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Chlorpyrifos: It's bioremediation in agricultural soils

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Abstract
Organophosphorous pesticides are widely used in agriculture to control major insect pests. Chlorpyrifos is one of the major organophosphorous pesticides which have been used since 1960s, to control insects including termites, beetles. The widespread use of these pesticides is hazardous to the environment and also toxic to mammals. Moreover, given the persistence and toxicity of chlorpyrifos towards life forms, it is essential to remove the same from the environment. Among many physical, chemical, and biological methods for the removal of organophosphorus pesticides from ecosystems, biodegradation is preferred because of its environmental compatibility and cost-effectiveness. Considering the havoc created by chlorpyrifos in food chain as well as in the ecosystem, it is urgent to develop a bioremediation technology for minimising its side effects. Keeping this in view, the present article is aimed to provide a detailed study on chlorpyrifos and witness the significant research conducted by various researchers for its bioremediation.

Keywords: chlorpyrifos, biodegradtion, bioremediation, organophosphorous pesticides

Introduction
Bioremediation is the use of living organisms to minimize or eliminate the environmental hazards resulting from accumulation of toxic chemicals and other hazardous wastes. It is an innovative technology that is frequently being chosen for the cleanup of sites on the National Priority List (NPL). Recent research is expanding the capabilities of this technology, which, along with its generally lower cost, has led to bioremediation becoming an increasingly attractive cleanup technology. Bioremediation is a promising alternative to physico-chemical methods of remediation, because it is less expensive and can selectively achieve complete destruction of organic pollutants (Alexander 1999) [8]. The use of microorganisms for the degradation and detoxification of numerous toxic xenobiotics, especially pesticides, proved to be an efficient tool to decontaminate the polluted sites in the prevailing environment (Mervat 2009) [63]. Bioremediation methodology to treat xenobiotics such as pesticides in soil has gained considerable attention owing to its ecofriendliness and has been employed successfully in many countries (Enrica 1994, Ritmann *et al.* 1988) [26, 82]. Pesticides in soil and water can be biodegraded and is the primary mechanism of pesticide breakdown and detoxification in many soils (Surekha *et al.* 2008) [96]. Conventional approaches (e.g. landfilling, recycling, pyrolysis and incineration) for the remediation of contaminated sites are inefficient, costly and may lead to the formation of several toxic intermediates (Sayler *et al.* 1990) [83]. Thus, biological decontamination methods are preferable to conventional approaches because, in general, microorganisms degrade numerous environmental pollutants without producing toxic intermediates (Pieper and Reineke 2000, Furukawa 2003) [73, 30].
Biodegradation and bioremediation are matching processes to an extent that both of these are based on the conversion or metabolism of pesticides by microorganisms. The difference between these two is that, the biodegradation is a natural process whereas bioremediation is a technology. In bioremediation, we use microbes to degrade the pesticides *in situ*. A successful bioremediation technique requires an efficient bacterial strain that can degrade largest pollutant to minimum level. Adequate rate of biodegradation is required to attain the acceptable level of pesticide residues or its metabolites at contaminated site in a limited time frame.
Degradation of endosulfan by three bacterial species namely *Staphylococcus* sp., *Bacillus circulans*-I, and *Bacillus circulans*-II, was studied by Kumar and Philip (2006) [49] both in mixed isolate and pure isolate. In mixed isolate, after four weeks of incubation degradation of 71.82 ± 0.2 per cent and 76.04 ± 0.2 per cent of endosulfan in aerobic and facultative anaerobic conditions, respectively was observed. In pure isolate a degradation potential of 93.3

± 0.15 per cent, 93.4 ± 0.15 per cent and 89.95 per cent of α endosulfan and 75.96 ± 0.05 per cent, 76.73 ± 0.05 per cent and 82.9 ± 0.05 per cent of β endosulfan by strains *Bacillus circulans*-I, *Bacillus circulans*-II and *Staphylococcus* sp. respectively was observed after 14 days of incubation.

Genetic studies of microbial degradation indicated that plasmid is the main place for the gene of interest throughout the microbial community. Esd gene that catalyzes the oxygenation of β-endosulfan to endosulfan monoaldehyde to endosulfan hydroxyether was reported by Sutherland *et al.* (2002) [39] in plasmid of *Mycobacterium smegmatis.*

There is a major problem when doing in-situ remediation which is the nature of the organisms. Most bioremediation organisms do their job under environmental conditions that suit their needs. Consequently, some type of environmental modification is needed to encourage the organisms to degrade or take up the pollutant at an acceptable rate. When using bacteria and fungi, it is usually necessary to add fertilizer or oxygen to the material containing the pollutant. This can be disruptive to other organisms when done in situ. In situations where simple compounds and metals are being taken up it is likely that these pollutants are at toxic levels for the organisms. Overall, the organisms do not always live as well on the pollutant diet as on other nutrients found more commonly in their environment.

Pesticide scenario in India

The use of pesticides to eradicate the harmful pests is not a new concept, but still the best option so far, because these cause rapid control measures. Pesticides constitute the key control strategy for crop pests and disease management and have been making significant contribution towards improving the crop yields per hectare. The use of pesticides has made an enormous contribution towards increasing the yield and quality of world's food supply, improving public health etc. (Knowles and Vander 2008) [47]. More than 890 synthetic chemicals and 20,700 products are available in the world market and the global usage of pesticides is 2.5 billion kg annually (Stenerson 2004) [94]. Indian pesticide market is largest in Asia and 12th largest in the world with a value of US $ 0.6 billion, which is 1.6 percent of the global market. However, per hectare consumption of pesticides is very low at 0.22 kg, when compared to developed countries. In India, 229 pesticides are presently registered for use and 69 technical grade pesticides are manufactured indigeneously and average growth rate of pesticides in India is 12.5% (Arora *et al.* 2011) [11]. In current agricultural practices, farmers apply pesticides (including herbicides, insecticides and fungicides) of various chemical groups indiscriminately to control the pests and phytopathogens which are detrimental for crop productivity. Consequently, a large amount of pesticides reach the soils and persists for long periods and destabilizes the soil-ecosystem (Ahmed *et al.* 2009) causing harm to plant growth promoting rhizobacteria (PGPR) and eventually the plant growth (Guo *et al.* 2007, Fox *et al.* 2007) [38, 28]. In addition, organic pesticides applied to soil may be used as substrates by the tolerant microorganisms and undergo degradation, resulting in the formation of new compounds which may be far more deleterious to the growing plants and the parent molecule (Ahemad and Khan 2011) [4]. Pesticides and their degraded products in soils interact with PGPR including rhizobia and cause DNA, protein, oxidative or membrane damage (Pham *et al.* 2004) [74].

