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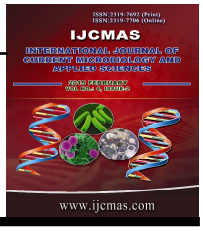

Original Research Article

Bioremediation of Persistent Pesticides in Rice field Soil Environment Using Surface Soil Treatment Reactor

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Keywords

Bioremediation, Surface soil treatment reactor, Pesticides, Microbial consortia

A B S T R A C T

The indiscriminate use of pesticides in agricultural field has resulted into contamination of soil environment leading to toxicity in the biological diversity. In the present study, the soil samples were collected from the rice field of Alampur village, Gandhinagar district of Gujarat state, wherein repeated application of pesticides such as chlorpyrifos and methyl parathion were observed. In the rice field pesticides degrading bacteria were identified as *Acenetobactor* sp., *Moraxella* sp., *Pseudomonas* sp., *Yersinia* sp., *Enterobacter*sp. and *Photobacterium*sp. A Surface Soil Treatment Reactor (SSTR) has been designed and developed for bioremediation of commonly used pesticides namely chlorpyrifos and methyl parathion using developed indigenous microbial consortium (*Acenetobactor* sp., *Pseudomonas* sp., *Enterobacter* sp. and *Photobacterium* sp.) under simulated environmental conditions. The pesticide degradation was studied by HPLC. The results of bioremediation of pesticides contaminated soil using microbial consortium in surface soil treatment reactor shows the 62.72 % degradation of chlorpyrifos and 65.99% degradation of methyl parathion within a period of 10 days. Thus bioremediation is found to be an effective technology for treatment of pesticides polluted soil using microbial consortium.

Introduction

India is primarily an agriculture based country with more than 60-70% of its population dependent on agriculture and covers maximum portion of its economy (Sachdeva, 2007). Due to fast growing population, a huge portion of agricultural land is being depleted each year by industries and urban encroachments, which creates food scarcity. After green revolution in 1960, the scenario of Indian agriculture has changed drastically. The promotion of high yielding varieties of crops that marked the green revolution has led to large scale use of chemicals as pesticides. The increased demand of agro-products in changing regional climate has resulted in an increase in consumption and application of pesticides (Shetty *et al.*, 2008). One of the main strategies to increase crop productivity is effective pest management because more

than 45% of annual food production is lost due to pest infestation (Abhilash and Singh, 2009).

Undoubtly, agriculture sector has been successful in keeping pace with rising demand for food, but the indiscriminate use, high biological activity and in some cases persistence in the environment, the pesticides has inflicted serious harm and problems to humans as well as to the biodiversity (Hussain *et al.*, 2009). The extensive use of pesticides leads to an accumulation of a huge amount of pesticide residues in the environment, therefore causing a substantial environmental health hazard due to uptake and accumulation of these toxic compounds in the food chain and drinking water (Mohammed, 2009).

According to the World Health Organization data, only 2–3% of these pesticides applied for mitigation of pests are utilized at target point whereas rest remains in the environment causing surface runoff, leaching and percolation into soil water environment leading toxicity to biota and human being through food chain (EPA, 2005). Improper handling and unsafe spraying of the agrochemicals cause high risk of health hazards (Bag, 2000; Gupta, 2004). Increased use of pesticides can result in various health and environmental problems like pesticides poisoning in farmers and farm workers, neurological and skin disorders, cardiopulmonary, miscarriages, foetal deformities and lowering the sperm counts in applicators (Bag, 2000).

The above hazardous effects of pesticides draw attention towards its removal from the environment. Although, various conventional methods such as chemical treatment, recycling, pyrolysis, incineration are able to degrade persistent pollutants hazardous to human health as well as the environment, but they are less efficient. The microbial action in the environment causes the natural degradation of the pesticides which might convert parent compounds to intermediates or comparatively less toxic compounds. However, the process of natural bioremediation is slow and needs to enhance the biodegradation of contaminants in the environment by the action of the potential microorganisms (Fulekar, 2005a). The adaptability of microorganisms during bioremediation releases certain enzymes, which metabolizes wide spectrum of anthropogenic chemicals (Fulekar, 2005b). Many bacteria that are able to degrade organophosphate pesticides have been isolated from soil around the world (Zhongli*et al.*, 2001; Horne *et al.*, 2002; Chang *et al.*,2005).*Pseudomonas aeruginosa*is known to be the most common Gram negative soil bacterium having potential to degrade chlorpyrifos (Geetha and Fulekar, 2008).

