



Unveiling Eco-Friendly Solutions: A Comprehensive Review on Leveraging Mechanism-Based Pathways for Sustainable Mitigation of Pesticide Metabolite Contamination and Enhanced Public Health

Soumita Maji, Debalina Samanta, and Sandhimita Mondal*

¹ Department of Biotechnology, Brainware University, 398 Ramkrishnapur Road, Barasat, North 24 Pgs, Kolkata 700125, West Bengal, India

*Correspondence E-mail: sandhimita@gmail.com

Abstract

The harmful consequences of several pesticides, such as Benzene Hexachloride (BHC), chlorpyrifos, cypermethrin and atrazine on the environment and human health are examined in this study. Through a variety of exposure pathways, these pesticides carry concerns including neurotoxicity, carcinogenicity, and reproductive problems. Even though traditional degrading methods like fire and hydrolysis exist, their usefulness is limited and they may produce hazardous materials. Alternative methods, such as bacteria-mediated remediation, have the potential to efficiently and sustainably break down pesticides. Pesticides can be converted into non-toxic chemicals by the enzymes and metabolic pathways found in bacterial species such as *Sphingomonas* sp., *Clostridium* sp. and *Pseudomonas* sp. BHC degradation is aided by enzymes like LinA and LinB, whereas the breakdown of chlorpyrifos is mediated by organophosphorus hydrolase (OPH) and methyl parathion hydrolase (MPH). *Pseudomonas alcaligenes* and *Bacillus thuringiensis* SG4 are effective at breaking down cypermethrin and break down atrazine using enzymes such AtzA, AtzB, and AtzC. This work highlights the potential of bioremediation to mitigate pesticide pollution and provides ecologically friendly alternatives for sustainable farming practices and ecosystem preservation by providing an understanding of bacterial mechanisms.

Keywords: Bacteria, Pesticides, Pathway, Public health

Introduction

In Pesticides, which encompass a wide range of chemical substances designed to combat insects, pests, fungi, rodents, and microbes, are essential for plant growth and enhancing agricultural yields. However, their widespread use raises concerns about their negative impacts on human health and the environment (Bondareva & Fedorova, 2021). In India, Benzene hexachloride (BHC) was the first commercialized pesticide produced in 1952, marking the beginning of extensive pesticide utilization. Despite their benefits in crop protection and yield improvement, pesticides have significant drawbacks, prompting scrutiny of their applications across different scale (Rajmohan *et al.*, 2020; Gupta *et al.*, 2004).

Annually, approximately two million tons of pesticides are consumed worldwide, with Europe accounting for 45%, the USA for 25%, and the remaining portion distributed across the globe. India's consumption constitutes merely 3.75% of the global total (Kumar *et al.*, 2013). The production of pesticides in India commenced in 1952 with BHC, followed by DDT, leading to a substantial increase

in synthesis. By the mid-1990s, India was manufacturing over 85,000 metric tonnes of pesticides, with insecticides being the primary category. More than half of global pesticide usage occurs in Asia, with India ranking 12th globally and 3rd in Asia after China and Turkey (Sharma et al, 2019). The use of agricultural pesticides has surged over the decades, with Maharashtra (13243 tonnes) and Uttar Pradesh (11557 tonnes) registering the highest consumption in 2020-21 (Nayak & Solanki, 2021).

Conversely, Punjab (5193 tonnes) and Haryana (4050 tonnes) have experienced a slight decrease compared to the previous year. Other states and union territories collectively contribute 35.10% and 6.97% to pesticide usage, respectively. Pesticides tend to accumulate in fruits and vegetables, posing potential hazards to human health (Kumar et al, 2013). Although widely available, conventional degradation methods such as incineration and hydrolysis have notable disadvantages. The use of organophosphorus insecticides is restricted in soil food, and plant samples due to the production of toxic compounds. Additionally, method including hydrolysis, face challenges regarding their efficacy and environmental impact (Kannan et al, 2023). However, one promising approach for efficiently decomposing pesticides with minimal environmental impact is bacteria-mediated remediation

Significant pesticides, its' application and presence in food

Benzene Hexachloride

Benzene Hexachloride (BHC), previously a widely employed pesticide, faced global limitations starting from the 1960s due to its propensity for bioaccumulation and environmental persistence. Despite restricted use, HCB remains a concern for soil and air contamination due to its strong soil adhesion and potential for long-distance transport. Recognized by the IARC as potentially carcinogenic (Group 2b), its carcinogenic properties are supported by rodent studies. HCB's limited water solubility contributes to its accumulation in aquatic sediments, heightening environmental apprehensions (Casadó *et al.*, 2019). With its substantial insecticidal properties, BHC or benzene hexachloride has experienced increased utilization in India, accounting for nearly half of all insecticides employed in the country. Its primary application revolves around safeguarding fruits and vegetables like bottle gourd, sponge gourd and kiwi fruit (Kumar et al, 2013) (Figure1a).

Hexachlorocyclohexane (HCH), also known as BHC, encompassed a group of pesticides with lindane (gamma-HCH) notably utilized for its insecticidal efficacy. Especially prominent in agriculture, lindane's broad-spectrum insecticidal action made it a preferred choice for combating various pests such as beetles, lice, mites, and ticks, owing to its cost-effectiveness compared to alternatives (Deering *et al.*, 2020).

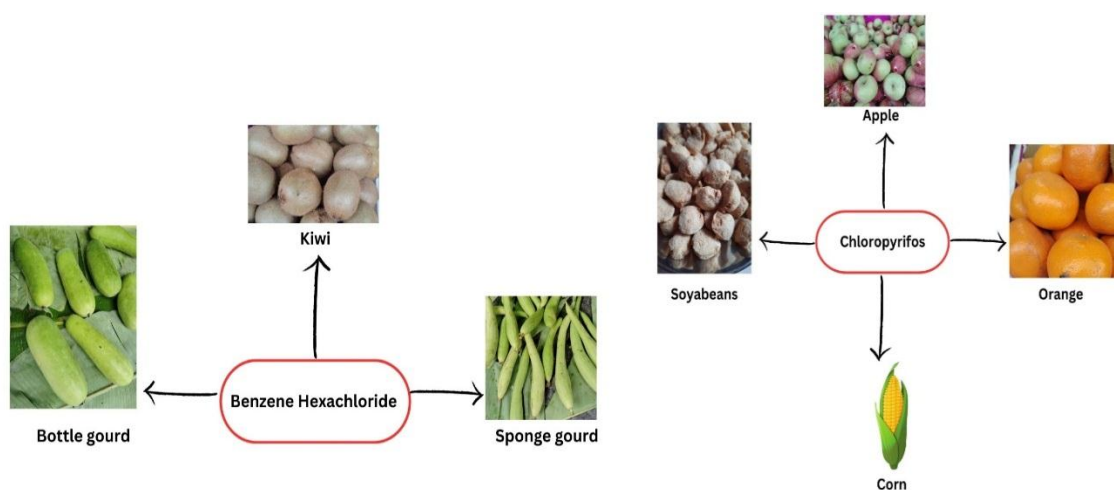


Figure 1. (a) Application of Benzene Hexachloride (b) Application of Chloropyrifos

Chlorpyrifos

Chlorpyrifos (CPF) is an organophosphate insecticide commonly used to treat fruit and vegetable crops. Until 2020, Chlorpyrifos (CPF) was extensively utilized in food production across the European Union (EU), despite its ongoing use in other global regions. National pesticide surveillance indicates CPF presence in soil, food, and water sources, raising concerns over potential hazards to consumers, farmers, and animal welfare (Wolejko *et al.*, 2022). Chlorpyrifos (CP), a member of the organophosphorus (OP) pesticide category, serves as an acaricide, insecticide, and termiticide, but its widespread usage prompts worries about environmental contamination and disruptions in biogeochemical processes (Figure1b). Associated with neurological issues and adverse impacts on immune and psychological health (Wolejko *et al.*, 2022). Chlorpyrifos effectively combats a broad spectrum of insects and mites, including foliar and soil-borne pests. Prior to regulatory restrictions, chlorpyrifos was extensively applied across various crops such as apples, oranges, corn, and soybeans (Joshi *et al.*, 2023). Its applications encompass agricultural, industrial, and public health sectors, covering food and feed crops, livestock care, pest control, and structural management (John & Shaik, 2015).

