Appl Biochem Biotechnol (2011) 164:1257–1277
DOI 10.1007/s12010-011-9211-5

# Bioremediation of Single and Mixture of Pesticide-Contaminated Soils by Mixed Pesticide-Enriched Cultures

**K. Rama Krishna · Ligy Philip**

Received: 25 January 2011 / Accepted: 1 March 2011 /
Published online: 19 March 2011


**Abstract** In the present study, degradation efficiencies for individual as well as mixed pesticide in different Indian soils, by mixed pesticide-enriched cultures, were evaluated under submerged and unsaturated conditions, Lindane (L), methyl parathion (MP), carbofuran (C), and a mixture of L, MP, and C were used in the study. For all the various conditions considered, methyl parathion degradation was the maximum and lindane degradation was the minimum. The degradation kinetics of the pesticides in sandy, clayey, compost, and red soils by various microbial isolates were studied. It was observed that adsorption was maximum and degradation of pesticides was minimum in compost soil. The degradation efficiencies of pesticides in liquid phase associated with soil sediment were less than those under the normal liquid phase conditions as leaching of pesticides from soil phase was continuous. Pesticide degradation was more in submerged soils compared to that in unsaturated soils. The degradation by-products of individual and mixed pesticides in liquid, unsaturated, and submerged soils were identified. Different metabolites were produced under submerged and unsaturated conditions.

**Keywords** Lindane · Methyl parathion · Carbofuran · Mixed pesticides · Soil and liquid phase

## Introduction

Many pesticides are being used in India for controlling insects and increasing agricultural production. The total pesticide consumption in India has increased from a low value of 154 MT in 1954 to an alarming value of 41,250 MT in 2005. Though several insecticides like lindane, parathion, etc. have been banned in many other developing countries, they are still being used in India. Many studies have reported the increased pesticide residues in all spheres of environment because of excessive application of these xenobiotics in soils. The

K. Rama Krishna · L. Philip (✉)
Department of Civil Engineering, Environmental and Water Resources Engineering Division,
IIT Madras, Chennai 600 036, India
e-mail: ligy@iitm.ac.in

indiscriminate and unplanned use of agricultural chemicals over the years has caused serious environmental problems [1]. Residues of these agrochemicals can damage the ecosystem [2], gain entry into the food chain either directly or indirectly, and cause health hazard to animals and plant life. The residual effects include carcinogenicity, mutagenesis, and reproductivity problems [3].

The movement and fate of these hydrophobic organic chemicals in the soil involves complex mechanisms including volatilization, adsorption, and chemical as well as biological degradations. Of all these processes, adsorption, desorption, and degradation processes are the key processes regulating the concentrations of contaminants in soils [4]. Many agricultural pesticides, which are applied to the soil, will interact with non-target soil organisms, including those microorganisms which are capable of degrading pesticide compounds. Microbial degradation is perhaps the single most important factor in preventing the buildup of pesticides in soils leading to potential environmental problems. It has been reported that microorganisms could degrade both soil-bound and soluble pesticides [5]. The ability of microorganisms to degrade pesticides is controlled by the bioavailability of chemicals as well as by the capacity of microorganisms to develop the ability to utilize available chemicals.

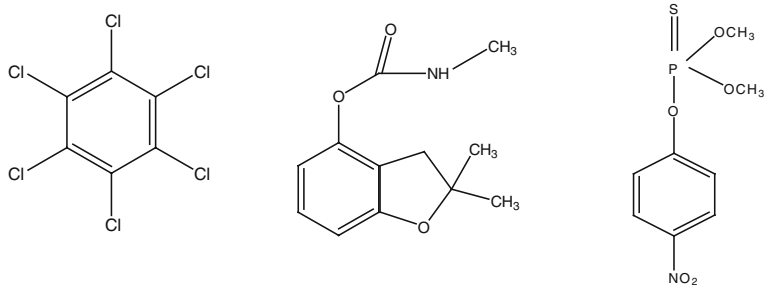
Microorganisms including bacteria, fungi, and algae are the major agents involved in biological degradation of pesticides. There have been many reports regarding the degradation of different insecticides by soil bacteria [6–9] and fungi [10–12]. Druzina and Stegu [13] studied the degradation of 15 organophosphorus insecticides in drinking, ground, and surface waters under different laboratory-controlled and environmental conditions. Fenlon et al. [14] reported the development of catabolism in indigenous soil microflora from four organically and one conventionally managed soils which were exposed to cypermethrin and diazinon pesticides. Rose et al. [15] investigated the dissipation of five cotton pesticides—endosulfan, chlorpyrifos, aldicarb, prometryn, and diuron—in cotton field runoff water contained in glasshouse columns under light or dark conditions.

Different types of pesticides are normally used to control specific pests of various crops. As a result, agricultural soils are contaminated with a mixture of pesticides; therefore, it is essential to develop a biosystem which can effectively degrade various groups of pesticides. However, research on biodegradation of mixed pesticides of different groups is very limited and most of the earlier research focused on the degradation of either an individual pesticide or a series of pesticides belonging to the same group. The main objective of this paper was to investigate the degradation potential of single as well as mixture of pesticides in different Indian soils by various enriched microbial cultures. An attempt has been made to postulate the degradation pathway of various pesticides under different operating conditions.

## Materials and Methods

### Pesticides and Chemicals

Technical grades of lindane and methyl parathion were supplied by Tamilnadu Pollution Control Board, Chennai, and commercial grade carbofuran (carbofuran 3G) was purchased from the local agricultural market. Stock solutions (1 g/L) of methyl parathion in acetone, lindane (1 g/L) in ethanol, and carbofuran (300 mg/L) in distilled water were prepared. High-purity (99.7%) lindane, methyl parathion, and carbofuran were purchased from Occua Standards (USA). Chemical structure of lindane, methyl parathion, and carbofuran are shown in Fig. 1. Chlorobenzene (CB), dichlorobenzene isomers (1,2-DCB, 1,3-DCB, 1,4-DCB), and trichlorobenzene isomers (1,2,3-TCB, 1,2,4-TCB, 1,3,5-TCB), carbofuran-3-hydroxyl, 3-keto




Lindane Carbofuran Methyl parathion

**Fig. 1** Structure of lindane, methyl parathion, and carbofuran pesticides

carbofuran, *para*-nitrophenol, amino phenol, and dimethyl thiophosphate (99–99.9% purity) were procured from Sigma Aldrich, USA. Organic solvents of high-pressure liquid chromatography (HPLC) grade were procured from Ranbaxy Chemicals, India.

## Soils

Most common Indian soils were selected for the biodegradation experiments. These soils were classified as clayey soil (CL, lean clay with sand); red soil (GM, silty gravel with sand); composted soil (PT, peat); and sandy soil (SM, silty sand with gravel). The soils were sieved through IS sieve no. 10 (2-mm aperture as per IS 2720 (Part 4), 1987). The fraction passing through the sieve was collected and preserved in air tight plastic containers for biodegradation studies. Similar soils were used in our earlier studies also [16, 17].

## Bacteria

Bacterial strains enriched and isolated from pesticide-contaminated sites were used for the present study. *Pseudomonas aeruginosa* (MTCC 9236), a methyl parathion degrader, was employed for the degradation of methyl parathion, and *Bacillus* sp. (MTCC 9235) was used for lindane degradation. Carbofuran degradation studies were carried out using *Chrysseobacterium joostei* (MTCC 9237). Mixed pesticide degradation studies were conducted with an enriched bacterial consortium consisting of *P. aeruginosa*, *Bacillus* sp., *C. joostei*, and *Klebsiella pneumonia*, along with two other unidentified microbial strains.

## Extraction of Pesticides from Soil and Liquid Phases

One gram of moist soil sample was added to 5 mL of distilled water and the mixture was sonicated for 15 min. The soil sample was vigorously agitated with 5 mL of acetone for 15 min followed by centrifugation at 5000×*g* for 10 min. The supernatant was decanted and diluted to 50 mL using distilled water. The extraction procedure was repeated for two more cycles using 5 mL of hexane. Finally, the extracted mixture was concentrated by heating the mixture at 45 °C for 1 min. The extraction efficiency of pesticide varied with the soil type. Extraction efficiency was maximum (92%) for sandy soil and was minimum (81%) for compost soil.

Liquid samples were analyzed by solvent extraction method. The procedure for the extraction of lindane, methyl parathion, and carbofuran residues in liquid samples is as follows: Aliquots of 5 mL of homogenized liquid samples were collected at different time

intervals and centrifuged at 6000×*g* for 6 min, and supernatant sample of 4 mL was transferred to 15-mL volumetric tubes and mixed with 4 mL of 1:1 hexane/acetone solution. The tubes were sealed and shaken for 10 min in a vortex mixture to facilitate transfer of pesticides and their metabolites, and waited for 5 min until the clear organic phase was separated from the liquid phase. The average extraction efficiencies were 89.4±3.8%, 91.8±1.8%, and 84.7±2% for lindane, methyl parathion, and carbofuran, respectively. Finally, extracted samples were filtered through anhydrous sodium sulfate to remove the moisture content.

