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Effect of pH and Inoculum Concentration on Biodegradation of DDT and Lindane by defined Microbial Consortium

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ABSTRACT

Microorganisms were found to be potential bio-degraders of organochlorine pesticides such as Lindane and DDT (dichlorodiphenyltrichloroethane) that have been used for agricultural purposes primarily for pest management. These are slowly degrading molecules and recalcitrant in nature which can pose adverse health effects to the environment and community.

In this study, the effects of pH and Inoculum Concentration were studied on the biodegradation capability of the Microbial Consortium that was isolated from river aquatic systems. The 16S amplicon sequencing identified 871 species in the consortium and established the biodiversity of the consortium. The defined consortium was able to degrade DDT and Lindane up to 30 ppm simultaneously in varying order of pesticide concentrations. The study revealed that a higher inoculum size of OD600 0.075 increased the degradation of both DDT and Lindane and pH 7 was found to be optimum for the simultaneous degradation of these insecticides.

Keywords: Inoculum Concentration, pH, Microbial Consortium, Biodegradation, Dichlorodiphenyltrichloroethane (DDT), Hexachlorocyclohexane (HCH), Lindane, Metagenomics

I INTRODUCTION

Organochlorine Pesticides (OCPs) have been widely used for agricultural purposes primarily for pest management and OCPs such as DDT (dichlorodiphenyltrichloroethane) is the potential agent used for public health programs to control vector-borne diseases. Although OCPs were banned in several countries; being persistent organic pollutants and recalcitrant in nature, they pose many environmental and health concerns [1, 2]. It is the need of the hour to identify sustainable methods and strategies for removing them from the environment [3].

DDT and Lindane (gamma-hexachlorocyclohexane) were the major OCPs that have been ubiquitously used in developing nations [4]. One of the sinks for discharging persistent organic pollutants are the

environmental ecosystems primerily water ecosystem comprising rivers and lake beds [5]. Endrin aldehyde, Endosulfan sulfate and DDT were detected in highest percentage in River Yamuna which demonstrates the pollution of the river with pesticide residues [6]. DDT, Trans-chlordane and Endo-sulfansulfate were the dominant OCPs in soil sediments from River Godavari [7]. Microorganisms are found to be potential degraders of organochlorine compounds, notably soil habitants belonging to genera *Bacillus, Pseudomonas, Arthrobacter and Micrococcus* were found to be effective bio-degraders [8]. Several persistent organochlorine pesticides were detected in other rivers where higher concentrations of Endosulfan sulfate and DDT were detected and even found their presence in drinking and bottled water [9]. Hence, it becomes imperative to remove these pollutants from the environment, from the sinks primarily water ecosystems to eliminate their residues.

In this study, the effects of pH and Inoculum Concentration were studied on the biodegradation capability of DDT and Lindane by Microbial Consortium that was isolated from Godavari and Yamuna rivers. The 16S amplicon sequencing identified 871 species in the consortium and established the biodiversity of the consortium. The defined consortium was able to degrade DDT and Lindane up to 30 ppm simultaneously in varying order of pesticide concentrations. The study revealed that a higher inoculum size of OD600 0.075 increased the degradation of both DDT and Lindane and pH 7 was found to be optimum for the simultaneous degradation of 15 ppm of these insecticides. This consortium was characterized using metagenomics, 16S amplicon sequencing in Illumina Next Generation Sequencing (NGS) platform [10, 11, 12]. The metagenomic analysis identified 871 species in in the defined Microbial Consortium.

II MATERIALS AND METHODS

Chemicals

Lindane (γ-HCH) was of 97% purity and obtained from Sigma- Aldrich, USA. DDT, 99.4% pure, was donated by Hindustan Insecticides Ltd, India. All other chemicals and reagents used in the study were of analytical grade and were purchased from standard manufacturers.

