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Magnificent Bio-Management of Pesticides in Agriculture Soil

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ABSTRACT: Following World War II, the agrochemical industry burgeoned, introducing diverse chemical agents like pesticides and herbicides to enhance crop production. These compounds, including herbicides, insecticides, and fungicides, are toxic and persist in the environment, causing soil, water, and air pollution and disrupting ecological balance. Farmers, heavily reliant on soil, initially use these chemicals to boost productivity, but over time, they become detrimental, accumulating in organisms and harming trophic levels. Addressing this challenge involves improving soil quality and eco-friendliness for maximum crop production. Indigenous microbial consortia, comprising bacteria and fungi, offer a cost-effective and eco-friendly solution by metabolizing and bio-remediating toxic compounds. This review focuses on their role in removing commercial xenobiotic pesticides that harm soil.

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KEYWORDS: Pesticides, Pollution, Agrochemical, Degradation, Soil

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1. INTRODUCTION

Soil pollution has emerged as a critical global concern, primarily driven by anthropogenic activities associated with urbanization and industrialization. The heightened demand for food production has led to the widespread application of chemical agents, such as heavy metals and polycyclic aromatic hydrocarbons, as soil contaminants. In the context of India, where agriculture plays a pivotal role in the economy, the adoption of high-yielding crop varieties during the "green revolution" has intensified the use of fertilizers, resulting in imbalanced soil conditions. Cereal cultivation, particularly rice, a staple globally and a major crop in India, is impacted by these practices. The preference for flooded soil in rice cultivation, influenced by the need for hot and humid climatic conditions, exacerbates the consequences of extensive chemical use. According to a 2011 study by the Central Soil Salinity Research Institute, Karnal (CSSRI), a significant rise in soil salinity is attributed to the excessive use of chemical agents such as fertilizers and pesticides (Barh *et al*. 2015). Pesticides, applied in substantial quantities, exert adverse effects on the soil's microbiological consortia, thereby significantly influencing plant growth. Some noteworthy consequences include the alteration of the ecological balance within the soil's microflora and the suppression of nitrifying bacteria (Shah 2017). These findings underscore the intricate relationship between agricultural practices, soil health, and the broader environmental implications, emphasizing the need for sustainable approaches to address the challenges posed by soil pollution.

The intensification of agriculture and expansion of manufacturing capacities have contributed to the contamination of natural resources, particularly soil, with various hazardous substances. In urban settings, there is a notable surge in the release of industrial waste into soil and water, resulting in the accumulation of toxic contaminants. The gradual diminution of these contaminants occurs through processes such as leaching, plant uptake, erosion, and deflation (Dixit *et al*. 2015). The degradation of detrimental contaminants within agricultural soil poses a significant challenge to the quality of soil on an international scale, as emphasized by Ansari *et al*. (2018). This phenomenon underscores the urgency of addressing the environmental issue associated with the presence of harmful substances in soil, acknowledging the intricate interplay between anthropogenic activities, contamination pathways, and the broader implications for ecosystem health.

Agricultural soil represents a highly intricate and dynamic ecosystem that sustains and fosters an exceptionally diverse array of micro and macro flora, exerting a profound influence on its inherent properties. It comprises inorganic and organic mineral nutrients, coexisting with vast populations of living organisms that collectively uphold equilibrium among biological, physical, and chemical factors within the soil (Doran and Safley 1997). Beyond its widely recognized role as a substrate for plant growth, soil performs multifaceted functions, including facilitating the exchange of gases, nutrients, energy flow, and the detoxification of pollutants, water, and various other substances, as elucidated (Larson and Pierce 1994). Consequently, the meticulous management of soil health emerges as a critical imperative to ensure sustainable agriculture and preserve soil biodiversity, encompassing the vital microbial diversity within the ecosystem.

In consideration of this, a widely applicable and promising approach for mitigating soil contamination involves the utilization of bioremediation techniques (Mosa *et al*. 2016). This method proves to be more cost-effective and promising than traditional reclamation approaches. Bioremediation employs biological agents, such as microorganisms, yeast (e.g., *Saccharomyces cerevisiae*), fungi (e.g., *Aspergillus tereus*), or bacteria (e.g., *Pseudomonas* sp.), to extract contaminants from soils (Strong and Burgess 2008). Microbes leverage the contaminants as sources of nutrients and energy within this process (Mosa *et al*. 2016).

Remediation methods like excavation and landfill, electroreclamation, thermal treatment, and acid leaching are often deemed unsuitable due to their high costs, low efficiency, substantial disruption of soil structure and fertility, and dependence on specific contamination conditions, soil properties, and site conditions. In the presence of enzymes, microorganisms play a pivotal role in degrading contaminants present in the soil, facilitating the purification of soil from xenobiotic compounds introduced during agricultural activities. The genetic diversity exhibited by microorganisms demonstrates metabolic versatility, enabling the transformation of contaminants into less toxic forms that integrate into biogeochemical cycles, as discussed by Alexander in 1994. Numerous biotic and abiotic factors, including the presence and activity of contaminant-degrading microorganisms, competitiveness, chemical and nutrient availability and concentration, salinity, and temperature, among others, can influence the efficacy of chemical contaminant degradation (Santos 2011).