## Analytical Techniques

Samples of lindane and methyl parathion were analyzed by Perkin Elmer Clarus 500 gas chromatograph with electron capture detector. Carbofuran samples were analyzed by reverse phase HPLC (Jasco) with UV detector (MD 2010 PDA). Gas chromatography mass spectrum (Agilent GC-MS) was used for separation and identification of intermediate products [18].

## Biodegradation of Single and Mixture of Pesticides in Submerged Soils

To find out the distribution and degradation of pesticides in soil as well as in liquid phases, miniature reactors were employed. Soils used for the biodegradation studies were sterilized in a hot air oven at 150 °C for 2 h. Each miniature soil reactor was a 50-mL capacity conical flask in which a total of 25 g of soil (dry weight) was mixed with technical grade pesticide (e.g., lindane) to give a target concentration of 2 mg active ingredient per gram of soil. Lindane-amended soil samples were inoculated with 75 mg/g of *Bacillus* sp. cells mixed with 25 mL of nutrient broth (NB). The composition of NB was $KH_2PO_4$ 1.0 g/L; $K_2HPO_4$ 1.0 g/L; $NH_4NO_3$ 1.0 g/L; NaCl 1.0 g/L; $MgSO_4 \cdot 7H_2O$ 0.2 g/L; $CaCl_2$ 0.02 g/L; Fe $(SO_4)_3$ 0.02 g/L; and 1 mL of trace metal solution added to 1 L of NB [19]. The reactors were operated in submerged condition. Simultaneously, blank rectors (with lindane and without bacterial cells) were also operated. All the experiments were conducted in duplicate. From the homogenized contents of the reactor, liquid as well as soil samples were collected at intervals of 0, 2, 4, 7, 14, 21, 28, 35, 42, 56, 70, and 84 days, extracted, and analyzed for residual lindane concentration and bacterial growth, respectively. Similar studies were carried out for the degradation of methyl parathion-contaminated soils by *P. aeruginosa* and carbofuran-contaminated soils by *C. joostei*. The experiments were conducted in aerobic and facultative anaerobic conditions at room temperature (28 °C).

Mixed pesticide (lindane, methyl parathion, carbofuran) degradation studies were carried out with mixed pesticide-enriched cultures in different environmental conditions. Degradation studies were conducted at 28±2 °C with a total pesticide concentration of 2 mg (0.4 mg of lindane, 0.8 mg of methyl parathion, and 0.8 mg of carbofuran) per gram of soil. Each miniature reactor was filled with 25 g of soil (dry weight) containing 50 mg technical grade of mixed pesticides to give a target concentration of 2 mg active ingredient per gram of soil.

Mixed pesticide-amended soil samples were inoculated with 75 mg/g of mixed pesticide-enriched cultures in 25 mL of NB. Identical reactors without microbes in both aerobic and facultative anaerobic conditions were also employed to check the stability of pesticides in the liquid and soil phases. One milliliter of supernatant liquid and 1 g of soil samples were collected at 0, 2, 4, 7, 14, 21, 28, 35, 42, 56, 70, and 84 days from each reactor and were analyzed for residual concentrations of lindane, methyl parathion, carbofuran, and their metabolites.

### Biodegradation of Mixture of Pesticides in Unsaturated Soils

Bench-scale reactor studies were conducted in the laboratory to simulate the pesticide-contaminated soil in an agricultural field, which usually exists in unsaturated condition [20]. The reactors were fabricated using 3-mm-thick acrylic transparent sheets. The top compartment was 10 cm in diameter and 25 cm in height, with three sample collection ports located at 10, 15, and 20 cm from the top. The bottom compartment was 12 cm in diameter and 10 cm in height, and was used as a leachate collector. Two red soil reactors were used for the degradation of mixed pesticides in unsaturated conditions.

Soils employed for all biotransformation studies were sterilized by keeping them in a hot air oven at 150 °C for 2 h. Soil was spiked with mixed pesticides in two steps: 1 g of pesticide (mixed) was dissolved in 100 mL of hexane/acetone (9:1) solvent and added to 1,000 g of soil and mixed well. After evaporation of the solvent, an additional 1,000 g of soil was mixed with it so that finally the total pesticide concentration in the soil was 2 mg/g. The final microbial concentration was estimated as $2\times10^6$ cells/mL (75 mg/g of soil), and the final moisture content was adjusted to 38% with NB. Glass beads were used as a supporting media, placed in the bottom of the reactor for a depth of 5 mm. The performance of the reactors was monitored for 70 days. At the end of 0, 2, 7, 14, 21, 28, 42, 56, and 70 days, samples were collected from each port (top, middle, and bottom) of the reactors. The collected soil samples were homogenized in a shaker. The homogenized soil samples were extracted with acetone/*n*-hexane and analyzed for lindane, methyl parathion, and carbofuran concentrations. Similar methodology was adopted for leachate collection. Five milliliters of leachate was collected from the bottom of the reactor extracted with *n*-hexane/acetone (1:1) and was analyzed for residual mixed pesticide concentration. The moisture content in the reactor was monitored regularly and the decrease in moisture content due to evaporation was compensated by supplying the required amount of NB. The top of the reactor was covered with wet cotton, and thereby, the system was maintained at uniform moisture content.

## Results and Discussion

### Biodegradation Potential of Individual Pesticide in Submerged Soils

#### *Lindane*

Lindane degradation studies were conducted using *Bacillus* sp., with an initial lindane concentration of 2 mg/g of soil in aerobic condition, for a period of 70 days. These studies have been carried out in four different soils—namely, red, sandy, clayey, and compost soils—in submerged condition. The results are presented in Fig. 2a, b. Immediately after application, 98% (1.96 mg/g) of applied lindane was retained by soil in the case of red and compost soils and 95% of applied lindane was attached in the case of clayey soils. The remaining pesticide was present in the liquid phase. However, sandy soil was able to hold only 92% (1.84 mg/g) of applied lindane. The observed concentrations of lindane in soil reactors matched with the adsorption study results of these pesticides in the respective soils [17]. The overall degradation efficiency of lindane in red, clayey, and sandy soils were 21.25±1.3%, 25.39±1.6%, and 29.09±2.2%, respectively, after 70 days of incubation. As discussed earlier, the initial concentrations of pesticides present in various soils were different. Minimum (16.49±1.1%) lindane

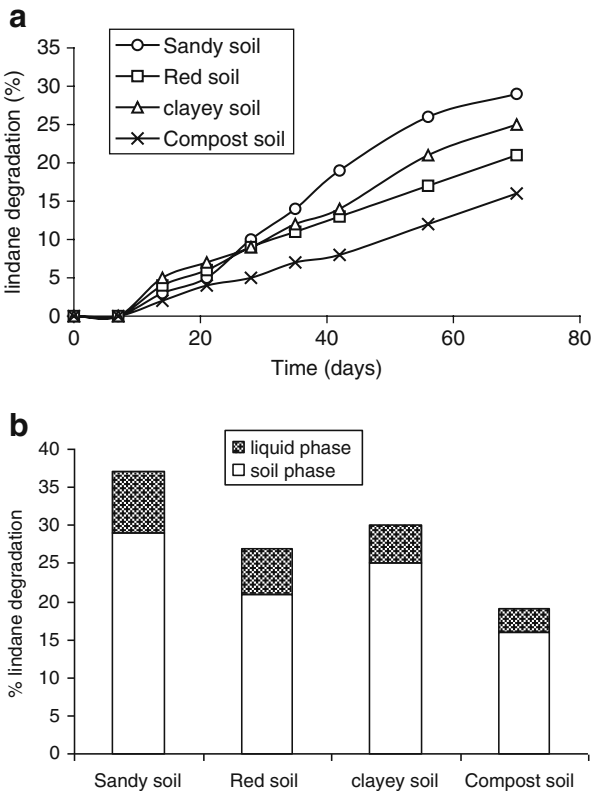



**Fig. 2** **a** Degradation kinetics of lindane in different Indian soils under aerobic condition. **b** Degradation of lindane in miniature soil reactors at the end of the 70th day in aerobic condition

degradation was observed in compost soil, whereas maximum (29.09±2.2%) degradation was achieved in sandy soil. The variation in lindane degradation in different soils was due to the difference in the availability of pesticides to microbes. Desorption of lindane by different eluents from various soils is discussed elsewhere [18]. Low degradation rates of lindane in red and compost soils might be due to the presence of organic matter in these soils. Organic matter can form complexes with pesticides and can compete with pesticides during adsorption and degradation.