Screening of DDT and Lindane Tolerant Microbial Consortium

Water samples from the rivers Yamuna (North India) and Godavari (South India) were collected in clean bottles and brought to the lab in sealed condition. These water samples were mixed and incubated with 1% (w/v) peptone in a rotary shaker maintained at 150 rpm and run in ambient conditions. Once the microbial growth was sufficient to make the broth highly turbid, the culture was starved for a week

followed by addition of 0.5% (w/v) peptone, 2 ppm Lindane and 2 ppm DDT. The growing culture was left shaking for a month followed by addition of 0.1% peptone, 5 ppm Lindane and 5 ppm DDT. After shaking for another month, the culture was continuously shaken only in presence of gradually increasing concentrations of Lindane and DDT for many months till a stable Lindane and DDT tolerant population were established in the flask [13]. These populated microbial cells formed the consortium that was used in this study.

The microbial population established over a period of many moths of enrichment was screened and tested for its potential to degrade mixture of 4, 4'-DDT and Lindane. Once the screening was done, the pH optima and effect of inoculum was studied at 15 ppm concentrations [14].

Estimation of Residual Pesticide Concentration

Thin Layer Chromatography

TLC was performed on 0.25 mm thick silica gel G plate with cyclohexane mobile phase. The thin layers were air- dried before detecting the pesticide residual spots using *o*-tolidine (2% as acetone solution) spray in bright sunlight. The spots appeared as peacock green. The area under the spot was used for quantifying the residual Lindane and DDT using the relationship that the square root of the area is directly proportional to the log of concentration [15]. The results were further confirmed using GC-MS/MS.

Gas Chromatography-MS/MS

The residual pesticide was quantified by Gas Chromatography using instrument GCMS/MS Triple quad; Model 7000D (Agilent Technologies Ltd) [16]. The column HP-5ms, Agilent 19091S EPC was used for analysis of residual pesticides. These columns have low bleed characteristics, excellent inertness for active compounds, and improved signal-to-noise ratio for better sensitivity. The sample was dissolved in 1 ml MS grade ethyl acetate and appropriate dilutions were used for analysis. The injector was maintained at 70°C initial set point to post run temperature of 280°C, while the column was programmed with pressure 30.797 psi, flow of 3.1793 mL/min, Average Velocity of 54.506 cm/sec and initial temp of 70°C. The ion source was electron ionized (EI) with source temperature of 300°C for triple quadrupole acquisition method.

RESULTS AND DISCUSSION

The microbial population established over a period of many moths of enrichment was screened and tested for its potential to degrade mixture of 4, 4'-DDT and Lindane. The established consortium was incubated

with 5ppm Lindane and 5ppm DDT mixture in 25 mL sterile RO water and whole flask samples were drawn at 0 h and 72 h. The residual pesticides dissolved in a known volume of HPLC grade acetone for analysis by thin layer chromatography (TLC) and GCMS/MS. The consortium was able to degrade effectively DDT and Lindane in varying order of pesticide dissipation simultaneously (Figures-1, 2). Once the screening was done, the pH optima and effect of inoculum was studied.

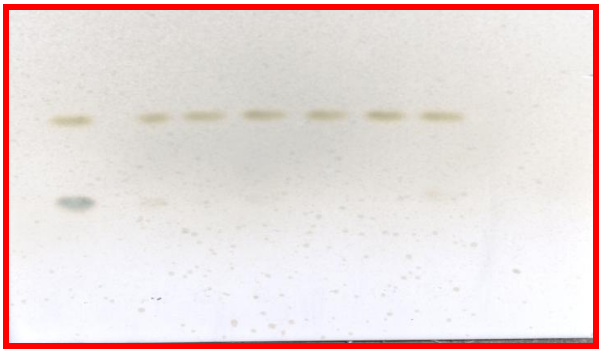

Fig. 1: Screening of RM Consortium for its potential to degrade 4, 4'-DDT and Lindane