Chlorpyrifos

Currently, among the various groups of pesticides that are being used the world over, organophosphorus group forms a major and the most widely used group. Organophosphate (OP) pesticides were first developed in Germany by Schrader in 1930 during World War II in the form of tetraethyl pyrophosphate as a by product of nerve gas development. Organophosphates are acutely toxic and act by inhibiting acetylcholine-esterase, an important enzyme in the nervous system (Kanekar *et al.* 2004) [45]. On exposure to organophosphates, the enzyme is unable to function hence causing accumulation of acetylcholine, which interferes with the transmission of nerve impulses at the nerve endings.

Chlorpyrifos (O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is a broad-spectrum moderately toxic organophosphate insecticide, acaricide and nematicide and is widely used in the prevention of both agriculture-related pests and urban public health pests (Fang *et al.* 2006) [111]. It has been used to control cockroaches, fleas, mosquitoes and termites and has also been an active ingredient in some pet flea and tick collars. Chlorpyrifos is used on agricultural food and feed crops, cattle ear tags, golf course turf, industrial plants and vehicles, non-structural wood treatments including processed wood products, fence posts and utility poles. Organophosphates (OP) are generally regarded as safe for use on crops due to their relatively fast degradation rate, which varies as a function of microbial components, pH, temperature, hydrolysis, photolysis and other factors and is effective by contact, ingestion and vapour action but not systemically active.

Chlorpyrifos is a white crystal-like solid with a strong odour. It may also be applied to crops in a microencapsulated form. It has low solubility in water (2 mg/ L) but is readily soluble in most organic solvents so it is usually mixed with oily liquids before it is applied to crops or animals. It has a high soil sorption co-efficient, has acute oral LD50; 135–163 mg kg/L for rat and 500 ppm for guinea pig and is stable under normal storage conditions (Racke 1993) [75].

Chlorpyrifos, a pesticide that can easily enter the human food chain has more victims to its credit than carcinogenic air pollutants such as polycyclic aromatic hydrocarbons (PAHS). A study conducted by researchers from US based Columbia University found a strong link between prenatal exposure to Chlorpyrifos and low birth weight and smaller head size of infants. Several studies correlate the smaller size of the head with lower Intelligence quotient (IQ) and poor functioning (Bhagobaty *et al.* 2007) [14].

Extensive use of chlorpyrifos contaminates air, ground water, rivers, lakes, rainwater and fog water. The contamination has been found up to about 24 kilometres from the site of application. Symptoms of acute poisoning include headache, nausea, muscle twitching and convulsions and even death in extreme cases. Human birth defects have also been associated with exposure to chlorpyrifos and its products. It also affects the male reproductive system. Chlorpyrifos is toxic to a variety of beneficial arthropods including bees, ladybird beetles and parasitic wasps. It kills fishes at concentrations as low as a few parts per trillion. Birds are also susceptible with effects ranging from reduced weight of nestlings, deformities and death. In plants there have been reports of delayed seedling emergence, fruit deformities and abnormal cell division upon prolonged exposure to chlorpyrifos (NCAP 2000) [72].

The use of chlorpyrifos has been vastly restricted in US and some European countries, even for agricultural purposes. However, it is still widely used in developing countries like India, where in the year 2000, it was the fourth highest consumed pesticide after monocrotophos, acephate and endosulfan. (Ansaruddin and Vijayalakshmi 2003) [10].

Environmental fate of Chlorpyrifos

Once a pesticide enters the environment, it is transported from one compartment to another and its transformation within a given compartment occurs. These processes, in turn, mediate biological significance by determining the quantity of pesticides that will be present in anyone compartment for a certain period of time. The sorptive behaviour of chlorpyrifos and its major metabolites in aqueous (Felsot and Dahm, 1979) [27], soil or sediment systems were extensively investigated (Macalady and Wolfe 1985, Kanazawa 1989) [57, 44]. For chlorpyrifos, adsorption kd values ranged from as low as 13.4 ml/g to as high as 1862.0 ml/g, while the TMP (3,5,6-trichloro-2-methoxypyridine) metabolite of chlorpyrifos had an affinity for sorption lower than that of chlorpyrifos itself. The TCP (3,5,6-trichloro-2-pyridinol) metabolite had a moderate affinity for sorption.

Racke (1993) [75] studied the environmental fate of chlorpyrifos found that due to high sorption rate of chlorpyrifos, its movement through and over the soil profile was limited. He further reported chlorpyrifos to be relatively immobile vertically in soil so it had not proved to be a groundwater contaminant. He recorded, low, surface runoff and erosion mobility of chlorpyrifos, less than 0.3% of soil surface application had been observed to move even under the heaviest simulated precipitation conditions. Chlorpyrifos had an intermediate vapour pressure (2 x 10(-5) mm Hg, 25°C), and under some conditions volatility was a significant mechanism of dissipation. This was especially true for plant foliage, from which it was the major means of loss, to some extent from water surfaces, and to a lesser degree from moist soil surfaces. He further reported that chlorpyrifos is a degradable compound, and both abiotic and biotic transformation processes effect its degradation within environmental compartments and the major pathway of transformation involves cleavage of the phosphate ester bond to form 3,5,6-trichloro-2-pyridinol (TCP).

Environmental fate of the dissipation and distribution of chlorpyrifos residue in citrus orchard soil was studied by Redondo *et al.* (1997) [80] who further reported that the pesticide concentration was always the highest in the upper layer, and its degradation half life was 10 days.

Similarly the fate of chlorpyrifos in seawater was examined by Schmimmel *et al.* (1983) [85] and they reported the Chlorpyrifos half-life of less than 2 days and nearly 63 per cent of it had volatilised.

Chlorpyrifos mobility in sprinkler irrigated fields was examined by Wauchope *et al.* (1991) [107]. They found that when chlorpyrifos was applied at the rate of 0.56 kg ai/ha, the average residuction in the top 3 cm and 3-15 cm level of soil were 0.28 ppm and 0.05 ppm respectively.

Environmental behaviour, movement, distribution, persistence and runoff of chlorpyrifos under field conditions were studied by Konda and Pasztor (2001) [48]. They detected the chlorpyrifos at a depth of 5-20 cm during the whole experimental interval of 5 months.

Chlorpyrifos was relatively stable and had lower water solubility as reported by Murray *et al.* (2001) [69]. They reported average half life of chlorpyrifos of 385 days, with retarded degradation rate when an initial soil concentration of chlorpyrifos was 1000 ppm for termite control as compared with chlorpyrifos concentration of 100 and 10 ppm in soil with average half lives of 155 and 41 days.