In the present study the widely used pesticides have been taken for bioremediation under controlled environmental conditions. The surface soil treatment reactor has been designed to develop the techniques for bioremediation of surface soil containing pesticides by monitoring and maintaining environmental parameters under simulated conditions. This technique has been found effective for bioremediation of pesticides persistent in agricultural soil environment.

Materials and Methods

Soil collection

The soil samples were collected from rice field up to a depth of 15 cm from Alampur village, Gandhinagar district of Gujarat. The collected samples were air dried, ground, passed through 2 mm sieve and stored in the sealed plastic bags at room temperature.

These stored samples were used for further experimentation. The important physico-chemical properties of the soil, viz. temperature, pH, electrical conductivity, moisture contents, water holding capacity, bulk density, hardness, chloride, alkalinity, total organic carbon, total organic matter, sulphate, nitrate, nitrite, ammonium, available phosphorus and total phosphorus were analysed using standard methods (APHA,1995).

Isolation and identification of bacterial isolates

Pour plate technique was used for the isolation of pesticide degrading bacteria in nutrient agar. Well grown bacterial colonies were picked and further purified by streaking. Identification of these seven different bacterial isolates were carried out by the routine bacteriological methods i.e., by the colony morphology, preliminary tests like Gram staining and biochemical analysis (Bergrey's Manual of Determinative Bacteriology, 1994).

Development of microbial consortium for bioremediation of pesticides

The microbial consortium was prepared from bacterial cultures, which were compatible with each other in order to concomitantly produce all those enzymes required for the degradation of pesticides from agricultural field. For this, all isolated bacteria were grown on nutrient broth and incubated at 37°C at 120 rpm. The combination of bacterial isolates was based on permutation combination. The compatibility of the bacterial strains within the consortium was checked by increasing optical density at 600 nm by spectrophotometer (Dynamica CE, model no. DB 20).

Bioremediation of pesticides in surface soil treatment reactor

A surface soil treatment reactor (SSTR) was designed and fabricated with the dimension of 26 x 16 x 8 cm. The reactor was developed in such a way that continuous aeration was provided with the help of aerator (Fig. 1). The soil sample collected from rice field was taken in the reactor. Pre developed microbial consortium was added to the soil and mixed properly. Bioremediation conditions like moisture content, temperature were monitored and maintained in the surface soil treatment reactor. During the period of experiment of 10 days soil sampling was done every day for analysis of physico-chemical parameters.

Extraction of pesticides from soil samples

Soil samples (10g) drawn at the interval of two days were dried for pesticide extraction using 200 ml dichloromethane and acetonitrile in a soxhlet extraction assembly. The solvents dichloromethane and acetonitrile were selected according to the solubility of methyl parathion and chlorpyrifos respectively. The 200 ml soxhlet extract was concentrated with a rotary evaporator to 10 ml for HPLC analysis

Analysis of chlorpyrifos and methyl parathion degradation by High Performance Liquid Chromatography (HPLC)

All reagents were of analytical or HPLC grade. Acetonitrile (CH_3CN), Dichloromethane, Chlorpyrifos and Methyl parathion were purchased from Sigma Aldrich. The water used in HPLC was from milli/Q system. The mobile phase was filtered through a Whatman filter paper (90 mm, 0.45µmpore size). All data for

quantification of chlorpyrifos and methyl parathion were obtained by applying the gradient elution program shown in Table 1. Chlorpyrifos and Methyl parathion were analysed with Agilent 1260 series LC system with UV detector at 225nm and 273 nm respectively having flow rate 1ml/minute.

Results and Discussion

The surface soil contamination with pesticides is a common environmental problem posed by repeated and continuous use in agricultural field. The present study was carried out with the aim of establishment of highly effective remediation method for remediation of persistent pesticides (chlorpyrifos and methyl parathion) using identified microbial consortium in surface soil treatment reactor. On the basis of morphological characteristics and biochemical tests isolates were identified and further taken for bioremediation studies purpose in surface soil treatment reactor.

