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Microbial degradation of pesticides in surface soil using native strain in Iran

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Abstract. The manufacturing and use of pesticides has been rising tremendously in Iran. The available data indicates that pesticide residues remain in surface soil, leading to toxicity in the soil water environment.Bioremediation has widely focused on insitu bacterial degradation of organophosphorus residues in the world. In the present study, a Surface Soil Treatment Unit has been designed wherein bioremediation of commonly used pesticides namely chlorpyrifos, cypermethrin, fenvalerate, and trichlopyr butoxyethyl ester at varying concentration viz. 25, 50 and 100 mg/kg have been carried out using soil microbial strain under simulated environmental conditions. The results presented here highlight the potential of sparking of soil using microbial consortia for bioremediation of soil contaminated with pesticides in surface soil treatment unit. The present surface soil treatment technique used for bioremediation of pesticides using soil microflora would be an effective treatment technology for other group of pesticides and its effluents.

Keywords: Pesticide, Biodegradation, surface soil, Native strain

1. Introduction

Since the middle of last century, the use of organic synthetic pesticides became a widespread practice, in order to better prevent, control and destroy pests. Despite their usefulness in the increment of food production, the extensive use of pesticides during production, processing, storage, transport or marketing of agricultural commodities can led to environmental contamination and to the presence of residues in food. Real and perceived concerns about pesticide toxicity have promoted their strict regulation in order to protect consumers, environment and also the users of pesticides. Thus, reliable and accurate analytical methods are essential to protect human health and to support the compliance and enforcement of laws and regulations pertaining to food safety. The pesticides dichlorodiphenyltrichloroethane (DDT), 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), plasticizers, pentachlorophenol, and polychlorinated biphenyls, among others are examples of halogenated aromatic compounds. Their stability and toxicity are cause for concern for the environment and public health. The halogenated aliphatic compound, position, and number of halogens are important in determining both rate and mechanism of biodegradation (Mulligan 2005). Among biological approaches, the use of microbes with degradative ability is considered the most efficient and cost-effective option to clean pesticide-contaminated sites. 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. 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). A vast

number of pollutants and waste materials including heavy metals are disposed into the environment per annum. Approximately 6 x 106 chemical compounds have been synthesized, with 1,000 new chemicals being synthesized annually. Almost 60,000 to 95,000 chemicals are in commercial use. According to Third World Network reports, more than one billion pounds (450 million kilograms) of toxins are released globally in air and water. The contaminants causing ecological problems leading to imbalance in nature is of global concern.

2. MATERIALS AND METHODS

Chemical

Technical grade chlorpyrifos, cypermethrin, fenvalerate and trichlopyr butoxyethyl ester (TBEE) were purchased from Sigma-Aldrich, USA.

Soil

Samples were collected with completely sterile containers during three season of summer, autumn and winter. Then, 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. Soil microbial status was also analyzed. For 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 (350 g); the flasks were closed for 10 min to let the solvent disperse. Thereafter the solvent is evaporated for 18 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.

Experimental set up

A surface soil treatment unit was designed and fabricated (42 x 20 x 8 cm).The soil (1 kg) spiked with 25, 50 and 100 mg/kg pesticide respectively, was taken in the treatment unit. 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, and P) were monitored and maintained in the surface soil treatment unit. 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 (Table4, 5). Microbial growth was checked and monitored by streaking the serial dilution of soil sample on a nutrient agar plate.

Extraction

Soil samples drawn every day (20 g) were dried for pesticide extraction using 400ml 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, cypermethirn, fenvalerate and TBEE) from soil was found to be around 75%.

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–100oC, Injection temperature– 250oC, detector temperature –280oC.

3. RESULTS

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) has been designed wherein; technical grade pesticide cypermethrin, fenvalerate, chlorpyrifos and TBEE wereamended separately in alluvial soil at three different concentrations viz. 25, 50 and 100 mg/kg.The physico chemical characteristics of soil were carried out. The data indicates presence of organic carbon, nitrogen, phosphorus, sulphate, calcium, chloride, sodium, potassium and magnesium in soil (Table1). The data indicates the presence of bacteria, fungi and actinomycetes in soil (Table2). The presence of nutrients as well as microorganisms in soil has been found to have great influence on the bioremediation of pesticides. The concentration of chlorpyrifos and its intermediates during the bioremediation of 25, 50 and 100 mg/kg chlorpyrifos amended soil is estimated. The analyses carried out on GC-MS showed that chlorpyrifos was rapidly hydrolyzed to 3, 5,6 trichloro- 2-pyridinol (TCP) in 25 and 50 mg/kg chlorpyrifos amended soil while in 100 mg/kg chlorpyrifos amended soil it was present till the 5rd 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 45 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 10days 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. The concentration of cypermethrin and its intermediates during the bioremediation experiment carried out and analyzed quantitatively and qualitatively on GC-MS. The data showed that cypermethrin was hydrolyzed to 3-phenoxy benzaldehyde and 3-phenoxy-benzyl alcohol. The degradation of fenvalerate and detection of intermediate metabolites are presented in Figure 4. 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. After duration of one week, at 100 mg/kg concentration, fenvalerate was still detected in the soil. However, at 50 mg/kg, fenvalerate was found completely metabolized into its intermediates by the action of microorganisms. The concentrations of TBEE and its intermediates during the course of bioremediation of TBEE contaminated surface soil at 25, 50 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. 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 mg/kg TBEE contaminated soil respectively; results suggest that; TBEE has been converted into trichlopyr acid within 12 h. In 200 mg/kg TBEE contaminated soil trichlopyr acid and 3, 5, 6 trichloropyridinol (TCP) were found throughout the ten days of the experiment.