Lindane degradation studies were also conducted in facultative anaerobic condition under similar experimental conditions. The distribution and degradation pattern of lindane was similar to that in aerobic condition. Removal efficiencies of lindane in facultative anaerobic condition were much higher than those in aerobic conditions. The results are shown in Fig. 3a, b. The degradation efficiency of lindane in compost, clayey, red, and sandy soils were 23.07%, 29.88%, 26.63%, and 36.05%, respectively. The redox potential of facultative anaerobic system was measured at different time intervals, and the maximum value observed was −100 mv. At low redox potential (−100 mv), the halogen atom separation from aromatic ring is very effective. As a result, the toxicity of the compounds will be reduced drastically, which in turn enhances lindane biodegradation. A control reactor for each soil sample was operated, without any microorganisms, to quantify the abiotic loss of lindane. The abiotic loss was only 8%. This clearly shows that majority of the degradation occurred through microbial activity.

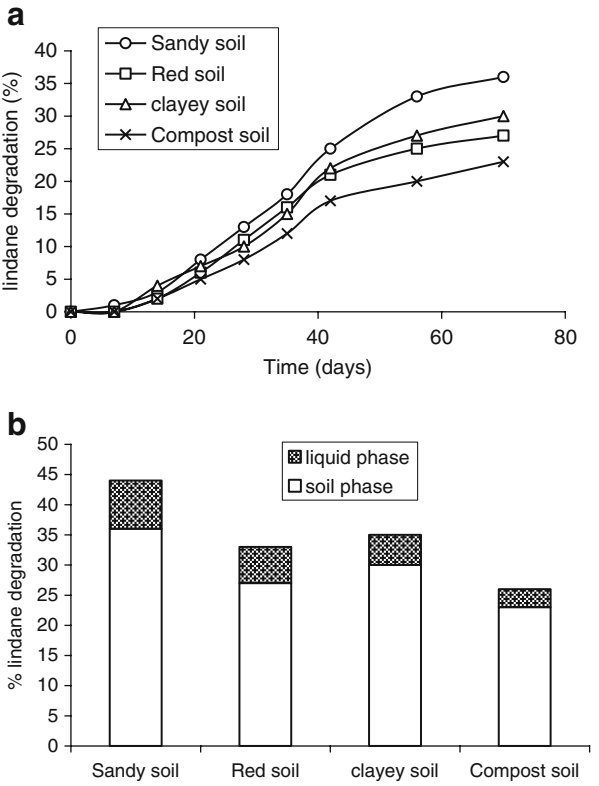



**Fig. 3** **a** Degradation kinetics of lindane in different Indian soils under facultative anaerobic condition. **b** Degradation of lindane in miniature soil reactors at the end of the 70th day in facultative anaerobic condition

The miniature soil reactors were operated in submerged condition. Hence, the concentration of lindane in the liquid phase was also monitored to quantify the lindane degradation by microbes in liquid phase. One milliliter of liquid (leachate) sample was collected from each reactor and was analyzed for lindane concentration in liquid phase. The initial concentrations (immediately after addition) of lindane in the leachate of compost, clayey, red, and sandy soil reactors were 0.8, 1.2, 4.6, and 8.3 mg/L, respectively. The degradation rates of lindane in liquid phase were high in the case of compost soil reactor followed by red and clayey soil reactors. Low concentration of lindane and availability of dissolved organic matter must have increased the biodegradation of lindane in red and compost soils. However, the overall degradation of lindane was more in soil than in the liquid in submerged miniature soil reactors. Similar results were reported by Kumar and Philip [20] during endosulfan degradation.

The degradation efficiency of pesticides in soil sediment liquid phase was less than the normal liquid phase conditions. It took 10 weeks to achieve 90% of 10 mg/L of lindane degradation in the liquid phase of miniature soil reactor. On the other hand, the same percentage of degradation was achieved in 7 weeks with the same bacterial concentration in normal liquid phase conditions. This may be due to the continuous desorption of lindane from solid phase to the liquid phase during the course of study, which could not be quantified. In this study, the loss of lindane concentration either from the liquid or soil phase is considered as degradation.

*Methyl Parathion*

Methyl parathion (MP) degradation studies were performed with an initial MP concentration of 2 mg/g in aerobic condition using *P. aeruginosa*, and the results are presented in Fig. 4a, b. About 95% (1.9 mg/g) of applied methyl parathion was attached in the case of the red and compost soils and 87% (1.74 mg/g) of applied MP was attached in the case of clayey soils. However, sandy soil was able to hold up to 82% (1.64 mg/g) of applied methyl parathion. Methyl parathion degradation in sandy soil was very high. More than 61±1.3% degradation was achieved in 28 days. Degradation efficiencies in red, clayey, and compost soils were 48±1.9%, 52±1.28%, and 40±1.57%, respectively. Methyl parathion degradation studies were also carried out in facultative anaerobic condition. In facultative anaerobic condition, degradation rates of methyl parathion were much higher than those in aerobic condition. A similar trend was observed in the case of lindane degradation also. In facultative anaerobic condition, 61±1.1% degradation of methyl parathion occurred in 28 days in sandy soils. Initial concentrations of methyl parathion in the leachates from compost, clayey, red, and sandy soil reactors were 1.7, 3.9, 9.4, and 18.5 mg/L, respectively. More than 96% of applied methyl parathion was adsorbed on red and compost soils, which resulted in low concentration of MP in the leachate. In sandy soil, only 85% of applied methyl parathion was adsorbed. As a result, high concentration (18.5 mg/L) of methyl parathion was found in the liquid phase. One milliliter of the supernatant was collected from each reactor and was analyzed for methyl parathion concentration. *P. aeruginosa* showed different degradation rates of methyl parathion in

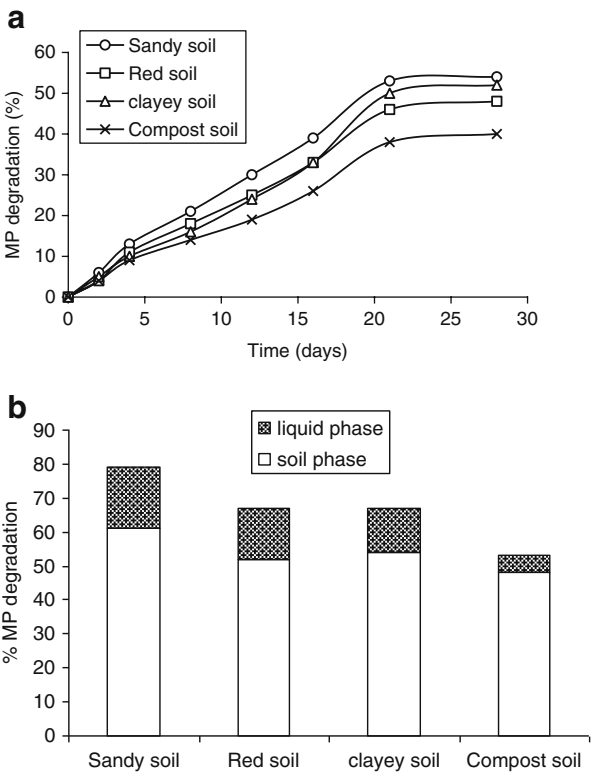



**Fig. 4** **a** Degradation kinetics of methyl parathion in different Indian soils under aerobic condition. **b** Degradation of methyl parathion in miniature soil reactors at the end of the 28th day in aerobic condition

liquid and soil phases. The percentage degradation of methyl parathion in sandy soil, red soil, clayey soil, and compost soil reactors were 15±1:18±1.3%, 12±1.2:15±1.1%, 10±1.1:13%±0.9%, and 5±0.5: 7±0.6% in aerobic and facultative anaerobic conditions, respectively. Lower degradation rates of methyl parathion were observed in soil phase compared to that in liquid phase. However, the overall degradation of methyl parathion was higher in soils than the liquid. This may be due to the leaching of MP from the soil phase to the liquid phase in all the reactors. The overall degradation efficiencies of different soil reactors in soil and liquid phases are presented in Fig. 5a, b. Maximum degradation of methyl parathion was observed in sandy soil reactor.