Chlorpyrifos (14C) fate in the tropical estuarine environment was investigated by Nhan *et al.* (2002) [71] who reported that chlorpyrifos rapidly adsorbed onto sediment. The accumulation of chlorpyrifos in flora and fauna was maximum i.e. 5.8 per cent and 2.2 per cent respectively of the initial chlorpyrifos activity, observed on second and third day after application. It was also observed by Gamon *et al.* (2003) [31] during a study on distribution of pesticide in a soil profile that the pesticide was always higher in the top soil

Chlorpyrifos underwent a number of degradation pathways while in the environment and its metabolites were susceptible to photo-degradation, with a half-life of approximately 3 days and in the presence of hydroxyl radicals in the atmosphere the half-life was lowered to 6 h. Upon entering surface water, Chlorpyrifos degradation was associated with abiotic hydrolysis or photosensitized oxidation. In soil, photo-degradation played role in hydrolysis, dechlorination, and oxidation of chlorpyrifos. However, in indoor environments, chlorpyrifos could persist for several months because of the relative lack of sunlight, water, and soil microorganisms that contributed to its rapid degradation in the outdoor environment (ATSDR 1997) [12].

In animals and human body, chlorpyrifos was oxidized to oxon form, which is regarded as the principal toxic metabolite, and was responsible for inhibition of cholinesterases. Chlorpyrifos-oxon was either enzymatically or spontaneously hydrolysed to form the diethylphosphate and 3,5,6-trichloro-2- pyridinol (TCPy). In addition to the formation of chlorpyrifos-oxon, chlorpyrifos was oxidized via cytochrome (s) P-450 to an unstable intermediate that spontaneously hydrolysed to diethylthiophosphate and TCPy. These metabolites were excreted in the urine, or form glucuronide and sulfate conjugates, which were also excreted in the urine (Eaton *et al.* 2008) [23].

Mode of action

Toxicological properties and mode of action of chlorpyrifos was studied by Caroline (1994) [19] who found that chlorpyrifos was directly toxic to the nervous system. It was transformed inside animals to chlorpyrifos-oxon which was about 3000 times more potent against the nervous system as chlorpyrifos itself. Like all organophosphates, chlorpyrifos and chlorpyrifos-oxon killed insects and other animals, including humans, because of their toxicity to nervous system. They inhibited enzyme, acetyl cholinesterase (AChE) that broke down acetylcholine, a chemical involved in transmitting nerve impulses across the junctions between nerves. Without AChE functioning, acetylcholine accumulated, produced rapid twitching of involuntary muscles, convulsions, paralysis, and ultimately death. Chlorpyrifos exposure was also shown to inhibit enzymes other than AChE. It impeded respiration (production of energy within a cell) in the livers of laboratory animals. This resulted from the effect of chlorpyrifos on the activity of ATPase, an enzyme important in cellular respiration.

Isolation of insecticides tolerant bacteria from soil

A bacterial strain *Bacillus licheniformis* capable of utilizing chlorpyrifos as the sole carbon sources and energy was isolated by Zhu *et al.* (2010) [113] from soil samples obtained

from Wuqi Farm in Shanghai, China which were exposed to chlorpyrifos for more than 10 years.

A chlorpyrifos resistant bacterium was isolated by Ajaz *et al.* (2005) [6] from cotton cultivated field Pakistani soils. Out of twenty isolates three chlorpyrifos hyper resistant bacteria were finally selected for follow up studies. The screening was performed by replica device. Three isolates viz., *Pseudomonas putida*, *Aeromonas* sp., and *Klebsiella* sp., were found resistant to 2mg/mL, 4mg/mL and 8mg/mL of Chlorpyrifos while *Ps. putida* and *Aeromonas* sp., also resisted the 10mg/mL and 20mg/mL doses.

Bacterial strains responsible for the biodegradation of profenofos in a soil from Hubei province of central China was isolated through enrichment technique by Malghani *et al.* (2009) [59]. Two pure bacterial isolates, were isolated, one of them showed 96% similarity to the 16S rRNA gene of a *Pseudomonas putida* unlike other gave 99% similarity to the 16S rRNA gene of *Burkholderia gladioli*.

A pentachlorophenol (PCP) degrading *Acinobacter sp.* was isolated by Sharma *et al.* (2009) [89] from a effluent discharge site. Sediment sample was collected from the effluent discharge site of a pulp and paper mill at Nainital, Uttarakhand state. Of the three isolated bacterial strains, one pure isolate of *Acinobacter* sp., ISTPCP-3 with highest PCP degradation efficiency was selected for further study.

Poly-aromatic hydrocarbon (PAH) degrading bacteria was isolate by Al-Thani *et al.* (2009) [9] from contaminated soil samples collected from the industrial zone at Umm-Saied city, state of Qatar. Isolation was done by enrichment using naphthalene, phenanthrene or anthracene as the sole source of carbon and energy. Molecular identification of the isolates based on partial 16S rDNA gene sequences assigned them to *Pseudomonas geniculata* and *Achromobacter xylosoxidans*, respectively. This study indicated that the contaminated soil samples contain a diverse population of PAH-degrading bacteria and the use of soil associated microorganisms had the potential for bioremediation of PAH contaminated sites.

Four diazinon-degrading bacteria were isolated from agricultural soil by using an enrichment technique by Abo-Amer and Aly (2011) [1]. Based on phylogenetic analysis isolated strain DI101 was identified to belong to the *Serratia marcescens* group and the ability of the strain to utilize diazinon as a source of carbon and phosphorus was investigated under different isolation conditions. The further reported that DI101 strain was able to completely degrade 50 mg/l diazinon in MSM within 11 days with a degradation rate of 0.226 day^{-1}.

Chlorpyrifos degrading bacteria, *Serratia marcescens* was isolated by Xu *et al.* (2007) [108] by an enrichment isolate technique using mineral salts medium and cultured it in a continuous reactor system. They reported complete disappearance of 50 mg chlorpyrifos l^{-1} caused by *Serratia marcescens* within 4 days and TCP was detected as the only major metabolites of chlorpyrifos degradation.

A soil bacterium, *Providencia stuartii* capable of utilizing chlorpyrifos as sole carbon source was isolated by Rani *et al.* (2008) [78] through selective enrichment on mineral medium containing chlorpyrifos. The individual bacterial colonies that grew on the medium were sub-isolated onto mineral agar containing chlorpyrifos until pure cultures were obtained. They reported that *P. stuartii* strain MS09 utilized chlorpyrifos in Luria-Bertani broth containing different concentrations of chlorpyrifos at 50 -700 mg/L. However, the optimum concentration that supported bacterial growth over 24 h was found to be 50 – 200 mg/L chlorpyrifos.

A chlorpyrifos utilizing bacterium, closest to members of the *Bacillus firmus* group was isolated by Agus Sabdono (2007) [3] from coral surface of Teluk Awur North Java Sea. They recorded the organism to utilize chlorpyrifos up to 25 mg l^{-1}. On the basis of morphological features, colonies were randomly picked and purified by making streak plates. They recorded the organism to utilize chlorpyrifos up to 25 mg l^{-1}.

Four chlorpyrifos tolerant bacteria were isolated by Bhagobaty and Mallick (2008) [15] from waste water irrigated agricultural soils from industrial area of Western Uttar Pradesh, using enrichment isolate technique by using minimal salt media. The isolates showed promising capability to utilize chlorpyrifos as a carbon source for their growth.

