth and energy (Singh *et al*., 2004).

GC–MS data illustrates that the most persistent intermediate was found to be benzo pyridine and TCP. In the surface soil treatment unit containing 25mg/l chlorpyrifos spiked soil, when the duration of the experiment was eight days it has been found that TCP was persisting in soil for 2 days and benzo pyridine for 3days & then was subsequently disintegrated into other simpler compounds. In the case of 50mg/l chlorpyrifos amended soil, the study shows that TCP was persisting in the soil for a period of 5 days and very low concentration of benzo pyridine was found remaining in the soil till the 7th day. In the case of 100 mg/l chlorpyrifos amended soil, the data indicates that both TCP and benzo pyridine were

persistent in the soil till the 8th day of the experimental study. It is evident from the study that primary metabolite TCP persist for longer duration in soil (Baskaran *et al*., 2003). Studies done by Racke *et al*. (1997) states that, formation and accumulation of TCP has antimicrobial properties and retards the proliferation of chlopyrifos degrading microorganisms. The surface soil treatment unit containing 100mg/l chlorpyrifos amended soil shows (Fig. 3) accumulation of TCP. Thus, variation in percentage increase and decrease of COD in the three different chlorpyrifos amended soil (25mg/l, 50mg/l and 100mg/l) as compared to the control is attributed to accumulation of TCP.



(i)



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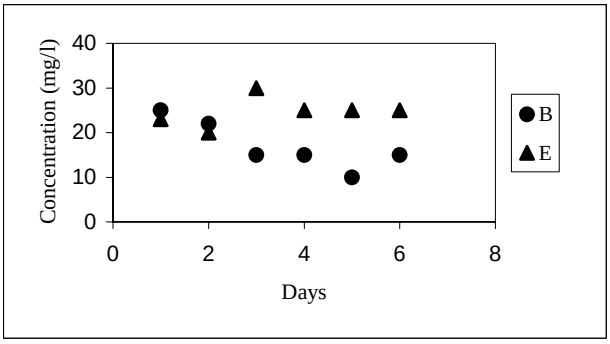


(iii)

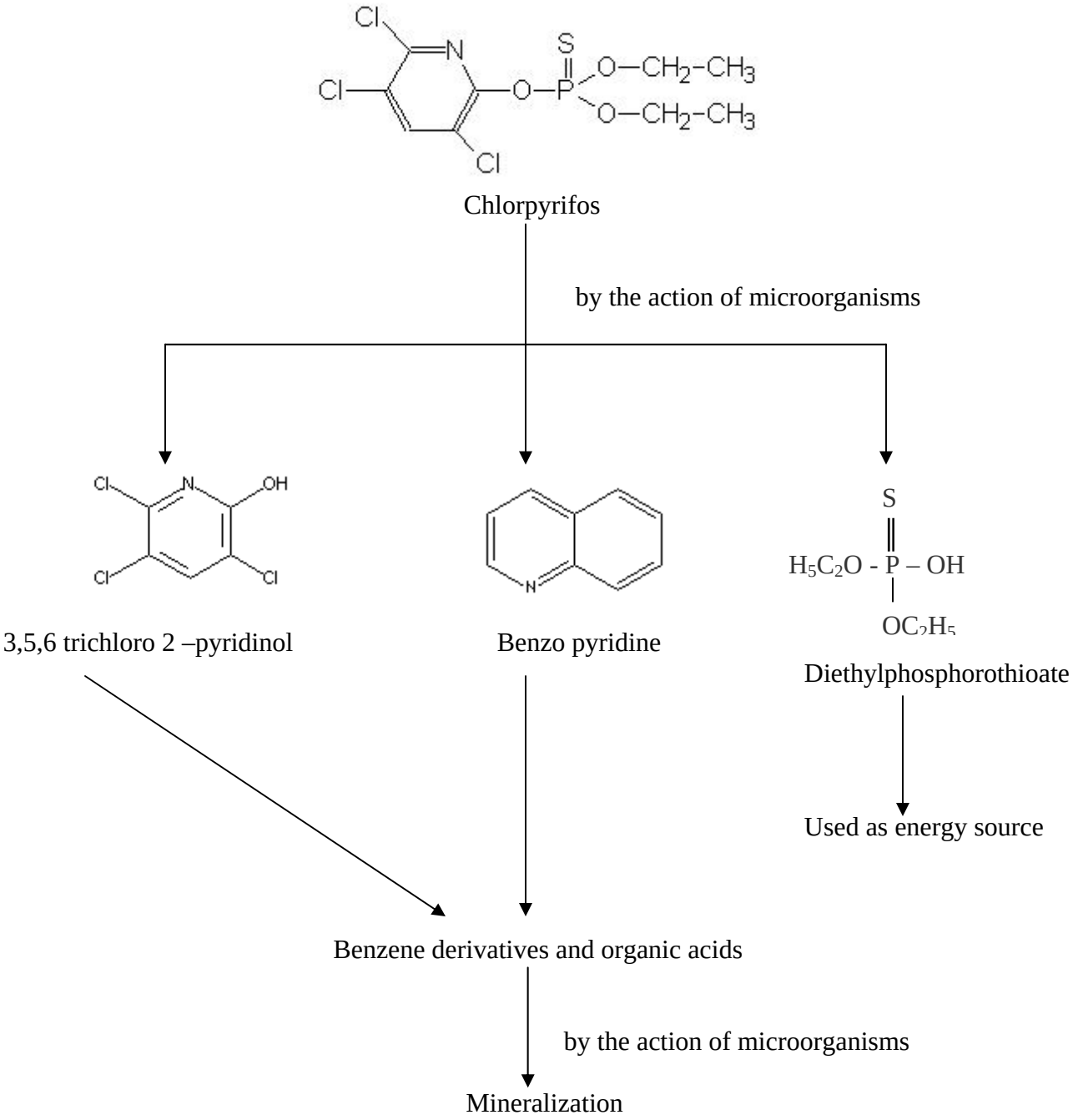
Fig. 3. Concentration of intermediates found every day during the bioremediation of chlorpyrifos amended soil (1) 25mg/l chlorpyrifos amended soil (2) 50mg/l chlorpyrifos amended soil (3) 100 mg/l chlorpyrifos amended soil.