Cypermethrin

Cypermethrin, categorized as a type II pyrethroid according to the World Health Organization (WHO), is considered moderately hazardous due to its increased toxicity (Shilpakar & Karki, 2021). Cypermethrin, with the IUPAC name cyano-(3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate, is a synthetic pyrethroid insecticide extensively employed in both agricultural and environmental settings. It finds application in agriculture for insect control in various crops including vegetables, fruits, paddy, cereals, cotton, and ornamental plants (Figure1c). In households and environmental contexts, it serves as a means of controlling ants and cockroaches (IAERI, Pati 59182, Indonesia *et al.*, 2019).

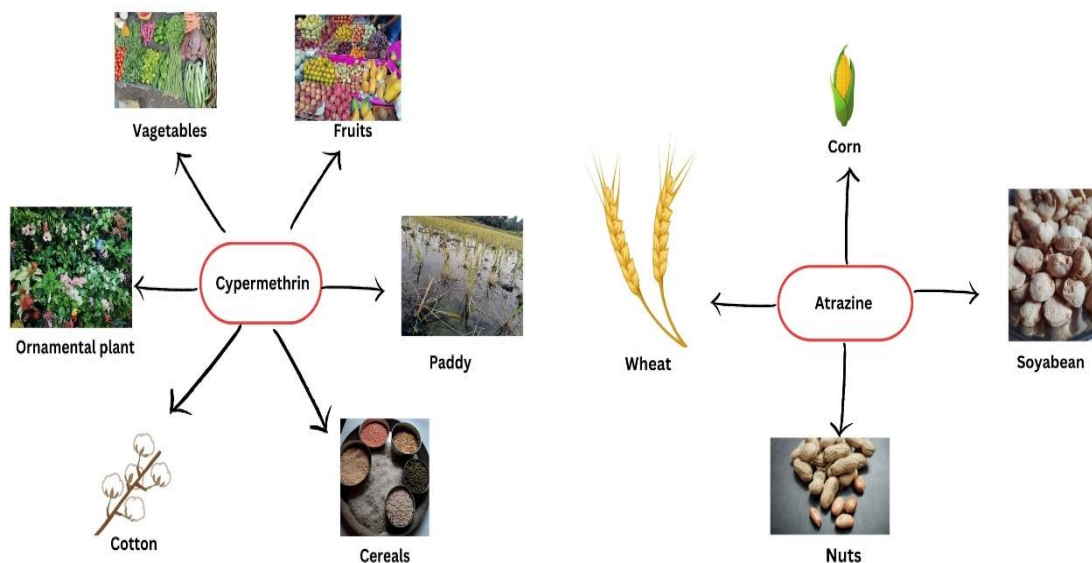


Figure 1. (c) Application of Cypermethrin (d) Application of Atrazine

Atrazine

Atrazine, scientifically known as 2-chloro-4-(ethylamine)-6-(isopropyl amine)-s-triazine, is a widely used herbicide, despite its prohibition in the European Union since 2004 (Billet *et al.*, 2019). Renowned for its affordability and efficacy, atrazine serves as both pre- and post-emergence herbicide to safeguard major crops such as conifers, macadamia nuts, pineapples, and chemical fallows, as well as for industrial weed control, particularly in sorghum, corn, and sugarcane production (El-Bestawy *et al.*, 2013; Ghazi Alattas *et al.*, 2023a). Belonging to the triazine group of herbicides, atrazine is employed to regulate weed growth in various crops including corn, soybean, sugarcane,

wheat, and macadamia nuts (Figure1d). Moreover, it finds utility in non-agricultural settings like lawns and golf courses. Atrazine remains among the most extensively utilized pesticides globally, notably in countries such as the U.S., Canada, and Australia (Singh *et al.*, 2018).

Metabolites of pesticides

β -benzene hexachloride (β -BHC), a primary derivative of benzene-hexachloride (BHC), exhibits weak estrogenic properties and is recognized as a persistent organic pollutant, with documented toxicity to male reproductive systems (Shi *et al.*, 2011). Chlorpyrifos (CPF) undergoes degradation, yielding 3,5,6-trichloro-2-pyridinol (TCP) as its principal metabolite, notable for its heightened water solubility compared to CPF (Elzakey *et al.*, 2023a). Investigation into the enantioselective degradation of α -cypermethrin, a widely utilized chiral insecticide in soils, reveals its major metabolites, cis-3-(2',2'-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis-DCCA) and 3-phenoxybenzoic acid (3-PBA), both posing potential environmental concerns (Yao *et al.*, 2021). Diaminochlorotriazine (DACT) and desethylatrazine emerge as crucial metabolites for assessing exposures to ATZ-related chemicals (Barr *et al.*, 2007) (Table1).

Table 1. Pesticides and its metabolites

Pesticides	Chemical Nature	Name of Metabolites	Reference
Benzene Hexachloride	Organochlorine	(346/5)-1,3,4,5,6-pentachlorocyclohex-1-enes (PCCHE), (36/45)-1,2,3,4,5,6-hexachlorocyclohex-1-ene (HCCHE)	Tanaka, 2019
Cypermethrin	Pyrethroid	3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylate and 2-hydroxy-2(3-phenoxyphenyl) acetonitrile	Bhatt <i>et al.</i> , 2020a
Chloropyrifos	Organophosphate	Diethylphosphate (DEP), diethylphosphorothioate (DETP) and 3,5,6-trichloro-2-pyridinol (3,5,6-TCP)	Yu <i>et al.</i> 2022
Atrazine	Chlorinated triazine	Dealkylated metabolites, hydroxyatrazine and nonextractable residues	International Agency for Research on Cancer 1991

Impact of pesticides Benzene hexachloride (BHC)

γ -Benzene hexachloride, commonly referred to as hexachlorocyclohexane (HCH) or lindane, is a widely employed insecticide belonging to the organochlorine group. Its adverse effects encompass seizures, ataxia, confusion, and other central nervous system dysfunctions (Paul *et al.*, 2013). BHC, a synthetic organic compound, comprises a benzene ring with six chlorine atoms, posing as a persistent and toxic pollutant capable of contaminating soil and water (Kumar *et al.*, 2013). Exposure to HCH can lead to various health issues, including respiratory irritation, headache, nausea, vomiting, dizziness, convulsions, liver and kidney damage, abnormal heart rhythm, and potential fatality if inhaled, ingested, or absorbed through the skin (Olivero-Verbel *et al.*, 2011). Furthermore, HCH exhibits potential carcinogenic, teratogenic, mutagenic, and genotoxic effects, acting as a neurotoxic agent that affects the nervous system, causing tremors, convulsions, and central nervous system depression (Nicolopoulou-Stamati *et al.*, 2016). Moreover, HCH has been associated with neurotoxicity, carcinogenicity, immunotoxicity, hepatotoxicity, oxidative stress, and blood disorders upon exposure to significant doses (Figure2a). The widespread usage of HCH, particularly its γ -isomer lindane, has led to the global accumulation of HCH waste, posing substantial environmental and health risks due to its toxic properties (Sharma *et al.*, 2024).