A chlorpyrifos-methyl degrading bacterium, KR100, related to members of the *Burkholderia cepacia* group was isolated by Kim amd Ahn (2009) [46] from a Korean rice paddy soil on mineral salts basal medium for enrichment culture of agricultural soils and liquid isolate of isolated bacteria. Peptone–tryptone–yeast extract–glucose (PTYG) agar medium was used for bacterial purification and colony production for the PCR. They reported Strain KR100 to hydrolyze CM to TCP which was the sole source of carbon for its growth. They further reported the isolate to degrade chlorpyrifos, dimethoate, fenitrothion, malathion, and monocrotophos at 300 µg/ml. Five cypermethrin utilizing bacteria were isolated by Murugesan *et al.* (2010) [70] from brinjal cultivated soil using enrichment technique, with varying concentrations of cypermethrin in the medium.

Morphological and biochemical characterization of chlorpyrifos tolerant bacteria

A chlorpyrifos utilising bacterial strain was identified as *Bacillus licheniformis* by Zhu *et al.* (2010) [113] based on morphological, physiological and biochemical properties, which had concordance with the result of 16S rRNA analysis made by TaKaRa Biotechnology (Dalian) Co., Ltd. The strain ZHU-1 was straight or curvulate bacillus, 0.7 - 0.8 × 2.0 - 2.5 um in size, Gram-positive, motile, facultative anaerobe, central spore or subterminal spore, and formed opaque and rough colonies on Nutrient Broth plates. It was positive in tests for catalase, Voges- Proskauer, gelatin liquefaction and starch hydrolysis, but negative for indole test. The optimal temperature and pH for the growth of ZHU- 1 were 35°C and pH 7.5 respectively.

A chlorpyrifos and TCP degrading bacteria was isolated and identified as *Serratia marcescens* by Xu *et al.* (2007) [108]. The bacterial strain (coded TCR) was Gram-negative, aerobic, catalase and oxidase positive was motile lustrous red rod (0.6-0.8 mm by 1.0-2.0 mm) that formed occasional filaments.

Four bacterial isolates isolated by Rani *et al.* (2008) [78] were subjected to morphological, cultural and biochemical studies for the identification. The four isolates were identified as *Providencia stuartii*, *Serratia marcescens*, *Klebsiella oxytoca* and *Bacillus subtilis*, respectively, according to Bergey's Manual of Systematic Bacteriology. *Providencia stuartii* displayed the highest Chlorpyrifos-hydrolyzing capability and biochemically it was indole, catalase, citrate, MR and phosphatase positive but urease negative.

Four Chlorpyrifos utilizing bacteria were isolated by Bhagobaty and Mallick (2008) [15] that utilized Chlorpyrifos as a carbon source for their growth. Biochemically it utilized carbohydrate, amino acid and indicated that they belong to the genus *Pseudomonas.* Thin layer chromatography and tetrazolium reduction assay showed that the strains were capable of degrading chlorpyrifos.

A Chlorpyrifos-methyl (CM) degrading bacterium, KR100 isolated by Kim amd Ahn (2009) [46] from a Korean rice paddy soil was further tested for its sensitivity against eight commercial antibiotics. Based on morphological, biochemical tests and molecular characteristics, this bacterium was shown to be most closely related to members of the *Burkholderia cepacia* group. The bacterium was nonmotile, Gram negative and non-sporeforming bacterium with a small straight rod shape (0.3-0.5 µm in length and 0.2-0.3 µm in width). Biochemically, the strain was catalase positive, oxidase negative, and urease positive. It metabolized glucose oxidatively and did not reduce nitrate. This organism was able to utilize for growth the following compounds as the carbon source: fructose, glucose, glycerol, mannitol, mannose, melibiose, raffinose, sorbitol, and xylose. It was not able to grow on arabinose, erythrose, sorbose, and Simmon's citrate.

Four diazinon-degrading bacteria were isolated by Abo-Amer and Aly (2010) from agricultural soil by using an enrichment technique. These strains were morphologically and biochemically characterized and phylogenetic analysis based on 16S rDNA sequencing indicated that one out of the four isolates, belonged to the *Serratia marcescens* group

A chlorpyrifos and 3,5,6-trichloro-2 pyridinol (TCP) tolerant bacterium *Alkaligenes faecalis* strain DSP3 isolated by Yang *et al.* (2005) [110] was further characterized morphologically and biochemically and was found to be gram negative, rod shaped, catalase and oxidase positive.

Five cypermethrin utilizing bacteria isolated by Murugesan *et al.* (2010) [70] were identified and characterized using morphological, cultural and biochemical tests as described by Colins and Lyne (1985) up to the stage of genus. On the basis of morphological, cultural and biochemical characteristics, the bacterial isolates were identified as a member of the genus *Pseudomonas aeruginosa*, *Klebsiella* Sp, *Escherichia coli*, *Bacillus* Sp, and *Corynebacterium* Sp according to, Bergey's Manual of Determinative Bacteriology (1994) [17].

Pesticide (atrazine and oxamyl) tolerant bacteria isolated by Aguiree *et al.* (2010) were further studied for their biochemical profiles using API 20E® strips. API 20E® strips included enzymatic tests for fermentation or oxidation of glucose, mannitol, inositol, sorbitol, rhamnose, saccharose, melibiose, amygdalin and arabinose, along with nitrate reduction to nitrite and nitrate reduction to nitrogen gas. API 20E® strips also tested for the presence of β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urease, tryptophan deaminase, indole production, acetoin production (Voges-Proskauer) and gelatinase.

Out of the twenty Chlorpyrifos resistant bacteria from Pakistani soils isolated by Ajaz *et al.* (2005) [6] three Chlorpyrifos hyper resistant bacteria were finally selected for follow up studies. The screening was performed by replica device. For the identification of these bacterial isolates, the characters such as morpho-colonial bases, utilization of various carbohydrates and synthesis of enzymes i.e. oxidase, catalase, esculin, nitrate, urea and gelatin hydrolysis test were studied and the isolates identified were *Klebsiella* sp, *Pseudomonas putida* and *Aeromonas* sp. all of which were Gram negative rods and catalase positive.

Five chlorpyrifos degrading bacterial strains from pesticide contaminated soil in Egypt isolated by Awad *et al.* (2011) [13] were identified as *Pseudomonas stuzeri*, *Enterobacter aerogenes*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas maltophila* and *Pseudomonas vesicularis* on the basis of molecular genetics and biochemical characteristics. All the strains were Gram negative, methyl red, catalase, oxidase and citrate positive and Voges-Proskauer negative. All were motile and none of them had spores.

Degradation of chlorpyrifos

The processes by which pesticides are transformed or degraded in environmental compartments and the factors that modulate the kinetics are critical from both pest control efficacy and non target organism toxicity points. Both abiotic and microbiological transformations of chlorpyrifos have been reported and are found to contribute significantly to its degradation.

