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Short Communication

Activity Assay of Local Bacterial Culture in Biological Treatment of Organochloropesticides

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The present study examined the efficacy of bacterial isolates as mixed culture to remove pesticide residues in situ. Pesticides are mostly non-selective, widespread applied, possess toxic properties, and in some cases are very refractory. These features entitle pesticide to be one of the most fearful group of substances, as far as biological communities and humans are concerned. The search aims to elaborate the potential application of bacterial isolates in decontamination of agricultural soils, which have been polluted with doses of pesticides (propachlor) through the process of biodegradation. Degradation rates of five concentrations of pesticides were studied. Changes in depth were probably caused by interactive effects of changes in soil microbial activities and in organic matter content (and thus pesticides sorption) in the different soil layers. Results showed that the concentration of pesticide introduced into the soil drastically affected the growth phase of the soil microbes), which increased from $9*10^6$ cfu/g in the control soil to $6*10^8$ cfu/g in the pesticide-contaminated soil after two weeks of incubation, but subsequently decrease to $7*10^3$ cfu/g on the last week. The rate of biodegradation was faster within the first two weeks, result recording 88% reduction in pesticide residue.

Keywords: Chlorinated organ pesticides, Biodegradation, Bacteria, Soil.

INTRODUCTION

Biodegradation is an eco-friendly, cost effective, highly efficient approach and can be considered as a superior alternative to physical and chemical methods which are not only technically laborious and costly, also are not sufficient to completely degrade organic toxins (12). In the last decade In situ biodegradation possess have focused on the role which microorganism may play alleviating environment pollutants (2). The use of bacteria for the degradation and detoxification of numerous toxic chemicals such as pesticides is an effective tool to decontaminate the polluted sites (1, 16).

Bacteria mainly of the genus *Alcaligenes, Pseudomonas, Flavobacterium* and *Rhodococcus* are the potent degraders of pesticides (5, 16, and 13). *Actinomycetes* also show impressive ability to biodegrade pesticides. Isolation of indigenous bacteria capable of metabolizing pesticides provides environmentally friendly means in situ detoxification (15). Decontamination of polluted areas is the need of the hour. The conventional means (physical & chemical methods) of degradation of toxic recalcitrant chemicals are not only expensive, labor intensive, less efficient but may harm the natural micro-environment of soil as well (8,9).

Pesticides are organic compounds manufactured and used for pest control. When pesticides are dispensed in the environment, they become pollutants. Remediation of environmental pollution is caused by both excessive and continuous use of pesticides & begins when these compounds enter the environment by various means such as accidental spills, direct applications, residues from cleaning of containers, state of equipment used and methods used to apply the products. The quality of soils, ground water, inland and coastal water, and air are all affected by pesticides contamination, (11).

The rate at which a pesticide is degraded in both the surface and sub-surface soils is an important factor in determining the ground water contamination. The present study examined the efficacy of bacterial isolates as a mixed culture to remove pesticide residues in situ. Pesticides are mostly non-selective, widespread applied, possess toxic properties, and in some cases are very refractory. These features entitle pesticide to be one of the most fearful group of substances, as far as biological communities and humans are concerned. Microbial degradation of pesticides applied to soil is the principal mechanism which prevents the accumulation of these chemicals in the environment, (3, and 7).

MATERIALS AND METHOD

Soil sample: model of soil were brought from AL-Jadirya gardens and at depth of (10-30) cm. Sample was dried aerobically and sift sieve with holes 2mm and stored in plastic containers until use.

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Table 1: Rate of changes of Soil Physical Characteristic with time of the Biodegradation of Pesticide

Parameter	Characteristic of soil before treatment	After treatment with Pesticide
pH	5.8	6.9
conductivity	183.7	162.8
Nitrogen (ppm)	0.16	0.31
Phosphorus (ppm)	0.121	5.202
Organic matter(ppm)	4.04	5.04
Total organic carbon(ppm)	6.64	7.64
Silt	1.23	1.30

Table 2: Influence of time of incubation on growth rate of soil microbes and pesticide degradation

Time/days	TOC ppm	Dry weight of pesticide mg/kg	Viable count/ cfu
0	650	95.0	$9*10^6$
7	502	6.3	$8*10^7$
14	67	4.3	$6*10^8$
21	65	4.0	$5*10^6$
28	60	3.3	$2*10^6$
35	54	3.0	$4*10^4$
42	50	3.0	$7*10^3$

Table 3: Rate of change of TOC and viable count with concentration of propachlor.