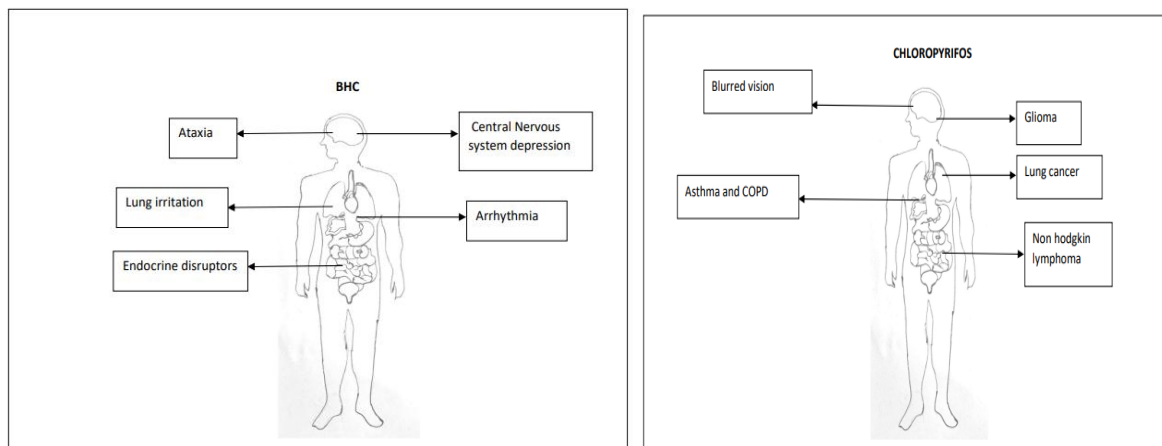


Figure 2. (a) Impact of Benzene Hexachloride on human health, (b) Impact of Chlorpyrifos on human health

Chlorpyrifos

Chlorpyrifos, a synthetic insecticide classified within the organophosphate group of pesticides, is extensively employed for pest control across diverse agricultural, animal, and structural contexts. Its mechanism involves targeting insects, mites, and ticks by inhibiting the acetylcholinesterase enzyme, thus affecting their nervous systems (John & Shaik, 2015). Despite its efficacy, chlorpyrifos presents significant risks to human health, particularly concerning the nervous system. High doses can lead to acute poisoning with symptoms including nausea, vomiting, headache, dizziness, muscle twitching, convulsions, respiratory paralysis, and potentially fatal outcomes. Chronic exposure to low doses can result in toxicity affecting various organs such as the brain, liver, kidneys, heart, blood, and hormones. Exposure during pregnancy or early childhood can lead to developmental issues including reduced birth weight, delayed growth, abnormal reflexes, lower IQ, attention deficit disorder, autism spectrum disorder, and learning disabilities (Figure 2b). Additionally, chlorpyrifos exposure is associated with an increased cancer risk as classified by the International Agency for Research on Cancer (John & Shaik, 2015). Furthermore, chlorpyrifos may impact changes in the population of fungi, bacteria, and actinomycetes in soil and inhibit nitrogen mineralization, with its mechanisms based on the inhibition of acetylcholinesterase (AChE) activity (Wolejko *et al.*, 2022). It also exhibits reproductive toxicity, neurotoxicity, and genotoxicity. The discrepancy between observed effects and final conclusions regarding chlorpyrifos approval highlights the ongoing importance of monitoring its influence, despite its withdrawal from the EU market. This review outlines the scientific findings describing chlorpyrifos effects on environmental and cellular levels in humans and animals, as well as the hazards and risks to human health in consumer products where chlorpyrifos has been detected (Wolejko *et al.*, 2022). CPF can cause severe adverse effects on body organs including the liver and central nervous system. CPF affects the nervous system by reversibly inhibiting the activity of cholinesterase (ChE), an enzyme that's necessary for the proper functioning of the nervous system. CPF also inhibits the neuropathy target esterase (NTE) enzyme, which plays a role in placental development, blood vessel development, and protein synthesis in the central nervous system.

Cypermethrin

Type II pyrethroids (cypermethrin) have an alpha-cyano group attached to them which result in salivation, choreoathetosis, coarse tremors, seizures and effects on the skeletal and cardiac muscles also known as the choreoathetosis–salivation or the CS syndrome (Figure 2c). It is considered comparatively safer for humans than for insects due to its slow dermal absorption in humans owing to the large body surface area and rapid metabolism in the liver to the nontoxic metabolites which are excreted from the urine (Shilpakar & Karki, 2021). During the degradation of cypermethrin, six intermediate metabolites were identified (Bhatt *et al.*, 2022). Cypermethrin, an insecticide belonging to the pyrethroid class, is widely used in agricultural and domestic settings, posing environmental

concerns due to its persistence and easy dissemination to soil and water ecosystems (Bhatt *et al.*, 2022). Through its interactions with sodium channels, It mainly affects the central nervous system, causing hyperexcitable behavior. Moreover, it impacts the ATPase system and voltage-dependent sodium channels in neuronal membranes, which causes DNA binding, instability, and unwinding. Although it is a broad-spectrum pesticide, it kills beneficial as well as target insects, which may cause insect resistance. Its use may have detrimental effects on the environment and human health, such as decreased levels of red blood cells and blood proteins and significant toxicity to fish and aquatic invertebrates. Cypermethrin has been identified as a potential human carcinogen and has been linked to a number of adverse health consequences, such as endocrine disruption, allergic skin reactions, eye irritation, neurotoxicity, immunotoxicity, genotoxicity, and reproductive toxicity (Indonesian Agricultural Environment Research Institute (IAERI), Pati 59182, Indonesia *et al.*, 2019)

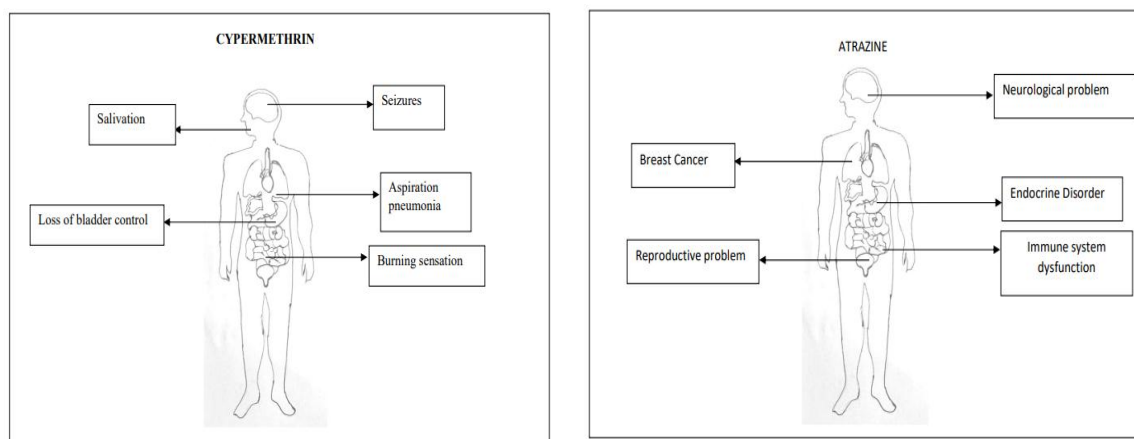


Figure 2(c) Impact of Cypermethrin on human health, (d) Impact of Atrazine on human health