2. ROLE OF PESTICIDES IN AGRICULTURE

Globally, pesticide consumption has reached millions of tons, and in India, the usage stands at approximately 0.5 kg/hectare, with a significant contribution from organochlorine pesticides such as aldrin, endosulfan, methoxychlor, heptachlor, and others. Organophosphorus pesticides, including diazinon, chlorpyrifos, dimethoate, parathion, carbamates like aldicarb, methiocarb, carbaryl, carbofuran, and nitrogen-based pesticides like picloram, atrazine, and diquat are extensively employed due to humid climatic conditions and nutritional requirements (Uquab *et al*. 2016).

The introduction of pesticides into the soil has direct repercussions on the microbiological aspects of the soil, subsequently influencing plant growth (Shah 2017). Soil, being an integral component of ecosystems, becomes susceptible to contamination through the excessive use of pesticides. This contamination adversely impacts agricultural ecosystems, microbial populations, bacterial diversity, the nitrogen-fixing process, and soil enzymes. The fungicide azoxystrobin, for instance, exhibits a detrimental effect on soil microbial biodiversity. Furthermore, pesticides can permeate underground water in crop fields, leading to an increasing prevalence of pesticide contamination in arable soils, particularly in developing countries like China and India.

3. TYPES OF PESTICIDES

Herbicides, insecticides, fungicides, and rodenticides represent a subset of compounds within the overarching category of pesticides (see Fig. 1). Pesticides are commonly stratified according to their structural composition, with classifications such as organochlorine, organophosphorus, carbamates, and nitrogen-based insecticides delineated in Table 1. Furthermore, pesticides undergo classification based on their intended use, leading to distinct types such as insecticides, herbicides, rodenticides, and fungicides (Akashe *et al*. 2018).

Table 1. Classification of pesticides (Uqab *et al*. 2016)

S.No.	Classification of Pesticides on the basis of use	Chemical constitution of Pesticides	
1.	Insecticides	i)	Pyrethroids:
		ii)	Organophosporus: Parathion Diazinon, dimethoate, malathion, dichlorvos,
		iii)	Carbamates: Aldicarb, methiocarb, Carbaryl, propoxur,
		iv)	Organochlorine: Toxaphene, DDT, Kepone methoxychlor, mirex
		v)	Manganese compounds.
		vi)	Cyclodienes: Aldrin, heptachlor, dieldrin, chlordane, endrin, endosulfan
2.	Fungicides	i)	Nitrogen-containing: Triazines, dicarboximides, phthalimide
		ii)	Thiocarbamates, dithiocarbamates,

		iii) Cupric salts, iv) tiabendazoles, triazoles, v) Dicarboximides, vi) Dinitrophenols vii) Wood preservatives: Creosote, hexachlorobenzene viii) Botanicals: permethrin, Perethrin
3.	Herbicides	Bipyridyis, chlorophenoxy, acetanilldes, triazines. Chlorophenoxy acids, hexachlorobenzene (HCB) Nitrogen based: Atrazine, Picloram, diquat, paraquat Organophosphates : glyphosate
4.	Rodenticides	i) Warfarines ii) Indanodiones
5.	Fumigants	Methyl bromide, ethylene dibromide
6.	Insects repellents	Diethyltoluamide.
7.	Antimicrobial Chlorine	Quaternary alcohols

