REVIEW

Recent advancements in pesticide mitigation using engineered *Escherichia coli* strains

R. Anju Ishwarya[1], **M. Kamaraj**[1 *], **J. Aravind**[2]
[1] Department of Biotechnology, Faculty of Science and Humanities, SRM Institute of Science and Technology, Ramapuram, Chennai 600089, Tamil Nadu, India
[2] Department of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences, Chennai 602105, Tamil Nadu, India

Abstract: Pesticides have considerably increased agricultural output, but their overuse presents serious threats to human health, food safety, and the environment. Alarmingly, only around 1% of pesticides used reach their intended pests, with the remainder polluting soil, water, and air. This causes broad environmental contamination and negative consequences on non-target animals, including people. Top pesticide-consuming countries, including China, the United States, and Brazil, confront considerable issues due to residual pesticide buildup. Recent biotechnology developments provide intriguing pesticide mitigation strategies. Engineered *Escherichia coli* (*E. coli*) strains have developed as very efficient bioremediation agents. These genetically engineered microbes are intended to convert hazardous chemicals into harmless metabolites. *E. coli* strains are tailored for increased expression of pesticide-degrading genes using modern genetic and metabolic engineering, dramatically enhancing their ability to break down hazardous chemicals. Studies have shown that modified *E. coli* may degrade persistent pesticides such as Paraoxon and p-nitrophenol (PNP), turning them to harmless molecules. These bacteria may reach great densities, making them ideal for large-scale detoxifying operations. Furthermore, recombinant DNA technology enables the development of *E. coli* strains with several copies of degradation genes, which improves their bioremediation capacities. Despite these advances, obstacles persist, including biosafety issues and the need for regulatory supervision. Ongoing research is critical for addressing these concerns and developing safer, more sustainable agriculture techniques. Engineered *E. coli* strains represent a substantial advancement in pesticide mitigation, providing a feasible approach for reducing environmental pollution and protecting human health.
Keywords: Pesticides, *E.coli*, metabolic engineering, recombinant DNA technology, detoxification, bioremediation

Correspondence to: Department of Biotechnology, Faculty of Science and Humanities, SRM Institute of Science and Technology, Ramapuram, Chennai 600089, Tamil Nadu, India; E-mail: drkamarajm@gmail.com

Received: September 15, 2024; **Accepted:** September 30, 2024; **Published Online:** October 11, 2024
Citation: Ishwarya, R.A., Kamaraj, M., Aravind, J., 2024. Recent advancements in pesticide mitigation using engineered *Escherichia coli* strains. *Applied Environmental Biotechnology*, 9(2): 16-29. http://doi.org/10.26789/AEB.2024.02.003
Copyright: Recent advancements in pesticide mitigation using engineered *Escherichia coli* strains © 2024 R. Anju Ishwarya et al. This is an Open Access article published by Urban Development Scientific Publishing Company. It is distributed under the terms of the Creative Commons Attribution-Noncommercial 4.0 International License, permitting all non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited and acknowledged.

1 Introduction

Pesticides have transformed agricultural output globally, but their widespread and heavy usage poses major environmental and health risks. Although the capacity of plants, particularly crops, to store and metabolize pesticides has been extensively investigated, full data on plant chemical and metabolic processes is still lacking (Sun et al., 2018). The astounding number that only roughly 1% of pesticides used efficiently reach their intended targets demonstrates the magnitude of pesticide application inefficiency (Lozowicka et al., 2016; Degeronimo, 2015; Feld et al., 2015). The remaining 99% makes its way into the surrounding environment, damaging soil, water, and air. These residues persist for long periods of time, frequently transforming into more stable and potentially harmful chemicals known as transformation products (TPs), which can remain in soils for more than a decade, as evidenced by studies in Switzerland, where 47% of TPs were detected in topsoil (Erinle et al., 2016). Pesticide pollution has far-reaching consequences, including serious health hazards. Groundwater pollution from pesticide leaching has been related to negative health consequences, such as higher cancer rates in areas known as “cancer villages”, where pesticides are widely used (Lu et al., 2015). Chronic pesticide exposure, particularly those designated as endocrine disruptors, has been linked to higher rates of obesity and neurological abnormalities in exposed populations, highlighting the far-reaching health effects (Schmidt et al., 2017). Specific pesticides, such as Chlorpyrifos, a neurotoxic organophosphate, have been demonstrated to harm children’s cognitive development through food crop contamination, emphasizing direct health dangers (Grijalbo et al., 2015). Pesticides like Benfuracarb can cause cytotoxicity in human cells, raising concerns about long-term health implications (Erdoğmuş et al., 2015).

Pesticide usage is concentrated in a few important nations throughout the world, with projections of 3.5 million tons

Table 1. Main information about the data retrieved for the document search in Scopus

Description		Results
Timespan		2023:2024
Sources (Journals, Books, etc)		3144
Documents		11939
Average citations per doc		2.884
DOCUMENT CONTENTS		
Keywords Plus (ID)		41527
Author's Keywords (DE)		27336
AUTHORS		
Authors		40980
Authors of single-authored docs		456
AUTHORS COLLABORATION		
Single-authored docs		541
Co-Authors per Doc		5.8
International co-authorships %		24.23
TOP 5 LIST IN EACH CATEGORY		
Document types	Article	8,593
	Review	1,532
	Book chapter	917
	Conference paper	681
	Note	82
Subject Area	Environmental Science	4,351
	Agricultural and Biological Sciences	4,091
	Chemistry	2,201
	Biochemistry, Genetics and Molecular Biology	1,725
	Medicine	1,702
Source title	Science of The Total Environment	373
	Environmental Science and Pollution Research	241
	Chemosphere	181
	Pest Management Science	147
	Encyclopedia Of Toxicology Fourth Edition Volume 1 9	132
Affiliation	Ministry of Education of the People's Republic of China	397
	Ministry of Agriculture of the People's Republic of China	241
	Chinese Academy of Agricultural Sciences	222
	Chinese Academy of Sciences	213
	China Agricultural University	197
Funding sponsor	National Natural Science Foundation of China	1,588
	National Key Research and Development Program of China	486
	Tecnológico	302
	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior	301
	European Commission	154

Table 2. Top 20 Corresponding authors country wise details for the documents retrieved from the Scopus database

Country	Articles	Single country publications (SCP)	Multiple country publication (MCP)	Frequency	MCP_Ratio
China	4586	3852	734	0.384	0.324
INDIA	1262	1016	246	0.106	0.195
USA	715	562	153	0.06	0.214
BRAZIL	506	386	120	0.042	0.237
IRAN	308	227	81	0.026	0.263
ITALY	263	188	75	0.022	0.285
FRANCE	239	151	88	0.02	0.368
GERMANY	226	147	79	0.019	0.35
SPAIN	218	146	72	0.018	0.33
EGYPT	183	128	55	0.015	0.301
TURKEY	177	157	20	0.015	0.113
PAKISTAN	172	94	78	0.014	0.453
KOREA	171	124	47	0.014	0.275
INDONESIA	164	139	25	0.014	0.152
UNITED KINGDOM	132	62	70	0.011	0.53
CANADA	130	83	47	0.011	0.362
MEXICO	120	101	19	0.01	0.158
JAPAN	115	86	29	0.01	0.252
POLAND	109	87	22	0.009	0.202
AUSTRALIA	103	52	51	0.009	0.495

per year by 2020 (Zhang, 2018). Japan leads in pesticide application per capita, closely followed by China, suggesting regional differences in agricultural practices and regulatory settings (Alengebawy et al., 2021). Pesticides enter the environment through a variety of mechanisms, including direct application, spray dispersion, volatilization, and runoff, all of which contribute to their global distribution and persistence (Pérez-Lucas et al., 2019). Pesticides' various chemical characteristics and breakdown mechanisms contribute to environmental deterioration (Tudi et al., 2021). Despite being prohibited for decades, organochlorine chemicals such as Dichloro-diphenyl-trichloroethane (DDT) remain in the environment due to their stability and resistance to degradation (Kim et al., 2017). Organophosphate insecticides, albeit less persistent, carry acute toxicity hazards that necessitate rigorous monitoring and mitigation techniques (Damalas and Eleftherohorinos, 2011). Microbial degradation is a promising strategy for reducing pesticide contamination. Several bacterial species, including *E. coli*, have been shown to breakdown a variety of pesticides, suggesting possible solutions for environmental remediation (Murugesan et al., 2010). Advances in recombinant DNA technologies have allowed the creation of modified *E. coli* strains capable of successfully converting harmful pesticides such as Paraoxon into non-toxic metabolites (Mali et al., 2023). This biotechnological strategy uses microorganisms' metabolic capacities to improve pesticide cleanup efforts, demonstrating the use of genetically modified organisms in environmental sustainability activities.

The bibliometric analysis was conducted by searching the Scopus archive for all published findings which will help with future perspectives. Bibliometric analysis of the literature investigates the relationships between the documents and the terms they include. According to Merigó et al. (2015), bibliometric analysis takes a quantitative look at all the research in a particular field. Structured talks and article phases are outlined below in this piece. The bibliometric exploration using a keyword 'pesticide'. Data was derived from study titles, abstracts, and keywords, with the time frame limited to 2023 year alone which contains 11939 documents. The year restricted to last 2023 to explore the recent trends and patterns instead of instead of analysing older data. The R studio bibliometric software (Aria and Cuccurullo, 2017) is utilized to import the documents data as BibTex format that were obtained in the Scopus search using the given query keywords in the given time frame. The overview of the analysed document data is listed in (Table 1). It is observed that 11939 documents are published in 3144 sources with the involvement of 40980 authors from various countries. The corresponding authorship countries data are indicated that the highest documents are published from China (4586), India (1262) and USA (715) with the progressive country collaboration (Table 2). The increased documents in the year indicates the growing interest on pesticides among the researchers.