Chemical degradation of Chlorpyrifos

Half lives of 120 days and 53 days of chlorpyrifos at pH values of 6.1 and 7.4 respectively was reported by Freed *et al.* (1979) [29]. Further it was reported by Meikle and Youngson (1978) [62] that the rate of hydrolysis increased on an average 3.5 fold for each 10°C rise in temperature. Brust (1966) [18] first identified 3,5,6-trichloro-2 pyridinol (TCP) as a hydrolytic metabolite and hydrolysis of chlorpyrifos was studied by Macalady and Wolfe (1983) [56] in aqueous buffers and polar solvent mixtures. The only products they detected at pH 9.7-12.9 were TCP and diethyl thiophosphate.

The chlorpyrifos photodegradation on glass and natural surfaces was reported by Walia *et al.* (1988) [105]. They found that upon exposure to low pressure mercury lamp irradiation, a degradation half life of chlorpyrifos was 13.7 days on glass, 17.2 days in moist soil and 52.6 days on the leaf surface of *Polystichum setiferum*. On dry soil surface chlorpyrifos was reported to be less susceptible to photolytic degradation (Getzin 1981b) [35].

Several studies reported that TCP was formed as a photodegradative metabolite of chlorpyrifos under aqueous conditions (Smith 1966) [93] and in surface soil (Walia *et al.* 1988) [105]. Meikle *et al.* (1983) [61] found that at least five unknown metabolities were formed from exposure of chlorpyrifos in aqueous solution to an artificial light source. A sandy loam was treated with chlorpyrifos at 3 ppm by Tashiro and Kuhr (1978) [99] and observed a degradation half life of 7-16 days. Degradation half lives of less than 1 week and 17 weeks in a mineral and an organic soil respectively was noted by Miles *et al.* (1979) [65], that had been treated with 10 ppm chlorpyrifos. Degradation in silt loam and clay loam soils treated with 16.7-20 ppm was studied by Getzin (1981a) [34].

The study on dissipation of various chlorpyrifos formulations in a mineral and an organic soil was examined by Chapman and Chapman (1986) [21] and reported the half lives was 31.5-49.5 days for mineral soil and 14.1-38.5 days for the organic soil. Similarly degradation in several soils treated with 1 ppm of technical chlorpyrifos was investigated by Racke *et al.* (1990) [76] and found that degradation half lives varied greatly and ranged from 3.8 to 43 days. Further work on degradation of chlorpyrifos in air dried soils was conducted by Getzin (1981b) [35] who reported the detection of TCP as the major metabolite. After 48 hrs of soil incubation at 30°C, 47.9 to 67.9 per cent of the applied chlorpyrifos was found to be converted to TCP.

Degradation of 2 µg/g of chlorpyrifos in Turkish soil with 74 KBq radioactively per 100 g soil flask was evaluated by Yucel *et al.* (1999) [112] for about 3 months. They observed that the time required for 50 per cent loss of the parent chemical in surface and subsurface soil was 10 days, and reported that the short persistence was due to high soil pH (7.9-8.1).

Microbial degradation of chlorpyrifos

When organophosphate pesticides are released in to the environment, their fate is decided by various environmental conditions and microbial degradation. Microbial degradation is the key factor for the disappearance of these pesticides. Micro-organisms possess the unique ability to completely mineralize many aliphatic, aromatic and heterocyclic compounds.

In general, microorganisms demonstrate considerable capacity for the metabolism of many pesticides. Although they are capable of catalyzing similar metabolic reactions as mammals and plants, they possess the unique ability to completely mineralize many aliphatic, aromatic, and heterocyclic compounds. There are two major types of microbial degradation of organic chemicals. The first type is catabolic degradation in which the organic chemical or a portion is completely degraded i.e. mineralized and the energy gained contributes to cell growth. The second is co-metabolism which involves the partial degradation of an organic chemical with no net benefit to the organism, the compound being merely caught up in some metabolic pathway during the normal metabolic activities of the microorganisms (Racke 1993) [75]. Studies conducted in soil have generally reported significantly longer dissipation half–lives of pesticides under sterilized than natural conditions and led to the conclusion that microbial activities were important in the degradation of chlorpyrifos in soil (Miles *et al.* 1984). Evidence from soil degradation studies indicated that cleavage and mineralization of the heterocyclic ring occured in soil due to the activities of microorganism (Racke & Coats 1990) [76]. However, the singularly most important microbial role in the chlorpyrifos degradation pathway was mineralization of 3, 5, 6- trichloro-2-pyrinidinol (TCP) and 3, 5, 6-trichloro-2-methoxypyridine (TMP) metabolites (Racke 1993) [75].

Microbial enzymes hydrolysed chlorpyrifos under controlled conditions. The ability of parathion hydrolase, an organophosphorus ester-hydrolyzing enzyme isolated from a mixed microbial culture hydrolysed chlorpyrifos was first reported by Munnecke and Hsieh in 1975. The metabolism of 50-ppm chlorpyrifos in isolates of several forest fungi *Trichoderma harzianum, Penicillium vermiculatum, and Mucor sp* was reported by Jones and Hastings (1981) [42]. Chlorpyrifos degradation by several microbial isolates maintained in liquid media containing 10 ppm chlorpyrifos was studied by Ivashina (1986) [41] who reported that dissipation was more rapid in a sucrose-supplemented media containing *Trichoderma sp*. and glucose supplemented media containing *Bacillus sp*. than in control media containing no microorganisms and the chlorpyrifos disappeared from the microbial isolates in a linear fashion over a 2-week period.

Microbial degradation of organophosphate pesticides was of interest because of their high mammalian toxicity. Two parathion degrading bacterial strains, *Flavobacterium* sp. strain ATCC 27551 and *Pseudomonas diminuta* strain, were isolated from soils in the Philippines and United States, respectively (Serdar *et al.* 1982, Sethunathan 1973) [86, 84]. In addition, studies by Lakshmirani and Lalithakumari (1994) [52] found that a strain of *Pseudomonas putida* could hydrolyze methyl parathion and use *p*-nitrophenol as a sole source of carbon. Although chlorpyrifos was widely used for agricultural and household pest control since 1965, it was difficult to isolate a degrading strain for this organophosphate. Several attempts to isolate a chlorpyrifos-degrading microbial system by repeated treatments or enrichment of soils and other media with chlorpyrifos have not been successful (Mallick *et al.* 1999, Racke *et al.* 1990) [58, 76]. The resistance of chlorpyrifos to enhanced degradation in soil was attributed for this failure. Chlorpyrifos was reported to be degraded co-metabolically in liquid media by *Flavobacterium* sp. and also by an *Escherichia coli* clone with an organophosphate degrading (opd) gene (Mallick *et al.* 1999, Richnis *et al.* 1997, Wang *et al.* 2002) [58, 106]. However these microbes did not utilize chlorpyrifos as a source of energy. Mallick *et al.* (1999) [58] reported degradation of chlorpyrifos in mineral salt medium by an *Arthrobacter* species that was initially isolated from methyl parathion-enriched soil.