*Carbofuran*

Biodegradation of carbofuran in contaminated soil by carbofuran-enriched culture, *C. "joostei*, in aerobic and facultative anaerobic conditions was studied. Immediately after the addition of carbofuran in soil reactors, concentrations of carbofuran in soil phase in compost, clayey, red, and sandy soil reactors were 1.83, 1.72, 1.68, and 1.38 mg/g, respectively. Concentrations of carbofuran in the liquid phase of compost, clayey, red, and sandy soil reactors during this period were 6.1, 8.9, 17.9, and 32.8 mg/L, respectively. The distribution of carbofuran

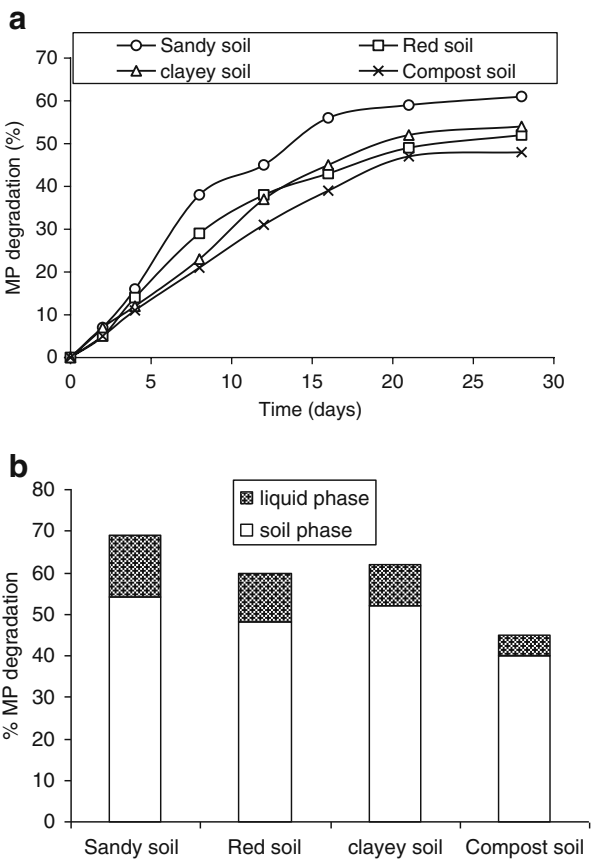



**Fig. 5** **a** Degradation kinetics of methyl parathion in different Indian soils under facultative anaerobic condition. **b** Degradation of methyl parathion in miniature soil reactors at the end of the 28th day in facultative anaerobic condition

between soil and liquid phases was different from that for lindane and methyl parathion. The liquid phase concentrations of carbofuran were four times higher than lindane concentrations, and a high concentration of carbofuran was observed in sandy soil. The degradation kinetics of carbofuran in different soils is shown in Fig. 6a, b. These results clearly demonstrate that among all the soil reactors, maximum degradation of carbofuran occurred (facultative anaerobic condition) in sandy soils, whereas minimum degradation occurred in compost soils under aerobic condition. In liquid phase, the degradation efficiencies of carbofuran in sandy soil, red soil, clayey soil, and compost soil reactors were 28±1.1:31±1.5%, 14±0.8:16±1.4%, 11±1.1:15±1.6%, and 8±0.9:10±1.5% in aerobic and facultative anaerobic conditions, respectively, in 42 days. Abiotic loss of carbofuran was 8%. The overall percentage degradation of carbofuran in sandy soil, red soil, clayey soil, and compost soil reactors were 86±2.5:92±1.8, 59±1.4:75±2, 61±1.7:77±1.1, and 47±2.3: 63±2.8 in aerobic and facultative anaerobic conditions, respectively (Fig. 7a, b).

## Biodegradation Kinetics of Mixed Pesticides in Submerged Soils

Mixed pesticide degradation studies were conducted in aerobic and facultative anaerobic conditions by mixed pesticide-enriched culture (MEC) with a total pesticide concentration of 2 mg/g of soil. After the addition of pesticide, compost soil retained 1.96 mg/g, clayey soil retained 1.85 mg/g, and red soil retained 1.90 mg/g of applied pesticides, whereas

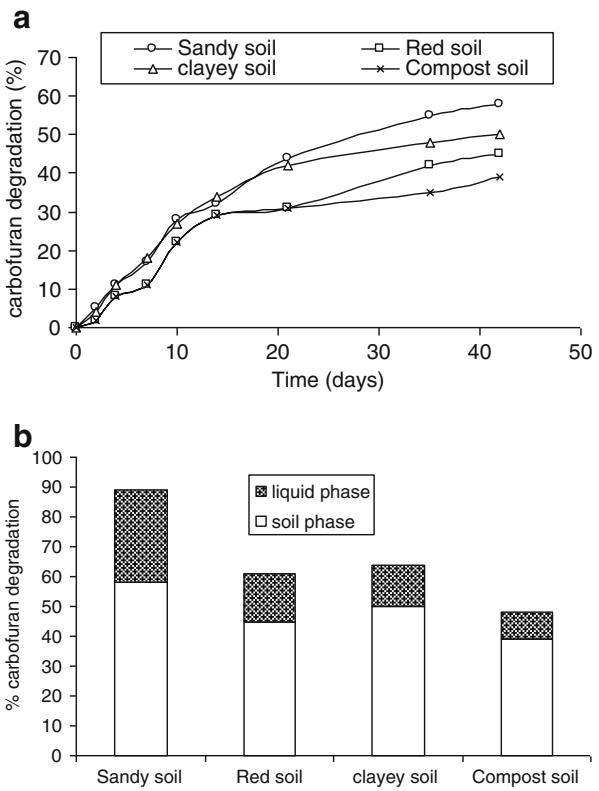



**Fig. 6** **a** Degradation kinetics of carbofuran in different Indian soils under aerobic condition. **b** Degradation of methyl parathion in miniature soil reactors at the end of the 42nd day in anaerobic condition

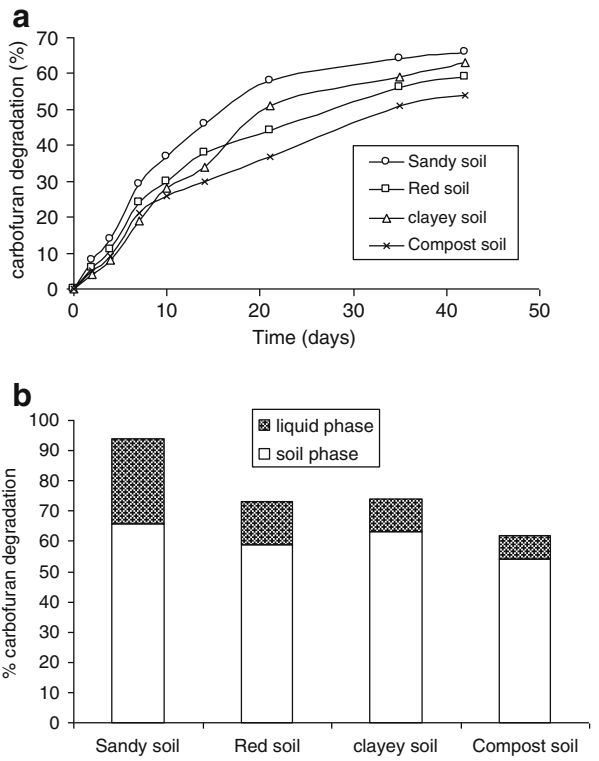



**Fig. 7** **a** Degradation kinetics of carbofuran in different Indian soils under facultative anaerobic condition. **b** Degradation of methyl parathion in miniature soil reactors at the end of the 42nd day in facultative anaerobic condition

sandy soil could retain only 1.72 mg/g of pesticide. The remaining pesticides were found in the liquid phases. The observed total mixed pesticide concentration in liquid phase in compost, clayey, red, and sandy soil reactors were 1.0, 5.2, 8.2, and 21.2 mg/L, respectively. Mixed pesticide degradation was studied in aerobic and facultative anaerobic conditions in soil and liquid phases in all the reactors. Degradation efficiencies of mixed pesticides in both soil and liquid phases were given in Fig. 8.