Despite these advances, there are still obstacles to improv-

ing the efficacy and safety of pesticide remediation solutions. Soil pH, temperature, and moisture all have a substantial impact on microbial degradation rates, emphasizing the need of tailoring techniques to local environmental circumstances (Qian et al., 2014). Regulatory frameworks must also adapt to assure the safe deployment of biotechnological solutions while limiting unforeseen environmental repercussions. Pesticides have played an important role in increasing global food production, but their uncontrolled use has had serious environmental and health consequences. Addressing these issues necessitates a multidisciplinary strategy that combines scientific inquiry, technology innovation, and rigorous regulatory monitoring. By improving our understanding of pesticide destiny and establishing long-term remediation solutions, we may reduce the hazards of pesticide contamination and pave the road for safer and more sustainable farming practices. This article discusses current advances in designing *E. coli* strains to reduce pesticide contamination.

2 Sources and Toxicity

2.1 Sources

Pesticide contamination is a widespread environmental and health problem resulting from numerous agricultural and industrial operations. The many contamination paths, including transportation, spray dispersion, inappropriate disposal, and runoff from treated regions, highlight pesticide pollution's complexity and broad nature (FAO, 2019). These toxins not only have an influence on agricultural output, but also represent major dangers to human health and ecosystems, particularly in less regulated areas where weak enforcement exacerbates the risks (Imoro et al., 2019; Bornman et al., 2017). Organophosphorus insecticides, which are widely employed in agriculture due to their efficiency, have high water solubility and adsorption rates in soil, resulting in their buildup in agricultural runoff (Kankam, 2021). The organic composition of the soil, as well as its hydrophobic or hydrophilic character, have a substantial impact on chemical adsorption, with hydrophilic soils absorbing less than hydrophobic soils. The presence of trace elements such as iron and aluminum oxides hamper the adsorption process (Diagboya et al., 2021). Human exposure to organophosphates is predominantly via food intake of contaminated crops and skin absorption, emphasizing the direct health concerns linked with agricultural practices (Ansari et al., 2021). Agricultural runoff, which transports pesticides into adjacent water bodies, not only harms aquatic life but also increases human exposure through contaminated water supplies.

Pesticides are classified into systemic and contact forms, which further explains their method of action and related dangers. Systemic herbicides, such as Glyphosate and 2,4-Dichlorophenoxyacetic acid (Whithaus and Blecker, 2021), pass through plants and animals, affecting untreated areas and potentially entering food chains. Contact pesticides, such as Diquat dibromide and Paraquat, operate directly on target organisms via epidermal penetration (Hassaan and El Nemr, 2020; Saari et al., 2019; Yadav and Devi, 2017). Urban and industrial activity also have a key role in pesticide pollution of water supplies. Urban pesticide usage, particularly in residential areas, introduces pesticides that can accumulate in water systems, impacting both urban habitats and downstream ecosystems (Yadav and Devi, 2017). Industrial usage in industries such as wood treatment increases environmental loads by releasing chemicals that pollute surface waterways and worsen ecological concerns. The various actions of pesticides on the environment is illustrated in Figure 1.

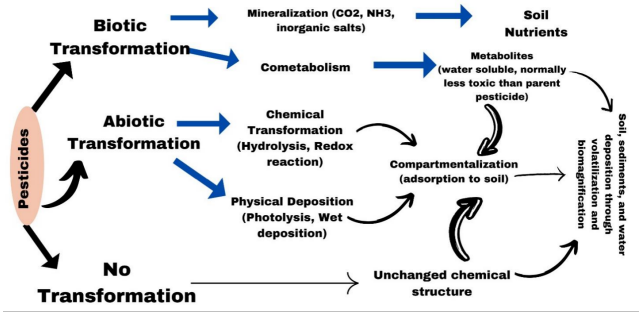

Figure 1. Fate of pesticides released into the environment (Recreated from Syafrudin et al., 2021).

Pesticide pollution has long-term consequences for human health, in addition to acute environmental problems. Pesticides disturb oxidative processes in the human body, causing dementia, respiratory problems, carcinogenesis, reproductive abnormalities, and endocrine disruptions (Botwe et al., 2012; Brühl and Zaller, 2019). Notorious pesticides like as Dichloro-diphenyl-trichloroethane (DDT), although being prohibited in many countries, continue to be found in environmental matrices and biological tissues, illustrating the long-term legacy of pesticide usage and the ongoing problems of environmental management. In response to these issues, regulatory structures and educational activities play critical roles in reducing pesticide dangers. To reduce pesticide contamination and protect the environment and public health, it is critical to strengthen laws, promote sustainable farming practices, improve farmer education and training, and develop pest control innovation.

2.2 Toxicity

While trace pesticide residues have minimal immediate effects on non-target organisms due to their high selectivity, their long-term accumulation poses significant threats to various physiological systems, including the nervous, endocrine, and reproductive systems (Tan et al., 2023; Ruomeng et al., 2023, Bansal, 2011). Pesticide residues have a significant influence, according to research that includes epidemiological studies, model animal studies (e.g., zebrafish, rats, mice), and multi-omics data analysis. For example, Glyphosate and Chlorpyrifos induce oxidative stress and mitochondrial damage in nerve cells, resulting in DNA damage (Xiong et al., 2023). Organophosphorus insecticides are linked to Parkin-

son's disease and autism, and the fungicide Maneb impairs neurotransmitter biosynthesis, resulting in Parkinson's disease (Madani and Carpenter, 2022; Liu et al., 2022). Even though pyrethroids have modest acute toxicity in animals, prolonged exposure can produce nerve dysfunction, particularly in fat-rich tissues (Bhatt et al., 2021; Pitzer et al., 2021). Pesticides, as endocrine disruptor chemicals (EDCs), disrupt endocrine homeostasis; for example, Dicholoro-diphenyl-dichloroethylene (DDE) antagonizes androgen receptors and Dichloro-diphenyl-trichloroethane (DDT) inhibits thyroid hormone function, resulting in extensive malfunction (Molina et al., 2022). Paraquat is a multi-target pesticide that harms many organs and tissues, including the kidneys, lungs, heart, gastrointestinal tract, neurological system, and immune system (Chen et al., 2021; Sule et al., 2022). However, our understanding of pesticides' systemic harmful effects remains inadequate. Some studies have found correlations between pesticides and malignancies, including liver, breast, and testicular cancers caused by Dichloro-diphenyl-trichloroethane (DDT) and its metabolites (Molina et al., 2022; McGlynn et al., 2008; Cohn et al., 2007). Huang et al., (2017) studied the increased risk of hypothyroidism after due to prolonged exposure to antichlorinesterase. Because of the scarcity of research, determining particular tissue damage caused by pesticides is difficult, however oxidative stress is a prevalent element, as seen by increased protein oxidation, lipid peroxidation, nucleic acid oxidation, and altered antioxidant levels (Ravula and Yenugu, 2021).

Pesticides such as Thiomethoxam and Cypermethrin have been shown to drastically inhibit microbial activity in soil. Sander et al. (2019) studies the decrease in cell desities of wild-type microbes, and also feedback inhibition of their metabolism. Thiomethoxam lowers phosphatase activity by 6.5% and nitrifying bacteria by 58.1%, whereas Cypermethrin lowers dehydrogenase activity by 32.8% and nitrifying bacteria by 74% (Filimon et al., 2015). Insecticides such as Miraj and Malathion lower soil CO_2 generation by up to 52% and soil microorganism populations (Al-Ani et al., 2019, Yousaf et al., 2013). Pesticides' influence in aquatic settings is determined by their solubility. Water-soluble pesticides travel more easily, but fat-soluble pesticides biomagnify in the food chain (Mojiri et al., 2020). Goldfish exposed to pesticides at 32°C exhibit more severe genotoxic effects and permanent cellular damage than those at 22°C (Jacquin et al., 2019). insecticides, particularly organochlorine and organophosphorus insecticides, have been found in fish species such as *Oreochromis mossambicus* and *Clarias gariepinus* (Pérez-Parada et al., 2018). 30 distinct pesticides were found in South American fish, with amounts ranging from less than 1 mg/kg to 194 mg/kg. The presence of these pesticides is associated with their K_{ow} values, environmental persistence, mobility, usage intensity, and agricultural land use. The most common pesticides found were Trifloxystrobin, Pyraclostrobin, and Metolachlor (Ernst et al., 2018). Pesticides can also fast and directly harm aquatic life, including salmon (Marie et al., 2017; Murthy et al., 2013). The effects of pesticide residues in a few organisms are listed in Table 3. This research highlights the critical necessity to manage pesticide contamination in order to preserve ecological balance, protect diverse living forms, and safeguard public health.

3 Role of *E. coli* in Pesticide Degradation

Recent research has concentrated heavily on using microbial diversity from natural sources such as sewage and soil to counteract pesticide contamination (Huang et al.2017). Pesticide degradation usually occurs via microbial, chemical, or photodegradation pathways (Luo et al., 2018, Su et al., 2017). Huang et al. (2018) focused the isolation and screening of a wide range of microbial strains, including bacteria, fungi, actinomycetes, and algae, to assess their potential for pesticide breakdown. For example, Kafilzadeh et al. (2015) discovered five bacterial genera—*Klebsiella*, *Acinetobacter*, *Alcaligenes*, *Flavobacterium*, and *Bacillus*—that successfully digest endosulfan, particularly in areas with high agricultural activity. Jayabarath et al. (2010) identified seven actinomycetes strains capable of surviving and efficiently decomposing carbofuran in Maharashtra's saline soils, demonstrating microbial adaptability to harsh settings. Mishra et al. (2020) suggested that the degradation of such pesticide residues may occur through cleavage of ester bonds or hydrolytic reactions. Satapute et al. (2016) worked on *Burkholderia* sp. strain BBK_9, which showed potential in degrading pesticide-based pollutants. Fungal species have also showed promise in pesticide remediation; Elgueta et al. (2016) found that white-rot fungus may substantially lower atrazine's half-life to only six days, highlighting their potential for environmental cleaning efforts. Algae, including *Chlamydomonas mexicana*, have been studied for their capacity to absorb and breakdown chemicals within their cells. Kabra et al. (2014) found that this microalga's activity resulted in considerable atrazine degradation rates ranging from 14% to 36%, indicating a viable biotechnological strategy to pesticide detoxification. Many potential strains have been found to degrade pesticide residues such as 2,4-D and monocrotophos (Itoh et al., 2002).