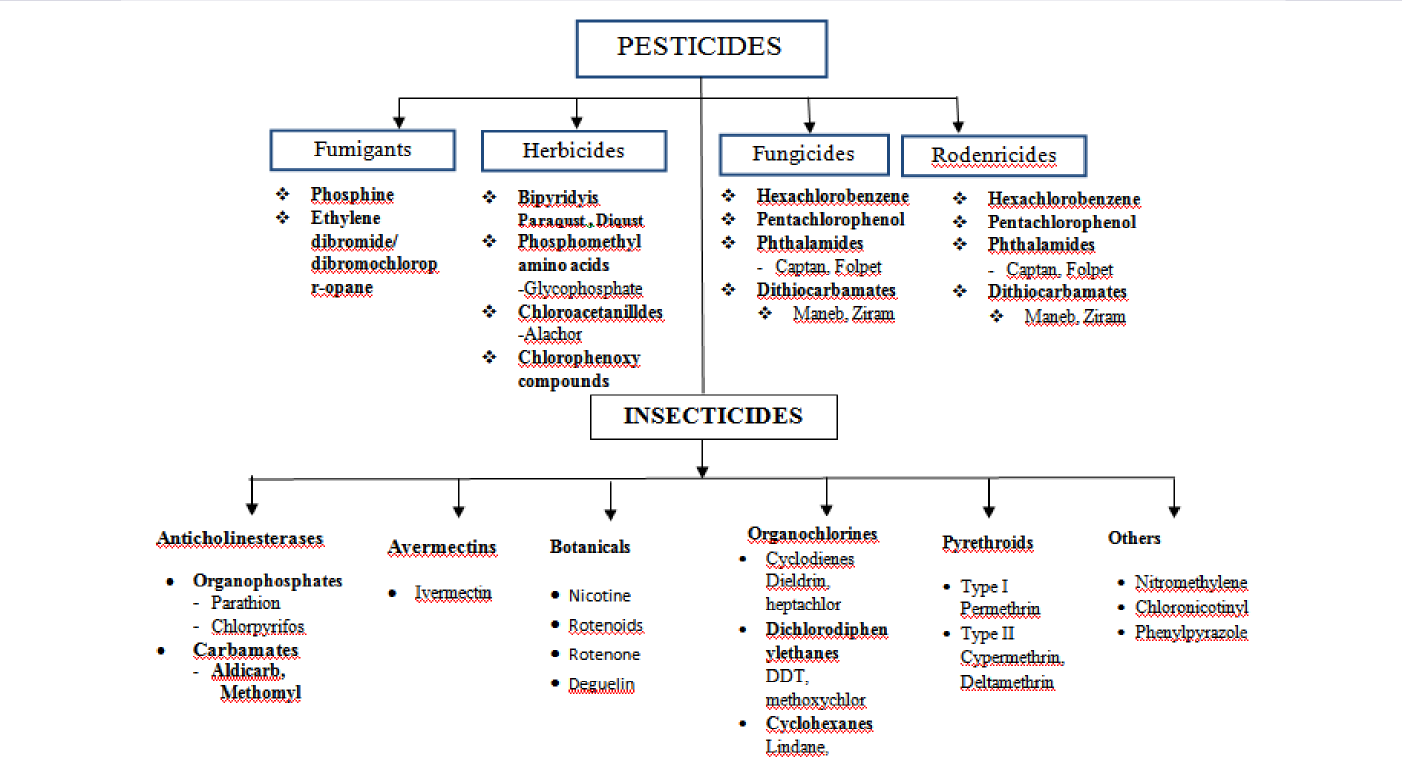

Fig. 1 Classification of Pesticides on the basis of mode of action (Singhal *et al*. 2021)

4. EFFECT OF PESTICIDES TOXICITY

The toxicological impact of pesticides on pests is contingent upon their chemical composition, influencing their interactions with soil components (Singh 2012). Elevated concentrations of pesticides in soil can be detrimental to nematodes, arthropods, and earthworms, vital for soil fertility. Some pesticides, like chlorpyrifos, exhibit prolonged persistence in the soil, potentially affecting seed germination (Tarla *et al*. 2020).

Pesticide persistence, behavior, and mobility exhibit significant variability due to diverse mechanisms involved in their degradation and retention in soils, encompassing processes like adsorption–desorption, volatilization, chemical and biological degradation, plant uptake, and leaching (Arias-Estévez *et al*. 2008) (see Table 2).

Several parameters, including water solubility, soil sorption, and half-life, determine the adherence and movement of pesticides and their transformation products in soil. Pesticides and their transformation products can be classified into two categories: (a) hydrophobic and bioaccumulable pesticides, strongly bound to soil, including organochlorines like endosulfan and heptachlor; and (b) polar pesticides, including carbamates, certain organophosphorus insecticides, and fungicide transformation products, which can be washed off by runoff and leaching, posing a threat to drinking water (Andreu and Pico' 2004, Aktar *et al*. 2009). The soil's ability to adsorb pesticides and their transformation products is significantly influenced by the organic matter content. Higher organic matter content enhances adsorption. For positively charged herbicides like paraquat, the soil's capacity to retain positively charged ions in an exchangeable state is crucial. Additionally, the adsorption of ionizable pesticides, such as picloram and atrazine, increases with decreasing soil pH, underscoring the significance of soil pH (Andreu and Pico' 2004, Aktar *et al*. 2009).

Table 2 Effect of pesticides on plants

S. No.	Class of pesticides	Types of pesticides used	Effect on plants	References
1.	Organophosphorus	Parathion, Chlorpyrifos, Diazinon, Dimethoate, Triazophos.	It kills plant through variety of mechanism, including the inhibition of biological processes such as photosynthesis, mitosis, cell divison, root growth or leaf formation.	Talat *et al.* 2016, Rodriguez *et al.* 2018.
2.	Organochlorine	Diazinon, chloropyrifos, dimethoate, parathion.		Sharma *et al.* 2019, Talat *et al.* 2016.
3.	Carbamates	Aldicarb, methiocarb.	It interfere the synthesis of pigments proteins or DNA destruction of cell membrane (Talat *et al.* 2016).	Rodriguez *et al.* 2018, Talat *et al.* 2016.

