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# Chlorpyrifos Bioremediation in the Environment: A Review Article

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***ABSTRACT***

***Introduction:*** Chlorpyrifos is an organophosphorus pesticide that is commonly used in agriculture. This toxin is harmful to a wide range of organisms, including living organisms, useful arthropods, fish, birds, humans, animals, and plants. There are many physical, chemical, and biological methods for the removal of organophosphorus pesticides from ecosystems, among which biodegradation is preferable because of environmental compatibility and cost-effectiveness. Identifying the effective genes and enzymes in the specific functional groups of pesticides and understanding the kinetics of biodegradation is essential for successful biorefining.

***Materials and Methods:*** This study was a narrative review article. For this purpose, relevant studies indexed in a variety of databases such as Google Scholar, Elsevier, Scopus, Science Direct, Magiran, and SID which published between 2004 and 2018 were retrieved using the key words *Bioremediation*, *chlorpyrifos*, *Dursban*, and *microorganism*. Finally, a total of 51 articles were studied.

***Results:*** The major processes of chlorpyrifos destruction are evaporation, photolysis, chemical hydrolysis, and microbial degradation. Biodegradation is an environmentally friendly and highly efficient process that can be used as an alternative to chemical and physical methods. In this process, the microbial population is used to convert complex toxic compounds into less toxic ones.

***Conclusion:*** Chlorpyrifos, which was previously thought to be resistant to advanced biodegradability, has currently shown to undergo advanced biodegradation by bacterial and fungal species. In the future, studies of genes that are highly capable of biodegradation will lead to a complete degradation method that is involved in the microbial destruction of this toxin.

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## Introduction

Many efforts have been made around the world, especially in developing countries, to provide food for the growing population of the world and the management of food shortage. Meanwhile, fertilizers, pesticides, and herbicides are used for nutrition and to protect food [1-4].

It is estimated that about 30% of agricultural products are lost due to pest attack. Meanwhile, excessive use of pesticides can lead to significant destructive effects. Pesticide is a chemical that is used to kill, break down, control, or repel pests that are harmful to agriculture, wood products, animals, food, and humans. Pesticides are classified into



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herbicides, insecticides, fungicides, and rodent repellents according to a classification. Among them, insecticides are widely used in developing countries; however, in industrialized countries the share of herbicides is higher. It is estimated that three million people are exposed to pesticides and 0.2 million people each year die because of pesticides [2, 5-6].

Based on chemical compounds, pesticides are divided into four main categories: Organochlorines, organophosphorus, carbamates, and pyrethroids.

About 38% of organophosphorus compounds in the world are used to control agricultural and domestic pests. Organophosphorus compounds are classified into four main subgroups:

Phosphates, phosphorothioates, phosphorodithioates, and phosphorothiolates. Organophosphorus compounds generally contain organic compounds with a phosphorus atom. Tetraethyl pyrophosphate, the first organophosphorus insecticide, was produced in 1937. Organophosphorus toxin subtypes include about 100 different types of organophosphorus toxins, many of which have been prohibited due to high toxicity [2, 7-8].

Quinalphos, monocrotophos, chlorpyrifos, malathion, and parathion are some of the most commonly used organophosphorus compounds. Furthermore, chlorpyrifos (O, Odiethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate), with the brand name *Dursban*, widely used in agriculture as a commercial insecticide and as a synaptic toxin, has a wide range of insecticidal activities to control the insect pests of corn, cotton, citrus fruits, fruits, nuts, potatoes, beets, legumes, etc. [3, 9-10].

According to the WHO classification, chlorpyrifos belongs to the second class of pesticides with moderate toxicity that entered the soil in 1965 with a half-life of 10-20 days. Its molecular weight is 350.6 g/mol, its melting point 43.5-41 °C, and solubility in water is 1.2 mg/L at 25 °C. Chlorpyrifos is the fourth leading used organophosphorus pesticide after monocrotophos, acephate, and endosulfan [2, 4, 11-12]. Some physicochemical properties of chlorpyrifos are shown in Table 1 [1].

