amidases, phosphates, arylsulphatase, cellulases, and phenol oxidases are studied. They are well-documented and quick assays are available for measuring the enzyme activities. Dehydrogenase an enzyme occurring in all the living microbial cells, which is linked with microbial respiratory processes. It gives an indication of overall microbial activity of soils. In the literature we can find the reports on the impact of pesticides on dehydrogenase activity [4]. The majority of them are either neutral toward this activity or they inhibit it. Dehydrogenase activity of endosulfan gets stimulated 100 to 200 times at its standard rate of application. Herbicides such as Butachlor have a repressive effect on dehydrogenase activity. It is not possible to detect a single type of response of this activity to fungicides: the enzyme was alternately stimulated and inhibited. The effect of a pesticide on soil microorganisms is governed not only by the chemical and physical properties of the pesticide itself, but also soil parameters like type, properties and prevailing environmental conditions. At higher doses of fungicides the dehydrogenase activity gets inhibited. The literature survey showed whatever the dose considered, fungicides, herbicides and insecticides show inhibitory effects or no effects on the dehydrogenase activity, except endosulfan and mancozeb. The present study focuses on the isolation, screening and identification of bacterial cultures for biodegradation of pesticides commonly used in the agriculture and we also checked in for pH activity of soil sample, conductivity measurements and dehydrogenase activity of the soil samples.

Materials and methods

Chemicals and reagents

Bovine Serum Albumin (BSA), D-glucose, Isopropyl-1-thio-β-D-Galactopyranoside (IPTG), Tris base, Ammonium sulphate, potassium dihydrogen orthophosphate, Di-potassium hydrogen orthophosphate, Sodium chloride were procured from Sigma Aldrich Company, Mumbai. The standards pesticides were procured from Sigma Aldrich Company, Mumbai. Glycerol, Ferrous sulphate, Magnesium chloride, Zinc sulphate, Tetracycline, Agar-agar, Phenol, Chloroform, Isoamylalcohol, Tris-HCL, Acetic acid were obtained from Hi-Media, Mumbai, India. Tris-saturated phenol from Genei, Bangalore, India., DNA ladders and the enzymes used in the study, *NdeI* and *BamHI*, were supplied by Fermentas, DNTPs, T4 DNA ligase, Taq Buffer, Taq DNA polymerase, Ribonuclease, TBE-buffer was obtained from New England Biolabs (NEB), Inc., Beverly MA, and USA. Plastic and Glass wares were procured from Axygen Inc, USA and Tarson Pvt Ltd., India. Primers, enzymes and all other reagents used in this study were procured from Fermentas Life Sciences, Bangalore, India. The reagents used here for the analysis were of AR grade.

Soil sample collection

Dicofol is used against variety of fruit, vegetable, ornamental and field crops and it is an organochlorine miticide. It is used on a wide variety of fruit, vegetable, ornamental and field crops. Field application of dicofol can be used as emulsifiable concentrate and wettable powder formulations. Dicofol has little effect on insects. 2, 4 DDT is commonly used for the disease control. Metachlor is an organic compound. It is widely used as an herbicide and is used for grass and broadleaf weed control in corn, soybean, peanuts, sorghum, and cotton. For its action it needs other combination of herbicide. Diuron is used as a substituted urea herbicide and is used against wide variety of annual and perennial broadleaf and grassy weeds. Screening and isolation of Pesticide degrading bacteria was carried out by using Minimal Salt Yeast (MSYM) media:

For the isolation of pesticide degrading bacteria (MSYM) media was prepared. The media components consisted of Na_2NO_3 4gm, Na2HPO4 0.5gm, KHPO4 1.5gm, $CaCo_3$ 0.01gm, $Fecl_3$ 0.05 gm, MgSO40.2 gm, yeast extract 0.01% and distilled water 100 ml. To 100 ml MSYM 25ppm pesticide and ten grams of soil sample were added. The samples were incubated on rotary shaker at (150 rpm) at 30°C for 7 days. 7^{th} day media components were then transferred to a fresh medium and under same conditions incubation was followed. The given conditions were successively sub cultured for every 3-4 days until increased turbidity was observed. After successive sub-culturing, 0.1 ml culture broth was pipetted and spreaded on MSYM and pesticide agar media. Colonies were selected and streaked on Nutrient Agar (NA) supplemented with 25, 50, 75 and 100 ppm of the pesticide. Cultures were incubated at 30°C for 3 days. Pesticide degrading isolates were selected from isolates which developed when grown on NA supplemented with 25, 50, 75 and 100 ppm of the pesticide [5].

Characterization of the bacterial isolates

Bacterial isolates were grown in MSYM+pesticide and incubated at 30°C until either turbidity or colony was observed. 16S rDNA analysis. Genomic DNA was extracted by boiling method. In Nutrient Broth (NB) one ml of cell culture was grown in at 30°C for 18-24 hrs. It was centrifuged at 10, 000 rpm for 10 min and then washed 2 times with pH 7.8 TE-buffer. In 0.3 ml TE-buffer (pH 7.8) the washed cell was resuspended and then boiled at 100°C for 10 min, tracked immediately by cooling at 0oC for 5 min. This cycle of boiling/ cooling step was repeated 3 times.

PCR was done for 16S rDNA which was extracted from the cell extract obtained with the above procedure was amplified by PCR using 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1492R (5'-ACGGCTACCTTGTTACGACTT-3') primers. It consisted of reaction components 0.2 mM each dNTP, 0.2 M of each primer, 5 l 10x PCR buffer, 10 l cell extract, 2.5 units Taq DNA polymerase and sterile deionized water to a final volume of 50 l. Following a hot start (94°C for 3 min), 25 cycles of amplification were used (94°C for 1 min, 50°C for 45 sec, 72°C for 2 min) followed by a final single extension of 72°C for 10 min. For PCR products electrophoresis was done on a 1% agarose gel and visualized under UV light after staining with ethidium bromide. The amplified PCR product was purified using QIA quick PCR Purification Kit according to the manufacturer's instruction. DNA sequencing was performed by Macrogen, Inc.

Sample preparation for conductivity measurements

Determination of conductivity 20 g of the ground soil sample was taken into a beaker and 100 cm^3 of distilled water was added and mixed thoroughly. The sample was allowed to stand for 10 minutes. The sample solution was then decanted into another clean beaker. The conductivity meter of the sample solutions were determined using a model ELE 470 conductivity meter. This conductivity meter was turned on and the probe was inserted into the decanted suspended solution of soil samples. The conductivity of each solution was taken and recorded. The probe was removed from the samples and thoroughly rinsed with distilled water. Enzyme activities have been reported to be long-sensitive indicators of soil ecological stress in both natural and agricultural ecosystems. Dehydrogenases contribute to the

respiratory activity of microorganisms; hence, DHA has been used to assess microbial activity in soil treated with pesticides. However, this assay should be used with caution because of confounding alternative electron acceptors in soil that can lead to overestimation of DHA. For measuring dehydrogenase activity, 1 g of dry soil was incubated with 1 mL of 0.4% (w/p) Triphenyltetrazolium Chloride (TTC) in Tris-HCl (0.1 M) buffer at pH 7.6 for 16 h at 25°C. The Triphenylformazan (TPF) formed was extracted with 5 mL of acetone by shaking vigorously for 2 h and filtered. TPF was measured spectrophotometrically at 546 nm.