In facultative anaerobic condition, MEC had shown better degradation efficiency of mixed pesticides. Mixed pesticide degradation efficiency was very high in sandy soil

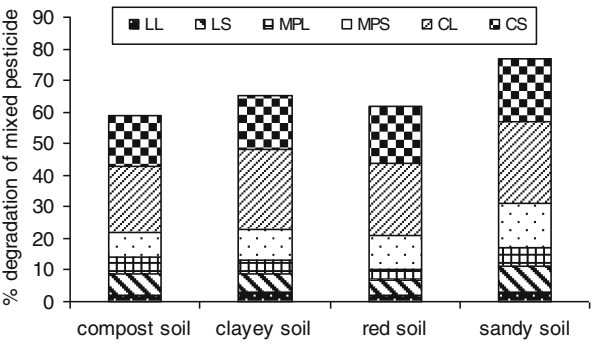



**Fig. 8** Biodegradation of mixed pesticides in facultative anaerobic condition in liquid and soil phases in different soils on the 70th day. LL- Lindane in liquid phase, MPL- Methyl parathion in liquid phase, CL- Carbofuran in liquid phase, LS- Lindane in soil phase, MPS- Methyl parathion in soil phase, CS- Carbofuran in soil phase

compared to the other three soils. The overall mixed pesticide degradation in sandy soil, red soil, clayey soil, and compost soil were 77±3.1%, 62±2.4%, 66±1.8%, and 59±1.1%, respectively. In the mixed pesticide system, among the three pesticides, carbofuran degradation was maximum both in the soil phase (20%) and the liquid phase (26%). Minimum degradation was observed for lindane both in the soil phase (7%) and the liquid phase (2%). In this system, the order of pesticide degradation was carbofuran > methyl parathion > lindane. Low degradation efficiencies of methyl parathion, lindane, and carbofuran were observed in the mixed pesticide system compared to individual pesticide degradation system. Degradation of lindane in liquid and soil phases in the first 3 weeks was insignificant. But methyl parathion and carbofuran degradations were 32% and 28% (including soil and water phases), respectively. The low degradation efficiency in mixed pesticide system may be due to the less number of specific microorganisms for the degradation of individual pesticides. Though the overall microbial concentration of mixed pesticide system was equivalent to individual pesticide degradation system, the individual microbial concentration specific to each pesticide was only one third. Degradation behavior of pesticides by MEC varied from the liquid to the soil phase. In the liquid phase, removal efficiencies of mixed pesticides were significantly high, especially in the case of methyl parathion and carbofuran. In this study, loss due to dissipation was monitored with the help of control reactors (without bacterial cultures). It was found that the abiotic loss of total pesticides in the system during a 2-month period was 12±1%.

## Biodegradation Kinetics of Mixed Pesticides in Unsaturated Soils

Though pesticide degradation was promising for individual pesticides in miniature soil reactors, the actual field condition would be different from these laboratory conditions. In miniature soil reactor studies, the reactors were operated separately in aerobic and anaerobic condition, which gave an idea about the efficiency of pesticide degradation in the above conditions. However, in prevailing environmental conditions in real life, field situations could be a combination of both. This often leads to the failure of a laboratory-designed system. Hence, it is always advisable to simulate a reactor which can represent actual field condition. To investigate this aspect, the degradation studies were carried out in bench-scale reactors. The results of these studies are summarized in Tables 1 and 2. During the operation, the simulated bioreactor has shown two distinct zones, i.e., top of the reactor was in aerobic condition whereas the bottom layers were in anaerobic condition. The entry of air to the bottom layers was restricted due to the compaction of soil, and no attempt was made to supply air to the bottom zone of the reactors. The loss in moisture content in the reactor was compensated by the supply of nutrients from the top layer of the reactor. Due to this, an increase in pesticide concentration was observed in the middle and bottom layers of the reactors during the study period (3 months).

The bottom layers of the soil containing mixed pesticides showed higher degradation rates than the two top layers. In the actual field condition, maximum degradation of pesticides will occur in the top layers due to the availability of high organic matter and microbial population. However, in the bench-scale reactor, microbial population and mixed pesticides were uniformly distributed throughout the reactor. Due to anaerobic conditions prevailing in the bottom layer, mixed pesticides were getting degraded effectively by the microbes. From the top to bottom of the soil reactor, the redox potential of the soil increased.

**Table 1** Percentage degradation of mixed pesticides in (unsaturated) bench-scale reactor at different ports

| Pesticide | Time (days) | % Degradation of mixed pesticide | | | |
|---|---|---|---|---|---|
| | | Top layer (4 cm from top) | Middle layer (8 cm from top) | Bottom layer (12 cm from top) | Leachate |
| Lindane | 0 | 0 | 0 | 0 | 0 |
| | 7 | 9±1.0 | 14±2.0 | 10±1.2 | 12±0.8 |
| | 21 | 21±2.8 | 26±1.5 | 34±2.0 | 38±3.0 |
| | 35 | 29±1.5 | 32±3.3 | 48±2.9 | 53±1.5 |
| | 49 | 32±3.0 | 41±2.1 | 59±3.0 | 62±2.6 |
| | 63 | 42±4.0 | 58±3.0 | 72±3.6 | 78±2.1 |
| Methyl parathion | 0 | 0 | 0 | 0 | 0 |
| | 4 | 12±1.3 | 17±0.6 | 11±2.6 | 16±2.2 |
| | 8 | 23±3.0 | 31±2.0 | 31±1.7 | 36±1.1 |
| | 14 | 34±2.9 | 45 ±1.8 | 54±3.2 | 68±1.9 |
| | 21 | 46±1.8 | 61±1.1 | 68±2.8 | 81±3.4 |
| | 28 | 59±2.6 | 72±3.5 | 81±4.2 | 98±1.8 |
| Carbofuran | 0 | 0 | 0 | 0 | 0 |
| | 4 | 6±1.2 | 8±2.0 | 5±1.5 | 11±0.6 |
| | 7 | 12±1.0 | 16±2.2 | 15±0.9 | 27±1.8 |
| | 14 | 21±2.8 | 28±2.0 | 29±1.8 | 41±2.6 |
| | 21 | 29±3.5 | 34±3.2 | 42±2.1 | 60±1.4 |
| | 28 | 38±4.2 | 46±1.6 | 54±1.4 | 72±3.6 |
| | 35 | 52±3.4 | 61±2.6 | 76±2.0 | 89±2.8 |

During the first week, 7% of lindane, 23±1.8% of methyl parathion, and 12±1.1% of carbofuran degradations were observed in the top layer of the red soil reactor. By the end of the fourth week, 62±3% of methyl parathion, 24±2% of lindane, and 38±1% of carbofuran were degraded. At end of the sixth week, more than 90±2% methyl parathion, 68±3% of carbofuran, and 28±3% of lindane were degraded in the top layer of the mixed pesticide system. By the end of the tenth week, 100% degradation of methyl parathion, 92±3% degradation of carbofuran, and 71±2.4% degradation of lindane were observed in the top layer.

In the middle layers, 81±3% of methyl parathion, 38±2% of lindane, and 54±1% of carbofuran were degraded by the end of the fourth week. At end of the sixth week, almost all methyl parathion was degraded in the mixed pesticide system, whereas 91±3% carbofuran and 54±3% of lindane degradation were observed during this period. As anaerobic conditions prevailed in the bottom layers of the reactor, the rate of degradation increased. The rate of degradation of these pesticides in the top layers (considered as aerobic condition) in unsaturated condition were less than those in submerged conditions. Degradation of the mixed pesticides by enriched cultures varied with the initial concentration of individual pesticides and the environmental conditions. Maximum degradation of mixed pesticide was observed in liquid phase followed by submerged soil sediment under anaerobic condition. The least degradation was observed in contaminated soil in unsaturated condition. Similar results were observed by Belden and Lydy [21] while studying the degradation pattern of atrazine and parathion. However, Manish et al. [9] have reported a higher HCH degradation at lower

