Chapter 15
Pesticide Interactions with Soil Microflora: Importance in Bioremediation

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Abstract Modern application of insecticides belonging to different chemical families to boost agricultural productivity has led to their accumulation in soils to levels that affect, directly and indirectly, soil enzyme activities and physiological characteristics of nontarget soil microflora including plant growth-promoting rhizobacteria, and, consequently the performance of crop plants. Various biological strategies can be applied for removing toxic substances, including insecticides, from the environment and are collectively known as bioremediation. Among biological approaches, the use of microbes with degradative ability is considered the most efficient and cost-effective option to clean pesticide-contaminated sites. The present review focuses on the role of naturally occurring rhizosphere microbes involved in degradation or transformation of insecticides.

15.1 Introduction

During cultivation, the majority of economically important crops are infested by insect pests including pod borers, aphids, jassids, and pod flies, which cause a substantial reduction in yields (Mukherjee et al. 2007). In current agronomic operations, pesticides, including insecticides are therefore applied, sometimes excessively or indiscriminately to crops and soils to combat insect problems and consequently to increase productivity of agro-ecosystems. After repeated application, a significant proportion of insecticides may accumulate in upper soil layers (0–10 cm) and exert damaging impacts, not only on the diversity but also on the functionality of ecologically and agronomoically important soil microflora (Das et al. 2005). Subsequently, abnormally high concentrations of insecticides may lead to a considerable loss in soil fertility (Pal et al. 2006).

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Soil microorganisms, specifically rhizospheric bacteria including plant growth-promoting rhizobacteria (PGPR), facilitate plant growth (Khan et al. 2010) by (i) solubilizing insoluble phosphates; (ii) fixing atmospheric N and transporting it to plants; (iii) facilitating uptake of other plant nutrients; and (iv) synthesizing siderophores and phytohormones (Zaidi et al. 2009). Documented results have, however, shown that insecticide concentrations above recommended values adversely affects both the physiological traits of rhizobacteria and various metabolic activities of plants leading to losses in biomass, symbiotic attributes, nutrient (N and P) uptake, and quantity and quality of seeds of plants (Ahemad and Khan 2010). To circumvent such losses and to reduce dependence on chemical additions to soil, biological methods (i.e., microbes and plants) may be applied to detoxify/remove insecticides from soils.

15.2 Toxicity of Pesticides to Soil Microorganisms and Plants

Soil microbial communities play a critical role in cycling of soil elements and, in turn, affect soil fertility and plant growth. However, beneficial microbial communities are greatly influenced by factors including the application of agrochemicals (Ahemad and Khan 2009), which are applied in modern agricultural practices to attain optimum crop yields. Of the various agrochemicals, pesticides, in particular, include any substance intended for preventing, destroying, repelling, or mitigating a pest. Microorganisms can, however, be tolerant or resistant (e.g., slightly or not affected) to pesticides. If, microorganisms are indeed sensitive, pesticides will interfere with vital metabolic activities of microbes.

The consistent and injudicious use of synthetic pesticides has, nevertheless, become a major threat to beneficial soil microbes (Zahran 1999; Srinivas et al. 2008) and in turn affects the sustainability of agricultural crops. Globally, the greater concern is how to minimize or reduce the effects of pesticides so that the potential negative impacts of these chemicals on microorganisms involved in nutrient cycling, vis-a-vis the productivity of crops could be preserved. In the following section, an attempt is made to highlight the impact of insecticides on soil microflora and agronomic crops.

15.2.1 Insecticidal Impact on Rhizobacteria and Crops

In modern high-input agricultural practices, insecticides belonging to diverse chemical groups (Table 15.1) are used as seed and/or soil treatments to prevent losses due to insect pests. Following application, such insecticides accumulate in soils to undesirable levels and affect, either directly or indirectly, soil enzyme activities and physiological characteristics of nontarget soil microbiota (Table 15.2), thereby leading to loss in productivity of soils. For example, Gundi et al. (2005)

Table 15.1 Examples of insecticides and their mode of action

Mode of action	Chemical type	Examples
Acetylcholinesterase inhibitors	Carbamates	Aldicarb, carbaryl, carbofuran, propoxur, carbosulfan
	Organophosphates	Phorate, chlorpyrifos, omethoate, parathion, methmidophos, malathion, diazinon
GABA-gated chloride channel antagonists	Cyclodienes and other organochlorines (OC)	Lindane, aldrin, endosulfan
	Phenylpyrazoles (fiproles)	Fipronil
Sodium channel modulators	OC	DDT
Acetylcholine receptor antagonists	Neonicotinoids	Imidacloprid, thiamethoxam
Acetylcholine receptor antagonists allosteric	Spinosyns	Spinosad
Voltage-dependent sodium channel blocker	Oxadiazine	Indoxacarb

Adapted from http://www.irac-online.org/

Table 15.2 Impacts of selected insecticides on soil biota

Insecticide	Effects	References
Fipronil and pyriproxyfen	Reduced synthesis of IAA and siderophores in *Rhizobium leguminosarum* and *Mesorhizobium.*	Ahemad and Khan (2009)
Malathion, dimethoate, phorate	Aerobic bacteria, among all groups of microflora, were most adversely affected by all insecticides at normal or four times more the normal rate and phorate was found to be most toxic	Aamil et al. (2005)
Chlorpyrifos	Reduced bacterial numbers, but significantly increased fungal numbers	Pandey and Singh (2004)
Carbofuran	Significant impacts on acetylcholinesterase activity in earthworms	Panda and Sahu (2004)
Dimethoate	Short-term reduction in microarthropod numbers (Collembola), but recovery in numbers after time	Martikainen et al. (1998)
DDT	Reduced bacterial and soil algal populations, but may have increased fungal counts	Megharaj et al. (2000)
Malathion	Short-term impacts on earthworm population	Panda and Sahu (1999)
BHC, phorate, carbofuran, and fenvalerate	Stimulated proliferation of aerobic nonsymbiotic N_2-fixing bacteria and phosphate-solubilizing microorganisms and also their biochemical activities, such as nonsymbiotic N_2-fixing and phosphate-solubilizing capacities, which resulted in greater release of available N (NH_4^+ and NO_3^-) and P in soil	Das and Mukherjee (2000)