Atrazine

Atrazine, characterized as a non-polar toxic compound, is widely recognized as a significant environmental contaminant, polluting water resources and soil worldwide due to its extensive use in crop production. The environmental fate of atrazine, with a half-life ranging from 13 to 261 days, is influenced by various factors including its attachment to polar soil colloids, uptake, transportation through runoff and leaching, and biodegradation. Both the original compound and its biodegradation byproducts frequently reach raw drinking water sources or are washed out from the root zone into groundwater, particularly following irrigation or heavy rainfall. Detection of atrazine, even decades after application, sometimes surpasses the maximum permissible limit of 3 mg/L set by the United States Environmental Protection Agency (USEPA). While atrazine is comparatively less toxic to humans than other chlorinated herbicides, it still poses risks such as reduced biodiversity, damage to future crops, and contamination of food sources. It exhibits long-term effects on reproduction and endocrine disruption, interfering with regular hormonal functions and causing birth defects, reproductive tumors, and weight loss in both humans and amphibians. Atrazine is associated with low birth weights, decreased sperm counts in men, menstrual problems, and is considered a probable human carcinogen (Ghazi Alattas *et al.*, 2023a). In summary, atrazine, a pesticide, poses potential risks to both human health and the environment. In terms of human health, it can negatively impact the reproductive system and has been linked to various cancers such as breast, ovarian, and uterine cancers, as well as leukemia and lymphoma (Figure 2d). Additionally, atrazine exposure may lead to birth defects, weight loss, and reproductive tumors. Furthermore, it poses risks of liver, kidney, and heart damage, particularly through exposure via drinking water. Regarding the environment, atrazine exhibits a half-life ranging from 14 to 109 days in the atmosphere (Pathak & Dikshit, 2012).

Conventional methods to eliminate pesticides

Similar to burning, hydrolysis is another conventional and readily accessible chemical degradation technique. Organophosphorus, organochlorine, and organonitrogen pesticides typically contain

heteroatoms, which can undergo hydrolysis under alkaline conditions, facilitated by metal compounds acting as catalysts. However, the hydrolysis of organophosphorus pesticides may generate harmful substances. Additionally, the necessity for alkaline conditions and metal catalysts poses limitations on the applicability of pesticide residue degradation, particularly in soil, food, and plant samples. (Ruomeng *et al.*, 2023).

The chemical degradation methods mentioned above involve aggressive reactions that can adversely impact the environment and lead to secondary pollution. In contrast, biodegradation is viewed as a gentle, cost-efficient, and environmentally friendly approach to breaking down pesticide residues. (Ruomeng *et al.*, 2023). Another tactic employed is phytoremediation, which involves using plants to purify contaminated soil and water. This approach is acknowledged as a cost-effective, visually appealing, and environmentally sustainable "Green technology." However, plants are constrained by their inability to possess catabolic pathways for the complete degradation or mineralization of externally introduced organic compounds (Laura *et al.*, 2013).

Bacteria mediated remediation

Benzene Hexachloride (BHC)

Soil bioremediation primarily utilizes biostimulation and bioaugmentation methods. Biostimulation encourages indigenous bacteria growth by adding oxygen and nutrients, while bioaugmentation introduces additional bacteria capable of degrading contaminants. The Lin pathway, associated with sphingomonads, is a key pathway for aerobic HCH detoxification (Lal *et al.*, 2010a). LinA, a dehydrochlorinase and LinB, a hydrolytic dechlorinase are crucial enzymes in this pathway, but their biochemistries require further understanding for improved bioremediation strategies (Figure 3a, Table 2). Various bacteria, like *Sphingomonas* sp. strain BHC-A3, *Clostridium* sp. strain GZ294, and *Pseudomonas* sp. strain P515, employ different pathways and enzymes for HCH degradation, depending on environmental conditions. These bacteria are essential for effective HCH contamination mitigation. The lin genes essential for breaking down γ -HCH aerobically were first found in *Sphingobium japonicum* UT26 and later in *Sphingobium indicum* B90A. Similar Lin genes have been discovered in other sphingomonads capable of degrading HCH. (Lal *et al.*, 2010a). The first step in the process is the conversion of benzene hexachloride (BHC) to benzene hexachloride diol (BHD), which is made possible by the monooxygenase enzyme LinA. Following this, BHD is subjected to dehydrochlorination, which is facilitated by the enzyme LinB. This process yields 1,2,4-trichlorobenzene (TCB). Then, another dehydrochlorinase enzyme called LinC further dechlorinates TCB to produce 1,2-dichlorobenzene (DCB). Ultimately, the dioxygenase enzyme LinD breaks down DCB into catechol and chloride ions (Sefidi-Heris & Hajizadeh, 2022).

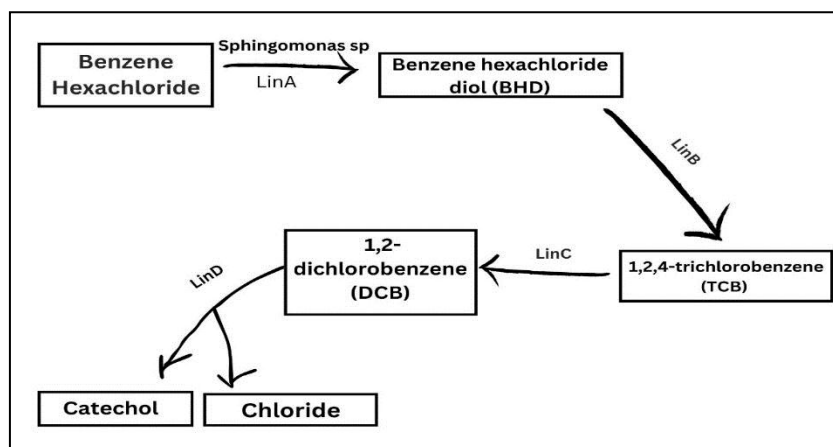


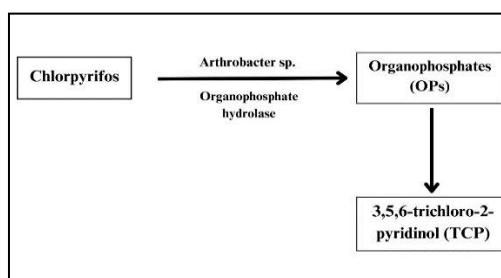
Figure 3a. Pathway of bacteria mediated degradation of Benzene Hexachloride