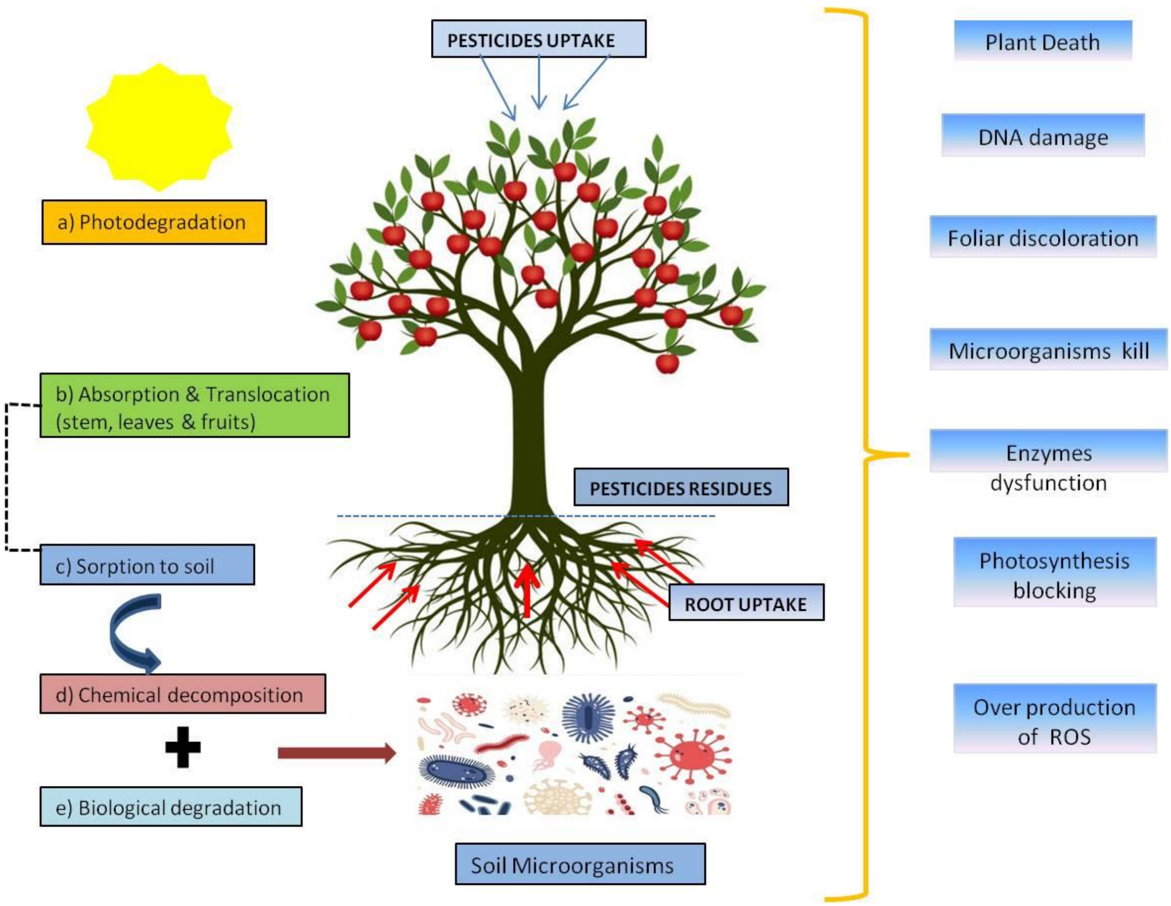

Fig. 2 Pathway of pesticides toxicity in soil and plant. (Alengebawy *et al*. 2021)

The effective management of soil quality is a critical factor for sustainable agriculture and soil biology. Soil microbes play a crucial role in maintaining soil fertility through the decomposition of organic matter and nutrient cycling. However, they can be adversely affected by stress factors such as high temperature, pH, and salinity (Paz-Ferreiro and Fu 2016).

The persistence, behavior, and mobility of pesticides are diverse, involving mechanisms like sorption–desorption, volatilization, chemical and biological degradation, plant uptake, and leaching (Weber *et al*. 2004, Laabs *et al*. 2007, Arias-Estévez *et al*. 2008, Hussain *et al*. 2009) (see Fig. 2). Pesticide transformation is illustrated in Fig. 3 (Nandhini *et al*. 2021). The interaction of degraded pesticides with native microorganisms and each other alters soil microbial diversity, metabolic processes, and enzymatic activity (Hussain *et al*. 2009, Munoz-Leoz *et al*. 2011). Numerous studies have demonstrated the negative effects of pesticides on soil microorganisms and soil respiration (Dutta *et al*. 2010, Sofo *et al*. 2012). Exogenous pesticide treatments may impact the proliferation, colonization, metabolic processes, and other characteristics of beneficial root-colonizing microbes, including bacteria, fungi, and arbuscular mycorrhiza (AM), affecting their ability to colonize roots.

Pesticides applied to the soil can have various effects on soil microflora, potentially negatively impacting their development, microbial diversity, or biomass. For example, certain herbicides have been observed to inhibit the growth of luminous bacteria Pseudomonas strains isolated from agricultural soil. This decline in Pseudomonas spp. may negatively affect soil fertility due to their significant ecological role (Boldt and Jacobson 1998).

The application of pesticides may suppress soil respiration and impede or kill specific microorganisms, allowing certain groups to dominate by eliminating competitors. For instance, endosulfan treatment increased bacterial biomass but decreased fungal biomass (Xie *et al*. 2011). Pesticides may serve as an energy source for specific microbial groups, promoting their growth and causing changes in the soil ecosystem. The functional diversity and structure of microorganisms may be altered by pesticides, affecting microbial biomass. For instance, methamidophos and urea reduced soil microbial biomass while increasing functional diversity. Pesticides have the potential to negatively impact essential biochemical processes in the soil, such as nitrogen fixation, nitrification, and ammonification. They can also influence the mineralization of soil organic matter, a critical factor for soil quality and productivity.

The enzymatic pool in soil, comprising free enzymes, immobilized extracellular enzymes, and enzymes secreted by microorganisms, serves as an indicator of biological equilibrium, including soil fertility and quality. Pesticides applied to the soil can potentially affect local metabolism or alter enzymatic activity, highlighting the importance of monitoring enzyme activity as a biological indicator for assessing the impact of chemical agents, including pesticides, on the biological functions of soil (Pathak *et al*. 2022).