Saccharomyces cervisiae was reported to degrade chlorpyrifos to some extent by Lal and Lal (1987) [51]. Two lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were reported by Shaker *et al.* (1988) [88] to degrade chlorpyrifos. Strains of *Aspergillus flavus* and *Aspergillus niger* isolated from agricultural soil with previous history of chlorpyrifos use were, also reported to bio-mineralise chlorpyrifos in liquid isolate medium (Swati & Singh 2002) [98].

A fungal strain capable of degrading chlorpyrifos was isolated and identified as *Verticillium* sp. by Yu *et al.* (2006) [111]. They found that the degradation of chlorpyrifos in by the fungal strain in mineral salt medium increased linearly with increasing concentrations of chlorpyrifos (r_2 =0.9999), suggesting that the degradation was subjected to pseudo-first order kinetics. With the first order kinetic function, the DT50 of chlorpyrifos at concentrations of 1, 10, and 100 mg/l, was calculated to be 2.03, 2.93, and 3.49 days, respectively. In the controls the hydrolysis percentages of chlorpyrifos was found to be less than 5%. They further used the cell free extracts of the strain to detoxify chlorpyrifos in vegetables and reported that the cell free extracts of the fungus could be used for enhanced degradation in vegetables. Some evidence also indicated that, the metabolites of chlorpyrifos were degraded and mineralized by soil microorganisms.

Racke and Robbins (1991) [79] investigated twenty-five soil samples and found that out of the twenty-five only two displayed significant degradation of TCP within 21 days of inoculation into mineral salts medium containing 5-ppm TCP as the sole carbon source. Isolation of a pure isolate of bacteria capable of using 3, 5, 6-trichloro-2-pyridinol (TCP) as the sole source of carbon and energy under aerobic conditions was reported for the first time by Feng and his co-workers in 1998. The bacterium was identified as a *Pseudomonas sp*. and designated as ATCC 700113. The TCP degradation yielded CO_2, chloride and some unidentified polar metabolites. They further reported that the degradation of the parent compound, TCP, by the *Pseudomonas sp*. involved a reductive de-chlorination mechanism.

Several studies conducted in soil indicated significantly longer dissipation half-lives under sterilized versus natural conditions, and led to the conclusion that microbial activities were important in degradation of chlorpyrifos (Getzin 1981a, Miles *et al.* 1983) [34]. Schmimmel *et al.* (1983) [85], based on laboratory degradation studies of insecticides aqueous solution and sediments, concluded that microorganisms play an important role in degradation of insecticides.

Chlorpyrifos is characterized by phosphate-oxygen-carbon (P-O-C) linkage as in other organophosphate pesticides, such as diazinon, parathion (Sethunathan& Yoshida 1973) [84], methyl parathion and fenitrothion (Mishra *et al.* 1992) [66]. Guha *et al.* (1997) [37] reported the involvement of plasmids in degradation of malathion and Chlorpyrifos by *Micrococcus*

sp. isolated from soil. Rapid degradation of Chlorpyrifos was reported by Mallick *et al.* (1999) [58], in mineral salt medium by the *Flavobacterium sp*. ATCC 27551 isolated from diazinon retreated rice fields (Sethunathan& Yoshida 1973) [84] and an *Arthrobacter sp*. isolated from a flooded soil retreated with methyl parathion (Mishra *et al.* 1992) [66].

The degradation of chlorpyrifos in poultry and cow-derived effluents was studied by Huang *et al.* (2000) [40] who reported that chlorpyrifos was degraded by aerobic microbial processes in animal derived lagoon effluents.

The effects of soil pH on the biodegradation of chlorpyrifos in United Kingdom and Australian soils was studied by Singh *et al.* (2003) [91] and reported that the dissipation of chlorpyrifos in United Kingdom soils varying in pH from 4.7 to 8.4 was mediated by the cometabolic activities of the soil microorganisms. A robust bacterial population that utilized chlorpyrifos as a source of carbon was detected in an Australian soil and this bacterial population was transferred to the United Kingdom soils. It was found that only soils with a pH of ≥ 6.7 were able to maintain this degrading ability 90 days after inoculation.

The enhanced degradation of chlorpyrifos by an *Enterobacter* strain B-14 was reported by Singh *et al.* (2004) [92] and found that the strain responsible for enhanced biodegradation of chlorpyrifos showed greatest similarity to *Enterobacter asburiae* based on 16s rRNA studies of the bacterium. This strain was shown to utilize chlorpyrifos as a sole source of carbon and phosphorus and hydrolysed chlorpyrifos to diethylthiophosphoric acid (DETP) and 3,5,6-trichloro-2-pyrinidol (TCP). It was also revealed that the strain possessed a novel phosphotriesterase enzyme system, as the gene coding for this enzyme had a different sequence from the widely studied organophosphate degradative gene (opd). The authors also reported that addition of the strain B-14 to chlorpyrifos contaminated soils resulted in higher degradation rate than that observed in non-inoculated soils.

Yang *et al.* (2005) [110] isolated *Alcaligenes faecalis* strain DSP3, capable of degrading both Chlorpyrifos and 3,5,6-trichloro-2- pyridinol (TCP). Further Yang *et al.* (2006) [109] successfully cloned the methyl parathion degrading (mpd) gene from a chlorpyrifos degrading bacterium and used it for bioremediation of contaminated soil. They isolated six chlorpyrifos degrading bacteria by enrichment procedure and found strain YC-1 to have highest degrading capability and this strain was identified as the genus *Stenotrophomonas*. The strain YC-1 degraded 100 mg/l of chlorpyrifos within 24 hour to diethylthiophosphoric acid (DETP) and TCP. DETP was utilized as a source of carbon and phosphorus, but it did not degrade TCP.

Bacillus licheniformis strain ZHU-1 isolated by Zhu *et al.* 2010 [113] when inoculated in soil resulted in a higher degradation rate than non-inoculated soils, the degradation rate of chlorpyrifos (100 ppm) reached ninty-nine per cent after fourteen days and they further suggested that the microbial manure added by strain ZHU-1 could be applied not only as fertilizer, but also in degrading chlorpyrifos residue in soil. This study provided basis for prevention and control of pesticides pollution**.**

Ghanem *et al.* (2007) [36] studied the biodegradation of chlorpyrifos by *Klebsiella* isolated from an activated sludge sample which was collected from the Damascus waste water treatment plant, Syria was studied by and reported that when *Klebsiella* sp. was maintained by culturing in a poor medium consisting of mineral salts and chlorpyrifos as a sole carbon source chlorpyrifos was degraded within 4 days. By comparison, within 4 days the isolated *Klebsiella* sp. was found to break down 92% of CPY when co-incubated in a poor mineral medium in which chlorpyrifos was the sole carbon source (13.9 g/L poor medium). It was further found that isolated *Klebsiella* sp. was able to tolerate upto 17.3 g of CPY in the poor medium.