The physico chemical properties of rice field soil were carried out and presented in Table 2, which indicates presence of chloride, sulphate, phosphorus, nitrogen, total organic carbon and total organic matter. The microbial analysis of soil was also carried out, which includes isolation and identification of seven different types of bacteria presented in Table 3 and Figure 2. The presence of nutrients as well as bacterial consortium in soil has been found to have great influence for the bioremediation of pesticides. The bacterial consortium identified (*Acenetobactor* sp., *Pseudomonas* sp., *Enterobacter* sp. and *Photobacterium* sp.) from agriculture field was used for the remediation of pesticides in surface soil treatment reactor.

Bioremediation of chlopyrifos and methyl parathion by isolated bacterial consortia in surface soil treatment reactor

The naturally occurring bacterial isolates capable of metabolizing pesticides were isolated from pesticides polluted agricultural soil. The bacterial isolates identified by various biochemical analyses are *Acenetobactor* sp., *Moraxella* sp., *Pseudomonas* sp., *Enterobacter* sp., *Yersinia* sp. and *Photobacterium* sp. A surfacesoil treatment reactor (SSTR) has been designed wherein; bioremediation of chlorpyrifos and methyl parathion polluted agricultural soil was carried out using developed bacterial consortium with a combination of four bacterial species (*Acenetobactor* sp., *Pseudomonas* sp., *Enterobacter* sp. and *Photobacterium* sp.). The environmental condition such as temperature (25-28°C) and moisture contents (60 -70%) were continuously monitored during the whole process. The degradation of chlorpyrifos and methyl parathion was determined by means of High Performance Liquid Chromatography (HPLC). The HPLC chromatograms of the respective pesticides are shown in Figure 4 and 5. The reduction in concentration of chlorpyrifos and methyl parathion during bioremediation in SSTR were mentioned in Table 4. During this period up to 62% degradation of chlorpyrifos and 65%degradation of methyl parathion was achieved shown in Table 4 and Figure 3.

Variation in environmental parameters during bioremediation of pesticides using microbial consortium

Bioremediation of chlorpyrifos and methyl parathion was carried out using identified bacterial consortium of *Acenetobactor* sp., *Pseudomonas* sp., *Enterobacter* sp. and *Photobacterium* sp. in surface soil treatment

reactor under controlled environmental conditions.

The environmental parameters monitored and assessed were pH, electrical conductivity, nitrate, nitrite, ammonium, sulphate, phosphate and total organic carbon as mentioned in Table 5. During the process of bioremediation, it was found that pH values were gradually deceasing from 7.7 to 7.1, while the values of electrical conductivity increase from 132 µs cm^{-1} to 199 µs cm^{-1}. The reason behind the reduction in pH value is the organic acids produced from intense fermentation of carbohydrates (Dibble and Bartha, 1979), while the increase in electrical conductivity was due to the aeration and moistening during remediation, which cause release of dissolved solutes and increase in electrical conductivity (Akpan *et al.,* 2013).

In the case of nitrate, decreased value was recorded during bioremediation process, which decreased from 8.5 mg/kg to 5.67 mg/kg, while nitrite value and value of ammonium found to be increased. The value of nitrite increased from 0.081 mg/kg to 0.1 mg/kg and the ammonium value increased from 2.02 mg/kg to 3.03 mg/kg. The reason behind the lowering of nitrate-N concentration is reduction of nitrate into nitrite and finally into ammonia during bioremediation, resulting increased value of nitrite-N and ammonium-N (Francis *et al.,* 2007; Hayatsu *et al.,* 2008).

The sulphate value decreased from 2.84 mg/kg to 1.88 mg/kg, while phosphate value increased from 0.65 mg/kg to 0.97 mg/kg during bioremediation. The reason behind the lowering of sulphate was sulphate reducing bacteria that can obtain energy by oxidizing organic compounds or molecular hydrogen (H_2) while reducing sulfate (SO_4^{-2}) to hydrogen sulphide, which reduces sulphate by producing hydrogen sulphide gas (Schulze and Mooney, 1993), while the reason behind the increase in phosphate concentration was release of PO_4^- ion during bioremediation of organic compounds such as pesticides (Mishra *et al.,* 2001). Similarly, a decrease in total organic carbon value was recorded during bioremediation process, which decreased from 1.26 % to 0.93% due to bacterial metabolic activities (Chefetz *et al.,* 1998).