The ability of *E. coli* to grow in far higher densities, when compared to similar species of *Pseudomonas* and *Flavobacterium*, makes it a better choice for degrading pesticides such as Methomyl at larger scales (Kulkarni and Kaliwal 2018, 2011, 2009). Advances in recombinant DNA technology have increased these capacities. Xu et al. (2022) created an *E. coli* strain to breakdown Methyl Parathion by inserting six synthetic genes, including organophosphate acid hydrolase from *Flavobacterium* sp. ATCC 27551 and PNP-degrading enzymes from *Pseudomonas putida*, into its genome. These genes were easily incorporated into *E. coli*'s genetic framework using the pCAMBIA1301 vector, illustrating how ge-

Table 3. Effects of pesticides on cells/tissues of different organisms

Cell/Tissue	Pesticide	Stress caused	References
Human colon carcinoma (HCT116)	Bifenthrin	Increased production of Reactive Oxygen Species (ROS); increased lipid peroxidation	Bouaziz et al., 2020
Bubalus bubalis (Water buffalo)	Carbaryl	Decrease in GSH level	Jawad et al., 2017
Human umbilical vein endothelial cells (HUVECs)	Bifenthrin	Increase in apoptosis	Park et al., 2020
Ramalina fraxinea	MCPA (2-Methyl-4-chlorophenoxyacetic acid)	Increase in lipid peroxidation	Sujetovienė et al., 2019
Male mice (Pre-puberty)	Malathion	Increase in ROS levels	Selmi et al., 2018
Allium cepa	Malathion	Increased DNA damage	Srivastava and Singh, 2020
Liver cells of *Labeo rohita* (Rohu)	Malathion	Increase in lipid peroxidation	Ullah et al., 2018
Zebrafish	Bifenthrin	Increase in intestinal ROS levels	Park et al., 2020
Wistar rats	Cypermethrin	Increase in plasma IL-6 and TNF-α levels	Afolabi et al., 2019
Male Wistar rats	Malathion	Decrease in testicular antioxidant level	Jalili et al., 2019
Male Swiss Albino mice	Malathion	Decrease in GSH level	Ali and Ibrahim, 2018
Male Wistar rats	Deltamethrin	Increase in lipid peroxidation	Uchendu et al., 2018
Ovary of Female Wistar rats	Malathion	Decrease in GSH level	Arab et al., 2018

netic alteration might improve pesticide degradation processes. These findings highlight the growing potential of genetically engineered microorganisms and wild microbial strains in pesticide cleanup techniques. These biotechnological breakthroughs not only contribute to environmental sustainability, but also open the way for future applications in agricultural operations and pollution management, emphasizing the importance of microbial biotechnology in tackling global environmental issues.

4 Degradation Mechanisms of Different Pesticides Using Various Engineered *E. coli* Strains

Engineered *E. coli* strains present a viable strategy for reducing pesticide contamination via specific breakdown pathways. These genetically edited bacteria use advanced genetic and metabolic engineering to convert hazardous chemicals into non-toxic metabolites. Several recent research are described below.

4.1 Carbaryl bioremediation using engineered *E. coli*

Liu et al. (2022) studied the functional display of the enzyme carboxylesterase CarCby on the surface of *E. coli* cells in order to improve the biodegradation of Carbaryl, a carbamate insecticide. The designed system degraded 30 mg/L of Carbaryl in 12 hours, exhibiting CarCby's high activity and stability on bacterial surfaces, simplifying bioremediation by eliminating the requirement for enzyme extraction and purification. To make the biocatalyst, the CarCby gene was inserted into the pET-28a(+) plasmid. INPN and GFP genes were then inserted, yielding constructs pET-28a(+)/CarCby, pET-28a(+)/CarCby/INPN, pET-28a(+)/CarCby/GFP, and pET-28a(+)/CarCby/INPN/GFP using specialized primer amplification and digestion-ligation procedures. The plasmids were successfully integrated into *E. coli* DH5α cells, as validated by DNA sequencing. The plasmids were introduced into *E. coli* BL21(DE3) cells and grown in LB broth with 50 μg/mL kanamycin until an optical density value of 05-0.7 (at 600 nm) was obtained at 37°C. Induction with 0.6 mM IPTG was followed by an 18-20-hour incubation period at 16°C. After culture, cells were taken, rinsed, and disrupted using ultrasonication on ice. CarCby in the supernatant was purified with Ni-NTA agarose, and non-target proteins were removed with an 0-80 mM imidazole gradient in a solution.

The target CarCby-His6 was eluted with 500 mM imidazole, which was removed by ultrafiltration. The purity, molecular mass, and concentration of CarCby were determined using SDS-PAGE and a BCA Protein Assay Kit. *E. coli* BL21(DE3) cells carrying pET-28a(+)/CarCby/INPN were further examined, and cell localization was validated using fluorescence microscopy with a control strain (BL21(DE3)[pET-28a(+)/CarCby/GFP]), showing CarCby's effective targeting to the cell surface. Further investigations revealed that the whole-cell biocatalyst degraded 30 mg/L (43%) of Carbaryl in 12 hours, whereas the purified CarCby enzyme reached 15 mg/L (21%) under same circumstances. Carbaryl degradation was insignificant in control processes without biocatalyst. Mass spectrometry found 1-naphthol as the major intermediate and Carbaryl, so verifying the breakdown route. The whole-cell biocatalyst's exceptional efficiency and durability indicate that it is well-suited for practical environmental bioremediation. This work also illustrates the possibility of employing such biocatalysts to degrade numerous contaminants, implying that more research into the specific functions of enzymes in these pathways might broaden bioremediation tactics. The mechanism of Carbaryl degradation by engineered *E. coli* BL21 (DE3) is depicted in Figure 2.

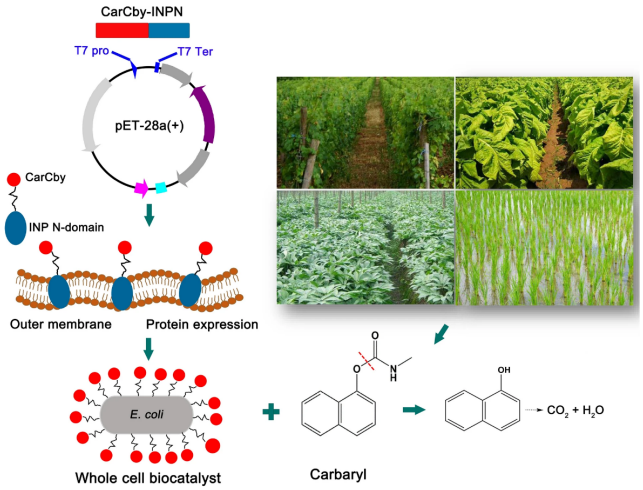

Figure 2. Schematic mechanism of Carbaryl degradation by engineered *E. coli* BL21 (DE3), using CarCby carboxylesterase (Reused from Liu et al. (2022) under a Creative Commons Attribution 4.0 International License).

4.2 Methyl Parathion (MP) degradation

Xu et al. (2022) improved *E. coli*'s capacity to digest Methyl Parathion (MP), an organophosphate pesticide, by adding a set of six synthetic genes: opdS, pnpAS, pnpBS, pnpCS, pnpDS, and pnpES. The gene cluster was initially cloned onto the pGEM-T easy plasmid using *E. coli* DH5α and expressed in *E. coli* BL21-AI using the pCAMBIA1301 vector. The genes were optimized for expression in *E. coli*. The gene expression cassette T7opdS-T7pnpAS-T7pnpBS-T7pnpCS-T7pnpDS-T7pnpES, flanked by EcoRI and HindIII restriction sites, was created using polyacrylamide gel electrophoresis (PAGE)-mediated overlap extension PCR (Peng et al., 2006) and inserted into pCAMBIA1301. Upon transformation into *E. coli* BL21-AI, the construct (BL-MP) expressed the synthetic operon required for MP biodegradation. The work addressed the difficulty of maintaining stability over

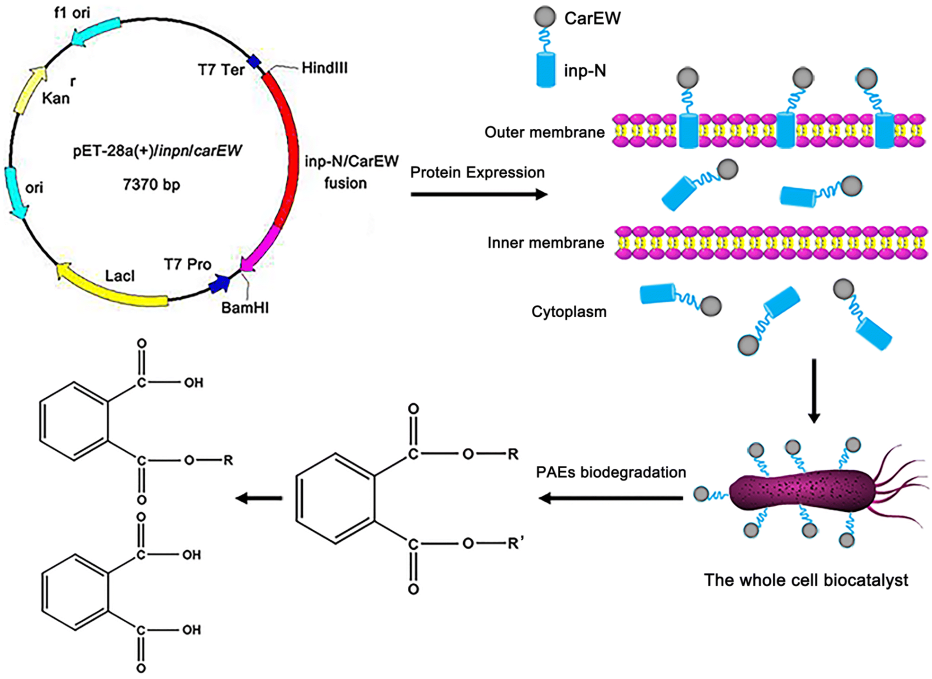

Figure 3. Microscopic view of Schematic representation of DiBP degradation using engineered *E. coli* BL21 (DE3) (Reused from Ding et al. (2022) under a Creative Commons Attribution 4.0 International License).

numerous gene transfers and established the effectiveness of the monocistronic transcriptional model. PCR and RT-PCR studies revealed that the six BL-MP genes were correctly constructed and expressed. Opd degrades MP into para-nitrophenol (PNP), which is then enzymatically transformed to β-ketoadipate and enters the TCA cycle via intermediates like acetyl-CoA and succinyl-CoA (Figure 3).