Substrate concentration mg\l	TOC ppm	Viable count cfu	Pesticide conception %
100	86.2	7*104	54
75	56	5*105	64
50	53	7*106	72
25	50	9*107	88
12	43	2*106	60

Some physical and chemical soil properties were estimated based on the methods described in, (1).

Pesticide description

2-Chloro-N-Isopropylacetanilide (Propachlor) is an acetamide, herbicide widely used to protect corn, onion, cabbage rose bushes and ornamental plant. With a molecular weight of 135.592 g \mol and its molecular formula is $C_5H_{10}ClNO$.

Preparation of inoculum

Streptomyces albus and *Rhodococcus* was inoculated in mineral medium broth containing 25 mg/l of propachlor in 100ml volume in 250 ml Erlenmeyer flask, incubated at 30° c for 7 days at 100 rpm in a shaking incubator.

Soil treatment with organ pesticide used in the experiment

Soil was treated with five levels of the pesticide (100, 75, 50, 25 & 12) mg/Kg. Pesticide different concentration mentioned are mixed with soil volume half Kg each treatment.

Extraction

Discard any foreign objects from the soil sample such as sticks and leaves. Air dry the sample at room temperature for 48 hours in a glass tray, add dichloromethane and dry this sample at 105°c and into desiccators before weighing, then calculate the % dry weight.

RESULTS AND DISCUSSION

Results showed that the concentration of pesticide introduced into the soil drastically affected the growth phase of the soil microbes, which increased from $9*10^6$ CFu/g in the pesticide-contaminated soil on the first day. Optimum growth $6*10^8$ was gained after two weeks, but subsequently decrease to $7*10^3$ CFU/g at the last week.

The rate of biodegradation was faster within the first two weeks, results recording 88% reduction in pesticide residue. According to the rate of degradation of chemicals, pesticides can be categorized as sensitive or tolerant to decomposition. Their destruction might be occurred under exposing to the normal atmospheric conditions or by biological activity of the soil microorganisms such as *Pseudomonas, Flavobacterium, Alcaligenes, Rhadococcus, Gliocladium, Trichoderma* and *Penicillium*. These microorganisms use the pesticides as their carbon and energy sources (1).

Conductivity value recorded 183.7 in the control soils due to the presence of soluble polar mobile solute in the soil. Particle size distribution of the soil was unaffected as with pesticide from (6.64 to 7.64 ppm). While a significant increase in pH, nitrogen and phosphorus was recorded as seen in table (1). The silt was (1.23-1.30). The pH for unpolluted soil fell within the range between (5-7) which is suitable for most good agricultural soil. A reduction in conductivity from183.7 to 162.8. Increasing in total organic carbon were observed in treated soil.

The concentration of propachlor introduced into the soil affected the growth rate of the microorganisms. It seems to be associated with the adaptation to the pesticide environment. An increase in viable count of the pesticide utilizing bacteria

was recorded in the first two weeks but thereafter decreased progressively as evident in table (3), with increasing concentration of pesticide. Availability of sufficient nutrients and carbon source explain the increase of bacterial cell accounts: The six weeks study on the bioremediation of propachlor contaminated soil showed a reduction of 88% of the substrate with initial concentration of 25 mg \Kg after two weeks of treatment.

CONCLUSIONS

1. Establishment of database for the pollution status existing in Iraq, Include data honest about pesticide residue and other contaminants such as heavy metal in environmental components and compared level allowable limits internationally.
2. Subjecting Pesticides used in all their uses for the control and development of laws for ways to deal with them and how to get rid of pesticide residues.

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