4. 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 soil contains robust mixed community of microorganisms like bacteria, fungi and actinomycetes, which was found effective in biodegradation of pesticide amended soil. The presence of high concentration of nutrients in soil further enhanced microbial activities in surface soil treatment unit (SSTU).The bioremediation conditions pH (6.5 – 7.5), C: N: P ratio (100: 10: 1), DO (18 – 22 mg/l), moisture (70 – 80%) and temperature (22 – 26oC) have been monitored and maintained during the bioremediation of each pesticide at varying concentrations (Table3). During bioremediation, it was found that chlorpyrifos was rapidly hydrolyzed to 3, 5, 6 trichloro-2-pyridinol (TCP) at all concentrations studied. Report on *Enterococcus* 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; 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) that degradation of chlorpyrifos was rapid in alkaline soils with pH 7.3 and 8.1. 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 unitcontaining 200 mg/kg chlorpyrifos amended soil, TCP and benzyl pyridine was partially degraded andfound accumulated and persistent till the 10th day of theexperiment, 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. 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. 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 ester linkage to yield 3- phenoxybenzoate.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. 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 Achromobacter 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-MSquantitative analysis showed that TBEE was rapidlybroken down into trichlopyr acid via hydrolysis of theester functional moiety. It was observed that hydrolysisand reduction reactions were the principal mechanismsoccurring during the course of bioremediation of TBEE insurface soil treatment unit. The compounds trichlopyracid and 3, 5, 6-trichloro-pyridinol were found to be theprincipal metabolites of TBEE biodegradation. In the treatment unit containing 25, 50 and 100 mg/kgTBEE contaminated soil respectively; TBEE has beenbiotransformed into trichlopyr acid within 24 h. Studiesdone by Bidlack (1978) also state that TBEE disintegratesrapidly into trichlopyr acid by virtue of hydrolysiswith a half-life of three hours. Aerobic bioremediation was carried out in SSTU usingcontinuous symmetric aeration with the help of electric airpump. The BOD measured during the bioremediation ofeach pesticide showed some variation in concentration due to the growth and proliferation of prominent microorganisms in the presence of high nutrient availability of soil under simulated conditions (Table 4).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 periodof time (Table 5). Previous research studies also reported that COD is a direct indicator of bioremediation (Singh and Fulekar, 2007).The physico-chemical parameter were also monitored and maintained for the bioremediation of chlorpyrifos, cypermethrin, fenvalerateand 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 transportof 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 surface soil treatment technique used for bioremediation of pesticides using soil microflora would be an effective treatment technology for other group of pesticides and its effluents.

Table 1. Physico-Chemical characteristics of soil

Parameters	soil
PH	7.4±0.3
Moisture	65%±0.4
Alkalinity/100gms	0.9meq
Dissolved Oxygen	11mg/kg
BOD	3±0.5mg/kg
COD	245±0.2mg/kg
Temperature	26^{0C}±3
Cation Exchange Capacity/100gms	100meq
Magnesium	11440mg/kg
Potassium	244mg/kg
Chloride	2130mg/kg
Calcium	6727mg/kg
Sulphate	3.5mg/kg
Kjeldahl Nitrogen	1100mg/kg
Phosphorus	0.45mg/kg
%Organic Carbon	3.18

Table 2.Microbial characteristics of soil

Parameters	Soil
Total viable count/g	1176
Total coliform count/g	490
Total Yeast and Mould count/g	113
Actinomycetes count/g	46
Pseudomonas count/g	<87
E.coli count/g	Nill
Anaerobic bacterial count	Nill
Thermophilic bacterial count	Nill
Anaerobic spore count	<10
Thermophilic spore count	<70
Anaerobic thermophilic spore count	Absent
Salmonella	<50
Enterobacter spp.	<200
Pseudomonas putida	Absent
Corynebacterium	463
Flavobacterium	Present
Alcaligenes	Present
Nocardia	Present
Serratialiquefaciens	Present
Bacillus pumilis	Present
Bacillus	Present
Alcaligenes	Present
Providencia	Present
Agrobacterium	Present
Mucor spp.	Present
Aspergillus	present
Penicillium	

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 – 7.5
Temperature	22 – 26^{0C}
Moisture	70 – 80%
Dissolved Oxygen	18 – 22 mg/kg
Microbial Growth	Present

Table 4.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
TBEE	29%	22%	14%	47%
Chlorpyrifos	36%	30%	24%	43%
Fenvalerate	23%	20%	16%	38.33
Cypermethrin	28%	22%	19%	31.2%

Table 5.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	73%	54%	42%	83%
Fenvalerate	59%	43%	40.5%	64%
TBEE	60%	57%	52%	67%
Cypermethrin	71.5%	62%	51.4%	75%

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