Table 2. Name of bacteria responsible for pesticides degradation

Name of the pesticides	Name of the bacteria
Benzene Hexachloride	<i>Clostridium sp.</i> , <i>Sphingomonas sp</i> <i>Sphingomonas sp.</i> , <i>Clostridium sp.</i> , <i>Pseudomonas sp</i> (Alvarez <i>et al.</i> , 2012; Zhang <i>et al.</i> , 2020)
Chlorpyrifos	<i>Pseudomonas stutzeri</i> , <i>Pseudomonas putida</i> , <i>Agrobacterium sp</i> , <i>Bacillus sp</i> , <i>Burkholderiasp</i> (Mali <i>et al.</i> , 2022)
Cypermethrin	<i>Bacillus thuringiensis</i> , <i>Stenotrophomonas</i> , <i>Acinetobacter</i> (Bhatt <i>et al.</i> , 2022), <i>Bacillus</i> , <i>Raoultella</i> , <i>Pseudomonas</i> , and <i>Brevibacterium</i> (Bhatt <i>et al.</i> , 2020a)
Atrazine	<i>Bacillus pacificus</i> , <i>Bacillus paramycoides</i> , <i>Bacillus cereus</i> , <i>Pseudomonas sp</i> (Seffernick <i>et al.</i> , 2000; Ghazi Alattas <i>et al.</i> , 2023a)

Chlorpyrifos

Several bacterial isolates, including *Bacillus cereus* strain PC2 (GenBank accession No. MZ314010) and *Streptomyces praecox* strain SP1 (GenBank accession No. MZ314009), have demonstrated proficient degradation of chlorpyrifos (CP) (Elzakey *et al.*, 2023a). *Pseudomonas stutzeri* and *Pseudomonas putida* are known to degrade CP, with *Arthrobacter sp.* strain HM01 exhibiting notable efficiency (Gene Accession No.: MT079332), reclassified from *Pseudomonas sp.* (Mali *et al.*, 2022). Various bacterial species, such as *Flavobacterium sp.*, *Pseudomonas sp.*, *Agrobacterium sp.*, *Bacillus spp.*, and *Burkholderia sp.*, utilize CP as a sole carbon source for degradation (Mali *et al.*, 2022). These bacteria employ enzymes like organophosphorus hydrolase (OPH) and methyl parathion hydrolase (MPH) to break down CP into intermediates like 3,5,6-trichloro-2-pyridinol (TCP), diethyl thiophosphate (DETP), and diethyl phosphate (DEP), ultimately leading to the production of less toxic compounds (Mali *et al.*, 2022). *Bacillus licheniformis* BHUJP-P3 and *Bacillus cereus* BHUJP-P4 exhibit significant ability to degrade chlorpyrifos, with their degradation genes *opdA* and *opd* being isolated from these respective strains (Huang *et al.*, 2021). Chlorpyrifos can undergo a reaction called nucleophilic addition substitution when acted upon by hydrolase, resulting in the production of a secondary metabolite known as 3,5,6-trichloro-2-pyridinol (3,5,6-TCP). Research indicates that the residual levels of 3,5,6-TCP initially rise in various components of wheat, followed by a subsequent decline over time and degraded (Figure 3b, Table 2) (Yu *et al.*, 2022)

**Figure 3b.** Pathway of bacteria mediated degradation of Chlorpyrifos

Cypermethrin

The biodegradation of cypermethrin is gaining attention as an environmentally friendly method for large-scale treatment. A novel binary bacterial combination-based approach, utilizing *Bacillus thuringiensis* strain SG4 and *Bacillus sp.* strain SG2, was investigated for enhanced cypermethrin degradation, achieving degradation rates of 80% and 85% in the presence of external nitrogen sources (KNO₃ and NaNO₃) (Bhatt *et al.*, 2022).

During cypermethrin degradation, six intermediate metabolites were detected, indicating a sequential degradation process involving hydrolysis of the carboxyl ester bond, diaryl linkage cleavage, and subsequent metabolism (Bhatt *et al.*, 2022). Three isolates, namely *Pseudomonas alcaligenes*, *Bacillus amyloliquenfaciens*, and *Pseudomonas aeruginosa*, exhibited significant cypermethrin residue reduction of up to 95% with fast half-lives and robust growth capability, highlighting their potential for biodegradation processes and soil remediation (Indonesian Agricultural Environment Research Institute (IAERI), Pati 59182, Indonesia *et al.*, 2019).

The bacterium *Bacillus thuringiensis* strain SG4, isolated from pesticide-contaminated soil, demonstrated the ability to degrade 78.9% of cypermethrin (50 ppm) after 15 days of growth in minimal media. Bacteria play a crucial role in cypermethrin breakdown by rupturing its ester link and producing acid and alcohol byproducts. Intermediate alcohol compounds are further metabolized into 3-phenoxybenzoic acid (3-PBA), with different bacterial species employing various enzymes such as hydrolases, monooxygenases, and dioxygenases to degrade cypermethrin's benzene or cyclopropane rings. This degradation process not only provides carbon and energy sources for bacteria but also reduces cypermethrin's toxicity and environmental persistence (He *et al.*, 2022). Microbial cultures like *Acinetobacter calcoaceticum* MCm5, *Brevibacterium parvum* FCm9, and *Sphingomonas* sp. RCM6 can degrade up to 85% of cypermethrin (initial concentration 100 mg/L) within 10 days. The degradation process is primarily governed by laccase and esterase genes (Gangola *et al.*, 2018). Reports stated that some bacteria effectively break down cypermethrin while generating innocuous intermediary compounds. According to the results, cypermethrin is first converted into two metabolites: 2-hydroxy-2(3-phenoxyphenyl) acetonitrile and 3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylate (Bhatt *et al.*, 2020a). Then it breaks down, acid and alcohol are produced as a result of ester bond cleavage (Figure 3c, Table 2).

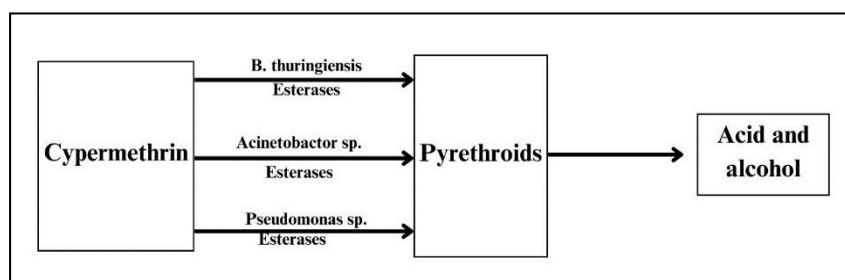


Figure 3c. Pathway of bacteria mediated degradation of Cypermethrin

Atrazine

Specific enzymes produced by bacteria facilitate the breakdown of the chemical structure of atrazine herbicide. These enzymes target the triazine ring of atrazine, which is adorned with chlorine, ethylamino, and isopropylamino groups. Bacteria hydrolyze the chlorine-carbon link and subsequently remove the ethyl and isopropyl groups to produce cyanuric acid. This substance further breaks down into carbon dioxide and ammonia. The genes encoding the enzymes AtzA, AtzB, and AtzC, responsible for these processes, are located on a transferable plasmid within the bacteria. Some bacteria also possess the enzyme TrzN, which functions similarly to AtzA. These unique enzymes enable bacteria to utilize atrazine as a source of carbon and nitrogen. Among the tested isolates, *Bacillus pacificus* strain MCCC 1A06182 (E7 and E8), *Bacillus cereus* strain ATCC 14579 (E9), and *Bacillus paramycoides* strain MCCC 1A04098 exhibited the most efficient atrazine biodegradation activity (Henn *et al.*, 2020), (Ghazi Alattas *et al.*, 2023a). The discovery of eight genes, including atzA, atzB, atzC, atzD, atzE, atzF, trzN, and trzD, within the atrazine metabolic pathway highlights a significant finding. Specifically, the identification of atzABC genes in *Pseudomonas* sp. strain ADP, which exhibit homology to five microorganisms capable of degrading atrazine, strongly suggests that these genes are widely distributed among different microbial isolates (Mili *et al.*, 2022). (Figure 3d, Table 2). The atrazine is metabolized by *Pseudomonas* strain ADP through three enzymatic stages

that are encoded by the genes *atzABC*. The result is cyanuric acid, which is a source of nitrogen for numerous bacteria (De Souza *et al.*, 1998).