In the surface soil treatment unit containing 100mg/l chlorpyrifos amended soil, TCP and benzo pyridine was partially degraded and found accumulated and persistent till the 8th day of the experiment, whereas in 50 mg/l and 25mg/l chlorpyrifos amended soil TCP and benzo pyridine were completely disintegrated into simpler benzene derivatives like 3-methyl phenol, chloromethylethyl benzene, benzene acetic acid which would be mineralized further into nutrient, biomass and inorganic on sufficient acclimatization. Thus, activated cow dung consortium has different impact on different concentrations of chlorpyrifos under the same environmental conditions. Therefore, the variation in the bioremediation of intermediates and the persistence of some compounds during the bioremediation. The bioremediation of Chlorpyrifos in soil was found proportional to decrease in COD concentration.

The study shows that due to the cumulative effect of microbial consortium present in soil and cow–dung slurry, the persistent intermediates like TCP and benzo pyridine were disintegrated into simpler organic compounds and other benzene derivatives. Thus, the results are explained considering the higher nutrient availability and the larger microbial population of the cow- dung slurry and soil. This is in agreement with the finding that animal-derived lagoon effluents are a good source of inorganic nutrients & 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 anthropogenic chemicals (Fulekar, 2005).

A Chlorpyrifos
B Benzo pyridine
C Phenol 4-(2- amino ethyl)
D 2-Butenedioic acid
E TCP
F Diethylphosphorothioate
G Benzothiazole, 2-(methylthio)
H 2-Pyridinamine, 5-methyl
I Acetic acid, 4-phenylmethyl ester
J Phenol, 4,4’ methylenebis
K Phenol, 3-methyl
L 2 Isoamyl- 6- methylpyrazine
M 2,4,6 Trichloro Benzeneamine
N Chloromethyl, ethyl benzene
O 2-3 Dimethoxy-6-methyl Flurobenzene
P 2-(4-cyanophenyl)-5-Hydroxypyrimidine
Q 1,1-Dimethylethyl, benzene
R Alpha hydroxy benzeneacetic acid
S 4-methyl-2-benzyl phenol
T 4 methyl, benzene

Fig. 4. Bioremediation pathway of Chlorpyrifos in Surface Soil treatment Unit.