**Table 1:** Physico-chemical properties of chlorpyrifos

| **Characteristics of Chlorpyrifos** | |
|---|---|
| Chemical name | O, O-diethyl O-(3,5,6-trichloro-2- pyridinyl)-phosphorothioate |
| Chemical formula | $C_9H_{11}C_{l3}NO_3PS$ |
| Molecular weight | 350.6 a.m.u. |
| Physical appearance | White crystalline solid |
| Melting point | 42-43.5 °C |
| Vapor pressure | $1.8 \times 10^{-5}$ mm Hg at 25 °C |
| Henry's law constant | $2.9 \times 10^{-6}$ atm $m^{-3}$mole at 25 °C |
| Solubility | Water 0.002 g $l^{-1}$ at 25 °C |
| | 0.0014 g $l^{-1}$ at 25 °C |
| | Methanol 450 g $l^{-1}$ at 25 °C |
| | Acetone > 400 g $l^{-1}$ at 20 °C |
| | Dichloromethane > 400 g $l^{-1}$ at 20 °C |
| | Ethyl acetate > 400 g $l^{-1}$ at 20 °C |
| | Toluene > 400 g $l^{-1}$ at 20 °C |
| | n-Hexane > 400 g $l^{-1}$ at 20 °C |
| Partitioning coefficient | Log $K_{ow}$ 4.96 - 5.11 |
| | 3.78 soil slurry |
| | Log $K_{oc}$ 3.78 |
| Half-life | pH 4.5, 25 °C 77 days |
| | pH 6.0, 25 °C 49 days |
| | pH 7.0, 15 °C 100 days |
| | pH 8.0, 25 °C 19 days |
| | pH 8.5, 4 °C 27 days |

Chlorpyrifos can exert its effect through contact, ingestion, and respiration. Absorption of chlorpyrifos is 70% by mouth and less than 3% by the skin. Organophosphorus compounds are easily biotransformed in the liver and do not accumulate in the human body. They are usually excreted through urination within 24 hours and less likely to exit through defecation. Common formulations include emulsifiable concentrate (EC), granule (GR), and wettable powder (WP). Human contamination sources of chlorpyrifos include its aerial and direct application on soil, land, fruits, and vegetables [2, 13].

By exposing to chlorpyrifos, acetylcholine accumulations occur which leads to high irritation and sometimes nerve compression. This inhibition leads to seizures, paralysis, and ultimately death of insects and mammals. The acetylcholine is responsible for transmitting nerve impulses across body parts, for example, skeletal muscles and brain. The acetylcholine accumulation interferes with the transmission of the nerve impulses at the nerve ending; therefore it should be hydrolyzed to prevent the hyperstimulation of the nervous system. Acetylcholine hydroxylation is catalyzed by an enzyme called acetylcholinesterase. The substrate binds to acetylcholinesterase in the active site of Serine 203 and converts acetylcholine to choline and acetyl CoA, which ultimately leads to the death of insects and mammals. The excessive use of chlorpyrifos on plants may delay plant emergence, deformation of the fruit, and abnormal cell division. The harmful effects of chlorpyrifos in humans include muscle sprain, skin irritation, depression, respiratory problem, seizure, and death. Microbial populations are also affected by the use of chlorpyrifos, including stimulatory or inhibitory effects. In animals' oxidative stress, abnormally high level of blood sugar has been increased due to chlorpyrifos exposure. Chlorpyrifos exerts adverse effects on non-target systems, such as nervous system disorders, birth defects, low birth weight, immune system disorders, and endocrine system impairments. Chlorpyrifos exposure is also associated with bladder cancer and chromosomal damage [2, 14].

Pesticides are naturally degraded by microorganisms in the environment, resulting in the conversion of main toxic compounds into less toxic or non-toxic products. Biorefining refers to the process of breakdown of organic compounds into less harmful mineral molecules by using microorganisms. In biotechnology, biorefining is considered as an efficient and cost-effective way to correct the contaminated environment [15- 17]. The purpose of this study was to investigate the methods of chlorpyrifos biodegradation.

## Materials and Methods

To conduct this narrative review, the relevant evidence was retrieved using the key words *biorefining*, *chlorpyrifos*, *Dursban*, and *microorganism* from the publications published in Persian and indexed in databases include Google Scholar, SID, Medlib (Iran Doc), Iran Medex, and Magiran as well as those published in English language and indexed in PubMed, Scopus, Google Scholar, and Web of Sciences between 2004 and 2018. Finally, 51 articles were selected for the analysis.