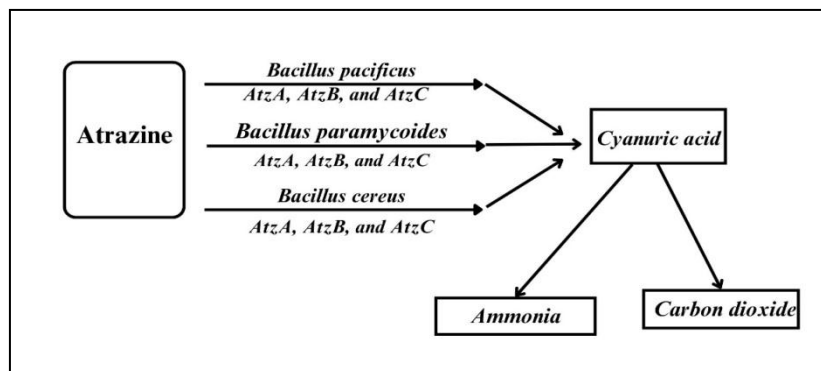


Figure 3d. Pathway of bacteria mediated degradation of Atrazine

Bacteria mediated method for pesticide remediation

Hydrolytic Deamination Reactions: Bacteria can facilitate pesticide degradation through hydrolytic deamination reactions. An example is the degradation of hydroxyatrazine, where aminohydrolases catalyze the deamination of the compound. This process involves the removal of an amino group from the pesticide molecule (Huang *et al.*, 2018).

Catabolism and Assimilation: Bacteria have the ability to assimilate certain components of pesticide molecules into their biomass. For instance, in the case of atrazine, the ring nitrogen is assimilated by bacteria, while carbon is not. This process involves incorporating specific pesticide components into bacterial biomass through metabolic pathways. (Laura *et al.*, 2013).

Ortho- or Meta-Cleavage Pathways: Bacteria utilize ortho- or meta-cleavage pathways to degrade synthetic pyrethroid insecticides like cypermethrin. These pathways involve enzymatic cleavage of the pesticide molecule, resulting in its breakdown into simpler, less toxic compounds. This process is a key mechanism for the degradation of specific pesticide classes by bacteria (Bhatt *et al.*, 2020a).

Anaerobic Degradation: Certain bacteria, such as *Clostridium sp.*, are capable of degrading highly ionic pesticides such as benzene hexachloride (BHC) under anaerobic conditions. When exposed to air, degradation stops, but resumes when returned to anaerobic conditions. This process highlights the role of bacterial metabolism in anaerobic environments for pesticide degradation (Lal *et al.*, 2010a).

Organophosphorus Pesticide Degradation: Environmental microbes, such as *Aspergillus*, *Pseudomonas*, *Chlorella*, and *Arthrobacter*, are capable of coupling a variety of physical and biochemical mechanisms for the degradation of organophosphate pesticides, including adsorption, hydrolysis of P–O alkyl and aryl bonds, photodegradation, and enzymatic mineralization. Enzymes, such as esterase, diisopropylfluorophosphatase, phosphotriesterase, somanase, parathion hydrolase, and paraoxonase, have been isolated from microbes to study and understand the catabolic pathways involved in the biotransformation of these xenobiotic compounds. This review highlights various aspects of biodegradation of organophosphate pesticides along with biological and molecular characterization of some organophosphate pesticide-degrading bacteria (Kumar *et al.*, 2018). These processes showcase the diverse strategies employed by bacteria for the degradation of pesticides and highlight the potential for bioremediation in addressing pesticide contamination in the environment (Figure 4).

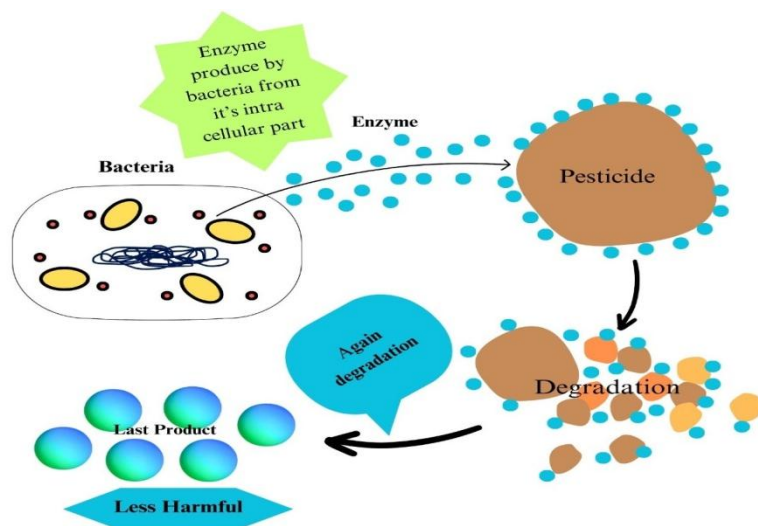


Figure 4. Mechanism of bacteria for pesticide degradations

Conclusion

The study underscores the pivotal role of bacteria in the remediation of pesticides, showcasing their diverse enzymatic pathways for degradation. Bacteria utilize specialized enzymes like LinA and LinB for benzene hexachloride degradation and organophosphorus hydrolase (OPH) and methyl parathion hydrolase (MPH) for chlorpyrifos breakdown. Bacterial strains like *Bacillus thuringiensis* SG4, *Bacillus amyloliquefaciens*, and *Pseudomonas alcaligenes* exhibit robust capabilities in breaking down pesticides like cypermethrin, while enzymes like AtzA, AtzB, and AtzC mediate atrazine degradation. These bacteria employ hydrolysis, cleavage, and subsequent metabolism to dismantle complex pesticide structures, yielding less toxic byproducts. By elucidating bacterial mechanisms, this study highlights the promising potential of bioremediation in mitigating pesticide pollution, offering environment friendly solutions for sustainable agricultural practices and ecosystem preservation.

Acknowledgement

We express our deep gratitude to the Honourable Chancellor of Brainware University, West Bengal, for generously providing us with the necessary space and infrastructure.

Conflict of Interest

The authors declare no conflict of interest regarding the publication of this paper.