BL-MP degraded 1 mM MP in 2 hours, with PNP peaking at 0.7 mM after 2 hours and becoming undetectable by 24 hours; 96% was degraded by 20 hours. Hydroquinone (HQ) peaked at 0.64 mM after 8 hours and was completely destroyed after three days. Minimal β-ketoadipate levels suggest efficient intermediate production and breakdown. BL-MP outperformed other microorganisms such as *Pseudomonas* sp., *Flavobacterium balustinum*, *Plesiomonas* sp., and *Serratiq* sp. in PNP degradation (Pakala et al., 2007; Zhongli et al., 2001; Somara and Siddavattam, 1995; Chaudhry et al., 1988), achieving a 50% reduction in PNP within 8 hours and complete degradation within 24 hours. This study highlights the potential of genetically modified microbes for environmental cleanup and urges future investigation of similar technologies for dealing with additional organophosphate contaminants. Future research should investigate the effectiveness of BL-MP in environmental situations such as soil or plant detritus in order to validate it's in situ use.

4.3 Di-isobutyl (DiBP) phthalate degradation

Ding et al. (2020) studied how to construct plasmids to produce esterase activity in *E. coli* BL21 (DE3), with a focus on the carboxylesterase (CarEW) enzyme that is expressed on the surface. The study used modern molecular biology techniques for plasmid creation, enzyme production, and rigorous enzymatic tests to assess catalytic efficiency and stability. The inak gene was originally cloned into pMD18-T, yielding pMD18-T/inak. Following BamHI/HindIII digestion, carEW from pEASY-E2/carEW was inserted into pET-28a(+), resulting in pET-28a(+)/carEW. gfp was then added to HindIII-digested pET-28a(+)/carEW, resulting in pET-28a(+)/carEW/gfp. Further changes involved incorporating inpn into BamHI-digested pET-28a(+)/carEW, resulting in pET-28a(+)/inpn/carEW, and then reintroducing gfp into BamHI-digested pET-28a(+)/inpn/carEW to produce pET-28a(+)/inpn/carEW/gfp. Esterase activity was evaluated by detecting PNP release at 405 nm. One unit of enzyme activity (U) is defined as releasing 1 μM PNP per minute. This test offered a reliable assessment of enzymatic function under a variety of settings. The optimal conditions for surface-displayed CarEW activity were investigated, revealing strong enzymatic activity across pH ranges: citrate/phosphate (pH 5.0-8.0), Tris/HCl (pH 8.0-9.0), and boric acid/borax (pH 9.0-10.0), emphasizing enzyme flexibility. Temperature profiling indicated 45°C as the ideal temperature for maximum activity, which is critical for thermal stability in industrial applications.

CarEW successfully hydrolyzed a wide range of PNP esters (PNPC2 to PNPC16), demonstrating its flexibility (Ding, 2020). Kinetic studies were performed on the PNPC2 substrate at 45°C and pH 9.0 to estimate Michaelis-Menten constants (Km) and maximum velocities (Vmax). Stability tests revealed that *E. coli* expressing pET-28a(+)/inpn/carEW re-

tained about 100% of its initial activity over 23 days at 45°C and doubled it over a month at 4°C. This stability outperformed free CarEW, demonstrating the benefits of surface displays. Catalytic studies for diisobutyl phthalate (DiBP) breakdown demonstrated effective removal (1.5 mg/ml within 120 min at 45°C and pH 9.0), demonstrating CarEW's potential for bioremediation. The proposed pathway is shown in Figure 3. Surface-displayed CarEW on *E. coli* BL21 (DE3) demonstrated higher catalytic efficiency, substrate adaptability, and stability, indicating potential for industrial and environmental applications. This study advances enzyme engineering and biotechnological innovations, paving the way for future research.

4.4 Degradation of organophosphate pesticides

Karbelkar et al. (2021) proposed a novel method for effectively removing organophosphate (OP) pesticides by combining microbial electrochemistry with surface-displayed enzyme technology. Their research used modified strains of *E. coli* and *S. oneidensis* that functioned within a self-sustaining bioremediation system. Initially, *E. coli* BL21 (DE3) was genetically engineered to express *Pseudomonas diminuta*'s surface-displayed organophosphate hydrolase (OPH). To improve stability and avoid the cytotoxicity associated with traditional membrane protein tags like OmpA, OPH was designed with an INPNC ice-nucleation sequence for membrane anchoring (Furst et al., 2017). This surface display method aided in the lyophilization of OPH-expressing cells after manufacture, improving their shelf life and suitability for real environmental applications. The performance of surface-displayed OPH-*E. coli* in degrading OPs was examined by measuring paraoxon degradation, a model OP molecule, using a colorimetric assay to quantify PNP generation. The Michaelis constant (KM) for paraoxon degradation by OPH-*E. coli* was found to be 197.9 $\pm$ 81.7 μM, slightly higher than values reported for purified OPH (Dumas et al., 1989). This is due to reduced substrate diffusion to enzymes immobilized on cell surfaces compared to enzymes in solution (Richins et al., 2000). OPH-*E. coli* also degraded additional OPs, including Parathion and Paraoxon-methyl. Parathion, with its phosphorus-sulfur bond, displayed a catalytic efficiency (Vmax/KM = 0.104 min^{-1} $\pm$ 0.013) equal to Paraoxon (Vmax/KM = 0.079 $\pm$ 0.13 min^{-1}). This highlights the enzyme's capacity across structurally varied OP compounds.

The incorporation of *S. oneidensis* into the bioremediation system highlighted its involvement in microbial electrochemistry. *S. oneidensis* activated transcription factor DmpR when exposed to PNP, an OP breakdown product, resulting in the production of CymA—a critical protein that enables extracellular electron transfer (EET) via the MtrABC conduit. This enabled electron transport to a carbon cloth electrode in a bioreactor setting, resulting in quantifiable anodic current. This microbial electrochemical system effectively detects and utilizes OP breakdown products without external stimulation, as evidenced by a considerable charge production of 1.32 $\pm$ 0.48 mA·h after 24 hours with 20 μM PNP. Figure 4 (a) and (b) show the mechanism. This work stresses the strength and possibility of integrating surface-displayed enzyme technology and microbial electrochemistry for effective OP pesticide cleanup. The surface enzyme display of *E. coli* and the electron transfer capabilities of *S. oneidensis*, combine to form a self-sustaining system with interesting applications in environmental bioremediation. Future study might improve enzyme kinetics, widen substrate selectivity, and maximize system performance across a variety of environmental conditions, advancing practical application and scalability.

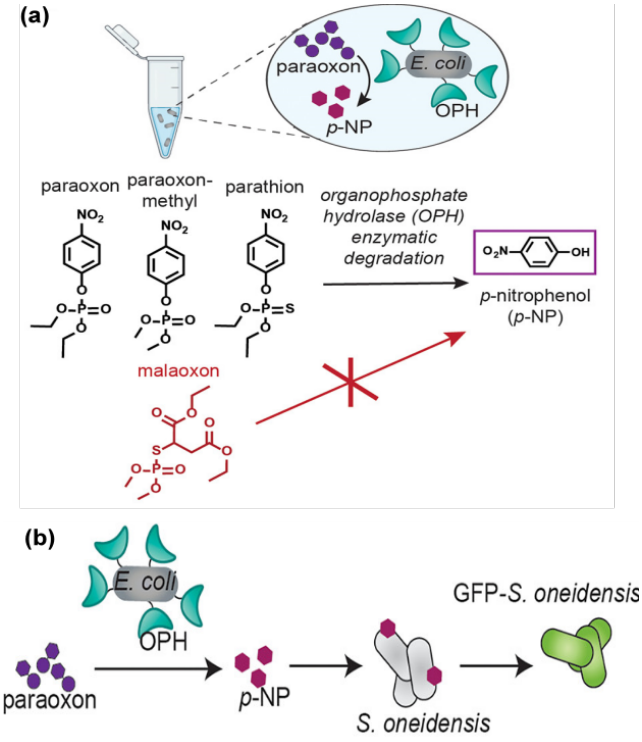

Figure 4. (a) Mechanism of Organophosphate (OP) pesticides by engineered OPH-*E. coli*. (b) Combined mechanism of OPH-*E. coli* and engineered *S. oneidensis* for detection and degradation of OP compounds (Reused from Karbelkar et al. (2021) under the privilege licensed CC-BY-NC-ND 4.0).