observed that a mixture of monocrotophos or quinalphos and cypermethrin had additive, synergistic, and antagonistic effects toward bacteria and fungi and dehydrogenase activity in a black clay soil. Application of monocrotophos, quinalphos, and cypermethrin at different rates used either singly or in combination to soil significantly enhanced proliferation of bacteria and fungi and soil dehydrogenase activity even at the highest level of 25 $\mu g\, g^{-1}$. Antagonistic interactions were, however, more pronounced for soil microflora and dehydrogenase activity when monocrotophos or quinalphos were applied with cypermethrin to soil at the highest rate (25 + 25 μg/g). Synergistic or additive responses, on the other hand, occurred at lower application rates with the same combination of insecticides. Some insecticide-tolerant strains of PGPR are also known. For example, Nazarian and Mousawi (2005) identified strains belonging to *Pseudomonas* and *Flavobacterium* which tolerated concentrations of 2.5, 4, and 8 g/L of guthion, methyl parathion, and dimethoate, respectively. The resistance in these bacteria against such organophosphorus pesticides was probably due to the presence of organophosphorous-degrading plasmids that have the ability to express hydrolytic enzymes.

In a follow-up study, Vasileva and Ilieva (2007) carried out pot trials to determine the effect of pre-sowing treatment of seeds with insecticides promet 400 SK (furathiocarb) at a dose of 3 L/100 kg seeds, and carbodan 35 ST (carbofuran) at 1, 2 and 3 L/100 kg seeds on nodulating ability, nitrate reductase activity, and plastid pigments content of lucerne (cv. obnova). It was found that the insecticides did not depress nodulation; instead, nodule numbers and specific nodulation ability of carbodan 35 ST (3 L/100 kg seeds)-treated plants increased by 23 and 7%, respectively, compared to control. Root length for the variants with pre-sowing treatment of seeds was higher than the control by 7–26%. The variant with carbodan at 2 and 3 L/100 kg seeds and promet increased nitrate reductase activity in roots and that with carbodan at 1 L/100 kg seeds increased nitrate reductase activity in leaves. Total content of plastid pigments increased in all variants with carbodan and was lower than the untreated control in the variant with promet.

Das et al. (2003) investigated the effects of phorate and carbofuran at 1.5 and 1 kg active ingredient per hectare, respectively, on the population and distribution of bacteria, actinomycetes, and fungi as well as the persistence of insecticidal residues in rhizosphere soils of rice (*Oryza sativa* L., variety IR-50). Application of insecticides stimulated populations of bacteria, actinomycetes, and fungi in rhizosphere soils. Stimulation was more pronounced with phorate when compared with carbofuran. Neither insecticide, however, markedly affected *Streptomyces* or *Nocardia* in the rhizosphere soils. Total numbers of *Bacillus*, *Escherichia*, *Flavobacterium*, *Micromionospora*, *Penicillium*, *Aspergillus*, and *Trichoderma* treated with phorate and that of *Bacillus*, *Corynebacterium*, *Flavobacterium*, *Aspergillus*, and *Phytophthora* with carbofuran increased. On the other hand, numbers of *Staphylococcus*, *Micrococcus*, *Fusarium*, *Humicola*, and *Rhizopus* under phorate stress and *Pseudomonas*, *Staphylococcus*, *Micrococcus*, *Klebsiella*, *Fusarium*, *Humicola*, and *Rhizopus* under carbofuran stress were inhibited. Similarly, phorate at 100 and 500 μg/mL substantially reduced IAA production by phosphate-solubilizing bacteria belonging to genera *Serratia*, *Pseudomonas*, and *Bacillus* isolated from

various rhizospheric soils, while P-solubilizing activity of PSB was marginally affected (Wani et al. 2005).

The effect of lindane on microbial populations was analyzed in soil with a history of contamination with various chemicals, including pesticides, by Rodríguez and Toranzos (2003). Soil microcosms were amended with 100 mg lindane/kg soil and microbial populations were monitored for 70 days. A 50% reduction in bacterial cell concentrations in lindane-amended microcosms was observed during the second week of the experiment. Overall, no effect of lindane was observed on the metabolic versatility and genetic diversity in these soils, demonstrating the ability of the bacterial populations to tolerate the stress generated by the addition of pesticides. In another report, pencycuron at field rate (FR), 2FR, and 10FR affected microbial biomass C (MBC), soil ergosterol content, and fluorescein diacetate-hydrolyzing activity (FDHA) differentially. Changes in microbial metabolic quotient (qCO_2) and microbial respiration quotient indicated pencycuron-induced disturbance at 10FR. This study revealed that the metabolically activated microbial population was more suppressed compared to the dormant population (Pal et al. 2006).

The effect of increasing rates of lindane (156.0, 244.0, and 312.0 g/ha), unden (propoxur) (125.0, 187.5, and 250.0 g/ha), dithane and karate (166.6, 209.8, and 333.3 g/ha) on garden eggs (*Solanum melongena*), okra (*Abelmoschus esculentus*), and tomatoes (*Lycopersicum esculentus*) was studied by Glover-Amengor and Tetteh (2008). Yields of garden eggs were suppressed by all rates of lindane. In tomatoes, lower lindane rates increased yields, whereas higher rates suppressed yields below the control. In okra, yields were higher than the control at all lindane levels though yield increments were low. Unden application had the greatest effect on garden egg yields followed by tomatoes, and the least on okra. In the garden egg and tomato treatments, increasing concentrations of unden resulted in decreased yields, though yields were higher in the control plots. The optimum unden rate for garden egg and tomato was U20 (125.0 g/ha). Increasing rates of unden on okra did not have any significant effect. Pesticide application reduced soil fungal populations by 50–70%, while bacterial populations declined by 23–38%. In general, dithane suppressed bacterial populations considerably, whereas karate suppressed fungal populations. Lindane did not have any advantage over other pesticides as it caused the lowest increase in yield. Singh and Singh (2006) evaluated the impacts of diazinon, imidacloprid, and lindane treatments on ammonium-, nitrate-, and nitrite-nitrogen and nitrate reductase enzyme activities in a groundnut field for 3 consecutive years (1997–1999). Diazinon was applied for both seed and soil treatment but imidacloprid and lindane were used for seed treatments only at recommended rates. Diazinon residues persisted for 60 days in both the cases. Average half-lives ($t_{1/2}$) of diazinon were found to be 29.3 and 34.8 days in seed and soil treatments, respectively. In the diazinon seed treatment, NH_4^+, NO_3^-, and NO_2^- nitrogen and nitrate reductase activity were not affected. However, diazinon soil treatment resulted in a significant increase in NH_4^+-N in a 1-day sample which continued until 90 days. Some declines in NO_3^--N were detected from 15 to 60 days. Along with this decline, significant increases in NO_2^--N and nitrate reductase activity were found between 1 and 30 days. Imidacloprid and lindane