Racke *et al.* 1994 isolated *Pseudomonas* sp. strain ATCC 700113 from an agricultural soil and demonstrated that it was able to mineralize 3,5,6-trichloro-2- pyridinol (TCP) by the release of $14CO_2$ and chloride ions.

Bacterial degradation of chlorpyrifos (10 ppm) in pure isolates and in soil was reported by Mallick *et al.* (1999) [58]. They found that *Arthrobacter* sp. degraded the insecticide completely within twenty-four hours of incubation while *Flavobacterium* sp. ATCC 27551 degraded Chlorpyrifos within 48 hrs of incubation. Further they reported that under flooded conditions degradation by these bacteria was faster as compared to non-flooded soil conditions.

Forty-three isolates of bacteria from various soil samples of Punjab were isolated by Kahlon and Saurabh (2003) [43] and were screened for the degradation of chlorpyrifos. They found four isolates growing efficiently which were further selected for the bioaugmentation of pesticide degradation in the contaminated soil.

The degradation of contrasting pesticides diuron, atrazine, metalaxyl and chlorpyrifos by white rot fungi was reported by Bending *et al.* (2002) [16]. Degradation of chlorpyrifos by two soil fungi, *Trichoderma viridae* and *Aspergillus niger* was reported by Mukherjee and Gopal (1996) [67].

Tortilla *et al.* (2010) [102] studied the degradation of the insecticide chlorpyrifos using a biomix of a biobed system biostimulated with inorganic fertilizer (NPK). They evaluated three concentrations of the fertilizer (0.1%, 0.5% and 1.0% w/w) on chlorpyrifos degradation, TCP accumulation and biological activity of the biomix and reported that chlorpyrifos was dissipated efficiently (>75%) after 40 days of incubation and no additional dissipation was obtained with increasing concentration of NPK after 20 days of incubation. they further demonstrated that the biomix prepared with Andisol and biostimulated with NPK nutrient could be recommended in biobeds as a viable alternative of chlorpyrifos dissipation avoiding soil and water contamination probability.

Thengodkar and sivakami (2010) [100] reported that *Spirulina platensis* could grow in media containing 80 ppm chlorpyrifos due to an alkaline phosphatase (ALP) activity that was detected in cell free extracts of *Spirulina platensis*.

Similarly Lia *et al.* (2008) [53] observed that bacterial strains *Sphingomonas sp., Stenotrophomonas sp., Bacillus sp.* and *Brevundimonas sp*., *Pseudomonas sp*. were the chlorpyrifos-degrading strains and had the potential to clean up the organophosphate pesticide-contaminated environment.

Agus Sabdono (2007) [3] isolated *Bacillus firmus* which could degrade 25 mg/l chlorpyrifos rapidly and further reported that at this concentration 50% of chlorpyrifos was degraded after 20 hours, following which there was a period of rapid loss, with almost constant degradation after 32 hours.

Genetics of degradation

The molecular basis of degradation of certain organophosphates was studied by Horne *et al.* 2002 [39] and he isolated organophosphate-degrading gene (*opd*) from geographically, and biologically different species. In most of the studies, *opd* genes were found to be plasmid based and had similar DNA sequences. They isolated an *opd* gene from

Agrobacterium radiobacter, which was located on the chromosome and had similar sequence to the *opd* gene from other bacteria.

Two mixed bacterial isolates utilizing methyl parathion and parathion were described by Chaudhary *et al.* (1988) [22]. The hybridisation data showed that the DNA from *Pseudomonas* sp. and from mixed isolate had homology with opd gene from a previously reported parathion hydrolysing bacteria *Flavobacterium* sp.

Microbial degradation of pesticides with special emphasis on the role of catabolic genes and the application of recombinant DNA technology in the development of an organism which could simultaneously degrade several xenobiotics was reviewed by Kumar *et al.* (1996) [50]. Possible involvement of plasmids in degradation of malathion and chlorpyrifos was reported by Guha *et al.* (1997) [37] and they found two plasmids harbouring strains of *Micrococcus* sp. (M-36 and AG-43) to degrade malathion and parathion.

Stenotrophomonas sp. isolated by Yang *et al.* (2006) [109] utilized chlorpyrifos as the sole source of carbon and phosphorus for its growth and hydrolysed chlorpyrifos to 3,5,6-trichloro-2-pyridinol (TCP). Parathion, methyl parathion, and fenitrothion also were degraded by strain YC-1 when provided as the sole source of carbon and phosphorus. The inoculation of strain YC-1 (106 cells/g) to soil treated with 100 mg kg^{-1} chlorpyrifos resulted in a higher degradation rate than in non-inoculated soils. The bacterium was detected to have mpd gene and the results highlighted the potential of this bacterium to be used in the cleanup of contaminated pesticide waste in the environment.

Use of live biocatalysts for pesticides detoxification was studied by Chen and Mulchandani (1998) [24]. They discussed the use of a genetically engineered *E. coli* with surface expressed organophosphorus hydrolase and suggested the ultimate creation of super biocatalyst capable of degrading several pesticides rapidly and cost effectively. *Moraxella* sp. growing on P-nitrophenol was genetically engineered for the simultaneous degradation of organophosphate pesticides and P-nitrophenol. The truncated ice nucleation protein anchor was used to target the organophosphorus hydrolase on to the surface and the resulting *Moraxella* spp. degraded organophosphates rapidly, all within an hour (Shimazu *et al.* 2001) [90].

The prevalence of plasmid mediated pesticide resistant bacteria was studied by Umamaheswari and M. Murali (2010) [103] in three field crops paddy, sugarcane and tomato. These crops were exposed to bavistin, monocrotophos and kinado plus respectively to study the bacterial population and degradation of pesticides. Significant variations in the bacterial population were observed evident between the treatments in sugarcane field and tomato field exposed to monocrotophos and kinado plus, respectively. In addition, significant variations between total heterophillic bacteria, *Staphlyococci* and *Enterococci* population were recorded in both the sugarcane and tomato fields. The dominant pesticide resistant bacteria, *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeuroginosa* harboured plasmids and the resistant trait observed was found to be plasmid borne.

Ajaz *et al.* (2009) [7] isolated a chlorpyrifos degrading bacterium *Pseudomonas putida* MAS-1 from the cotton grown soil of NIAB, Faisalabad, Pakistan. Genetic study based on plasmid curing and electroporation mediated transformation was performed on this bacterium. The bacterium lost the property to grow on the Nutrient agar containing 10mg/mL chlorpyrifos after acridine orange mediated curing and further observed that chlorpyrifos degradation *Pseudomonas putida* MAS-1 was accomplished by the combined action of plasmid and chromosomal genes.

Bioremediation of pesticide contaminated soils

Bioremediation includes the gainful utilization of microorganism for the metabolism of target pollutants into safer and innocuous products among the potent technology being used globally for the restoration of contaminated sites.