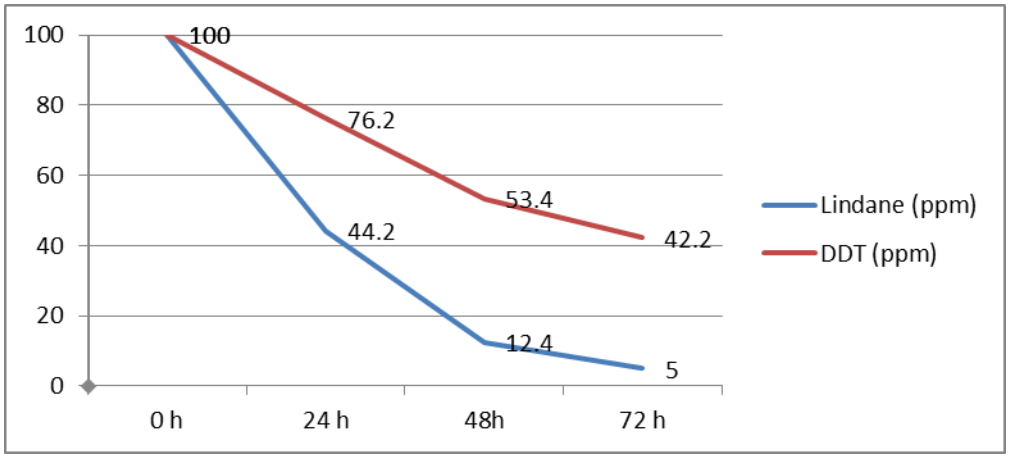

Fig. 2: Biodegradation Capability of RM Consortium to degrade 4, 4'-DDT and Lindane

Effects of pH on Simultaneous Degradation of DDT and Lindane

The effect of pH on degradation of 15 ppm 4, 4'-DDT and Lindane was studied in the range 4-9. The visual results are presented in the Fig. 3 wherein substantial degradation of both the insecticides is seen at pH 7 and 8. Among the two, pH 7 was found to optimum for the simultaneous degradation of these insecticides.