Table 3. Overview of the interactions between pesticides and soil enzymes

S.No.	Enzyme (Function in soil)	Examples of the pesticides a pplied	Comments
1	Nitrogenase (An enzyme used by organisms to fix atmospheric nitrogen gas).	Carbendazim, Imazetapir, Thiram, Captan, 2,4-D, Quinalphos, Monocrotophos, Endosulfan, γ-HCH, Butachlors	Nitrogenase activity is inhibited by pesticides (Niewiadomska 2004, Niewiadomska and Klama 2005, Prasad *et al*. 2011).
2	Phosphatase (hydrolyzes organic P compounds to inorganic P)	2,4-D, Nitrapyrin, Monocrotophos, Chlorpyrifos, Mancozeb and Carbendazim	Phosphatase activity increased, but higher concentration or increase in incubation period has an inhibitory effects (Madhuri and Rangaswamy 2002, Srinivasulu *et al*. 2012).
3	Urease (catalyzes the hydrolysis of urea into CO_2 and NH_3 and is a key component in the nitrogen cycle in soils)	Isoproturon, Benomyl, Captan, Diazinon, Profenofos	Increase in urease activity (Chen *et al*. 2001, Nowak *et al*. 2004), Pesticide reduced/inhibited urease activity (Ingram *et al*. 2005).
4	Dehydrogenase (DHA): (an oxidoreductase enzyme that catalyzes the removal of hydrogen)	Azadirachtin, Acetamiprid, Quinalphos,Glyphosate	Positive/stimulatory influence on the DHA (Singh and Kumar 2008, Kizilkaya *et al*. 2012).
5	Invertase (hydrolyzes sucrose to fructose and glucose)	Atrazine, Carbaryl, Paraquat	Inhibited invertase activity (Sannino and Gianfreda 2001).
6	β-glucosidase (hydrolyzes disaccharides in soil to form β-glucose)	Metalaxyl, Ridomil gold plus copper	β-glucosidase activity increased and then decreased or inhibited (Sukul, 2006, Demanou *et al*. 2004).
7	Cellulase (hydrolyzes cellulose to D-glucose)	Benlate, Captan, Brominal	Inhibited cellulose activity (Omar and Abdel-Sater 2001).
8	Arylsulphatase (an enzyme that hydrolyzes aryl sulfates)	Cinosulfuron, Prosulfuron, Thifensulfuron methyl, Triasulfuron	Arylsulphatase activity reduces (Sofo *et al*. 2012).

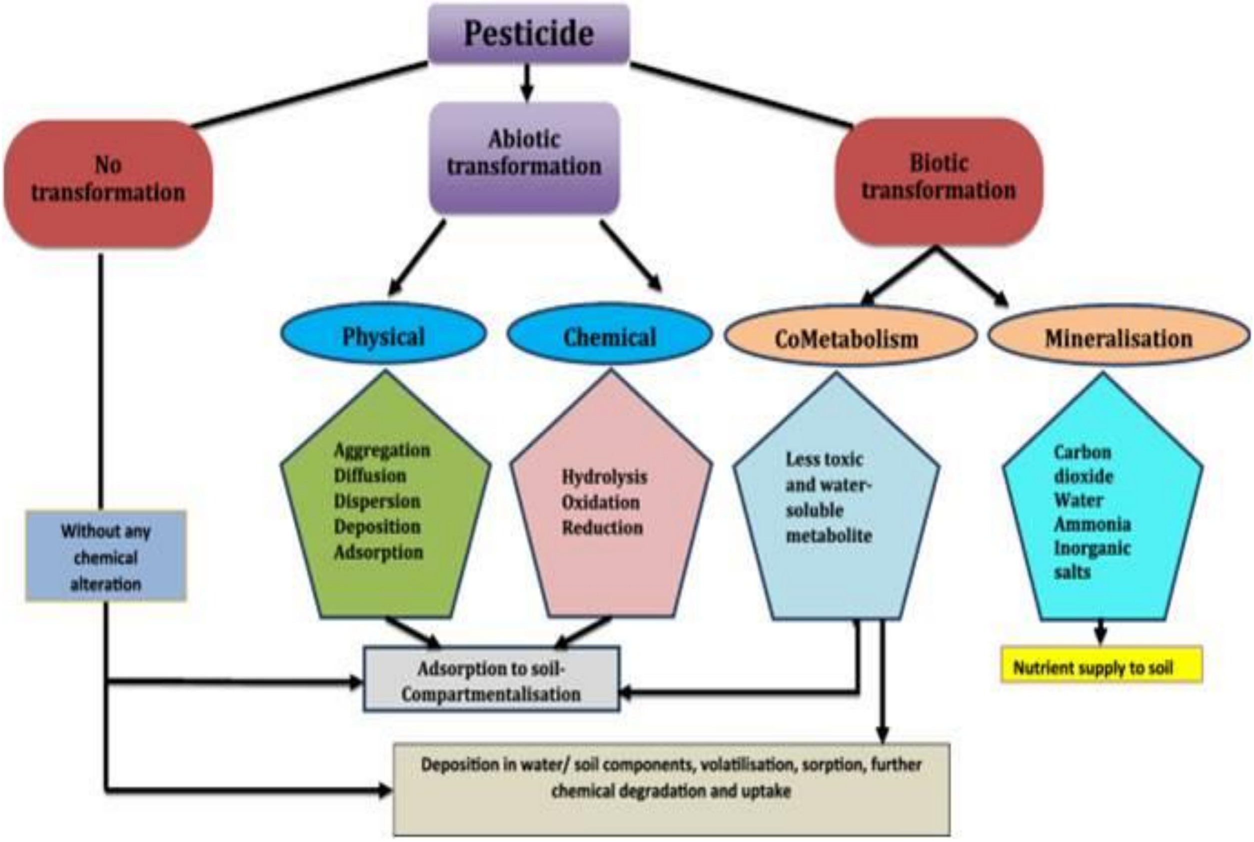

Fig. 3 Transformation of pesticides (Bose *et al*. 2021)