Table.1 HPLC conditions used for analysis of Chlorpyrifos and Methyl parathion

HPLC Conditions	Chlorpyrifos	Methyl parathion
Column	C18	C18
Flow rate	1ml/min	1ml/min
Column temperature	25°C	25°C
Injection volume	10 µl	20 µl
Mobile phase	Acetonitrile : Water (70:30)	Acetonitrile:Water (90:10)
Retention time	6.9 minute	1.1 minute
Wavelength	225 nm	273 nm

Table.2 Physico-chemical properties of rice field soil

Parameters	Site 1	Site 2	Site 3	Site 4	Site 5	Average	SD
Temperature (°C)	27	27.2	26.8	26.8	27.1	26.98	0.178885
pH	7.8	7.9	7.8	8.0	7.7	7.84	0.114018
Electrical conductivity (µs cm^{-1})	120	100	150	136	127	126.6	18.62257
Moisture content (%)	33.81	33.77	33.49	33.51	33.47	33.61	0.165529
Water holding capacity (%)	37.44	36.57	37.25	36.94	36.93	37.026	0.333961
Bulk density (g/ml)	1.74	1.73	1.73	1.74	1.74	1.736	0.005477
Hardness (mg $CaCO_3$/kg)	30	30	28	32	26	29.2	2.280351
Chloride (mg/kg)	116.5	100	130	115	115	115.3	10.62779
Alkalinity ($CaCO_3$ mg/L)	150	180	140	140	130	148	19.23538
Sulphate (mg/kg)	2.92	2.92	2.52	2.39	2.84	2.718	0.246617
Inorganic phosphorus (mg/kg)	0.59	0.63	0.66	0.54	0.67	0.618	0.053572
Total phosphorus (mg/kg)	8.12	7.77	8.32	8.12	8.12	8.09	0.198746
Nitrate-N (mg/kg)	9.02	9.21	9.16	8.50	8.88	8.954	0.28457
Nitrite N (mg/kg)	0.081	0.084	0.077	0.082	0.082	0.0812	0.002588
Ammonium -N (mg/kg)	2.07	2.05	1.99	1.99	2.10	2.04	0.04899
Total organic carbon (%)	1.2	1.05	1.2	1.05	0.9	1.08	0.125499
Total organic matter (%)	1.71	1.71	1.72	1.71	1.71	1.712	0.004472

Table.3 Identification of bacteria isolated from rice field soil environment

Isolates	Identification
R1	*Acenetobactor*sp.
R2	*Moraxella* sp.
R3	*Pseudomonas* sp.
R4	*Photobacterium* sp.
R5	*Yersinia* sp.
R6	*Enterobacter*sp.
R7	*Photobacterium* sp.

Table.4 Bioremediation of Chlorpyrifos and Methyl parathion in SSTR

Pesticides	0^{th} day	2^{nd} day	4^{th} day	6^{th} day	8^{th} day	10^{th} day	Degradation (%)
Chlorpyrifos (mg/kg)	1.02	0.98	0.88	0.73	0.58	0.38	62.72
Methyl parathion (mg/kg)	1.15	1.09	0.98	0.80	0.56	0.39	65.99

Table.5 Variation in environmental parameters during bioremediation of pesticides

Parameters	**0th Day**	**2nd Day**	**4th Day**	**6th Day**	**8th Day**	**10th Day**
pH	7.7	7.6	7.5	7.3	7.2	7.1
Electrical conductivity(µs cm^{-1})	132	138	142	160	178	199
Nitrate(mg/kg)	8.50	7.93	7.37	6.66	6.23	5.67
Nitrite(mg/kg)	0.081	0.083	0.088	0.092	0.097	0.10
Ammonium(mg/kg)	2.02	2.12	2.25	2.45	2.72	3.03
Sulphate (mg/kg)	2.84	2.68	2.52	2.36	2.20	1.88
Phosphate (mg/kg)	0.65	0.73	0.75	0.80	0.85	0.97
TOC (%)	1.26	1.125	1.02	0.99	0.96	0.93