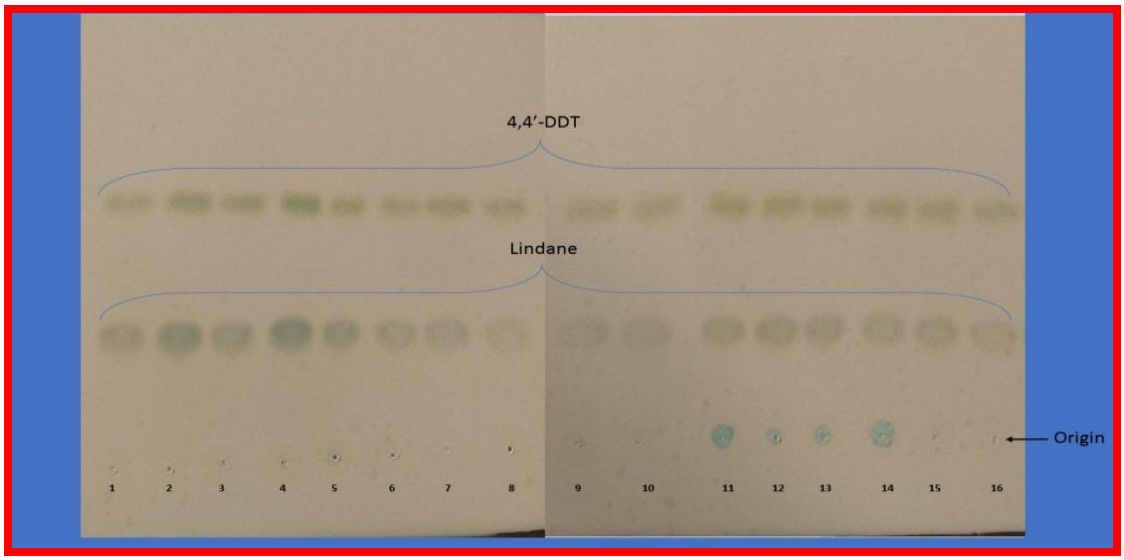

Fig. 3: Thin Layer Chromatogram for 4,4'-DDT and Lindane. (Spots 1-10 belong to pH): 1= pH 5- 0 h; 2= pH 5- 72 h; 3= pH 6- 0 h; 4= pH 6- 72 h; 5= pH 7- 0 h; 6= pH 7- 72 h; 7= pH 8- 0 h; 8= pH 8- 72 h; 9= pH 9- 0 h; 10= pH 9- 72 h; (Spots 11-16 belong to inoculum size OD_{600}): 11= 0.025- 0 h; 12= 0.025- 72 h; 13= 0.05- 0 h; 14= 0.05- 72 h; 15= 0.075- 0 h; 16= 0.075- 72 h

The residual pesticide was quantified by Gas Chromatography using instrument GCMS/MS Triple quad; Model 7000D (Agilent Technologies Ltd), and the following are results of the residual pesticides when a 15 ppm of Lindane and DDT is subjected to biodegradation at varying pH ranges from pH 5 to pH 9. Table 1 and Figure 4 show the degradation percentages of 4,4'-DDT and Lindane at various pH ranges.

pH Range	At 0 Hour		At 72 Hour	
	Lindane (ppm)	DDT (ppm)	Lindane (ppm)	DDT (ppm)
pH 5	14.94	14.72	14.91	14.98
pH 6	14.94	14.8	13	12.2
pH 7	14.84	14.93	8.06	6.02
pH 8	15.27	14.93	10.2	11.11
pH 9	14.96	14.52	14.96	14.38