References

- Alvarez, A., Benimeli, C. S., Saez, J. M., Fuentes, M. S., Cuozzo, S. A., Polti, M. A., & Amoroso, M. J. (2012). Bacterial bio-resources for remediation of hexachlorocyclohexane. *International Journal of Molecular Sciences*, 13(11), 15086-15106.. <https://doi.org/10.3390/ijms131115086>
- Barr, D. B., Panuwet, P., Nguyen, J. V., Udunka, S., & Needham, L. L. (2007). Assessing Exposure to Atrazine and Its Metabolites Using Biomonitoring. *Environmental Health Perspectives*, 115(10), 1474–1478. <https://doi.org/10.1289/ehp.10141>
- Bhatt, P., Huang, Y., Zhang, W., Sharma, A., & Chen, S. (2020a). Enhanced Cypermethrin Degradation Kinetics and Metabolic Pathway in *Bacillus thuringiensis* Strain SG4. *Microorganisms*, 8(2), 223. <https://doi.org/10.3390/microorganisms8020223>
- Bhatt, P., Rene, E. R., Huang, Y., Wu, X., Zhou, Z., Li, J., Kumar, A. J., Sharma, A., & Chen, S. (2022). Indigenous bacterial consortium-mediated cypermethrin degradation in the presence of organic amendments and Zea mays plants. *Environmental Research*, 212, 113137. <https://doi.org/10.1016/j.envres.2022.113137>

- Billet, L., Devers, M., Rouard, N., Martin-Laurent, F., & Spor, A. (2019). Labour sharing promotes coexistence in atrazine degrading bacterial communities. *Scientific Reports*, 9(1), 18363. <https://doi.org/10.1038/s41598-019-54978-2>
- Bondareva, L., & Fedorova, N. (2021). Pesticides: Behavior in Agricultural Soil and Plants. *Molecules*, 26(17), 5370. <https://doi.org/10.3390/molecules26175370>
- Casadó, L., Arrebola, J. P., Fontalba, A., & Muñoz, A. (2019). Adverse effects of hexachlorobenzene exposure in children and adolescents. *Environmental Research*, 176, 108421. <https://doi.org/10.1016/j.envres.2019.03.059>
- De Souza, M. L., Seffernick, J., Martinez, B., Sadowsky, M. J., & Wackett, L. P. (1998). The Atrazine Catabolism Genes atzABC Are Widespread and Highly Conserved. *Journal of Bacteriology*, 180(7), 1951–1954. <https://doi.org/10.1128/JB.180.7.1951-1954.1998>
- Deering, K., Spiegel, E., Quaisser, C., Nowak, D., Rakete, S., Garí, M., & Bose-O'Reilly, S. (2020). Exposure assessment of toxic metals and organochlorine pesticides among employees of a natural history museum. *Environmental Research*, 184, 109271. <https://doi.org/10.1016/j.envres.2020.109271>
- El-Bestawy, E., Sabir, J., Mansy, A. H., & Zabermaawi, N. (2013). Isolation, identification and acclimatization of Atrazine-resistant soil bacteria. *Annals of Agricultural Sciences*, 58(2), 119–130. <https://doi.org/10.1016/j.aosas.2013.07.005>
- Elzakey, E. M., El-Sabbagh, S. M., Eldeen, E. E.-S. N., Adss, I. A.-A., & Nassar, A. M. K. (2023a). Bioremediation of chlorpyrifos residues using some indigenous species of bacteria and fungi in wastewater. *Environmental Monitoring and Assessment*, 195(6), 779. <https://doi.org/10.1007/s10661-023-11341-3>
- Gangola, S., Sharma, A., Bhatt, P., Khati, P., & Chaudhary, P. (2018). Presence of esterase and laccase in *Bacillus subtilis* facilitates biodegradation and detoxification of cypermethrin. *Scientific Reports*, 8(1), 12755. <https://doi.org/10.1038/s41598-018-31082-5>
- Ghazi Alattas, S., Zabermaawi, N. M., & El Bestawy, E. (2023a). Biodegradation of atrazine using selected marine bacteria: Possibilities for treating pesticide - contaminated wastewater. *Journal of King Saud University - Science*, 35(6), 102721. <https://doi.org/10.1016/j.jksus.2023.102721>
- Gupta, P. K. (2004). Pesticide exposure—Indian scene. *Toxicology*, 198(1-3), 83-90. <https://doi.org/10.1016/j.tox.2004.01.021>
- He, J., Zhang, K., Wang, L., Du, Y., Yang, Y., & Yuan, C. (2022). Highly efficient degradation of cypermethrin by a co-culture of *Rhodococcus* sp. JQ-L and *Comamonas* sp. A-3. *Frontiers in Microbiology*, 13, 1003820. <https://doi.org/10.3389/fmicb.2022.1003820>
- Henn, C., Monteiro, D. A., Boscolo, M., Da Silva, R., & Gomes, E. (2020). Biodegradation of atrazine and ligninolytic enzyme production by basidiomycete strains. *BMC Microbiology*, 20(1), 266. <https://doi.org/10.1186/s12866-020-01950-0>
- Huang, Y., Xiao, L., Li, F., Xiao, M., Lin, D., Long, X., & Wu, Z. (2018). Microbial Degradation of Pesticide Residues and an Emphasis on the Degradation of Cypermethrin and 3-phenoxy Benzoic Acid: A Review. *Molecules*, 23(9), 2313. <https://doi.org/10.3390/molecules23092313>
- Huang, Y., Zhang, W., Pang, S., Chen, J., Bhatt, P., Mishra, S., & Chen, S. (2021). Insights into the microbial degradation and catalytic mechanisms of chlorpyrifos. *Environmental Research*, 194, 110660. <https://doi.org/10.1016/j.envres.2020.110660>
- Indonesian Agricultural Environment Research Institute (IAERI), Pati 59182, Indonesia. (2019). Degradation of Cypermethrin by Indigenous Bacteria from Contaminated Soil. *Makara Journal of Science*, 210–216. <https://doi.org/10.7454/mss.v23i4.7998>
- International Agency for Research on Cancer, International Agency for Research on Cancer, Weltgesundheitsorganisation (eds) (1991) Occupational exposures in insecticide application, and some pesticides: this publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 16 - 23 october 1990. IARC, Lyon
- John, E. M., & Shaik, J. M. (2015). Chlorpyrifos: Pollution and remediation. *Environmental Chemistry Letters*, 13(3), 269–291. <https://doi.org/10.1007/s10311-015-0513-7>
- Joshi, V., Jindal, M. K., & Sar, S. K. (2023). Approaching a discussion on the detachment of chlorpyrifos in contaminated water using different leaves and peels as bio adsorbents. *Scientific Reports*, 13(1), 11186. <https://doi.org/10.1038/s41598-023-38471-5>
- Kannan, S., Perumal, V., Yuvaraj, A., Pittarate, S., Kim, J. S., & Krutmuang, P. (2023). Biodegradation of pesticide in agricultural soil employing entomopathogenic fungi: Current state of the art and future perspectives. *Heliyon*. <https://doi.org/10.1016/j.heliyon.2023.e23406>
- Kumar, B., Verma, V. K., Naskar, A. K., Chakraborty, P., Kumar, S., & Mukherjee, D. (2013). Human health risk from hexachlorocyclohexane and dichlorodiphenyltrichloroethane pesticides, through consumption of vegetables:

Estimation of daily intake and hazard quotients. *Journal of Xenobiotics*, 3(1), 6. <https://doi.org/10.4081/xeno.2013.e6>

Kumar, S., Kaushik, G., Dar, M. A., Nimesh, S., López-Chuken, U. J., & Villarreal-Chiu, J. F. (2018). Microbial Degradation of Organophosphate Pesticides: A Review. *Pedosphere*, 28(2), 190–208. [https://doi.org/10.1016/S1002-0160\(18\)60017-7](https://doi.org/10.1016/S1002-0160(18)60017-7)

Lal, R., Pandey, G., Sharma, P., Kumari, K., Malhotra, S., Pandey, R., Raina, V., Kohler, H.-P. E., Holliger, C., Jackson, C., & Oakeshott, J. G. (2010a). Biochemistry of Microbial Degradation of Hexachlorocyclohexane and Prospects for Bioremediation. *Microbiology and Molecular Biology Reviews*, 74(1), 58–80. <https://doi.org/10.1128/MMBR.00029-09>