## Results

The biodegradation rate and the initial amount of toxin are effective factors on the amount of pesticide residues in the environment.

The fate of chlorpyrifos not only depend solely on its physical and chemical properties, but also depends on the characteristics of the soil, environmental conditions, and environmental activities. Chlorpyriphos binds to plants, soil particles, and sediments, and then, most of which evaporates, hydrolyzes, or biodegrades. The half-life of chlorpyrifos in soil, estimated by EPA (1996), is about 360 days. Chlorpyriphos is relatively soluble in water and its half-life has been estimated to be 35-78 days. Chlorpyrifos in the atmosphere reacts with hydroxyl radicals produced by photochemical processes and turns into chlorpyrifos oxon with a half-life of 4.2 hours. The highest levels of chlorpyrifos in human urine have been observed 6-7 hours after oral administration and 17-24 hours after skin contact [1].

The destruction of chlorpyrifos depends on four variables: (a) The availability of pesticides or metabolites microorganisms; (b) the physiological conditions of microorganisms; (c) the survival and proliferation of microorganisms that degrade pesticides at the contaminated site; and (d) the sustainable microbial population in the soil.

Advanced degradation of a specific pesticide may not be carried out for the following reasons. One possibility is the inability of the microbial flora to destroy the primary pesticide that will slow down the biodegradation process. The second possibility is that inadequate pesticide degradation can be caused by adverse environmental conditions. The third and most likely possibility is that pesticide metabolites may not be sufficiently catalyzed by soil microorganisms [1-2, 18].

### *Biodegradation of Chlorpyrifos in Water and Soil*

Biodegradation is a common process for the removal of organic pollutants due to its low cost and lower risk for natural organisms. Naturally, microorganisms can significantly affect the metabolism of many pesticides; furthermore, it can be enhanced by the production of genetically engineered microorganisms.. Microorganisms are able to completely mineralize many of the aliphatic, aromatic, and heterocyclic compounds. There are two major types of organic chemical degradation by microorganisms. In the first type of degradation, organic chemicals are completely decomposed into their metabolites, along with the release of energy and nutrients that are used by microorganisms. This type of degradation is called *catabolism*, while in the second type, called *co-metabolism*, organic chemicals are not completely metabolized; however, they may either convert or engaged in some part of the metabolic pathway along the normal metabolic activity without producing any beneficial effect on microbial population [1- 2, 12, 19].

Among all the microorganisms, bacteria and fungi have the highest growth potential in the chlorpyrifos substrate, which is why they have been considered in degradation studies. In Table 2, a number of studies that have been carried out on the use of bacteria and fungi in the degradation of chlorpyrifos are cited. Microorganisms are capable of decomposing artificial chemicals in the soil and using them as the only source of carbon and energy. Chlorpyrifos can be directly used by bacteria as sources of C, P and N. Fungal strains that are mainly important for the capability of degrading chlorpyrifos include *Coriolus versicolor*, *Hypholama fascicularae*, and white rot fungi. Recently, *JAS4*, a species of *Ganoderma* has been isolated from agricultural soil that is highly capable of reducing chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol (TCP). The bacterial species that degrade chlorpyriphus in aquatic environments belong to the genus *Enterobacter*, *Alcaligenes*, *Klebsiella*, *Providencia*, *Pseudomonas*, *Leuconostoc*, *Lactobacillus*, *Serratia*, and *Synechocystis*. In addition to bacteria and fungi, plants such as water lettuce and duckweed can remove chlorpyrifos from aquatic environments [1, 18, 20- 21].

In order to increase our understanding of different mechanisms and pathways of enhanced biodegradation, isolation and specification of the pesticide-degrading microorganisms is important. One of the new and emerging technologies for this toxic compound and its related neurological agents, is the use of organophosphorus hydrolysis (OPH), phosphotriesterase or aryldialkylphosphatase (PTE), organophosphorus acid anhydrolase (OPAA), and methyl parathion hydrolase (MPH) that are produced by the microorganisms degrading this toxin. Two of the important isolated genes from the chlorpyrifos-degrading microorganisms are *mpd* and *pod* present in chromosomes and plasmid [22- 23].