Table 1: Effect of pH on DDT and Lindane Simultaneous Degradation through GC MS

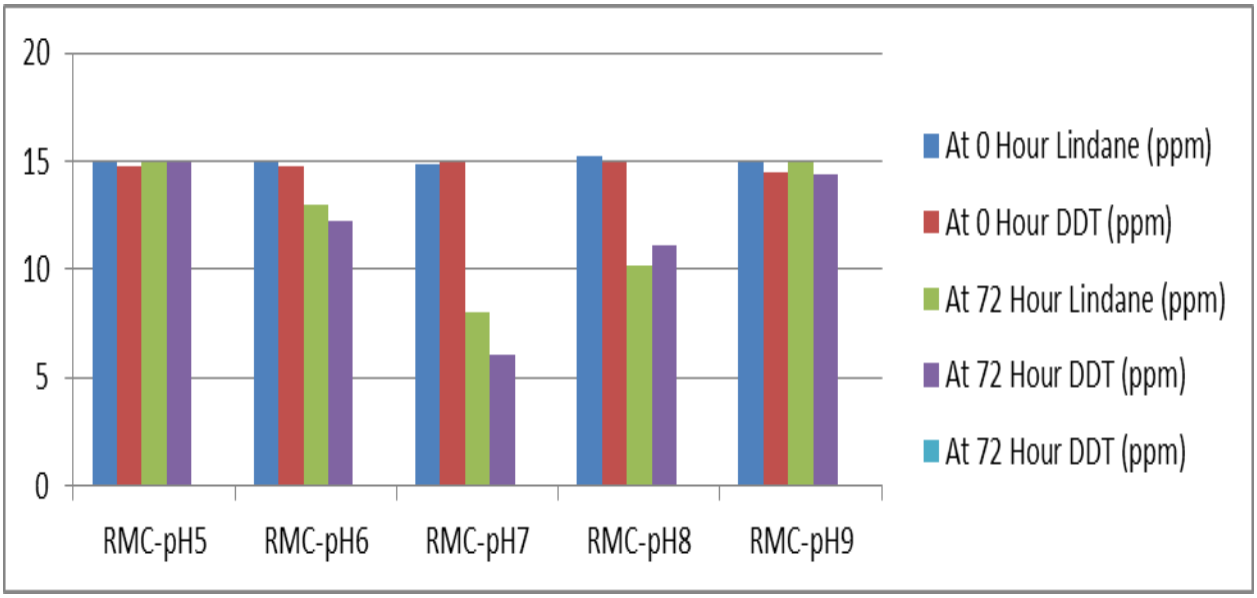

Fig 4: Effect of pH on DDT and Lindane Simultaneous Degradation through GC MS

Effect of Inoculum concentration on Simultaneous Degradation of DDT and Lindane

The effect of Inoculum concentration on degradation of 15 ppm 4, 4'-DDT and Lindane was studied in the ranges OD_{600} 0.025 to OD_{600} 0.075. A 72 h sample showed that the inoculum concentration if higher from OD_{600} 0.025 to OD_{600} 0.075 increased the degradation of both DDT and Lindane. Table 2 and Figure 4 represent the degradation percentage of the two insecticides after 72 h by different initial inoculum size.

OD_{600}	At 0 Hour		At 72 Hour	
	Lindane (ppm)	DDT (ppm)	Lindane (ppm)	DDT (ppm)
0.025	14.86	14.72	8.12	6.09
0.05	15.07	14.96	7	4.83
0.075	14.84	14.78	2.61	3.38

Table 2: Effect of Inoculum Concentration on Simultaneous Degradation of DDT and Lindane by GC-MS

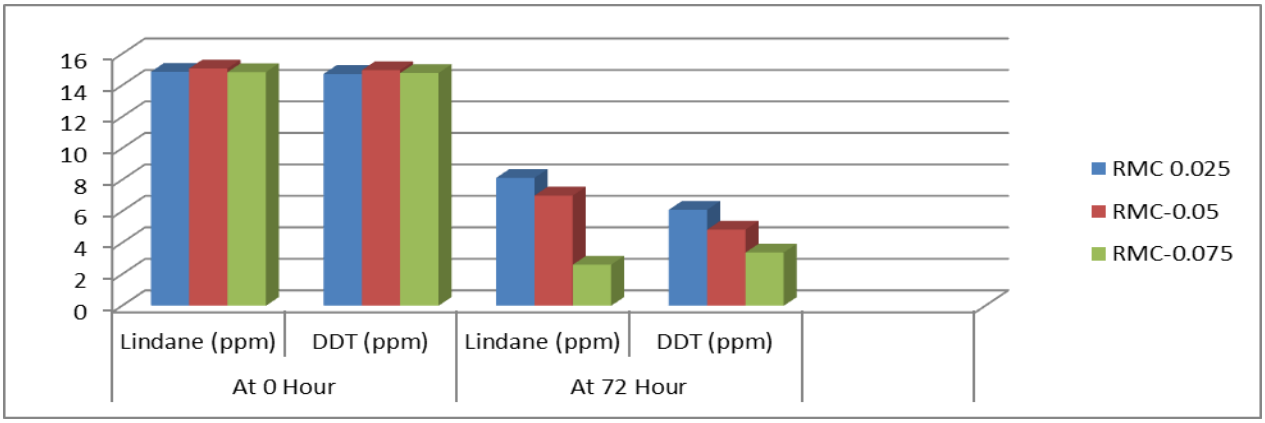

Fig 4: Effect of Inoculum Concentration on Simultaneous Degradation of DDT and Lindane by GC-MS

A great deal of research has been carried out using single organism and a single compound of OCPs in the realm of bioremediation [17, 18]. This study determined the optimized pH and Inoculum concentrations that can increase the rate of simultaneous degradation of DDT and Lindane insecticides. The study concludes that a higher inoculum size of OD600 0.075 increased the degradation of both DDT and Lindane and pH 7 was found to be optimum for the simultaneous degradation of these insecticides. The defined consortia at these optimized conditions may facilitate higher degrading capability, a crucial phenomenon which can be a promising solution for removal of DDT and Lindane pesticide mixtures in aquatic ecosystems by eliminating pesticide residues thereby enhancing environmental conditions.

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