**Table 2** Percentage degradation of mixed pesticides in the leachate from bench-scale reactor (unsaturated)

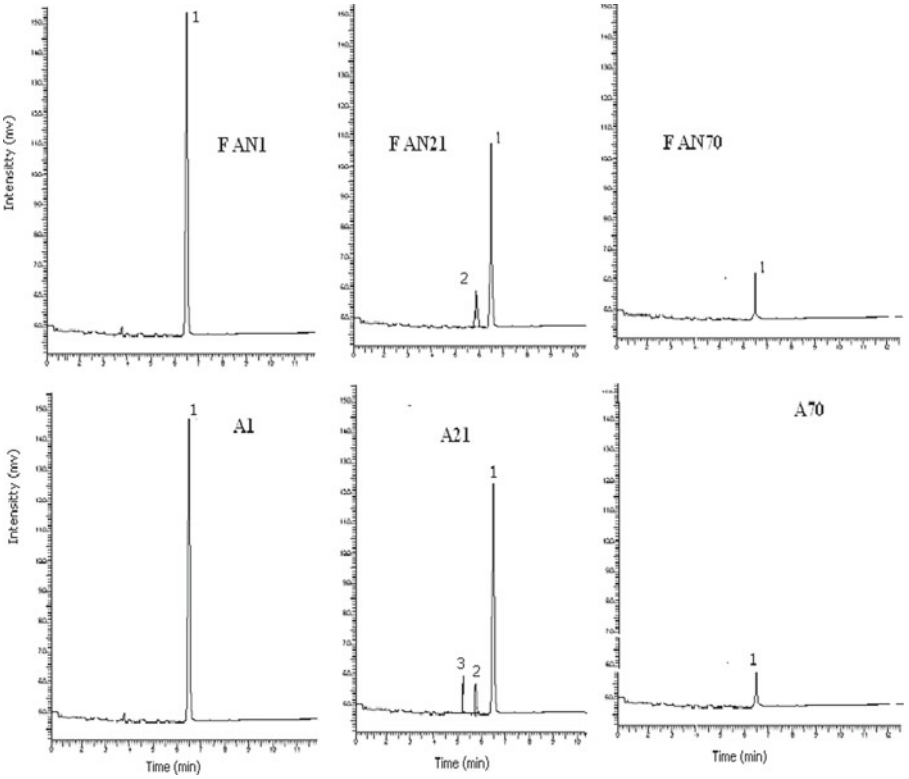
| Pesticide | Time (days) | Leachate | | |
|---|---|---|---|---|
| | | Volume (mL) | Concentration (mg/L) | Degradation (%) |
| Lindane | 0 | 480 | 36±1.1 | 0 |
| | 7 | 463 | 29±2.2 | 12±0.8 |
| | 21 | 495 | 23±1.8 | 38±3.0 |
| | 35 | 506 | 20±1.2 | 53±1.5 |
| | 49 | 489 | 16±0.8 | 62±2.6 |
| | 63 | 519 | 10±1.2 | 78±2.1 |
| Methyl parathion | 0 | 480 | 78±2.6 | 0 |
| | 4 | 471 | 66±1.7 | 16±2.2 |
| | 7 | 463 | 50±1.5 | 36±1.1 |
| | 14 | 454 | 31±1.0 | 68±1.9 |
| | 21 | 495 | 8±1.4 | 81±3.4 |
| | 28 | 487 | 3±0.9 | 98±1.8 |
| Carbofuran | 0 | 480 | 194±2.6 | 0 |
| | 4 | 471 | 170±2.5 | 11±0.6 |
| | 7 | 463 | 142±1.8 | 27±1.8 |
| | 14 | 454 | 118±1.6 | 41±2.6 |
| | 21 | 495 | 81±1.2 | 60±1.4 |
| | 28 | 487 | 56±0.8 | 72±3.6 |
| | 35 | 506 | 20±1.8 | 89±2.8 |

moisture contents. As the anaerobic condition prevailed in the bottom layers of the reactor, lindane degradation efficiency has been enhanced drastically in this zone compared to methyl parathion and carbofuran.

## Metabolic Pathway of Biodegradation of Pesticides

### *Individual Pesticides in Submerged Soils*

*Lindane* During degradation of lindane in the soil phase under facultative anaerobic condition, a single metabolite of lindane, γ-PCCH, was identified by gas chromatography mass spectrum, whereas in the liquid phase, two metabolites of lindane—namely, dichlorobenzene (DCB) and monochlorobenzene (MCB)—were observed. During the liquid phase biodegradation, dechlorination products of lindane were found, whereas in the soil phase, dehydrochlonation product (γ-PCCH) was observed. These results show that either the degradation metabolism of lindane in soil differs from that in the liquid phase or that metabolites of lindane (DCB and MCB) were trace amounts and disappeared from the soil system. In the aerobic condition, three metabolites (PCCH, TCCH, TCB) of lindane were observed in the liquid phase and two metabolites (PCCH, TCCH) were observed in the soil phase. The low peak intensity of pentachlorocyclohexane (PCCH, <1%; Fig. 9) compared to lindane indicates the possibility of multiple steps in the formation of PCCH. It has also been reported that PCCH is 1,000




F AN1 – Facultative anaerobic, day 1 A1- Aerobic, day 1

F AN21 – Facultative anaerobic, day 21 FA6 - Aerobic, day 21

F AN70 – Facultative anaerobic, day 70 FA12 - Aerobic, day 70

1- Lindane 2 - PCCH 3 – TCCH

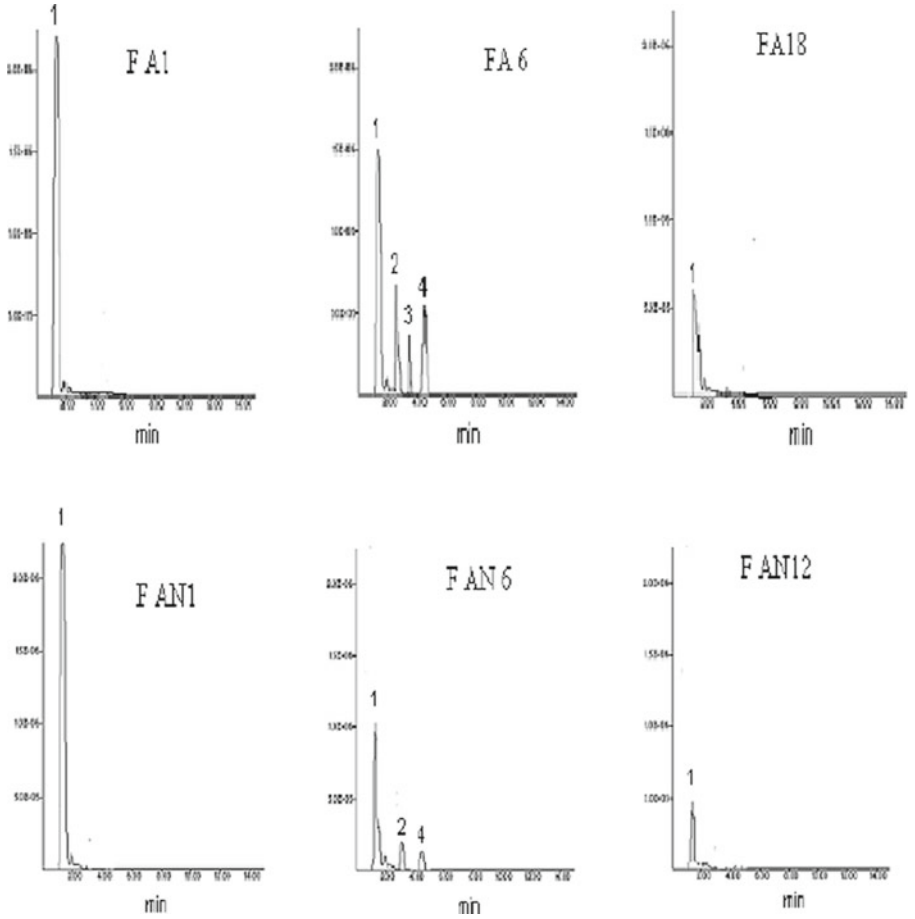
**Fig. 9** Gas chromatograph of lindane and its metabolites in facultative anaerobic and aerobic conditions in soils

times less toxic than lindane [22]. Two unknown compounds were observed in blank as well as in the bioreactors. However, none of these compounds contained chlorine. These compounds may be the organic matter present in the soil. Though many metabolites were formed during the degradation of lindane in both the soil and liquid phases, none of the metabolites were accumulated in the system. No metabolite of lindane was observed in blank reactor.

*Methyl Parathion* In facultative anaerobic condition, three metabolites of methyl parathion, i.e., *para*-nitrophenol, methyl amino phenol, and dimethyl thiophosphate (DMTP) were observed during soil extraction. Hydrolysis and nitro group reduction products of methyl parathion were observed in the facultative anaerobic condition. Ramanathan and Lalithakumari [23] reported the formation of methyl amino phenol in the flooded soils. At high redox potential (−150 mv), formation of amino phenol was found by many other researchers also [24, 25]. Both oxic and anoxic products of methyl parathion were observed in the facultative anaerobic condition. In the liquid phase, only two metabolites—namely,

*para*-nitrophenol and DMTP—of methyl parathion were observed in both the aerobic and facultative anaerobic conditions (Fig. 10).