### *Products of Chlorpyrifos Degradation*

A number of destruction products such as TCP, DETP, chlorodihydro-2-pyridone, dihydroxypyridine, tetrahydro-2-pyridone, and semialdehyde maleamide are produced by bacteria and fungi during degradation of chlorpyrifos. Chlorpyrifos-oxon and TCP are two major products of chlorpyrifos conversion that


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are found in groundwater. According to the United States Environmental Protection Agency (USEPA), TCP with a half-life of 65-360 days has greater toxicity, antimicrobial property, and mobility than chlorpyrifos [1, 24- 25].

**Table 2:** Studies on the Use of Bacteria and Fungi in Chlorpyrifos Degradation [17]

| Name of Microorganisms | Type of Microorganisms | References |
|---|---|---|
| Enterobacter sp. | Bacteria | Singh et al. 2006 [26] |
| Stenotrophomonas sp. | Bacteria | Yang et al. 2006 [27] |
| Pseudomonas fluorescens<br>Brucella melitensis<br>Bacilus subtilis<br>Bacilus cereus<br>Klebsiella sp.<br>Serratia marcescens<br>Pseudomonas aeruginosa | Bacteria | Lahkshmi et al. 2008 [28] |
| Sphingomonas sp | Bacteria | Li et al. 2008 [29] |
| Pseudomonas aeruginosa<br>Bacillus cereus<br>Klebsiella sp.<br>Serratia marscecens | Bacteria | Lakshmi et al. 2009 [30] |
| Synechocystis sp. | Bacteria | Singh et al. 2011 [31] |
| Streptomyces sp.<br>AC5 & AC7 | Bacteria | Briceno et al. 2012 [16] |
| Pseudomonas putida<br>(NII 1117)<br>Klebsiella sp. (NII 1118)<br>P. stutzeri (NII 1119)<br>P.aeruginosa (NII 1120) | Bacteria | Sasikala et al. 2012 [13] |
| Lactobacillus fermentum<br>L.lactis<br>Escherichia coli | Bacteria | Harishankar et al. 2013 [32] |
| Cellulomonas fimi | Bacteria | Barathidasan et al. 2014 [33] |
| Mesorhizobium sp. HN3 | Bacteria | Jabeen et al. 2015 [34] |
| Pseudomonas putida | Bacteria | Pradeep and Subbaiah, 2015 [35] |
| Staphylococcus sp. ES-2 | Bacteria | Supreeth and Raju 2016 [36] |
| Streptomyces sp. HP-11 | Bacteria | Supreeth et al. 2016 [37] |
| Bacillus subtilis inaquosorum<br>strain KCTC13429<br>B. cereus ATCC 14579<br>B. safensis F0-36b | Bacteria | Ishag et al. 2016 [38] |
| Verticillium sp | Fungi | Yu et al. 2006 [39] |
| Trichosporon spp | Fungi | Xu et al. 2007 [40] |
| Verticillus sp. | Fungi | Fang et al. 2008 [3] |
| Acremonium sp.<br>strain (gfrc-1) | Fungi | Kulshrestha and Kumari 2011 [41] |
| Cladosporium<br>cladosporioides Hu-01 | Fungi | Chen et al. 2012 [42] |
| Aspergillus sp.<br>Penicillium sp.<br>Eurotium sp.<br>Emericella sp | Fungi | Maya et al. 2012 [43] |
| Aspergillus terreus | Fungi | Silambarasan and Abraham 2013 [44] |

In previous studies, no evidence on the advanced degradation of chlorpyrifos has been found, but it is currently assumed that soil microorganisms are degraded to 3, 5, 6-trichloro-2-pyridinol (TCP) and diethylthiophosphate (DETP). TCP is more resistant than chlorpyrifos itself, and is one of the factors that limits the complete mineralization of chlorpyrifos in aqueous environments. The extensive mineralization of TCP into carbon dioxide in soil has been observed in several studies. Soil microorganisms mineralize 65-85% of the TCP within 14 days. The products of chlorpyrifos destruction are inherently toxic and have accumulation properties. TCP has antimicrobial properties that prevent the proliferation of chlorpyrifos degrading microorganisms. The most important role of microorganisms in the pathway of chlorpyrifos degradation is the metabolization and mineralization of TCP and DETP, which are toxic compounds in nature [12, 15].