persisted for 90 and 120 days with average half-lives of 40.9 and 53.3 days, respectively. Within 90 days, imidacloprid residues decreased by 73.17–82.49%, while lindane residues declined by 78.19–79.86% within 120 days. In imidacloprid seed-treated field, stimulation of NO_3^--N and decline in NH_4^+-N, NO_2^--N, and nitrate reductase activity were observed between 15 and 90 days. However, lindane seed treatment resulted in significant increases in NH_4^+-N, NO_2^--N, and nitrate reductase activity and decline in NO_3^--N between 15 and 90 days. Fox et al. (2007) concluded, via study on interaction of agrochemicals with crop plants, that organochlorine pesticides and other environmental contaminants induce a symbiotic phenotype of inhibited or delayed recruitment of rhizobia bacteria to host plant roots, fewer root nodules produced, lower rates of nitrogenase activity, and a reduction in overall plant yield at time of harvest. Moreover, Evans et al. (1991) reported that omethoate was toxic to some *Rhizobium* strains on direct contact when diffused through agar seeded with these bacteria or mixed in broth cultures containing the bacteria. Omethoate mixed with peat-based legume inoculant and applied to seed of subterranean clover or lucerne significantly reduced number of nodules formed over 3 weeks on seedlings grown in pots of sand, compared with inoculated controls. Rhizobia numbers were reduced markedly by mixing with omethoate. Seed pretreatment with omethoate before inoculation had no effect on nodule number (9–11 weeks after sowing), compared with inoculated controls. In another experiment, Evans et al. (1993) found that the effectiveness of inoculation with *Rhizobium meliloti* was significantly reduced when inoculant was applied to seeds pretreated with omethoate. Nodule numbers and shoot mass per plant were reduced by 6 and 22%, compared to untreated plants.

15.3 Bioremediation

Injudicious use of natural resources has resulted in the contamination of land and water with hazardous substances to a considerable extent in many parts of the world. Contaminated sites continue to be discovered due to increasing urbanization and industrialization. Contaminated sites pose a serious threat to human health and also to the environment. Many biological, physical, and chemical strategies are available to clean up contaminated land or water. Some of the commonly used methods for removing pollutants from soil are presented in Table 15.3.

One of the more promising and cost-effect approaches to address soil contamination problems is bioremediation. Bioremediation is defined as the engineered use of biological agents such as microbes or plants to remove/neutralize/degrade/transform contaminants present in soil, sediments, or water. Bioremediation can take place both in situ and ex situ (Hussain et al. 2009). In situ bioremediation does not require excavation of soils; generally, in situ bioremediation is applied for degradation of pollutants present in saturated soils and groundwater. This method has considerable appeal over other bioremediation strategies due to its low cost and employment of innocuous microflora to biodegrade hazardous chemicals and their derivatives. In this technology, chemotaxis is an important attribute since microbial

Table 15.3 Remediation strategies for contaminated soils

Remediation strategy	Advantages	Disadvantages
Chemical inactivation (immobilization/oxidation)	Rapid	Use of chemicals may be costly and may give rise to added contamination
Incineration	Rapid Reduction in waste volume	High costs of transportation, problems such as combustibility of soil matrix and toxic emissions
In situ vitrification	Reduces leaching and soil volume	High costs to generate required temperature (1,600–2,000°C)
Stabilization/solidification (binding to resins)	Reduces leaching	The cost of binding resins may be very high
Thermal desorption(high temperatures in the absence of oxygen to vaporize or destroy pesticides)	Required less heat than incineration The matrix is not incinerated Reduced emissions	Gaseous emission controls required
Vapor stripping (vacuum is applied to contaminated soil, removing volatile waste)	Generates little wasteFairly cost effective	Only suitable for volatile contaminants
Bio- and phyto-remediation	Low cost and maintenance, environment-friendly, suitable for in situ	Slow compared to incineration/chemical deactivation

Adopted from Atterby et al. (2002)

communities with chemotactic traits migrate toward a site enriched with contaminants (chemoattract). Therefore, by enhancing the chemotactic abilities of cells, in situ bioremediation can be made safer for degrading harmful compounds. The benefits of application of in situ bioremediation include: (i) it does not require excavation of the contaminated soils and is consequently cost-effective and (ii) there is minimal site disruption resulting in simultaneous treatment of soil and groundwater. Conversely, in situ bioremediation also has drawbacks: (i) the method is time-consuming compared to other remedial methods and (ii) the potential efficiency of microbes is subject to seasonal variation and environmental factors. In addition, microorganisms perform better when contaminant molecules provide nutrients and energy for growth. If these conditions are not favorable, the ability of microbes to degrade pollutants is decreased. Alternatively, genetic manipulation of microbes is required to accelerate degradability of the pollutants even though stimulating indigenous microflora is generally preferred.

Ex situ bioremediation processes require excavation of contaminated soils or pumping of groundwater to facilitate microbial degradation. Depending on the state of the contaminant to be removed, ex situ bioremediation is classified as (i) a solid-phase system (including land treatment and soil piles) and (ii) slurry-phase systems

(including solid–liquid suspensions in bioreactors). Solid-phase treatment may be applied to organic wastes (e.g., sewage sludge, animal manures, and agricultural wastes) and problematic wastes (e.g., domestic and industrial hazardous wastes, municipal solid wastes). Solid-phase soil treatment processes include landfarming, soil biopiles, and composting. Slurry-phase bioremediation is a relatively rapid process compared to the other biological treatment processes. In slurry-phase bioremediation, contaminated soil is combined with water and other additives in a large vessel termed a bioreactor and mixed to keep the soil microorganisms in contact with contaminants. Nutrients and oxygen are added, and conditions in the bioreactor are monitored and controlled to create the optimum environment for the microorganisms to degrade the contaminants. When treatment is complete, water is removed from the solids, which are disposed or treated further, if they contain additional pollutants (Sasikumar and Papinazath 2003).

