Summary and Conclusions
DDT is one of the first anthropogenic pesticides used by man. Though it was synthesised in 1874, its insecticidal properties were discovered in the 1930s. Since then it was used for controlling vector borne diseases and pests. At a later stage it was found to be an environmental hazard for its toxicity towards many non-target species. It was banned completely in few countries while its use was restricted to public health programmes and emergencies. Even though its use was restricted, developing countries still use it for both agriculture and health programmes as no other alternative to DDT is available. Usage of DDT since many years and the long persistence of the compound in the environment has led to its accumulation in the environment. DDT is suspected to be teratogenic, carcinogenic and mutagenic. It enters human body via food chain. Therefore, detection of DDT in the environment, degradation of this pesticide in a short time and in an inexpensive way becomes important.

Detection of DDT is usually done by chromatographic techniques like TLC, GLC and HPLC. These techniques require sample pre-treatment, well-equipped laboratory and trained personnel to perform the test. The non-chromatographic techniques like ELISA are therefore gaining importance. In our laboratory, an attempt was made to develop a Dot-ELISA technique for the detection of DDT in food and water samples. A hapten was prepared using DDA, a metabolite of DDT. The prepared hapten, 3-[bis(4-p-chlorophenyl)acetylamino]propionic acid was conjugated to BSA and used for further immunisation studies. Six weeks old New Zealand white rabbits and 20 week old single comb white leg horn poultry (layers) were immunised with hapten-BSA conjugate and antibodies were isolated from egg yolk (IgY) and rabbit blood (IgG). The protein and antibody titres were tested. The 6th week antibody from rabbit and 19th week antibody from poultry showed highest protein and antibody titres. These antibodies were used for further studies. The antigen and antibody concentrations were optimised by checkerboard analysis. An antibody dilution of 452 pg protein/µL and an antigen dilution of 80 pg protein/µL for IgG and an antibody dilution of 355 pg protein/µL and an antigen dilution of 40 pg protein/µL for IgY gave the
best immunogenic reaction. A competitive Dot-ELISA was done with different concentrations of DDT from 1 µg through 1 pg. The DDT level up to 1 ng could be visually differentiated by this method. To enhance the sensitivity of the assay, signal amplification was done with electron rich casein, biotinylated tyramine and avidin-ALP conjugate. The limit of detection was enhanced and DDT level up to 1 pg could be detected visually. The specificity of the assay was done by measuring the cross-reactivity of some of the DDT- metabolites and other organochlorine pesticides. Cross-reactivity was highest with DDA with both IgG and IgY. The cross reactivity was lesser with other organochlorines.

Food and water samples were analysed using the Dot-ELISA technique. The matrix effect in all the samples analysed was very low. An Immuno-DDT kit was developed in our laboratory for the on-field detection of DDT in food and water samples. The detection time was 30 min excluding the extraction time. Thus, an economical, faster, on-field detection system for DDT was developed.

The presence of DDT residues in the environment poses many health problems. Hence, its removal from the environment is of prime importance. Microbial remediation is one method of removing DDT from the environment. Microbial remediation is safer and economical. In our laboratory, a consortium capable of degrading DDT was isolated from DDT-contaminated soil by long term enrichment technique. The consortium constituted seven different strains of Pseudomonas, one each of Flavobacterium, Vibrio and Burkholderia sp. The established consortium was inoculated to 10 ppm DDT. By 10 days of incubation, 65% of DDT was found to be degraded. To further enhance the degradation, co-metabolitic degradation was studied. Among 12 different co-substrates used, yeast extract at a concentration of 1% gave best degradation with 74.56% degradation by 72 h at the rate of 0.0174 µg/mL/day. Response surface methodology (RSM) was done to optimise the degradation parameters. The maximum predicted percentage degradation of 96.69, 96.37, 97.97, 92.44 and 98.19 was obtained respectively for 5, 10, 20, 30 and 35 ppm initial DDT concentrations at different pH levels 7.82, 6.59, 6.92, 7.06 and 8.00 with an incubation temperature of 25°C and inoculum
concentration of 1500 μg protein/mL. The degradation of DDT by the microbial consortium increased with time. The degradation reached 95% by 72 h of incubation.

Primers designed for identifying DDT-dehydrohalogenase genes gave positive amplification with genomic DNA and plasmid DNA of Flavobacterium sp. The PCR product of genomic DNA of Flavobacterium sp. was ligated to pTZ57R/T vector and cloned in to E.coli DH5α. The sequence analysis of Flavobacterium species T₆ DDT-dehydrohalogenase gene PCR amplified insert indicated that the protein had 272 residues. This amounted to a reverse translation product of 757 base sequences of most likely codons. The molecular weight of the reverse translated protein has been given as 30,823 with a theoretical pI of 6.42. The amino acid composition of the reverse translated protein indicated that the total number of negatively charged residues in the protein (Asp + Glu) is 28 and the number of positively charged residues (Arg + Lys) is 30. The ExPASSy blast results for finding out the homology of DDT- dhl 1 gene with Pseudomonas species genome indicated that the sequence had 24- 26% identity with Ps. putida genome. The DDT-dhl 1 gene showed homology with acetolactate synthase gene sequence. The southern blot analysis of genomic DNA from Flavobacterium species for the DDT- dhl 1 probe was done. The genomic DNA of Flavobacterium species yielded positive hybridisation. The cloned PCR product was expressed in E. coli BL21 using pET28a vector. The expressed protein showed positive amplification in western blot analysis.

Bioremediation is an interdisciplinary technology, involving microbiology, engineering, ecology, geology, chemistry, etc. Microbes are the primary stimulants in the bioremediation of contaminated environments. Investigations in to the microbial degradation of DDT are useful in the development of methods for the remediation of the contaminated environments. Laboratory studies have shown that microbes have the machinery to metabolise DDT and other xenobiotics. However, little is known about the conditions, which favour the degradation of DDT and other pollutants. In situ remediation may be limited because of the presence of
complex sets of environmental conditions. The knowledge of the biological contribution to the effect of bioremediation and its impact on the eco-system is limited. The microbial communities are considered as a “Black Box” and will make remediation a more reliable and safer technology as and when the information hidden within it is deciphered and used in a constructive way.

Nature has provided with every facility and given complete rights to each form of life to live and survive in the environment. Man has taken this liberty of this right to improve his survival with more comforts and luxuries and while doing so; he not only has created hazards for himself but even for other life forms and nature itself. Instead of looking into the welfare of man if we start monitoring the environment for the safe ecology we can survive with more security, happiness and satisfaction that we need not pollute the environment and nor create dangers to the other lives which are of same significance as ours.