### *Kinetics and Modeling of Chlorpyrifos*

Knowledge about biodegradation kinetics is essential for understanding the stability of the organic pollutant and assessing human's exposure to it. The rate of biodegradation depends on the type and concentration of target compound and also active microorganisms in those environmental conditions. Primary pest concentration is crucial to determine the kinetics of biodegradation. If the microorganisms do not grow with the use of the target compound, the Michaelis Menten kinetic model is used to describe the kinetics of the biodegradation. If the microorganisms directly use the target compound as a carbon source, the Monod model is used to describe the kinetics of biodegradation. According to studies, chlorpyrifos biodegradation follows the first order kinetics [1].

### *Effective Biological Effects of Chlorpyrifos*

Biodegradation depends on several factors such as temperature, solar radiation, soil type, pH, the presence of oxygen or other oxygen receptors, nutrient, chemical structure and availability of the target compound, as well as the type and number of decomposer population. Concentration, solubility, and availability of pesticides for microbes are essential factors for the rate of biodegradation. Optimization of environmental factors plays a vital role in accelerating the biodegradation of chlorpyrifos. Because of the low chlorpyrifos solubility in water (2 mg/L) and the strong affinity to organic compounds and soil, it is less susceptible to microbial decomposition. The use of carbon sources increases the availability of the target compounds for microbes due to the production of biosurfactants and the increase in the growth of microbes [1, 45- 46].

## Discussion

Due to the adverse effects of chlorpyrifos on all living organisms, there is a need to refine contaminated sites through chemical purification, recovery, pyrolysis, burning, and landfilling. These methods are costly and difficult to perform; moreover, toxic compounds are likely to form. Pesticides are naturally degraded by the activity of microorganisms in the environment [38]. Microorganisms convert toxic compounds into moderately and less toxic compounds through the biorefining process. The natural process of biorefining is relatively slow; therefore, there is a need to increase the potential to destroy microorganisms. Throughout the biorefining process, microorganisms release certain enzymes that contribute to the metabolism of a wide range of human-manufactured chemicals [2, 25, 45, 47].

The destruction of chlorpyrifos by microorganisms is an efficient, cost-effective, and inexpensive process to improve the quality of the environment. Destruction of pesticides is carried out through both biological and non-biological pathways; however, the destruction of pesticides by microorganisms is the primary mechanism of decomposition and detoxification in the soil. In 1971, the first evidence of biodegradation of pesticides was reported. Understanding the physiological, microbiological, ecological, biochemical, and molecular aspects of contaminant conversion is required for the use of microbial biorefining [7, 19, 38, 48].

One of the main steps in the destruction of chlorpyrifos is the metabolization and mineralization of 3, 5, 6-trichloro-2- and 3, 5, 6 - 3, 5, 6-trichloro- 2- methoxypyridine. Soil microorganisms play a vital role in the degradation and mineralization of these metabolites.



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The distribution of endophytic bacteria was first reported by Gardner et al. *Pseudomonas* (40%) and *Enterobacter* (18%) are the dominant endophytic species.

*Pseudomonas* is one of bacteria in the soil and plays a vital role in the mineralization of organic compound. They are metabolically compatible and can degrade many aromatic hydrocarbons, oils, petroleum products, and pesticides. German et al. reported the degradation of herbicides by endophytic *Pseudomonas*. In addition, *Pseudomonas* is able to mineralize phenolic compounds. Different types of low weight molecular compounds, including chlorinated aliphatic hydrocarbons, are also metabolized by *Pseudomonas* due to a diverse range of catabolic pathways; therefore, they have been widely used in the destruction process. The individual bacterial species of *Pseudomonas* genus can use a number of substrates as foods. For example, *P. cepacia* alone is able to use over 100 different types of substrates as the only source of C, N, and S [2, 19, 29].