CONCLUSION

The surface soil treatment unit designed for bioremediation of Chlorpyrifos at varying concentration using cow–dung slurry consortium under controlled environmental conditions has been found effective for biodegradation of Chlorpyrifos into less toxic or harmless compounds. The persistence of intermediates like 3 – methyl phenol, chloromethylethyl benzene, alpha hydroxy benzene acetic acid, TCP and benzo pyridine in longer duration will ultimately get converted into biomass, inorganic and nutrients. The present research has proved that the nutrient availability and larger microbial population in the activated cow dung slurry as a source of biomass and its application to pesticide-contaminated soil is an effective treatment technology for pesticide industry and other pesticide contaminated lands.

The present surface soil treatment technique used for bioremediation of chlorpyrifos using activated cow dung and soil microflora would be an effective technology. Further, investigating the pathway for degradation, enzymes and identifying the genes involved in this technique opens scope for research.

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REFERENCES

Baskaran, S., Kookana, RS. and Naidu. R. 2003. Contrasting behavior of chlorpyrifos and its primary metabolite, TCP (3,5,6-trichloro-2-pyridinol) with depth in soil profiles. Australian Journal of Soil Research. 41(4):749 -760.

Brinch, UC., Ekelund, F. and Jacobsen. CS. 2002. Method for spiking soil samples with organic compounds. Applied and Environmental Microbiology. 68 (4):1808-1816.

Environmental Protection Agency. 1997. Review of chlorpyrifos poisoning data. Washington, DC, USA.

Environmental Protection Agency. 2003. Soxhlet Extraction Method 3540, Test Methods, Washington, DC, USA.

Extoxnet. 1996. Extensive Toxicology Network, Pesticide Information Profiles, www.extoxnet.orst.edu

Fulekar, MH. 2005. Bioremediation Technologies for Environment. Indian Journal of Environmental Protection. 25(4):358-364.

Fulekar, MH., 2005. Environmental Biotechnology. Oxford and IBH Publishing House, New Delhi, India.

Getzin, LW. 1981. Degradation of chlorpyrifos in soil: influence of autoclaving, soil moisture, and temperature, J.Econ. Entomology. 74:158-162.

Horne, I., Sutherland, TD., Harcourt, RL, Russell, RJ. and Oakshott, JG. 2002. Identification of an opd gene in an agrobacterium isolate. Applied and Environmental Microbiology. 68 (7):3371-3376.

Huang, Xinjiang, Lee, Linda S. and Nakatsu, C. 2000. Impact of Animal Waste Lagoon Effluents On Chlorpyrifos Degradation In Soils**.** Journal of Environmental Toxicology and Chemistry. 19 (12):2864-2870.

Jackson, ML. 1973. Soil Chemical Analysis. Prentice-Hall of India, New Delhi, India.

Yang, Li., Yu-hua Zhao, Bing-xin Zhang, Ching-Hong Yang. and Xin Z. 2005. Isolation and characterization of a chlorpyrifos and 3,5,6-trichloro-2-pyridinol degrading bacterium. FEMS Microbiology Letters. 251 (1):67-72.

McTernon, WF. and Pereira, JA. 1991. Biotrasformation of lindane and 2,4 – D in Batch Environment Cultures. Water Research. 25(11):1417-1423.

Racke, KD., Skidmore, MW., Hamilton, DJ., Unsworth, JB., Miyamoto, J. and Cohen, SZ. 1997. IUPAC report on Fate of Pesticides in tropical soil. Pure and Applied Chemistry. 69 (6):1349-1371.

Report of analysis of pesticide residues in soft drink samples. 2003. Central Food Laboratory, Central Food technological Research Institute (C.F.T.R.I), Mysore, India.

Singh, BK., Walker, A., Morgan, AW. and Wright, DJ. 2003. Effects of soil pon the biodegradation of chlorpyrifos and isolation of a chlorpyrifos degrading bacterium. Applied and Environmental Microbiology. 69 (9):5198-5206.

Singh, BK., Walker, A., Morgan, AW. and Wright, DJ. 2004. Biodegradation of chlorpyrifos by Enterobacter strain B-14 and its use in Bioremediation of contaminated soils. Applied and Environmental Microbiology. 70(8):4855-4863.

Singh, BA., Kuhad, RC., Singh, A., Lal, R. and Tripathi, KK. 1999. Biochemical and molecular basis for pesticide degradation by microorganisms. Critical Review Biotechnology.19 (3):197-225.

Standard Methods for the examination of water and wastewater. 1975. APHA – AWWA – WPCF.

The Energy and Resource Institute (TERI). 2003. Report on Hazardous Waste Management in India.