*Carbofuran* Two metabolites of carbofurn, namely, 3-hydroxy carbofuran and 3-ketocarbofuran, were observed during the aerobic degradation of carbofuran in the soil phase. One metabolite, 3-ketocarbofuran, was observed in the soil phase extractions of reactor contents, which were maintained in the facultative anaerobic condition. Under aerobic conditions, carbofuran underwent hydrolysis and oxidation pathway in both the soil and liquid phases. However, in the facultative anaerobic condition, only hydrolysis product




F AN1 – Facultative anaerobic, day 1 FA1- Aerobic, day 1

F AN6 – Facultative anaerobic, day 6 FA6- Aerobic, day 6

F AN12 – Facultative anaerobic, day 12 FA12- Aerobic, day 12

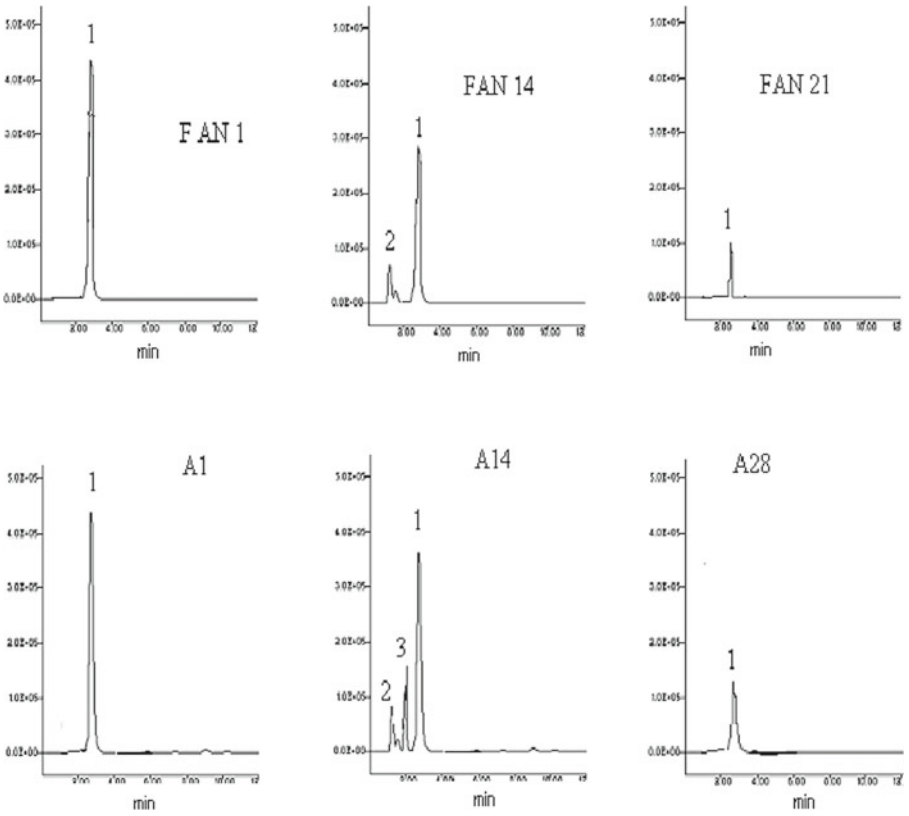
1-. Methyl parathion 2 - methyl amino phenol 3- paranitro phenol 4- DMTP

**Fig. 10** Gas chromatograph of methyl parathion and its metabolites in facultative anaerobic and aerobic conditions in soils

of carbofuran (3-hydroxy carbofuran) was observed in both the soil and liquid phases (Fig. 11). Carbofuran degradation pathway varied more with the environmental conditions than with the phases [26].

## Mixed Pesticide Degradation in Submerged Soils

Metabolites were analyzed during the mixed pesticide biodegradation in submerged soils, and the results are presented in Fig. 12. During the biodegradation of mixed pesticides in the facultative anaerobic condition, along with *para*-nitrophenol and dimethyl thiophosphate, amino phenol was also observed as methyl parathion metabolites in the soil phase. Similar results were observed in liquid phase degradation of mixed pesticides in facultative anaerobic conditions. Besides methyl parathion metabolites, tetrachloro-cyclohexane



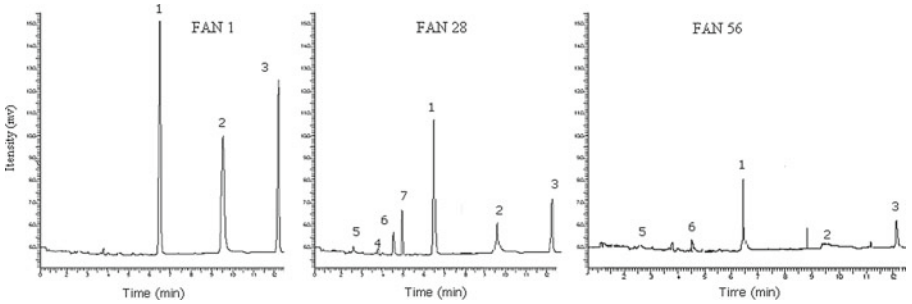

F AN1 – Facultative anaerobic, day 1 A1- Aerobic, day 1

F AN14 – Facultative anaerobic, day 14 FA6- Aerobic, day 14

F AN22 – Facultative anaerobic, day 22 FA12- Aerobic, day 22

1. Carbofuran 2- 3 hydroxy carbofuran 3- 3 keto-carbofuran

**Fig. 11** Gas chromatograph of carbofuran and its metabolites in facultative anaerobic and aerobic conditions in soils



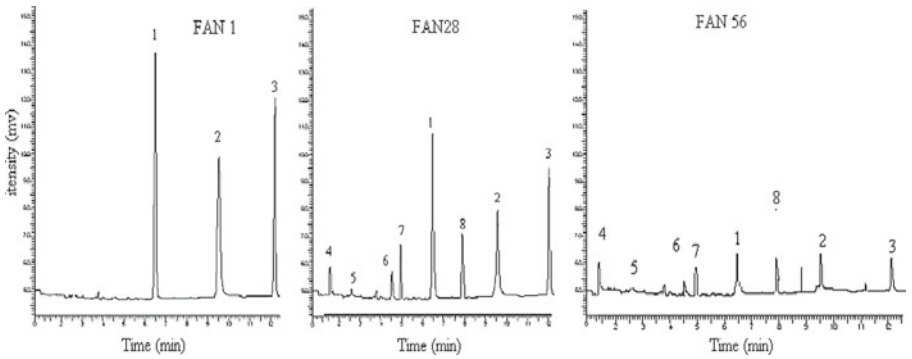

1 lindane, 2-methyl parathion, 3- carbofuran, 4-MCB, 5-TCCH, 6- Paranitro phenol, 7-DMTP

**Fig. 12** Gas chromatograph of mixed pesticides and its metabolites in facultative anaerobic conditions in saturated red soil

(TCCH) and MCB, the known metabolites of lindane, were also identified during mixed pesticide degradation under facultative anaerobic condition. Formation of PCCH during the biodegradation of lindane in anaerobic condition in soils was reported by many researchers [27, 28]. Though PCCH was observed during the degradation of lindane in the single pesticide system, it was totally absent in the mixed pesticide system. In the facultative anaerobic system, methyl amine and 3-hydroxyl carbofuran 7-phenol, the metabolites of carbofuran, were observed during the initial stages of degradation. As the time progressed, methyl amine completely disappeared, and by the end of the fourth week, almost all the metabolites were degraded.

## Mixed Pesticides in Unsaturated Soils

Metabolites were analyzed during the mixed pesticide biodegradation in unsaturated soils, and the results are presented in Fig. 13. Metabolites observed during the mixed pesticide degradation under submerged conditions and unsaturated conditions were different. During the biodegradation of mixture of pesticides, various metabolites were observed at different




1 lindane, 2-methyl parathion, 3- carbofuran, 4-MCB, 5-TCCH,6- Paramitro phenol, 7-DMTP,7- 3 Hydroxy carbofuran, 8- 3 Keto carbofuran

**Fig. 13** Gas chromatograph of mixed pesticides and its metabolites in facultative anaerobic conditions in unsaturated red soil

time intervals. Most of these metabolites were in trace levels and volatile in nature, and many of them were non-persistent. In non-flooded soils (bench scale reactor studies), trace concentrations of PCCH and trichlorobenzene (TCB), metabolites of lindane, were observed in GC-MS analysis during the first 2 weeks of degradation study. The other metabolites of lindane appear to be more volatile than the parent compound and might have escaped into the atmosphere immediately after formation. Metabolites like TCCH, DCB, and benzene, which were observed in liquid phase degradation of lindane by mixed pesticide-enriched cultures [18], were not found in soil phase degradation studies. Many researchers observed the formation of PCCH and TCB during the biodegradation of lindane [28, 29]. However, Gupta et al. [30] reported that no peaks were observed while studying HCH biodegradation by *Bacillus* sp. *para*-nitrophenol was the only metabolite of methyl parathion observed during the degradation of mixed pesticides in unsaturated condition. However, in our earlier studies of mixed pesticide degradation in liquid phase, two metabolites of methyl parathion, namely, *para*-nitrophenol and dimethyl thiophosphate, were found. Dimethyl thiophosphate seems to be accumulating in the soil system. *para*-Nitrophenol formation is a very common phenomenon during the degradation of methyl parathion, and this mechanism was reported by Ramanathan and Lalithakumari [23]. Three metabolites of carbofuran were found in the mixed pesticide system during the second and fourth weeks of incubation, and these metabolites were identified as 3-hydroxycarbofuran 7-phenol, carbofuran 7-phenol, and 3-keto carbofuran. In our earlier studies of carbofuran degradation, carbofuran 7-phenol and 3-keto carbofuran were not observed. Carbofuran 7-phenol and 3-keto carbofuran were formed by the oxidative pathway of carbofuran degradation [31, 32].