Results

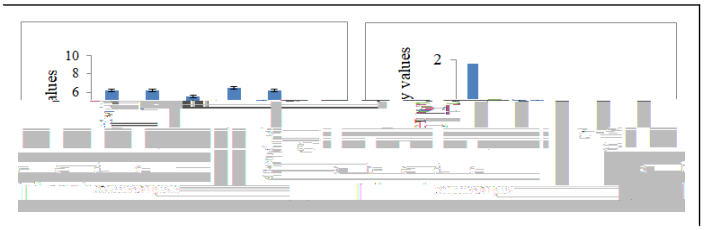
Sample collection and sampling sites

Soil samples were collected from different regions of agriculture campus which from the past several years have agricultural activity. The soil samples in which crops were grown and the pesticides used for isolation of microorganisms from those soil samples is given in the (Table 1).

S.No	Pesticide used	Crops under cultivation
1	Dicofol	Cauliflower
2	2,4 DDT	Pigeon pea
3	Metachlor	Pepper
4	Diuron	Cardamom

Table 1: Sampling sites and crops under cultivation.

Electrical conductivity of soil ranges from 0.10 to 1.95 dS/m. pH was found in between 5 to 7, indicating the nature to be acidic (Figure 1). The ambient temperature was 30.0 ± 0.5°C throughout the study period.



Dechlorination of DDT has been reported by *Enterobacter aerogenes*, Escherichia coli, and *Kleibsiella pneumonia* under anaerobic conditions. This genus was also reported to have the ability of degrading organophosphorous insecticides like phosphonate, glyphosate and chlorpyrifos.

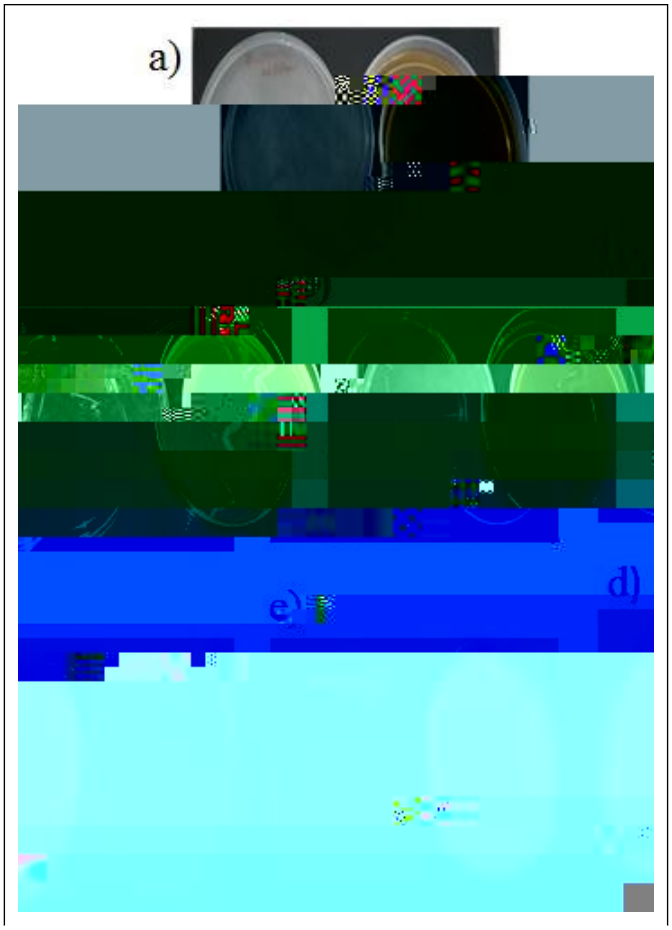


Figure 2: Bacterial growth images where a) control (without dicofol pesticide); b, c, d, e) represents dicofol pesticide +agar agar).

Conclusion

Based on the sequence characterization and analysis the organism capable of degrading Dicofol and 2, 4 DDT pesticides was identified as Leclercia adecarboxylata and Pseudomonas aeruginosa. The given bacterial sample was isolated from agriculture university field which had cauliflower as the given crop. Previous studies have shown endosulfan degradation, organ phosphorous insecticide degradation such as diazinon. Living organisms that resides in the soil break down the organic materials and soil bacteria thrive at a pH of 6.3 to 6.8.