## Conclusion

Organic organophosphorus pesticides are a highly toxic kind of neurotoxins and cause severe toxicity in insects, mammals, and other animals. These compounds inhibit acetylcholinesterase and prevent the transmission of nerve signals. Chlorpyrifos is one of the organophosphorus compounds that are widely used as insecticide in agriculture. This toxin is harmful to a wide range of organisms, including organisms living in the soil, beneficial arthropods, fish, birds, animals, humans, and plants [2, 7].

Different physical, chemical, and biological methods are used to remove these contaminants from the environment. Today, biorefining techniques and using microorganisms are considered as appropriate methods of choice to reduce or eliminate these pollutants from the environment due to low costs, environmental compatibility, and high efficiency [25, 49].

Encoding the plasmid of the chlorpyrifos-degrading bacterial strains is one of the proper methods used for complete mineralization of chlorpyrifos. Molecular markers will be effective in detecting and tracking further chlorpyrifos degrading microbial strains. The induction of catabolic genes and the expression of appropriate pesticide-degrading enzymes will increase the use of biodegradation in the removal of environmental contaminants [1, 48, 50- 51].

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## Conflict of Interest

The authors declare no conflict of interest.



## References

1. Chishti Z, Hussain S, Arshad KR, et al. Microbial degradation of chlorpyrifos in liquid media and soil. J Environ Manage. 2013; 114: 372-80.
2. Gilani RA, Rafique M, Rehman A, et al. Biodegradation of chlorpyrifos by bacterial genus Pseudomonas. J Basic Microbiol. 2016; 56(2): 105-19.
3. Fang H, Qin Xiang Y, Jie Hao Y, et al. Fungal degradation of chlorpyrifos by Verticillium sp. DSP in pure cultures and its use in bioremediation of contaminated soil and pakchoi. Int Biodeterior Biodegradation. 2008; 61(4): 294-303.
4. Anwar S, Liaquat F, Khan QM, et al. Biodegradation of chlorpyrifos and its hydrolysis product 3,5,6-trichloro-2-pyridinol by Bacillus pumilus strain C2A1. J Hazard Mater. 2009; 168(1): 400-5.
5. Farhan M, Khan AU, Wahid A, et al. Chlorpyrifos biodegradation in laboratory soil