4.5 Degradation of p-nitrophenol

Xu et al. (2021) created BL-PNP, a modified *E. coli* strain designed to biodegrade PNP, a toxic nitroaromatic chemical present in the environment due to its use in pesticides and pharmaceutical manufacture. The host organism for this genetic engineering was *E. coli* BL21-AI from Invitrogen. To promote gene expression, M9 medium was supplemented with glycerin, casamino acids, thiamine hydrochloride, IPTG, and arabinose, and cells were maintained at 37°C with shak-

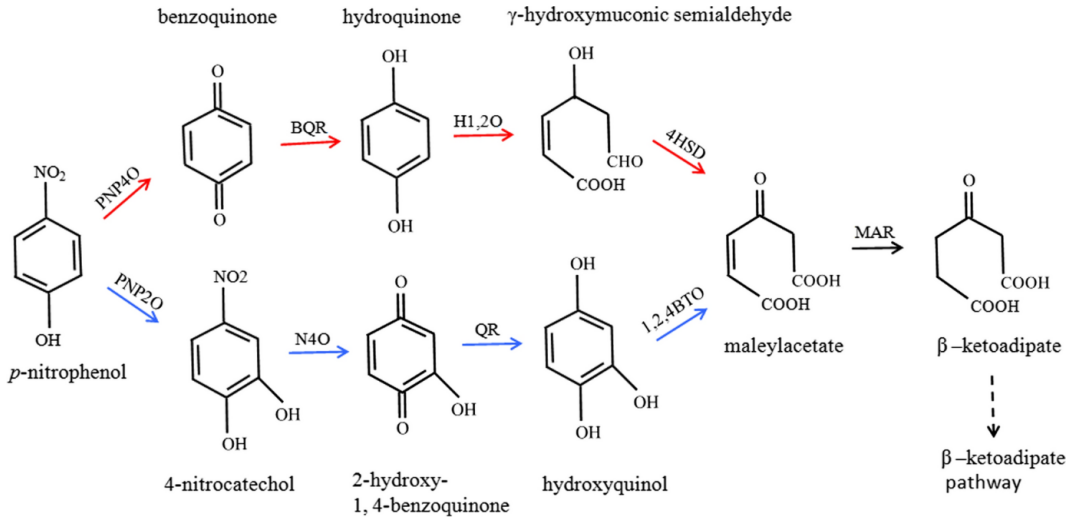

Figure 5. Degradation of PNP by engineered *E. coli* BL21-AI (Reused from Xu et al. (2021) under a Creative Commons Attribution 4.0 International License).

ing. After culture, cells were centrifuged, washed, and resuspended at an optical density value of 0.5 (at 600 nm) for the next assays. The modified BL-PNP strain was created to breakdown PNP by inserting a gene cluster from *Pseudomonas putida* that included five optimized genes (pnpAS, pnpBS, pnpCS, pnpDS, and pnpES). These genes were chemically synthesized to improve mRNA stability, optimize codon use, and remove possible regulatory regions. The gene cassette, which is connected between T7 promoters and terminators, was created using PAGE-mediated overlap extension PCR and inserted into the pCAMBIA1301 vector, yielding the plasmid pYB3847. *E. coli* BL21-AI was transformed with pYB3847, resulting in the engineered strain BL-PNP.

BL-PNP demonstrated significant PNP degradation at varied doses. Despite an early lag phase, 1 mM PNP was completely destroyed in 8 hours, whereas 5 mM PNP was totally degraded in 24 hours. At higher doses, such as 10 mM, breakdown was less effective, indicating that BL-PNP development is inhibited under these circumstances. During the degradation process at 1 mM PNP, Hydroquinone (HQ) concentration first increased, peaking at 8 hours with PNP depletion, then gradually decreasing, with 74% destroyed by 24 hours and becoming undetectable thereafter. The ultimate hydrolysis product, β-ketoadipate, progressively grew over the first 8 hours, peaked at 24 hours, and then gradually dropped. The control strain (BL-control) did not degrade PNPs and had no detectable HQ or β-ketoadipate, indicating the selectivity and effectiveness of BL-PNP degradation. The potential of BL-PNP for bioremediation of phenolic pollutants is demonstrated by its effective breakdown route via the HQ pathway in *E. coli*, which produces β-ketoadipate. This route connects to the bacterial metabolic network, allowing β-ketoadipate to join many anabolic pathways (Figure 5). While BL-PNP was successful in degrading PNP at varied concentrations, its inhibition at higher PNP concentrations (10 mM) implies that additional modification is required to improve performance under high pollutant levels. This work proposes a viable bioremediation technique for PNP and perhaps other phenolic pollutants based on genetically enhanced *E. coli* strains, which will contribute to improved environmental health and safety.

5 Merits and Demerits

5.1 Merits

The use of modified *E. coli* strains for pesticide bioremediation has various advantages and disadvantages that must be considered while developing and deploying this technique. In this section, we will go further into these issues. Engineered *E. coli* strains can be programmed to express specific enzymes that breakdown pesticides with great efficiency. For example, the modified strain BL-PNP produces *Pseudomonas putida* genes that allow it to convert PNP to less hazardous chemicals, thereby reducing this persistent pollutant (Xu et al., 2021). Engineered *E. coli* strains reduce the environmental and health concerns associated with pesticide residues by degrading toxic pesticides into non-toxic or less damaging compounds. The capacity to insert and optimize various genes inside *E. coli* enables the breakdown of a diverse spectrum of pesticides. For example, codon optimization and stability modifications to the pnpA-pnpE gene cluster provide successful expression in *E. coli*, allowing for strong biodegradation pathways (Xu et al., 2021). Engineered strains may be created to degrade a variety of organophosphates and nitroaromatic compounds, as demonstrated by strains capable of decomposing Paraoxon, Parathion, and PNP. *E. coli* is a well-known, simple-to-cultivate bacteria that grows quickly on cheap media. The investigations employed a basic M9 medium supplemented with glycerin, casamino acids, and thiamine hydrochloride, which is quite inexpen-

sive (Xu et al., 2021). The capacity to lyophilize modified strains for long-term preservation without loss of functioning, as proven with OPH-displaying *E. coli*, lowers the costs of maintaining live cultures and facilitates transport and deployment (Karbelkar et al., 2021). Engineered *E. coli* strains provide a more environmentally friendly alternative to chemical pesticide breakdown processes. They can be used in situ, at contaminated locations, minimizing the requirement for chemical treatments that may have negative environmental consequences. Degradation products like β-ketoadipate can safely integrate back into the environment through natural anabolic processes. Combining *E. coli* with other microbial species, such as *S. oneidensis* (Karbelkar et al., 2021), for electrical current production highlights novel techniques in which biodegradation can create beneficial byproducts such as energy, hence increasing the bioremediation process's sustainability.

5.2 Demerits

The introduction of genetically modified organisms (GMOs) into natural ecosystems raises serious concerns about horizontal gene transfer, unanticipated ecological effects, and long-term ramifications for microbial communities. Strict regulatory frameworks and robust containment methods are required to reduce these dangers. Public apprehension about GMOs due to safety concerns may impede the acceptance and deployment of engineered *E. coli* strains for bioremediation. Engineered strains frequently require precise environmental parameters, such as pH, temperature, and nutrition availability, to function well, which may not necessarily coincide with various contamination locations. High concentrations of target pesticides or pollutants can prevent engineered strains from growing and functioning properly. For example, BL-PNP growth suppression was seen at higher PNP concentrations (10 mM) (Xu et al., 2021). Furthermore, the stability of inserted genetic constructs may deteriorate with time due to mutations or plasmid loss, thereby lowering degradation efficiency. Continuous monitoring and maybe re-engineering may be required to keep functionality. The production of many foreign genes can place a metabolic strain on host cells, jeopardizing their fitness and survival in competitive natural settings. Deploying modified microbes necessitates negotiating complex regulatory approval processes that are both time-consuming and expensive. Compliance with national and international biosafety rules is a major concern. Ethical problems surrounding the alteration of microbial genomes and the introduction of GMOs into the environment necessitate open discussion, balancing technical developments with social concerns. Despite encouraging achievements in controlled laboratory conditions, scaling up modified *E. coli* strains to real-world applications presents significant obstacles. Environmental variables can influence the efficacy and consistency of bioremediation activities. Field applications demand continual monitoring to verify the continued activity and efficacy of modified strains, adding complexity and cost to operations. Using modified *E. coli* strains for pesticide bioremediation is a very promising strategy with advantages such as efficiency, adaptability, cost-effectiveness, and environmental sustainability. However, addressing issues such as biosafety assurance, operational optimization, genetic stability maintenance, regulatory compliance, and effective field deployment are critical. Finding a balance between these factors will be critical for the effective and appropriate use of modified *E. coli* strains in bioremediation programs.

6 Future Prospects

Engineered *E. coli* strains have enormous potential to revolutionize pesticide bioremediation, providing several advantages across technological, regulatory, environmental, and application domains. Future advances are projected to improve genetic engineering techniques, increasing the strength and efficacy of *E. coli* strains. CRISPR-Cas9 and other gene-editing tool advancements may allow for precise alterations, enhancing degradation pathways to treat a larger range of pesticides more efficiently. Synthetic biology integration might aid in the development of specialized metabolic pathways, allowing *E. coli* to breakdown various pesticide kinds simultaneously, giving a comprehensive pollution mitigation method. Prospective study attempts to fine-tune *E. coli* metabolic networks to increase tolerance to high pesticide concentrations and related byproducts. Strategies can include developing routes that reduce bacterial toxicity, improving their resistance and functioning in contaminated settings. Targeted deployment of modified *E. coli* might concentrate on site-specific remediation, using biobarriers and bioreactors to trap and treat toxins directly at pollution hotspots, reducing environmental dispersion and limiting ecological damage.

In agricultural situations, these bacteria might be included into everyday procedures using biofertilizers, encouraging crop development while removing leftover soil pesticides. Advanced biosensors and monitoring systems are ready to track *E. coli* presence and activity, assuring safe and efficient operation and limiting any environmental threats. Establishing strong regulatory frameworks is critical for monitoring genetically modified microbe deployment, assuring bioremediation performance, and addressing environmental and health problems. Public acceptability is dependent on open information regarding advantages, hazards, and safety measures. Ethical issues, such as the effects on natural microbial ecosystems and the hazards of gene transfer, demand thorough scientific study and societal debate. Beyond agriculture, applications include a wide range of industries dealing with chemical pollution, thanks to adaptive bioremediation systems that are suited to regional environmental circumstances and pesticide profiles.