Laura, Ma., Snchez-Salinas, E., Dantn Gonzlez, E., & Luisa, M. (2013). Pesticide Biodegradation: Mechanisms, Genetics and Strategies to Enhance the Process. In R. Chamy (Ed.), Biodegradation—Life of Science. *In Tech*. <https://doi.org/10.5772/56098>

Mali, H., Shah, C., Patel, D. H., Trivedi, U., & Subramanian, R. B. (2022). Degradation insight of organophosphate pesticide chlorpyrifos through novel intermediate 2,6-dihydroxypyridine by *Arthrobacter* sp. HM01. *Bioresources and Bioprocessing*, 9(1), 31. <https://doi.org/10.1186/s40643-022-00515-5>

Mili, C., Kalita, S., & Roy, S. (2022). Microbes as a potential bioremediation tool for atrazine-contaminated soil: A review. *Journal of Applied Biology & Biotechnology*. <https://doi.org/10.7324/JABB.2023.110102>

Nayak, P., & Solanki, H. (2021). Pesticides And Indian Agriculture- A Review. *International Journal of Research - Granthaalayah*, 9(5), 250–263. <https://doi.org/10.29121/granthaalayah.v9.i5.2021.3930>

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., & Hens, L. (2016). Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. *Frontiers in Public Health*, 4. <https://doi.org/10.3389/fpubh.2016.00148>

Olivero-Verbel, J., Guerrero-Castilla, A., & Ramos, N. R. (2011). Biochemical Effects Induced by the Hexachlorocyclohexanes. In D. M. Whitacre (Ed.), *Reviews of Environmental Contamination and Toxicology* Volume 212 (Vol. 212, pp. 1–28). Springer New York. https://doi.org/10.1007/978-1-4419-8453-1_1

Pathak, R. K., & Dikshit, A. K. (2012). Atrazine and Human Health. *International Journal of Ecosystem*, 1(1), 14–23. <https://doi.org/10.5923/j.ije.20110101.03>

Paul, R., Talukdar, A., Bhattacharya, R., & Santra, G. (2013). -Benzene hexachloride poisoning leading to acute hepatorenal decompensation. *Case Reports*, 2013(aug07 1), bcr2013009851–bcr2013009851. <https://doi.org/10.1136/bcr-2013-009851>

Rajmohan, K. S., Chandrasekaran, R., & Varjani, S. (2020). A Review on Occurrence of Pesticides in Environment and Current Technologies for Their Remediation and Management. *Indian Journal of Microbiology*, 60(2), 125–138. <https://doi.org/10.1007/s12088-019-00841-x>

Ruomeng, B., Meihao, O., Siru, Z., Shichen, G., Yixian, Z., Junhong, C., Ruijie, M., Yuan, L., Gezhi, X., Xingyu, C., Shiyi, Z., Aihui, Z., & Baishan, F. (2023). Degradation strategies of pesticide residue: From chemicals to synthetic biology. *Synthetic and Systems Biotechnology*, 8(2), 302–313. <https://doi.org/10.1016/j.synbio.2023.03.005>

Seffernick, J. L., Johnson, G., Sadowsky, M. J., & Wackett, L. P. (2000). Substrate Specificity of Atrazine Chlorohydrolase and Atrazine-Catabolizing Bacteria. *Applied and Environmental Microbiology*, 66(10), 4247–4252. <https://doi.org/10.1128/AEM.66.10.4247-4252.2000>

Sefidi-Heris, Y., & Hajizadeh, N. (2022). Bacterial Biodegradation of Phenolic Hydrocarbons. In S. I. Mulla & R. N. Bharagava (Eds.), *Enzymes for Pollutant Degradation* (Vol. 30, pp. 139–162). Springer Nature Singapore. https://doi.org/10.1007/978-981-16-4574-7_7

Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G. P. S., Handa, N., Kohli, S. K., Yadav, P., Bali, A. S., Parihar, R. D., Dar, O. I., Singh, K., Jasrotia, S., Bakshi, P., Ramakrishnan, M., Kumar, S., Bhardwaj, R., & Thukral, A. K. (2019). Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences*, 1(11), 1446. <https://doi.org/10.1007/s42452-019-1485-1>

Sharma, M., Singh, D. N., Uttam, G., Sharma, P., Meena, S. A., Verma, A. K., & Negi, R. K. (2024). Adaptive evolution of *Sphingopyxis* sp. MC4 conferred degradation potential for persistent β - and δ -Hexachlorocyclohexane (HCH) isomers. *Journal of Hazardous Materials*, 461, 132545. <https://doi.org/10.1016/j.jhazmat.2023.132545>

Shi, X., Gu, A., Ji, G., Li, Y., Di, J., Jin, J., Hu, F., Long, Y., Xia, Y., Lu, C., Song, L., Wang, S., & Wang, X. (2011). Developmental toxicity of cypermethrin in embryo-larval stages of zebrafish. *Chemosphere*, 85(6), 1010–1016. <https://doi.org/10.1016/j.chemosphere.2011.07.024>

Shilpakar, O., & Karki, B. (2021). Cypermethrin poisoning manifesting with prolonged bradycardia: A case report. *Toxicology Reports*, 8, 10–12. <https://doi.org/10.1016/j.toxrep.2020.12.005>

Singh, S., Kumar, V., Chauhan, A., Datta, S., Wani, A. B., Singh, N., & Singh, J. (2018). Toxicity, degradation and analysis of the herbicide atrazine. *Environmental Chemistry Letters*, 16(1), 211–237. <https://doi.org/10.1007/s10311-017-0665-8>

Tanaka, K. (2019). Studies on the metabolism, mode of action, and development of insecticides acting on the GABA receptor. *Journal of Pesticide Science*, 44(1), 71–86. <https://doi.org/10.1584/jpestics.J18-04>

Wołejko, E., Łozowicka, B., Jabłońska-Trypuć, A., Pietruszyńska, M., & Wydro, U. (2022). Chlorpyrifos Occurrence and Toxicological Risk Assessment: A Review. *International Journal of Environmental Research and Public Health*, 19(19), 12209. <https://doi.org/10.3390/ijerph191912209>

Yao, S., Ye, J., Yang, Q., Hu, Y., Zhang, T., Jiang, L., Munezero, S., Lin, K., & Cui, C. (2021). Occurrence and removal of antibiotics, antibiotic resistance genes, and bacterial communities in hospital wastewater. *Environmental Science and Pollution Research*, 28(40), 57321–57333. <https://doi.org/10.1007/s11356-021-14735-3>

Yu, L., Li, J., Feng, M., Tang, Q., Jiang, Z., Chen, H., Shan, T., & Li, J. (2022). Identification and Dissipation of Chlorpyrifos and Its Main Metabolite 3,5,6-TCP during Wheat Growth with UPLC-QTOF/MS. *Metabolites*, 12(12), 1162. <https://doi.org/10.3390/metabo12121162>

Zhang, W., Lin, Z., Pang, S., Bhatt, P., & Chen, S. (2020). Insights Into the Biodegradation of Lindane (γ -Hexachlorocyclohexane) Using a Microbial System. *Frontiers in Microbiology*, 11, 522. <https://doi.org/10.3389/fmicb.2020.00522>