15.3.1 Bioremediation of Insecticides

Numerous processes occur during dissipation of insecticides in the environment, for example: (i) volatilization into the air, (ii) sorption to soil components, (iii) movement in soils through runoff, (iv) leaching into soils, and (v) upward movement in soils through capillary forces (Fig. 15.1) (HCN 1996). Degradation, the principal method for insecticide loss, is the primary process affecting the dynamics of insecticide residues in the environment including persistence in soils. The degradation of insecticides is carried out both by physico-chemical methods

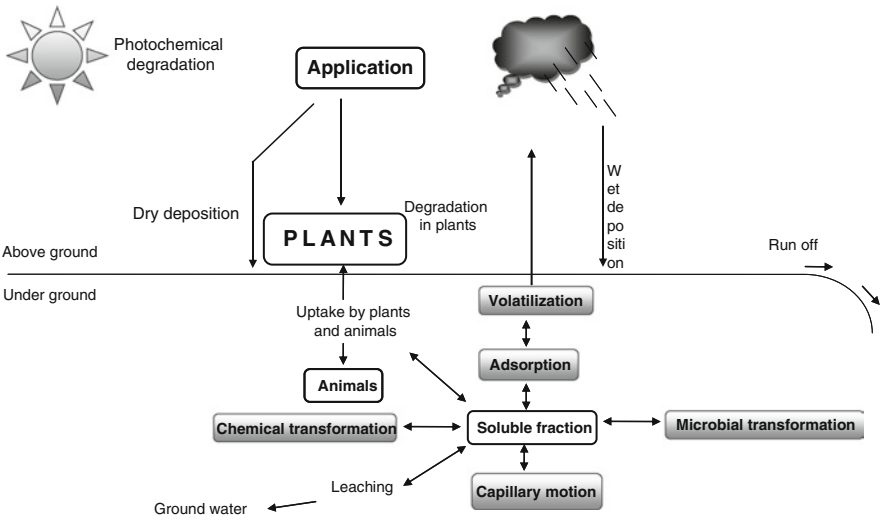

Fig. 15.1 The fate of the pollutants in the environment [modified from HCN (1996)]

and by organisms (microbes and plants). Microbial degradation of insecticides is characterized by enzymes that mediate the splitting of the molecules through different metabolic pathways and is ultimately dependent on the viability, density, and enzymatic activity of soil microorganisms. Furthermore, physico–chemical properties of soils, and types and concentrations of insecticides either applied or previously accumulated in soils, greatly influence degradation rate (Cáceres et al. 2010). In this section, the microbial degradation of insecticides, in particular lindane, chlorpyrifos, and monocrotophos, is discussed primarily because of their toxicity and extensive usage in agricultural production systems.

15.3.1.1 Lindane and Its Isomers

Lindane (1, 2, 3, 4, 5, 6-hexachlorocyclohexane, γ-HCH), a broad-spectrum organochlorine pesticide, is a persistent organic pollutant (POP) and enters soil by direct application, disposal of contaminated waste, or wet/dry deposition from the atmosphere. Immediately following application, HCHs are adsorbed to the soil particles, volatilized to the atmosphere or leached into groundwater, or enter crop plants along with contaminated water. HCHs are strongly adsorbed to soil organic matter (SOM) and, consequently, remain immobile in soils. Nevertheless, under conditions of low SOM and consistent rainfall, lindane, and other HCH isomers pose a significant threat to groundwater (Wauchope et al. 1992). Conventionally, three methods like chemical degradation, physical adsorption, and bioremediation have been reported for the removal of lindane from the contaminated sites. Chemical treatments include the use of microwave irradiation (Salvador et al. 2002), degradation with NaOH-modified sepiolite (Salvador et al. 2002), and addition of hydrogen peroxide (Ahlborg and Thunberg 1980). These treatments involve the use of corrosive chemicals and hence are not eco-friendly. In contrast, physical methods involve thermal desorption and incineration, which provide sufficient degradation but require huge infrastructure and are expensive. In addition, they generate high toxic gases (phosgene). Biological treatments including the use of microbes often called bioremediation, are even though a relatively slow process but are an attractive option due to its inherent eco-friendly characteristics and low cost. In this context, the isolation of microorganisms with lindane degrading potential has confirmed specific strains, which degrade lindane and other HCH isomers (Anupama and Paul 2010) either aerobically or anaerobically. Some strains grow well in media supplemented with HCH as a sole source of C and energy.

Anaerobic Biodegradation Pathway

The first anaerobic lindane-degrading bacterium isolated was *Clostridium sphenoides* UQM780 (MacRae et al. 1969). Subsequently, several other degrading microorganisms were reported, which include genera of *Clostridium*, *Bacillus*, and *Enterobateriaceae* (Kuritz and Wolk 1995; Middeldorp et al. 1996; Boyle et al.

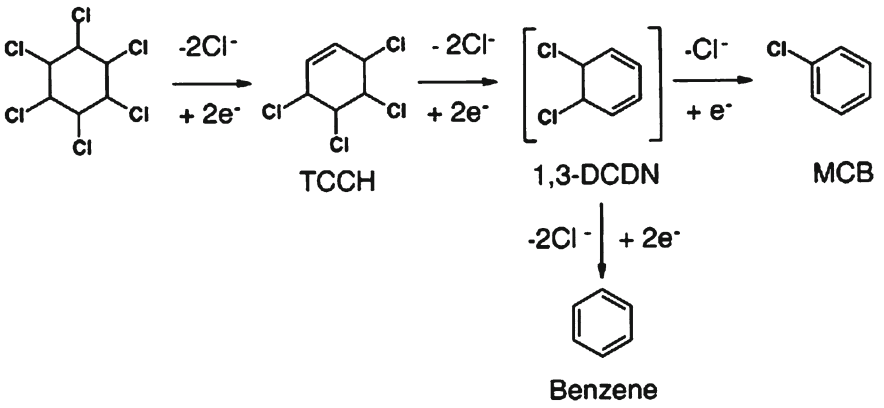

Fig. 15.2 Pathway for anaerobic degradation of HCH [adopted from Middeldorp et al. (1996)]

1999). There is however, inconsistency in results reported for the degradation of different HCH isomers, predominantly owing to varied genera of microorganisms in test soils and degree of tolerance and resilience to contaminants (Moreno and Buitron 2004). Haider and Jagnow (1975) reported that γ-HCH was degraded significantly (up to 90% after 5 days), whereas α- and β-HCH were found to be resistant under both methanogenic and sulfate-reducing conditions (Bachmann et al. 1988). Moreover, the degradation of all four HCH isomers (α-, β-, γ-, and σ-HCH) by mesophilic anaerobic sludges was reported by Buser and Muller (1995) and Quintero et al. (2005). In an anaerobic degradation pathway of HCHs, tetrachlorocyclohexene (TCCH) is identified as an intermediate, even though it is assumed that the primary intermediate was pentachlorocyclohexane (PCCH), which is too unstable to be detected. The complete pathway is shown in Fig. 15.2.