7 Conclusion

Engineered *E. coli* strains have considerable potential for environmental biotechnology because they efficiently degrade pesticides like Paraoxon into innocuous molecules, providing a targeted and cost-effective solution to environmental and health problems. Biosafety problems, as well as ethical considerations for genetic changes and environmental repercussions, necessitate stringent regulation and monitoring. Future advances in genetic engineering and synthetic biology are predicted to improve *E. coli*'s bioremediation capacities even more. Creating site-specific remediation procedures and building public confidence via open communication are critical for widespread adoption and effective environmental stewardship. Overall, modified *E. coli* strains provide a disruptive approach to pesticide bioremediation, with the goal of considerably reducing environmental pollution and promoting sustainable practices in agriculture and beyond.

Acknowledgement

The authors thankful to all the authors whom work is reviewed in this article.

Author Contributions

RA & MK conceptualized, designed, and wrote the manuscript. JA analysed and interpreted the data. All authors reviewed the manuscript.

Conflict of Interest

No conflict of interest.

References

Afolabi, O.K., Aderibigbe, F.A., Folarin, D.T., Arinola, A., Wusu, A.D., 2019. Oxidative stress and inflammation following sub-lethal oral exposure of cypermethrin in rats: Mitigating potential of epicatechin. Heliyon, 5(8). https://doi.org/10.1016/j.heliyon.2019.e02274

Al-Ani, M. A., Hmoshi, R.M., Kanaan, I.A., Thanoon, A.A., 2019. Effect of pesticides on soil microorganisms. In Journal of physics: Conference series, Vol. 1294, No. 7, p. 072007. IOP Publishing. https://doi.org/10.1088/1742-6596/1294/7/072007

Alengebawy, A., Abdelkhalek, S.T., Qureshi, S.R., Wang, M.Q., 2021. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. Toxics, 9(3): 42. https://doi.org/10.3390/toxics9030042

Ali, R.I. and Ibrahim, M.A., 2018. Malathion induced testicular toxicity and oxidative damage in male mice: the protective effect of curcumin. Egyptian Journal of Forensic Sciences, 8: 1-13. https://doi.org/10.1186/s41935-018-0099-x

Ansari, I., El-Kady, M.M., Arora, C., Sundararajan, M., Maiti, D., Khan, A., 2021. A review on the fatal impact of pesticide toxicity on environment and human health. Global Climate Change, 361-391. https://doi.org/10.1016/B978-0-12-822928-6.00017-4

Arab, S.A., Nikravesh, M.R., Jalali, M., Fazel, A., 2018. Evaluation of oxidative stress indices after exposure to malathion and protective effects of ascorbic acid in ovarian tissue of adult female rats. Electronic physician, 10(5): 6789. https://doi.org/10.19082%2F6789

Aria, M. and Cuccurullo, C., 2017. bibliometrix: An R-tool for comprehensive science mapping analysis. Journal of informetrics, 11(4): 959-975. https://doi.org/10.1016/J.JOI.2017.08.007

Bansal, O.P., 2011. Fate of pesticides in the environment. Journal of the Indian Chemical Society, 88(10): 1525. https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/2664254

Bhatt, P., Zhou, X., Huang, Y., Zhang, W., Chen, S., 2021. Characterization of the role of esterases in the biodegradation of organophosphate, carbamate, and pyrethroid pesticides. Journal of Hazardous Materials, 411: 125026. https://doi.org/10.1016/j.jhazmat.2020.125026

Bornman, M.S., Aneck-Hahn, N.H., De Jager, C., Wagenaar, G.M., Bouwman, H., Barnhoorn, I.E., Heindel, J.J., 2017. Endocrine disruptors and health effects in Africa: a call for action. Environmental health perspectives, 125(8): 085005. https://doi.org/10.1289/EHP1774

Botwe, B.O., Ntow, W.J., Nyarko, E., Kelderman, P., 2012. Evaluation of occupational and vegetable dietary exposures to current-use agricultural pesticides in Ghana. Pesticides—recent trends in pesticide residue assay. In Tech, 46-62. http://dx.doi.org/10.5772/80105

Bouaziz, C., Graiet, I., Salah, A., Ben Salem, I., Abid, S., 2020. Influence of bifentrin, a pyrethriod pesticide, on human colorectal HCT-116 cells attributed to alterations in oxidative stress involving mitochondrial apoptotic processes. Journal of Toxicology and Environmental Health, Part A, 83(9): 331-340. https://doi.org/10.1080/15287394.2020.1755756

Brühl, C.A. and Zaller, J.G., 2019. Biodiversity decline as a consequence of an inappropriate environmental risk assessment of pesticides. Frontiers in Environmental Science, 7: 464007. https://doi.org/10.3389/fenvs.2019.00177

Chaudhry, G.R., Ali, A.N., Wheeler, W.B., 1988. Isolation of a Methyl Parathion-degrading *Pseudomonas* sp. that possesses DNA homologous to the opd gene from a Flavobacterium sp. Applied and Environmental Microbiology, 54(2): 288-293. https://doi.org/10.1128/aem.54.2.288-293.1988

Chen, J., Su, Y., Lin, F., Iqbal, M., Mehmood, K., Zhang, H., Shi, D., 2021. Effect of Paraquat on cytotoxicity involved in oxidative stress and inflammatory reaction: A review of mechanisms and ecological implications. Ecotoxicology and Environmental Safety, 224: 112711. https://doi.org/10.1016/j.ecoenv.2021.112711

Cohn, B.A., Wolff, M.S., Cirillo, P.M., Sholtz, R.I., 2007. DDT and breast cancer in young women: new data on the significance of age at exposure. Environmental health perspectives, 115(10): 1406-1414. https://doi.org/10.1289/ehp.10260

Damalas, C.A. and Eleftherohorinos, I.G., 2011. Pesticide exposure, safety issues, and risk assessment indicators. International journal of environmental research and public health, 8(5): 1402-1419. https://doi.org/10.3390/ijerph8051402

Degeronimo, K., 2015. Fragile ecosystems: pesticide use in conventional agriculture.

Diagboya, P.N., Mtunzi, F.M., Adebowale, K.O., Olu-Owolabi, B.I., 2021. Assessment of the effects of soil organic matter and iron oxides on the individual sorption of two polycyclic aromatic hydrocarbons. Environmental Earth Sciences, 80: 1-12. https://dx.doi.org/10.1002/elsc.200520098

Ding, J., Zhou, Y., Wang, C., Peng, Z., Mu, Y., Tang, X., Huang, Z., 2020. Development of a whole-cell biocatalyst for diisobutyl phthalate degradation by functional display of a carboxylesterase on the surface of Escherichia coli. Microbial Cell Factories, 19: 1-11. https://dx.doi.org/10.1186/s12934-020-01373-6

Dumas, D.P., Caldwell, S.R., Wild, J.R., Raushel, F.M., 1989. Purification and properties of the phosphotriesterase from *Pseudomonas diminuta*. Journal of Biological Chemistry, 264(33): 19659-19665. https://doi.org/10.1016/S0021-9258(19)47164-0

Elgueta, S., Santos, C., Lima, N., Diez, M.C., 2016. Immobilization of the white-rot fungus Anthracophyllum discolor to degrade the herbicide atrazine. Amb Express, 6: 1-11. https://dx.doi.org/10.1186/s13568-016-0275-z

Erdoğmuş, S F., Eren, Y., Akyıl, D., Özkara, A., Konuk, M., Sağlam, E., 2015. Evaluation of in vitro genotoxic effects of Benfuracarb in human peripheral blood lymphocytes. Fresenius Environmental Bulletin, 24(3): 796-799.

Erinle, K. O., Jiang, Z., Ma, B., Li, J., Chen, Y., Ur-Rehman, K., Zhang, Y., 2016. Exogenous calcium induces tolerance to atrazine stress in Pennisetum seedlings and promotes photosynthetic activity, antioxidant enzymes and psbA gene transcripts. Ecotoxicology and environmental safety, 132: 403-412. https://dx.doi.org/10.1016/j.ecoenv.2016.06.035

Ernst, F., Alonso, B., Colazzo, M., Pareja, L., Cesio, V., Pereira, A., Pérez-Parada, A., 2018. Occurrence of pesticide residues in fish from south American rainfed agroecosystems. Science of the total environment, 631: 169-179. https://dx.doi.org/10.1016/j.scitotenv.2018.02.320

FAO, W., 2019. Detoxifying Agriculture and Health from Highly Hazardous Pesticides: a Call for Action, vol. 24. FAO and WHO. Rome. https://apps.who.int/iris/bitstream/handle/10665/330659/9789241517065-eng.pdf

Feld, L., Hjelmsø, M. H., Nielsen, M. S., Jacobsen, A. D., Rønn, R., Ekelund, F., Jacobsen, C. S., 2015. Pesticide side effects in an agricultural soil ecosystem as measured by amoA expression quantification and bacterial diversity changes. PLoS One, 10(5): e0126080. https://dx.doi.org/10.1371/journal.pone.0126080

Filimon, M.N., Voia, S.O., Popescu, R.O.X.A.N.A., Dumitrescu, G.A.B.I., Ciochina, L.P., Mituletu, M., Vlad, D.C., 2015. The effect of some insecticides on soil microorganisms based on enzymatic and bacteriological analyses. Romanian Biotechnological Letters, 20(3): 10439-10447.