5. EFFECTS OF PESTICIDES IN AGRICULTURAL SOIL

When pesticides are applied to treat plants in a field, there is a potential for their exposure to the environment. Upon exposure, pesticides can undergo processes such as degradation or transformation, resulting in the generation of new chemical compounds (Liu *et al*. 2015, Marie *et al*. 2017). These pesticides may also be dispersed into non-target plants or the environment through processes like adsorption, leaching, volatilization, spray drift, and runoff. Many organochlorine compounds, which have been banned in numerous countries due to their long-term accumulation in soil, can cause harm to plants by damaging tissues. Presently, organophosphate pesticides are preferred for use due to their shorter persistence in soil (Damalas *et al*. 2011, Kim *et al*. 2017, Tudi *et al*. 2020).

Once pesticides are applied in the targeted area, they exert their effects and undergo degradation processes mediated by microbes, chemical reactions, or exposure to light, depending on environmental conditions and pesticide characteristics (Wu *et al*. 2018). The degradation of pesticides may take hours to days or even years, contributing to their control in soil and resulting in the formation of various metabolites (Tariq *et al*. 2018). For instance, in the case of chlorpyrifos, a major metabolite, 3, 5, 6-trichloro-2-pyridinol, was found to be more mobile and toxic than the original compound (Zhao *et al*. 2016). The breakdown of chlorpyrifos and its products has been observed in groundwater and soil sediments in several locations (Yue *et al*. 2017).

A novel herbicide called quintrione has been developed for rice cultivation. It inhibits p-hydroxyphenylpyruvate dioxygenase and alters hormone levels to control weeds. The primary ingredient of quinclorac, dichloroquinoline, modulates hormone levels, while mesotrione's primary chemical constituent, triketone, inhibits p-hydroxyphenylpyruvate dioxygenase. Quintrione engages in antioxidant defense and influences the synthesis and control of ethylene. Some evidence supports an additional action mechanism of quintrione inhibiting p-hydroxyphenylpyruvate dioxygenase (Peng *et al*. 2021).

6. MICROBIAL MANAGEMENT OF THE PESTICIDES IN AGRICULTURAL SOIL

There are few risk-free and universally effective strategies for managing pesticide resistance across diverse situations. Key priorities include monitoring pest populations before pesticide application, changing the modes of action of pesticides, restricting the frequency and spatial distribution of applications, creating or utilizing refugia, minimizing unnecessary persistence, targeting the most vulnerable stages of pest life cycles with pesticide applications, and incorporating synergists to enhance pesticide toxicity by inhibiting detoxification mechanisms. The challenge in managing resistance lies not in the lack of effective methods but in encouraging growers and pest control professionals to implement those (Dhaliwal *et al*. 2006).

Efficient pesticide application is crucial in the current scenario to reduce environmental pollution and enhance effectiveness against target pests, thereby mitigating issues related to pest recurrence and pesticide resistance. This has prompted a consideration of both the practical application of pesticides and their physiological and ecological selectivity. Physiological selectivity is defined by differential toxicity between species for a specific pesticide, while ecological selectivity involves modifying operational methods to minimize harm to non-target organisms (Dent 2000). Farmers should focus on using insecticides that are more toxic to the target species, which are often natural enemies, to reduce resurgence to some extent (Dhaliwal *et al*. 2006).

Beyond the permanence, concentration, and toxicity of applied pesticides, environmental conditions such as vegetation, soil texture, organic matter content, and cultural practices also influence the bioavailability, degradation, and impact of pesticides on soil microorganisms. For example, a laboratory evaluation revealed that a compost and straw mixture had the ability to biodegrade several fungicide mixes commonly used in vineyards. The herbicide imazapyr exhibited varying persistence in three Argentinean soils, and its half-life was inversely related to soil pH, iron and aluminum content, and positively correlated with clay content. Soil moisture, a crucial factor governing pesticide bioavailability and degradation, influences pesticide movement and diffusion, and is essential for microbial functioning (Gianelli *et al*. 2014).

Given these challenges, it is crucial to develop solutions for the safe, efficient, and cost-effective elimination of pesticides. Various approaches have been devised to mitigate the environmental and human health impacts of pesticides, remediate contaminated areas, and address pesticide residues and obsolete pesticides. Existing methods include physical treatments like adsorption and percolator filters, as well as chemical treatments like advanced oxidation, employing potent transient species, primarily the hydroxyl radical (Ferrusquía *et al*. 2008). Traditional physicochemical techniques often prove expensive and inadequately address the conversion of the parent substance to metabolites, which may be more persistent and equally or more hazardous to non-target organisms (Singh *et al*. 2006).