Aerobic Biodegradation Pathway

Complete mineralization of HCH occurs under only aerobic conditions. Microbial aerobic degradation of the four HCH isomers has been observed in both mixed soil cultures (Sahu et al. 1993) and pure cultures (Thomas et al. 1996; Johri et al. 1998). The majority of studies on the determination of an aerobic degradative pathway of lindane and other HCH isomers has concentrated on *Sphingomonas paucimobilis* UT26, a nalidixic acid-resistant mutant of *Sphingomonas* (previously classified as *Pseudomonas*) *paucimobilis* SS86 (Imai et al. 1989; Senoo and Wada 1989). This novel bacterial strain UT26 degrades α-, γ-, and σ-HCH and exploits γ-HCH as a sole source of carbon in the presence of oxygen (Nagasawa et al. 1993). The degradation pathway shown in Fig. 15.3 and involves several enzymes [Lin A (dehydrochlorinase), Lin B (halidohydrolase), Lin C (dehydrogenase), Lin D (reductive dehalogenase), Lin E (dioxygenase), Lin F (maleylacetate reductase), and Lin X (dehydrogenase)] encoded by genes (*linA*, *linB*, *linC*, *linD*, *linE*, *linF*, *linR*, and

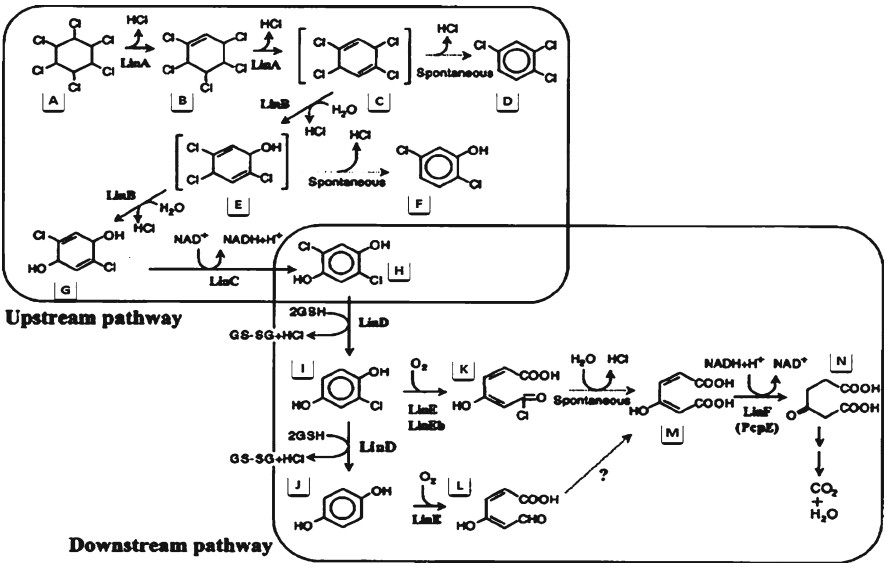

Fig. 15.3 Aerobic pathway of γ-HCH degradation by *S. paucimobilis* UT26. Compounds: *A* γ-HCH, *B* γ-pentachlorocyclohexene, *C* 1,3,4,6-tetrachloro-1,4-cyclohexadiene, *D* 1,2,4-trichlorobenzene, *E* 2,4,5-trichloro-2,5-cyclohexadiene-1-ol, *F* 2,5-dichlorophenol, *G* 2,5-dichloro-2,5-cyclohexadiene-1,4-diol, *H* 2,5-DCHQ, *I* CHQ, *J* HQ, *K* acylchloride, *L* γ-hydroxymuconic semialdehyde, *M* maleylacetate, *N* β-ketoadipate [modified from Endo et al. (2005)]

linX, respectively) and leads to eventual mineralization (Nagata et al. 2006). In addition to these catalytic enzymes, a putative ABC-type transporter system encoded by linKLMN is also essential for the γ-HCH utilization in UT26. After complete genome sequence analysis of UT26, it was found that lin genes for the γ-HCH utilization are dispersed on three large circular replicons of 3.5 Mb, 682 kb, and 191 kb. Nearly identical lin genes were also found in other HCH-degrading bacterial strains, and it has been suggested that the distribution of lin genes is mainly mediated by insertion sequence IS6100 and plasmids. Recently, it was revealed that two dehalogenases, LinA and LinB, have variants with small number of amino acid differences, and they showed dramatic functional differences for the degradation of HCH isomers, indicating these enzymes are still evolving at high speed (Nagata et al. 2007).

In a study, Böltner et al. (2007) isolated four *Sphingomonas* strains, all of which degraded α-, β-, γ-, and δ-HCH. Of these, two strains effectively colonized corn roots reaching a high cell density in cultivated soil and could partly remove γ-HCH. These bacteria, however, performed poorly in unplanted soils. It was suggested that the removal of persistent toxic chemicals can be accelerated by composite application of plants and bacteria, a process generally known as rhizoremediation. Pesce and Wunderlin (2004) reported the aerobic biodegradation of lindane by a consortium of bacteria, *Sphingobacterium spiritivorum*, *Ochrobactrum anthropi*, *Bosea thiooxidans*, and *S. paucimobilis*, from sediment at a polluted site on the Suquia