Fig.1 Schematic diagram of surface soil treatment reactor (SSTR)

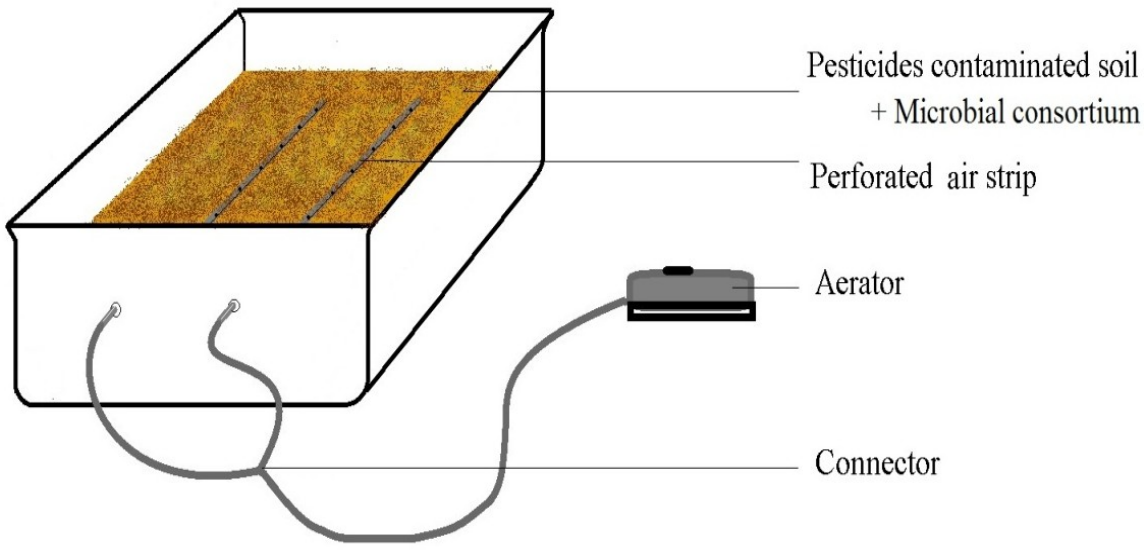


Fig.2 Bacteria isolated from Rice field (R1, R2, R3, R4, R5, R6 and R7 are pure culture of different isolates