## Conclusion

Among the three pesticides, methyl parathion degradation was the fastest while lindane degradation was the slowest in all the reactors. In the case of soils, minimum pesticide disappearance was observed in compost soil while maximum disappearance was achieved in sandy soils. Removal efficiencies of three pesticides in facultative anaerobic condition were much higher than those in aerobic conditions. Degradation efficiency of pesticides in the liquid phase along with soil sediment was less than that in the normal liquid phase as continuous leaching of pesticide from the soil phase was occurring. In the bench-scale reactor, the bottom layers showed higher degradation rates than the two top layers due to the prevailing anaerobic conditions in the bottom layers. The redox potential in the bench-scale soil reactor increased from top to bottom. The degradation products of individual and mixed pesticides in the liquid phase, unsaturated soils phase, and submerged soil phase were identified. Different metabolites were observed under submerged and unsaturated compost and clayey soil reactors. Most of these metabolic products were present in trace levels and were non-persistent in nature. Based on the metabolites, plausible degradation pathways for various pesticides under different environmental conditions were postulated.

## References

1. Kullman, S. W., & Matsumura, F. (1996). Metabolic pathways utilized by *Phanerochaete chrysporium* for degradation of the cyclodiene pesticide endosulfan. *Applied and Environmental Microbiology, 62*, 593–600.

2. Somich, C. J., Muldoon, M. T., & Kerney, P. C. (1990). On site treatment of pesticide waste and rinsate using ozone and biological active soil. *Environmental Science & Technology, 24*, 745–749.
3. Parsek, M. R., McFall, S. M., & Chakrabarty, A. M. (1995). Microbial degradation of toxic, environmental pollutants: Ecological and evolutionary considerations. *International Biodeterioration and Biodegradation, 35*(1–3), 175–188.
4. Dragun, J. (1998). Adsorption and mobility of organic chemicals. In J. Dragun (Ed.), *The soil chemistry of hazardous materials*. Massachusetts: Amherst Scientific Publishers.
5. Gevao, B., Semple, K. T., & Jones, K. C. (2000). Bound pesticide residues in soils: A review. *Environmental Pollution, 108*, 3–14.
6. Manonmani, H. K., Chandrashekaraiah, D. H., Sreedhar, R. N., Elcey, C. D., & Kunhi, A. A. M. (2000). Isolation and acclimatization of a microbial consortium for improved aerobic degradation of γ-hexachlorocyclohexane. *Journal of Agriculture and Food Chemistry, 48*, 4341–4351.
7. Okeke, B. C., Siddique, T., Arbestain, M. C., & Frankenberger, W. T. (2002). Biodegradation of γ-hexachlorocyclohexane (lindane) and α-hexachlorocyclohexane in water and soil slurry by a *Pandoraea* species. *Journal of Agriculture and Food Chemistry, 50*, 2548–2555.
8. Boltner, D., Moreno-Morillas, S., & Ramos, J. (2005). 16S rDNA phylogeny and distribution of lin genes in novel hexachlorocyclohexane degrading *Sphingomonas* strains. *Environmental Microbiology, 7*, 1329–1338.
9. Manish, K., Chaudhary, P., Dwivedi, M., Kumar, R., Paul, D., Jain, R. K., et al. (2005). Enhanced biodegradation of $\beta$- and $\delta$-hexachlorocyclohexane in the presence of $\alpha$- and $\gamma$-isomers in contaminated soils. *Environmental Science & Technology, 39*, 4005–4011.
10. Arisoy, M., & Kolankaya, N. (1998). Biodegradation of chlorinated organic compounds by white-rot fungi. *Bulletin of Environmental Contamination and Toxicology, 60*, 872–876.
11. Mougin, C., Pericaud, C., Dubroca, J., & Asther, M. (1997). Enhanced mineralization of lindane in soils supplemented with the white rot basidiomycete *Phanerochaete chrysosporium*. *Soil Biology and Biochemistry, 29*(9–10), 1321–1324.
12. Fragoeiro, S., & Magan, N. (2002). Influence of fungal inoculant type and water potential on degradation of pesticides. 17th WCSS, Thailand.
13. Druzina, B., & Stegu, M. (2007). Degradation study of selected organophosphorus insecticides in natural waters. *International Journal of Environmental Analytical Chemistry, 87*(15), 1079–1093.
14. Fenlon, K. A., Jones, K. C., & Semple, K. T. (2007). Development of microbial degradation of cypermethrin and diazinon in organically and conventionally managed soils. *Journal of Environmental Monitoring, 9*, 510–515.
15. Rose, M. T., Crossan, A. N., & Kennedy, I. R. (2007). Dissipation of cotton pesticides from runoff water in glasshouse columns. *Water, Air, and Soil Pollution, 182*(1–4), 207–218.
16. Mathava Kumar, S., & Philip, L. (2006). Adsorption, desorption characteristics of endosulfan in various Indian soils. *Chemosphere, 62*, 1064–1077.
17. Rama Krishna, K., & Philip, L. (2008). Adsorption and desorption characteristics of lindane, carbofuran and methyl parathion on various Indian soils. *Journal of Hazardous Materials, 160*(2–3), 559–567.
18. Rama Krishna, K., & Philip, L. (2008). Biodegradation of lindane, methyl parathion and carbofuran by mixed bacterial consortium. *Journal of Environmental Science and Health, 43*(2), 157–171.
19. Awasthi, N., Manickam, N., & Kumar, A. (1997). Biodegradation of endosulfan by a bacterial co-culture. *Bulletin of Environmental Contamination and Toxicology, 59*, 928–934.
20. Mathava Kumar, S., & Philip, L. (2006). Bioremediation of endosulfan contaminated soils. Optimization of operating conditions under laboratory scale reactors. *Journal of Hazardous Materials, 136*, 354–364.
21. Belden, B., & Lydy, M. J. (2000). Impact of atrazine on organophosphate insecticide toxicity. *Environmental Toxicology and Chemistry, 19*, 2266–2274.
22. Francis, A. J., Spanggord, R. J., & Ouchi, G. I. (1975). Degradation of lindane by *Escherichia coli*. *Applied Microbiology, 29*, 567–568.
23. Ramanathan, M. P., & Lalithakumari, D. (1999). Complete mineralization of methyl parathion by *Pseudomonas* sp. A3. *Applied Biochemistry and Biotechnology, 80*, 1–12.
24. Adhya, T. K., Sudhakar, B., & Sethunathan, N. (1981). Stability of commercial formulation of fenitrothion, methyl parathion, and parathion in anaerobic soils. *Journal of Agricultural and Food Chemistry, 29*, 90–93.
25. Chaudhry, G., Ali, A., & Wheeler, W. (1988). Isolation of a methyl parathion degrading *Pseudomonas* sp. that possesses DNA homologous to the *opd* gene from a *Flavobacterium* sp. *Applied and Environmental Microbiology, 54*(2), 288–293.
26. Kale, S. P., Murthy, N. B. K., & Raghu, K. (2001). Degradation of $^{14}$C-carbofuran in soil using continuous flow system. *Chemosphere, 44*, 893–895.

27. Kuritz, T., & Wolk, C. P. (1995). Use of filamentous cyanobacteria for biodegradation of organic pollutants. *Applied and Environmental Microbiology, 61*, 234–238.
28. Nagata, Y., Ohtomo, R., Miyauchi, K., Fukuda, M., Yano, K., & Takagi, M. (1994). Cloning and sequencing of a 2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase gene involved in the degradation of γ-hexachlorocyclohexane in *Pseudomonas paucimobilis*. *Journal of Bacteriology, 176*, 3117–3125.
29. Quintero, J. C., Moreira, M. D., Feijoo, G., & Lema, J. M. (2005). Anaerobic degradation of hexachlorocyclohexane isomers in liquid and soil slurry systems. *Chemosphere, 61*, 528–536.
30. Gupta, A., Kaushik, C. P., & Kaushik, A. (2001). Degradation of hexachlorocyclohexane isomers by two strains of *Alcaligenes faecalis* isolated from a contaminated site. *Bulletin of Environmental Contamination and Toxicology, 66*, 794–800.
31. Karpouzas, D. G., Morgan, J. A. W., & Walker, A. (2005). Isolation and characterization of 23 carbofuran degrading bacteria from soils from distant geographical areas. *Letters in Applied Microbiology, 31*(5), 353–358.
32. Felsot, J., Maddox, V., & Bruce, W. (1981). Enhanced microbial degradation of RA 565-A1BB carbofuran in soils with histories of carbofuran use. *Bulletin of Environmental Contamination and Toxicology, 26*, 781–788.