River, Cordoba, Argentina. The consortia of bacteria showed initial lindane degradation rates of 4.92×10^{-3}, 11.0×10^{-3}, and 34.8×10^{-3} mM h^{-1} when exposed to lindane concentrations of 0.069, 0.137, and 0.412 mM, respectively. Chloride concentration increased during aerobic biodegradation, indicating lindane mineralization. A metabolite identified as γ-2,3,4,5,6-pentachlorocyclohexene appeared during the first 24 h of biodegradation. Pure strains of *B. thiooxidans* and *S. paucimobilis*, however, degraded lindane after 3 days of aerobic incubation. The potential of different enriched bacterial cultures for degrading lindane, methyl parathion (*O*-dimethyl *O*-(4-nitro-phenyl) phosphorothioate) and carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) was assessed under various environmental conditions by Krishna and Philip (2008). Generally, the enriched cultures showed a variable level of degradation and differed with different pesticides. Degradation was more in a facultative anaerobic condition relative to those observed under aerobic condition. In aerobic cometabolic process, the degradation of lindane by lindane enriched cultures was 75 ± 3%, whereas 78 ± 5% of lindane degradation occurred in anaerobic cometabolic process. Degradation of methyl parathion by methyl parathion enriched culture was 87 ± 1% in facultative anaerobic condition. During degradation, many intermediate metabolites were observed, some of which were, however, disappeared after 4–6 weeks of incubation. Interestingly, it was found that the mixture of pesticide-enriched culture was more effective and degraded all the three pesticides more rapidly compared to the sole pesticide-enriched culture. This study suggested that the consortia of bacterial cultures capable of detoxifying the toxicity of multiple pesticides at one time could serve an interesting option for restoring the sites contaminated with multiple pesticides. In addition to bacterial communities, soil also harbors fungi, which are known to degrade lindane very effectively. For example, the degradation of lindane through secretion of certain enzymes has been reported for nonwhite-rot fungus *Conidiobolus* 03-1-56 (Nagpal et al. 2008), white-rot fungi *Cyathus bulleri* and *Phanerochaete sordid* (Singh and Kuhad 2000), and other fungus *Pleurotus ostreatus* (Rigas et al. 2005).

15.3.1.2 Biodegradation of Chlorpyrifos

Chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) is a broad-spectrum organophosphate insecticide displaying insecticidal activity against a wide range of insects and other arthropod pests. There are many reports on the degradation of chlorpyrifos by an extensive array of microbial communities inhabiting varied ecological niches and belonging to different genera including *Flavobacterium* and *Escherichia* (Wang et al. 2005; Richinis et al. 1997), *Enterobacter* (Singh et al. 2004), *Arthrobacter* (Mallick et al. 1999), and *Klebsiella* (Ghanem et al. 2007). The importance of microorganisms in the degradation of the organophosphorus insecticide chlorpyrifos during kimchi fermentation was investigated by Cho et al. (2009). Of the 30 mg chlorpyrifos L^{-1} used during the kimchi fermentation, 83.3% of chlorpyrifos was degraded rapidly until day 3, while after

9 days, it was degraded completely. The chlorpyrifos degrading lactic acid bacteria isolated from kimchi fermentation in the presence of 200 mg chlorpyrifos L^{-1} were identified as *Leuconostoc mesenteroides* (WCP907), *Lactobacillus brevis* (WCP902), *Lactobacillus plantarum* (WCP931), and *Lactobacillus sakei* (WCP904). All bacterial strains exhibiting degrading ability, used chlorpyrifos as the sole C and P source. Other insecticides, such as coumaphos, diazinon, parathion, and methyl parathion, were also degraded by the tested strains. In other study, a bacterial strain M-1 isolated from sludge collected from the wastewater treatment pool of a pesticide factory later identified as *Paracoccus* sp. by morphological and biochemical properties and 16 S rDNA sequence analysis was able to degrade 92.47% monocrotophos (100 mg/L) in 24 h. Monocrotophos was used as a sole C source by strain M-1. The biodegradation of monocrotophos was mediated by constitutively expressed cytosolic proteins, which had the greatest activity at pH 8 and 25 C, with its Michaelis–Mentn's constant (K_m) and maximum degradation rate (V_{max}) of 0.29 $\mu mol \times mL^{-1}$ and 682.12 $\mu mol\,(min \times mg)^{-1}$, respectively. The degrading enzyme was sensitive to high temperature, but was active at alkaline conditions (Jia et al. 2007). In a recent investigation, species of *Bacillus* and *Pseudomonas* were found to degrade 75% of chlorpyrifos and phorate and 50% of dichlorvos, methyl parathion, and methomyl within 7 days of incubation. However, dichlorvos and phorate were completely degraded by the end of 14 days and the order of microbial degradation was: phorate > dichlorvos > methyl parathion > chlorpyrifos > methomyl. Qualitative analysis of chlorpyrifos and methyl parathion residues by gas chromatography revealed the formation of one unidentified metabolite in inoculated samples, whereas no metabolite formation was detected in the case of other insecticides-inoculated samples (Madhuri and Rangaswamy 2009). Mallick et al. (1999) reported the rapid degradation of chlorpyrifos, added to a mineral salt medium, or applied to soil as a sole C source, by *Flavobacterium* sp. ATCC 27551 isolated from diazinon-retreated rice fields (Sethunathan and Yoshida 1973). Similarly, an *Arthrobacter* sp. isolated from a flooded soil retreated with methyl parathion has shown chlorpyrifos-degradating ability (Mishra et al. 1992). Moreover, Huang et al. (2000) studied the degradation of chlorpyrifos in poultry and cow-derived effluents and reported that chlorpyrifos was degraded by aerobic microbial processes in animal-derived lagoon effluents. Analysis of the microbial communities involved in the degradation process by denatured gradient gel electrophoresis of PCR-amplified 16 S rRNA genes showed that a single band became dominant in effluents during chlorpyrifos degradation, thus indicating that a single aerobic bacterial population is involved in chlorpyrifos degradation.