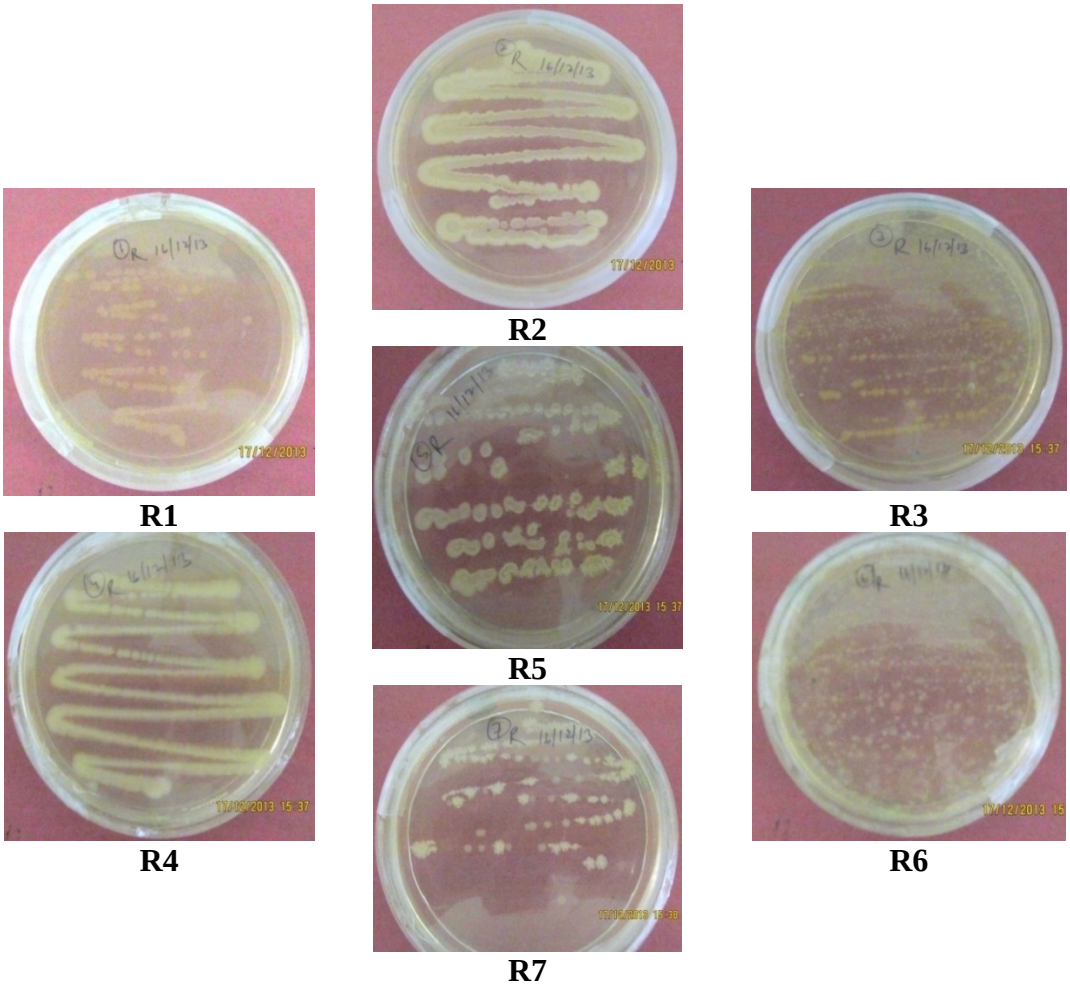


Fig.3 Biodegradation of chlorpyrifos and methyl parathion in SSTR

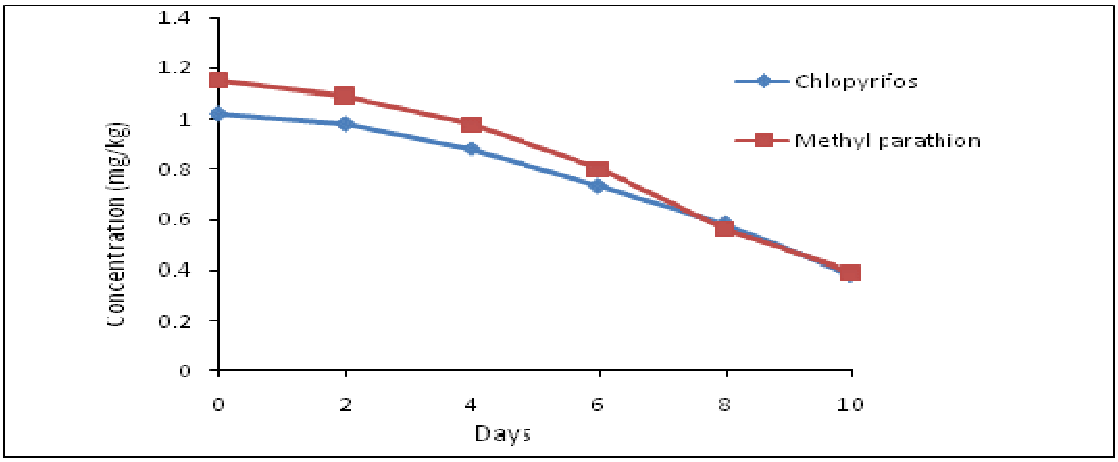


Fig.4 HPLC chromatogram of Chlorpyrifos
(A- Before bioremediation, B - After bioremediation)