Furst, A.L., Hoepker, A.C., Francis, M.B., 2017. Quantifying hormone disruptors with an engineered bacterial biosensor. ACS central science, 3(2): 110-116. https://dx.doi.org/10.1021/acscentsci.6b00322

Grijalbo, L., Gutierrez Mañero, F.J., Fernandez-Pascual, M., Lucas, J.A., 2015. Photosynthetic and ultrastructure parameters of maize plants are affected during the phyto-rhizoremediation process of degraded metal working fluids. International journal of phytoremediation, 17(12): 1183-1191. https://dx.doi.org/10.1080/15226514.2015.1045132

Hassaan, M.A., and El Nemr, A., 2020. Egyptian Journal of Aquatic Research. https://doi.org/10.1016/j.ejar.2020.08.007

Huang, D., Hu, C., Zeng, G., Cheng, M., Xu, P., Gong, X., Xue, W., 2017. Combination of Fenton processes and biotreatment for wastewater treatment and soil remediation. Science of the Total Environment, 574: 1599-1610. https://dx.doi.org/10.1016/j.scitotenv.2016.08.199

Huang, H.S., Lee, K.W., Ho, C.H., Hsu, C.C., Su, S. B., Wang, J.J., Huang, C.C., 2017. Increased risk for hypothyroidism after anticholinesterase pesticide poisoning: a nationwide population-based study. Endocrine, 57: 436-444. https://dx.doi.org/10.1007/s12020-017-1373-7

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://dx.doi.org/10.3390/molecules23092313

Imoro, Z.A., Larbi, J., Duwiejuah, A.B., 2019. Pesticide availability and usage by farmers in the northern region of Ghana. Journal of Health and Pollution, 9(23): 190906. https://dx.doi.org/10.5696/2156-9614-9.23.190906

Itoh, K., Kanda, R., Sumita, Y., Kim, H., Kamagata, Y., Suyama, K., Tiedje, J.M., 2002. tfdA-like genes in 2, 4-Dichlorophenoxyacetic acid-degrading bacteria belonging to the Bradyrhizobium-Agromonas-Nitrobacter-Afipia cluster in α-Proteobacteria. Applied and Environmental Microbiology, 68(7): 3449-3454. https://dx.doi.org/10.1128/AEM.68.7.3449-3454.2002

Jacquin, L., Gandar, A., Aguirre-Smith, M., Perrault, A., Le Hénaff, M., De Jong, L., Jean, S., 2019. High temperature aggravates the effects of pesticides in goldfish. Ecotoxicology and Environmental Safety, 172: 255-264. https://dx.doi.org/10.1016/j.ecoenv.2019.01.085

Jalili, C., Roshankhah, S., Salahshoor, M.R., Mohammadi, M.M., 2019. Resveratrol attenuates malathion induced damage in some reproductive parameters by decreasing oxidative stress and lipid peroxidation in male rats. Journal of Family & Reproductive Health, 13(2): 70. https://dx.doi.org/10.18502/jfrh.v13i2.1912

Jawad, N., Kaur, R., Gouri, S.S., Dumka, V.K., Saini, S.P., 2017. Carbamate pesticide Carbaryl induces alterations in antioxidant and oxidative stress related responses of Indian buffalo (Bubalus bubalis) after sub-chronic exposure. Toxicology International (Formerly Indian Journal of Toxicology), 198-202. https://dx.doi.org/10.22506/ti/2017/v24/i2/162425

Jayabarath, J., Musfira, S.A., Giridhar, R., Arulmurugan, R., 2010. Biodegradation of carbofuran pesticide by saline soil actinomycetes. International Journal of Biotechnology & Biochemistry, 6(2): 187-193.

Kabra, A.N., Ji, M.K.; Choi, J., Kim, J.R., Govindwar, S.P., Jeon, B.H., 2014. Toxicity of Atrazine and Its Bioaccumulation and Biodegradation in A Green Microalga, Chlamydomonas Mexicana. Environ. Sci. Pollutr. 21: 12270–12278. https://doi.org/10.1007/s11356-014-3157-4

Kafilzadeh, F., Ebrahimnezhad, M., Tahery, Y., 2015. Isolation and identification of endosulfan-degrading bacteria and evaluation of their bioremediation in Kor River, Iran. Osong public health and research perspectives, 6(1): 39-46. https://dx.doi.org/10.1016/j.phrp.2014.12.003

Kankam, F., 2021. Causes and management of pesticides contamination in agriculture: A review. Ghana Journal of Science, Technology and Development, 7(2): 103-118. https://dx.doi.org/10.47881/265.967x

Karbelkar, A.A., Reynolds, E.E., Ahlmark, R., Furst, A.L., 2021. A microbial electrochemical technology to detect and degrade organophosphate pesticides. ACS Central Science, 7(10): 1718-1727. https://dx.doi.org/10.1021/acscentsci.1c00931

Kim, K.H., Kabir, E., Jahan, S.A., 2017. Exposure to pesticides and the associated human health effects. Science of the total environment, 575: 525-535. https://dx.doi.org/10.1016/j.scitotenv.2016.09.009

Kulkarni, A.G. and Kaliwal, B.B., 2009. Methomyl induced effects on free and immobilized Escherichia coli. Int J Biotechnol Res (IJBR), 2(2): 97-101.

Kulkarni, A.G. and Kaliwal, B.B., 2011. Effect of Methomyl on protease activity in free and immobilized Escherichia coli. International Journal of Microbiology Research, 3(2): 74. https://dx.doi.org/10.9735/0975-5276.3.2.74-78

Kulkarni, A.G. and Kaliwal, B.B., 2018. Bioremediation of Methomyl by Escherichia coli. Toxicity and Biodegradation Testing, 75-86. https://dx.doi.org/10.1007/978-1-4939-7425-2_4

Liu, C., Liu, Z., Fang, Y., Du, Z., Yan, Z., Yuan, X., Zhang, Z., 2022. Exposure to the environmentally toxic pesticide maneb induces Parkinson's disease-like neurotoxicity in mice: A combined proteomic and metabolomic analysis. Chemosphere, 308: 136344. https://dx.doi.org/10.1016/j.chemosphere.2022.136344

Liu, Y., Wang, X., Nong, S., Bai, Z., Han, N., Wu, Q., Ding, J., 2022. Display of a novel carboxylesterase CarCby on Escherichia coli cell surface for Carbaryl pesticide bioremediation. Microbial Cell Factories, 21(1): 97. https://doi.org/10.1186/s12934-022-01821-5

Lozowicka, B., Jankowska, M., Hrynko, I., Kaczynski, P., 2016. Removal of 16 pesticide residues from strawberries by washing with tap and ozone water, ultrasonic cleaning and boiling. Environmental monitoring and assessment, 188: 1-19. https://dx.doi.org/10.1007/s10661-015-4850-6

Lu, Y., Song, S., Wang, R., Liu, Z., Meng, J., Sweetman, A. J., Wang, T., 2015. Impacts of soil and water pollution on food safety and health risks in China. Environment international, 77: 5-15. https://dx.doi.org/10.1016/j.envint.2014.12.010

Luo, C., Huang, Y., Huang, D., Liu, M., Xiong, W., Guo, Q., Yang, T., 2018. Migration Transformation Characteristics of Niclosamide in a Soil–Plant System. ACS Omega, 3: 2312–2321. https://dx.doi.org/10.1021/acsomega.8b00071

Madani, N.A. and Carpenter, D.O., 2022. Effects of Glyphosate and Glyphosate-based herbicides like Roundup™ on the mammalian nervous system: A review. Environmental Research, 214: 113933. https://dx.doi.org/10.1016/j.envres.2022.113933

Mali, H., Shah, C., Raghunandan, B.H., Prajapati, A.S., Patel, D.H., Trivedi, U., Subramanian, R.B., 2023. Organophosphate pesticides an emerging environmental contaminant: Pollution, toxicity, bioremediation progress, and remaining challenges. Journal of Environmental Sciences, 127: 234-250. https://dx.doi.org/10.1016/j.jes.2022.04.023

Marie, L., Sylvain, P., Benoit, G., Maurice, M., Gwenaël, I., 2017. Degradation and transport of the chiral herbicide S-metolachlor at the catchment scale: combining observation scales and analytical approaches. Environmental Science & Technology, 51(22): 13231-13240. https://dx.doi.org/10.1021/acs.est.7b02297

McGlynn, K.A., Quraishi, S.M., Graubard, B.I., Weber, J.P., Rubertone, M.V., Erickson, R.L., 2008. Persistent organochlorine pesticides and risk of testicular germ cell tumors. Journal of the National Cancer Institute, 100(9): 663-671. https://dx.doi.org/10.1093/jnci/djn101

Merigó, J. M., Mas-Tur, A., Roig-Tierno, N., Ribeiro-Soriano, D., 2015. A bibliometric overview of the Journal of Business Research between 1973 and 2014. Journal of Business Research, 68(12): 2645-2653. https://dx.doi.org/10.1016/j.jbusres.2015.04.006

Mishra, S., Zhang, W., Lin, Z., Pang, S., Huang, Y., Bhatt, P., Chen, S., 2020. Carbofuran toxicity and its microbial degradation in contaminated environments. Chemosphere, 259: 127419. https://doi.org/10.1016/j.chemosphere.2020.127419

Mojiri, A., Zhou, J.L., Robinson, B., Ohashi, A., Ozaki, N., Kindaichi, T., Vakili, M., 2020. Pesticides in aquatic environments and their removal by adsorption methods. Chemosphere, 253: 126646. https://dx.doi.org/10.1016/j.chemosphere.2020.126646

Molina, E.M., Kavazis, A.N., Mendonça, M.T., Akingbemi, B.T., 2022. Effects of different DDE exposure paradigms on testicular steroid hormone secretion and hepatic oxidative stress in male Long-Evans rats. General and Comparative Endocrinology, 317: 113963. https://dx.doi.org/10.1016/j.ygcen.2021.113963

Murthy, K.S., Kiran, B.R., Venkateshwarlu, M., 2013. A review on toxicity of pesticides in Fish. International Journal of Open Scientific Research, 1(1): 15-36.