Biodegradation of chlorpyrifos is dependent on numerous abiotic factors. Soil pH plays a crucial role in the process. Singh et al. (2003) studied the effects of soil pH on biodegradation of chlorpyrifos in the UK and Australian soils and reported that the dissipation of chlorpyrifos in the UK soils varied at pH values from 4.7 to 8.4 and was mediated by the cometabolic activities of soil microorganisms. A robust bacterial population that utilized chlorpyrifos as a sole source of C was detected in an Australian soil. Transmission and propagation of chlorpyrifos-degrading microorganisms from the Australian soil to UK soils was monitored by

molecular fingerprinting of bacterial 16sRNA genes by PCR-denaturing gradient gel electrophoresis. A heightened ability to biodegrade chlorpyrifos was increased in the UK soils. In addition, only soils with pH ≥ 6.7 were able to maintain this degrading ability 90 days after inoculation. The rate of degradation in chlorpyrifos-degrading bacteria-inoculated soils increased with increasing soil pH from 4.3 to 7.0, but there was no significant difference in degradation rate with pH 7.0–8.4. The degradation rate of chlorpyrifos in acidic soils was slower than in neutral and alkaline soils (Yang et al. 2006). In another study, Singh et al. (2004) reported the enhanced degradation of chlorpyrifos by an *Enterobacter* strain B-14 and found that the strain responsible for enhanced biodegradation of chlorpyrifos showed greatest similarity to *Enterobacter asburiae* based on 16 s rRNA analysis. This strain utilized chlorpyrifos as a sole source of C and P and hydrolyzed it to diethylthiophosphoric acid (DETP) and 3,5,6-trichloro-2-pyridinol (TCP). Further studies with B-14 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 (*opd*) gene (Singh et al. 2004). The authors also concluded that the application of the strain B-14 as bioinoculant in chlorpyrifos-contaminated soil led to substantial increase in the degradation rate of chlorpyrifos than that observed for noninoculated soils. Yang et al. (2005) isolated *Alcaligenes faecalis* DSP3, which has the ability to degrade both chlorpyrifos and TCP. Moreover, Yang et al. (2006) were successful in cloning the *mpd* gene from a chlorpyrifos-degrading bacterium and applying it to bioremediation of contaminated soils. Six chlorpyrifos-degrading bacteria were isolated using chlorpyrifos as the sole source of carbon by enrichment procedure. Their strain, YC-1, showed the highest degrading capability and was putatively identified as the genus *Stenotrophomonas*. The strain YC-1 degraded 100 mg/L chlorpyrifos within 24 h. When chlorpyrifos-degrading strain YC-1 was used as bioinoculant in fumigated and nonfumigated soils, the inoculated soils experienced a more rapid rate of chlorpyrifos degradation compared to the noninoculated control. The initial concentration of 100 mg/kg chlorpyrifos was completely degraded within 15 days. Degradation of chlorpyrifos in control nonfumigated soils (without inoculation) was considerably lower. According to Guha et al. (1997), the *opd* gene for the degradation of chlorpyrifos occurs on plasmids as observed in *Micrococcus* sp. isolated from soil. In contrast, the presence of plasmids was not detected in chlorpyrifos-degrading *Stenotrophomonas* strain YC-1 by the alkali lysis method, which inferred that the *opd* gene was located on the chromosome (Yang et al. 2006). However, both *mpd* and *opd* genes have also been found located variably on chromosome and plasmid. For example, Ajaz et al. (2009) suggested that the biodegradation of chlorpyrifos is mediated by split location of the genes (located on the plasmid and the chromosome) in the *Pseudomonas putida* MAS-1.

In a follow-up study, Li et al. (2007) isolated a highly effective chlorpyrifos-degrading bacterium strain Dsp-2 from the polluted treatment system of a chlorpyrifos manufacturer. This strain identified as *Sphingomonas* sp. by morphological, physiological, biochemical tests, and employing molecular tool (16 S rDNA) could utilize chlorpyrifos as a sole C source for growth by hydrolyzing chlorpyrifos to

3,5,6-trichloro-2-pyridinol (TCP). It could also utilize parathion, parathion-methyl, fenitrothion, and profenofos, but not phoxin and triazophos. Subsequently, the bioremediation ability of this strain was tested under soil environment. When strain Dsp-2 was added to soil treated with 100 mg kg^{-1} chlorpyrifos, it showed a higher degradation rate relative to control soils (without inoculation).The moderate pH, moisture, and inoculum density were found to promote degradation. The gene encoding the chlorpyrifos-hydrolytic enzyme was found as having 99% similarity to *mpd* (a gene encoding the parathion-methyl hydrolyzing enzyme in *Plesiomonas* sp. M6). The hydrolytic efficiency of mpd for chlorpyrifos was significantly greater than the wild-type *mpd* from strain M6.

The degradation of chlorpyrifos is, however, influenced by various factors. In order to assess the impact of variable culture conditions, such as pH, inoculum density, presence of added carbon/nutrient sources, and pesticide concentration, Anwar et al. (2009) conducted an experiment employing *Bacillus pumilus* C2A1 for chlorpyrifos degradation. Chlorpyrifos was utilized by strain C2A1 as the sole source of C and energy as well as it was cometabolized in the presence of glucose, yeast extract, and nutrient broth. Chlorpyrifos was degraded maximally at pH 8.5 and high-inoculum density. Degradation was, however, further enhanced in the presence of other nutrients probably due to high growth on easily metabolizable compounds which in turn increased degradation. The strain C2A1 also showed 90% degradation of TCP (300 mg/L) within 8 days of incubation. In a similar study, Lakshmi et al. (2009) observed that the degradation of chlorpyrifos in soil by three aerobic bacterial consortia, AC, BC, and DC, was greater (50, 56, and 64%, respectively) at 30 days compared to those observed after 21 days (54, 46, and 61%, respectively) growth in basal medium treated with 50 mg chlorpyrifos L^{-1}. *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella* sp., and *Serratia marscecens* when grown alone in basal medium supplemented with 50 mg chlorpyrifos L^{-1} degraded chlorpyrifos by 84, 84, 81, and 80%, respectively, after 20 days and 92, 60, 56, and 37%, respectively, after 30 days. Formation of 3,5,6-trichloro-2-pyridinol, the major metabolite of chlorpyrifos degradation, was observed during the degradation of chlorpyrifos by *P. aeruginosa*, which disappeared to negligible amounts. This and other associated studies are thus likely to help overcome chlorpyrifos toxicity in contaminated environment.