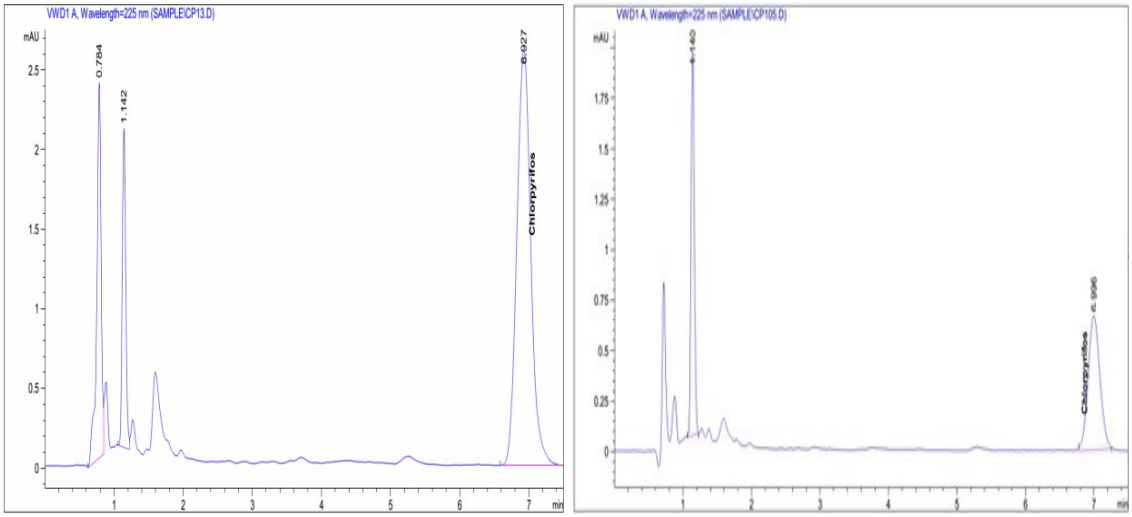
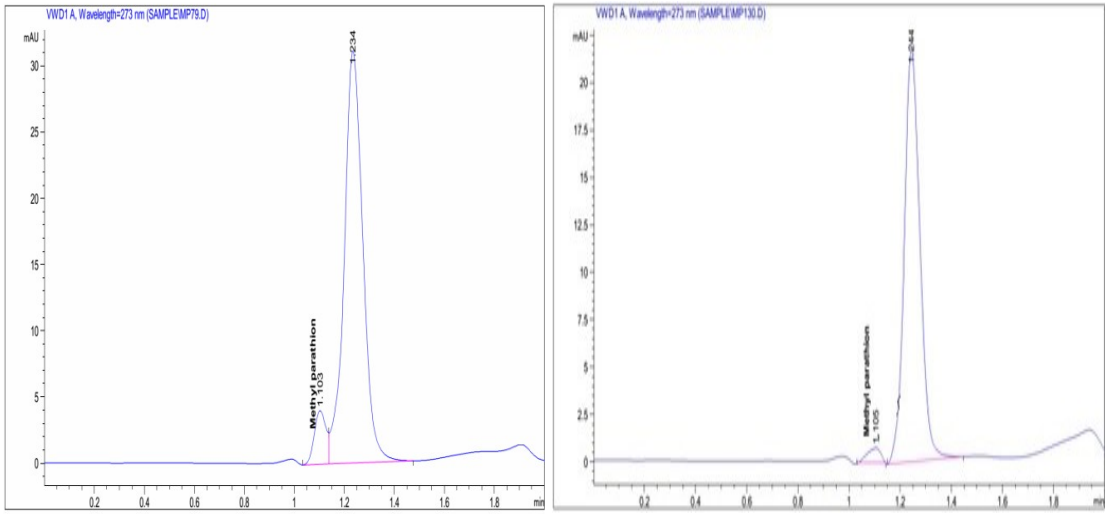


Fig.5 HPLC chromatogram of Methyl parathion
(A- Before bioremediation, B - After bioremediation)



The overall results presented in this study show that bacterial consortia play the key role in the degradation of pesticides. The presence of a natural microbial community is a necessary and prerequisite for an effective remediation of pesticides. The use of bacterial consortia makes it possible to evaluate the natural microbial potential to degrade persistent pesticides in soil. The choice of the bioremediation strategy should be made on the basis of type and properties of pesticide, environmental matrix and the organisms present in the environment. The research study proved that indigenous microbial consortium is effective for remediation of persistent pesticides in agriculture environment.

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