through bio-augmentation and its kinetics. Asian J Chem. 2013; 25(17): 9994.
6. Prabha R, Singh D, Verma M. Microbial interactions and perspectives for bioremediation of pesticides in the soils. Plant-Microbe Interactions in Agro-Ecological Perspectives: Springer; 2017; 27: 649-71.
7. Bhagobaty RK, Joshi SR, Malik A. Microbial degradation of organophosphorous pesticide: chlorpyrifos (mini-review). Internet J Microbiol 2010; 4(1): 1-6.
8. Abraham J, Shanker A, Silambarasan S. Role of Gordonia sp JAAS1 in biodegradation of chlorpyrifos and its hydrolysing metabolite 3,5,6-trichloro-2-pyridinol. Lett Appl Microbiol. 2013; 57(6): 510-6.
9. Sharma S, Singh PB, Chadha P, et al. Chlorpyrifos pollution: its effect on brain acetylcholinesterase activity in rat and treatment of polluted soil by indigenous Pseudomonas sp. Environ Sci Pollut Res. 2017; 24(1): 381-7.
10. Pant A, Rai J. Bioremediation of chlorpyrifos contaminated soil by two phase bioslurry reactor: processes evaluation and optimization by Taguchi's design of experimental (DOE) methodology. Ecotoxicol Environ Saf. 2018; 150: 305-11.
11. Dhanya M. Advances in microbial biodegradation of chlorpyrifos. J Environ Res Dev. 2014; 9(1): 232.
12. Tang X, Yang Y, Huang W, et al. Transformation of chlorpyrifos in integrated recirculating constructed wetlands (IRCWs) as revealed by compound-specific stable isotope (CSIA) and microbial community structure analysis. Bioresour Technol. 2017; 233 (Supplement C): 264-70.
13. Sasikala C, Jiwal S, Rout P, et al. Biodegradation of chlorpyrifos by bacterial consortium isolated from agriculture soil. World J Microbiol Biotechnol. 2012; 28(3): 1301-8.
14. KUMAR S. Bioremediation of chlorpyrifos by bacteria isolated from the cultivated soils sumit Kumar. Int J Pharma Bio Sci. 2011; 2(3): 359-66.
15. Vidya Lakshmi C, Kumar M, Khanna S. Biotransformation of chlorpyrifos and bioremediation of contaminated soil. Int Biodeterior Biodegradation. 2008; 62(2): 204-9.
16. Briceno G, Fuentes MS, Palma G, et al. Chlorpyrifos biodegradation and 3,5,6-trichloro-2-pyridinol production by actinobacteria isolated from soil. Int Biodeterior Biodegradation. 2012; 73(Supplement C): 1-7.
17. Supreeth M, Raju N. Biotransformation of chlorpyrifos and endosulfan by bacteria and fungi. Appl Microbiol Biotechnol. 2017; 101(15): 5961-71.
18. Rayu S, Nielsen UN, Nazaries L, et al. Isolation and molecular characterization of novel chlorpyrifos and 3,5,6-trichloro-2-pyridinol-degrading bacteria from sugarcane farm soils. Front Microbiol. 2017; 8: 518.
19. Sharma A, Pandit J, Sharma R, et al. Biodegradation of chlorpyrifos by pseudomonas resinovarans strain AST2. 2 isolated from enriched cultures. Curr. World Environ. 2016; 11(1): 267.
20. Karolin KP, Meenakumari KS, Subha P. Isolation and characterization of novel chlorpyrifos degrading fungus isaria farinosa. J Chem. 2015;9:403-7.
21. Akbar S, Sultan S. Soil bacteria showing a potential of chlorpyrifos degradation and plant growth enhancement. Braz J Microbiol. 2016;47(3):563-70.
22. Jaiswal S, Bara JK, Soni R, et al. Bioremediation of chlorpyrifos contaminated soil by microorganism. Int J Environ Agr Biotechnol. 2017;2(4):1624-30.
23. Duraisamy K, Muthusamy S, Balakrishnan S. An eco-friendly detoxification of chlorpyrifos by Bacillus cereus MCAS02 native isolate from agricultural soil, Namakkal, Tamil Nadu, India. Biocatal Agric Biotechnol. 2018;13:283-90.
24. Khalid S, Hashmi I, Khan SJ. Bacterial assisted degradation of chlorpyrifos: The key role of environmental conditions, trace metals and organic solvents. J Environ Manage. 2016; 168(Supplement C):1-9.