15.3.1.3 Monocrotophos

The degradation of a widely used organophosphorus insecticide, monocrotophos (dimethyl (E) 1-methyl-2-methylcarbamoyl vinyl phosphate) in two Indian agricultural soils, i.e., a black vertisol and red alfisol, was studied in the laboratory by Gundi and Reddy (2006). The insecticide was applied at two concentrations, 10 and 100 μg g^{-1} soil, under aerobic conditions at 60% water-holding capacity at 28 ± 4°C. The degradation of monocrotophos (MCP) at both concentrations was rapid, accounting for 96–98% of the applied quantity following first-order kinetics with rate constants (k) of 0.0753 and 0.0606 day^{-1} and half-lives ($t_{1/2}$) of 9.2 and

11.4 days, respectively. Degradation of MCP in soils proceeded by hydrolysis, with the formation of *N*-methylacetoacetamide. Even three additions of MCP at 10 μg g^{-1} soil did not result in enhanced degradation. However, there was cumulative accumulation of *N*-methylacetoacetamide in soils pretreated with MCP, i.e., 7–15 μg g^{-1} soil. Both biotic and abiotic factors were involved in MCP degradation. In one study (Bhalerao and Puranik 2009), soil fungi capable of degrading MCP were isolated from various geographical sites. Twenty-five strains were isolated by an enrichment method using MCP as a carbon and phosphorus source. On the basis of MCP tolerance capacity exhibited in gradient agar plate assay, the isolate M-4, identified as *Aspergillus oryzae* ARIFCC 1054, was selected for further studies. The ability of the isolate to mineralize MCP was investigated under different culture conditions. The isolate was found to possess phosphatase activity. The course of the degradation process was studied using high-performance thin layer chromatography (HPTLC) and FTIR analyses. The results suggest that this organism could be used for bioaugmentation of soil contaminated with MCP and for treatment of aqueous wastes.

Degradation of MCP in soils was found to be enhanced by light, moisture (more in flooded soils than in dry loam soils), and type of water (greater in tap water than distilled water) (Dureja 1989). Biodegradation of MCP and other organophosphates by soil bacteria was studied by Rangaswamy and Venkateswarlu (1992). They isolated several strains of *Bacillus* and one isolate of *Azospirillum lipoferum*, which were capable of degrading MCP. Microbial degradation was more pronounced and rapid than chemical decomposition.

Bhadbhade (2001) studied microbial degradation of MCP; microorganisms capable of degrading MCP were isolated from ten soil samples collected from Maharashtra. Among 54 isolates, 74% (32 isolates) were obtained from exposed soils, whereas 26% (22 isolates) were from soils not exposed to MCP. This revealed the predominance and ease in isolating MCP-degrading bacteria from exposed soils. The cultures belonged to the genera *Bacillus* (62%), *Arthrobacter* (22%), *Pseudomonas* (12%), and 2% each to *Planococcus* and *Stomatococcus*. Three cultures identified as *Arthrobacter atrocyaneus*, *Bacillus megaterium*, and *Pseudomonas mendocina* showed 80–90% degradation to MCP at maximum initial concentration of 500 mg/L in synthetic medium within 48 h. The cultures tolerated MCP up to a concentration of 2,500 mg/L and could utilize MCP as a sole source of carbon in synthetic media. The isolates showed maximum degradation of MCP under different environmental conditions; for example, pH values of 7.0–8.0, temperatures of 30–35ºC, MCP concentrations ranging from 100 to 500 mg/L, and an inoculum density of 108–109 cells/mL, in synthetic medium under aerated culture condition in 48 h. The removal of MCP ranged between 77 and 78% (Bhadbhade 2001).

Biodegradation of MCP to phosphates, ammonia, and carbon dioxide was brought about through the formation of intermediate compounds; namely, one unidentified metabolite, methylamine, and volatile fatty acids such as acetic acid or *n*-valeric acid. The isolates were found to exhibit two enzymes, namely phosphatase and esterase, which were involved in the degradation of MCP. The microbial metabolic pathway for the degradation of MCP has been proposed

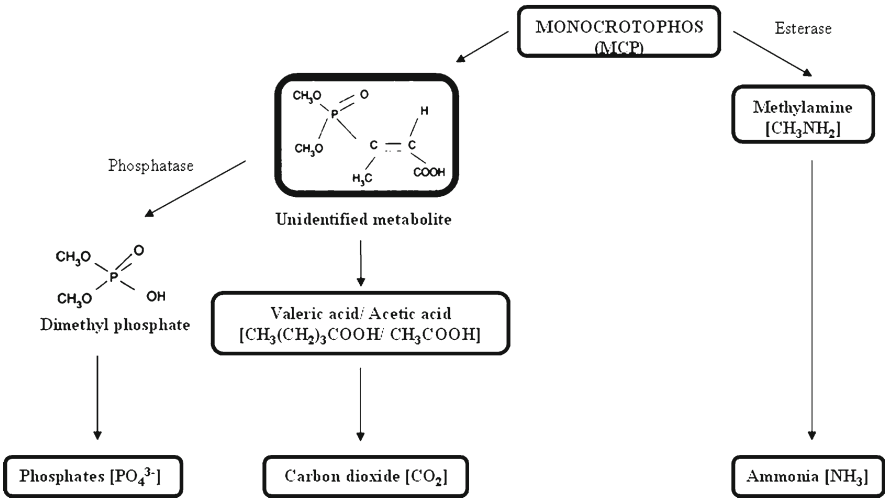

Fig. 15.4 Pathway for degradation of monocrotophos (Bhadbhade et al. 2002a)

based on experimental results (Fig. 15.4) (Bhadbhade et al. 2002a). In addition, MCP degradation genes are now known to be located on plasmids (Bhadbhade et al. 2002b). In a similar study, Subhas and Singh (2003) observed *Pseudomonas aeruginosa* F10B and *Clavibacter michiganense* subsp. *insidiosum* SBL 11 able to degrade technical MCP in shake-flask culture up to 98.9 and 86.9%, respectively, and pure MCP up to 79 and 80%, respectively, within 24 h at 37°C. The optimal concentration of MCP required for the normal growth was 500 ppm. Tris-*p*-nitrophenyl phosphate was found as the most preferred substrate followed by paraoxon. The enzyme involved in the degradation of MCP was phosphotriesterase, which was localized on the membrane-bound fraction of the disrupted cells. The gene responsible for the production of phosphotriesterase (*opd*) in *P. aeruginosa* F10B was plasmid-borne.

15.4 Conclusion

Insecticides in general adversely affect metabolic activities of both soil microflora and crop plants. At recommended dose rates, the toxic effects of insecticides on beneficial activities of rhizobacteria and plant growth parameters are, however, less severe. Rates higher than recommended field rates have been found to decrease nitrogen fixing ability, production of phytohormones, and other regulatory substances in soil microorganisms and photosynthesis, dry biomass accumulation and the general nutrient status of crop plants. Therefore, natural, inexpensive, and eco-friendly microbes endowed with insecticide-degrading potential could be an ecologically sound alternative to detoxify persistent and excessive quantities of residual insecticides in soils.

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