The ability of a soil bacterium, *Agrobacterium radiobacter* J14a to degrade herbicide atrazine was examined by Struthers *et al.* (1998) [95] who found that addition of J14a cells (105/gm) into soil with a low indigenous population of atrazine degraders resulted in two to five times higher mineralization than in the uninoculated soil. The soils treated with 200 µg of atrazine/g and inoculated with J14a mineralized the atrazine continuously throughout the 68 days period.

The fate of varying mixture of pesticides chlorpyrifos, atrazine and monosodium methanearsonate was recorded by Lytle and Lytle (2002) [55] who observed that chlorpyrifos levels in the leaves of the common fresh water macrophyte *Juncus effuses* was highest on the first day. However, atrazine remained near nominal concentrations in water through day 16 and reached maximum accumulation in the leaves on the same day.

A study on bioremediation of technical HCH contaminated soil and removal of seed germination toxicity was conducted by Manonmani *et al.* (2002) [60] who reported that when some of the seeds were exposed to tech-HCH the germination was delayed and root and shoot length was effected and when HCH spiked soil was bioremediated with HCH degrading microbial consortium. The germination was improved and plants were healthy in bioremediated soil.

Pseudomonas aeruginosa isolate NCIM 2074 was subjected to varying concentrations of chlorpyrifos in incubator shaker at 37 °C and 150 rpm. The entire scale up process continued for a period of 70 days. *Pseudomonas aeruginosa* (NCIM 2074) was adapted to increased chlorpyrifos concentration upto 50 mg/l, but higher concentrations (75 and 100 mg/l) were inhibitory to the organism (Geetha and Fulekar 2010) [33].

Biodegradation of chlorpyrifos by *Enterobacter* strain B-14 and its use in bioremediation of contaminated soils was observed by Singh *et al.* (2004) [92] who observed that the addition of strain B-14 (106 cells/g) to soil with a low indigenous population of chlorpyrifos degrading bacteria treated with 35 mg of chlorpyrifos/kg resulted in a higher degradation rate.

A bacterium named LZ-1 identified as *Stenotrophomonas sp.* capable of utilizing high concentrations of p-nitrophenol (PNP) (up to 500 mg/L) as the sole source of carbon, nitrogen and energy was isolated from an activated sludge by Liu *et al.* (2007) [54]. Other P-substituted phenols such as 4-chlorophenol (4-CP) were also degraded by strain LZ-1, and both PNP and 4-CP were degraded via the hydroquinone pathway exclusively. It was further reported that an indigenous plasmid was responsible for phenols degradation. In soil samples, 100 ppm of PNP and 4-CP in mixtures were removed by strain LZ-1 (106 cells/g) within 14 and 16 days respectively, and degradation activity was maintained over a wide range of temperatures (4–35 1C). Therefore, strain LZ-1 can potentially be used in bioremediation of phenolic

compounds either individually or as a mixture in the environment.

Biobed, a biological technology was developed in Sweden and widely used in Europe to minimize point source contamination by pesticides in agricultural system (Torstensson and Castillo 1997) [101]. Typical Swedish biobeds are built with simple and cheap materials and a biomix mainly composed of a volumetric proportion of straw (50%), peat (25%) and soil (25%) (Torstensson and Castillo 1997) [101]. Straw is the main component for ligninolytic fungi growth, soil provides sorption capacity and favors microbial activity, and peat contributes to sorption capacity and moisture control in the biomix. Several studies have demonstrated that these biological systems can effectively retain and degrade pesticides including Chlorpyrifos (Castillo *et al.* 2008, Coppola *et al.* 2007, Vischetti *et al.* 2008) [20, 25, 104].

Microbial consortium effective for the treatment of pesticides in soil was reported by Geetha and Fulekar (2008) [32]. They designed surface soil treatment unit where bioremediation of commonly used pesticides namely Chlorpyrifos, cypermethrin, fenvalerate, and trichlopyr butoxyethyl ester at varying concentration viz. 25, 50 and 100 ppm using cow-dung microbial consortia under simulated environmental conditions and reported that cowdung slurry consortia had potential for bioremediation of soil contaminated with pesticides in surface soil treatment unit.

A chlorpyrifos-methyl (CM) degrading bacterium (designated strain KR100) identified as *Burkholderia cepacia* was isolated by Kim and Ahn (2009) [46] from a Korean rice paddy. Based on morphological, biochemical, and molecular characteristics, this bacterium was shown to be most closely related to members of the *Burkholderia cepacia* group which hydrolysed CM to 3,5,6-trichloro-2-pyridinol (TCP) and utilized TCP as the sole source of carbon for its growth. It was further found that this isolate was also able to degrade Chlorpyrifos, dimethoate, fenitrothion, malathion, and monocrotophos at 300 lg/ml and diazinon, dicrotophos, parathion, and parathion-methyl at low concentration i.e. 100 lg/ml. The study further revealed that ability to degrade CM was found to be encoded on a single plasmid of 50 kb, pKR1. Genes encoding resistance to amphotericin B, polymixin B sulfate, and tetracycline were also located on the plasmid.

The DI101 Strain isolated from agricultural soil by Abo-Amer and Aly (2011) [1] was able to completely degrade 50 mg/l diazinon in MSM within 11 days with a degradation rate of 0.226 day^{-1}. The inoculation of sterilized soil treated with 100 ppm of diazinon with 106 CFU/g DI101 resulted in a faster degradation rate than was recorded in non-sterilized soil. The diazinon degradation rate by DI101 was efficient at temperatures from 25 to 30ºC and at pH from 7.0 to 8.0. The degradation rate of diazinon was not affected by the absence of a phosphorus supplement, and addition of other carbon sources (glucose or succinate) resulted in the slowing down of the degradation rate. The maximum degradation rate (V_{max}) of diazinon was 0.292 day^{-1} and its saturation constant (Ks) was 11 mg/l, as determined by a Michaelis-Menten curve. The strain was able to degrade diethyl-thiophosphate containing organophosphates such as chlorpyrifos, coumaphos, parathion, and isazofos when provided as a source of carbon and phosphorus, but not ethoprophos, cadusafos, and fenamiphos. These results proposed useful information for the potential application of the DI101 strain in bioremediation of pesticide-contaminated environments.

Conclusion

Indiscriminate use of insecticides has resulted in contamination of biodiversity and ecological systems causing environmental pollution and poisoning. Biodegradation using microorganisms is one of the safest and cheapest ways of reducing pesticide levels in the environment. Use of pesticide degrading microbial systems for bioremediation thus receives attention because of its cost effectiveness and eco-friendly nature. Microorganisms have the enzymatic capacity to utilize virtually all naturally and synthetically occurring compounds as their sole carbon and energy source. The metabolism of chlorpyrifos by microorganisms in soil has been reported by many researchers. Farmer's awareness about the ill-effects caused by pesticide abuse and more research on this emergent issue is need of the hour.

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