7. THE REMOVAL OF PESTICIDES THROUGH BIOTECHNOLOGICAL APPROACH

Bioremediation, accomplished through the biodegradation of chemical compounds, has emerged as a globally significant alternative for pesticide treatment. This method leverages the capacity of microbes to convert organic pollutants into simple and environmentally friendly chemicals. Bioremediation surpasses the limitations of conventional hazardous compound disposal methods, offering a cost-effective means to dismantle various organic toxins. Consequently, bioremediation technology has evolved into a virtual powerhouse, exploring the degradation of a diverse range of pollutant chemicals in recent years. It proves to be a cost-effective and efficient approach for decontaminating affected environments and eliminating pesticides (Singh *et al*. 2006, Dua *et al*. 2002). The efficiency, cost-effectiveness, and ecological benefits of biodegradation have positioned it as a viable alternative to traditional procedures, although the biodegradation processes of many pesticides remain inadequately researched (Sun *et al*. 2010).

i. Involvement of Microorganisms in Pesticide Removal Microorganisms capable of breaking down pesticides can be sourced from areas predominantly subjected to pesticide applications, particularly agricultural soil. Since pesticides are commonly sprayed on agricultural crops, soil serves as the primary reservoir for these toxins. Microorganisms identified as pesticide degraders have been isolated from agriculturally polluted areas. Various laboratories worldwide maintain collections of microorganisms characterized by their ability to identify, grow, and degrade pesticides. Pesticides used in agriculture include organic phosphorus, organic chlorine, carbamate, pyrethroid, chloronicotinyl insecticides, and various fungicides. Microbial strains, such as bacteria, fungi, actinomycetes, algae, among others, can be screened from natural sewage or soil. Notable examples include *Pseudomonas*, *Klebsiella* sp., *Bacillus subtilis*, *Trichoderma* sp., *Aspergillus* spp., white rot fungus, and other fungi. Bacterial species like *Flavobacterium*, *Arthobacter*, *Aztobacter*, *Burkholderia*, and *Pseudomonas* are recognized for pesticide breakdown. Recently, *Raoultella* sp. has been identified for its pesticide-degrading capabilities. Introducing pesticide-degrading microflora to soils has proven effective in degradation when the native microbial community struggles to manage pesticides (Uqab *et al*. 2016).

ii. Microbial Action in Pesticide Removal Many scholars argue that ex-situ remediation is not a universally viable option for cleanup due to its limitations (Barupal *et al*. 2019). Its profitability may vary at specific locations, and the microbes that successfully cleaned up contaminants in vitro may not replicate the same success in vivo.

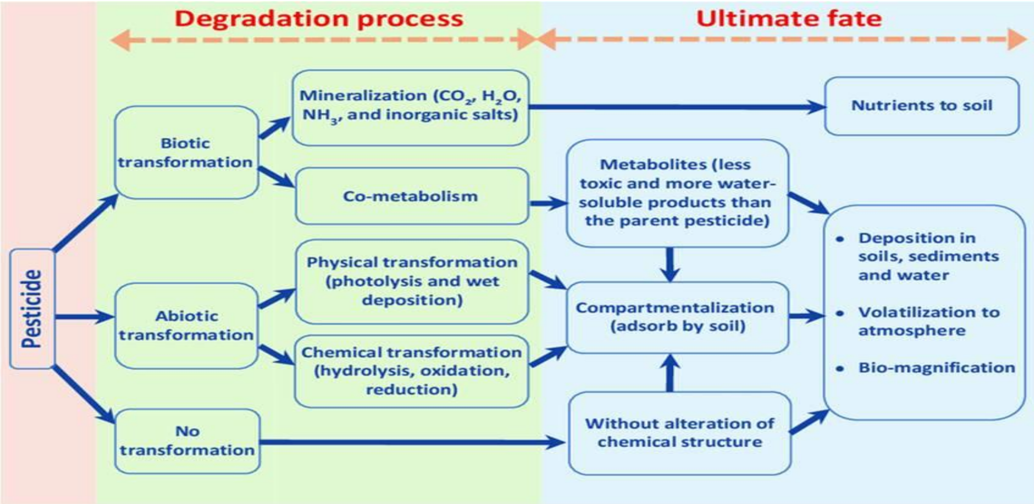

Fig. 4 Microbial degradation of recalcitrant pesticide (Bose *et al*. 2021)

Progress in the field has been facilitated by the utilization of molecular tools, enabling the development of resistant genotypes contributing to Integrated Pest Management (IPM) and a reduction in pesticide applications through innovative biotechnological and molecular methods. Traditional breeding faces challenges such as time requirements, linkage drag, and the scarcity of resistant genotypes in the gene pool when creating resistant genotypes. In contrast, the application of biotechnology in crop improvement expedites the generation of pest-resistant genotypes and mitigates the effects of linkage drag. Notably, the creation of transgenic plants, involving the modification of plant traits by introducing foreign DNA from another species, exemplifies the successful application of biotechnology in generating resistant genotypes (De la Pena *et al*. 1987).

Various crop genotypes, including cotton, rice, mustard, and maize, have been engineered to resist specific biotic stressors. An illustrative example involves introducing the Bt toxin from the bacterium *Bacillus thuringiensis* (Bt) into cotton, enabling cotton genotypes to produce the toxin in their tissue. This exemplifies the synthesis of a transgenic genotype resistant to pests, as Lepidopteran larvae feeding on the transgenic plants were effectively controlled with reduced insecticide application in the field. Several transgenic crops have been developed to potentially reduce pesticide use, such as potato lines against the potato tuber moth (*Phthorimaea operculella*) expressing Cry1Ab and rice against yellow stem borer (*Scirpophaga incertulas*) expressing potato proteinase inhibitor 2 (Kumar *et al*. 2010). However, the cost and complexity of molecular tools pose significant challenges, making microbial consortia a highly effective, efficient, and less time-consuming alternative. The conventional method of bioremediation stands out as a sustainable, cost-effective, less time-consuming, and easily achievable approach.