25. Lu P, Li Q, Liu H, et al. Biodegradation of chlorpyrifos and 3,5,6-trichloro-2-pyridinol by Cupriavidus sp. DT-1. Bioresour Technol. 2013;127(Supplement C):337-42.
26. Singh BK, Walker A, Wright DJ. Bioremedial potential of fenamiphos and chlorpyrifos degrading isolates: influence of different environmental conditions. Soil Biol Biochem. 2006;38(9):2682-93.
27. Yang C, Liu N, Guo X, et al. Cloning of mpd gene from a chlorpyrifos-degrading bacterium and use of this strain in bioremediation of contaminated soil. FEMS Microbiol Lett. 2006;265(1):118-25.
28. Lakshmi CV, Kumar M, Khanna S. Biotransformation of chlorpyrifos and bioremediation of contaminated soil. Int Biodeterior Biodegradation. 2008;62(2):204-9.
29. Li X, Jiang J, Gu L, et al. Diversity of chlorpyrifos-degrading bacteria isolated from chlorpyrifos-contaminated samples. Int Biodeterior Biodegradation. 2008;62(4):331-5.
30. Lakshmi CV, Kumar M, Khanna S. Biodegradation of chlorpyrifos in soil by enriched cultures. Curr Microbiol. 2009; 58(1): 35-8.
31. Singh D, Khattar J, Nadda J, et al. Chlorpyrifos degradation by the cyanobacterium Synechocystis sp. strain PUPCCC 64. Environ. Sci. Pollut. Res. 2011;18(8):1351-9.
32. Harishankar M, Sasikala C, Ramya M. Efficiency of the intestinal bacteria in the degradation of the toxic pesticide, chlorpyrifos. 3 Biotech. 2013;3(2):137-42.
33. Barathidasan K, Reetha D, Milton DJ, et al. Biodegradation of chlorpyrifos by co-culture of Cellulomonas fimi and Phanerochaete chrysosporium. Afr J Microbiol Res. 2014;8(9): 961-6.
34. Jabeen H, Iqbal S, Anwar S. Biodegradation of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol by a novel rhizobial strain Mesorhizobium sp. HN3. Water Environ J. 2015;29(1): 151-60.
35. Pradeep V, Subbaiah UM. Repeated batch and continuous degradation of chlorpyrifos by Pseudomonas putida. J Environ Sci Health B. 2015; 50(5): 346-60.
36. Supreeth M, Raju N. Bio-Mineralization of organophosphorous insecticide-chlorpyrifos and its hydrolyzed product 3, 5, 6-Trichloro-2-pyridinol by staphylococcus Sp. ES-2. Curr. World Environ. 2016; 11(2): 486.
37. Supreeth M, Chandrashekar M, Sachin N, et al. Effect of chlorpyrifos on soil microbial diversity and its biotransformation by Streptomyces sp. HP-11. 3 Biotech. 2016; 6(2): 147.
38. Ishag AESA, Abdelbagi AO, Hammad AMA, et al. Biodegradation of chlorpyrifos, malathion, and dimethoate by three strains of bacteria isolated from pesticide-polluted soils in sudan. J Agric Food Chem. 2016; 64(45): 8491-8.
39. Yu YL, Fang H, Wang X, et al. Characterization of a fungal strain capable of degrading chlorpyrifos and its use in detoxification of the insecticide on vegetables. Biodegradation. 2006; 17(5): 487-94.
40. Xu G, Li Y, Zheng W, et al. Mineralization of chlorpyrifos by co-culture of serratia and trichosporon spp. Biotechnol Lett. 2007; 29(10): 1469-73.
41. Kulshrestha G, Kumari A. Fungal degradation of chlorpyrifos by Acremonium sp. strain (GFRC-1) isolated from a laboratory-enriched red agricultural soil. Biol Fertil Soils. 2011; 47(2): 219-25.
42. Chen S, Liu C, Peng C, et al. Biodegradation of chlorpyrifos and its hydrolysis product 3, 5, 6-trichloro-2-pyridinol by a new fungal strain Cladosporium cladosporioides Hu-01. PLOS One. 2012; 7(10): e47205: 1-12.
43. Maya K, Upadhyay S, Singh R, et al. Degradation kinetics of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol (TCP) by fungal communities. Bioresour Technol. 2012; 126: 216-23.
44. Silambarasan S, Abraham J. Ecofriendly method for bioremediation of chlorpyrifos from agricultural soil by novel fungus Aspergillus terreus JAS1. Water Air Soil Pollut. 2013; 224(1): 1369.

45. Das S, Adhya TK. Degradation of chlorpyrifos in tropical rice soils. J Environ Manage. 2015; 152(Supplement C): 36-42.
46. Medo J, Makova J, Kovacsova S, et al. Effect of Dursban 480 EC (chlorpyrifos) and Talstar 10 EC (bifenthrin) on the physiological and genetic diversity of microorganisms in soil. J Environ Sci Health B. 2015; 50(12): 871-83.
47. Rokade KB, Mali GV. Biodegradation of chlorpyrifos by Pseudomonas desmolyticum NCIM 2112. Int J Pharm Bio Sci. 2013; 4(2): 609-16.
48. Fulekar M, Geetha M. Bioremediation of chlorpyrifos by pseudomonas aeruginosa using scale up technique. J Appl Biosci. 2008; 12: 657-60.
49. Eissa FI, Mahmoud HA, Massoud ON, et al. Biodegradation of chlorpyrifos by microbial strains isolated from agricultural wastewater. J Am Sci. 2014; 10(3): 98-108.
50. Singh BK, Walker A, Morgan JAW, et al. Biodegradation of chlorpyrifos by Enterobacter strain B-14 and its use in bioremediation of contaminated soils. Appl Environ Microbiol. 2004; 70(8): 4855-63.
51. John EM, Shaike JM. Chlorpyrifos: pollution and remediation. Environ Chem Lett. 2015; 13(3): 269-91.