Murugesan, A.G., Jeyasanthi, T., Maheswari, S., 2010. Isolation and characterization of cypermethrin utilizing bacteria from Brinjal cultivated soil. African Journal of Microbiology Research, 4(1): 010-013.

Pakala, S.B., Gorla, P., Pinjari, A.B., Krovidi, R.K., Baru, R., Yanamandra, M., Siddavattam, D., 2007. Biodegradation of Methyl Parathion and p-nitrophenol: evidence for the presence of ap-nitrophenol 2-hydroxylase in a Gram-negative Serratia sp. strain DS001. Applied microbiology and biotechnology, 73: 1452-1462. https://doi.org/10.1007/s00253-006-0595-z

Park, S., Lee, J.Y., Park, H., Song, G., Lim, W., 2020. Bifenthrin induces developmental immunotoxicity and vascular malformation during zebrafish embryogenesis. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 228: 108671. https://dx.doi.org/10.1016/j.cbpc.2019.108671

Peng, R.H., Xiong, A.S., Yao, Q.H., 2006. A direct and efficient PAGE-mediated overlap extension PCR method for gene multiple-site mutagenesis. Applied microbiology and biotechnology, 73: 234-240. https://dx.doi.org/10.1007/s00253-006-0583-3

Pérez-Lucas, G., Vela, N., El Aatik, A., Navarro, S., 2019. Environmental risk of groundwater pollution by pesticide leaching through the soil profile. Pesticides-use and misuse and their impact in the environment, 17: 1-28. https://dx.doi.org/10.5772/intechopen.82418

Pérez-Parada, A., Goyenola, G., de Mello, F.T., Heinzen, H., 2018. Recent advances and open questions around pesticide dynamics and effects on freshwater fishes. Current Opinion in Environmental Science & Health, 4: 38-44. https://dx.doi.org/10.1016/j.coesh.2018.08.004

Pitzer, E.M., Williams, M.T., Vorhees, C.V., 2021. Effects of pyrethroids on brain development and behavior: Deltamethrin. Neurotoxicology and teratology, 87: 106983. https://dx.doi.org/10.1016/j.ntt.2021.106983

Qian, J., Li, J., Fang, D., Yu, Y., Zhi, J., 2014. A disposable biofilm-modified amperometric biosensor for the sensitive determination of pesticide biotoxicity in water. RSC Advances, 4(98): 55473-55482. https://dx.doi.org/10.1039/c4ra08468c

Ravula, A.R. and Yenugu, S., 2021. Pyrethroid based pesticides–chemical and biological aspects. Critical Reviews in Toxicology, 51(2): 117-140. https://dx.doi.org/10.1080/10408444.2021.1879007

Richins, R.D., Mulchandani, A., Chen, W., 2000. Expression, immobilization, and enzymatic characterization of cellulose-binding domain-organophosphorus hydrolase fusion enzymes. Biotechnology and bioengineering, 69(6): 591-596. https://dx.doi.org/10.1002/1097-0290(20000920)69:6%3C591::AID-BIT2%3E3.0.CO;2-X

Ruomeng, B., Meihao, O., Siru, Z., Shichen, G., Yixian, Z., Junhong, C., Baishan, F., 2023. Degradation strategies of pesticide residue: From chemicals to synthetic biology. Synthetic and Systems Biotechnology, 8(2): 302-313. https://dx.doi.org/10.1016/j.synbio.2023.03.005

Saari, S., Näreaho, A., Nikander, S., 2019. Chapter 5-Nematoda (roundworms). Canine Parasites and Parasitic Diseases, 83-149. https://doi.org/10.1016/B978-0-12-814112-0.00005-2

Sander, T., Farke, N., Diehl, C., Kuntz, M., Glatter, T., Link, H., 2019. Allosteric feedback inhibition enables robust amino acid biosynthesis in E. coli by enforcing enzyme overabundance. Cell systems, 8(1): 66-75. https://dx.doi.org/10.1016/j.cels.2018.12.005

Satapute, P. and Kaliwal, B., 2016. Biodegradation of propiconazole by newly isolated Burkholderia sp. strain BBK_9. 3 Biotech, 6(1): 110. https://dx.doi.org/10.1007/s13205-016-0429-3

Schmidt, R.J., Kogan, V., Shelton, J.F., Delwiche, L., Hansen, R.L., Ozonoff, S., Volk, H.E., 2017. Combined prenatal pesticide exposure and folic acid intake in relation to autism spectrum disorder. Environmental health perspectives, 125(9): 097007. https://dx.doi.org/10.1289/EHP604

Selmi, S., Rtibi, K., Grami, D., Sebai, H., Marzouki, L., 2018. Malathion, an organophosphate insecticide, provokes metabolic, histopathologic and molecular disorders in liver and kidney in prepubertal male mice. Toxicology reports, 5: 189-195. https://dx.doi.org/10.1016/j.toxrep.2017.12.021

Somara, S. and Siddavattam, D., 1995. Plasmid mediated organophosphate pesticide degradation by*Flavobacterium balustinum*. Biochemistry and molecular biology international, 36(3): 627-631.

Srivastava, A.K. and Singh, D., 2020. Assessment of malathion toxicity on cytophysiological activity, DNA damage and antioxidant enzymes in root of Allium cepa model. Scientific reports, 10(1): 886. https://dx.doi.org/10.1038/s41598-020-57840-y

Su, W., Hao, H., Wu, R., Xu, H., Xue, F., Lu, C., 2017. Degradation of mesotrione affected by environmental conditions. Bulletin of environmental contamination and toxicology, 98: 212-217. https://dx.doi.org/10.1007/s00128-016-1970-9

Sujetovienė, G., Gasauskaitė, K., Žaltauskaitė, J., 2019. Toxicity of a phenoxy herbicide on the lichen Ramalina fraxinea. Toxicological & Environmental Chemistry, 101(9-10): 497-507. https://dx.doi.org/10.1080/02772248.2020.1747466

Sule, R.O., Condon, L., Gomes, A.V., 2022. A common feature of pesticides: oxidative stress—the role of oxidative stress in pesticide-induced toxicity. Oxidative medicine and cellular longevity. https://dx.doi.org/10.1155/2022/5563759

Sun, S., Sidhu, V., Rong, Y., Zheng, Y., 2018. Pesticide pollution in agricultural soils and sustainable remediation methods: a review. Current Pollution Reports, 4: 240-250. https://dx.doi.org/10.1007/s40726-018-0092-x

Syafrudin, M., Kristanti, R.A., Yuniarto, A., Hadibarata, T., Rhee, J., Al-Onazi, W.A., Al-Mohaimeed, A.M., 2021. Pesticides in drinking water—a review. International journal of environmental research and public health, 18(2): 468. https://dx.doi.org/10.3390/ijerph18020468

Tan, H., Wu, Q., Hao, R., Wang, C., Zhai, J., Li, Q., Wu, C., 2023. Occurrence, distribution, and driving factors of current-use pesticides in commonly cultivated crops and their potential risks to non-target organisms: A case study in Hainan, China. Science of the Total Environment, 854: 158640. https://dx.doi.org/10.1016/j.scitotenv.2022.158640

Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., Phung, D.T., 2021. Agriculture development, pesticide application and its impact on the environment. International journal of environmental research and public health, 18(3): 1112. https://dx.doi.org/10.3390/ijerph18031112

Uchendu, C., Ambali, S.F., Ayo, J.O., Esievo, K.A.N., 2018. Chronic co-exposure to Chlorpyrifos and deltamethrin pesticides induces alterations in serum lipids and oxidative stress in Wistar rats: mitigating role of alpha-lipoic acid. Environmental Science and Pollution Research, 25: 19605-19611. https://dx.doi.org/10.1007/s11356-018-2185-x

Ullah, S., Li, Z., Hasan, Z., Khan, S.U., Fahad, S., 2018. Malathion induced oxidative stress leads to histopathological and biochemical toxicity in the liver of rohu (Labeo rohita, Hamilton) at acute concentration. Ecotoxicology and Environmental Safety, 161: 270-280. https://dx.doi.org/10.1016/j.ecoenv.2018.06.002

Whithaus, S.M. and Blecker, L.A., 2021. Pesticide Safety: A Study Manual for Private Applicators. UCANR Publications.

Xiong, Y., Wang, C., Dong, M., Li, M., Hu, C., Xu, X., 2023. Chlorphoxim induces neurotoxicity in zebrafish embryo through activation of oxidative stress. Environmental Toxicology, 38(3): 566-578. https://dx.doi.org/10.1002/tox.23702

Xu, J., Wang, B., Wang, M.Q., Gao, J.J., Li, Z.J., Tian, Y.S., Yao, Q.H., 2022. Metabolic engineering of Escherichia coli for Methyl Parathion degradation. Frontiers in Microbiology, 13: 679126. https://dx.doi.org/10.3389/fmicb.2022.679126

Xu, J., Wang, B., Zhang, W.H., Zhang, F.J., Deng, Y.D., Wang, Y., Yao, Q.H., 2021. Biodegradation of p-nitrophenol by engineered strain. AMB Express, 11: 1-8. https://dx.doi.org/10.1186/s13568-021-01284-8

Yadav, I.C. and Devi, N.L., 2017. Pesticides classification and its impact on human and environment. Environmental science and engineering, 6: 140-158.

Yousaf, S., Khan, S., Aslam, M.T., 2013. Effect of pesticides on the soil microbial activity. Pakistan Journal of Zoology, 45(4).

Zhang, W., 2018. Global pesticide use: Profile, trend, cost/benefit and more. Proceedings of the International Academy of Ecology and Environmental Sciences, 8(1): 1.

Zhongli, C., Shunpeng, L., Guoping, F.U., 2001. Isolation of Methyl Parathion-degrading strain M6 and cloning of the Methyl Parathion hydrolase gene. Applied and environmental microbiology, 67(10): 4922-4925. https://dx.doi.org/10.1128/AEM.67.10.4922-4925.2001