Numerous available in silico tools support data mining and understanding the metabolic pathways of cellular networks applied to facilitate cellular processes like biodegradation and bioremediation. Common methods for stoichiometric analysis of metabolic networks, such as Flux Balance Analysis (FBA), Metabolic Flux Analysis (MFA), and Metabolic Pathway Analysis (MPA), aid in studying flux and organization within a living system, as outlined by Gomez and Barton (2018) to explore microbial consortia applications (Jaiswal *et al*. 2019). The core applications of various tools are detailed in Table 4 below.

Table 4. List of Gene Editing Tools and its application

S.No.	Gene Editing Tool	Function	Reference
1	Bioinformatics	Application of computational biology in insilico prediction and function of various molecules. The construction of contemporary enzyme based mechanisms for bioremediation by analysis of biodegradation and bioremediation is generally analyzed using following tools namely 1. Proteomics and Metaproteomics: Proteomics tools such as Metaproteomics is the protein study derived from environmental samples where bacterial adaptation tactics in various contaminated sites, and other pollutants can be analyzed. The effect of contaminants such as pesticides on diverse bacterial consortia can also be studies with respect to enzymes and exopolymers.	Jaiswal *et al*. 2019 Gomez and Barton, 2018 Gong *et al*. 2018

		2. Genomics and Meta-genomics: The field of genomics focuses on the analysis of DNA using various molecular genetics techniques. The field of also uses these conventional methods. Application of the genomics tools such as metagenomics can be applied for analysis of bioremediation of different contaminants in the environment. Genomic tools are listed below. ➢ Cloning and sequencing of ribosomal DNA ➢ Quantitative PCR ➢ Second generation sequencing ➢ RFLP (restriction fragment length polymorphism), fingerprinting methods ➢ SIP (stable isotope probing) ➢ FISH (fluorescent in situ hybridization) 3. Transcriptomics: Study of transcripts and their function by RNA sequencing, Q- and RT-PCR. Organophosphates, pyrethroids, and carbamates degradation analysis in Pseudomonads have been analyzed.	
2	CRISPR-Cas	• Most efficient and productive gene editing tool • There are 3 types, namely, Types I, II, and III along with numerous subtypes of the CRISPR-Cas systems present. • Specific Cas such as Cas9, a DNA endonuclease guided by RNA that targets foreign DNA for obstruction. CRISPR is 30–40 bp direct repeat sequence is applied. • The gene of interest can be manipulated either by deleted or inserted within the system with the help of CRISPR/Cas9 by introducing double strand break (DSB) at the target site.	Zhu Y. *et al.* 2018 Mahas and Mahfouz, 2018 Zhang *et al.* 2018 Shapiro *et al.* 2018 Greene 2018
3	Transcription Activator-Like Effector Nucleases	TALENs stand for Transcription activator-like effector nucleases. ➢ Innovative tool for gene modification and editing. TALENs have TAL proteins. ➢ TAL proteins effectively bind to even very short sequence, (1–2 nucleotides). ➢ The nucleases are very efficient in binding. ➢ Gene knock out (non-homologous end joining), and gene knock in (Homology directed repair) of the target gene or gene of interest are now preferred with TALENs. ➢ Two protein domains, one for sequence cleavage and second for recognizing and binding the very particular and specific site ➢ Robust gene editing tool.	Jaiswal *et al.* 2019
4	Zinc Finger Nucleases	ZFNs stand for Zinc Finger Nucleases. ➢ It is most commonly used endonuclease. ➢ Artificial restriction enzyme.	Jaiswal *et al.* 2019

		➢ ZFPs are basically eukaryotic transcription factors having the ability to act as DNA binding domain. ➢ ZFNs have Folk1 (nucleotide cleavage domain) originated from Flavobacterium okeanokoites. ➢ Numerous ZFPs (usually four to six) surrounds the cleavage domain depending upon the target site. ➢ ZFPs have 18 bp specificity hence accurate target specific gene editing. ➢ ZFPs are 30 amino acids long with alpha-helix in opposition to two antiparallel β-sheets. ➢ This gene editing tool is gene knock out (non-homologous end joining) and knock in (Homology directed repair) ➢ Successful prokaryotic and eukaryotic gene editing.	

8. CONCLUSION

Selecting a bioremediation technique requires consideration of factors such as the pesticide type, environmental matrix, and ecosystem organisms. The organism's qualification is pivotal, necessitating a thorough understanding of their characteristics, benefits, and drawbacks for effective bioremediation. Careful selection and characterization of the optimal enzyme are crucial for maximizing the benefits. Biotechnological tools, particularly the omics approach, including metagenomics, proteomics, transcriptomics, and metabolomics, enable in-depth analysis of microbes and their biochemical attributes, aiding in predicting and characterizing newly synthesized products. This includes proteins and enzymes, their responsible genes, and specific enzyme protein engineering. A significant challenge lies in the slow degradation of pollutants, particularly pesticides in agricultural soil, and the stability of enzymes in non-native environments. To address this, computer-assisted applications or experiments (*in-silico* and high-throughput screening) precede field applications in-situ or ex-situ, guiding future environmental bioremediation efforts.

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